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Volume 18

Environmental Physiology

Editors

F. OBÁL
G. BENEDEK

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PHYSIOLOGICAL SCIENCES

Volume 18

Environmental Physiology

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Proceedings of the 28th International Congress of Physiological Sciences
Budapest 1980

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PHYSIOLOGICAL SCIENCES

Proceedings of the 28th International Congress of Physiological Sciences
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F. Obál
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Budapest, Hungary

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FOREWORD

This volume is one of the series published by Akadémiai Kiadó, the Publishing House of the Hungarian Academy of Sciences in coedition with Pergamon Press, containing the proceedings of the symposia of the 28th International Congress of Physiology held in Budapest between 13 and 19 July, 1980. In view of the diversity of the material and the "taxonomic" difficulties encountered whenever an attempt is made to put the various subdisciplines and major themes of modern physiology into the semblance of some systematic order, the organizers of the Congress had to settle for 14 sections and for 127 symposia, with a considerable number of free communications presented either orally or as posters.

The Congress could boast of an unusually bright galaxy of top names among the invited lecturers and participants and, naturally, the ideal would have been to include all the invited lectures and symposia papers into the volumes. We are most grateful for all the material received and truly regret that a fraction of the manuscripts were not submitted in time. We were forced to set rigid deadlines, and top priority was given to speedy publication even at the price of sacrifices and compromises. It will be for the readers to judge whether or not such an editorial policy is justifiable, for we strongly believe that the value of congress proceedings declines proportionally with the gap between the time of the meeting and the date of publication. For the same reason, instead of giving exact transcriptions of the discussions, we had to rely on the introductions of the Symposia Chairmen who knew the material beforehand and on their concluding remarks summing up the highlights of the discussions.

Evidently, such publications cannot and should not be compared with papers that have gone through the ordinary scrupulous editorial process of the international periodicals with their strict reviewing policy and high rejection rates or suggestions for major changes. However, it may be refreshing to read these more spontaneous presentations written without having to watch the "shibboleths" of the scientific establishment.

September 1, 1980

J. Szentágothai

President of the
Hungarian Academy of Sciences

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PREFACE

The more that has been learned about the physiological mechanisms in living organisms, the greater has become the interest in the responses of living organisms to environmental effects. It is logical that nowadays, in the age of chemicalization, intensive industrial development, cosmonautics and other extreme stress effects, this interest is progressively growing. The Organizing Committee of the 28th Congress of the International Union of Physiological Sciences therefore felt obliged to gather all studies scattered throughout the various fields of applied physiology into a separate section under the collective heading "Environmental Physiology". Indeed, the interest was equal to the expectations: a great many authors registered for participation in this section, which thereby became one of the most frequented. The large number of papers submitted moved the Organizing Committee to create a separate Gravitational Symposium too.

The incongruency between the many excellent studies representing the multilateral theme and the limited time available created an almost insoluble problem as regards the selection and grouping of the invited presentations and symposia. Hence, the themes appearing in this volume reflect only some of the most current problems in the field of environmental physiology. The study of circadian rhythms, which is at present yielding a wealth of new information, the problems and methods of thermoregulation, the complex problematics of osmoregulation, the environmental effects on sleep-wake cycles, as well as the effects of sport, training and work on physiological processes, were the points which were highlighted by the invited lectures and symposia.

I am convinced that the valuable presentations and discussions published in this volume have drawn new attention to the problems of environmental physiology and will promote the solution of the questions facing mankind.

I should like to express our gratitude to all participants for their valuable contributions, and primarily to the symposium chairmen and all authors.

Francis Obál

Chairman of the Local Organizing Committee
of the Section "Environmental Physiology"

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CIRCADIAN SYSTEM PROPERTIES

Jürgen Aschoff
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Én nem csalódom. Minden szervem óra,
mely csillagokhoz igazitva jár.
(I am not misled. All my organs are
clocks which run adjusted to the stars.)
Attila József, 1937

When Attila József wrote his poem 'Majd emlékezni jó lesz' (It will be good to remember) in which he refers to his inner clocks, the biological basis of such time measuring devices had yet to be discovered. As a true poet, József envisaged what was shown to be true about 25 years later: that there is a biological clock in man which measures time of day, and that it consists of a multiplicity of oscillating units, located in various organs and coacting as a complex entity called 'the circadian system'. In this lecture, I am attempting to introduce a few basic features of the circadian system to those who have become interested in the subject only recently. To a large extent, the survey is based on data which have been collected by my co-workers E.Gwinner, K. Hoffmann, H.Pohl, U. von Saint Paul, and R. Wever over the last 15 years.

1. FREERUNNING AND ENTRAINED RHYTHMS IN ANIMALS

By evolutionary adaptation to the temporal program of day and night, eucariothic organisms have developed endogenous periodic processes whose natural frequency approximates that of the earth's rotation and which persist in the absence of any periodic input to the organism. Since the period of the rhythm slightly deviates from 24 h under artificially constant conditions, the prefix circa has been introduced by Halberg (1959). Circadian rhythms, then, are characterized a) by their capability to freerun in constant conditions like self-sustaining oscillations, and b) by the way in which they are synchronized (entrained) by periodic factors in the environment, the zeitgebers. The two examples provided in Fig. 1 show rhythms in oxygen uptake of two chaffinches, kept initially in light-dark cycles of 12 h light and 12 h darkness (LD 12:12) and thereafter in conditions of constant dim illumination (LL). As dayactive animals, the birds have a high

level of oxygen uptake during L, and a low one during D. In LL, the rhythm persists undamped with a period, τ, which is longer than 24 h in the upper record, and shorter than 24 h in the lower record. This difference indicates that there can be a substantial interindividual variability in τ. In addition, τ is known to depend on the physiological state of the organism e.g. with regard to its reproductive functions, as well as on external factors such as intensity of illumination or ambient temperature. The effects of external factors show systematic differences between dayactive and nightactive species. (For a review, cf. Aschoff 1979a).

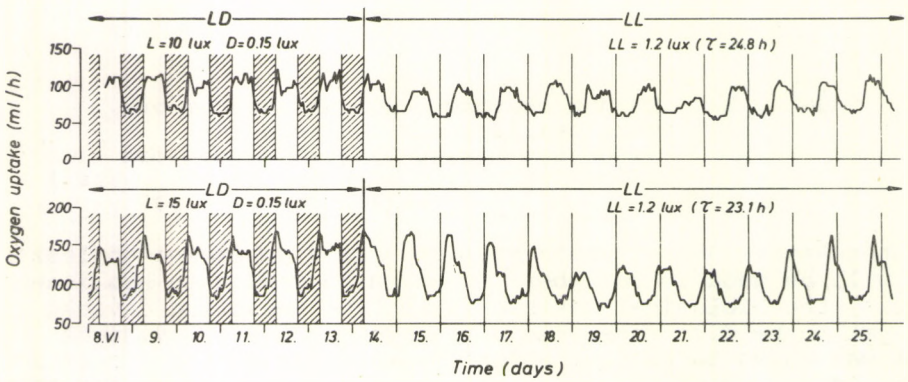

Fig. 1. Circadian rhythms of oxygen uptake in two chaffinches, Fringilla coelebs, kept first in a light-dark cycle (LD), thereafter in constant dim illumination (LD). τ = Mean circadian period. Shaded area: darkness. (From Pohl, published in Aschoff et al. 1980)

When entrained by a zeitgeber, the circadian rhythm maintains a distinct phase-relationship with the entraining signals. This phase-angle difference ψ might be measured between an arbitrary phase of the rhythm, e.g. a minimal value, and the time of 'light-on' of a LD-cycle. If in case of the records reproduced in Fig. 1 one takes as phase reference the point where oxygen starts to increase from its low D-level, it becomes evident that ψ has a small positive value (i.e. a leading phase) in the upper record, and a large positive value in the lower record. This difference in ψ is correlated with the difference in the τ-values (24.8 h in the upper record, 23.1 h in the lower record) and reflects the general rule that ψ depends on the ratio between the τ of a rhythm (as measured in constant conditions) and the period T of the entraining zeitgeber. In consequence of this rule, ψ also changes when a rhythm becomes entrained by zeitgebers with periods other than 24 h (cf. Fig.3). (For a discussion of these rules, cf. Aschoff 1965a, 1981a).

Next to a LD-cycle which is the prime zeitgeber for most organisms, a cycle of low and high temperature can entrain circadian rhythms at least in poikilothermic animals. In lizards, a cycle with a range of only 0.9°C suffices to entrain the activity rhythm of 1/3 of the animals tested (Hoffmann 1969). Homeiothermic animals are less easily entrained by temperature cycles. In squirrel monkeys, a range of 17°C has been found to be effective in about 50% of the animals tested (Fig. 2, left diagram). If entrainment is not achieved, the rhythm continues to freerun with a mean period which is continuously modulated by the signals from the zeitgeber. This relative coordination (Enright 1965) between the rhythm and a zeitgeber of insufficient strength is illustrated in the right diagram of Fig. 2. The period of the non-entrained activity rhythm is close to that of the zeitgeber when onset of activity coincides with the warm half of the temperature cycle, and it is lengthened when the onset of activity falls in the cold half of the cycle. Relative coordination also plays its role when various components of a freerunning circadian system loose their mutual coupling as in the case of internal desynchronization (cf. section 4, Fig. 9 and 10).

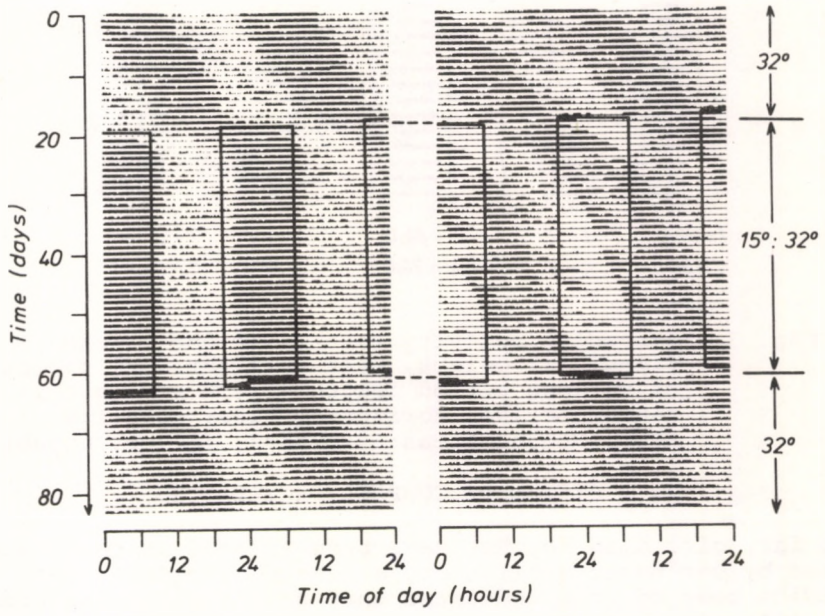

Fig. 2. Circadian activity rhythms in two squirrel monkeys, Saimiri sciureus, kept alternatively in constant temperature and in a temperature cycle. Original record duplicated along the abscissa. Within rectangles: time of higher temperature. (Tokura and Aschoff, unpubl.)

Circadian rhythms are only entrainable by zeitgebers within a limited range of periods. Within this range of entrainment the rhythm changes its phase-relationship to the zeitgeber according to the rule mentioned above: the rhythm phase leads a zeitgeber which has a relatively long period, and phase lags a zeitgeber with a short period. This is illustrated in Fig. 3 by the activity records from chaffinches. When kept in a LD-cycle with a period T = 24 h, the bird is dayactive but a late riser (onset of activity about 2 h after light-on). When T is changed to 25 h, the bird becomes an early riser, while in T = 23 h the normally dayactive bird becomes mainly nightactive. When T is lengthened to 26 h, or shortened to 22 h, entrainment is lost and the freerunning rhythm only shows relative coordination. It should be noted that a LD-cycle with 5 lux in L and 1 lux in D has been used in these experiments. Such a small range in intensity of illumination provides only a weak zeitgeber; hence the range of entrainment is very small, and the changes in ψ with changing T are large (cf. Aschoff and Pohl 1978).

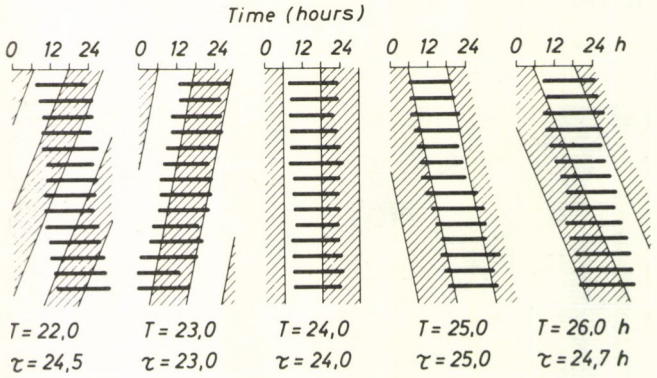

Fig. 3. Circadian activity rhythms of chaffinches, Fringilla coelebs, kept in light-dark cycles of various period length (T). τ = mean circadian period. Horizontal bars: activity time. Shaded areas: darkness. (Wever, unpubl.)

2. A SYSTEM OF COUPLED OSCILLATORS

So far, circadian rhythms have been treated as being controlled by one basic oscillator, a single circadian clock. A growing body of data indicates that such a one-oscillator model does not account for all the facts. Some evidence for a multi-oscillator system comes from the observation that freerunning rhythms of locomotor activity can be dissociated into two components which, for some time, run with different frequencies until they have reached a phase difference of 180°. Such splitting often occurs after a change in the intensity of illumination, as illustrated in Fig. 4. A for the tree shrew (Hoffmann 1971). Splitting was first described by

Pittendrigh (1960) for the arctic ground squirrel Spermophilus undulatus, and for the golden hamster Mesocricetus auratus (Pittendrigh 1967). Together with the fact that many activity records show two major peaks at the beginning and the end of each activity time (Aschoff 1957, 1966), the phenomenon of splitting prompted Pittendrigh (1974) to suggest that the circadian pacemaker is comprised of two separable oscillators which mutually entrain each other and whose period differentially depends on light intensity. An expansion of this idea and its implications can be found in Pittendrigh and Daan (1976).

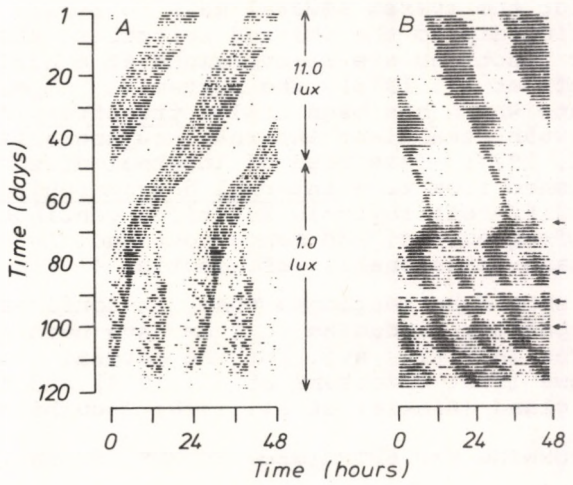

Fig. 4. Splitting of circadian activity rhythms in constant light; records duplicated along the abscissa. A) Tree shrew Tupaia belangeri (from Hoffmann 1971). B) European starling Sturnus vulgaris; arrows: injection of testosterone (from Gwinner 1974).

In discussing his findings with tree shrews, Hoffmann (1971) suggested that the endocrine system might be involved in the processes which result in splitting. This hypothesis is supported by Gwinner's (1975) observation that, in the European starling, the period of the freerunning rhythm and the activity time are subject to seasonal changes in correlation with the growth and regression of the testes, and that activity time can be lengthened by the administration of testosterone. At the time of maximal testes growth, Gwinner (1974) observed a splitting of the starling's activity rhythm, and was able to induce splitting in castrated birds by repeated injections of testosterone (Fig. 4 B). These results suggest that testosterone affects the mutual coupling of circadian oscillators.

Other data which, more indirectly, support the multi-oscillatory concept refer to the temporal interrelationship

between various rhythmic functions. If an organism is fully entrained by a zeitgeber, all rhythms usually maintain a distinct phase-relationship with each other. As has been shown by Pohl (1971) for three species of fringillid birds, these relationships can be altered when the birds are placed in constant conditions, and they are subject to changes when the period of the freerunning rhythm is altered due to a change in light intensity. This dependence of internal phase-relationships on experimental conditions will be discussed in more detail below (cf. section 3, Fig. 5 and 6). Drastic changes in the temporal patterns of circadian systems also occur during entrainment after a sudden shift of the zeitgeber. It usually takes for the system several days to regain its normal phase relationship to the shifted zeitgeber. Since different rhythmic functions are re-entrained at a different rate (cf. Aschoff et al. 1975), the system is temporarily out of order, a state which has been called transitory dissociation (Aschoff 1965b; transient internal desynchronization, Moore-Ede et al. 1977). This kind of internal dissociation is to be distinguished from true internal desynchronization, a state in which different rhythmic functions continue to run with different frequencies, and hence continuously change their mutual phase-relationship (cf. section 4, Fig. 8 to 10).

It finally should be mentioned that, in confirmation of József's antizipation, circadian rhythms have been documented in organ cultures in vitro, e.g. for the adrenal gland of hamsters (Andrews 1968; Shiotsuka et al. 1974) and for the chicken pineal gland (Binkley et al. 1978; Deguchi 1979)

3. FREERUNNING AND ENTRAINED RHYTHMS IN MAN

When enclosed in an isolation unit without time cues, human subjects usually show freerunning rhythms with periods somewhat longer than 24 h. In studies with 137 singly isolated subjects and 12 groups of two subjects each, a mean period ($\pm$ standard deviation) of 25.0 $\pm$ 0.50 h has been found (Wever 1979). The transition from the entrained to the freerunning state is accompanied by changes in the internal phase relationships. The left diagram of Fig. 5 exemplifies this for the rhythm of wakefulness and sleep and of oral temperature in a subject who was exposed to the natural zeitgebers for the first and last 7 days of the experiment, and was isolated from day 8 to 24. The rhythm of oral temperature remained close to 24 h during the first few days of isolation even though the activity rhythm was shortened for two cycles and then assumed a mean period of 26.1 h. As a consequence of this transitory internal dissociation (Aschoff and Wever 1976) the maxima of oral temperature were advanced relative to the sleep-wake cycle until, in the steady state of the freerunning rhythm, they occurred at the beginning instead of at the end of wakefulness. Similar changes in internal phase-relationships were observed in other autonomic functions, as shown in the right half of Fig. 5. In addition to the well expressed shifts in maxima and minima of the rhythms, the two diagrams indicate that the wave form was changed: the curves are skewed to the right in the entrained rhythm, and skewed to the left in the freerunning rhythm.

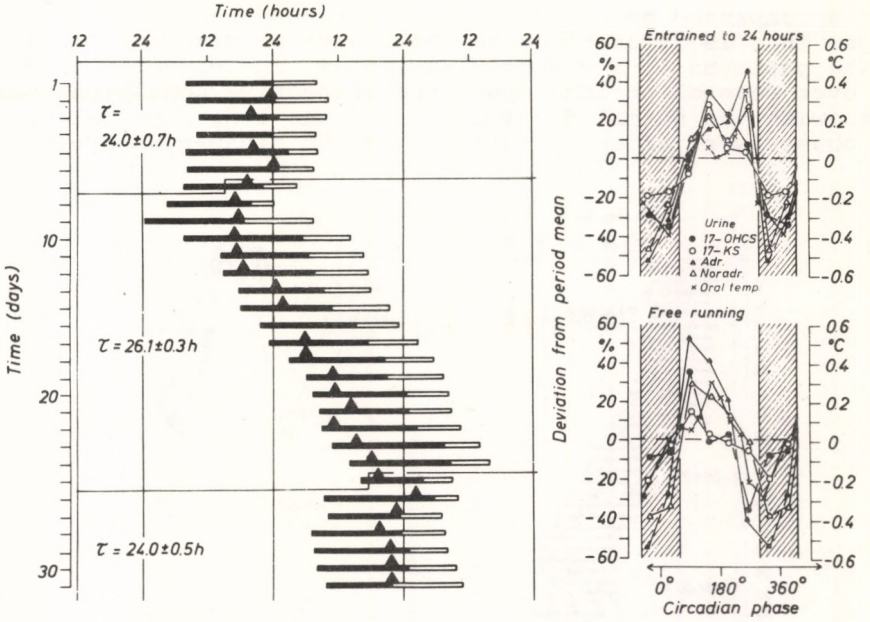

Fig. 5. Circadian rhythms of a subject exposed to the natural zeitgebers for the first and last 7 days, and isolated from day 8 to 24. Black and white bars represent wakefulness and sleep, triangles the maxima of oral temperature; $\bar{\tau}$ = mean circadian period ($\pm$ standard deviation). Right two diagrams: patterns of rhythms, averaged over 14 days under entrainment and 12 days during isolation. Shaded area: sleep. The abscissa represents 24 h in the upper diagram, and 26.1 h in the lower diagram. (Data from Kriebel 1974).

When exposed to an appropriate zeitgeber, e.g. a light-dark cycle complemented by regular gong signals (Wever 1970), human circadian rhythms can be entrained, within limits, to periods other than 24 h. In Fig. 6 the results are reproduced of an experiment in which the subject was able to make use of an additional reading lamp at will; hence, he was not forced to adher to the cycle of the main lights controlled from outside the chamber. Nevertheless, he remained entrained when the artificial 24-h day was lengthened to 26.67 h (left diagram of Fig. 6). At the same time he changed from a late riser to an early riser, a change in the external phase relationship that was to be expected in view of the rule mentioned above (cf. Fig. 3). When the zeitgeber period was shortened to 22.67 h, after an interlude of some 24-h days, entrainment was lost, and the subject's rhythms started to freerun with a period of 25.2 h.

The lengthening of the zeitgeber period from 24 to 26.67 h not only forced the subject to become an early riser, but was

also accompanied by changes in the internal phase-relationships. This is illustrated in the right two diagrams of Fig. 6 by the patterns of 3 rhythmic variables, individually averaged over several periods under the respective condition. Compared with the normal day (above), in the long day (below) all phases were advanced relative to the sleep-wake cycle.

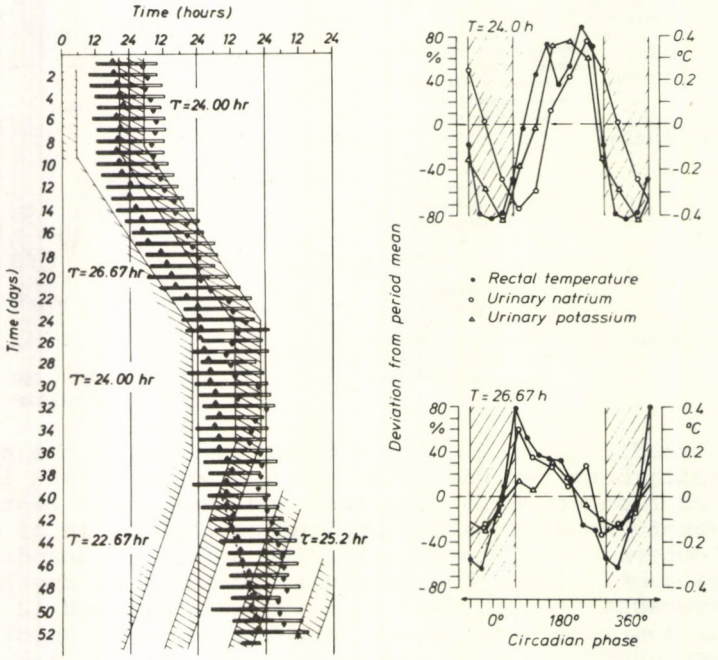

Fig. 6. Circadian rhythms of a subject exposed to an artificial zeitgeber, with reading lamp available. Left: Black and white bars represent wakefulness and sleep, triangles maxima (above bars) and minima (below bars) of rectal temperature; shaded area = darkness; τ = mean circadian period; T = zeitgeber period. Right two diagrams: patterns of rhythms, averaged over several periods under T = 24 and T = 26.67 h; shaded area = sleep (From Aschoff et al. 1969).

A summary of data from 6 subjects who were entrained to various zeitgeber periods is given in Fig. 7. The horizontal dashed line representing the middle of light time of the zeitgeber is used as a reference phase. Within the range of periods from about 23 to 27 h, all rhythms change their phase angle difference from large negative values (lagging phases) to less negative or even positive values. The slope of the curve is steeper for the autonomic functions than for the sleep wake cycle. This indicates that a smaller range of entrainment is to be expected for the autonomic functions than for the activity rhythm (Aschoff and Pohl 1978; Wever 1979).

The results obtained from 12 subjects in these and other experiments support this view: when exposed to a 26.67-h day or a 22.67-h day, some of the subjects remained entrained only with their sleep-wake cycle while the rhythms of body temperature and urinary excretion started to freerun (Aschoff et al. 1969)

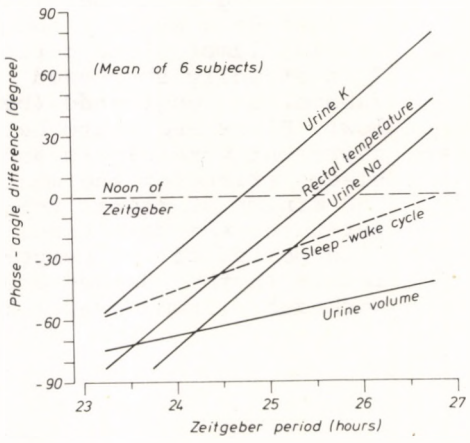

Fig. 7. Phase-angle differences between circadian rhythms and zeitgeber, derived from 6 singly isolated subjects who were entrained to various zeitgeber periods. Regression lines drawn through acrophases (computed maxima) as a function of zeitgeber period (From Wever 1972).

4. INTERNAL DESYNCHRONIZATION

That the entrainment of autonomic rhythmic functions is limited to a narrow range of periods can be easily demonstrated in subjects who are exposed to light-dark cycles but do not have access to reading lamps. Under those conditions, the subject can hardly do anything as long as the room is dark, and, hence, is more or less forced to adjust his activity-rest cycle to the cycle of light and dark. Consequently, it is possible to entrain the activity rhythm beyond the limits found with weaker zeitgebers as described above (cf. Fig. 6). The two examples provided in Fig. 8 show entrainment to light-dark cycles whose period is either steadily shortened (above) or lengthened (below) in small daily increments. In the upper record, the rhythm of rectal temperature (indicated by the triangles) becomes desynchronized from the activity rhythm when the zeitgeber has reached a period of about 22 h; in the lower record the same happens when the zeitgeber period approaches 27 h. Using this technique of forced internal desynchronization, Wever (1980) has been able to demonstrate that different overt rhythms split away from the activity cycle at different periods, indicating differences in the range of entrainment between various functions. One further comment has to be made regarding the two records reproduced in Fig. 8. As indicated in the diagrams, the τ-values of the freerunning temperature rhythms differ by 0.5 h. This should not be interpreted as indicating after-effects of the shortened or lengthened zeitgeber period on τ; at least, the data collec-

ted so far do not produce evidence for systematic effects of that kind (Wever 1980).

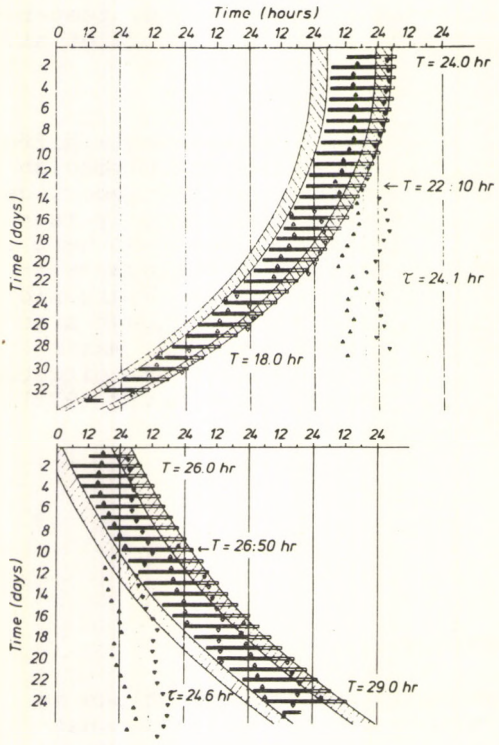

Fig. 8. Circadian rhythms of two singly isolated subjects under the influence of a strong zeitgeber (i.e. a light-dark cycle without reading lamp) whose period T is steadily shortened (above) or lengthened (below). Black and white bars represent wakefulness and sleep, triangles the maxima and minima of rectal temperature. τ = mean circadian period of the temperature rhythm. Shaded area: darkness. (From Wever 1980).

Internal desynchronization can always be enforced by applying a strong zeitgeber, but it also occurs spontaneously in freerunning rhythms. As shown in Fig. 9, a sleep-wake cycle (indicated by the sleep times only) may first freerun with a period of approximately 25.7 h and then suddenly lengthen to a mean period of 33.4 h. When this happens, the rhythm of rectal temperature desynchronizes from the activity rhythm and continues to freerun with a period of about 25.1 h. Several points should be noted: a) When coupled to each other (internal synchronization, day 1 to 13), the two rhythms maintain a distinct phase-relationship, the minima of temperature occurring at the beginning of each sleep time (cf. Fig. 5); b) During internal desynchronization, the minima of rectal temperature continuously change their phase-relationship to the sleep wake cycle; c) The duration of sleep continuously changes as the rhythm of rectal temperature crosses through the rhythm of sleep and wakefulness. The last observation points to the fact that, although synchronization between the two rhythms is lost there still is an interaction due to the coupling forces which are no longer strong enough to achieve entrainment. This persisting interaction between the uncoupled oscillators is indicated by the continuous modulation of the mean period, i.e. by relative coordination (Wever 1968; Aschoff 1974).

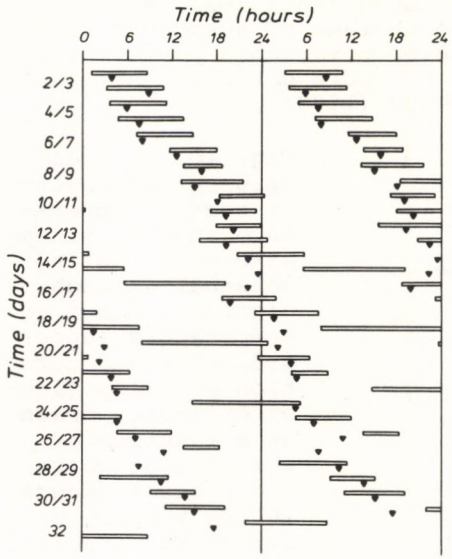

Fig.9. Freerunning circadian rhythms of a singly isolated human subject, showing spontaneous internal desynchronization on day 14 of the experiment. White bars: sleep time. Triangles: minima of rectal temperature. Data plotted twice along the abscissa. (Data from Wever 1979)

During internal desynchronization, the interaction between the uncoupled rhythms has a twofold consequence: it continuously modulates the duration of sleep, and it also modulates the amplitude of the rhythm of rectal temperature as documented earlier (Aschoff et al. 1967). Both these phenomena are illustrated in Fig. 10 on the basis of data from 10 subjects who all showed spontaneous internal desynchronization of their freerunning rhythms. From the lower diagram if can be seen that duration of sleep is longest when onset of sleep occurs several hours before the minimum of rectal temperature and sleep, hence, coincides with decreasing temperatures; duration of sleep is short when sleep coincides with increasing temperatures. The upper diagram of Fig. 10 demonstrates that the range of the rhythm of rectal temperature depends on the phase relationship between the two rhythms: sleep at the time of decreasing temperatures results in large ranges of the temperature rhythm, sleep at increasing temperature in small ranges. From the two diagrams, it is also clear that the occurrence of sleep, although not bound to a certain phase, is not equally distributed over al phases of the temperature rhythm. As a consequence of relative coordination, sleep times are accumulated at certain phases, and are less represented at other phases (cf. also Fig. 9 in Aschoff 1981b). In other words, when the two rhythms cross through each other, sleep times tend to lock on to the rhythm of temperature for some periods (accumulation of sleep times) and then drift away again (scarce representation of sleep).

All in all, these findings strongly support the concept that the circadian system has a multi-oscillatory structure with at least two groups of oscillators (pacemakers) which influence all overt rhythms but to a different extent. One

group with a high degree of persistence and a small variability of period mainly controls the rhythms of autonomic functions; the other group with a much more variable period mainly controls the sleep-wake cycle. For more details, the reader is referred to the monograph of Wever (1979).

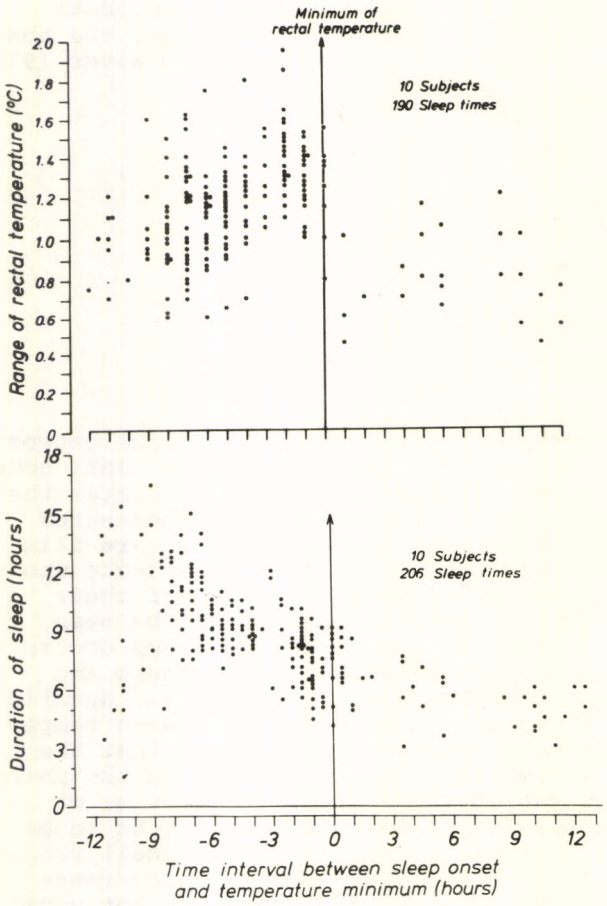

Fig. 10. Interaction between the rhythm of rectal temperature and the sleep-wake cycle during internal desynchronization. Range of temperature (upper ordinate) and duration of sleep (lower ordinate) drawn as a function of the time at which sleep onset occurs in the temperature cycle. The abscissa represents a full circadian cycle of rectal temperature with a mean period of 25 h. (Data from Zulley 1980 and Zulley et al. 1981)

A final example should suffice to elucidate the complex nature of the circadian system. In Fig. 11, data are presented from an experiment in which a rat alternatively was kept in one of two conditions: either with an ad libitum access to food, or a 4-h daily restriction of food availability. In continuous dim illumination and with food available all the time, the activity rhythm of the rat is freerunning with a period of about 24.6 h. This band of activity continues for some time after day 18 when food is only uncovered for 4 h per day. At the same time, a second component of activity appears with a phase lead to the feeding time which persists

as a 24-h band for several days after the feeding restriction is terminated. The fact that activity 'anticipates' feeding indicates that this component is not just a passive response to a stimulus; it must be locked to some clock which runs according to a 24-h day (i.e. the feeding schedule). On the other hand, the original freerunning rhythm of activity has not been entrained by this schedule, as is evident from its continuation after feeding restriction is terminated (see the oblique lines). The record demonstrates that there are 'distinct and dissociable components' (Pittendrigh 1960) within the same overt rhythm - some which can be entrained by a zeitgeber and others which continue to freerun.

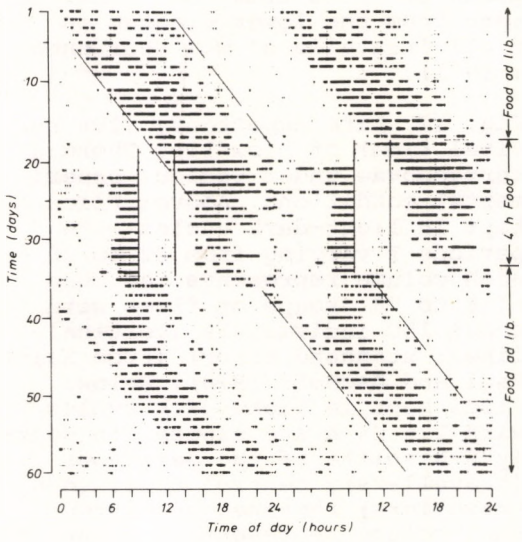

Fig. 11. Circadian activity rhythm of a rat kept in constant dim illumination with either an ad libitum access to food or a 4-h daily food availability (within vertical lines). Original record duplicated along the abscissa. (From Honma and Aschoff unpublished).

5. CONCLUDING REMARKS

In two of the foregoing sections, emphasis was placed on the multi-oscillator structure of the circadian system. This infers that there is more than one central pacemaker, and that each pacemaker may consist of two or more self-sustaining oscillators. The concept also implies that self-sustaining oscillators exist in the periphery, e.g. in single organs, and that there may be a kind of hierarchical order among the constituents of the system. In view of this complexity, it is remarkable to see to what a degree temporal order is maintained in the circadian system under normal conditions (Aschoff 1979b; Aschoff and Wever 1980). This order is provided by the mutual coupling among the constituents, as well as by the entraining signals from the zeitgebers. It can well be assumed that maintenance of the circadian temporal order is a prerequisit for well being, and that disturbances of the order can have harmful effects. Two sets of data obtained with the blowfly Phormia terraenovae support this view

Blowflies which are kept in light-dark cycles with a period T = 24 h have a life expectancy of about 125 days. When T is shortened to 23 h or less, or when it is lengthened beyond 26 h, the survival time of the flies is shortened by 10 to 30% (Fig. 12, upper diagram). The observation that optimal T-values seem to extent from 24 to 26 h, may be explained by the fact that blowflies tend to have freerunning activity rhythms with periods longer than 24 h (Saint Paul and Aschoff 1980). The second example concerns flies which always had been kept in light-dark cycles with T = 24 h, but which were phase-shifted (by shifts of the zeitgeber) for 6 h every week. By this procedure, 'Jet flights' were simulated in eastward or westward direction, or to and fro across the Atlantic. In each experimental series, a group of flies remained unshifted (non travellers). As can be seen from the lower diagram in Fig. 12, the survival time of all 'travellers' was shortened by about 25%, (Aschoff et al. 1971).

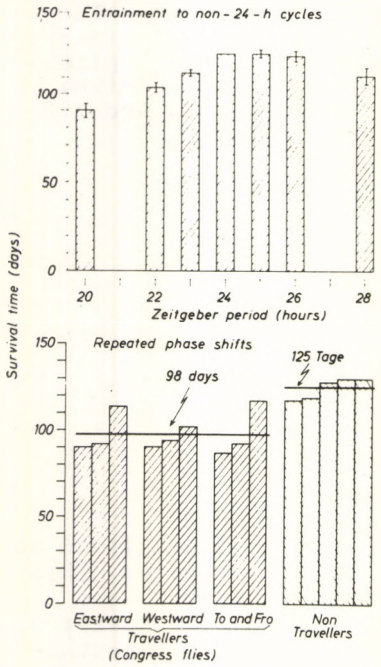

Fig. 12. Life expectancy (10% survival time) of blowflies Phormia terraenovae under various experimental conditions. Above: Flies kept in light-dark cycles with periods T varying from 22 to 28 h; each column represents the mean of 8 to 28 groups of flies with about 150 individuals (vertical lines: standard error) (From Saint Paul and Aschoff 1978). Below: Flies kept in light-dark cycles with T = 24 h and exposed to weekly 6-h shifts of the zeitgeber (travellers); control flies (non travellers) remained unshifted. Each column represents a group of 250 flies. (From Aschoff et al. 1971).

The circadian temporal organization has many implications for theoretical and practical medicine; it would need a further lecture to discuss all the aspects which are of relevance here. In essence, there seems to be hardly an area in etiology or in therapy as well as in the applied sciences such as ergonomics where the circadian system properties and its consequences for efficiency and responsiveness of the organism have not to be taken into account. The temporal structure provided by clocks which 'run adjusted to the stars' is of equal importance as the morphological structure.

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ANALYSING THERMOREGULATION WITH THERMODES AND ELECTRODES

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Thermoregulation is one of the most complex control systems in the body involving, as it does, behavioural and autonomic components. In this review I will concentrate on the autonomic aspects and discuss recent advances which have come from the use of combinations of three techniques: lesion making, local thermal stimulation and microelectrode recording.

The mechanisms for heat exchange with the environment are well understood. The various avenues of heat flow can be measured accurately in the laboratory, although the physics of heat transfer in a natural situation is very complex (Mitchell, 1974). The unsolved problems lie in understanding the neural control processes which govern the effector mechanisms and, at an earlier stage, the various inputs which feed into the control system.

It is generally agreed that in engineering terms the control system for temperature is of the negative feedback type as proposed by Hardy (1961) and elaborated by others. Opinions differ as to whether a reference signal is present or even necessary. The block diagram in Fig.1 shows a control system without a reference, which depends upon the balance between the two opposing feedback elements. Since it is well established that there are two such types of thermal sensor and since it is difficult to conceive or test for a fixed reference signal in neural terms, Fig.1 seems to be the preferred circuit for the temperature controller.

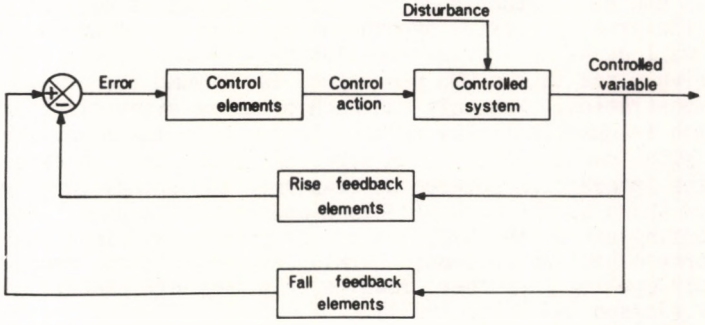

Fig.1. Block diagram of a feed-back control system without a reference signal but with two opposing inputs (From Mitchell, Snellen and Atkins, 1970).

Lesion making

This technique was an early one in attempts to discover which parts of the central nervous system contained the control elements (Fig.1). It has largely fallen into disuse. One cannot know if the lesion has destroyed relevant cells or fibres or both; the functions of the lesioned part can be taken over by the remaining tissue. However when used with finesse, lesioning can still provide useful information. By making micro-cuts in the rat hypothalamus, Gilbert and Blatteis (1977) have demonstrated that the connexions of the preoptic area are essential for cold-induced vasoconstriction, but not for cold thermogenesis. Another recent use of small electrolytic lesions (Taylor, 1980) is concerned with an input pathway. The thermoreceptors in the rat scrotum send information to the thalamus (Hellon and Misra, 1973; Jahns, 1975) and the hypothalamus (Nakayama et al., 1979). Neurones in both these areas give characteristic abrupt increases in their activity for a very slight increase in scrotal temperature. The creation, in an acute experiment, of a very small lesion confined to one of the brain stem raphe nuclei can eliminate the responses of thalamic and hypothalamic cells. Clearly the ascending pathway must pass through or relay in this raphe nucleus rather than the conventional spinothalamic tracts through the lemnisci. It remains to be demonstrated whether the thermal information from the trunk skin in general also uses this route, but trunk thermal information certainly reaches the raphe nuclei (Dickenson, 1977).

Thermode studies

By implanting localized thermodes in various central regions of the body, investigators have, over the years, been able to show which regions have a thermosensitivity which can drive the thermoregulatory system in the expected direction: local heating excites heat loss responses so lowering other central temperatures and local cooling has the opposite effects.

The classical work of Ranson's group established the exact site of warm-sensitivity in the cat's preoptic region. Since then many others have implanted thermodes in the hypothalamus and other regions of the CNS (for reviews see Hammel, 1968; Hellon, 1972; Reaves and Hayward, 1979). It is now well established that local cooling as well as warming in the preoptic area, posterior hypothalamus, midbrain, medulla, and spinal cord will evoke appropriate regulatory responses. The relevant thermosensitive sites in the CNS now extend far beyond the original preoptic demonstration. Not only can each of these sites elicit a response which is quantitatively related to the stimulus magnitude, but the several sites can interact in an algebraic function. An excellent example is the interaction between hypothalamic and spinal cord temperatures in dogs shown by the work of Jessen and his colleagues. For instance, cooling either the hypothalamus or spinal cord in a conscious dog will increase heat production; warming either of these regions while simultaneously cooling the other, results in a complete cancellation of the response (Jessen and Simon, 1971).

Besides this widespread and interactive sensitivity in the CNS, it is now established that other central regions can also provide inputs to

the thermoregulatory controller. Rawson and Quick (1972) were the first to demonstrate that abdominal heating in sheep could drive heat loss responses. Comparable sensitivity exists in the rabbit (Riedel et al., 1973). At the moment the exact whereabouts of the relevant receptors remains uncertain. The most recent evidence suggests that thermosensitivity outside the CNS may be more widespread and more powerful than had been realised. By implanting tubular heat exchangers within the great veins of goats and at the same time clamping the CNS temperature, Mercer and Jessen (1978) have clearly shown that the body core, exclusive of the CNS, can provide a strong input to the temperature controller. Furthermore, as another example of integration, the core and CNS signals were found to interact.

The total evidence indicates that many regions of the body core can provide potential drives to thermoregulation under experimental circumstances. A vital question is, do they do so in real life, and if so, under what conditions.

Sadly, we know very little about natural fluctuations in CNS or core temperature. Most is known about the hypothalamus and this evidence shows that only very slight changes in temperature occur when powerful responses like shivering or panting are activated. The slight natural changes are far smaller than would be necessary to drive the same responses if only one particular site was being warmed or cooled in an experiment. But in real life it would be very unusual for just one region to be affected. All central regions would tend to warm or cool together due to the mixing action of the circulation. Hence it may be the combined input of all the core and CNS thermosensitive sites which provides the input signal. The new technique of manipulating the whole core temperature (Jessen, et al., 1977) suggests that this may be so.

There seems to be an urgent need for more measurements of central temperatures during thermoregulatory responses. The observations which have been made (eg. Necker, 1979) suggest there are random changes in CNS temperature which could not provide any recognizable thermal input, at least in resting animals. All the recent evidence points to multiple inputs from widespread internal sites acting in concert with the other major input which comes from the skin thermoreceptors. While the warm and cold receptors alone could not provide the sufficient inputs for stable thermoregulation, once again an interaction between skin temperature and core temperature occurs. Numerous studies have shown how the responses to imposed changes of central temperature are modulated by varying skin temperature (eg. Brück and Wünnenburg, 1967; Stitt, 1976; Hellstrom and Hammel, 1967).

Microelectrode studies

What more can we learn about the processes of thermoregulation by studying the single elements of the system and recording from individual neurones? Writing on this question eight years ago Eisenman (1972) stated '....electrophysiological studies have served mainly to confirm data obtained by other methods, such as thermal stimulation and ablations. Thus, we have confirmed the presence of highly thermosensitive cells in the preoptic area. Inputs from spinal cord to hypothalamus and convergence of activity in the posterior hypothalamus have likewise been confirmed'.

Eisenman, with Nakayama and Hardy (Nakayama et al., 1961) was the first to make single neurone recordings in the anterior preoptic hypothalamus and found a number of cells whose activity was very sensitive to local temperature. The correlation between discharge rate and temperature

was positive - as temperature rose so did activity. Since then others have found, in addition, the opposite kind of response with a negative correlation (see Reaves and Hayward (1979) for a review of recent work). So it is very reasonable to postulate that these neurones, a small percentage of those in the hypothalamus, are responsible for the effects of thermode warming or cooling in a conscious animal.

What is the cellular basis for this central thermosensitivity? Is the neuronal membrane itself showing a high degree of temperature dependence or is the sensitivity a synaptic property and therefore involving a number of neurones? The answer will come from in vitro preparations of the hypothalamus in which intracellular recordings will be possible. Hori et al. (1980) have already shown that extracellular recordings in hypothalamic slices show the same kind of thermal sensitivity as in whole brains.

Besides the recordings in the anterior hypothalamus, temperature neurones have been characterized in other parts of the CNS where thermodes have shown there is thermosensitivity capable of driving effector responses. These extra-hypothalamic areas include the posterior hypothalamus, the midbrain and pons, the medulla, and the spinal cord (Reaves and Hayward, 1979). The responses of these neurones to their local temperature is much the same as in the anterior hypothalamus. Furthermore, just as in the thermode studies already described, investigators have found interactions between one thermosensitive site and another, so with microelectrodes, the same can be demonstrated. For example, a preoptic cell, besides its own thermal sensitivity, might respond also to imposed temperature changes in the spinal cord or in the skin. Endless permutations are possible and have been found (eg. Hardy and Guieu, 1971).

Ingenious though these schemes and models are, they have serious weaknesses and their predictive value is limited. In the first place, it is probable that the imposed temperature changes are many times larger than occur in real life, so the responses may not be physiological. Next, it is only possible to guess at the function of a particular neurone. Is it playing an afferent, integrative or efferent role? In such a diffuse system, answers to this question can only be tentative. The new anatomical techniques such as horseradish peroxidase tracing and metabolic marking with 2-deoxyglucose will undoubtedly provide valuable new information.

One area where neurophysiology can be useful is in analyzing the thermal input from the skin. One example has already been given of the very dramatic central response to slight warming of the rat's scrotum. This indicates that there can be much processing of the incoming thermal information, but the scrotal pathway is probably not representative of the general skin input. In the trigeminal system, higher order neurones behave very like the skin temperature sensors (Poulos and Molt, 1976; Dostrovsky and Hellon, 1978). But like most sensory systems, these trigeminal thermal neurones are under inhibitory control generated by the activity of non-thermal mechanosensitive afferents from the face as Fig.2 demonstrates.

The present situation, at least in the writer's understanding of thermoregulation, is that we still need to define the physiological inputs to the temperature controller, where they come from and when they are activated; especially those outside the CNS. For this task the blunt weapon of the thermode must come before the rapier of the microelectrode.

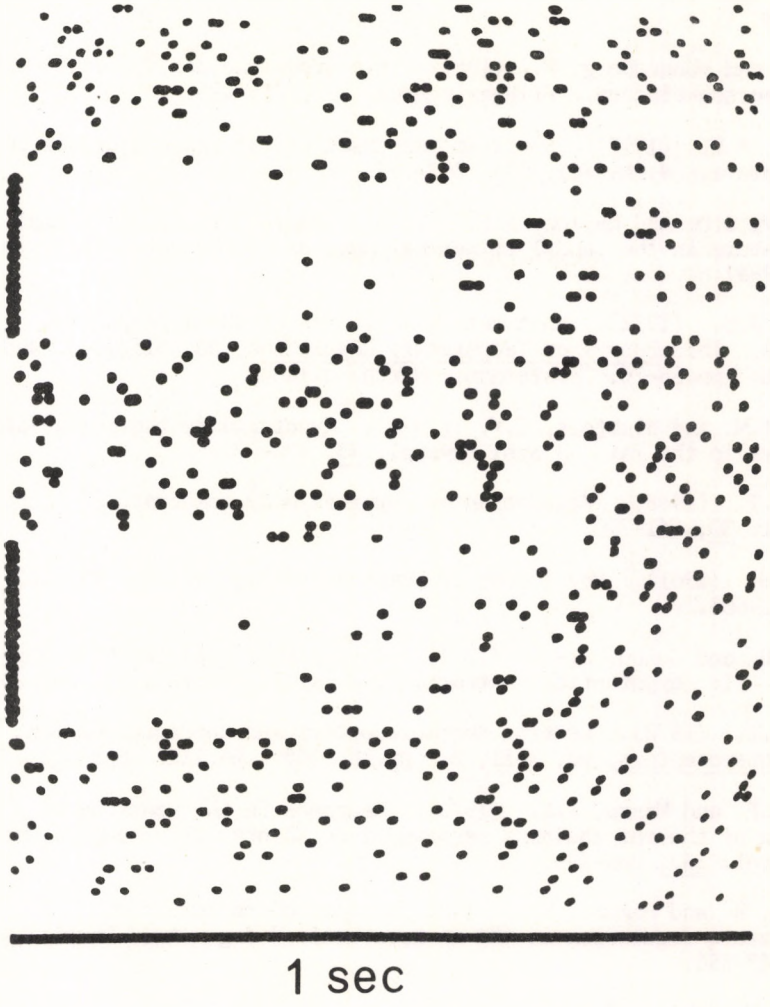

Fig.2. Raster display of the continuous activity of a neurone in the rat trigeminal nucleus caudalis excited by cold receptors on the whisker pad. Each dot corresponds to one action potential. The sweep length and sweep interval are both 1 sec. The two groups of artifacts on the left indicate electrical stimulation of large fibres in the whisker pad. Each stimulus inhibited the 'cold' activity for about 500msec. (unpublished result of Dawson, Dickenson, Hellon and Woolf).

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OSMOREGULATION

Chairman:

H. T. HAMMEL (USA)

Adv. Physiol. Sci. Vol. 18. Environmental Physiology
F. Obál, G. Benedek (eds)

OSMOREGULATION, INTRODUCTORY QUESTION: CAN WE OUTLINE THE NEURAL NETWORK FOR OSMOREGULATION IN VERTEBRATES?

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When solutes or water are in excess in the fluids of the body, they are excreted by osmoregulatory processes. Since the amount of any solute is the product of its concentration and the fluid volume in which it is dissolved, we may anticipate that an excess is sensed by receptive elements sensitive to concentration as well as to the appropriate volume. Further, since the system functions to eliminate only the excess, we may anticipate that the system recognizes a threshold concentration and/or a threshold volume which exceeded, signals a corresponding organ to eliminate the excess. Similarly, when solutes or water are deficient in the fluids of the body, we may anticipate that receptors sense the concentration as well as the volume and the system recognizes these values to be less than threshold values and activates responses to minimize the deficiency by ingestion, reduced elimination or mobilization of substances from reserves. The question to be considered by this symposium on osmoregulation is: to what extent can we describe the neural network which maintains the amounts of solutes and water within the ranges that are optimal for the body tissues? Essential aspects of an exploration of osmoregulation are to determine: 1) the properties of the body fluids which are transduced to neural signals, 2) the location of the receptive sites in the peripheral as well as the central nervous system, 3) how and where the several neural and humoral signals are integrated, 4) how the integrated information is communicated by neuro-humoral connections with the responding organs and 5) how this central control interacts with local and systemic regulatory processes not involving the CNS. An ultimate goal is to describe the neural network which accomplishes osmoregulation in each of the vertebrate classes and enables central coordination of osmoregulation with other centrally controlled homeostatic functions. The contributing authors were invited to describe the condition in the vertebrate class most familiar to them in so far as they perceive that to be possible.

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CONTROL OF SECRETION IN REPTILIAN SALT GLANDS

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THE SALT GLANDS

Types and Phylogenetic Distribution

In the following discussion a small number of studies relating to the question of control or regulation of salt gland function will be evaluated. For a broader treatment of salt glands and reptilian osmoregulation, the reader is referred to a series of recent reviews (Peaker and Linzell, 1975; Minnich, 1979; Dunson, 1976, 1979).

There are five different kinds of head glands in reptiles that have become modified into salt glands (Dunson, 1976, 1979; Dunson and Dunson, 1979; Taplin and Grigg, submitted; Table 1).

Table 1. The occurrence of salt glands in reptiles

Family	Generic Example	Gland Type
Turtles		
Dermochelyidae	*Dermochelys*	Lachrymal
Cheloniidae	*Chelonia*	Lachrymal
Emydidae	*Malaclemys*	Lachrymal
Crocodilians	*Crocodylus*	Lingual
Lizards		
7 families		Nasal
Snakes		
Hydrophiidae	*Pelamis*	Posterior sublingual
Acrochordidae	*Acrochordus*	Posterior sublingual
Homalopsidae	*Cerberus*	Premaxillary

When one considers that birds (nasal glands) and elasmobranchs (rectal glands) only have a single gland type, the diversity among reptiles is remarkable. This must reflect the independent evolution of salt glands at least five times. In fact the appearance of homologous glands in separate families (such as the sea turtles Dermochelyidae and Cheloniidae and the pond turtles Emydidae) probably also represents independent development since these three groups are so distinct in other ways. Among the snakes, convergent evolution is also the most likely explanation for the appearance of posterior sublingual salt glands in two very dissimilar

families. All known lizard salt glands are nasal, yet it is impossible to say at present if any of the families involved represent examples of independent development of this character. The diverse evolutionary history of reptilian salt glands needs to be recalled when one is tempted to generalize for all reptiles on the basis of findings derived from the studies of only a few species. As will become apparent in the subsequent remarks, our general knowledge of reptilian salt glands is fragmentary. It is often necessary to use data derived jointly from studies of the salt glands of turtles, lizards and snakes to construct a unified view of function. This should be viewed merely as a temporary expedient to be utilized until the data base enlarges and allows a realistic appraisal of similarities and differences between gland types and major taxonomic groups.

The adaptive significance of salt glands in the various reptilian lineages is an interesting topic that can only be briefly mentioned here. It is apparent that the greatest degree of salt gland development has occurred among marine reptiles, especially those of pelagic habits or those whose diets are especially high in salts. The former situation is well exemplified by the yellow-bellied sea snake (Pelamis) which feeds on fish and apparently never drinks sea water. The maximum secretory rate of the salt gland (140 μmoles Na/100 g body wt·h) is about 20 times the balanced whole body influx and efflux of Na (Dunson, 1968). Thus any increase in extracellular fluid Na can readily be excreted. The Galápagos marine iguana (Amblyrhynchus), which lives on rocks along the coast and feeds on marine algae, is an example of the latter situation. Its large nasal salt gland can secrete more Na and K (up to 255 and 51 μmoles/100 g·h respectively) than that of Pelamis (Dunson, 1969). Desert lizards face similar problems in gaining free water and in excreting salts. However K and anions other than Cl are often more important in terrestrial ecosystems and the overall value of extracloacal excretion is diminished in comparison with many marine species. The nasal glands of many terrestrial lizards have a great degree of lability in varying the secretory output to match the intake of ions (Shoemaker et al, 1972). The extent to which this lability is related to environmental stresses is not yet well established. However it is clear that there is considerable diversity in the composition and rate of secretion of reptilian salt glands. Thus caution should be exercised in generalizing from specific cases without a careful evaluation of the habits that presumably led to the development of extracloacal salt excretion.

Structure of Reptilian Salt Glands

All reptilian salt glands have one feature in common, the presence of a major cell type, the principal cell (Abel and Ellis, 1966; vanLennep and Komnick, 1970; Dunson, 1976). Less numerous cell types are probably either early stages of the principal cells, or involved in minor functions of the salt gland. There is the possibility that an additional cell type in desert lizard salt glands, the tuft cell, is also directly involved in salt transport (Ellis and Goertemiller, 1974).

Two main features of the principal cells are of particular interest, (1) the degree to which they differ from avian salt gland principal cells in surface membrane architecture, and (2) the nature of the apical junctions between cells. These two points are important in elucidating the mechanism by which the hyperosmotic secretion occurs. A commonly encountered view in the literature (see Berridge and Oschman, 1972) is

that avian and reptilian principal cells are extremely similar; confusion is often evident over the homologies of reptilian glands which have been described above. It is true that avian and reptilian principal cells are similar in that they both possess large numbers of mitochondria and greatly elaborated cell surfaces. However the avian glands achieve this by deep invaginations along the basal surfaces of the cells; these infoldings extend in some cases all the way to the nucleus and include mitochondria within them. In contrast it is mainly the lateral portions of reptilian principal cells that are covered with numerous slender evaginations lacking mitochondria. VanLennep and Komnick (1970) were the first to point out that a similar amount of surface amplification occurs in both cases, but that the avian basal infoldings are associated with a much higher rate of fluid flow. Such a relationship between structure and function even occurs within a single species. The fresh water adapted adult mallard duck has a reptilian type ultrastructure without basal invaginations (Martin and Philpott, 1973). The basal infoldings only develop after salt adaptation. Similarly, salt glands of terrestrial birds that secrete at low rates have a reptilian appearance (Dunson et al, 1976).

Ions moving from the blood to the lumen of the salt gland could pass either through the principal cells or through the intercellular spaces and the apical junctions. Most early observers (for example see vanLennep and Komnick, 1970) reported "tight" apical cell junctions or zonula occludens in reptilian salt glands, apparently ruling out the intercellular (paracellular) route. However recent freeze fracture studies (Riddle and Ernst, 1979) have shown that the avian zonula occludens is similar in structure to junctions of epithelia known to be "leaky" to ions. Yet the rectal salt gland analyzed with similar techniques shows a junctional configuration that is considered to be "tight" (Forrest et al, 1979). It is apparent that each type of reptilian salt gland should be examined by freeze fracture techniques to determine the junctional structure. It may finally be possible to compare the properties of the apical cell interactions in different salt glands on a more quantitative basis than has previously been possible, and to improve the accuracy of rather speculative cell transport models.

Secretory Characteristics

Few presumed reptilian salt glands have actually been cannulated (Dunson, 1976). Thus some scepticism regarding reported secretion concentrations is necessary, especially for lizards where salt encrustations can build up on the nares or in the nasal cavity. The most reliable comparative data on concentration among genera in a single family have been obtained on sea snakes (Dunson and Dunson, 1974). After injection of a salt load the NaCl concentration of the gland secretion rises to a plateau level which is maintained for hours (Fig. 1). Plateau concentrations are fairly constant within the same genus, despite wide interspecific variations in flow rates. For example, members of the genus Aipysurus secrete a fluid near 800 mM Cl, whereas Hydrophis species produce a fluid only about 500 mM Cl. Yet flow rates overlap and it appears that change in salt gland weight is the major factor regulating the total rate of excretion within a genus. The genus Aipysurus shows a ten-fold variation in Cl excretion rate, from 24 to 222 μmoles/100 g·h (Dunson and Dunson, 1974).

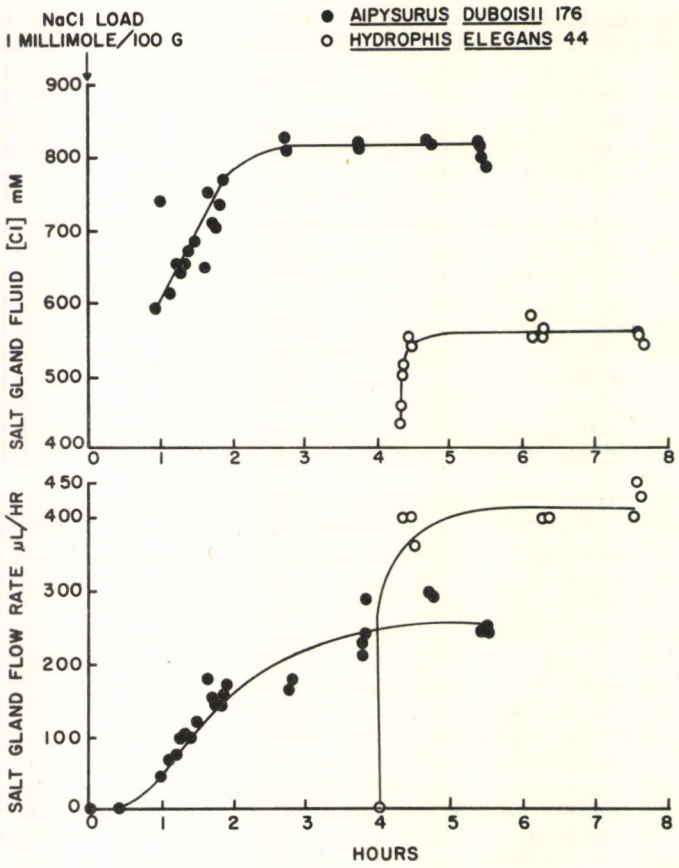

Fig. 1. Changes in secretion concentration and flow rate of sea snake salt glands after injection of salt loads at time 0 (from Dunson and Dunson, 1974).

The secretory fluid in sea snakes is predominantly NaCl; only 2% is K. Of course this is in response to NaCl injections. It would be interesting to test the effect of K loading on secretion composition. The ionic composition of lizard nasal fluid is considerably different, as will be discussed below.

LEVELS OF FUNCTIONAL CONTROL

Embryonic Differentiation

This topic is unstudied in reptilian salt glands. It could be assumed that the young are born or hatched in a state of physiological readiness for the environmental stresses to be encountered. Can there be a relation between the degree of salt gland development of the young and the state of hydration of the mother? We do know that the young of marine

species such as the sea turtles (Evans, 1973; Kooistra and Evans, 1976) and the file snake (Dunson and Dunson, 1973) are quite capable of osmoregulating in sea water. The effect of dehydration of sea turtle eggs on the physiology of the developing embryo would also be an interesting subject for experimentation.

Acclimation Effects

The effects of acclimation to fresh or saline water conditions on salt gland function can be examined either by measuring the secretory ability of the gland or some aspects of the gland tissue that reflect this ability. Avian salt glands undergo marked increases in size, and in RNA and Na-K ATPase concentrations on exposure of the bird to saline loading (Peaker and Linzell, 1975). Few reptilian comparisons are available. Results of histochemical localization of gland ATPases have not proven to be reliable and are not considered here. There are three published studies of more definitive biochemical assays of Na-K ATPase and K-stimulated p-nitrophenylphosphatase (NPPase) (Cowan, 1974; Dunson and Dunson, 1974, 1975). The levels of Na-K ATPase and NPPase in reptilian salt glands are high and similar to those of some avian glands. However differences in techniques between studies usually obviate detailed comparisons. Cowan (1974) found no difference in NPPase concentration in the lachrymal salt glands of diamondback terrapins (Malaclemys) kept in sea water or 10 days in fresh water. In previous studies he had found no increase in the weight of the gland, nor in cellular proliferation upon sea water exposure (Cowan, 1969, 1973). However Dunson (1970) demonstrated that the capacity of the gland to excrete NaCl was lost after prolonged fresh water acclimation. Dunson and Dunson (1975) confirmed the lack of any absolute increase in gland weight, and were able to demonstrate a direct relation between plasma Na concentration and gland Na-K ATPase content (Fig. 2).

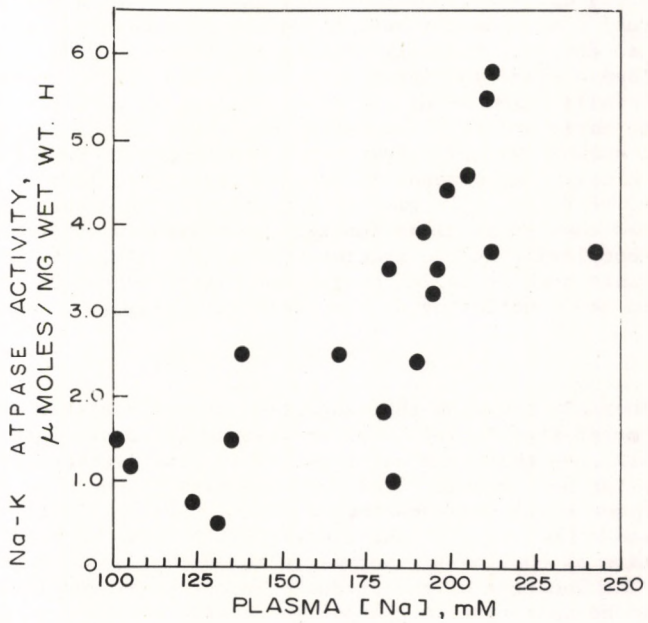

Fig. 2. The relation between diamondback terrapin (Malaclemys) lachrymal salt gland Na-K ATPase activity (whole homogenate) and plasma Na concentration (from Dunson and Dunson, 1975).

There is approximately a three-fold increase in enzyme activity on long term acclimation to sea water. However Malaclemys has a very low rate of net Na uptake from sea water and changes in plasma Na occur very slowly (Robinson and Dunson, 1976). Indeed plasma Na must exceed about 200 mM before a pronounced stimulation of Na-K ATPase occurs. Cowan (1974) did not measure plasma Na and his "fresh water" and "sea water" acclimated animals probably had very similar plasma values. Robinson and Dunson (1976) also found that plasma Na is directly related to bladder urine K concentrations. Thus bladder K levels can also be used to predict Na-K ATPase activity of the lachrymal salt gland.

Malaclemys is an estuarine turtle that only uses its salt gland when dehydration reaches extreme levels. The pelagic sea snake Pelamis apparently maintains an active salt gland even when plasma Na is relatively low (Dunson and Dunson, 1975). Na-K ATPase remained high even in snakes placed in fresh water for 48 days (plasma Na 140 mM), and the salt gland secreted when stimulated by NaCl injections. Another intriguing result was that levels of Na-K ATPase/mg tissue were very similar in salt stimulated glands of a desert lizard (Dipsosaurus), a turtle (Malaclemys), and a snake (Pelamis). This suggests that reptilian salt gland tissue has a certain maximum enzymatic capacity and that the very different maximum secretory rates of these three glands are set by other factors. The Malaclemys gland is about the same relative size as that of Pelamis, despite its much lower secretory rate. Yet among sea snakes, gland weight seems to be well correlated with the maximum salt gland secretory rate; neither Na-K ATPase activity nor ultrastructure seemed to vary between glands differing ten-fold in their rates of Cl excretion (Dunson and Dunson, 1974).

The recently discovered premaxillary salt gland in a marine homalopsid snake (Cerberus) does show ultrastructural changes associated with fluctuations in external salinity (Dunson and Dunson, 1979). This very small salt gland de-differentiates when snakes are placed in fresh water, and plasma Na falls below about 150 mM. Cerberus is probably a good example of the early stages of evolution of a salt gland. The gland only secretes when snakes are quite dehydrated and exogenous NaCl loads are cleared very slowly. At present it appears that the effect of salinity acclimation on reptilian salt glands is variable. Some estuarine species show pronounced changes in gland function as plasma Na varies; this may not occur in completely marine species such as Pelamis. It is obvious that considerable work is needed to further clarify the role of salinity acclimation in the functioning of reptilian salt glands.

On-Off Control

It is generally accepted that secretion in avian salt glands is mediated by neural signals initiated by changes in osmotic pressure of the plasma, although there are other points of view (Peaker and Linzell, 1975). Reptilian salt glands, like most exocrine glands, are innervated by both parasympathetic and sympathetic fibers (Dunson, 1976). Secretion can be elicited by injection of the acetylcholine mimic mecholyl, indicating that stimulation of cholinergic nerves is the normal mode of gland activation. The location of the presumed receptors in reptiles is unknown; they appear to be sensitive to changes in osmotic pressure of the extra-

cellular fluid in one species (Fig. 3).

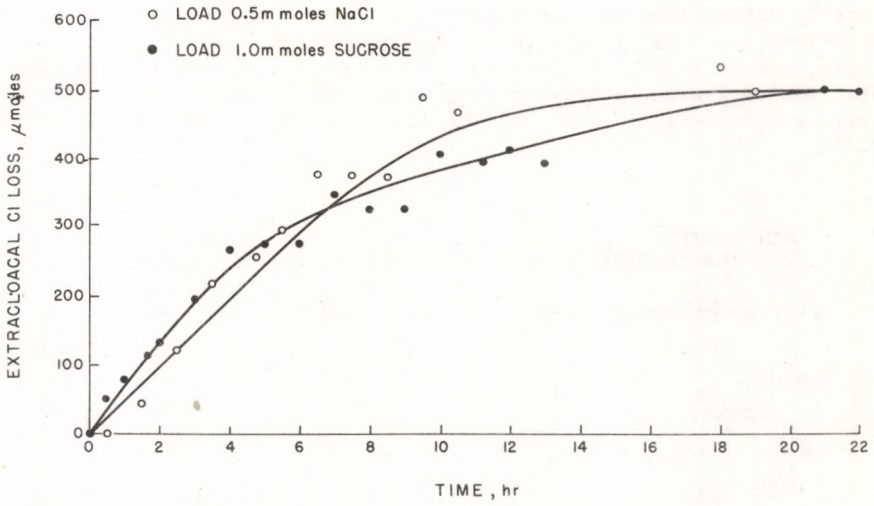

Fig. 3. Effect of NaCl load and an osmotically equivalent sucrose load on the extracloacal (salt gland) Cl excretion of the sea snake Pelamis (from Dunson, 1968).

The responses of the sea snake salt gland to subcutaneous injections of salt and sucrose were virtually identical. Shoemaker et al (1972) obtained a different result when they injected desert iguanas (Dipsosaurus) intraperitoneally with Na, K, and Rb salts, in comparison with mannitol and sucrose. The salts stimulated nasal gland secretion, but the sugars did not. They concluded that the receptor in Dipsosaurus is sensitive not to osmotic pressure, but specifically to alkali metal ions. This is indeed an exciting new finding, it if proves to be true. However Peaker and Linzell (1975) have argued that the peritoneal sugar solutions may not have been absorbed well, resulting in little change in plasma osmolarity. This is a valid point and the experiments will have to be repeated with careful monitoring of plasma concentrations, preferably with intravenous rather than intraperitoneal injections.

Secretory Fluid Composition

The salt gland secretions of marine snakes and turtles are essentially pure NaCl and are probably subject to little change in composition (Dunson, 1976). In lizards, secretory fluid composition is much more labile. Most attention has been focused on relative changes in Na and K. Data have often been collected in a haphazard fashion, so that reported values for Na or K excretion are unlikely to represent maxima. However there is good evidence that the ability to switch between Na and K excretion is relatively limited in a given species. For example the desert iguana (Dipsosaurus) has a maximum excretion rate of 35 μmoles Na + K/100 g body

wt·h, with Na/K ratios variable between 0.02 and 3 (Shoemaker et al, 1972; Dunson and Dunson, 1975). A coastal monitor lizard (Varanus semiremex) with a similar rate of Na + K excretion, had a higher range of Na/K ratios, 0.14-21.0 (Dunson, 1974). Thus inland desert herbivores mainly need to excrete K, rather than Na, and their salt gland is apparently adapted for this purpose. A coastal carnivore, that may also be found in inland habitats, has an ability to excrete either K or Na, as does the exclusively coastal marine iguana (Amblyrhynchus) that feeds on algae. The process by which a changeover in the ionic ratios of secreted fluid occurs is slow (Fig. 4).

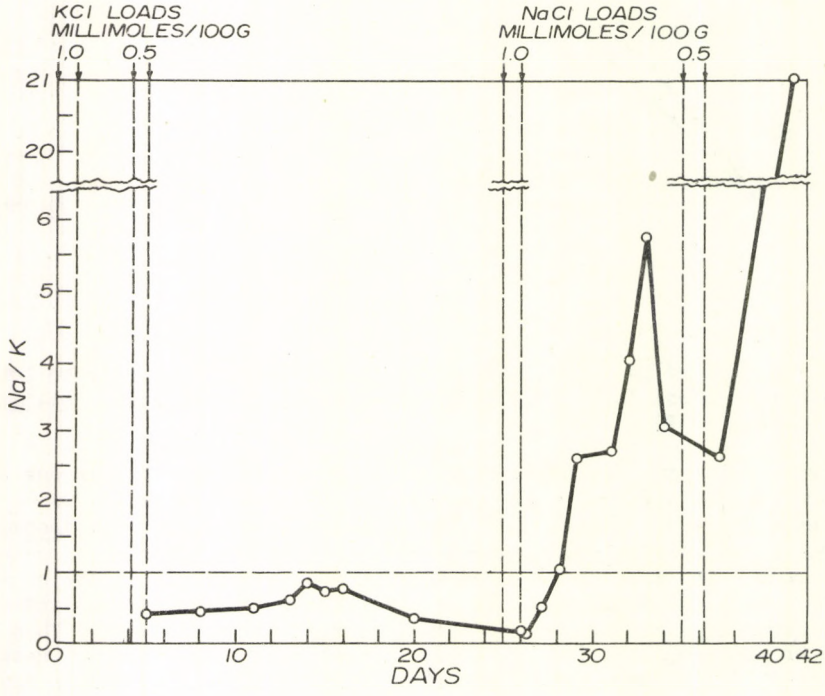

Fig. 4. Variation in the Na/K ratio of nasal encrustations of a mangrove monitor lizard (Varanus semiremex) injected first with KCl and then NaCl (from Dunson, 1974).

This leisurely time course suggests that hormonal regulation is involved. An interesting parallel can be found in the composition of the parotid saliva of sheep (Blair-West et al, 1964). The normal Na/K ratio of 30 can be greatly diminished (to about 0.2) by Na depletion, and aldosterone appears to be involved in this process of Na retention. Templeton (1972) and Templeton et al (1972) reported that aldosterone caused retention of Na by the nasal salt gland of Dipsosaurus, but had no effect on K. Shoemaker et al (1972) obtained similar results and in addition they found that dexamethasone abolished Na excretion. This synthetic steroid is

normally used to eliminate ACTH secretion, but it apparently also has mineralocorticoid activity of its own in lizards. Corticosterone and ACTH each had a lesser effect on nasal gland Na excretion, and cortisol had no effect. The salt gland of green sea turtles (Chelonia) also appears to be under some adrenal control. Holmes and McBean (1964) reported that adrenal insufficiency reduced Na and K excretion, which was partially restored by corticosterone therapy.

Lizard nasal fluid shows variation in anionic as well as cationic ratios. Early studies demonstrated that bicarbonate was a major anion in certain herbivorous forms (Schmidt-Nielsen et al, 1963; Norris and Dawson, 1964). Shoemaker et al (1972) showed that Cl was the only secreted anion following injections of Cl salts in Dipsosaurus. However injections of K acetate, K succinate, and $KHCO_3$ led to the secretion of bicarbonates or carbonates as one quarter of the total anions.

FUTURE RESEARCH

It is readily apparent that many aspects of the biology of reptilian salt glands need to be investigated further. Since their first description in 1958 by Schmidt-Nielsen and Fange, reptilian salt glands have received much less research attention than those of birds. This is only natural since ducks and geese are easier to work with than giant sea turtles and venomous sea snakes! However numerous smaller and more readily available lizards and turtles are now known to be useful in salt gland research. It is my hope that physiologists, endocrinologists, and biochemists will consider studying reptilian salt glands as model systems of ionic and osmotic regulation. The diversity of gland types, secretion concentrations and rates, as well as the variability in cationic and anionic ratios offer challenging opportunities for the investigation of control mechanisms and the correlation of ultrastructure and function. Research opportunities are not limited to the cell and organ level. Salt glands and mechanisms of osmoregulation often represent a major component of a reptile's adaptation to rigorous environments or to special foods. As such they deserve increasing attention from ecologists interested in strategies of adaptation.

ACKNOWLEDGMENTS

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PROPERTIES OF THE BODY FLUIDS AFFECTING NASAL SALT GLAND SECRETION AND URINE FORMATION IN BIRDS

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INTRODUCTION

The extracerebral body fluid (ECF) may be considered, in a first approximation, as a solution of Na^+ salts in water, the latter determining its volume and the former its tonicity. Homeostasis of the ECF implies that the amounts of salt and water in this fluid compartment remain constant. This may be achieved by the control of salt and water ingestion as well as of their elimination.

In marine mammals and birds, the intake of salt and water often cannot be independently controlled, as these animals are forced to ingest salt with the food or sea water. Consequently, regulation of the ECF relies only on the control of salt and water excretion. While the marine mammals meet this challenge with the high concentration capability of their kidneys, possibly in association with some control of the diet in several species, the kidneys of the marine birds cannot concentrate sufficiently. These birds eliminate salt with their supraorbital salt glands as first discovered by Schmidt-Nielsen et al. (1957). The secreted fluid is discharged from the nares and its main constituents are sodium and chloride. Fluid concentration may be well in excess of that of sea water, and salt secretion may exceed 1.5 milliosmoles (mO) per min (Hammel et al., 1977).

In birds with salt glands, salt and water output may, to a considerable degree, be controlled by separate organs: The salt glands eliminate the salt and the kidneys the water. The efferent mechanisms controlling these organs are also separate: The salt glands are under parasympathetic control; renal water excretion is controlled by arginine vasotocin (AVT), the avian ADH. This unique separation of salt and water excreting systems offers favourable conditions for the investigation of the ECF properties determining the output of its main constituents. This review describes methods and results of recent studies which aimed at the evaluation of the roles tonicity and volume of the ECF play in avian osmoregulation and of the mechanisms by which these properties are monitored.

METHODS

Detailed investigations of the afferent control of salt gland and kidney functions in the osmoregulation of marine birds have been carried out mostly in domestic species like geese and ducks which possess functioning salt glands, particularly when adapted to chronic salt loading. In our own

studies on Pekin ducks, the animals were reared on saline as the only water supply, the final concentration being 1.9%. These adapted animals were able to excrete up to 0.8 ml/min of salt gland fluid with a maximum osmolality of 1200 mO/kg. Their maximum urine concentration was about 600 mO/kg. Compared to the freshwater ducks with plasma osmolalities between 280 and 300 mO/kg, the plasma osmolalities of saltwater ducks were chronically elevated with a physiological range between 300 and 360 mO/kg (Deutsch et al., 1979).

During the experiments, various test solutions were infused, either intravenously (i.v.) into a wing vein or by means of a gastric tube, to induce steady state salt gland secretion or diuresis. When studying renal water excretion, the animals were fasted 24 h prior to the experiments to keep the cloaca free of feces. A perforated bulb was inserted into the cloaca to withdraw the urine by continuous suction into graded cylinders which were exchanged every 5-15 min. Water diuresis was induced by i.v. infusion of 1-2 ml/min of hypoosmotic (200 mO/kg) glucose solution or by intragastric infusion of 1 ml/min tap water. In some experiments, a diuresis was induced by i.v. infusion of 1 ml/min isotonic saline or mannitol solution. - When studying salt gland secretion, the secreted fluid was continuously withdrawn by suction from the nares through silastic tubing into vials which were exchanged every 5-15 min. The amount of fluid was determined by weighing. Salt secretion was induced by i.v. infusion of 0.2-0.4 ml/min of NaCl solution at 800-1300 mO/kg.

For both, the urine and salt gland fluid, the secretion rates and osmolalities were determined for each sampling interval. In some experiments, the Na^+, K^+ and Cl^- concentrations were measured. Blood samples for analysis were drawn from a cannulated leg vein. Plasma osmolality and hematocrit were regularly determined. Plasma Na^+, K^+ and Cl^- were measured in special cases. In two series of experiments, the concentrations of arginine vasotocin (AVT) were determined in 2 x 1 ml serum samples with a radioimmunoassay established by Möhring et al. (1980).

All experiments were carried out in conscious animals. The investigations included intracarotid (i.c.) osmotic stimulations by means of chronically implanted catheters, intracerebroventricular (i.c.v.) microinfusions into the lateral or third ventricle by means of chronically implanted devices, and temporary blockade of the vagus nerves by local anesthesia.

RESULTS AND DISCUSSION

ECF properties influencing osmoregulation

Homeostasis of the ECF is considered as the goal to be achieved by the osmoregulatory processes. Accordingly, tonicity and volume of the ECF or of particular compartments of it have to be investigated as to the effects which changes have on both the control of renal water excretion by AVT and on neural control of salt gland secretion.

Tonicity

In agreement with observations in mammals, a positive correlation between serum AVT and serum osmolality was found to exist in ducks. The relationship between serum AVT (fmol/ml) and plasma osmolality (mO/kg) could be described with a common regression line for freshwater and saltwater animals for the osmolality range between 280 and 360 mO/kg:

$$AVT_{serum} = 0.39 \times osmolality_{serum} - 109.28;\ r = 0.89 \qquad (1)$$

This relationship was virtually identical with that found for man in the osmolality range of 280-310 mO/kg (Robertson et al., 1977). In the duck,

the control of renal water excretion by serum AVT was determined in experiments in which AVT was infused for 30 min periods at different rates during water diuresis induced by continuous i.v. infusion of 2 ml/min hypoosmotic glucose solution. At steady state conditions, the relationship between urine flow rate (ml/min) and serum AVT (fmol/ml) was determined:

$$\text{flow rate}_{urine} = -1.07 \times \log \text{AVT}_{serum} + 1.66;\ r = -0.91 \qquad (2)$$

The relationship between urine osmolality (mO/kg) and serum AVT (fmol/ml) could be described best by the regression:

$$\log \text{osmolality}_{urine} = 0.60 \times \log \text{AVT}_{serum} + 1.81;\ r = 0.91 \qquad (3)$$

Concerning salt gland secretion, the importance of ECF tonicity has been convincingly demonstrated by many investigators as summarized by Peaker and Linzell (1975). Osmotically active substances with a predominantly extracellular distribution stimulate the salt glands when administered as hyperosmotic solutions, whereas rapidly permeating solutes like urea have no stimulating effect.

Volume

In birds, a volume dependence of AVT release and renal water excretion to a similar extent and in the same way as in mammals has been implicitly assumed. Our own studies have strengthened this assumption by the observation of a temporary antidiuretic effect of blood withdrawal and of a temporary enhancement of the diuresis upon blood re-infusion in ducks made diuretic by 1 ml/min isoosmotic mannitol solution i.v. (Simon et al., 1978). The influence of the ECF volume on salt gland secretion has been controversially discussed (Holmes, 1965; Zucker et al., 1977; Peaker, 1978). Recent studies showed that blood withdrawal inhibits and blood re-infusion stimulates salt gland secretion (Deutsch et al., 1979). This observation need not contradict the notion of Peaker (1978) that blood volume changes per se do not influence salt gland secretion, because blood volume changes are accompanied by parallel changes in other body fluid compartments.

Receptive mechanisms in avian osmoregulation

The assumption that the afferent control of avian and mammalian osmoregulation are basically identical (Schmidt-Nielsen, 1960) appears self-evident for the control of AVT release but less likely so for salt gland control, since this excretory system is phylogenetically independent from the renal system. Hanwell et al. (1972) presented evidence for a mainly extracerebral afferent control of salt gland activity in the goose. On the other hand, the assumption that ADH release in birds is controlled by cerebral osmoreceptors, as in mammals, has never been experimentally tested.

Control of renal water excretion

Cranial osmoreceptors influencing AVT release and diuresis in the duck were identified with the technique employed first by Verney (1947) in mammals. In ducks made diuretic by i.v. infusion of 2 ml/min hypoosmotic glucose solution, 2 ml/2min of NaCl solution at 1000 mO/kg were infused into the carotids (i.c.) or into a peripheral vein (i.v.). While i.c. infusion caused a temporary reduction of urine flow rate and an increase of urine osmolality, each by about 50%, the i.v. infusion had a very delayed and barely detectable antidiuretic effect (Hammel et al., 1980a). Conversely, the diuresis of ducks receiving a continuous i.v. infusion of 1 ml/min isotonic saline was enhanced, when 0.5 ml/min hypoosmotic glucose solution were i.c. infused for 10 min, while the saline infusion was reduced during this time by the same amount to keep the infused volume constant. The same

glucose infusion administered i.v. with the same simultaneous reduction of the saline infusion did not alter the urine formation (unpublished observation). When ducks under water diuresis due to i.v. infusion of 2 ml/min hypoosmotic glucose solution received an i.c. infusion of 2.4 ml/2 min of 1000 mO/kg NaCl solution, a significant rise of serum AVT from 3.3±0.7 to 10.8±1.7 fmol/ml (means with S.E.M) was found in 10 experiments in conscious ducks (Simon-Oppermann et al., 1980). The intracerebral location of the cranial osmoreceptors was confirmed by a similar rise of serum AVT in response to an intracerebroventricular (i.c.v.) microinfusion of 0.2 µl/min of 4.8% NaCl solution during 15 min. In ducks made diuretic by intragastric infusion of 1 ml/min tap water, i.c.v. microinfusions with hypertonic NaCl solutions consistently caused antidiuresis. Osmotically equivalent i.c.v. microinfusions with cations different from Na^+ (K^+, Li^+, Mg^{++}, Ca^{++}) also induced antidiuresis, however, with different time courses. On the other hand, i.c.v. microinfusions of osmotically equivalent mannitol solution induced a diuresis which could be converted into an antidiuresis by adding NaCl. Further, i.c.v. microinfusions of hyperosmotic urea solution were ineffective (Deutsch and Simon, 1980). Thus, in conformity with the concepts of central regulation of ADH release and renal water excretion in mammals (Andersson, 1977) intracerebral osmoreception in birds appears to be established by cation sensitive elements with Na^+ as the naturally involved cation which are localized at the CSF brain barrier. In addition, the observation in ducks that i.c.v. infusion of 0.5-2.5 pmol angiotensin II induced a dose dependent antidiuresis (Deutsch and Simon, 1980) suggests a similar organization of signal transmission to the ADH releasing structures as in mammals in which angiotensin II has the same effect.

Table 1: Urine formation of ducks under continuous i.v. infusion of 0.9% saline at 1 ml/min as influenced: (A) by additional i.c. and i.v. infusions of 0.5 ml/min 0.9% saline during 10 min; (B) by the same i.c. and i.v. infusions, however, with simultaneous reduction of the continuous i.v. infusion so as to keep the entire infusion rate constant. Means with S.E.M.

Urine formation	20 min, before infusion	20 min, after start of infusion	20 min, subsequent period
A: i.c. infusion, n = 9			
flow rate (ml/min)	0.80±0.03	1.02±0.04	0.82±0.03
osmolality (mO/kg)	359.2 ±8.7	328.8 ±6.2	338.5 ±8.9
i.v. infusion, n = 9			
flow rate (ml/min)	0.78±0.04	0.99±0.04	0.85±0.04
osmolality (mO/kg)	358.8 ±8.3	332.3 ±6.0	341.9 ±9.7
B: i.c. infusion, n = 9			
flow rate (ml/min)	0.78±0.04	0.75±0.02	0.81±0.04
osmolality (mO/kg)	363.5 ±8.9	371.6 ±4.8	367.8 ±8.3
i.v. infusion, n = 9			
flow rate (ml/min)	0.81±0.03	0.83±0.03	0.85±0.04
osmolality (mO/kg)	359.1 ±7.1	355.2 ±5.7	355.7 ±7.4

Renal excretion in ducks was found to be surprisingly sensitive to changes of volume loading under certain experimental conditions, for instance, when the animals were in a steady state diuresis due to continuous i.v. infusion of 1 ml/min isotonic saline. As shown by Table 1 (unpublished observations), urine flow increased and urine osmolality fell (part A of Tab. 1) in response to an additional i.c. or i.v. volume loading as small as 5 ml administered during 10 min. The onset of enhanced diuresis occurred within 5 min, i.e. after infusion of about half of the additional load. The same

i.c. or i.v. infusions, however, with a simultaneous reduction of the continuous infusion so as to keep the infused volume constant (part B of Tab. 1) did not alter the steady state diuresis. Consequently, the enhanced diuresis in response to temporarily increasing the isotonic volume load suggests the involvement of volume receptors mediating effects on renal excretion in response to changes of the ECF volume by less than 1%. Since the effects of i.c. and i.v. infusions did not differ, cerebral receptive structures appeared not to be involved. Whether the sensitive reactions of urine formation in ducks to changes of ECF volume were entirely or partially mediated by AVT remains to be elucidated. However, evidence for a great volume sensitivity of AVT release in ducks is contributed by the relationship between serum AVT (fmol/ml) and serum osmolality (mO/kg) found at steady state conditions in freshwater and saltwater ducks receiving various experimental i.v. infusions at rates between 0.4 and 2.0 ml/min (Simon-Oppermann et al., 1980). The regression:

$$AVT_{serum} = 0.28 \times osmolality_{serum} - 80.60;\ r = 0.93 \qquad (4)$$

differed significantly from the relationship (1) found for the non-infused control animals so that, at a given serum osmolality, serum AVT was lower in the infused than in the non-infused ducks.

An extracerebral location of the volume sensing mechanisms was suggested by the observation of Simon-Oppermann et al. (1980) that reversible vagus blockade in diuretic ducks caused antidiuresis associated with a rise of serum AVT from 2.6±0.7 to 7.1±0.4 fmol/ml (means with S.E.M.) in 8 experiments. This finding would conform to the idea promoted for mammalian osmoregulation (Gauer and Henry, 1976) that volume perception is mediated by vagal afferents transmitting the signals of extension receptors in the low pressure section of the circulation, especially in the left atrium. However, when considering the studies in mammals (Robertson et al., 1977) doubts may be raised, whether these receptors alone might account for the great volume sensitivity of the control of renal water excretion observed in the duck: The control system reacted measurably to a less than 1% volume increase of the ECF! No evidence exists from studies in mammals that such minute volume changes can be perceived by the atrial receptors.

Control of salt gland secretion

Schmidt-Nielsen (1960) originally hypothesized that the osmoreceptors controlling salt gland activity were located in the brain. In their studies on anesthetized and decerebrate geese, Hanwell et al. (1972) found no evidence for the involvement of cerebral osmoreceptors in salt gland control and postulated that the salt glands were driven by vagal osmoreceptors. Our own studies in ducks (Hammel et al., 1980a) partially confirmed this hypothesis by showing that a steady salt gland secretion induced by continuous i.v. infusion of 0.4 ml/min of 1000 mO/kg NaCl solution was equally enhanced by i.c. and i.v. infusion of 2 ml/2 min of 1000 mO/kg NaCl solution, i.e. no particular stimulating effect of the i.c. application was observed. However, animals secreting under the same continuously infused salt load reacted to additional i.c. infusion of 2 ml/2 min hypoosmotic glucose solution with a strong inhibition of salt gland secretion, whereas the same glucose infusion administered i.v. did not inhibit the salt glands. This observation is difficult to understand, at present, but it may be hypothesized that a reduction of the cerebral osmoreceptor signals may shut off the salt glands, while salt gland stimulation originates elsewhere. This points to something like a "permissive" action of cerebral osmoreceptors in salt gland control allowing salt gland secretion only above a certain tonicity threshold which may be influenced by other properties of the ECF.

Such an organization of the cerebral control of the salt glands would not appear unreasonable, since the salt glands need to be stimulated only when renal water conservation is already enforced. Whatever the true mode of interaction between the control systems for salt gland secretion and renal water excretion may be, a common central control of both systems is suggested by these findings. Central integration of both systems by hypothalamic neural structures was further confirmed by the observation that both, salt gland secretion and AVT release were inhibited in penguins and ducks by hypothalamic cooling (Hammel et al., 1977; Simon-Oppermann et al., 1979, 1980).

The involvement of stimulating vagal osmoreceptors in salt gland control was concluded by Hanwell et al. (1972) from their observation that vagus transection in geese caused a complete stop of salt gland secretion. Simon-Oppermann et al. (1980) showed that reversible vagus blockade in conscious ducks could induce a complete salt gland inhibition for the duration of the blockade. However, while the importance of vagal afferents for salt gland activation is, thus, firmly established, this activation might arise from vagal volume receptors as well as osmoreceptors, since an increase of extracellular tonicity always means an increase of ECF volume, either absolute or relative to the intracellular compartment. The question to be answered is whether the extracerebral afferent control of salt gland activity is achieved by volume receptors or osmoreceptors or by both.

As mentioned already, an important role of volume receptors in the stimulation of salt gland secretion in the duck was demonstrated by Deutsch et al. (1979). Hammel et al. (1980b) have presented evidence for the involvement of a mechanism sensing changes in the interstitial compartment of the ECF. This evidence was obtained in experiments on salt adapted ducks. The animals were first primed by temporary salt loading so that threshold conditions for salt gland secretion were established. Then, the animals were intermittently (for 90 min) loaded with 0.4 mO/min NaCl, given either as a control infusion of 0.4 ml/min at 1000 mO/kg or as an experimental infusion with a deviating composition or at an altered condition. Since the NaCl content of the control infusion was equal to that of the average salt gland fluid, the animals at threshold conditions reacted to this infusion by eliminating, within a few percent variability and with a latency of some 30 min, the entire amount of salt and water infused, i.e. the ECF was not systematically altered with respect to its volume (ECFV) nor its tonicity (ECFT). Concerning the experimental infusions, salt secretion was 1.) also stimulated when the salt load was administered as an isotonic infusion, however, salt and water were not totally eliminated (including the renal excretion) so that the ECFV increased and the ECFT decreased. Nevertheless, the animals were found to have established a new threshold condition after this experimental infusion, since a subsequent control infusion was eliminated 100%. Obviously, the increase of ECFV had compensated for the decrease of ECFT in establishing the new threshold; in other words, the increase of the ECFV by the isotonic infusion had stimulated the salt glands to eliminate more salt than necessary to maintain a constant ECFT. The salt gland response was 2.) reduced when the experimental infusion consisted of the control infusion preceded by blood withdrawal which had reduced both the intra- and extravascular fractions of the ECFV. The salt gland response was 3.) also reduced when the experimental infusion consisted of the control infusion made, however, hyperoncotic by 20% dextran (60,000) by which the intravascular fraction of the ECFV was greatly augmented at the expense of a reduction of the interstitial fraction. - When comparing these three series of experiments, the only possible common explanation for the

altered salt gland responses to the various experimental infusions appears to be that mechanisms sensitive to interstitial volume changes affected salt gland activity. While a direct effect on the salt glands cannot yet be excluded, it appears more likely that interstitial volume receptors are involved, stimulating the salt glands in response to an increase and inhibiting them in response to a decrease of the interstitial volume. The inhibitory effect of vagus blockade on salt gland secretion would point to an extracerebral location of these receptors.

Extracerebral osmoreceptors with signal transmission by vagal afferents, as proposed by Hanwell et al. (1972), may additionally be involved in salt gland control; at least their existence is not excluded by the available experimental data. This follows from experiments in which salt adapted ducks were continuously loaded with 0.3 mO/min NaCl in a 1300 mO/kg solution, i.e. at a concentration exceeding the maximum concentration of the salt gland fluid (Deutsch et al., 1979). The animals were able to balance the salt input and its output by the salt glands over more than 8 h. However, since they had to produce more salt gland fluid than the amount of fluid infused, ECFV was continuously decreasing and ECFT was rising. Since the decrease of ECFV should have reduced the drive for salt secretion, the constancy of the secretion rate must have been achieved by an increasing drive resulting from the increase of ECFT. As demonstrated by the i.c. infusion experiments reported above, this drive most likely could not originate from the cerebral osmoreceptors. Hence, an increasing drive from osmoreceptors outside of the brain appears likely. A similar conclusion may be drawn from dehydration experiments in ducks (Kaul and Hammel, 1979). However, more direct evidence appears necessary to establish the existence of extracerebral osmoreceptors and their role in avian osmoregulation.

PERSPECTIVES

The osmoregulation of marine birds employs two excretory systems with separate phylogeny. The question posed 1975 by Peaker and Linzell: "Do the kidneys know when the salt glands are switched on, and vice versa?" reflects a certain state of investigation at which it appeared as if not only the efferent control of salt gland secretion and renal water excretion were separate, but also the afferent control of each system might be accomplished by different sets of receptors. By analogy to mammalian osmoregulation and from the studies in geese and in ducks the conclusion was suggested that cerebral osmoreceptors and intravascular volume receptors would control AVT release, while extracerebral osmoreceptors and interstitial volume receptors would control salt gland function. Until recently, only the investigations of Simon-Oppermann et al. (1979) indicated a common integration by hypothalamic neurons of the control systems for renal water excretion and salt gland secretion.

The most recent results reported in this survey have, however, modified this picture. Cerebral osmoreceptors appear to be involved in the control of each excretory system, though in a different manner: While AVT release is influenced in a range from below to far above the normal body fluid osmolality, salt gland function appears to be influenced only below a certain threshold osmolality. This difference might still be ascribed to the existence of two different sets of brain osmoreceptors each controlling one system. However, it appears equally reasonable to assume a common set of receptors linked differently to the two excretory systems. In the pursuit of the idea that all receptors of ECF properties somehow affect both excretory systems, it may be speculated about the roles played by the various extracerebral receptors of ECF properties. In this respect, the postulated

interstitial volume receptors deserve special interest in view of the great volume sensitivity of renal water excretion in the duck. Is this phenomenon related to the involvement of interstitial volume receptors in the control of AVT release? If one would accept this as a working hypothesis, one would have to apply it also to mammalian osmoregulation, because all studies in birds have, thus far, supported the idea of an identical afferent control of ADH release in birds and mammals. In fact, the question of interstitial volume perception in mammalian osmoregulation was raised by Share (1974). Kaufman et al. (1980) have recently presented evidence that interstitial volume receptors might contribute to thirst control in both birds and mammals. In addition, extracerebral osmoreceptors which were postulated to participate in avian salt gland secretion (Hanwell et al., 1972) have to be considered also as input factors in the control of renal water excretion of both birds and mammals, although the experimental evidence is still equivocal for mammals (Moses and Miller, 1974) and appears not to exist for birds. It may, however, be difficult to delineate interstitial volume sensing elements from those sensing changes of tonicity in this ECF compartment because of the close interrelations between these two ECF properties. On the other hand, the results of the vagotomy and vagus blockade experiments in geese and ducks would suggest that a great fraction of the afferents conveying the signals of the various extracerebral receptors controlling salt gland function and AVT release in these birds are conducted in the vagus nerves. This would offer the possibility to identify the properties of the extracerebral receptors in avian osmoregulation also with neurophysiological techniques.

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CENTRAL NEURAL SUBSTRATES FOR OSMOREGULATION IN THE MAMMAL

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Disturbances in body fluid osmolarity are rapidly corrected by changes in water intake and renal water clearance. To effect these responses, the nervous system must appropriately modulate elaboration of the thirst motivational state and secretion from the posterior pituitary of the antidiuretic hormone vasopressin. The studies of Verney (1947) established that the forebrain functioned not only in effecting vasopressin release but also in detecting the relevant initiating stimuli. Direct sensitivity of the hypothalamic area was suggested by Andersson (1953) who observed motivated water intake after local intracranial application of a hypertonic stimulus. The organ cultured hypothalamo-neurohypophysial system studied by Sladek and Knigge (1977) demonstrated that neural elements required for some aspects of osmoregulation are completely contained within the preoptic-hypothalamic area since the organ explant increases or decreases vasopressin release in response to changes in bathing medium osmolarity.

In the following pages, current studies on the anatomical localization and functional organization of the central neural substrates for osmoregulation in mammals will be reviewed. Evidence implicating the preoptic periventricular tissue surrounding the anterior ventral part of the third ventricle (AV3V) as a necessary component in osmotic modulation of thirst and vasopressin release will be presented. The relevant stimuli and the nature of the receptor, whether osmoreceptor or Na^+ receptor, will be considered. Finally, the functional organization of the osmoregulatory system will be examined through analysis of its relationship to angiotensin-sensitive neural substrates involved in fluid regulation.

ANATOMICAL LOCALIZATION

The AV3V region was first identified in the rat as a sensitive and necessary site of action for intracranial angiotensin stimulation of thirst (Buggy et al., 1975). Although periventricular areas had also been implicated as osmosensitive from stimulation studies in goats (Andersson, 1977), the lateral preoptic area received most of the initial attention as the osmosensitive substrate in the rat (Blass & Epstein, 1971; Blass, 1974). A more extensive stimulation mapping of osmosensitive forebrain sites in the rat later indicated, however, that as many medial (within 1 mm of the midline) as lateral (more than 1 mm lateral of midline) cannula

sites were osmosensitive (Peck & Blass, 1975). When we directly compared in the same rats the effects of local injections of hyperosmotic solution into lateral preoptic area and the medially adjacent AV3V, more water intake was elicited after AV3V stimulation suggesting a greater sensitivity for that area (Buggy et al., 1979). In addition to arousing drinking, osmotic stimulation of AV3V in rats elicits a characteristc group of responses (Buggy et al., 1979). Within minutes of injection, the animals begin drinking, increase blood pressure and increase antidiuretic activity. Within the range of osmotic stimulation examined, these responses typically occurred together, representing coordinated behavioral, autonomic and endocrine facets of the same overall regulatory response. Thus, osmotic stimulation of the AV3V elicits a complete regulatory response rather than a fragment of the total response.

Brain Lesion Studies

To assess more completely the role of the AV3V region in fluid balance, the effects of AV3V ablation on osmoregulation were studied in the rat. Bilateral electrolytic destruction of periventricular tissue surrounding AV3V resulted in an acute hydrational crisis characterized by adipsia, the absence of voluntary water intake and rapid loss of body weight, 5-10%/day initially (Buggy & Johnson, 1977). It is unlikely that the lesion-induced adipsia is secondary to gross motor impairment or general motivational deficits since feeding continues normally as long as hydration is maintained. Indeed, one means of maintaining hydration is to present a sweet solution which is avidly drunk at a time when water alone is not accepted.

The acute hydrational crisis induced by AV3V lesion involved more than adipsia, however. If not maintained on some fluid replacement regimen, these adipsic animals would often die within 5 days while neurologically intact rats deprived of water survive for at least 2 weeks. When urine output of adipsic-lesioned rats was compared with that of water-deprived, sham-lesioned rats for the first 3 days after surgery, it was apparent that rats with AV3V lesions failed to compensate for insufficient water intake by reduction of renal water loss. Water-deprived, sham-lesioned rats manifested antidiuretic activity, a progressive decrease in daily urine volume coupled with a progressive increase in solute concentration of urine. Adipsic-lesioned rats, on the other hand, continued to elaborate a larger volume of more dilute urine, with a pattern of urine output similar to the pre-lesion pattern, as though they were insensitive or unable to respond to the developing dehydration after the lesion (Johnson and Buggy, 1978). It should be noted that lesioned rats did not manifest the loss of large volumes of very dilute urine characteristic of diabetes insipidus and the loss of all antidiuretic activity. Rather, rats with AV3V lesions continue to release basal amounts of vasopressin but fail to increase or decrease secretion in response to an appropriate osmotic signal (Johnson, Hoffman, and Buggy, 1979; Johnson et al., 1980).

The lesion-induced disruption of fluid intake coupled with failure of compensatory antidiuresis constitutes a profound disturbance of osmoregulation which is reflected in the massive increase in plasma osmolality and sodium concentration of adipsic-lesioned rats compared to water-deprived normals or normals with free access to water (see Figure 1). Some lesioned rats with several days of continued adipsia and

progressive dehydration die, but others spontaneously resume water intake adequate for survival. By providing lesioned rats access to palatable fluids during the adipsic period when water alone is not accepted, adequate levels of fluid intake can be achieved until spontaneous intake of water alone eventually returns (2-14 days).

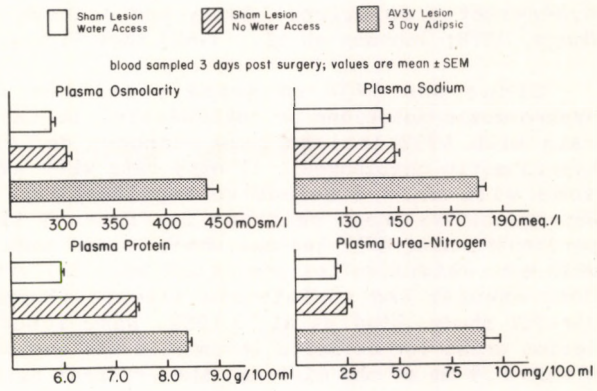

FIGURE 1. Fluid balance parameters in plasma of sham-lesioned rats with or without access to water, and AV3V lesioned rats adipsic for 3 consecutive days.

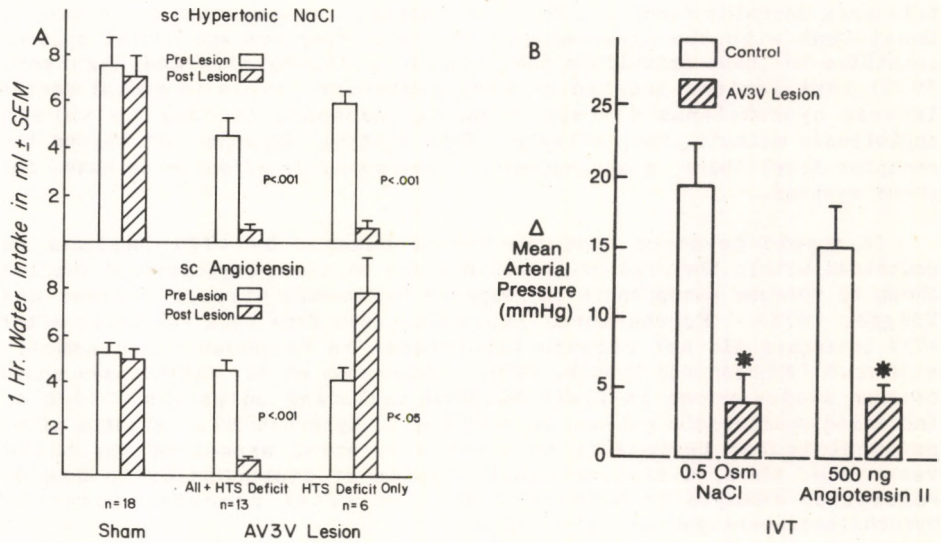

FIGURE 2. Panel A shows water intake to subcutaneous injections of angiotensin (AII, 1.5 mg/kg) and hypertonic NaCl (HTS, 4 ml of 12%/kg). AV3V lesioned rats are subdivided into groups with deficits to both AII and HTS or to HTS only. Panel B shows pressor responses in conscious rats to lateral ventricle injections of angiotensin and hyperosmotic-artificial cerebrospinal fluid. Tests were conducted several weeks post-lesion, after recovery from adipsia.

Even several weeks after recovery from adipsia, rats with AV3V lesions continue to manifest impairments in osmoregulation. Plasma osmolality and sodium are chronically increased compared to sham-lesioned rats, but the magnitude of the hypernatremia and hyperosmolality is less severe than during the acute adipsic period. Furthermore, there are several persisting deficits in thirst, pressor, and vasopressin release responses to challenges of systemic or intracranial injections of hyperosmotic solutions (Buggy and Johnson, 1977; Johnson, Hoffman and Buggy, 1978; Johnson et al., 1980) (See Figure 2).

Since the AV3V is quite sensitive to local stimulation by hyperosmotic solutions or angiotensin, it is not surprising that many rats with AV3V lesions show response deficits to both angiotensin and hyperosmotic challenges. If many rats with AV3V lesions are examined, some will show a selective impairment to only hypertonic stimuli. Representative brain sections in Figure 3 illustrate the location of periventricular AV3V lesions which caused such a selective deficit; rats with more extensive lesions in the saggital extent showed impairments to both osmotic and angiotensin stimuli (Buggy and Johnson, 1977). In a careful study, Lind et al. (1980) have found that while the critical lesion areas for deficits to osmotic and angiotensin stimulation are both within 0.4 mm of the midline, the critical lesion area for these deficits do not overlap completely and are neuroanatomically discriminable.

Since AV3V is sensitive to osmotic and angiotensin stimulation and since AV3V lesions disrupt responses to these stimuli, it is likely that this area contains receptors for both osmotic and angiotensin stimuli. Consistent with the hypothesis of separate receptors and neural systems sensitive to these stimuli is the observation (Kucharczyk and Mogenson, 1975) that lesions located at more posterior levels in medial versus lateral hypothalamus disrupt drinking responses to osmotic versus angiotensin stimuli, respectively. This finding suggests that beyond the receptor level there is an anatomical divergence of effector pathways for these systems.

It should be noted that the tissue damaged by AV3V lesions is contained within the organ-cultured hypothalamic-neurohypophyseal explant shown to release vasopressin in response to osmotic stimuli (Sladek and Knigge, 1977). Furthermore, explants taken from rats two weeks after AV3V lesioning did not release vasopressin in response to an osmotic stimulus (Johnson and Sladek, 1979). Andersson et al. (1975) have shown similar disturbances in fluid balance including adipsia and lack of increased vasopressin release in response to hypernatremia in goats after periventricular lesions in the rostral-ventral aspect of the third ventricle; thus, a similar constellation of disturbances in osmotic regulation results in both rat and goat after preoptic-anterior hypothalamic periventricular ablation.

While data implicating periventricular areas in osmotic regulation have accumulated, studies re-examining the effects of lateral preoptic area lesions in rats have not consistently demonstrated specific or persistent disturbances in osmoregulation (Coburn and Stricker, 1978; Almli and Weiss, 1974). There are difficulties of resolution and interpretation of localization studies using intracranial stimulation and lesioning techniques, however, especially when trying to discriminate closely adjacent areas that can be easily affected by manipulation of the

other. For these reasons, it was desirable to employ a technique which could non-disruptively assess neural activity of adjacent brain regions during osmotic regulation.

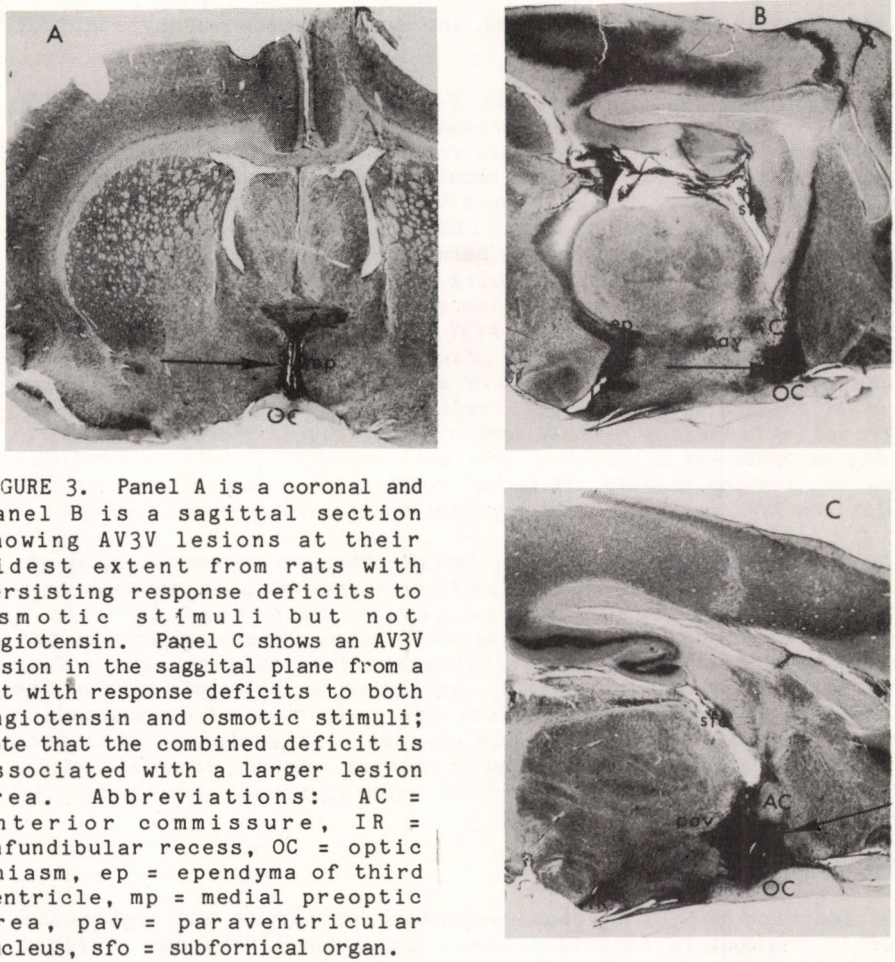

FIGURE 3. Panel A is a coronal and Panel B is a sagittal section showing AV3V lesions at their widest extent from rats with persisting response deficits to osmotic stimuli but not angiotensin. Panel C shows an AV3V lesion in the saggital plane from a rat with response deficits to both angiotensin and osmotic stimuli; note that the combined deficit is associated with a larger lesion area. Abbreviations: AC = anterior commissure, IR = infundibular recess, OC = optic chiasm, ep = ependyma of third ventricle, mp = medial preoptic area, pav = paraventricular nucleus, sfo = subfornical organ.

Regional Cerebral Metabolic Activity

A powerful research method would permit simultaneous sampling of neural activity without anesthesia in several brain regions during osmotic stimulation. One method which accomplishes these goals employs autoradiography of brain sections from rats injected with a marker for regional cerebral metabolic activity during a period of osmotic stimulation.

The glucose analog 2-deoxyglucose (2-DG), competes with glucose for cellular uptake and after partial metabolism remains trapped within the cell. Since neurons depend on glucose for energy, the accumulation of tracer amounts of 2-DG reflects the rate of glucose utilization which is

in turn highly correlated with functional neural activity. Using this autoradiographic-2-DG method in water- deprived rats injected with Carbon 14 radiolabeled 2-DG, Schwartz et al. (1979) observed increased glucose utilization in the posterior pituitary which they interpreted as resulting from increased activity of the hypothalamo-neurohypophyseal secretory system.

Our laboratory has modified this 2-DG regional metabolic activity method by using tritium label, a lower energy beta emitter than Carbon 14, and tissue perfusion before brain removal and freezing in an attempt to improve tissue quality and gain resolution of autoradiographs of brain sections (Wells et al., 1980). Figure 4 shows photographs taken from a reconstructed television monitor picture of brain section autoradiographs; this videoscan has been digitized and then enhanced for data analysis using computer imaging techniques. Water deprivation or injections of hypertonic solutions increased metabolic activity not only of posterior pituitary but also of AV3V periventricular tissue previously implicated in osmoregulation by the stimulation and lesion studies just discussed. Lateral preoptic area of other forebrain structures did not show great changes in glucose utilization during osmotic stimulation. Electrical stimulation of AV3V region also produced increased metabolic activity in posterior pituitary. This autoradiographic data on regional glucose utilization provide additional support for a link between osmotic stimuli, the AV3V region and the hypothalamo-neurohypophyseal system.

Using retrograde transport of horseradish peroxidase to study the supraoptic nuclei, Miselis et al. (1979) have demonstrated neurons from the organum vasculosum of the lamina terminals (OVLT), a circumventricular organ within the AV3V area, which project to the supraoptic nuclei containing vasopressin secreting neurons. This demonstration of neural connectivity, coupled with the sensitivity of the AV3V region to osmotic stimulation, the dysfunction of osmoregulation after AV3V lesions, and the increased glucose utilization of this area after osmotic stimulation provide a coherent picture establishing the AV3V region as an important component of central neural substrates for osmoregulation.

NATURE OF THE OSMORECEPTOR

In addition to his key observations implicating periventricular forebrain regions in osmoregulation, Andersson (1977) has provided several thought provoking hypotheses concerning the nature of osmoreceptors which have stimulated and focused research effects. Based on observations in the goat, Andersson proposed that juxtaventricular sodium-sensitive receptors rather than osmoreceptors mediate water regulation. Furthermore, on the basis of interactions of angiotensin and hypertonic NaCl stimuli, Andersson (1977) suggests as a working hypothesis that both stimuli act synergestically on the same juxtaventricular sodium-sensitive receptors to effect fluid regulatory responses. Finally, while acknowledging the importance of mechanisms governing water intake or loss, Andersson (1977) also stresses the role of cerebral mechanisms governing sodium intake or excretion in the overall neural regulation of body fluids.

The question of sodium receptors or osmoreceptors has now been pursued in several other species including rat (Buggy et al., 1979), sheep (McKinley et al., 1978) and dog (Thrasher et al., 1980a and b).

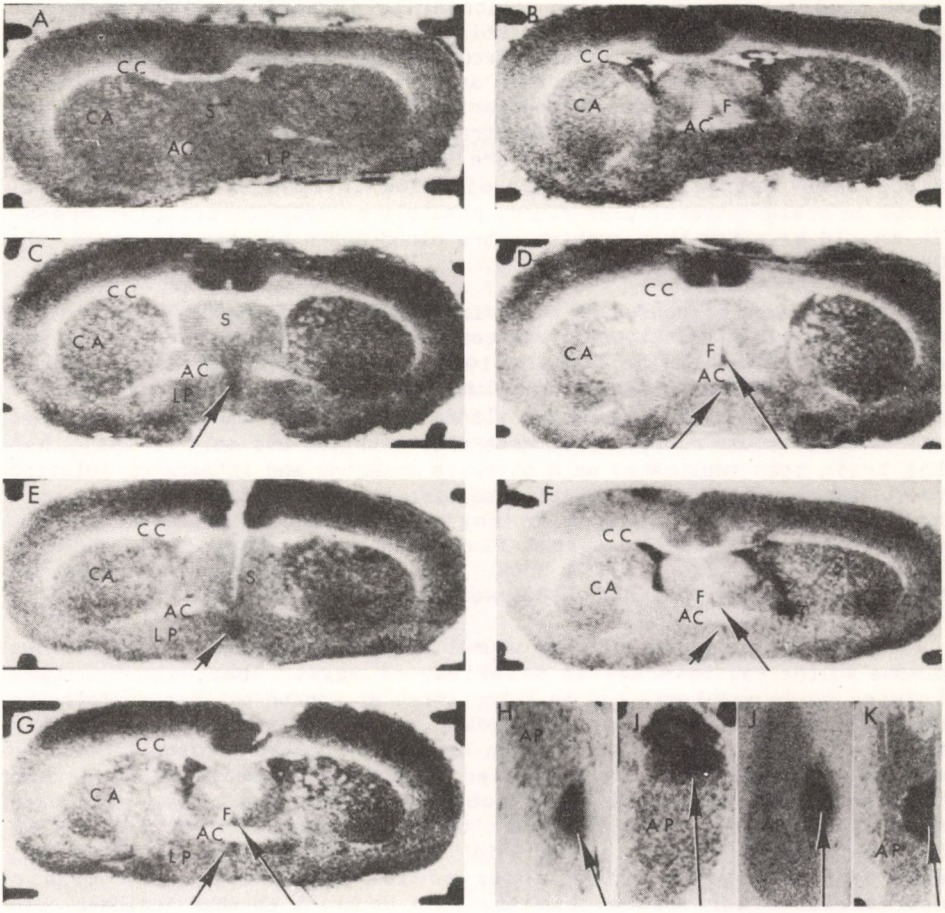

FIGURE 4. Photos of videoscans of brain section autoradiographs from rats injected with tritiated 2-DG to mark regional cerebral metabolic activity during various physiological conditions. Panels A-G are coronal sections through the AV3V region. Panels A and B are from a control rat in normal fluid balance. Panels C, D, and E are from Brattleboro rats with diabetes insipidus, water deprived for 48 hours to induce severe dehydration. The arrows point to a dark region in the AV3V which indicates increased metabolic activity in this region during dehydration. Panel F is from a rat that received an intracranial infusion of 600 mOsm artificial cerebrospinal fluid (star is adjacent to the infusion site); the arrow points to an area of increased metabolic activity in the AV3V region. Panel G is from a rat that received a subcutaneous injection of 3 ml of 12% NaCl/kg; the arrow shows increased metabolic activity in AV3V during this osmotic challenge. Panels H, I, J and K are pituitary sections with arrows pointing to increased metabolic activity in the posterior pituitary of the rats in panels C and D, E, F, and G, respectively. There was no darkening in the posterior pituitary of control rats. Abbreviations: AC = anterior commissure, AP = anterior pituitary, CA = caudate nucleus, CC = corpus callosum, F = fornix, LP = lateral preoptic area, S = septal region.

These studies have found that ventricular injections of hypertonic, artificial cerebrospinal fluid solutions elicit drinking and antidiuresis whether the hypertonicity results from addition of NaCl or other solutes such as sucrose; these studies then support an osmoreceptor rather than a sodium-receptor mechanism. By comparing latencies and threshholds after systemic versus ventricular administration, Thrasher et al. (1980a and b) have suggested that osmoreceptors are located in a brain region lacking a blood brain barrier yet capable of being influenced by cerebrospinal fluid or brain extracellular fluid, possibly a circumventricular organ. It should be noted that such an extra blood-brain barrier structure, the OVLT, is located in the AV3V region.

Once source of confusion regarding osmo- versus sodium-receptors may stem from a close relationship between central fluid balance mechanisms defending intracellular fluid volume versus extracellular or plasma volume. Sodium concentration or volume may be a significant stimulus for fluid balance systems regulating plasma volume. The importance of renal and cardiac volume receptors is generally accepted but only recently has the brain been implicated as a stimulus detector and integrator relevant to volume regulation. In addition to effects on thirst and antidiuretic hormone release, changes in angiotensin as well as in sodium concentration in cerebrospinal fluid can alter both renal sodium excretion (Andersson, 1977; Mouw, 1976) and sodium appetite (Buggy and Fisher, 1975; Bryant et al., 1980; Weisinger et al., 1979).

It should be apparent that neural systems defending cellular versus plasma volume can interact in a variety of ways. Thus, water intake induced by hypovolemia can be potentiated by concurrent hyperosmolarity or inhibited by hyposmolarity (Stricker, 1975). It is quite possible then that the response to a given osmotic or volume (sodium) challenge could vary depending on the parameter selected for measurement or the background physiological conditions. Moreover, there may be at least some neuroanatomical overlap between these systems; Bealer et al. (1979) have reported that AV3V lesions which disrupt osmotic regulation in rats also decrease sodium intake after sodium deprivation and impair natriuresis after volume expansion; in contrast to sham-lesioned rats that increased circulating natriuretic hormone after volume expansion, rats with AV3V lesions did not have levels of natriuretic hormone detectable by bioassay.

FUNCTIONAL ORGANIZATION

Since similarities and interactions exist between neural systems for fluid balance regulation, it has been a worthwhile strategy to search for differences in angiotensin and osmotic stimulated regulatory responses to establish in what ways the underlying neural substrates are common and in what ways distinct. On the basis of a synergestic interaction in goats to central stimulation with angiotensin or hypertonic NaCl, Andersson (1977) suggested that angiotensin in cerebrospinal fluid may act on the juxtaventricular receptors normally stimulated by sodium ions by influencing local ion transport to facilitate excitation of sodium receptors. With intraventricular injections or infusions of hypertonic NaCl combined with a range of angiotensin doses, however, we have not observed in the rat a drinking response to the combined stimuli greater than the sum of drinking to each stimulus alone. Thus, in the rat there is no available functional evidence which supports a synergestic central

action of angiotensin and hypertonic NaCl.

Furthermore, our experience with AV3V lesions in rats does not support the hypothesis that angiotensin and hypertonic NaCl act on the same juxtaventricular receptor. Although responses to both osmotic and angiotensin challenges are often blocked by AV3V lesions, this is not invariably the case. As discussed earlier and as shown in Figures 2 and 3, some AV3V lesions produce a selective deficit to angiotensin or osmotic stimuli only (Buggy and Johnson, 1977; Lind et al., 1979). This implies that while the receptors and neural substrates for these stimuli may be comingled, they are not identical nor do they overlap completely. As the next sections indicate, it is also possible to functionally dissociate response systems for hypertonic NaCl and angiotensin stimuli.

Sodium Preferences after Osmotic and Angiotensin Stimulation

When access to both water and dilute sodium chloride solutions is provided, rats will consume under ad lib conditions a relatively fixed ratio of water to saline. If drinking is stimulated instead by peripheral or central injections of hypertonic solutions or angiotensin, different preferences for saline are demonstrated. Figure 5 summarizes the general observation from studies examining both saline and water intake (Buggy and Fisher, 1975; Buggy et al., 1979). Water intake is induced by both angiotensin and hyperosmotic stimulation but significant intakes of saline are observed only after stimulation with angiotensin. Increased sodium intake after angiotensin stimulation has also been reported by Bryant et al. (1980). With respect to the saline preference obtained under ad lib conditions, angiotensin increases saline preference while hyperosmotic stimuli or cholinergic agonists decrease the preference for saline.

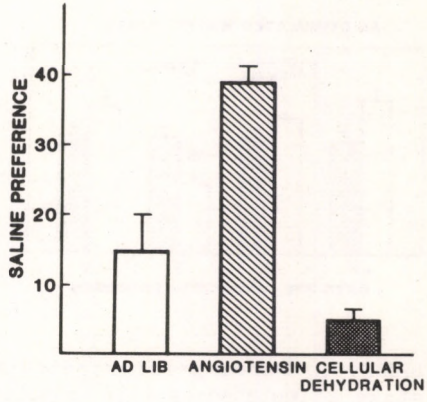

FIGURE 5. Saline preference for rats in 2 bottle test with both water and 1.8% NaCl solution to drink. The preference measure is the percent of total fluid intake accounted for by saline drinking. These changes in saline preference are evident after either central or peripheral administration of angiotensin or hypertonic NaCl solutions (cellular dehydration).

These responses to ingest or not ingest NaCl when water intake is stimulated are appropriate in both cases to correct the dehdration conditions represented by these stimuli. Hyperosmolality signals intracellular dehydration which is most efficiently repleted by addition of water alone whereas angiotensin signals decreased extracellular volume or plasma volume which is most effectively repleted by addition of sodium as well as water. The difference in saline preference is a fundamental

difference between fluid regulatory systems activated by osmotic versus angiotensin stimuli which must reflect basic differences in the organization of the underlying neural substrates.

Differences in Estrogen Modulation

Fluid regulatory systems sensitive to angiotensin and osmotic stimuli can also be discriminated on the basis of sensitivity to estrogen (Danielsen et al., 1980; Findlay et al., 1979). The volume of fluid intake is reduced in female rats on the day of estrus for ad lib or angiotensin stimulated fluid intake but not drinking stimulated by osmotic stimuli or cholinergic agonists. In male rats or ovariectomized female rats, fluid intake over days does not vary for any of the above drinking conditions. If saline and water is provided, the magnitude of saline as well as water intake varies through the estrous cycle, again for ad lib or angiotensin stimulated but not osmotic or cholinergic agonist stimulated conditions. The reductions in water intake are proportional to reductions in saline intake so that the overall saline preference remains steady (Danielsen et al., 1980).

Since the changes in fluid intake noted are greatest on the day of estrus, 24 hours after peak levels of plasma estrogen, and since removal of the ovaries and their production of estrogen abolishes the fluctuations in intake, it seemed possible to account for the observed effects by postulating a differential action of estrogen on the various neural substrates for fluid balance. To test this hypothesis, water intake and pressor responses under a variety of conditions were measured for several days before and after a single injection of 1 μg estradiol benzoate or vehicle in the AV3V of male or ovariectomized female rats (see Figure 6; Danielsen and Buggy, 1980).

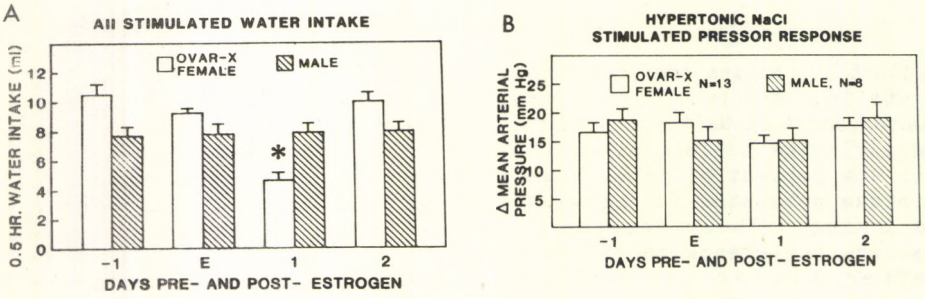

FIGURE 6. Panel A illustrates the transient depression in water intake stimulated by AV3V injections of angiotensin in ovariectomized female but not male rats observed on the day following AV3V administration of estrogen; pressor responses induced by AV3V injections of angiotensin were also attenuated on the day after central treatment with estrogen, again in females only. Panel B illustrates the absence of change in pressor responses induced by AV3V injections of hypertonic NaCl following AV3V estrogen administration; drinking responses to AV3V injections of hypertonic NaCl also remained constant after estrogen treatment.

Water intake for all conditions was not different from preceding days when measured 2 hours after estradiol injection. On the day following estradiol injection, however, ad lib water intake or drinking stimulated by angiotensin (but not cholinergic agonists or osmotic stimuli) was depressed in the ovariectomized female but not the male rats. When pressor responses induced by central injections of hypertonic NaCl, cholinergic agonists, and angiotensin were examined in this way, central estrogen treatment resulted in a smaller increase in arterial pressure to the angiotensin stimulus only, and only in females. The latency of about a day until visible effect on both thirst and pressor responses is consistent with the delayed time course of steroid action allowing time for expression of a steroid effect on gene transcription and protein synthesis. The next day, 2 days after estradiol injection, responses under all conditions returned to the magnitudes observed before AV3V estrogen treatment.

Thus, central treatment with estrogen produces in females only a reversible depression of response magnitude which differentially affects the neural substrate sensitive to angiotensin versus osmotic stimuli. This demonstration of differential sensitivity to estrogen coupled with the observations on sodium preferences provide a basis for discriminating the functional organization of neural systems sensitive to angiotensin versus those subserving osmotic regulation.

SUMMARY

The experiments reviewed here have the common theme of characterizing the central neural substrates for osmoregulation. Intracranial stimulation and ablation studies reinforced by mapping of regional cerebral metabolic activities have all implicated the preoptic periventricular tissues (AV3V) as a necessary component in osmoregulation, probably as a receptive or integrative area before divergence of effector pathways for thirst, antidiuretic, and pressor responses. Most studies concur that the relevant central receptor is an osmoreceptor rather than a sodium-receptor although sodium receptors in the brain may subserve extracellular fluid volume regulation via effects on sodium intake and sodium excretion. The neural substrate for osmoregulation may be contrasted to the neural substrate mediating central responses to angiotensin both anatomically and functionally. While the AV3V region is sensitive to both angiotensin and osmotic stimuli, lesion studies suggest that the critical neural substrates do not overlap completely. Functionally, angiotensin and osmotic sensitive neural substrates may be discriminated on the basis of sodium preference since compared to ad lib preference, angiotensin increases while osmotic stimuli decrease sodium preference. These neural substrates may also be distinguished on the basis of estrogen modulation since drinking and pressor responses to angiotensin but not osmotic stimuli are reversibly decremented in female rats after central estrogen administration.

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IS THE CONTROL OF SODIUM EXCRETION PARTLY DUE TO SIGNALS FROM RECEPTORS LOCATED IN THE LEFT ATRIUM OF THE HEART?

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In mammals osmolar concentration and volume of the extracellular fluid is kept fairly constant within narrow limits. Though many important informations concerning the osmo- and volume control have been published since the first description by Verney (1948) and Henry and Gauer (1956). Many questions remained unanswered (Goetz et al. 1975). The maintenance of a constant osmotic pressure is partly dependent on the control of sodium balance, because 90% of the extracellular osmolality are due to sodium salts. The control of sodium balance can be described under two different aspects:

1. After a loss of sodium, the organism must be able to retain Na by the kidneys - if it is available again - as long as the deficit is repaired and the extracellular volume is reexpanded. In conscious dogs, kept under the conditions of a high sodium intake, the upper limit of the extracellular volume seems to be in the range of 22% of the body weight (Reinhardt, H.W. and D.W.Behrenbeck 1967).
2. After the intake of an oral sodium load the osmotic and volume homeostasis should be regained as fast as it is possible.

This presentation will contribute to answer the question: What is the possible role of receptors, located in the left atrium or the pulmonal vascular bed in the short-term adjustment of sodium balance under these two aspects ?

Methods:

Experiments were performed on chronically instrumented dogs, kept under constant dietetic and environmental conditions. The sodium content of the diet was 0.5 and 14.5 mmol Na/kg bw/day. For details see: Kaczmarczyk et al. 1978 (6), Reinhardt et al. 1977, 1980 (10, 11, 12). Briefly: Female beagle dogs (10-12 kg bw) were instrumented with a carotid loop, a purse string around the left atrium and an implanted left atrial catheter. Pulling of the string, which was lead out on the left side of the thorax, elevated the left atrial pressure (eLAP ↑). Some dogs were additionally cardiac denervated (Drake et al. 1980), adrenalectomized (Reinhardt et al. 1980, 10), or fitted with an pneumatic cuff above the renal arteries. The degree of inflation or deflation of the cuff was automatically controlled to keep the renal perfusion pressure constant (Reinhardt et al. 1980, 12).

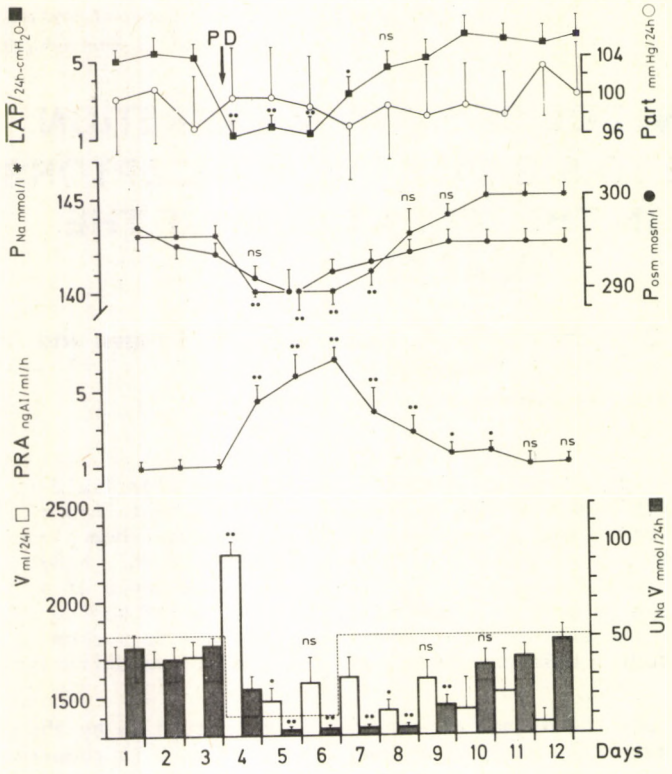


Fig. 1

Influence of a reduction of total body sodium (-91±8 mmol) (5 conscious dogs (8 expts)) on left atrial pressure (LAP), plasmaconcentration of Na (PNa), osmolality (Posm), plasma renin activity (PRA), and sodium and water excretion (UNaV, V̇). The dogs were kept under standardized conditions (water intake: 100 ml/kg/day). dashed line: sodium intake. 'osmocontrol' overrides 'volumecontrol'
Δ LAP and Δ PRA correlate well with sodium retention

Some mongrel dogs (10-21 kg bw) were instrumented only with chronic arterial, venous and left atrial catheters (Reinhardt et al. 1980, 11 *). These dogs received 0.5 or 2.0 mmol Na/kg/day and a constant daily water intake (100 ml/kg bw). In these dogs the left atrial pressure (LAP) was recorded over several weeks: The LAP-catheter was connected with two pressure transducers mounted on either side of the chest at coordinates

*These studies were performed in the Department of Physiology and Biophysics (Dr.A.C.Guyton) together with A.W.Cowley, jr. and E.W.Quillen, Jackson, Ms., USA

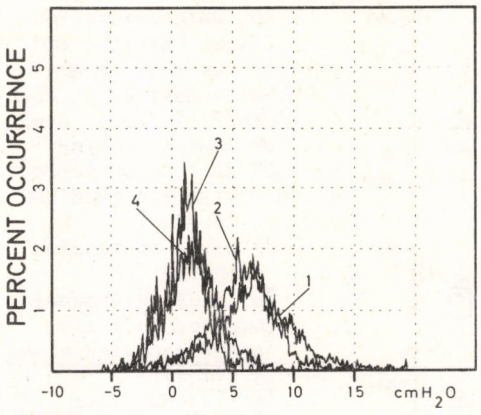


Fig. 2

Frequency distribution of LAP values, recorded every 60 sec (1 dog, 9 days)
1. mean of 3 days, control
3. first day after peritoneal dialysis
4. mean of 3 days after peritoneal dialysis
2. mean of 3 days after equilibration of the Na-deficit (compare fig. 1)

'LAP a not controlled variable ?'
LAP remains low as long as no sodium is available

representing the hemodynamic zero reference point. Continuous computerized averaging of digitalized signals from both transducers enabled corrections for posturally induced hydrostatic pressure changes. This procedure enabled quantification of short and long-term changes of LAP in the conscious dog. The arterial pressure (Part) was recorded over 24 h also. In these experiments total body sodium was diminished by peritoneal dialysis (PD). In a first set of 8 experiments 91 $\pm$ 8 mmol Na were withdrawn by PD.

Results to 1.:

After the removal of sodium a remarkable decrease in plasma sodium concentration (PNa), osmolar concentration (Posm) and left atrial pressure (LAP) occurred (PNa 143$\pm$1 to 135$\pm$2 mmol/l, Posm 295$\pm$4 to 279$\pm$4 mosm/l). Plasma renin activity ($\overline{P}$RA) increased (.96$\pm$.57 to 9.60$\pm$1.87 ng AI/ml/hour). But on the next morning (fig. 1), 20 hours after PD, Posm and PNa were in the range of the controls (98 %) though no sodium was available. The urine volume ($\dot{V}$) of all dogs was very high up to 20 hours after PD, resulting in a negative water balance by about 500 ml on the day of PD. This 'shrinking' could be demonstrated by an increase of hematocrit (+23 %). For further 3 days the dogs received no additional Na to the food and the LAP remained near +1.0 cm H_2O, this means that there was a decrease of LAP by about 4 cm of water for 3 days (fig.2). LAP remained lowered and PRA elevated as long as no Na was available. The arterial pressure (Part) was unchanged over the time. After 3 days 2 meq Na were added to the food. Thereafter for 2 days a complete Na retention was observed. After 5 days the Na-deficit, induced by PD, was equilibrated. LAP increased stepwise with Na retention, while PRA decreased in the same manner. These results clearly indicate that under defined conditions in conscious dogs the osmocontrol dominates the volumecontrol. But the organism should be able to feel a volume deficit by means of intrathoracic receptors: LAP seems to be in contrast to Posm or PNa not a controlled variable.

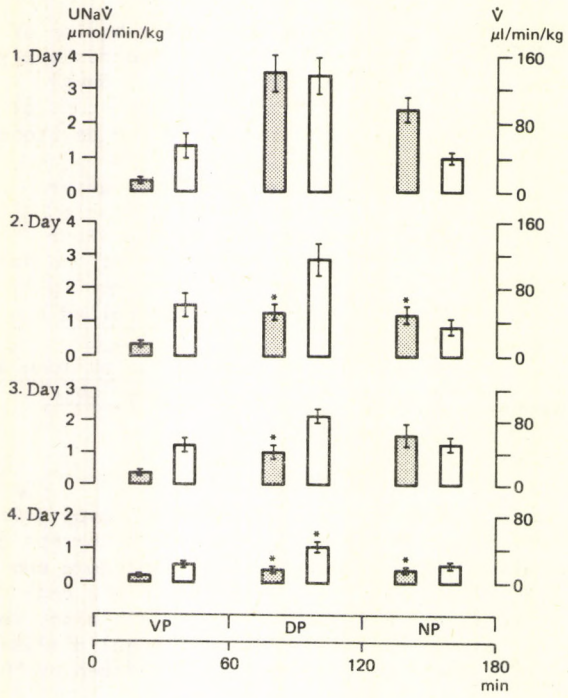


Fig. 3

Sodium (dotted bars) and water excretion (light bars), 6 conscious dogs, eLAP↗ for 60',4 days after each other (1. to 4. day)
VP control period
DP distension period with elevated LAP (eLAP↗)
NP after distension period
*$p < 0.05$ (Wilcoxon) compared to 1. day.
'after repeated eLAP↗ (3 times)' 'atrial natriuresis' is nearly abolished

For the mechanism of Na-retention, the renin-angiotensin-system (RAS) could be responsible. This is supposed because there is a very clear correlation between LAP-increase and PRA-decrease. The retention of Na did occur as long as PRA was elevated.

Results to 2.:

After the intake of a sodium rich meal in chronically instrumented dogs, a very good correlation between UNaV̇ and LAP could be demonstrated (Kaczmarczyk et al. 1979). Therefore a lot of experiments were performed in which LAP was elevated by means of a purse string (see methods or (Reinhardt et al. 1977)). By pulling the string LAP was elevated for 60' as high as it was observed postprandially (+10 cm H_2O (Kaczmarczyk et al. 1979)). This technic has the advantage that the experiments could be performed in conscious dogs without any changes of the Na-balance, because the last food and water intake was 20 hours before.
Under these defined conditions eLAP↗ was followed by an increase of UNaV̇ even under the conditions of a low sodium intake (Reinhardt et al. 1977). It could be clearly demonstrated that under defined conditions there is existing a very potent mechanism for sodium excretion which can be activated by stretching the wall of the left atrium or the vascular bed of the lungs. eLAP↗ was always followed by a pronounced decrease of PRA.

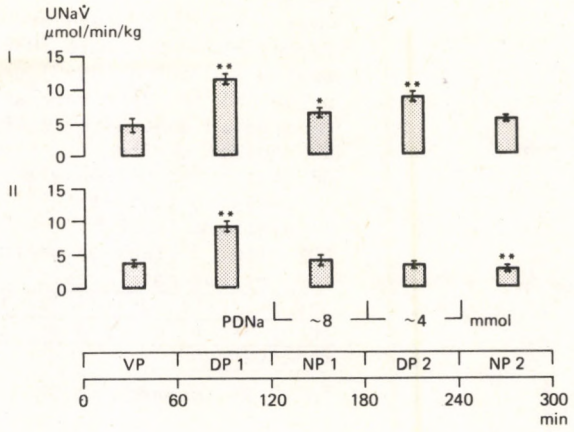


Fig. 4

Sodium excretion during eLAP↗, 2 times for 60 min (DP_1 and DP_2) in dogs kept on a high sodium diet.
I undiminished total body sodium (TBS)
II diminished TBS (12 mmol PD)
No 'atrial natriuresis' is present during DP_2 after acute slight reduction of TBS

This decrease was shown under different conditions of Na intake (Reinhardt et al. 1980, 10).

At first it was supposed that a decrease in mineralocorticoid activity, due to the decrease of PRA, was responsible for the 'atrial natriuresis' (AN), but the natriuretic effect of eLAP↗ could not be abolished after the removal of the adrenals (Reinhardt et al. 1980, 10). Therefore AN is not due to changes in mineralocorticoid secretion of the adrenals. In contrast to anesthetized dogs, in conscious dogs eLAP↗ is followed by an increase in Part. To exclude the possibility that the increase of renal perfusion pressure (Pren) causes the increase in UNaV̇, Pren was kept constant by means of an implanted pneumatic cuff above the renal arteries, which automatically controlled Pren. But this procedure did not modify AN. These results clearly indicate that AN is not produced by an increase of Part (Reinhardt et al. 1980, 12).
But on the other hand the infusion of very small amounts of angiotensin II (4 ng/min and kg), which did not change Pren, abolished the eLAP↗ induced natriuresis completely and diminished the diuretic response of eLAP↗.

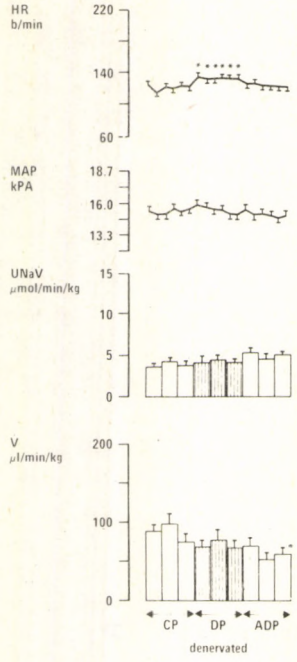


Fig. 5

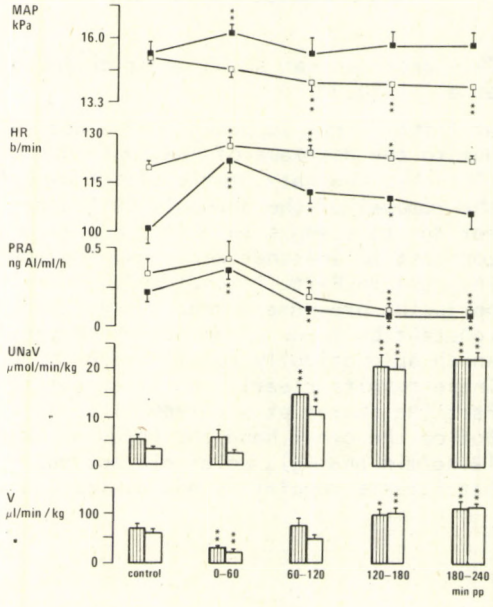
Heart rate (HR), mean arterial blood pressure (MAP), sodium excretion (UNaV̇), and urine volume (V̇) in cardiac denervated dogs after eLAP↗
CP control period
DP distension period with elevated left atrial pressure (eLAP↗)
ADP after distension period
The 'atrial natriuresis' and diuresis was completely abolished after cardiac denervation

*$p < 0.05$ compared to CP

Fig. 6

Mean arterial blood pressure (MAP), plasma renin activity (PRA), sodium excretion (UNaV̇) and urine volume (V̇) in conscious dogs after the intake of a sodium rich meal.
Intact dogs: striped bars
cardiac denervated dogs: light bars
Postprandial sodium and water excretion shows no significant differences between intact and cardiac denervated dogs

*$p < 0.05$ compared to
**$p < 0.01$ fasting control



Therefore it is possible that AII is a fast acting component for the sodium retention on renal level. But it should be pointed out, that this finding is not a valid argument against the existence of a 'natriuretic hormone' or other Na eliminating factors - if we assume that the antinatriuretic properties of AII would override natriuretic activities. It is now the question if further reliable experimental results are available which clarify the role of atrial receptors in controlling sodium balance. This seems to be very important because, as it was shown above, an expansion of extracellular fluid could not be obtained without retentionof sodium.

Results to 1. and 2.:

If the standard eLAP↗ experiment was repeated on 4 subsequent days without replacement of the eLAP↗ induced 'chronic' sodium deficit, the natriuretic eLAP↗-response decreased from day to day (fig.3). These results could be confirmed by acute withdrawl of very small amounts of sodium by means of peritoneal dialysis (PD) (fig.4). The same suppression of AN could be obtained by an acute reduction of blood volume by about 10% (unpublished observations). These very small acute and chronic changes of total body sodium were not accompanied with any detectable changes in Part, Posm or the plasmaconcentration of protein, but with a slightly decreased PNa in dogs kept on a high Na intake in the PD-studies.

If eLAP↗ was continued over several hours, UNaV̇ was still elevated after 4 hours (Tab.1).

Table 1: Adaption of atrial receptors or other reasons for Angiotensin II-escape ?
Urine volume (V̇), Na-excretion (UNaV̇), mean arterial blood pressure (Part), Plasma Renin Activity (PRA) during elevation of left atrial pressure (+ 10 cm H_2O)
(**$p<0.01$ compared to control *$p<0.05$)

	control: before eLAP↗	eLAP↗: after 1 hour	after 2 hours	after 3 hours	after 4 hours	when UNaV̇ was in the range of control*
V̇ µl/min/kg	59+13 (10)	194+16 ** (10)	126+12 ** (10)	68+6 * (10)	43+5 **(9)	23+6 ** (9)
UNaV̇ µmol/min/kg	3.7+0.9 (10)	12.4+1.8 ** (10)	12.6+1.2 ** (10)	9.6+0.5 ** (10)	6.5+0.9 **(9)	3.9+1.0 ns (7)
Part mmHg	116+3 (20)	125+4 ** (20)	123+3 ** (20)	120+3 ** (20)	116+3 ns(18)	116+5 ns (16)
PRA ng AI/ml/h	0.80+30 (10)	0.18+0.5 ** (10)	0.16+0.05 ** (10)	0.17+0.05 ** (10)	0.28+0.07 **(9)	0.61+14 ns (9)

*between 5. and 7. hour of eLAP↗.

Beginning at the end of the 4. hour and up to the end of the 5. hour UNaV decreased and reached the control level during a persistent eLAP ↑ (Tab.1). When UNaV had reached the control level PRA had been increased also. This means, that a withdrawl of 12-23 mmol of Na is followed by an 'escape' of UNaV and PRA from the regimen of the elevated LAP. Since PNa showed not always detectable changes and since Part was not below the controls we did not know the acting stimuli for renin release at present time. But it is supposed that sodium or osmotic receptoractivities are responsible for the PRA escape.
Another approach to evaluate the role of atrial receptors in the control of Na-balance was the dissection of the sympathetic and vagal nervous supply of the heart*. The results were very clear (fig.5): After complete cardiac denervation eLAP ↑ failed to induce a natriuresis and diuresis. No increase in heart rate or Part could be observed. This means, that the origin of AN and the cardiac reflexes are located in the heart and not in the vascular bed of the lungs.

Therefore signals, generated by cardiac receptors, are completely responsible for AN. Comparable results were obtained during the same time by D.C.Fater et al.(1980). This group inflated a balloon in the left atrium of conscious dogs before and after cardiac denervation.

But when we tested the postprandial sodium excretion (fig.6) we did not find significant differences between cardiac innervated and denervated dogs. These results indicate, that after the elimination of cardiac receptors the equilibration of Na-balance can be obtained as well as before denervation. Therefore, these results do not support the hypothesis that cardiac receptors are important for the control of sodium and water excretion. But they do not exclude any importance as long as the real physiological function of these receptors is not known.

Conclusions:
1. The left atrium is a suitable place to "measure" an extracellular volume deficit. The extracellular volume can only be reexpanded if external sodium is available. 2. Osmocontrol overrides volumecontrol. 3. Elevation of left atrial pressure reduces renin secretion by the kidneys. The renin-angiotensin-aldosterone-system has two antinatriuretic components: a faster acting one: AII, and a slower acting one: aldosterone. 4. Atrial natriuresis can be prevented by acute or chronic reduction of total body sodium (by means of an RAS-escape ?). 5. It is supposed, that the RAS is more responsible for Na-retention than for Na-elimination. 6. Cardiac denervation prevents atrial natriuresis also, but does not disturbe the postprandial adjustment of sodium balance. Therefore atrial receptors are not unique in the role of rapid elimination of an orally given sodium load. Their physiological importance is absolutely unknown.

*These experiments were performed together with M.I.M.Noble, A.J.Drake and J.Stubbs, The Midhurst Research Center, London

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F. Obál, G. Benedek (eds)

CONCLUDING REMARKS ON OSMOREGULATION

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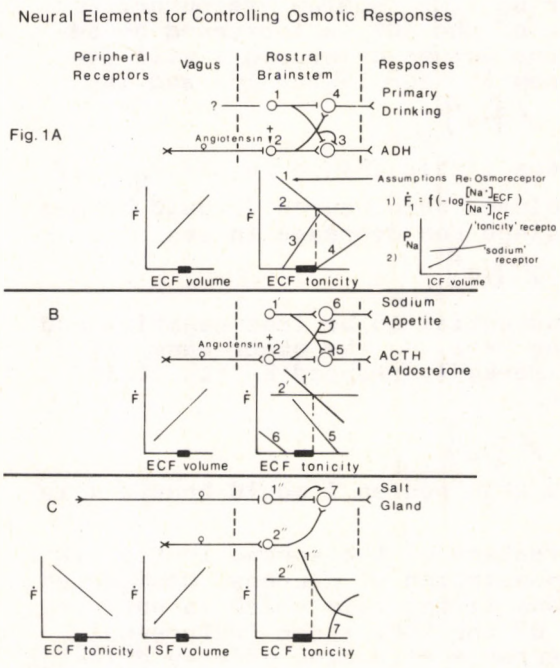
The aim of this symposium has been to outline the neural network for osmoregulation in vertebrates. The four major presentations of this symposium deal with available information for three vertebrate classes, reptiles, birds and mammals. This summary statement will be an attempt to describe a neural network which can account for most, but not all, of the known facts. No model of a complex regulatory system is adequate. At best, a model summarizes the known and invites an exploration of the unknown. Fig. 1, attempts to model the neural elements controlling some of the autonomic and behavioral responses affecting the osmolality of the body fluids. When the osmolality of the body fluid tends to exceed an optimal level, primary drinking is elicited and the release of antidiuretic hormone (ADH) is increased to recover more water from the urine. These are important responses in reptiles, birds and mammals for avoiding dehydration. Fig. 1.A suggests a

Fig. 1A



schema whereby they are activated. There are several features of this schema which require detailed comment. The first feature is to assume that there is a population of neurons concentrated in the region of the rostral brainstem where the blood brain barrier is lacking and which respond to changing tonicity of the extracellular fluid (ECF) surrounding them. Further, it is assumed that these neurons, designated 1 in Fig. 1A, exhibit a spontaneous firing rate, $\dot{F}_1$, which is some function of their membrane potential. That is

$$\dot{F}_1 = F_1 \left(- \log \frac{[Na^+]_{ECF}}{[Na^+]_{ICF}}\right)$$

since the membrane potential is determined primarily by the negative logarithm of the ratio of the extracellular to the intracellular sodium concentrations. Thus, adding NaCl to the ECF increases the ratio

$$[Na^+]_{ECF} / [Na^+]_{ICF}$$

of neuron 1 so that its membrane potential becomes more negative (hyperpolarized) and its firing rate decreases. In addition it must be assumed that the sodium permeability of the membrane of neuron 1 increases with increasing intracellular fluid volume (ICFV). As illustrated in Fig.1A, P_{Na^+} of neuron 1 may increase more or less rapidly as a function of ICFV, depending on whether neuron 1 behaves like a "tonicity" receptor or like "sodium" receptor. For example, when the tonicity of the ECF is increased by adding sucrose to the ECF, the volume of neuron 1 will decrease by loss of water from its ICF to the ECF and its ratio of

$$[Na^+]_{ECF} / [Na^+]_{ICF}$$

will decrease. If there were little change in the P_{Na^+} of neuron 1 at the reduced volume, then neuron 1 would behave like a "sodium" receptor since the decrease in its

$$[Na^+]_{ECF} / [Na^+]_{ICF}$$

would cause its membrane potential to be less negative and thereby increase its firing rate. On the other hand, if the P_{Na^+} of neuron 1 were markedly reduced by its diminished volume, then its ratio

$$[Na^+]_{ECF} / [Na^+]_{ICF}$$

could actually increase so that neuron 1 would behave like a "tonicity" receptor.

The second important feature of the schema in Fig. 1A is that there is another population of neurons, designated 2, which exhibit spontaneous firing rate which is not very dependent on the tonicity of the ECF. These "reference" neurons facilitate both neuron 4 effecting primary drinking and neuron 3 effecting the release of ADH whereas the receptor neurons like 1 inhibit both primary drinking and ADH release. The threshold tonicity for these two responses

differ. This feature is achieved in the schema simply by providing more inhibition than facilitation to neuron 3 effecting ADH release. This provision lowers the threshold tonicity and increases the sensitivity as well. An alternate provision to lower the threshold tonicity for ADH release without effecting to sensitivity would have been to provide more facilitation from neuron 2 than inhibition from neuron 1.

A third feature of the schema in Fig. 1A is a provision for the influence of other neural and humoral inputs. Prof. Simon demonstrated in his presentation that ADH release is quite sensitive to the ECF volume, that is, a small increase in ECFV is sufficient to lessen the release of ADH. Angiotensin II can also facilitate drinking and release of ADH.

Prof. Buggy's evidence that neurons adjacent to the anterior-ventral region of the third ventricle are more active during response to hypertonic solutions suggests that neurons like 3 and 4 are situated in this region. The schema illustrated in Fig. 1A suggests that neurons like 1 would be less active during hypertonic stimulation and would not be detectable by the C_{14} tagged 2-deoxyglucose method.

A possible partial schema for influencing the retention of sodium and increasing sodium appetite is illustrated in Fig. 1B. This schema is also based on the assumptions that the populations of receptor neurons and "reference" neurons reside in the region of the hypothalamus lacking the blood brain barrier and that these two populations act antagonistically. However, unlike the receptor neuron in Fig. 1A, receptor neuron 1' facilitates neuron 5 effecting release ACTH and in turn the release of mineralocorticoids so that more sodium is resorbed by the renal tubules. Neuron 1' also facilitates neuron 6 which elicits sodium appetite. The "reference" neuron 2' is illustrated as inhibiting both sodium appetite and sodium resorption. A separation of tonicity thresholds for these responses is provided for by differing degree of facilitation and inhibition from neurons 1' and 2', respectively. Illustrated also is a provision for the ECF volume and for angiotensin II to influence these responses.

Prof. Reinhardt has presented evidence that stretching the left atrial wall affects atrial receptors in such a way to reduce plasma renin and allow natriuresis, an effect which he calls "atrial natriuresis" (AN). An experimental increase of left atrial pressure (LAP) which elicits AN can be blocked by an infusion of non pressor levels of angiotensin II. Furthermore, in adrenalectomized dogs, an experimental increase in LAP elicits an undiminished AN; therefore these atrial receptors must influence the renin angiotensin system by a neural-humoral link other than that postulated in Fig. 1B. Dogs with denervated hearts cannot respond by AN. Nevertheless, their postprandial response to salt ingestion is not severely disturbed. This indicates that more than one connection exists between the CNS and the renal tubules including perhaps the one suggested in Fig. 1B.

Professor Dunson and Simon have discussed salt secretion by extra-renal glands found in reptiles and birds which in-

gest marine organisms. These glands receive parasympathetic ennervation and their activity is controlled by the CNS. Prof. Simon presented evidence that receptors reside both in the head and in the region of the heart, at least, in the duck and the goose. These features are incorporated into the schema for controlling salt gland secretion illustrated in Fig. 1C. A population of neurons designated 1'' are shown to inhibit neuron 7 which controls salt gland activity. Another population designated 2'' is shown to facilitate neuron 7. In these respects neurons 1'' and 2'' are similar to neurons 1 and 2.However, Prof. Simon discussed evidence which suggests that there must be distinct differences between neurons 1'' and 1. Neuron 1 behaves as if it were a "tonicity" receptor responding to an ECF tonicity increase by either sodium or mannitol and eliciting an antidiuretic response. The receptor responded equally to an increase and to a decrease in ECF tonicity. On the other hand, the receptor controlling salt gland secretion responded as if it were a "sodium" receptor. Furthermore, it responded weakly or not at all to an increase in ECF sodium concentration when the latter was sufficient to stimulate continous salt gland secretion. Under the same circumstances it responded promtly and strongly to a decrease in sodium concentration. These features are incorporated into Fig. 1C by suggesting that the firing rate of neuron 1'', $F_{1''}$, is a different function of

$$\left(-\log \frac{[Na^+]_{ECF}}{[Na^+]_{ICF}}\right)$$

than that of neuron 1 and that the P_{Na^+} for neuron 1'' depends weakly on its ICF volume as expected for a "sodium" receptor. There are other important distinctions between the control of urine formation and the control of salt gland secretion. In forming urine, the release of ADH could be inhibited by a small increase in ECF volume; whereas decrease in ECF volume inhibited salt gland secretion. Furthermore, a decrease in the interstitial fluid volume inhibited secretion with no effect from the plasma volume. These features are also incorporated into the schema illustrated in Fig. 1C.

In conclusion, osmoregulation is achieved in reptiles, birds and mammals by a complex system of neural elements which control thirst, salt appetite, the release the endocrines and the activity of salt secreting glands. These responses are controlled in anappropriate way to maintain the osmolality of the intracellular fluid at an optimal level. This summary schema for activating these responses in an appropriate sequence is, at best, only partially accurate and must be altered as more evidence becomes available.

REGULATION OF THE SLEEP-WAKING RHYTHM BY ENVIRONMENTAL AND ENDOGENOUS FACTORS

Chairman:

A. A. BORBÉLY (Switzerland)

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REGULATION OF THE SLEEP-WAKING RHYTHM BY ENVIRONMENTAL AND ENDOGENOUS FACTORS

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The daily pattern of sleep and waking corresponds closely to the circadian rest - activity rhythm which is characterized by highly regular endogenous cycles. Light and other environmental factors may influence the phase of the rhythm by non-linear mechanisms (1). Focussing on this rhythmic aspect of sleep, one may investigate interactions between the pacemaker and environmental influences, and compare them with the regulation of other circadian rhythms. There are indications for a certain degree of independence between the circadian rhythms of the sleep substates as well as between the rhythms of sleep and those of other physiological parameters (e.g. body temperature) (2). The internal phase-relationship between these different rhythms may be of functional significance for processes subserved by sleep.

In addition to its rhythmic aspect, sleep may also be regarded as a homeostatic processes, since a total or selective deprivation gives rise to compensatory changes. Neurotransmitters, and in particular the monoamines, are known to be critically involves in sleep regulation (3), although their precise role remains to be established. In addition, compelling evidence indicates that an as yet unidentified sleep-inducing factor derived from the brain of sleep-deprived animals, may play a crucial role in sleep-homeostasis (4). These developments may be important for the search of a physiological sleep remedy in medicine. The unexpected observation that a pineal hormone exerts a sleep-inducing action when administered in minute doses to the cat (5), may be viewed in a similar context. Do these potent chemical agents act by separate mechanisms or are their actions mediated by a common neurotransmitter such as serotonin?

The study of thermoregulation in relation to the sleep - -waking cycle constitutes a further promising approach to the physiological characterization of the sleep state. By exposing animals to various ambient temperatures, substates of sleep may be selectively influenced (6). Moreover, sleep recordings during the transition to hibernation may offer insights into the evolutionary aspects of sleep and its relations to homeothermy (7).

The two facets of sleep, the circadian rhythm and the homeostatic process, show clear differences in their regulatory mechanims which may brought into conflict by experimental schedules (8). It may be useful to keep these two facets in mind when studying the influence of environmental and endogenous factors on sleep regulation.

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THE SLEEP PROCESS: CIRCADIAN AND HOMEOSTATIC ASPECTS

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Sleep may be regarded as a state which is characterized by a well-defined set of values of behavioral or electrophysiological parameters. The sleep state is customarily subdivided into the substates non-REM sleep (NREMS) and REM sleep (REMS) on the basis of distinctive electrographic features. For human sleep, NREMS is even further subdivided into 4 different stages. The state concept has undoubtedly contributed to provide a general descriptive framework of the sleep process. However, the strict definition of sleep states may make them appear as homogenous physiological entities which succeed each other in an orderly manner. The undue emphasis of the state aspect obscures the fact that many parameters of the sleep process undergo continuous changes. The direction and rate of their change may be more closely linked to the funtional aspects of sleep than the invariant features which are commonly regarded as distinctive state indicators. In the present paper attention will be focused on the temporal structure of the sleep-waking cycle, and on the regulatory and adaptive properties of the sleep process.

1. Sleep as a circadian rhythm

The sleep period coincides largely with the rest period of the daily rest-activity cycle. Figure 1 illustrates the regular pattern of the rest-activity rhythm, and its similarity between man and rat. The cycles reflect a truly circadian rhythm since they persist with a period close to 24 h even in the absence of time cues (1,2). Like various other circadian rhythms, the sleep-waking rhythm is generated by a central pacemaker, and is abolished after lesioning the suprachiasmatic nucleus (4). On the other hand, the rhythm is little affected by interfering with the normal sleep process. Thus sleep-

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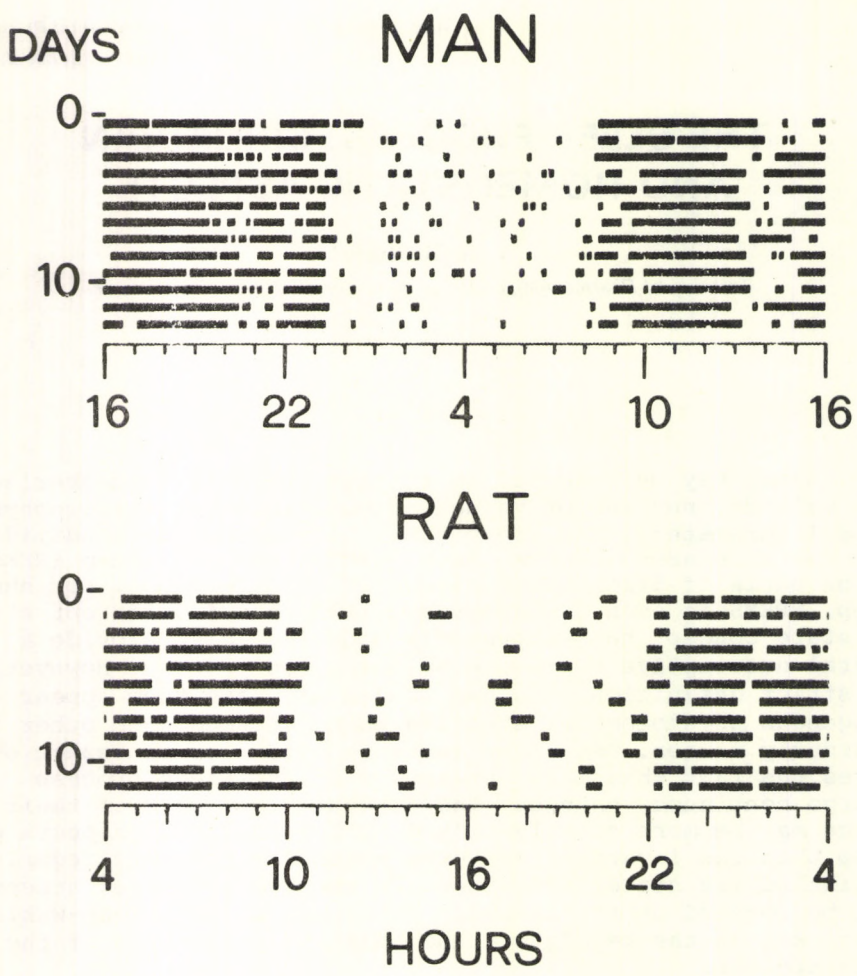

Figure 1. Rest-activity rhythm recorded in man and rat over a 11-12 day period. Lines indicate supra-threshold activity. In man recordings were made with a wrist-worn solid state activity monitor, in the rat with a mechano-electric transducing system under the cage. Note in the human record the reduced activity level in the evening, the sporadic movements during sleep at night, and the regular nap period in the afternoon.

deprivation for 24 h in a rat maintained under continuous darkness alters neither the period nor the phase of the circadian rest-activity rhythm (ref. 5 and manuscript in preparation). Viewed from the circadian vantage point, sleep appears therefore as a pre-programmed process which is under the rigid control of a circadian oscillator, and shows little capacity for adapting to environmental changes. On the other side, the existence of the circadian rest-activity rhythm in lower organisms that do not exhibit the typical electrographic sleep signs, may indicate an adaptive function from the evolutionary point of view. It is obvious that the timing of the circadian rest period to a specific part of the day-night cycle may reduce the danger from unfavorable environmental influences or predators, and minimize energy expenditure, thereby augmenting the chances for survival. It can be argued that the circadian rest period constitutes a phylogenetic precursor of sleep (6).

2. Sleep as a homeostatic process

The fact that sleep deprivation is followed by a compensatory increase in sleep, indicates that the sleep process is regulated relative to an internal reference level. The adaptive aspect of sleep homeostasis can be therefore contrasted with the rigid control of sleep by a circadian oscillator.* I have proposed elsewhere that the emergence of sleep may constitute a partial liberation from the limitations imposed by the circadian oscillator, since it allows an adaptive response to the momentary needs of the animal (3).

The homeostatic regulation of sleep is particularly obvious for slow wave sleep (SWS), the NREMS fraction with a low predominant EEG frequency. In animals and man, SWS predominates at the beginning of the daily sleep period, and then declines progressively (3,8). Sleep deprivation experiments have clearly shown that SWS increases as a function of prior waking time (3). Thus the slow waves in the EEG may represent an indicator for the intensity of the NREMS process. Moreover, if it is assumed that sleep mediates a restitutional process, SWS appears to be a good candidate for an electrophysiological correlate.

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* The term "homeostasis" has been applied also to the circadian rest-activity rhythm to characterize the tight regulation of its frequency (7).

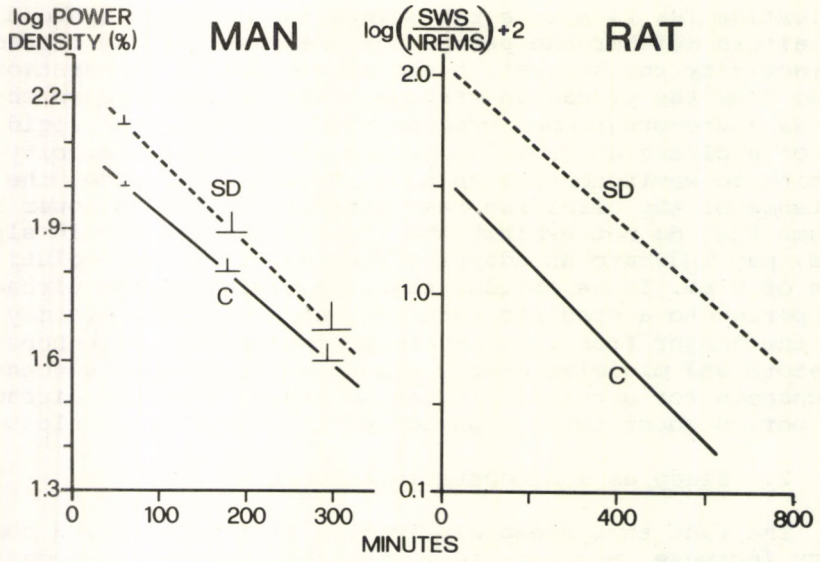

Figure 2. EEG power density (man) and slow wave sleep fraction of non-REM sleep (rat) plotted for a control sleep period (C; solid line) and a sleep period following sleep deprivation (SD; interrupted line; man: 40,5 h SD; rat: 24 h SD).
Man: regression lines computed for mean values plotted at the midpoints of the first three NREMS cycles (means with SEM for 8 subjects). The power density (0.75 - 25,0 Hz) of the first control NREMS cycle was defined as 100 % (Borbély, Baumann, Brandeis, Strauch and Lehmann; manuscript in preparation).
Rat: SWS was defined as the NREMS fraction with the lowest 10 % of EEG zero-crossing values (see ref. 3,5). Regression lines were computed for the mean hourly values (n=6).
The correlation coefficient r was for all computations significant at the 0.001 level.

Figure 2 illustrates the exponential decline of the EEG power density in man, and of SWS in the rat, during a control sleep period (C), and a sleep period subsequent to sleep deprivation (SD). Since the slopes of the regression lines do not differ significantly between control and SD, it can be assumed that they reflect a single process with different initial values. The slopes exhibit strikingly similar values in the two species ($t_{1/2}$ values: man: ca. 175 min; rat: ca. 150 min). Although the different computation procedures preclude a strict comparison of the records, the results may indicate that a similar process occurs during sleep in the two species. The data are also compatible with the assumption that

EEG slow waves indicate the presence of an endogenous sleep-promoting substance whose level depends on the prior waking period, and which is eliminated during sleep at an exponential rate. Pappenheimer and colleagues have shown that a sleep-promoting factor accumulates in the CSF as a function of prior waking time (9), and that deep SWS is induced upon its intraventricular administration (10 and this volume).

3. Sleep: a restitutional process?

The restitutional quality of a "good night's sleep" is a common experience. However, the failure to identify a specific recovery process subserved by sleep, leaves a major problem of sleep research unsolved. Various hypotheses relating to the functional significance of sleep and rest are outlined in the diagrams of Figure 3.

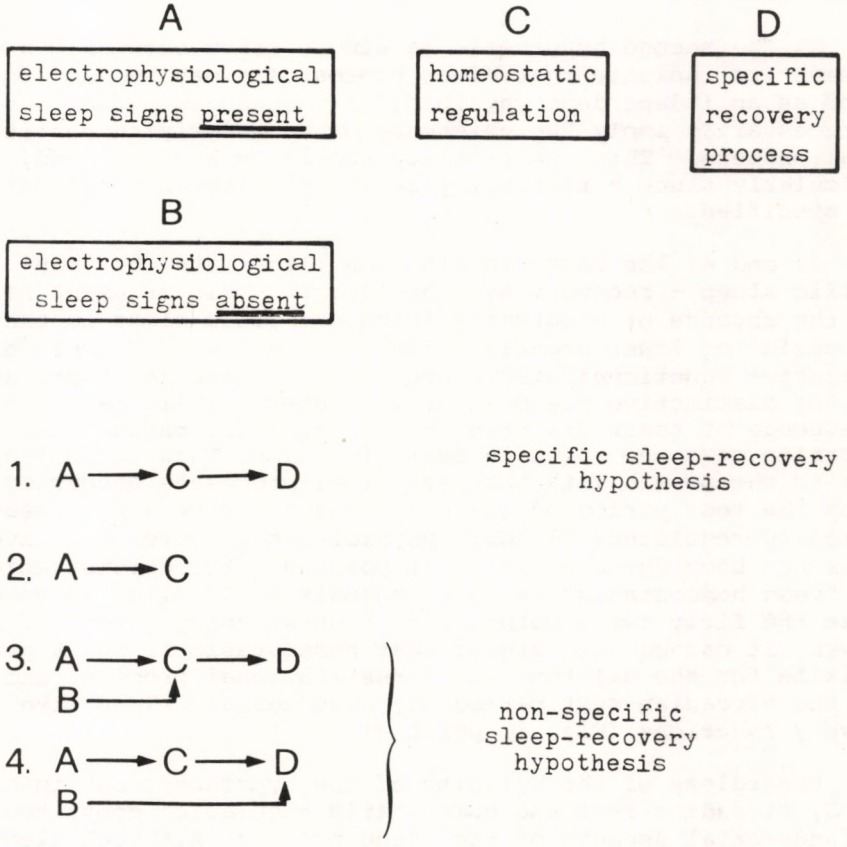

Figure 3. Diagrams outlining various hypotheses on the relationship between sleep and a recovery process.

1) The "specific sleep - recovery hypothesis" is based on the homeostatic regulation of sleep and its substates. Since homeostasis in other physiological systems serves to maintain essential body constituents within an optimal range (e.g. the extracellular level of sodium or glucose), an analogous function could be attributed to sleep. The periodic occurrence of sleep, and in particular its dependence on the prior waking time, may reflect a specific restitutional process. Although the level of REMS is also regulated, the SWS fraction of NREMS may be a particularly good indicator of the intensity of such a hypothetical recovery process (see preceding section). In addition, one could argue that although a circadian rest period is present also in lower organisms, it does not subserve a specific sleep-like recovery funtion, because no homeostatic regulatory mechanisms are evident. Thus, in contrast to sleep, the circadian rest period can be regarded as a pre-programmed 'forced immobility' state whose possible long-term adaptive significance has been mentioned above.

2) The second hypothesis (a single arrow between A and C) is meant to indicate that sleep homeostasis may be also regarded as an independent physiological mechanism which does not necessarily imply the existence of an underlying restitutional process. This possibility should be kept in mind, particularly since a recovery function of sleep has not yet been specified.

3) and 4) The last two diagrams illustrate the "non-specific sleep - recovery hypothesis". The main argument is that the absence of electrophysiological sleep signs in the rest period of lower organisms does not necessarily imply a qualitative functional difference between sleep and rest, and that the distinctive sleep signs in higher animals may be a consequence of their different brain structure rather than an indication of a new specific brain function. This reasoning leads to the possibility that essential processes occurring during the rest period of lower organisms may be also homeostatically regulated, although physiological correlates have so far not been detected (Fig. 3: point 3). The demonstration of a "rest homeostasis" in lower animals would allow to generalize the first two hypotheses to "non-sleeping" species. However, it can be also argued that homeostasis is not a prerequisite for the existence of a restitutional process, and that the circadian rest period may also mediate sleep-like recovery functions (Fig. 3: point 4).

Regardless of the validity of the hypotheses outlined in Fig. 3, circadian rest and homeostatic regulation constitute two fundamental aspects of the sleep process. Although sleep has been investigated principally in mammalian species,it is important to realize that the circadian rest-activity rhythm prevails throughout the animal kingdom, and is notably also

present in species with little or no brain. Are there homologies between sleep and rest and at what evolutionary level does homeostasis emerge? The experimental investigation of these problems may lead to a deeper understanding and to a more global concept of sleep.

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THE SEROTONINERGIC HYPOTHESIS OF SLEEP REVISITED

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Recent developments of the electrophysiology of 5HT neurons, of in vivo 5HT liberation as studied by push-pull or voltametric techniques and autohistoradiography of ^{3}H 5HTP in the PCPA reverseal of insomnia with 5HTP in the cat have permitted to differentiate multiple role of 5HT neurons in the sleep-waking cycle:

1) Unit activity of the N.Raphe Dorsalis increases during waking (W) and diminishes during slow wave sleep (SWS) to become almost totally silent during Paradoxical Sleep (PS). Push-pull or voltametric methods demonstrate that the extra cellular release of 5HT at the cortical level is also increased during W and decreased during PS. Thus release of 5HT from the rostral raphe is not synchronously linked with SWS or PS. On the contrary these findings support a permissive role for this group of 5HT neurons for PS.However, the pattern of firing of caudal raphe is different since unitary activity of N.Raphe Magnus increases dramatically during PS and since release of 5HT from the terminals of this nucleus increases during PS as compared with W. Thus there is a differential 5HT liberation during the sleep--waking cycle according to rostral and caudal raphe. These results are in accordance with hypnogenic or waking effects induced by reversible cryogenic inactivation of rostral or caudal raphe in the sleep states. Possible direct or indirect control between different subsystems of 5HT neurons are suggested but not yet proved.

2) The PCPA-5HTP paradigm is still the only one suggesting a causal relationship between the restored 5HT liberation and sleep. Extensive investigations using intraventricular (IVT) or cisternal injections of low dose (150-500 ug of 5HTP) in PCPA pretreated cats have permitted to study in details the latency of the induction of the different sleep states and to delimit some possible targets for the hypnogenic effects of 5HT. The shortest effect which follows IVT injection of 5HTP (1-2 min) is the abolition of PCPA induced PGO activity. Then return of SWS occurs after a 2o min. latency and PS after 50-60 min. Shortest latencies occur after IVT injection in the caudal part of the fourth ventricle or in the cisterna magna. Longer laten-

cies are observed if the IVT injection is performed in the rostral part of the fourth ventricle. The duration of induced SWS and PS is dose-dependent up to maximal dose of 800-1000 ug. IVT injections of similar dose of 5HT induce also SWS and PS while 5 HIAA has no effect. Chloramphenicol does not supress the induction of SWS but totally suppress the return of PS. Since small doses of either 5HTP or 5HT injected in the Cisterna Magna reach mostly the ventral superficial portion of the brain stem, this suggests that 5HT acts upon ventrally situated structures for inducing SWS and PS. Autoradiography of brain slices have been performed by sacrificing PCPA pretreated cats 90 min. after intravenious injection of a mixture of ^{3}H 5HTP and 5HTP, when the animals were in PS. Although only a few reactive perikarya were identified in control non PCPA pretreated cats, numerous reactive perikaryas inside or outside the raphe system corresponding to the topography of 5HT neurons of the cat were seen in PCPA treated cats.
These results suggest that 5HT neurons contributed differentially to the regulation of the sleep-waking cycle according to their topography. A permissive role upon PGO activity and PS played by rostral raphe neurons as a neurotransmitter or neuromodulator is suggested. A neurohormonal action of caudal raphe neurons initiating PS inducing factor (S) will also be discussed.

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PROPERTIES OF SLEEP-PROMOTING FACTOR S DERIVED FROM HUMAN URINE*

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Sleep-promoting Factor S was originally extracted and purified from cerebrospinal fluid and from whole brains of sleep-deprived animals (1,2,3). However, the yield of purified material [less than 1 nanogram per gram brain (3)] was insufficient for systematic studies of its chemical and physiological properties. We have recently discovered that a sleep-promoting factor, similar to and perhaps identical with that from brain, can be obtained from human urine (5). The urinary factor behaves identically with that derived from brain in every chromatographic system we have used and its physiological effects on rats and on rabbits are indistinguishable from those of Factor S derived from brain. Methods for purifying Factor S from human urine have now been developed for use on a large scale and sufficient quantities of purified material have been obtained to start detailed chemical and physiological studies. For purposes of the present symposium we will describe briefly the purification procedures and then present data showing the effects of urinary Factor S on the sleep cycles of cats, rabbits and rats.

1). Outline of purification procedures

Figure 1 summarizes procedures used for preparation of Factor S from large quantities of human urine. The physiological data to be described were obtained with Factor S purified as in Figure 1 through the step labeled Biological Studies. At this stage of purification the amount of material required to induce excess slow wave sleep in a rabbit is equivalent to that obtained from about 100 ml. of original urine. This amount of partially purified product contains almost no detectable ninhydrin reacting material (less than 15 picomols of any known free amino acid). After acid-hydrolysis the highest concentration of any of the liberated amino acids is less than 300 picomols. Although further fractionation reduces this concentration to less than 80 picomols per effective rabbit dose, we have used only the partially purified material for the physiological experiments described below.

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**Career Investigator, American Heart Association

2. Effects of Urinary Factor S on the Sleep Cycle of Cats

Five cats were provided with chronically implanted ventricular guide tubes and electrodes for recording EEG, EOG and EMG. The cats were adapted to living in a sound proof box (60 x 60 x 80 cm) on a 10-14 hour light-dark cycle. Sleep states were analyzed on each cat for 32 hour periods under each of the following conditions 1) without infusion 2) after 30 minute intraventricular infusion of 0.3 ml. artificial CSF and 3) after 30 minute infusion of artificial CSF containing 500-750 mlu Factor S (i.e. Factor S purified as in Figure 1 from 500-750 ml. human urine.

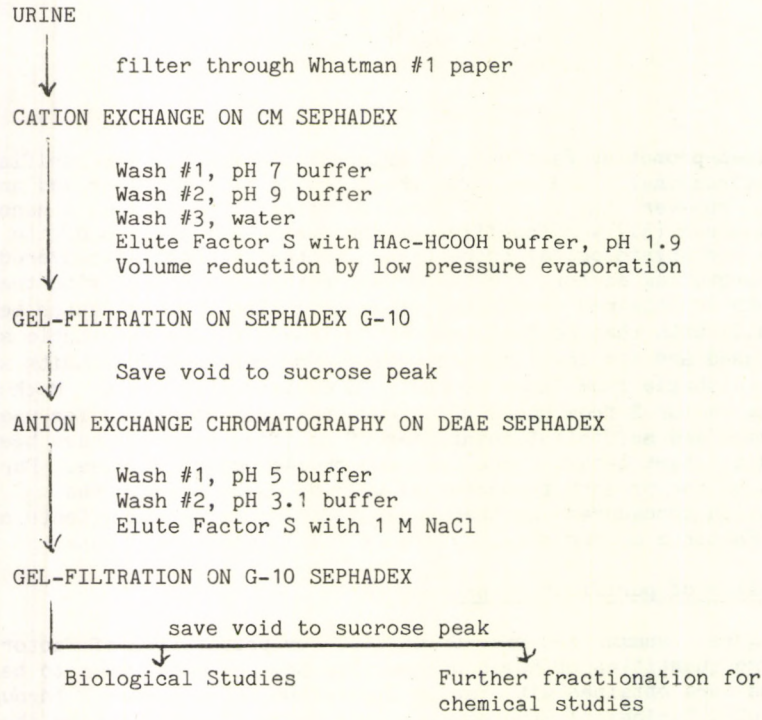

Figure 1. Outline of procedures used to purify Factor S for the studies on cats and rabbits reported at this symposium. These procedures are modified from those used previously (5) to prepare Factor S for studies on rabbits and rats. A high degree of purification is made possible by the fact that Factor S changes its charge without loss of biological activity during elution from Sephadex CM-25. It is thus possible to use both cationic and anionic exchange resins for the isolation. Details of the charge change have been described (5).

In four of the cats each of the above conditions was repeated at least once. Infusions were carried out from 8:30 to 9:00 a.m. and recordings continued for 32 hours thereafter except for a 10 minute interruption at 5 p.m. and a 30 minute interruption at 8 a.m. the next day. The records

were scored visually using conventional criteria but in addition the SWS component was determined electronically by the filter-rectifier-integrator system described elsewhere (6,7). Table 1 shows that the principal effect of Factor S is to increase the amount of SWS during the first 8 hours after infusion. The effect was large and it was present in every cat; on average, the amount of SWS increased from about 40% to 60% after

TABLE I

EFFECTS OF FACTOR S ON SLEEP OF CATS

Means ± S.E.

	HOURS AFTER INFUSION (Day 1) 0 - 8	(Night) 8 - 22	(Day 2) 24 - 32
I. % SWS			
a) No infusion	37 ± 1.7	41 ± 1.9	---
b) Art. CSF	42 ± 1.9	39 ± 2.3	37 ± 1.9
c) Factor S	59 ± 2.7	46 ± 3.1	38 ± 2.8
II. % REM			
a) No infusion	10.1 ± 0.8	17.1 ± 0.7	---
b) Art. CSF	9.1 ± 1.1	16.8 ± 0.9	9.5 ± 1.2
c) Factor S	7.9 ± 0.8	19.2 ± 1.2	9.7 ± 0.6

Factor S, while wakefulness decreased from about 50% to 30%. The amount of REM remained constant at about 10%. The peak effect of Factor S on SWS occurred about 4 hours after the infusion; at this time the cats slept an average of more than 70% of the time in SWS. The effects of Factor S subsided gradually about 12 hours after the infusion; during the next 20 hours there was no detectable "rebound" of wakefulness to make up for the 2 hours of excess SWS (loss of wakefulness) during the first 12 hours after the infusion.

In contrast to the effects on SWS there were no significant changes in amount of REM averaged over periods of 8 hours or more. Episodes of REM began during periods of SWS and the normal alternation of W-SWS-REM periods was retained. A complete analysis of these extensive experiments will be given elsewhere; for purposes of the present symposium we need only emphasize that in cats, the primary effect of Factor S is to increase SWS at the expense of wakefulness, without major changes of REM or of normal periodicity. Table 1 also shows that in cats, as in rabbits (6), the intraventricular infusion of artificial CSF has little effect on sleep states under the conditions of our experiments. This is in contrast to rats, which respond to intraventricular infusion of control solutions with a significant increase in duration of sleep (1,3)

3) Effects of Urinary Factor S on Sleep in Rabbits

Development of procedures for extracting and purifying Factor S from brain or urine involved many biological assays for Factor S. Initially we used the nocturnal locomotor activity of rats for routine

assays (3) but since 1974 we have utilized 6 hour daytime recording of EEG in rabbits. Rabbits have several advantages over rats for screening large numbers of fractions generated by various kinds of ion exchange, gel-filtration, electrophoresis, HPLC etc. Rabbits readily adapt to the experimental cages, there is little day-to-day variation in the amount of SWS and REM sleep is a negligible fraction (less than 5%) of sleep time. Rabbits are well suited to automatic analysis of SWS by the filter-rectifier-integrator technique we have described (6,7); the mean, rectified EEG potentials during SWS are usually 3-5 fold greater than during wakefulness and the absolute values in any given rabbit are usually stable within 10% for several weeks.

Rabbits sleep in short SWS episodes which last a few seconds to several minutes. These episodes typically occupy from 35-45% of the daylight hours in rabbits that are kept on a 12 hour light-dark cycle. Rabbits respond to Factor S by increasing the amount of SWS to values as high as 90% of each hour. The response usually peaks 2-3 hours after infusion and it subsides gradually during the subsequent 4-6 hours. For standardization of assays we take the average %SWS in the period 2-6 hours after infusion. The response is dose dependent but the maximum response has never been more than 85% SWS averaged over the 5 hour period. Even during a maximal response the animals wake occasionally to eat, drink or groom, and they can be easily aroused at any time by noise or touch. During SWS induced by Factor S in the rabbit, the amplitude of cortical slow waves is about 50% greater than during normal SWS in the same rabbit (6). Similar changes of EEG amplitude are observed in rabbits and in rats (2) allowed to sleep following sleep deprivation. However, we have not observed changes in EEG amplitude during SWS induced by Factor S in the cat.

4) Effects of Urinary Factor S on Sleep of Rats

Rats have a marked day-night activity cycle and the normal variations in their sleep pattern are large compared to those in cats or rabbits. For these and other reasons it is more difficult to assay Factor S in rats. A further complication arises from the fact that intraventricular infusions of control solutions cause significant reduction of nocturnal locomotor activity and increase in SWS for the first 6 hours of the dark cycle (2,3). Nevertheless, it is possible to show that intraventricular infusion in rats of CSF from sleep-deprived animals induces more SWS than similar infusions of CSF from normal animals (3,9). Similarly, we find that infusion of 20-50 mlu urinary Factor S in 0.1 ml artificial CSF just prior to the dark cycle results in a 50% increase in SWS for the ensuing 6 hours. Thus 5 rats receiving control artificial CSF just prior to the dark cycle slept 25 $\pm$ 1% of the next 6 hours in SWS; the same rats slept 38 $\pm$ 2% of the time following infusion of Factor S (4). At present we have no information concerning the effects of urinary Factor S on REM in rats.

5) Summary and Conclusions

Procedures have been developed to obtain Sleep-promoting Factor S from large volumes of human urine. Chemical and physiological properties of this material are similar to those of Factor S purified from brain. Emphasis is being placed on the use of this material for studies of its chemical structure but sufficient purified material has been obtained to

start systematic physiological studies. In the present paper we describe the effects of urinary Factor S on the sleep cycle of cats, rabbits and rats. The primary effect of Factor S is to increase SWS at the expense of wakefulness, while leaving the periodicity and amount of REM unaffected. The excess SWS induced by Factor S is not followed by increased wakefulness during the 32 hour recording periods we have employed for the analysis.

The chemical and physiological properties of Factor S differ from those of DSIP (10), arginine vasotocin (8) and other putatative sleep factors; however, Factor S may be related to the sleep-promoting substance extracted from brainstems of sleep-deprived rats by Uchizono et al (11). Further progress toward understanding the normal function and mechanism of action of Factor S will depend upon elucidation of its chemical structure and the development of methods for analysis of its distribution and concentration in brain.

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PINEAL VASOTOCIN AND SLEEP

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Introduction

We first demonstrated by specific differential bioassay, the presence of the nonapeptide hormone arginine vasotocin (AVT) in the pineal gland of all mammals so far investigated including man (27). Our work was confirmed by mass spectrometry (4), indirect radioimmunoassay (42), immunocytochemistry (3) as well as by a specific AVT anti-serum (6). AVT is synthesized both in the fetal and adult pineal gland of mammals including man, by specialized ependymal cells of the pineal recess (27) and released into cerebrospinal fluid (CSF) by its releasing hormone, the pineal indole melatonin (26, 31). In the pineal gland of the rat, AVT displays a circadian rhythm with high levels at noon and low levels at midnight, suggesting its release into CSF during the night in the dark (27). AVT represents the most active hormone so far known, able to inhibit the release of hypothalamic releasing and inhibiting hormones at concentrations equivalent to only several hundreds of molecules (27). Extremely small amounts of synthetic or purified pineal AVT, corresponding to about 600 molecules AVT, injected into the pineal recess of unanesthetized cats, induce NREM sleep and suppress REM sleep (32). The high potency of AVT in inducing NREM sleep has been recently confirmed in rabbits (J.R.Pappenheimer, personal communication). AVT differs both in its structure and effects from all known sleep inducing peptides (19, 22, 25). However, in the absence of a chemical characterization, the problem remains open, whether the CSF factor affecting motor activity in the rat (43), is identical or not with AVT. Compared on a molar basis with the synthetic delta nonapeptide (19), AVT is at least six thousand milliard times more potent, being thus by far the most active sleep inducing substance so far known.

Melatonin as releasing hormone

Since in spite of its quite different structure, AVT mimics all known effects of melatonin (27), and since melatonin injected either intravenously or intraventricularly, induces the release of AVT into the CSF of cats, it was advanced the

hypothesis that melatonin represents the releasing hormone for pineal AVT (26, 31). Not only in cats, but also in man, we demonstrated recently (36), that the suboccipital CSF AVT levels in 3 prepuberal boys admitted for minor neurological disorders,increase at 15 minutes, from 9 ± 3 pg/ml after the intravenous injection of the diluent alone, to 77 ± 5 pg/ml after the intravenous injection of 50 mg melatonin. In cats, it has been shown that AVT induces NREM sleep and suppresses REM sleep (28, 32), while melatonin also induces NREM sleep (18). However, because these authors (18) injected pharmacological amounts of melatonin (30 ug) in the preoptic area and no mention was made about REM sleep, we reinvestigated recently (9) the effects of melatonin on REM sleep in cats and demonstrated that 10 ng of melatonin, an amount comparable to the content of a pineal gland (50), induces NREM sleep and suppresses REM sleep in an identical manner as AVT, when injected into the pineal recess of unanesthetized cats. Both after melatonin (9) and AVT (28), the reappearance of REM sleep occurs with a marked rebound, like that observed after the administration of 5-hydroxytryptophan (15). In man, however, in contrast to cats, both melatonin (2, 5, 34, 36) and AVT (29, 34, 35, 36, 39) increase incidence and duration of REM sleep in healthy adults and induce and dramatically increase the amount of REM sleep in infants, some prepuberal boys and narcoleptics. The identical effects of both melatonin and AVT in inducing NREM sleep and in suppressing REM sleep in cats as well as the opposite effects of both melatonin and AVT on REM sleep in cats and man, further supports the postulate that melatonin represents the releasing hormone for pineal AVT.

Sleep deprivation

The lumbar CSF AVT levels were measured in 3 young male volunteers at 08.00h, after 24h of sleep deprivation or at 08.00h, at approximately 2h after awakening from a normal night of sleep. The CSF AVT levels markedly increase from the control levels of less than 5 pg/ml, to 85 ± 13 pg/ml after 24h of sleep deprivation. A similar increase of the urinary excretion of melatonin has been reported in sleep deprived subjects (14, 49).

REM sleep-dependent release

Lumbar CSF of healthy young males, contains detectable AVT levels when CSF was removed after awakening from REM sleep (33). No detectable AVT levels were found in the CSF of the same subjects when CSF was removed after awakening from NREM sleep, regardless the stage of NREM sleep from which they were awakened (33). The release of AVT into CSF after REM sleep is apparently independent of lighting conditions of hour of the day. The above data, demonstrating a strong REM sleep-dependent release of AVT into CSF of man, provide the first evidence of a physiological stimulus for the release of AVT into CSF (33). Whether AVT is released into CSF during the REM sleep period or in the period immediately preceding

REM sleep, cannot be deduced from these experiments. However, the increase by AVT of incidence and duration of REM sleep in healthy adults (29, 34), the induction by AVT on REM sleep in infants (35), some prepuberal boys (36) and narcoleptics (34, 39) as well as the increase of CSF AVT levels in sleep deprived subjects, strongly suggests the involvement of AVT in the induction of REM sleep, and consequently its release into CSF in the period immediately preceding REM sleep. Although the relationship between the nocturnal rise in melatonin secretion and sleep stages in humans is not well defined (47, 48), a REM sleep dependent release has been reported in epileptic patients (44).

Mechanism of action(see also Addendum)

The effects of AVT are highly specific because neither vasopressin nor oxytocin, which differ from AVT by only a single amino acid, was able to induce NREM sleep and to suppress REM sleep in cats (28, 32) or to induce REM sleep in man (36). In view of the evidence for the involvement of 5-hydroxytryptamine (5-HT) containing midbrain rahe neurons in the regulation of the sleep-waking cycle (15), it was of crucial importance to determine whether the sleep inducing activities of AVT are or are not mediated by 5-HT containing neurons. The following available evidence strongly suggest that AVT induces its hypnogenic effects by interfering with 5-HT neurotransmission: a) AVT increases 5-HT and decreases 5-hydroxyindole acetic acid levels in the cat brain (28); b) fluoxetine, a specific and selective 5-HT uptake inhibitor which facilitates 5-HT neurotransmission (7), greatly enhances the AVT induced NREM sleep in cats, when administered in small amounts without any apparent effects on sleep (28); c) higher amounts of fluoxetine alone, completely mimics the effects of AVT both in inducing NREM sleep and in suppressing REM sleep in cats (28, 45); d) in rats, fluoxetine alone, mimics the effects of AVT both in inducing NREM sleep and in suppressing REM sleep during the night in the dark and in reducing REM sleep without affecting apparently NREM sleep during the dayligth hours (10, 45); e) in man, in contrast to cats and rats, small amounts of fluoxetine without any apparent effects on sleep, greatly enhance the AVT induced REM sleep, whereas higher amounts of fluoxetine alone, induce REM sleep (29, 36); f) methergoline, a selective central 5-HT receptor blocker (8), completely prevents AVT in inducing NREM sleep in cats (28) and REM sleep in man (36); g) raphe dorsalis lesions in cats, completely prevent AVT in inducing NREM sleep and in suppressing REM sleep (11). The opposite effects of both AVT and fluoxetine on REM sleep in cats and man, reflect a different involvement of 5-HT containing neurons in the organization of REM sleep in cats and man, as suggested by several workers (17, 23, 51). The present results disagree with the conclusions of a recent study in which no effects of AVT on sleep in rats could be detected (46).

Dreaming

Seventeen young male volunteers receiving intra-nasally 2.5 ug AVT or saline, were awakened from their first REM period and questioned about their dreams, giving particular emphasis to the vividness of visual imagery. Thirteen subjects reported vivid and coloured dreams after AVT but only four after saline, confirming our first study (29). In another experiments we demonstrated that at awakening from REM sleep, the amount of AVT detected in the CSF of the subjects which experienced vivid and emotive dreams,was significantly higher than the amount of AVT detected in the CSF of the subjects whose REM dreams were devoid of vividness and emotionality (33). In narcoleptics, AVT induces intense coloured dreams, completely devoid of their usual nightmare-like elements (34). Melatonin affects also the visual imagery of dreaming (2) and we could confirm this, both in healthy subjects (36) and narcoleptics (34). Since the hallucinogenic drug LSD, whose effects on 5-HT neurons are well established (1), decreases REM sleep in cats (13) and increases REM sleep in man (21) like AVT, and since fluoxetine greatly enhances the hallucinogenic effects of AVT (29, 36), suggests that AVT induces the hallucinatory imagery of REM dreaming by interfering with 5-HT neurotransmission.

REM sleep ontogenesis

The AVT content in the pineal gland of mammals (27) and CSF of man (30) markedly decreases from fetal to adult age, concomitantly with the reduction of the ependymal cells that synthesize AVT from the pineal recess and pineal gland (24). There is also a decrease in the amount of REM sleep from fetal to adult age (37, 41). This intriguing parallelism in the marked decrease with age of both AVT and REM sleep, promted us to investigate the effects of AVT on REM sleep in infants. Synthetic AVT administered intra-nasally to 7 full-term infants aged 7-9 weeks, induces REM sleep within 1 minute and dramatically increases the amount of REM sleep up to 100 percent of the recording time of 60 minutes (35). A dose--related effect could be established between 0.1, 0.5 and 2.5 ug synthetic AVT. The equivalent of 1 mg pineal extract from human fetuses aged 4-5 months and containing 13.5 hydroosmotic milliunits, the most specific biological activity of AVT (27), administered intranasally to another 3 infants, increases REM sleep from 17, 21 and 23 minutes respectively, to 31, 37 and 43 minutes respectively (35). The significance of the high potency of both synthetic and human fetal pineal AVT in inducing and in increasing REM sleep in infants is unknown. It has been proposed that REM sleep, a source of intense endogenous excitation, play an important role in neural and behavioral development (16, 41). If this postulate is true, then AVT would represent, at least in man, an important endogenous chemical stimulus for the maturation and differentiation of the brain. The delayed maturation of the brain by the impairment of myelin synthesis in neonatally pinealectomized rats (40), supports the above hypothesis.

Narcolepsy

Since narcolepsy represents essentially a pathology of REM sleep (12, 37) and pinealectomy, which removes the main source of melatonin (50) but leaves intact most of the ependymal cells that synthesize AVT (27), induces both in rats and man (20) a narcoleptic-like distribution of REM sleep, we investigated the effects of AVT and melatonin on REM sleep in narcoleptics. Both AVT and melatonin induce REM periods at sleep onset and dramatically increase the amount of REM sleep and decrease REM sleep latency in narcoleptics (34, 39). Although in the normal subjects the effects of AVT and melatonin were more variable, both AVT and melatonin significantly increase the amount of REM sleep and decrease REM sleep latency. However, in contrast to narcoleptics, in the normal subjects, neither AVT nor melatonin, was able to induce REM periods at sleep onset, or to increase the amount of REM sleep at the dramatic level of narcoleptics (34, 39). Since in healthy infants (35) and even in some healthy prepuberal boys (36), in contrast to healthy adults, AVT is extremely potent in inducing REM periods at sleep onset and in increasing the amount of REM sleep, suggests that the high sensitivity of narcoleptics to melatonin and AVT,reflects an immaturity of REM triggering centers. Since the AVT content in the pineal of narcoleptics, as deduced from its release into CSF (38), is apparently normal or even higher than in healthy subjects (33), it appears that the pineal of narcoleptics displays rather a melatonin deficiency, either in its synthesis or in its control over AVT release, with resultant alterations in the induction and circadian organization of REM sleep.

Conclusions

The present results suggest that the pineal gland by its indole melatonin as releasing hormone and by its nonapeptide AVT as specific effector in the brain, plays an important, still not elucidated role, in the induction and circadian organization of sleep. Compared with other sleep inducing peptides, AVT fulfills the following specific physilogical criteria: 1) it is synthesized both in the fetal and adult pineal; 2) it displays a circadian rhythm in the pineal; 3) its release into CSF is REM sleep dependent; 4) its levels in CSF markedly increase after sleep deprivation; 5) its decrease in the pineal and CSF from fetal to adult age, parallels the decrease of REM sleep; 6) it induces its hypnogenic effects within few minutes; 7) it is the most active sleep inducing substance so far known; 8) it induces its hypnogenic effects in different species according to the specific involvement of 5-HT containing neurons in the organization of their sleep states; 9) it induces vivid and coloured dreams; 10) it appears to be involved in the pathophysiology of narcolepsy.

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Addendum

Whether AVT acts directly or indirectly on 5-HT neurons is unknown. However, the failure of AVT to induce consistent changes in firing rate when applied by microintophoresis to 5-HT neurons in the dorsal raphe nucleus of the rat (G.K. Aghajanian, personal communication), the existence of a major gaba-ergic projection from the habenula to raphe dorsalis (1) as well as the increase of GABA levels in the brain by melatonin (2), suggests a possible gaba-ergic mediation of the AVT effects on raphe dorsalis neurons. This, would also agree with the results obtained after raphe dorsalis cooling (Cespuglio et al. 1979).

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F. Obál, G. Benedek (eds)

THE CONTRIBUTION OF WARM RECEPTORS TO THE REGULATION OF SLEEP-WAKING CYCLES

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Several papers have been reported which suggest the existence of a close interaction between thermoregulation and sleep-waking activity (Heller and Glotzbach, 1977, Schmidek et al.,1972, Valatx et al., 1973). The conflicting findings concerning the influence of a warm environment on sleep-waking activity can be attributed to the fact that, for example in rats, several mechanisms opposite in action are activated by a warm exposure. On the one hand, warm stimuli put into action behaviours with increased motor activity to protect the animals against overheating of the body (Hainsworth, 1967). On the other hand, behavioural deactivation (prone extension of the body, sleep) can also been observed (Roberts and Mooney, 1974, Roberts and Robinson, 1969), which reduces thermogenesis and favours heat dissipation.

The study of the acute and chronic consequences of capsaicin administration offers useful means to elucidate some effects of warm stimuli on behaviour. Acute administration of capsaicin excites warm receptors, which anyone who has come into contact with red pepper can attest to as a common experience. Fractionated administration of large doses of capsaicin, however, results in a state characterized by irresponsiveness to warm stimuli (desensitization, Jancsó-Gábor et al., 1970).

If rats are put into a warm environment, they respond with intensive saliva spreading and escape reactions. Desensitized rats fail to produce such reactions, as if they

were insensitive to warm stimuli. Due to these failures, their body temperature increases to a great extent and soon reaches a lethal limit (Obál et al., 1979).

As a first step in our experiments, the effects of a warm ambient temperature on the sleep-waking activities of capsaicin-desensitized and untreated rats were studied (Benedek et al., 1980). Eleven pairs of desensitized and control rats were kept at 22-24 °C for base-line recordings, then at 32 °C and at 34 °C for 24 hours each. A light-dark cycle of 12:12 hours was maintained, and recordings were performed during the light phase. The sleep-waking activities of both groups were influenced by the elevation of the ambient temperature (Fig. 1). In the control rats, the amount of wakefulness (W) increased parallel to the ambient temperature. The time spent in light slow wave sleep (LSWS) also decreased at the expense of deep slow wave sleep (DSWS). It seems that a warm ambient temperature exerts a powerful activating effect on the control rats, resulting in both increased wakefulness and lighter

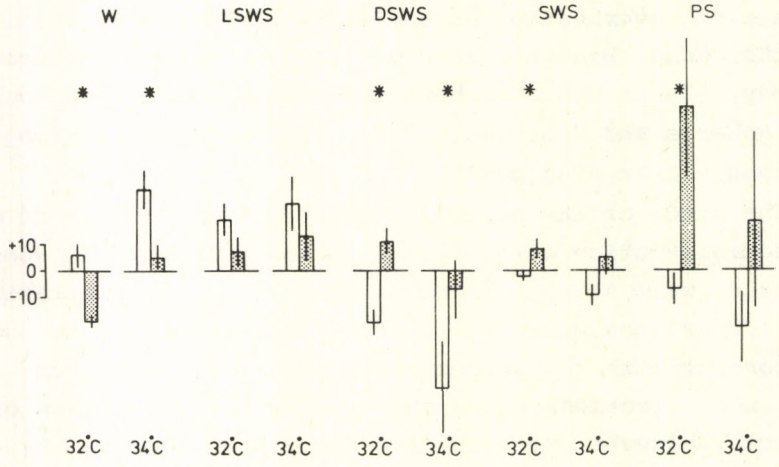

Fig. 1. Effect of 32 °C and 34 °C ambient temperature on the amount of sleep-waking stages in control (white columns) and desensitized (shaded columns) rats. The percentage deviation of each stage from the values observed at room temperature

sleep. In desensitized rats, however, a deactivation was obtained at 32 °C.
The amount of wakefulness decreased and that of deep slow wave sleep increased. There was a pronounced increase in the amount of paradoxical stage (PS).

The study of cyclicity in the appearance of paradoxical phases revealed conspicuous differences between the two groups (Fig. 2). The cyclicity was calculated by the method of Globus (1970), taking into account the daytime paradoxical episodes. In the control rats, a definite cyclicity with a maximum at 18 min cycle length appeared at 22-24 °C. On elevation of the ambient temperature, this cyclicity greatly deteriorated. In capsaicin-desensitized rats, the cycle length was shortened and the cyclicity was less pronounced than in the controls, even at 22-24 °C. No characteristic changes

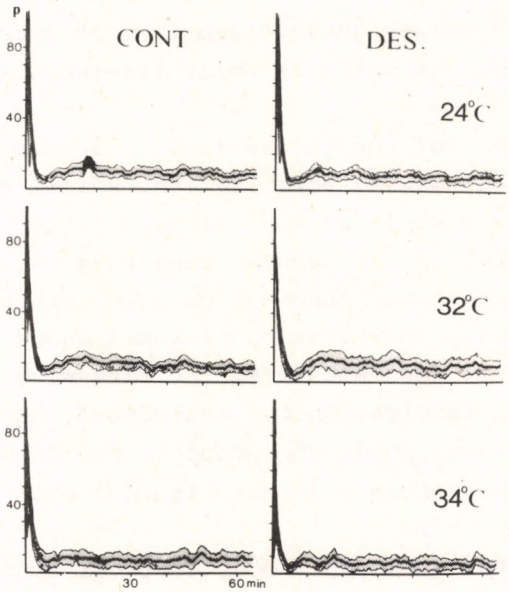

Fig. 2 Changes in the cyclicity of the occurence of paradoxical sleep under the effect of warm ambient temperature. The curves represent the mean values and confidence intervals obtained in eleven rats during daytime.

could be observed under the effect of a warm ambient temperature.

Two findings are worthy of emphasis:

i) The deactivating effect of heat did not manifest itself in the sleep-waking activity of untreated rats, at least on the first few days of exposure; indeed, an increase in wakefulness was observed. The facilitation of wakefulness may be due to behaviours enhancing heat dissipation: when vasodilatation itself is not enough for thermolysis, the rats are forced to wake up and spread saliva on their fur. This repeated awakening is reflected by the impairment of PS cyclicity; the need for thermolytic behaviours interferes with sleep cycles. The reason for alteration in PS cyclicity of desensitized animals at room temperature is far less understood. The alteration suggests that warm receptors may play a role in timing the sleep-waking cyclicity even at room temperature.

ii) Desensitized rats increase their sleep time at a moderate warm ambient temperature, in spite of their irresponsiveness to a warm environment.

For further analysis of the interaction of a warm environment and the sleep-waking activity, the effect of local preoptic heating on cortical electrical activity was studied in acute, immobilized unanaesthetized rats (Benedek et al., 1976). The animals were operated on under ether anaesthesia. After careful infiltration of wound edges and pressure points with local anaesthetics, the rats were immobilized, artificial respiration was introduced, and then the anaesthesia was discontinued. The preoptic region was heated by means of high-frequency alternating current through concentric bipolar electrodes.

Local heating of the RPO/AH brought about cortical synchronization in both control and desensitized animals (Fig. 3). This synchronization consisted of slow waves and frontal spindles, appearing progressively during the 5-6 min of heating which elevated the local temperature by 1-2 ^{o}C. This finding suggests that heating of the central thermosen-

sor area can elicit deactivation, and furthermore this deactivation can be brought about even after capsaicin desensitization.

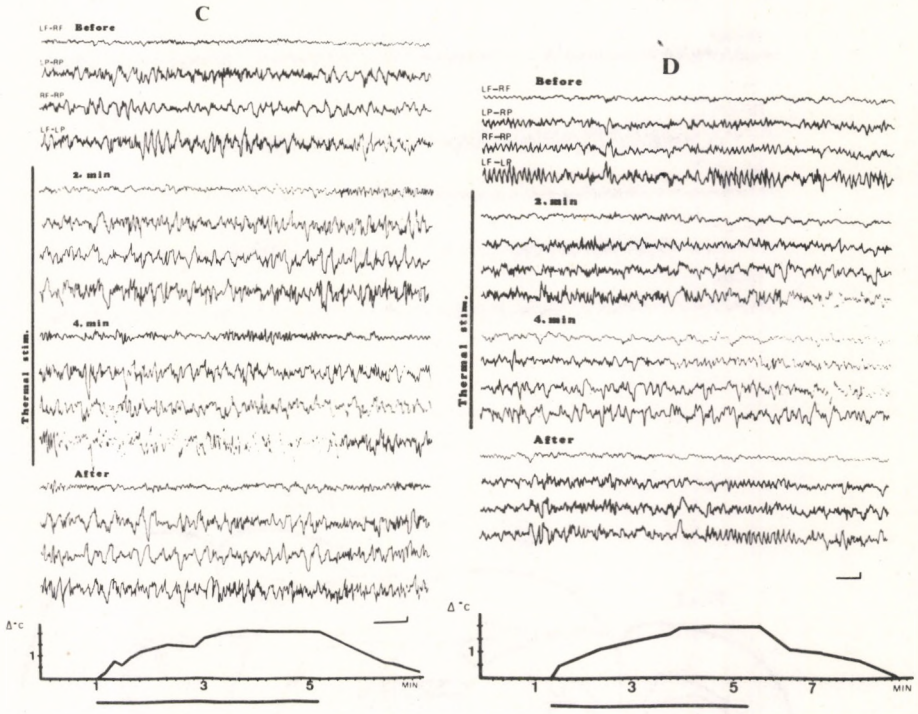

Fig. 3. The effect of preoptic heating on the EEG of control (C) and capsaicin-desensitized (D) unanaesthetized immobilized rats. LF:left frontal, RF:right frontal, LP: left parietal, RP: right parietal derivations. Bottom: change in preoptic temperature. Lines: heating period. Calibration: 1 sec, 50 μV.

To test whether these animals were really insensitive to the central influence of capsaicin, the EEG effects of application of the drug into the preoptic region was studied in control and desensitized rats. The experiments were carried out on 62 acute, immobilized rats. Capsaicin solution (1%) was injected in an amount of 1 μl. In response to the microinjection, cortical synchronization appeared after a 1-2 min desynchronization period. The synchronization

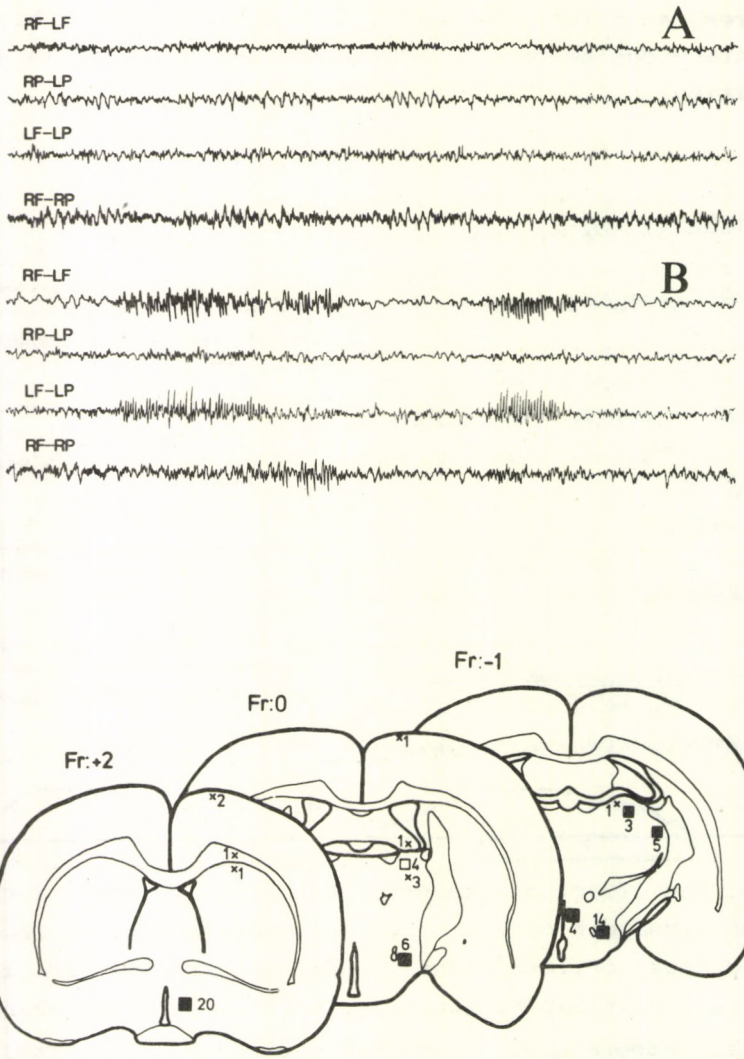

Fig. 4. The effect of local preoptic injection of capsaicin on the cortical electrical activity of an unanaesthetized immobilized rat. A: before capsaicin application. B: 4 min after 1 µl capsaicin. Bottom: schematic drawing of frontal sections (Pellegrino-Cushman atlas). Black squares: synchronizing sites, empty squares: desynchronizing sites, crosses: ineffective sites. The number of experiments is also indicated.

lasted for 8-10 min. Not only the RPO/AH but also the hypothalamus in its entire length was tested in these experiments. Every point was heated first, and when the effect had elapsed, capsaicin microinjection was applied. Points effective in eliciting cortical synchronization were found throughout the basal forebrain, from the level of the RPO to the mesencephalon (Fig. 4). In control animals, all points sensitive to warming reacted to capsaicin too. In capsaicin--desensitized animals, however, capsaicin microinjection failed to elicit cortical synchronization, while preoptic heating was still effective.

These results show that heat-sensitive structures which can contribute to the deactivating effect brought about by warming are not confined to the RPO/AH region. In spite of the fact that these areas are insensitive to capsaicin in desensitized animals, the desensitization does not influence the deactivating effect of warmth.

The manifestations of this deactivation were studied in freely-moving animals. The effect of preoptic heating on the behaviour of rats was tested in eight animals kept at 30 ^{o}C ambient temperature. Five minute preoptic heating periods were alternated with non-heating ones for an hour. In response to preoptic heating, characteristic large spindles were observed which accompanied an immobile pronal extension of the body (Fig. 5). This extended lying was often interrupted by grooming or drinking behaviour. The amount of sleep was not altered significantly by intermittent heating for one hour, but the time spent in extension and grooming significantly exceeded that observed during the control, prestimulation period.

To sum up, the effects of localized preoptic heating and a warm ambient temperature were studied in capsaicin-desensitized and control rats. It seems that warm stimuli affect capsaicin-sensitive and capsaicin-insensitive mechanisms which contribute to the activating and deactivating effects of a warm environmental temperature. While capsaicin pretreatment prevents the activating effect of heat, the deactivation seems to be unaltered. Since the synchronizing

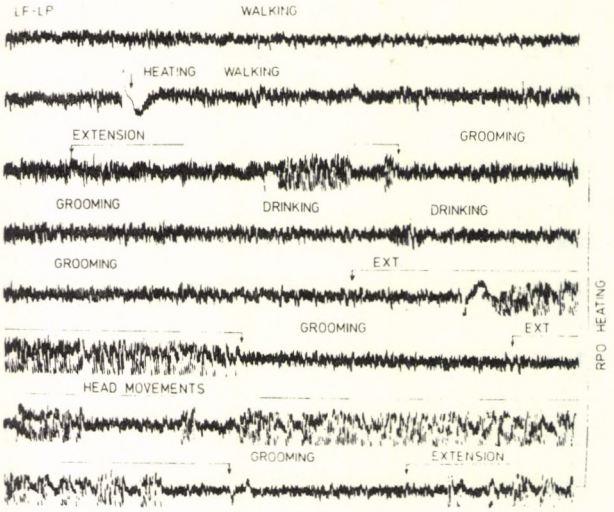

Fig. 5. The effect of preoptic heating on the EEG and behaviour of a freely-moving rat. Lines indicate prone extension of the body. Left fronto-parietal derivation. Calibration: 1 sec, 50 μV.

effect of preoptic warming survived capsaicin treatment, we may assume that it is the elevation of the preoptic temperature that causes synchronization and deactivation even in freely-moving animals. This assumption is in agreement with the findings of Heller and Glotzbach (1977) since the increase of slow wave sleep we noted in desensitized rats in a 32 ^{o}C environment was similar to that observed by them in rats exposed to an ambient temperature of 20 ^{o}C provided the preoptic temperature was clamped above normal. To explain this deactivating effect of warm stimuli in capsaicin-desensitized rats, a non-specific excitation of preoptic neurons can be taken into consideration. This explanation was first proposed by Parmeggiani et al. (1974) in their interpretation of the prolongation of PS elicited by preoptic heating. The role of capsaicin-insensitive, but still heat-sensitive units, and the contribution of cold-sensors, can not be ruled out either.

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INTERRELATION BETWEEN SLEEP AND TEMPERATURE REGULATION*

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That the thermal environment has an influence on sleep is a proposition we can accept from personal experience. This relationship has been explored in depth through a series of studies over the past 12 years (reviews, see Heller and Glotzbach 1977, Henane et al. 1977, Parmeggiani 1977). The results can be generalized as follows: a) total sleep time (TST) is maximal in a thermoneutral environment and progressively decreases above and below thermoneutral conditions, b) within the thermoneutral zone TST is increased by warming, c) the sleep stage most curtailed by thermal stress is paradoxical sleep (PS). So far these generalizations apply to cats (Parmeggiani et al. 1969, 1970), rats (Valatx et al. 1973, Schmidek et al. 1972), kangaroo rats (Sakaguchi et al. 1979) and man (Haskell et al. 1978, Buguet et al. 1976, 1979).

Investigation of arousal state related changes in the thermoregulatory system have suggested adaptive explanations for the observed influences of the thermal environment on sleep, especially the strong effect of high and low temperatures on PS. Reductions in thermoregulatory responses during sleep in comparison to wakefulness had been noted in many studies (review, see Heller and Glotzbach 1977). The most dramatic was the apparent total block of panting, shivering, and thermoregulatory vasomotor tone during PS in cats observed by Parmeggiani and Rabini (1967). It was then discovered that active thermoregulatory responses in other species, including man, were blocked during REM sleep (Glotzbach and Heller 1976, Henane et al. 1977, Shapiro et al. 1974). The central nervous system basis for these sleep related changes in the thermoregulatory system was revealed in studies of hypothalamic thermosensitivity as a function of arousal state in kangaroo rats (Glotzbach and Heller 1976). Since hypothalamic thermosensitivity is the dominant feedback loop in the thermoregulatory system of small mammals, its characterization provides a direct approach to reveal alterations in this central nervous regulatory system. Measures of the metabolic heat production response to hypothalamic cooling showed that hypothalamic sensitivity was much lower during slow wave sleep (SWS) than during wakefulness. This downward resetting of the thermoregulatory system explains decreases in heat production/conservation responses, increases in heat loss responses, and declines in body temperature (T_b) seen to be associated with sleep onset in a variety of studies. Hypothalamic thermosensitivity was completely eliminated during PS explaining observations of loss of thermoregulatory responses during this sleep stage.

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The strong inhibition of an autonomic homeostatic mechanism during PS is a puzzling phenomenon. It does, however, offer an adaptive rationale for the disproportionate disruption of PS in the face of thermal stress. If regulation cannot occur during PS, then this sleep stage should be minimized under conditions in which regulation is required. Carrying this line of speculation one step farther leads us to wonder whether a significant selective pressure in the evolution of insulated nest building behavior in mammals besides the overall conservation of metabolic energy might have been the need to preserve PS.

Similarly the reduction in sensitivity of the central thermoregulatory system during SWS might also contribute to the reduction of TST under thermal stress. A reduced driving signal to thermoregulatory effector mechanisms would result in an inadequate homeostatic response and a drift of T_b in the direction of the stress unless responses were periodically increased by episodes of wakefulness.

An adaptive explanation for the observed influences of the thermal environment on sleep leads to the question of whether these influences are mediated by the thermoregulatory system or whether they are direct effects of peripheral temperature reception on systems subserving arousal state control. The possibility of interaction between the thermoregulatory system and arousal state controlling systems requires us to take a new look at studies on the involvement of the hypothalamus on the control of arousal states. The effects of hypothalamic lesions and electrical stimulation have led to the postulation that the preoptic nuclei and anterior hypothalamus (POAH) are involved in producing cortical synchrony and sleep whereas the posterior hypothalamic areas are involved in producing cortical desynchrony and arousal (for reviews see Jacobs 1978 and Benedek et al. 1979). Also cited in support of the POAH as a sleep center is the fact that mild warming of the POAH is effective in producing cortical synchrony and sleep (Roberts and Robinson 1969, Benedek et al. 1976).

A very careful study by Benedek et al. (1979) has described the influences on cortical and hippocampal EEG synchrony of low (10 cps) and high (100 cps) frequency stimulation in precisely localized regions of the POAH. The results of this study are too extensive and complex to be discussed in detail here, however, two general findings are of interest for comparison with thermoregulatory studies of the hypothalamus. First, there is a distinct medial-lateral difference in the responses to electrical stimulation. Low frequency stimulation of lateral areas produces cortical synchrony whereas similar stimulation of medial areas produces cortical desynchrony. Second, the effect of the stimulation was dependent upon the frequency used. High frequency stimulation resulted in cortical synchrony only when applied in the laterobasal part of the preoptic region and produced cortical desynchrony and hippocampal theta in other locations where low frequency stimulation caused cortical synchrony.

One criticism of the lesion and stimulation studies of putative hypothalamic sleep centers is that the effects may be due to disruption or stimulation of fibers of passage rather than to hypothalamic nuclei. For example, the lateral hypothalamus includes the medial forebrain bundle which includes descending pathways from septal and olfactory nuclei plus olfactory, hippocampal and orbitofrontal cortices to the midbrain and possibly lower brainstem. Ascending fibers in the median forebrain bundle include those from several brainstem nuclei. The medial forebrain bundle also contains axons from various hypothalamic nuclei (Millhouse 1973).

Another possibility which must be considered in the interpretation of the hypothalamic lesion and stimulation studies is that various hypothalamic manipulations influence sleep indirectly because of effects on the thermoregulatory system or other hypothalamic homeostatic mechanisms.

The regional differences described for the effects of lesions, thermal stimulation, and electrical stimulations of the basal forebrain on sleep bear interesting relations with the thermoregulatory functions of the same areas, especially when one keeps in mind the fact that mild peripheral or central warming facilitates sleep but strong thermal stimuli interfere with sleep. The effects of hypothalamic lesions on thermoregulation have suggested a division of function with neurons in the POAH responsible for driving heat loss responses and neurons in the posterior hypothalamus responsible for controlling heat conservation/production responses. Studies using localized electrical stimulations have largely supported this functional division and have suggested reciprocal inhibitory interactions as well (Bligh 1973, Reaves and Hayward 1979). Another study has taken the approach of recording local EEG of the anterior and posterior hypothalamus while manipulating the temperature of the spinal cord (Wünnenberg 1973). The average frequency of the EEG of the anterior hypothalamus increased with spinal warming and that of the posterior hypothalamus increased with spinal cooling, thus supporting the concept that the anterior hypothalamus subserves heat loss and the posterior subserves heat production responses. It is therefore possible that mild electrical stimulation of the posterior hypothalamus mimics a cold stimulus and is therefore arousing whereas a mild electrical stimulus of the POAH mimics a warm stimulus and is hypnogenic. High frequency stimulation in either area may mimic intense thermal stimuli.

On another front, since the pioneering study of Nakayama et al. (1963), numerous investigators have recorded temperature sensitive neurons in the hypothalamus. A common feature in the results of many of these studies is that the highest numbers of temperature sensitive cells are found close to midline. In the studies of Nakayama et al. (1963) over 50% of thermosensitive cells were within 0.5 mm of midline. In a study on rabbits by Hellon (1967) 75% of the temperature sensitive cells were within 1 mm of midline, the rest within 2 mm. In a study by Jell and Gloor (1972) on cats most of the temperature sensitive cells were within 1 mm of midline. None of these studies describes number of penetrations in different areas or some other criteria which would indicate whether or not the reported results truly represent different densities of temperature sensitive cells, but the best assumption at this time is that temperature sensitivity is greatest in the most medial locations in the POAH. This means that the same level of electrical stimulation in medial and lateral POAH would have very different effects on thermoregulatory pathways. Whereas the laterally placed stimuli might be the equivalent of slightly warm inputs, medial stimulation might be the equivalent of intense thermal inputs.

Another reason for hypothesizing that mild electrical stimulation of the POAH might mimic a warm input to the thermoregulatory system has to do with the anatomy of temperature sensitive neurons. Although uncertainty remains, the differences in firing rate versus temperature curves for hypothalamic neurons have generally been interpreted to mean that primary thermodetectors are concentrated in the POAH; whereas the cells responding to temperature in the posterior hypothalamus are interneurons in integrative pathways. The important point, however, is that surveys of

temperature sensitivity of POAH neurons report a predominance of warm sensitive cells (10 to 50%) over cold sensitive (2 to 20%) (Reaves and Hayward 1979). It is therefore probable that electrical stimulation of this area would enhance the activity of more warm sensitive than cold sensitive thermodetectors and represent a net warm input.

A functional thermoregulatory differentiation between medial and lateral POAH is also evidenced in recent unpublished studies by Kilduff, Sharp and Heller in which ^{14}C-2-deoxy-d-glucose was used to measure glucose uptake in brain structures during heat stress. A ground squirrel was made hyperthermic in a 40°C environment before the label was infused into the external jugular vein through a chronic catheter. After 40 minutes of this central and peripheral thermal stress the animal was sacrificed with sodium pentabarbitol, its brain was frozen, sectioned, and autoradiographed. There was a much increased glucose uptake in the POAH area of this animal in comparison to controls. Of greater interest, however, was the sharp difference between the glucose uptake of the lateral and medial POAH areas. Although we cannot yet interpret this pattern, the medial-lateral difference is yet another strong coincidence between the electrophysiological studies of cortical synchronizing systems and the thermoregulatory activities of these same structures.

All of the coincidences between sleep related and thermoregulatory functions of the hypothalamus we have reviewed are circumstantial evidence that the thermoregulatory system has a strong influence on arousal state controlling mechanisms and that manipulations of hypothalamic structures may have their observed effects on arousal states indirectly through the thermoregulatory system. More direct investigations of these relationships are required. One such study is our work on the separate and combined effects of hypothalamic and peripheral temperature manipulations on arousal state distributions in kangaroo rats (Sakaguchi et al. 1979). The aim of this study was to see whether peripheral temperature influences arousal systems directly or through the output of the thermoregulatory system. We therefore used water perfused thermodes around the POAH to disassociate thermoregulatory drive from peripheral thermal stimulation. This was done by first knowing the threshold hypothalamic temperature for the metabolic heat production response at each T_a used in the study. We were then able to clamp T_{hy} at an appropriate level to maintain the desired difference between T_{hy} and threshold at each T_a. In this way we could produce a central thermoregulatory drive normally seen at the cold T_a (20°) in experiments conducted at the neutral T_a (30°). Similarly in experiments at the cold T_a we could suppress central thermoregulatory drive to levels normally occurring at the neutral T_a. Any combination of conditions was held for a 4-hour period falling in the middle of the animals' daily inactive period during which electrophysiological recordings were made to enable description of arousal state distributions.

The question was whether distributions of arousal states would follow peripheral temperature or thermoregulatory drive. The results were that TST was a function of thermoregulatory drive. In other words, the animal in the cold environment had a TST equal to that seen in the warm environment if the POAH were warmed to reduce thermoregulatory drive. Reciprocally the animal in a warm environment responded to POAH cooling, producing the same thermoregulatory drive as would result from exposure to a T_a of 20°C by showing a reduction in TST to a value seen in controls at a T_a of 20°C.

The distribution of TST between SWS and PS was not a simple function of thermoregulatory drive. Either central or peripheral thermal stimulation characteristic of nonneutral conditions caused a reduction of the PS/SWS ratio regardless of thermoregulatory drive. For example, the animals at T_a = 20°C had reductions of PS in comparison to values seen at 30°C even when thermogenesis was suppressed by warming the POAH.

These results argue for direct influence of thermal afferents on mechanisms influencing SWS/PS transitions in addition to the indirect influence of those afferents on wakefulness/SWS transitions via the thermoregulatory system. Neurophysiological and neuroanatomical studies show that the necessary circuitry exists to account for these results. There is some evidence for a limbic system midbrain circuit which receives inputs from hypothalamic thermoregulatory mechanisms and influences wakefulness/SWS transitions (Heller 1978). Hinckel et al. (1980) have recently reported evidence for direct projections from cutaneous cold receptors onto cells in the dorsomedial pontine reticular formation, an area known to contain noradrenergic cell bodies which project to the hypothalamus. Also, Dickenson (1977) has demonstrated specific responses of cells in the raphé nuclei to changes in skin temperature.

Whatever the neurophysiological mechanism involved, it seems rather certain from rodent studies that peripheral thermal stimulation is altering the PS/SWS ratio by influencing transitions from SWS to PS rather than by disrupting PS epochs. Statistical comparisons of the PS epochs in our kangaroo rat studies demonstrated that thermal stress primarily reduced the number rather than the average duration of PS epochs (Sakaguchi et al. 1979). Similar results can be seen in the data of Schmidek et al. (1972) on rats and partially at least in the data of Parmeggiani et al. (1969, 1970) on cats. The cats sometimes show a large number of "abortive" transitions into PS at low ambient temperatures which appear in the frequency distributions of single epoch durations as increases in the shortest epoch duration class. If these "abortive" transitions are set aside there appears to be no significant difference in the frequency distribution of PS epoch durations between neutral and cold temperatures, but there is a large reduction in number of PS epochs at the low temperatures (Parmeggiani and Rabini 1970). In another study on cats Parmeggiani et al. (1975) simply measured brain temperature during sleep at a low and at a neutral T_a and observed a correlation at the low T_a between brain temperature during SWS and whether or not that epoch of SWS was followed by PS or wakefulness. The conclusion was that active thermoregulatory processes during SWS were antagonistic to the transition into PS. Warming the skin after the onset of SWS has the effect of facilitating the transition to PS in rats (Szymusiak et al. 1978) which also suggests the thermal influences on PS are acting during SWS. However, we cannot rule out an influence of thermal input during PS epochs as it has been shown in cats that hypothalamic warming initiated after the transition from SWS to PS can significantly lengthen the PS epoch (Parmeggiani et al. 1974).

Reports of human sleep at nonneutral temperatures also show considerable disruption of PS. Haskell et al. (1978) showed decreased PS/TST at high and low T_a's, Karacan et al. (1978) showed that PS was more curtailed by high T_a's than was SWS, Buguet et al. (1976, 1979) reported that PS was the sleep stage most affected by cold exposure. Unfortunately, these reports do not permit the assessment of whether PS epochs are shortened or whether it is the transition into PS which is most influenced by thermal

stress. Muzet (personal communication) recently reported that low T_a's primarily cause an increase in the PS cycle length rather than a shortening of PS epoch duration.

Two species of hibernators seem to have no reduction in TST or PS at low ambient temperatures, golden mantled ground squirrels (Haskell et al. 1980) and pocket mice (Walker et al. in press). This may reflect either an extremely low peripheral thermosensitivity which is very likely the case in small hibernators (Heller et al. 1974) or it may reflect a special adaptation for hibernation. After all, since hibernation is entered through sleep it would be maladaptive for declining skin and central temperatures to disrupt sleep in hibernators. It has been documented, however, that during the early entrance into hibernation there is a great increase in SWS but a progressive decline in PS until PS completely disappears below a T_b of about 27°C (Walker et al. 1977, 1979). Possibly in the hibernator the influence of central but not peripheral cold stimuli disrupts transitions from SWS to PS, and as long as thresholds for thermoregulatory heat production response are low or declining there is no hypothalamic thermoregulatory error signal generated to disrupt TST. We might expect then in the hibernator in comparison to the nonhibernator a greater reduction in hypothalamic thermosensitivity between wakefulness and SWS. This adaptation along with the tendency to greater TST during the hibernation season (Walker et al. 1980) would facilitate the entry into hibernation.

In conclusion, we wish to emphasize that the clear functional relationships demonstrated between sleep and thermoregulation point to the possibility that hypothalamic manipulations which influence arousal states may be doing so because they are perturbing the thermoregulatory or some other homeostatic system. Conversely, studies of alterations of thermoregulatory responses must control for changes in arousal states. Our future progress in understanding the interactions of these two systems depends on advances in our understanding the detailed functional neurophysiology underlying them. This means more effort placed on working with unanesthetized chronic preparations in which normal thermoregulatory functions and arousal state control mechanisms are intact.

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F. Obál, G. Benedek (eds)

REGULARITY IN THE PATTERNS OF UNSYNCHRONIZED CIRCADIAN RHYTHMS IN MAN

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When activity-rest (A) and body temperature (T) cycles become unsynchronized in man, large variations in sleep duration and timing occur. We report that: 1) A and T have become unsynchronized (NS) in every subject free-running longer than 2 months; 2) The relative phase A and T usually drifted apart before NS.; 3) This was often followed by recurrent phase jumps in the timing of sleep (phase trapping); 4) A cluster line of sleep periods, 6-10 hrs.long, was synchronous with T; both had a shorter period than before NS; 5) The average period of A typically lengthened during the free-run; and 6) Sleep duration varied predictably with the phase of T at sleep onset, even in subjects with activity-rest cycles 24 hrs. The rate of REM sleep accumulation varied with the phase of T, as did plasma cortisol and subjective assessments of alterness. We postulate that the occurence of extremely long or short activity-rest cycle periods is a natural consequence of the progressively changing interaction of these oscillators in man.

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CONCLUDING REMARKS ON THE REGULATION OF THE SLEEP-WAKING RHYTHM BY ENVIRONMENTAL AND ENDOGENOUS FACTORS

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Monoamines

F.Obál jr. raised the point that according to the serotonin (5-HT) hypothesis of sleep, slow wave sleep (SWS) should be abolished in the chronic cerveau isolé preparation, since the 5-HT nerve terminals would be degenerated. M. Jouver replied that cortical slow waves can be generated by various nonspecific factors (e.g. drugs, change in CSF pressure), and that EEG synchronization does not indicate therefore necessarily the presence of SWS. He emphasized the need for a better definition of SWS on the basis of EEG criteria (e.g. by frequency analysis), a problem that has not received sufficient attention.

Sleep factor and arginine vasotocin (AVT)

J.R.Pappenheimer specified that the active dose of Factor S is approximately 30 pmol (question from J.Magnes), and that the factor causes no significant hypothermic effect (question from H.C.Heller). He stressed the fact that Factor S produces a behaviorally natural sleep in the cat which exhibits a strong urge to return to sleep when it is awakened (question from G.Benedek). In the rabbit, Factor S has been shown to increase the duration of the long SWS episodes (Am.J.Physiol., 238, E116-E123,1980). Due to the small amounts of the purified substance that had been available so far, the effect of a systemic administration has not been investigated (question from M.Kluger). A peptidic bond is present in Factor S, because its biological activity is abolished after treatment with carboxypeptidase (question from J.P.Rossier). Finally, M.Jouvet reported that the sleep factor of the Uchizono group enhanced both nonREM sleep and REM sleep in the mouse as shown by experiments conducted in the Lyon laboratory.

S.Pavel indicated that he has found the sleep-promoting effect of AVT in several species (question J.H.Wolstencroft). R.Goldstein, a coworker of Dr.Pavel, presented results from experiments carried out in the rat in which AVT depressed

REM sleep when given either in the light phase or in the dark phase, while it enhanced nonREM sleep only in the dark phase. S.Pavel did not see any other behavioral effects of AVT in his sleep studies (question from P.Johnson). J.R. Pappenheimer referring to Dr.Pavel's first results (Brain Res.Bull., 2, 251-254, 1977) expressed his reservations with respect to the claim that a few hundred molecules of AVT induce sleep. Finally, A.Borbély drew attention to the questionable ethical side of AVT experiments in infants, particularty since in view of the potent endocrinological actions of the compound, adverse effects on a developing organism cannot be excluded.

Thermoregulation

E.Simon raides the point that the changes of sleep observed by D.Heller after altering the hypothalamic temperature were not necessarily caused by a specific influence on thermosensitive units. H.C.Heller admitted that this possibility cannot be excluded. However, the fact that the experiments focussed on the differences between the actual hypothalamic temperature and the hypothalamic threshold, should largely rule out nonspecific effects. M.Kluger asked if the peripheral vasodilation caused by hypothalamic heating in rats exposed to 30°C could not effect sleep via the raised skin temperature. H.C.Heller emphasized in his response the large drop in core temperature in these animals. G.Benedek specified in response to Dr.Heller's question that capsaicin applied via a single cannula had an immediate desynchronizing action due to the mechanical stimulation of the preoptic region, an effect that was not seen when the drug was administered via a double cannula.

Circadian oscillators

C. Czeisler specified in response to a question from A. Borbély that the hypothesis of an environmental zeitgeber effect on the body temperature rhythm via a direct action on the sleep-waking oscillator, is based on computer simulation studies.

MAMMALIAN NERVOUS SYSTEM UNDER PRESSURE

Chairman:

M. HUGON (France)

Adv. Physiol. Sci. Vol. 18. Environmental Physiology
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MAMMALIAN NERVOUS SYSTEM AT DEPTH INTRODUCTION TO A ROUND TABLE (R.T.)

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We specifically considered here the case for "isopressure" diving : the breathed gas pressure and the hydrostatic water pressure are equal (approximately 10 M depth in sea water for 1 ATA, absolute pressure). Consequently high pressures of biological mixtures of gases in "dry chamber" can simulate the most important characteristic of the underwater dives, and make it possible to carry out experimental studies.

The ambient pressure is usually made up of added Helium, a light and relatively inert gas. The normal air in the chamber can be preserved, or washed out and replaced by Oxygen (PiO_2 = 200 to 400mb normally; He-O_2 mixtures). Adding Nitrogen (He-O_2-N_2 mixtures or Trimix) may result in ventilatory difficulties due to the high specific mass of the compressed gas, and in some, narcosis.

High rate of compression with He-O_2 (100M sw/H or more...) induces behavioral modifications in the mouse, rat, squirrel monkey (see review in Brauer et all.,1980, this R.T.), in the cat (Fagni et all., in preparation), in the Papio monkey and in Man (see Rostain thesis, 1980; also Burgess, thus T.R.).

In every species, with some relatively minor variations, a fast compression provokes : i) tremor (8-12 Hz) in active muscles by 20-30 ATA; 2i) jerks or short myoclonies (each lasting several hundred milliseconds; a few jerks a minute ...); tonico-clonic crisis can then develop. Thresholds for such epileptic modifications are about 40-60 ATA; 3i) a slowing in E.E.G. frequencies with a thêta frequency power increase (Rostain, 1980, p. 47-60), a facilitation in somatic evoked potentials (Hugon et all., 1980, the development of erratic, fast, spike-like events in the scalp EEG; 4i) defects in sensori-motor and cognitive performance (Seki, 1976; Lemaire, 1980).Burgess (this R.T.) has observed nausea, possibly from labyrinthine origin.

In contrast low mean rates of compression (10 M sw or less...), including possible interspersed stages at constant pressure provokes only small neurological disturbances, as long as the ultimate depth is moderate : less than 30 ATA for evident clinical modifications (Lambertsen et all., 1977; O'Reilly, 1977; Hugon et all., 1979; Hugon and Seki, 1979). If the final saturation pressure is high enough (e.g. 80 ATA for Papio ...) the subject may undergo a non-paroxystic lethal degradation (Rostain, 1980, p.71).

After fast compression, at constant depth, the hyperbaric modifications tend to reduce with time (Gruenau and Ackerman, 1978; Rostain, 1980, p.71; Burgess this R.T., Brauer this R.T.). Such reduction is small or

absent, when the constant depth is high (Berghage et all., 1975); in fact acute modifications may develop (mouse : Brauer et all., 1977).In any case, adaptation does appear to be not sufficient beyond a certain limit of depth.

Progressive addition of N2 (5 to 10% of the on-going pressure) diminishes the motor problems (Bennett et all., 1974; Brauer et all., 1974; Rostain et all., 1977; Bennett et all., 1980) without reducing the EEG disturbances (Rostain, 1980, p. 93 and sq; 157 and sq). Trimix is said to be better for fast, confortable and safe diving.

Behavioral modifications, reflex variations (Roll et all., 1978; Lacour et all., 1978; Harris, 1979), EEG and cognitive modifications under constant high pressure, all should be included in an comprehensive definition of the "High pressure nervous syndrome" revisited after the initial definitions (see Rostain, 1980).

HPNS is understood here to be the net result of the pressure strain, plus the biological effect of the diluent gas after tissue saturation, and perhaps molecular fixation, and after the adaptative response of the organism. In contrast a clinically different "compression syndrome" would be "a pure" pressure effect, with minimal gas action and adaptation. A "pure" compression syndrome is, of course, a theoretical concept because any compression needs time, and this time could be sufficient for some gas diffusion and adaptation by the organism.

Such syndromes are "nervous" as long as there are no (ventilatory) respiratory problems, no gas conductance impairment (Imbert et all., 1980), no blood and circulation problems (Rostain, 1980, p.170 and sq; but also Ornhagen, 1979), no cellular problems in oxidative processes (Geiger et all., 1976), nor ionic disturbances ... Then disorders in nerve excitation, impulses conduction, synaptic transmission... could be related to some specific neural diving effect from pressure and/or gas.

Neurological pressure effects are demonstrated by dysfunctions in organisms or preparations under water pressure (Lundgren and Ornhagen, 1976; Ornhagen and Hogan, 1977). Gases by themselves have biological effects. Nitrogen is far from being inert (Miller, 1972; Brauer et all.,1974; Rostain et all., 1977; Mac Donald, 1978; Gruenau and Ackerman, 1979; Cromer et all., 1979...). Helium,on the other hand, does not have much physiological effect (Lambertsen, 1967, 1978 and this R.T.; Fries and Durant, 1974; Kendig and Cohen, 1976 and in reviews by Mac Donald and Wan, 1978; Mac Donald, 1980). In many cases Helium actions seems to be negigible, after short time of exposure (under 62 ATA : Rostain, 1980, p. 150, Man and monkey). It is worth noting here that "bathyphysiology" of submammals and invertebrates is of interest to the understanding of progresses in mammal physiology.

In pressure and gas effects, the authors theorize about molecular modifications in single macromolecules or in multimolecules structures (membranes, organelles, enzymatic systems...). The fact that hydrostatic pressure rouses animals under light narcosis (Miller, 1972; Brauer et all., 1974) or reverses the effects of general anaesthetics on nerve fibers and neurones (Spyropoulos, 1957; Roth et all., 1976; Kendig and Cohen, 1975) led to the development of the membrane expansion theory (Seeman, 1972) and the critical volume hypothesis (Miller et all.,1973).Briefly,pressure is considered to diminish the fluidity of the phospho bilayers in the membranes, with a possible increase of the hydration and ionisation of the hydrophilic groups. Many anaesthetics (but not all of them) would have opposite effects. The ionic permeability of the membrane (Na^+, K^+...Ca^{++}) would be restored by anaesthetics (and nitrogen ?) after adverse pressure effects. Such a model could be valuable for small neurones in neuropiles,

but not for myelinated fibres in peripheral nerves, under 100 ATA. Under such pressures there is no detectable change in most of the membrane or action potentials (invertebrates). Similarly Hugon and Lemaire (1975) detect no variation in the recovery cycle of the human single motor axon in situ, a finding which suggests no impairment in the ionic environment and exchanges of the myelinated fibre. In line with this conclusion, the Helium pressure does not produce any detectable variation of velocity in sensory and motor fibres in Man (Roll et all., 1978) or in the monkey (99 ATA : Bonnet et all., 1973; Harris, 1979; Hugon et all., 1980). So the membrane of the (large) nerve fibre under such pressures seems to be sound - HPNS would result from modifications of the physiology in the neuropile (membrane properties of neurones and neuroglia, including synaptic transmission and related phenomenons).

At pressures less than 100 ATA, HPNS mainly consists of modifications of polyneuronal polysynaptic processes reflexes, EEG activity, evoked potentials and gross behavior. Focusing interest on the synaptic processes, it is possible to elaborate a rough outline of synaptic pressure effects, as in the work of Mac Donald (1980) and Lehmann (1978). Under pressure, molecular organization can undergo modifications if there is a (causal) decrease in molecular volume (Kettman et all., 1966) : pressure per se would induce a modification of the external conformation of the cloud of electron "gas" of the molecules. Hydration, ionisation, possible changes in inter atomic spatial arrangement... are quoted as examples of pressure effects.Because of such a change in the molecular "shape", interaction between a modified molecule and a specific substrate could be also modified. "Recognition" (or "stereo selectivity") could be impaired. Pressure could result for instance : i) in a modification of the conformation and properties of the membrane receptor for a neural mediator or hormone (Kendig et Cohen, 1975, 1976; Athey and Ackers, 1978); 2i) in modifications of the related adenyl-cyclase system (Fain, 1978; Fish et all., 1979); 3i) or in changes of properties (fluidity) of the phospho lipid matrix in which the receptors and cyclase systems are embedded (an other version of the membrane expansion theory ?). Defects in synaptic transmission will result from any such disturbances. This kind of hypothesis is often alluded to in papers (Martin et all.,1972 ; Friess et all.,1975 ; Henderson et all.,1977 ; Yeandle ,1977 ; Kaufmann et all.1979...). Hugon et all. (1980) tentatively explain the atropine-like effect of Helium on reflexes and evoked potentials as due to a post-synaptic anti-muscarinic pressure effect. Such an effect on synaptic transmission is not due to some problem in acetylcholine release in their experiments, and that suggests some post-synaptic receptor impairment.

Such a stereochemical hypothesis is very attractive because it is very suggestive : it could explain the relation of a variety of problems at depth to many different pressure-sensitive structures in the CNS (Naquet this R.T., 1980), in isolated spinal cord, as well as in other centers in nervous system (Kaufmann et all., 1979b). It suggest a rationale for understanding the exageration of the HPNS in higher biological forms (Brauer et all., 1980). Emphasis was placed, at the beginning of this paper, on the reduction of some problems during stages of moderate constant depth after fast compression. Such a reduction could be due to the effect of Helium on molecular systems when saturation develops (Mac Donald, 1980). But a model from Mandell (1980) provides us with another valuable tool for research : homeostasis at cellular level could explain the reduction of the problems through the reactions of the biological system. Yet, the permissive and regulatory effects of the hormones on the nerve cells (thyroid-

gluco-corticoids) develop using the membrane receptors, and consequently can be pressure-sensitive processes as well. Of course presynaptic receptors, endocellular systems (e.g. mitochondria, Wattiaux, 1974) and other molecular systems could be sensitive-pressure. One modification does not exclude another and any physiological and pharmacological analysis should take advantage of both neurophysiology in adaptative organism or preparation and biology at cellular or subcellular levels.

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PREDICTION OF PHYSIOLOGICAL LIMITS TO HUMAN UNDERSEA ACTIVITY AND EXTENSION OF TOLERANCE TO HIGH PRESSURE

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Man exposed as a diver in the deep sea experiences, in all of his physiological systems, greater environmental forces and stresses than in any other sustained working situation. These forces drastically modify his physiological processes and his performance.

The continuing question to physiology is "What are the specific limits of tolerance to high pressures?" The answers to be obtained will involve the most fundamental mechanisms in physiology and biophysics.

THE PHILOSOPHY OF LIMITS

Physiological limits of many forms do exist for diving. They exist at all depths, from the shallowest to deep diving. Some limits can be overcome or postponed by modification of the diving method. Some can be masked. Some can be eliminated by engineering. Most persist and must re-emerge with the increasing pressures and durations of deep diving.

Investigation and prediction of these limits require constant awareness of what diving actually is, in its many forms. The activity of diving is a linked composite of subconscious physiologic mechanisms which support the consciously purposeful functions (interpretation and thought, communication, work, manipulation) which are the intended aspects of diving. Diving is not simply passive exposure to gas pressure in a chamber, and it is not simply breathing or breath holding underwater. Therefore prediction of limitations must be concerned not only with the absence of convulsions or unconsciousness but also with the quality of thought and the capacity for useful physical action. The nature and degree of performance disruption can vary with any combination of sensory, mental, psychomotor and physical processes (Table 1). The disruptions can be of any degree, extending from undetectable to full physical incapacitation to unconsciousness.

Against this background it is the intent of this paper to offer the philosophy of limitations and prediction that has guided the Institute for Environmental Medicine's

series of Predictive Studies concerned with extending tolerance to work at high ambient pressures.

Oxidation	
Respiration	Sleep
Circulation	Rest
	Work
Sensation	
Mentation	
Manipulation	
Communication	

Table 1. Functional Components of Deep Diving.

The Diver

The working diver is unique in the spectrum of exposure to extreme physiological stress (Fig. 1). The athlete functions to physical exhaustion, but in an ideal and harmless environment. The astronaut is essentially unstressed, week after week, regardless of distance from earth, protected by engineering from most hazards or even need for severe exertion. The mountaineer suffers the cold and extreme hypoxia of Mount Everest, but after weeks of progressive adaptation prior to attempting his final ascent. Even the whale is not exposed to the full severities of human diving.

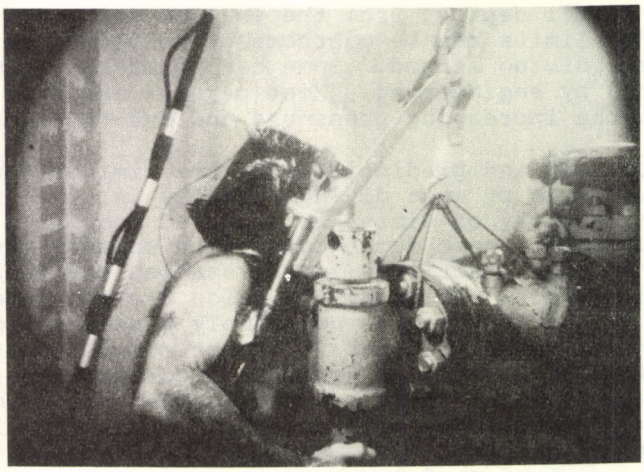

Fig. 1. Diver Breathing Helium-Oxygen Mixture While Performing Practical Work in Water-Filled Chamber at Pressure Equivalent to 488 Meters (1600 Feet of Sea Water) (8).

It does not have to ventilate its lungs, it has no narcotic, hyperoxic, decompression, temperature or strenuous exercise stress. Its exposures to hypoxia and pressure are acute, but the requirement for detailed performance is limited. For the human diver each of many forces or effects increases with the greater pressures of deep diving, and some increase with duration of exposure. Of all these examples he is the only one who becomes "physiologically" trapped by the high pressure environment and unable to leave it at will. It requires longer to decompress from saturation exposure to a helium pressure of 1000 feet of sea water than to return to earth from a lunar landing.

Fundamental Physiological Mechanisms Affected

In a search for limitations to deep diving it is not sensible to assume a precise pressure limit or a single limiting mechanism in any form of deep diving. The mammalian organism (mouse, man or whale) is infinitely complex. Moreover, the function of one process or system or organ or sensor or effector is intricately related to functions of other systems and processes. Ultimately all depend for their normal function upon fundamental biophysical and biochemical mechanisms, concerned with life factors of charge, reaction velocity, synthesis, binding and even physical diffusion of gases and ions.

Under the physical and chemical stresses of deep diving each of these many basic functions is a potential target, but all must differ greatly from each other in the conditions for producing initial disturbance (threshold ?) and also for rates of subsequent failure. It is even a large error to simplify the prediction of extreme pressure effects in diving by assuming that a specific site or structure is the primary limiting target. Even with equivalent effects upon the fundamental chemistry or membrane characteristics of many different cells, the measurable consequences of exposure can be expected to vary greatly. In great physiological systems, such as the entirety of our neurological assets, the more complex functions (with more components and steps in chemical and electrical activity) can be expected to fail at lower hydrostatic or gas pressures than will the simpler functions. The important study of impulse transmission in a peripheral nerve fiber or autonomic ganglion synapse will teach us the nature of their particular responses to pressurization. To learn of limiting effects upon judgment and vision it will be necessary to measure vision and judgment.

With this as general perspective it is clear that to learn the ultimate limits of diving requires two related forms of investigation and analysis. Fundamental mechanisms must be examined in any appropriate tissue or animal to hydrostatic and gas pressures well beyond those conceivably reachable by man. And man himself must be systematically examined, step-by-step, in minute physiologic detail under

conditions beyond those to be encountered in practical operations. Both approaches are honorable and absolutely necessary. There is no room for trial and error research in human exposure to environmental extremes.

PRIMARY STRESSES OF UNDERSEA ACTIVITY

A classical concept out of basic pharmacology and engineering is that response to a drug or physical stress is usually proportional to the drug dose or to the severity of the stress. The quantitative "dose-response" curve can often be used to describe basic cellular reactions or overall physiologic competence. It is the ideal predictive measure for specific effect and limitation. However, without precise quantitative studies no such predictions of limiting relationships can be described. Description is required for each function of interest, as affected by increasing degree of each stress of importance. Prediction derives from this description.

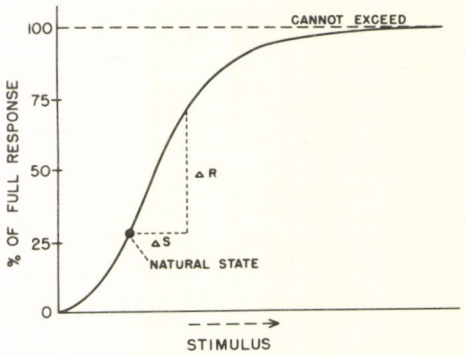

Fig. 2. Stimulus or Dose-Response Relationships in Physiology and Pharmacology

The Nature of the Stresses

The identities of several primary stresses are well known, even though their mechanisms and interactions are more conceptual than demonstrated. They include: Temperature, Physicochemical Effects of Inert Gases, Hydrostatic Pressure, Inert Gas Exchange, Hyperoxygenation, Hypoxia and Respiratory Gas Density. Each ultimately influences neural, circulatory, respiratory and other functions (Table 2).

Hypothermia	Hyperthermia
Hyperoxia	
Hypoxia	
Hypercapnia	
Hyperbaria	
Physicochemical Effects of High Inert Gas Pressure	
Communication Decrement	
Increased Respiratory Gas Density	
Respiratory Work	
Sleep	
Interactions	

Table 2. Major Pressure, Temperature and Atmospheric Stresses in Undersea Activity

Thermal Exchange

Temperature regulation, hypothermia and hyperthermia are the background factors against which nearly every other stress of undersea activity expresses itself.

Deep body temperature is a controlled component of the design of the internal environment of the mammalian organism, affecting such basic factors as hydrogen ion activity, calcium ionization, and the kinetics of numerous enzymatic reactions. Its control is not to be interfered with in diving.

Temperature is therefore of extreme practical limiting importance, regardless of depth, but not independent of it. Temperature deviations can be incapacitating or lethal or can contribute to lethal outcome in interactions with gas narcosis, very probably with hydrostatic pressure, and certainly with increased gas density. Search for tolerance to ambient temperature alteration is essential to the understanding of limitations of other stresses, and no study of effects of gases or pressure can ignore temperature as a critical variable.

Temperature stress increases severely with increasing ambient pressure, in water or in a gaseous environment. The compression of gas (e.g., helium) molecules increases heat capacity of the respired and ambient atmosphere, leading to excessive heat transfer between lungs and atmosphere or skin and atmosphere. Transfer may involve gain or loss of body heat, depending upon thermal differential between body and atmosphere. The result may be intolerable or incapacitating hypothermia or hyperthermia. Since this aspect of deep undersea activity involves physical exchange processes not adaptable to physiological modification, the limitations upon temperature regulation imposed by increased gas density and altered ambient temperature can be predicted to remain unless minimized by engineered systems for adjusting the temperature of ambient and respired gas. The precision of

regulation required for these systems increases with diving depth, and relates as well to forms of physical work and to individuals (Table 3). Even in dry, helium-filled chambers the spread of comfort temperature becomes close to 1°C at 1200 feet of sea water and, in the presence of physical activity, should be still less at still higher pressures. Temperature control is therefore a major technical component of any deep diving life support system. The real requirement is to eliminate temperature abnormality at all depths rather than to provide physiologic countermeasures in the presence of uncontrolled or abnormal body temperature.

Depth		Low Limit	High Limit
Meters	Feet	°C	°C
122	400	28.5	31.5
213	700	29.0	31.5
274	900	30.0	32.0
366	1200	32.5	33.5

Table 3. Thermal Comfort Ranges in a Helium Environment (1).

Physicochemical Effects of High Inert Gas Concentrations in Cellular Structures

It seems inevitable that at increasingly high pressures the increasing molecular concentration of any inert gas in cell structures will interfere with any function of any cell. The effects of inert gases are probably qualitatively numerous, even though the tendency persists in undersea physiology to designate "narcosis" as a single end result. The term "narcosis" is a loose one and it is very likely that the influence of high inert gas pressures is not a single one, even for a single inert gas. Effects upon membrane function, metabolic enzyme function and synthetic functions can all be conceived, with different dose-effect patterns, and different consequences or symptoms, with different gases. Inert gas effects on a retinal rod cell could affect vision. The same biophysical effect upon the smooth muscle cell of a retinal vessel could affect its contractility.

Gases differ in fundamental influence upon cell components. While nitrogen is distinctly narcotic at pressures less than 10 atmospheres, helium and neon produce no prominent depression of mental or sensory function at (38 ata pressure) 1200 feet of sea water (1). Since all indications are that inert gases should induce "dose-effect" patterns of functional change, it is probable but not at all certain that helium or neon will not induce disruptive effects on central nervous system function comparable in degree to those of nitrogen until ambient pressures much in excess of 3000 feet of sea water are experienced (1) (Fig. 3).

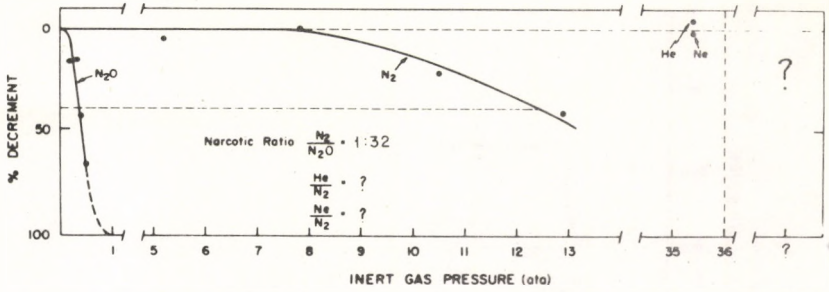

Fig. 3. Comparative Narcotic Effects of N_2O, N_2, Ne and He on Mental Function (Arithmetic Index) (1).

Faced with these forms of unawareness of the mechanisms of inert gas effects, prediction of limitations imposed by inert gases requires detailed study of individual gases over an extreme range of pressures. The true separation of helium and hydrostatic pressure effects will be extremely difficult in any specimen, and probably not possible in man.

Hydrostatic Pressure

Extreme increase in hydrostatic pressure itself is limiting, even without accompanying increase in solution of inert gases in tissues. Pressurization can produce myospastic immobilization, paralysis, convulsions, cardiac arrest and death in experimental animals (2, 3, 4, 5).

In man, "moderate" increase in hydrostatic pressure (e.g., to 10 meters of sea water) produces no clearly detectable effect. Higher pressures (e.g., 20 to 60 meters of sea water), especially when rapidly attained, induce increasing degrees of derangement, including temporary incapacitation (Fig. 4) (6, 7, 8). The bases for the varied symptoms and signs which include malaise, mental slowness, sleepiness, dizziness, nausea and vomiting, weakness, tremors and myoclonic spasms, and electroencephalographic changes (6, 7, 8, 11, 26), is not known. In the absence of an evident effect of helium itself (1), it is presumed that these derangements are due to hydrostatic pressure.

Since the most easily measurable effects of compression have been tremor and electroencephalographic changes, the designation "High Pressure Nervous Syndrome" has been applied to the pattern of abnormalities produced (6, 7). The term is useful but too specific for a phenomenon which is undoubtedly general in its effects, even on non-neural biological systems (9, 3, 10, 30, 31).

Limitations. Investigation of hydrostatic pressure effects has involved (a) study of rate and degree of compression in man to approximately 65 ata (11, 24), and (b) extension of hydrostatic pressure exposures to over 200 ata in animals and isolated tissues (12, 9, 10, 27, 28, 17, 16).

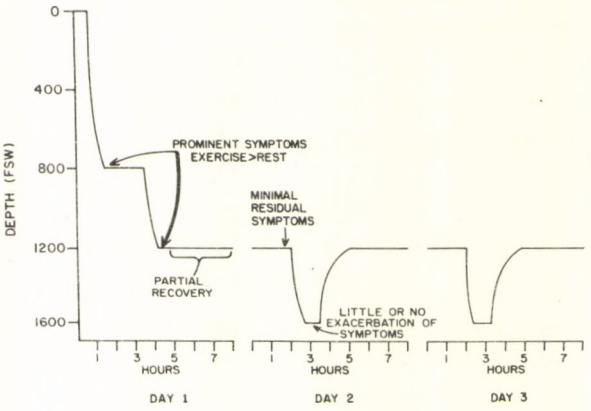

Fig. 4. Rapid Compression in Helium Atmosphere to Presure Equivalent of 244-366-488 Meters (800-1200-1600 Feet) of Sea Water (8).

Prediction of limits of tolerance to increased hydrostatic pressure requires the same philosophical reasoning as for the narcosis and/or oxygen toxicity encountered in diving. Each of these stresses must be considered as exerting effects on more than a single biophysical or chemical mechanism, at many sites and therefore on many functions (Fig. 5). Indication of multiple molecular sites for effects of hydrostatic pressure has actually been observed (12). While an obvious initial expression of hydrostatic pressure effect may be tremor, it should be considered that multiple, simultaneous other effects must be occurring during high pressure exposure. While within limits all such effects may be reversible, probably most still remain unidentified. Therefore, the desired dose-effect relationships have not been determined and prediction of limitations to hydrostatic compression in man is not now possible.

On gross and purely practical grounds it is evident from experiment in man with helium breathing that between 0 and 1200 feet of sea water no acute or lasting general handicaps develop when the rate of compression is slow or when a waiting period follows rapid compression (1, 6, 7, 8). Moreover, adaptation to rapid compression to this pressure appears to be complete (8), and in subsequent excursions from 1200 to 1600 feet of sea water general functions remain close to normal in spite of prolonged persistence of some electroencephalographic effects of compression (1, 13, 8). At helium pressures between 1600 and 2000 feet of sea water serious limitations of activity with helium breathing appear to persist for prolonged periods without full adaptation, even when compression is slow (11, 13, 14, 22, 30, 31).

The causes, the variety, and the reserve tolerance for these effects of hydrostatic pressure itself is not known. Therefore, at still higher pressures not yet fully explored in humans, it is not possible to predict which of many

PERFORMANCE
- PERCEPTUAL
- COGNITIVE
- PSYCHOMOTOR
- EXERCISE (AMBIENT GAS)
- WORK (UNDERWATER)

NEUROLOGICAL
- ELECTROENCEPHALOGRAM
- EVOKED POTENTIAL
- TREMOR
- POSTURAL FUNCTION
- ELECTROMYOGRAPHY
- NERVE CONDUCTION VELOCITY
- NEUROLOGICAL EXAMINATION

VISUAL
- ACUITY
- COLOR
- FIELDS
- EYE MOVEMENT

AUDIO-VESTIBULAR
- ELECTRONYSTAGMOMETRY
- AUDIOGRAM
- PHYSICAL EXAMINATION

METABOLIC
- O_2 CONSUMPTION
- CO_2 PRODUCTION
- RESPIRATORY EXCHANGE RATIO
- END-TIDAL P_{CO_2}, P_{O_2}

TEMPERATURE
- DEEP BODY TEMPERATURE (RECTAL)
- SKIN TEMPERATURES
- EXPIRED GAS TEMPERATURE
- ENVIRONMENTAL TEMPERATURE

ENDOCRINE FUNCTION
- URINE
- BLOOD

RESPIRATORY
- RESPIRATORY RATE
- TIDAL VOLUME
- EXERCISE RESPONSE
- DIAPHRAGMATIC ELECTROMYOGRAPH
- END-TIDAL P_{CO_2}, P_{O_2}

PULMONARY
- MAXIMUM VOLUNTARY VENTILATION
- FORCED VITAL CAPACITY (INSPIRATORY AND EXPIRATORY)
- VITAL CAPACITY
- TIDAL VOLUME
- INSPIRATORY CAPACITY
- EXPIRATORY RESERVE VOLUME
- FUNCTIONAL RESIDUAL CAPACITY
- RESIDUAL VOLUME
- TOTAL LUNG CAPACITY
- AIRWAY RESISTANCE
- LUNG COMPLIANCE
- ESOPHAGEAL PRESSURE

CARDIOVASCULAR
- ELECTROCARDIOGRAPH
- HEART RATE
- CARDIAC OUTPUT (IMPEDANCE)
- EXERCISE RESPONSE
- CIRCULATORY REFLEX

UNDERWATER WORK PERFORMANCE
- VIDEO RECORDING
- UNDERWATER RESPIRATION
- PERFORMANCE MONITORING

RENAL FUNCTION
- URINE VOLUME
- URINE COMPOSITION
- URINE ELECTROLYTES
- SERUM ELECTROLYTES

HEMATOLOGY
- COAGULATION STUDIES
- HEMOGLOBIN, HEMATOCRIT
- HISTOLOGY

Fig. 5. Scope of Correlated Measurements in Exposures to Rapid Compression, Breathing He-O_2 (8).

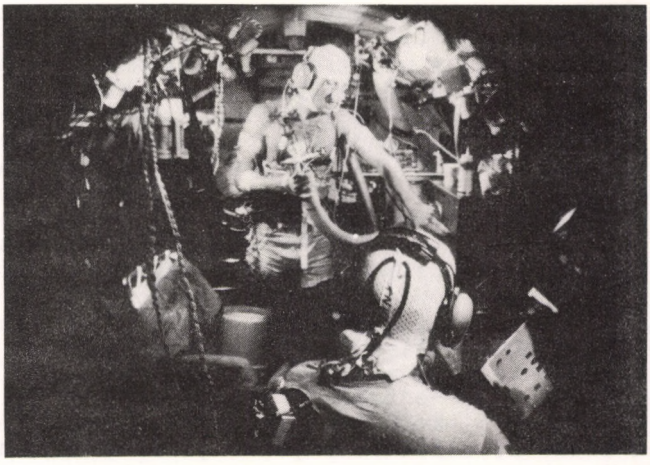

Fig. 6. Performance of Physiological Measurements <u>During</u> and <u>Following</u> Rapid Compression in a Helium-Oxygen Environment (Pressure Equivalent to 488 meters or 1600 Feet of Sea Water) (8).

affected functions have already reached their tolerable limits and which have only begun to be affected.

At the now relatively low pressures of 30 ata, where effects of helium itself have been shown to be innocuous (1), study of rapid rates of compression has indicated the occurrence of gross effects presumed to be due to the increasing hydrostatic pressure (8, 22, 30). Therefore, rapid compression to still higher pressures can be expected to induce more severe effects, including convulsions or other incapacitation. Prediction of tolerance to rapid compression requires consideration of the degree of compression, its rate and the patterns of time allowed for adaptation at stages during compression.

Extension of Tolerance to Pressure. Addition of narcotic substances (gases or other drugs) has been shown to prominently modify effects of very high hydrostatic pressure on isolated tissues (16) and in intact aquatic or terrestrial animals (5, 12, 15). Conversely, compression can at least partially overcome effects of depressant gases or drugs (2, 5). These classical findings in animals have been applied to undersea physiology, as by the addition of nitrogen to helium breathed by man at high pressure (22, 24, 25, 30), to increase tolerance to the effects of hydrostatic compression. Nitrogen modifies the pattern of effects produced by rapid compression (22, 24); it is not known whether it prevents or merely masks these effects (8).

Since determination of the scope and quantitative degree of effects produced by hydrostatic pressure alone and by compression with helium has been accomplished only in part, the specific influences of concurrent exposure to other inert gases along with helium cannot be defined. Any site of neurotransmission is a potential site where pressure or anesthetic may alter several functions (17). Some synapses are components of sensory or stimulant transmission pathways, others serve in depressant pathways. Since compression aggravates some neurophysiologic depressant effects of anesthetics and counteracts others, uniform or progressive benefit for all influences of compression cannot be predicted and is in fact unlikely. It therefore becomes necessary at pressures beyond those already explored for helium alone to "expect the unexpected" rather than simply to assume general amelioration of all compression effects.

This awareness is especially pertinent to neurological and respiratory effects of compression, since a convulsion in man at extreme gas density, with its composite of violent exercise and breathholding must inevitably result in death due to the failure to re-establish alveolar ventilation (35). The requirement therefore continues to exist for simultaneous study of critical physiological functions (Figs. 5 and 6) (8).

Inert Gas Exchange

There is as yet no indication that rate of uptake of inert gas during compression should be a limiting factor for ultimate diving depth (Fig. 6). Even if the concept of

osmotic forces related to local differential inert gas concentration (18) is eventually determined to have importance, its effects will most probably continue to be overshadowed by the more drastic influences of hydrostatic pressure. An exception, though not strictly limiting, is the arthralgia of compression which has been conceived as possibly related to osmotic influences of inert gas uptake (20).

Aspects of inert gas exchange will predictably continue at all pressures to be major limiting factors in decompression, in development of decompression sickness, in the multiple forms and consequences of isobaric inert gas counterdiffusion and in the therapy of decompression and isobaric sicknesses (23, 32). All of these attest to the physiological importance of inert gas exchange, and the necessary interactions among them make impractical prediction of definite improvement in safe decompression. It can be predicted that for each inert gas its exchange will continue to be governed by normal factors of anatomy, circulation, temperature and respiration, which themselves will continue to be limiting. Therefore the rates of inert gas elimination from critical sites are unlikely to be improved. For this reason it can be predicted that opportunities for extending diving without hazard of decompression or isobaric gas lesion diseases rests, not with a primary physiological speeding of gas elimination, but largely with (a) improvement in oxygen tolerance; and (b) improved understanding of the generation, growth and dissemination of gas bubbles, the interactions of bubbles and blood constituents, and the interplay among decompression, gas exchange, oxygen tolerance and isobaric gas exchange both in normal diving and in treatment situations.

Oxygen Toxicity and Oxygenation

Oxygen toxicity must be paired in importance with hydrostatic pressure in any ranking of major factors affecting predictions of ultimate diving capability. It presents limits to oxygenation as well as to attainable rates of inert gas elimination and effectiveness in "bends" therapy.

Oxygenation. At extreme pressures, beyond those yet reached by man, it has been considered on theoretical and indirect empirical grounds that large mammals are incapacitated through limitation of intrapulmonary diffusion of oxygen (19). This is not grossly evident in monkeys exposed to 100 ata or smaller mammals exposed even to 200 atmospheres and has not been found in man breathing dense gases at high pressures (1, 24), even in severe exercise (1).

Decompression and Isobaric Counterdiffusion. Extension of limits for excursion diving from saturation critically depends upon improvement in oxygen tolerance, as does increase in safety of decompression from each other form of diving, and improved therapeutic success in all forms of decompression sickness.

While substantial gains in extending oxygen tolerance are being made by programmed alternation of high and normal PO_2 (Fig. 7) (33, 34), prediction of further influence

upon diving depends in part both upon methods of oxygen use and upon resolution of the scope of acute and chronic effects of hyperoxia. Just as for narcosis and hydrostatic pressure, increased pressures and durations of supranormal oxygen exposure should be considered as causing multiple adverse effects upon multiple tissues. Determination of

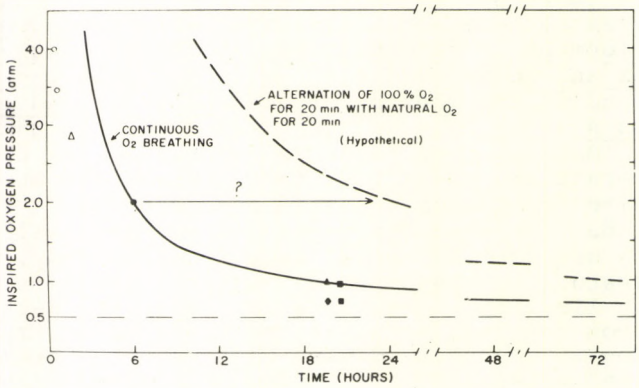

Fig. 7. Predicted Effect of Intermittent Oxygen Breathing on Pulmonary Oxygen Tolerance Limits in Man (34).

limits of oxygen tolerance and means for extending oxygen usage requires determination of the nature, onset, time course and reversibility of the several specific forms of enzymatic and related oxygen poisoning (34). Of critical importance is the determination of any as yet unquantified chronic or cumulative or residual effects of hyperoxia upon organs and their tissues.

Density of Respired and Ambient Gas

The linear increase in gas density which occurs with increasing pressure induces non-linear decrements in two forms of interchange between internal and external environments. It progressively modifies respiratory thermal exchange and ventilatory gas exchange, toward potential failure of each function.

Effects on Pulmonary Ventilation, Respiratory Control and Exercise Tolerance. Increased respiratory gas density increases respiratory resistance and work of breathing, with inevitable decrements in alveolar ventilation and capability for sustained effort by respiratory muscles. At any gas density each factor cited is related to the magnitude of pulmonary ventilation and hence to the degree and the duration of physical work being performed.

Tolerance to respired gas density has been extensively studied in man at increasingly high ambient pressures. In

the absence of prominent effects of hydrostatic pressure, acute exposure to increased gas density diminishes pulmonary ventilatory capacity in rest and in exercise (Fig. 8) (1, 20, 29). Such studies have indicated that density effects

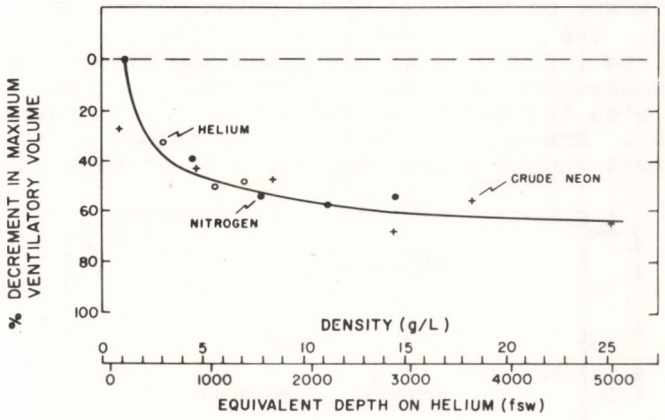

Fig. 8. Influence of Respiratory Gas Density and Airway Resistance on Ventilatory Capacity (1).

upon respiratory function (pulmonary ventilation and respiratory reactivity) at rest and in mild exercise should be tolerable even at gas density equivalent to helium breathing at 1500 meters (c.a. 5000 feet of sea water) (Fig. 9) (1).

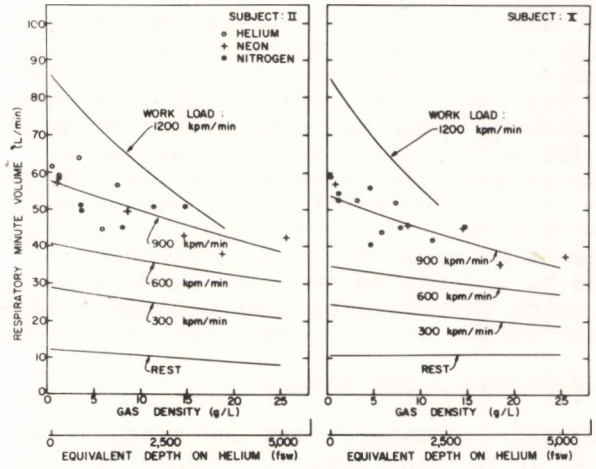

Fig. 9. Pulmonary Ventilatory Response to Exercise at Extreme Respiratory Gas Density (Equivalent to Helium-Oxygen Breathing at 1500 meters or to 1200, 2000, 3000, 4000, 5000 Feet of Sea Water) (1).

There is as yet no reason to revise this prediction for effects of gas density alone.

However, increase in gas density during compression in a helium atmosphere is inevitably accompanied both by increase in hydrostatic pressure and increase in any as yet unknown effect which may be produced by solution of helium in critical tissues. The interaction of such effects with the better defined influences of increased gas density appears to induce subjective respiratory distress not importantly associated with increased gas density at lower pressures (14, 21, 29). Precision in matching of method in such investigations should allow separation of density and

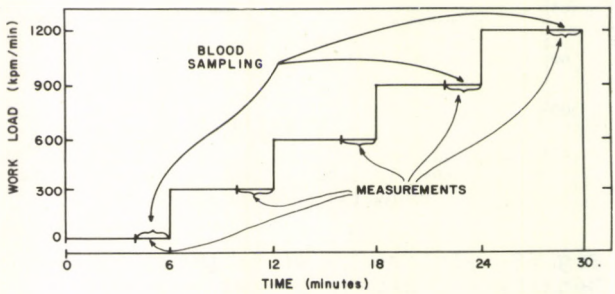

Fig. 10. Pattern of Continuous, Sustained, Increasing Workload in Studies of High Respiratory Gas Density (1).

pressure-related effects (Fig. 10). Since this separation is not now complete, prediction of diving limitations due to interactions of gas density and pressure now depends upon indications that even moderate exertion appears impractical in prolonged exposure to helium at pressures of 550 meters (1800 feet) of sea water (14). Since vigorous underwater work has been clearly shown to be practical in helium excursions to 488 meters (1600 feet) of sea water (8), a zone of sharply exaggerated decrement between 488 and 610 meters (1600 - 1800 - 2000 feet) of sea water breathing He-O_2 can be predicted. The subjective limitations encountered should be expected to be tolerable at rest, and to be magnified by increasing severity or duration of work. The degree to which these limitations are modified by use of gases other than helium must be quantitatively determined.

INTERACTIONS OF STRESSES AND EFFECTS

Throughout these considerations and predictions of physiological limits to pressurization, selected interactions of important stresses have been cited. Those interactions already mentioned are to be considered merely examples from an extensive pattern of inevitable cross-influences of physiological mechanisms, degrees of physical or mental activity, and severity of environmental forces. They include necessary interplay among work, oxygen pressure, gas den-

sity, exposure durations, hydrostatic pressure, temperature, inert gas and other factors (Fig. 11). They change qualita-

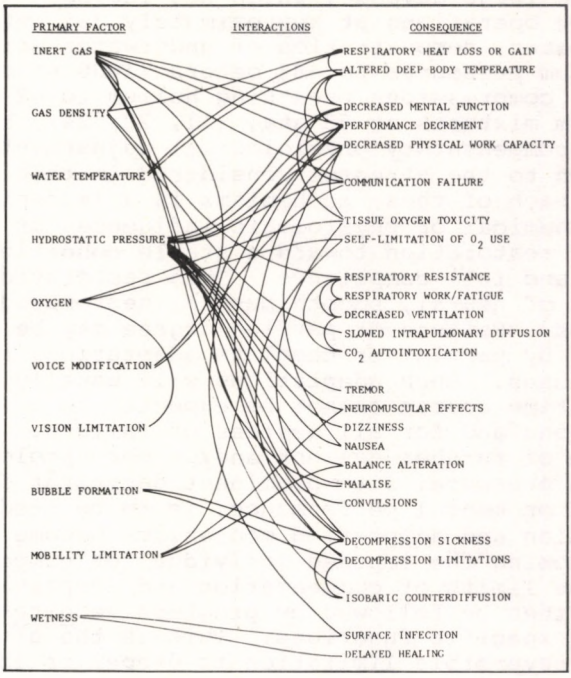

Fig. 11. Examples of Interacting Influences of Stresses and Effects in High Presure Exposures (35).

tively and in degree with increasing depth in the sea, and with duration of exposure. They confound any but gross prediction, making it necessary to explore men in controllable actual circumstances as well as to investigate basic mechanisms. Each stress alone can conceivably be tolerated better than can the composite of several or all stresses. The great progress of undersea physiology and activity of the past decade has derived from modification of the stresses and interactions (e.g., by choice of gas and compression rates, and improved life support systems) and not from modification of the basic physiological responses by drugs.

DETERMINATION, COMPENSATION, ADAPTATION, DETERIORATION

The fact that multiple limitations do exist to further extension of deep diving is less surprising than the fact that human pressurization to 122 to 615 meters (400-600-800-1000-1200-1400-1600-1800 and 2000 feet) of sea water, inconceivable very few years ago, has actually been possible. Extreme determination on the part of many of the "diver" subjects has allowed physiological exploration of the unknown in spite of sometimes severe symptoms.

Compensation and Adaptation

Part of the large advance, which has included sustained open sea diving operations at approximately 365 meters (1200 feet) of sea water, demonstration of underwater work capability at helium pressures to 488 meters (1600 feet) of sea water (8), and compressions breathing helium to 62 or nitrogen-helium mixtures to 65 ata, (11, 22, 24), has resulted from compensatory physiological adjustments, and from adaptation to the stresses considered in this analysis. The result of each of these mechanisms is a lessening of the biophysical, chemical or physiologic influences of the stresses, with restoration toward a stable condition of natural function and full competence. This restoration may include return of neural, smooth muscle, neuromuscular and other functions. Failure of partial degree may be expected to be followed by partial or complete adaptation, at least for some processes. Such adaptations will usually require time, and the time course cannot be expected to be the same for all functions and for all degrees of failure.

Limitation of further descent and/or more prolonged exposure to high pressure, with prominent decrement in physical or sensory or mental performance, is to be predicted when compensation and adaptation mechanisms become inadequate in overcoming the imposed individual or composite stress. At the limits of compensation and adaptation acute decrement can then be followed by progressive deterioration and failure of specific functions. This is the ultimate and potentially irreversible limitation to deeper or longer exposure.

Deterioration

In several situations exposure to the physical or toxic stresses of high pressure must predictably impose true limitations upon extension of diving depth. A proposed example is an increase in pressure and respired gas density to such a high degree that the work of breathing, even at rest, is severe. In this situation performance of useful physical activity will be impractical, even though discrete manipulation and sensory/mental functions are competent at rest. Continued excessive exertion by the muscles of respiration, even without considering the probable overlay of hydrostatic effects upon neuromuscular function, will necessarily result in progressive fatigue of these respiratory muscles, decompensation of the diaphragm and intercostal muscles and failure of ventilatory function (1, 8, 21). In the presence of hydrostatic pressure effects upon neuromuscular transmission or muscle contraction, the above-mentioned influences of gas density must inevitably be aggravated. This entire sequence must be exaggerated by any requirement for exercise, whether for practical purpose or emergency. Concurrent with this predicted respiratory deterioration, the act of sleep would predictably further diminish respiratory reactivity, accelerate the failure of ventilation and result in further hypoxia and hypercapnia.

Since decompression from prolonged exposure to high pressure cannot be rapid, any escape or withdrawal from a progressive respiratory decompensation can only be slow. The rate of withdrawal allowable by the requirements for inert gas elimination should be considered inadequate to allow recovery of capacity for ventilation. Continued deterioration, complete respiratory failure, severe hypoxia and death must therefore result (1, 8, 21).

At the limits of tolerance to extreme levels of respiratory gas density the use of pharmacologic therapy or substitution of another inert gas is not likely to overcome all effects of compression, even though it may mask some (8). Moreover, attempts to sustain survival at high gas density by hyperoxygenation should predictively further diminish ventilation while introducing oxygen toxicity as an additional respiratory complication.

The example is cited here again to indicate that, while capability for work at high pressures has been remarkably extended, limits of several forms can indeed ultimately be expected for rate and degree of compression, even if all factors but respiratory gas density and associated hydrostatic pressure are controllable. The limits must be expected to be more stringent in open sea operations than in laboratory chambers. At present, largely due to lack of quantitative knowledge concerning effects of hydrostatic pressure in man, it is not possible to project the depth range at which the irreversible respiratory failure described above should be considered inevitable. It surely exceeds 600 meters (or 2000 feet) at rest and may not be much greater for extended work.

CONCLUSIONS

Exposure of man to high ambient pressures has increased from the equivalent of approximately 100 to over 600 meters (300 to over 2000 feet) of sea water during little more than the past decade.

Detailed investigation of the physiologic influences of compression indicates that:

oxygenation will not be limiting at depths less than 1000 meters (c.a. 3000 feet).

increased respiratory gas density alone should be tolerable at rest at least to pressures equivalent to helium breathing at 1500 meters (c.a. 5000 feet), but will severely limit productive, sustained physical work at lesser pressures.

full functional competence for human physical, sensory and intellectual activity in water at depths between 365 and 610 meters (c.a. 1200 and 2000 feet) of sea water should be attainable.

the most clearly limiting stresses are hydrostatic pressure and temperature. In their

interactions with other stresses each is exaggerated and rendered unpredictably more limiting.

increased hydrostatic pressure with helium breathing induces prominent but ill-defined limitations upon physical activity and respiration at pressures between 490 and 550 meters (1600 and 1800 feet) of sea water. At 610 meters (2000 feet) of sea water neurological changes are sustained throughout exposure even at rest, and actual capacity for sustained physical work is not predictable.

decompression rates in saturation or excursion diving depend upon physical principles and will probably not be increased except by improvement in oxygen tolerance.

oxygen tolerance is susceptible to practical extension by programmed intermittency of exposure even though the several chemical mechanisms of oxygen toxicity will predictably remain active.

temperature limitation in diving will remain serious at all depths and will continue to impose extreme engineering requirements in open sea operation at pressures beyond 365 meters (1200 feet) of sea water.

the multiple and interacting influences of oxygen, hydrostatic pressure, inert gases and temperature upon fundamental cellular physicochemical and biochemical processes will continue to result in unpredictably changing patterns of limitation with increasing depth.

beyond 600 meters (c.a. 2000 feet) the composite effects of gas density and hydrostatic pressure upon respiratory function in sustained exposures can predictably result in progressive, inescapable hypoxia and hypercapnia, and irreversible respiratory failure in man, even at pressures tolerated in smaller animals.

the addition of narcotic drugs or gases to helium breathed at high pressures can be expected to diminish some symptomatic and neurologic expressions of hydrostatic pressure effects. Where this diminution is produced by masking rather than by prevention of the hydrostatic pressure effects, effects can be expected to re-emerge as pressure is further increased.

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PSYCHOMETRICS: QUESTIONS TO NEUROPHYSIOLOGY

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Introduction

For some years we at the Admiralty Marine Technology Establishment (AMTE) have been studying the effects of pressure on both men and animals, and for many of these years have been concerned with the neurological effects of pressure. In our early dives of the mid-1960s it was recorded that the divers showed signs of body tremor, and in one compression to 800 ft (244 m) in 8 minutes 12 seconds the performance impairment was accompained by dizziness, nausea and vomiting. From this series, together with the work of others, it was concluded that there was some form of barrier at 1100 ft (335 m) beyond which man could not go. This limit was however not fully believed by the workers of this Laboratory and in a dive using slow rates of compression, together with staging, a depth of 1500 ft (457 m) was achieved with only marginal signs of tremor etc. After this initial push into deep diving we had somewhat of a rest and it was not until September 1975 that we started on our present deep dive programme. In this programme we decided that what was needed was a full scientific investigation into the problems of pressure. Therefore, so as to eliminate a few of the problems, such as confinement, etc, our early dives (1 to 4) were carried out at low pressures and will not be mentioned further as they presented no neurological changes.

300 m Dives

By Dive 5 we were ready to extend our experiment depth down to 300 m and until this dive all had worked well. In this series, to eliminate any effects of normal air breathing, it had been our procedure to confine the volunteers in the chamber in helium at 3 m with 0,4 bar oxygen. At the end of this 2-day washout period compression was continued to the working depth at a rate of 1 m/min. On the third day, therefore, we began compression down to 300 m, when around 200 m one of the volunteers reported signs of nausea and vertigo;

20 m later he reported a sense of fatigue and muscular weakness with increased intensity of all other symptoms. At 250 m the other subject reported a sense of impeding loss of consciousness and the descent was halted. Nausea and vertigo, as well as a sense of rotation of the visual world, were the main symptoms, but tremulousness, feeling of faintness and extreme muscular weakness were equally distressing. The subjects looked sweaty and distressed, but their conversation with outside staff indicated a normal mental state. At no time did they show gross tremor, EEG changes or manifest nystagmus. One hour later both subjects felt better and compression was resumed at the previous rate (1 m/min). No worsening occurred and 300 m was reached 50 minutes later. Over the next 5 to 6 hours further improvement occurred, but in one subject the symptoms of nausea with vomiting persisted for the next 24 hours.

What then had caused this problem, as previous dives at the Deep Trials Unit had not shown these symptoms. As these dives had started from air, it was concluded, therefore, that perhaps the removal of dissolved nitrogen before compression could have been the cause. We therefore rescheduled our next 300 m dive (Dive 6) to start compression on Day 1, from an air-filled chamber. Compression rate was again continuous at 1 m/min. Again all was well until at 250 m one of the volunteers reported that he had signs of nausea and vertigo. The compression was stopped, and although he reported one hour later that he now felt better, we held at 250 m until the next day, before continuing to 300 m. No further symptoms were observed and there were no changes seen in any of the neurophysiological tests taken at depth. The volunteers performed well and completed the full work programme with no further problems, and were successfully decompressed after spending 7 days at 300 m.

420 m Dives

We now moved on to our next working depth of 420 metres and we concluded that perhaps the problem was due to the continuous compression, as normal working dives would have some built-in stages to test equipment etc. To test this hypothesis, therefore, we decided to compress at a very slow rate with not more than 60 m/day. Dive 7 therefore followed this routine and 420 m was reached with only a very slight passing sign of nausea on the last drop. We therefore considered that we had a successful profile and set out to repeat this dive with a full scientific programme of studies at depth. However, this time on the last stage one volunteer reported marked feelings of vertigo. During the next 24 hours he had repeated attacks of vertigo and vomiting which produced serious problems in our scientific programme. The next day he reported well and the dive was continued as planned. All neurological tests were negative in the ranges measured, but it was noted that the tape recorder had overloaded due to a marked increase in low frequency swings in both the EEG and tremor. Since at this time we had a low

frequency cut-off at 2 Hz in our analysis equipment, no comment could be made on this data.

In all our previous dives it had been observed that the first 180 m had produced no problems. Therefore in an attempt to speed up this part of the compression we scheduled a dive to 180 m (Dive 9A) with a compression rate of 3 m/min. At the end of the compression one volunteer reported well, but the other stated that he felt a little sick. Some short while later his symptoms became so bad that he stopped all experimental work and lay down on the floor of the chamber. During this period EEG and whole body tremor recordings were made, with the frequency range now extended down to 0,1 Hz. During the onset of this episode low frequency whole body tremor was seen; that later changed to bursts of higher frequencies, set at regular intervals, as the volunteer lay down. Low frequency changes were also observed in the EEG of the other volunteer which lasted for a period of some 5 minutes. However, during this time he did not report any signs of vertigo or nausea. This low frequency data was the first time, that we had observed any neurophysiological changes during an attack of high pressure vertigo in man.

Further studies

Since the 420 m series we have attempted three experiments to 520 m, only one of which, using professional divers, has reached the full depth. In each case there has been signs of vertigo and nausea, which in two of the cases, have been so bad that the dive has had to be cancelled. All compressions have used 0.4 bar oxygen in helium with no nitrogen added. Evidence suggests that without some form of diver selection for a lack of neurophysiological-related symptoms we will find it hard to reach depths in excess of 300 m using pure He/O_2 mixes. Neurological signs of low frequencies have been seen which gave some early warnings of later troubles. These signs are under further investigation at our Laboratory and will be studied in our continuing deep dive programme.

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MAN AND SUBHUMAN MAMMALS. HIGH PRESSURE NERVOUS SYNDROME, SINGULAR OR PLURAL?

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The high pressure nervous syndrome (HPNS) in man, as described by Brauer et al. (1969) after several dives made to more than 300 meters with a helium-oxygen mixture, includes a whole group of clinical and electroencephalographic (EEG) signs.

The principal symptoms are the following :
- From a clinical point of view : tremor with rapid frequency (8 to 12 c/sec.), dysmetria, fasciculations and myoclonia (occurring without any EEG modifications) and daytime drowziness.
- From an EEG point of view : the appearance of slow waves in bursts (most often theta frequency, occupying especially the anterior regions of the scalp), the diminishing of the amplitude of the posterior alpha rhythm and the occurrence of microsleep accompanying the lowering in the level of vigilance noted in the clinical observations.

Complementary symptoms have subsequently been added, in particular the possibility of the occurrence of dizziness and nausea, a relative lowering of the psychomotor performance (Bennett and Towse, 1971) and a lightening of night-time sleep (see Bennett et al., 1976 ; Rostain, 1980).

Epileptic seizures, preceded by myoclonia accompanied by EEG paroxysmal discharges, are not found in the HPNS in man, whereas they are one of the characteristics of the HPNS in the subhuman mammal (Rostain, 1973) ; the fact that man has not yet had any epileptic seizures in deep dives can certainly be explained in part by the fact that man was submitted to lesser depths than the animal.

In 1969, at the time HPNS was described, the various symptoms were thought to be due to the pressure, without taking into account the role of other parameters such as the composition of the gas mixture, or the compression speed and profile.

It was even thought that man could not go beyond depths of 365 meters without too much damage. Thanks to research carried out in different hyperbaric centers in France, in England and in the U.S. it has been demonstrated that this limit could be extended and it is known at the present time that man can work in the sea without too much difficulty at a 500 meter depth (french experiment in 1978) and work and live correctly in a chamber under pressure beyond 600 meters (610 meters in France in 1971 and 1974 ; 650 meters in the U.S.A. in 1980).

These experiments have shown that the intensity and the type of symptoms varied according to the experimental conditions and the subjects tested, and they allowed to establish several general rules :

1) For a given gas mixture and a given depth, the HPNS symptoms depend on the rate and mode of compression (Rostain and Naquet, 1974).

In a helium-oxygen mixture it has been shown that :

a. in the Papio papio baboon, at a constant compression speed of 200 m/h, the epileptic seizure occurs between 650 and 750 meters ; it does not occur until 1000 m with a speed of 40 m/h and close to 900 m for a decreasing speed of 120 to 40 m/h (Rostain, 1980).

b. in man, the compression curves without intermediate stages are less favorable than those interrupted by stages ; when stages exist, the compression curves are more favorable when they are slow and exponential than when they are linear ; with equivalent compression speeds, and especially at high compression speeds, the effects increase at greater depths (Corriol et al., 1973 ; Hunter and Bennett, 1974 ; Rostain and Naquet, 1974).

The following examples illustrate these data :

- During the Physalie VI and Sagittaire IV experiments (Rostain and Naquet, 1978) the compression speeds were identical up to 550 meters and the EEG modifications in the different subjects were similar from one experiment to another. Between 550 m and 610 m the compression speed was three times faster and the EEG modifications five times greater during the Sagittaire VI experiment.

- During the Physalie V experiment, compression was much faster between 350 and 400 m and between 460 and 490 m than between 400 and 460 m and 490 and 518 m. Up to 460 m, tremor did not increase more than 200 % for the two divers, during compression and at the stages. Between 460 and 490 meters for the two divers there was a very marked increase in tremor which exceeded 600 % for one diver (Rostain and Lemaire, 1973).

- During 11 compression experiments carried out between 300 and 610 m in 24 subjects, the results showed that

-- depending on the compression speed, the clinical and EEG symptoms appear at different depths. The variation is about 100 meters for tremor, and 200 meters for myoclonia, drowziness, theta activities, the depression of alpha, and microsleep,

-- that the degree of the symptoms depends on the compression curve

-- that the symptoms do not all occur at the same depth (tremor appears between 200 and 300 meters, theta activities between 200 and 400 meters, myoclonia, daytime drowziness and microsleep between 300 and 500 m (Naquet et al., 1975).

2) In a given subject, all the symptoms do not occur simultaneously, even for a given depth. This is true especially when compression is very rapid. For example it has been shown that for a compression to 180 m in 15 min., tremor appears immediately, reaches its maximum all at once, and eventually diminishes in several hours. The EEG modifications appear slowly and may not reach their maximum until 7 hours after the arrival at the bottom while the other symptoms have already begun to diminish (Rostain et al., 1980b).

3) If the subject stays at a given depth, certain symptoms, once they appear, show little variation during 24 hours, while others such as tremor are more pronounced in the morning than in the evening (Rostain et al., 1975 ; Rostain et al., 1977).

4) The symptoms are related to the type of gas mixture used. The addition of a given quantity of nitrogen to the helium-oxygen mixture modifies the electroclinical symptomatology of the HPNS (Bennett et al., 1974). With a mixture containing a sufficient percentage of nitrogen (9 %) tremor disappears. However, upon arrival at 300 m for rapid compression behavioral symptoms (euphoria) and increased fatigue occur ; the EEG modifications are greater than in a heliox mixture at the same depth ; the power spectra of the slow waves and also of the fast-activities is greater than at the surface. Daytime drowziness is very marked. However, even when the subject remains at a constant depth, the clinical and EEG symptomatology is more transitory than in a heliox mixture ; in general the clinical symptoms disappear in 24 hours (Rostain et al., 1980b).

These data, which were valid at 300 m were also valid for greater depths. This was confirmed recently by two experimental series in which 8 divers were carried to 400 and to 450 m with an exponential curve (Rostain et al., 1980a). During these 2 dives nitrogen was added progressively to the mixture and it seemed to have had a positive effect ; in spite of that the degree of the EEG modifications (increase in the slow waves, and the rapid frequencies) is greater at the beginning of the experiment where compression speed is rapid, than at the time when the subjects arrive at the bottom, due to a progressive slowing down of the compression rate. This means that even with a trimix mixture the compression rate and profile influence the symptoms of the HPNS.

5) The symptoms are not reproductible from one subject to another. This difference in sensitivity which was noted during the first dives with heliox, was confirmed during the dives with trimix. During the dive to 450 m which included 8 divers, it was shown that not only do differences in sensitivity exist from one subject to another but that for a given subject certain symptoms are more sensitive for the same type of compression.

For example, upon arrival at the bottom, in certain subjects, there were very marked EEG modifications and few changes in the sensorimotor performance, while in others, the EEG records were very slightly modified and there was a marked deficiency in the psychomotor tests (Lemaire, 1980 ; Rostain et al., 1980c). This means that each symptom of the HPNS of each subject has a sensitivity corresponding to the hyperbaric conditions to which he is submitted.

In conclusion, these data make us consider more closely the HPNS entity and the danger of using this sole term to cover all the multiple symptoms which may appear in dives in various experimental conditions. An analytical description of the evolution of each of the symptoms in relationship with the experimental conditions (gas mixture, pressure, compression profile) would perhaps contribute to a better comprehension of the origin, the significance and the value of each of them. It seems too that one cannot consider the disappearance of a given symptom due to the modification of one or several parameters of the compression signifies necessarily the disappearance of the HPNS. It has been seen in man that with an appropriate mixture, the tremor could disappear while other symptoms persist or increase ; it is also known that in the baboon using a particular compression profile and a progressive nitrogen addition, it is possible to retard the appearance of one of the most serious symptoms of HPNS, the convulsive epileptic seizure. However, for depths beyond 1000 meters, it has been noted that the global amplitude of the EEG decreases and violent tonic muscle spasms occur. These spasms, whose

origin is still unknown are not accompanied by paroxysmal EEG discharges (Naquet et Rostain, 1980 ; Rostain et al., 1979). The disappearance of this alarm signal, (the convulsive epileptic seizure), is dangerous since in its absence, access to greater depths may reveal other more signs with any warning.

Continued animal experimentation and the evaluation of the most significant alarm signals for each type of compression used in the animal and in man are necessary in order to avoid exposing subjects in the future to serious accidents during even deeper dives.

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Adv. Physiol. Sci. Vol. 18. Environmental Physiology
F. Obál, G. Benedek (eds)

SOME ASPECTS OF THE COMPARATIVE PHYSIOLOGY OF THE HIGH PRESSURE NEUROLOGIC SYNDROME

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Clinically, it seems to me, there is rather more unity than diversity in the response of a wide range of animals to progressive increase in hydrostatic pressure. Verbal descriptions of the responses in primates or in mice, or even in crayfish and in snails, sound remarkably alike: they all progress through one type or another of motor disturbances such as tremors or enhanced activity, to a stage characterized by more or less generalized muscular spasms, (which, in vertebrates, are quite properly described as convulsions), to a terminal or near terminal stage of paralysis or cardiac failure (cf. (1) for recent review). Yet, even in this grossly oversimplified description, one can recognize that more than one organ system is involved: while it seems to be generally true that the CNS plays a paramount role in the first two stages, it is virtually certain that the third group of effects must have their origin in disturbances of the contractile system.

More detailed analysis of the portion of the syndrome dominated by CNS effects, the high pressure neurologic syndrome (HPNS), reveals further evidence of complexity: Thus, for instance, in the convulsion stage of the vertebrate HPNS one can readily discern two substages which we have designated as Type I and Type II HPNS seizures. We as well as others, have called attention to the fact that the threshold pressures eliciting these two types of seizures show numerous differences in the ways in which they are affected by drugs (2, 3), by manipulation of compression conditions (2), by age (4), or by genetic factors (5) (Table 1). These differences are sufficiently marked to raise the question whether one should view the two types of HPNS convulsions merely as successive manifestations of a single common underlying biophysical change the severity of which increases with increasing pressure, or whether they should not rather be viewed as the end results of two divergent chains of cause and effect emanating from distinct biophysical events.

TABLE 1

DIFFERENCES BETWEEN TYPE I AND TYPE II HPNS SEIZURES IN CD-1 MICE

CRITERIA:	TYPE I:	TYPE II:
Clinical	Clonic Burst	Tonic/Clonic Sequence
EEG	Little Change	4-5 sec. spike and wave; post-ical silence
Heart Rate	No change; no atropine effect	80-90% decrease; atropine blocked partially
Compression Rate	Very: k = 11	None: k = 0 or negative
Phenobarbital	Protects	Protects - but to a much greater degree than I
Diphenylhydantoin	Sensitizes	Markedly protects
Trimethadione	Sensitizes early, protects slightly-late	Protects early, no effect late
Reserpine	Sensitizes, especially at low compression rate	Little effect
Ontogenetic	Mature, more resistant than newborn	Little change from birth to maturity
Spinal Animal	No seizures below transsection	Seizure also in isolated part of spinal cord
Mortality	None	29%

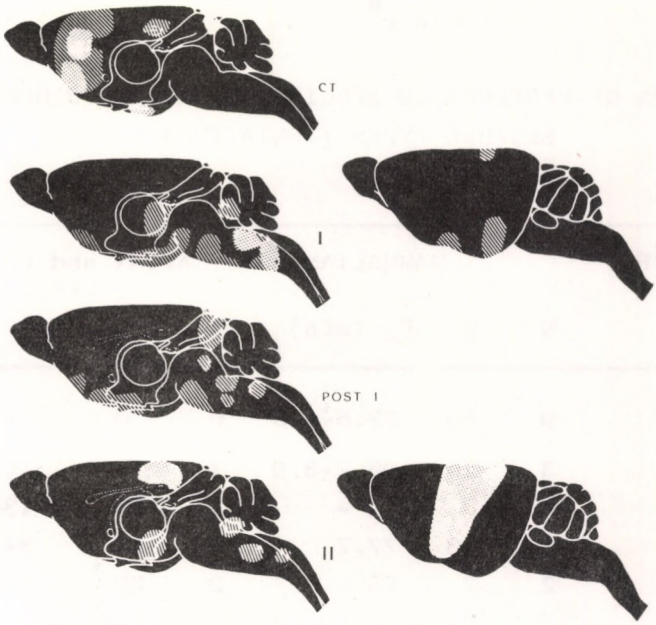


Fig.1 Mapping of distribution of 2 deoxy-1-C^{14}-d-glucose retention in mouse brain at successive stages of HPNS development. From top to bottom: Coarse tremor stage; Type I HPNS convulsion; interictal stage intervening between Type I and onset of Type II seizures; Type II HPNS seizures. Left column - sagittal sections (schematic); right column - surface views at the two seizure stages. Two degrees of C^{14} retention are shown. In order of increasing density these are: "Two" - ▥ , and "Three" ▩ .

While at this moment it seems to me that no one can provide a truly conclusive answer to this question, I believe that we do have some data that throw additional light on this matter: In a number of mammals it has been shown that localized paroxysmal activity of the CNS elicits a grossly exaggerated rate of glucose utilization in the affected areas (6). It is possible, therefore, by the use of a radioactive glucose analogue such as 2-deoxy 1-C^{14}-d--glucose to label such sites and subsequently by proper histologic manipulation to prepare radioautographic records which permit developing, as it were, a map of sites involved in any particular form of convulsive activity. So far as I can tell, while the basic methodology has been available for some time, experimental difficulties had precluded its

Table 2*

DISTRIBUTION OF VERTEBRATES SPECIES ACCORDING TO HPNS SEIZURE TYPES (TENTATIVE)

SEIZURE TYPE	MAMMALIAN			AVIAN and LOWER VERTB.		
	N	%	$\bar{P}_c$ (ata)	N	%	$\bar{P}_c$
I and II	9	6o	77.6±4.9	0	0	-
C	3	2o	96.7±3.9	1	10	108
II only	0	0	-	5	50	134± 20
I only	2	13	77.7	2	20	84 and 15
I or II	2	7	65	2	20	207
TOTAL:	16	100		10	100	

The basic seizure types discussed in the text are Types I and II for the mouse, and compound (C) for the majority of adult rats. Other symbols: N - number of species in category: % - fraction of all mammalian, or all non-mammalian species reviewed. $\bar{P}_c$ - mean convulsion threshold pressure for a given category at a compression rate of 135 atm/h.

follows the mouse rather than the rat pattern.

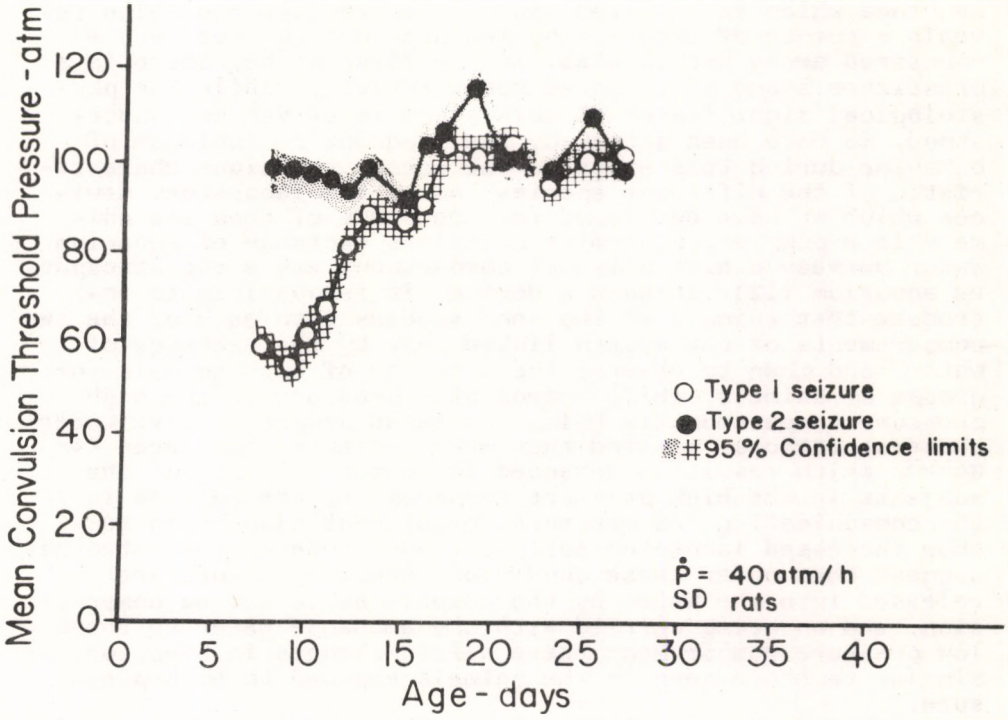


Fig.2 Pattern of changes of HPNS convulsion thresholds during maturation of the baby rat. After about the twentieth day of postnatal age the majority of rats of this strain show compound seizures. A small proportion (about 15%) continue to display the double seizure pattern, and it is the mean value for each type of seizure in this group which is represented by the open or filled circles shown after day twenty. The mean for the compound seizure group after day twenty is comprised within the space marked off by the lines drawn through the two families of points. Ṗ is the compression rate.

The situation for non-mammalian vertebrates is more complicated (cf. Table 2, right side). We have not been able to reproduce the double seizure pattern characteristic of the mouse and other mammalian species in birds, reptiles, amphibians, or fish. Among non-mammalian vertebrates, the first seizure event frequently is a tonic seizure, but sufficiently detailed information is not yet available to decide whether this pattern should be compared to the Type II seizure of mammals or whether it should rather be compared to the modified Type I seizure recently observed by collegues at Oxford University in mice after decerebration (S. Daniels - pers.

commun.).

Finally, I wish to comment briefly on the HPNS-like sequence which is observed among invertebrates and which reveals a number of interesting features not (or not yet) encountered among vertebrates. In the first place, there is a preseizure stage of enhanced motor activity. While the physiological significance of this stage is as yet not understood, we have been struck by the frequent resemblance of behavior during this stage to the escape reactions characteristic of the different species. Among the laboratory devices which we have developed for the study of deep sea animals is a pump which permits pulseless exchange of aquarium water between a high pressure compartment and a one-atmosphere aquarium (12). In such a device, it is possible to introduce test animals of the same species into each of the two compartments of the system linked only by the exchange of water, and then to observe the behavior of both animals, or groups of animals, while hydrostatic pressure in the high pressure compartment is being increased progressively. Under those conditions, we find that when pressures have been reached which result in enhanced locomotor activity of the subjects in the high pressure compartment, the animals in the communicating low pressure compartment also begin to show increased locomotor activity. Such observations strongly suggest that under these conditions chemical agents are released into the water by the animals subjected to compression, and on being carried with the exchange water to the low pressure compartment there elicit changes in behavior similar to those seen in the animals exposed to high pressure.

A second characteristic of the high pressure response in crustaceans is that it is subject to acclimatization. Thus, for instance, convulsion threshold pressures observed in gammarid crustaceans from Lake Baikal very clearly reveal a substantial difference between species inhabiting shallow waters and closely related ones inhabiting deep (i.e., 1,000--1,500 m) waters of the Lake (13). In the laboratory, it can be shown that this type of acclimatization can be reproduced to some extent by maintaining animals in the high pressure aquarium for periods of the order of two to three weeks at pressures of 100 atm. Acclimatization of this general type would account for observations made by our colleagues in Aberdeen, suggesting a linear relation between the depth at which a given crustacean was captured and its convulsion threshold pressure as observes on recompression after retrieval to the surface (14). It is particularly interesting to us to note that this acclimatization appears confined to the convulsion stage of the crustacean "HPNS": neither the activation nor the immobilization stages of gammarids show anything comparable to the increased pressure tolerance of the abyssal species for high pressure convulsions (13).

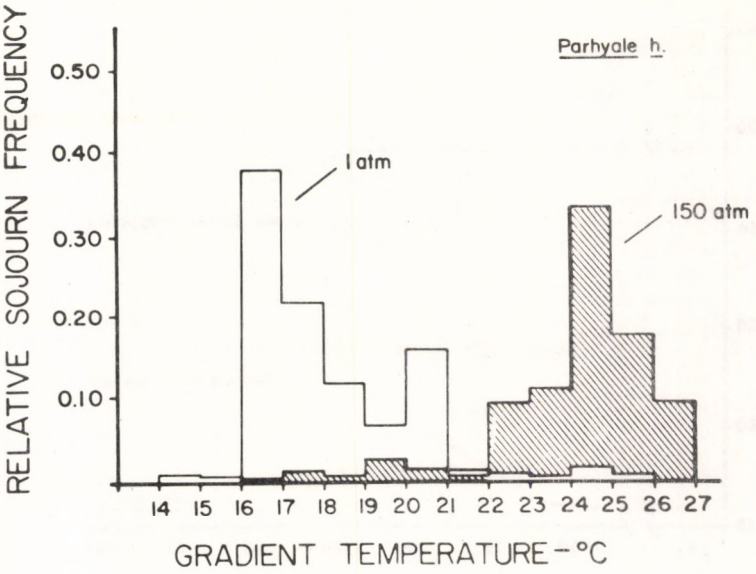


Fig.3A Effect of changes in hydrostatic pressure on temperature preference behavior in a marine gammarid, Parhyale h. Relative frequency of sojourn of animals in a given temperature interval within a dynamically stabilized temperature gradient tube. Open polygon - 1 atm control; cross hatched polygon- 150 atm.

The complexity of these events is sufficiently evident to make us tend to shy away from premature hypotheses regarding biophysical mechanisms. Nonetheless, some progress is being made toward understanding both, the acclimatization mechanisms, and the mechanisms giving rise to the neuromuscular disturbances. I would like to show you only one example of this kind of work: Many crustaceans, when placed in a temperature gradient, respond by showing a well defined preference for a narrow temperature band, the position of which varies as a function of acclimatization temperature and perhaps of specific life stages (cf. e.g., (15)). We have developed a device which permits carrying out such experiments at various pressures to study the interaction of hydrostatic pressure with preferred temperature. The results of such experiments reveal that in marine crustaceans increased pressure will elicit a surprisingly dramatic in-

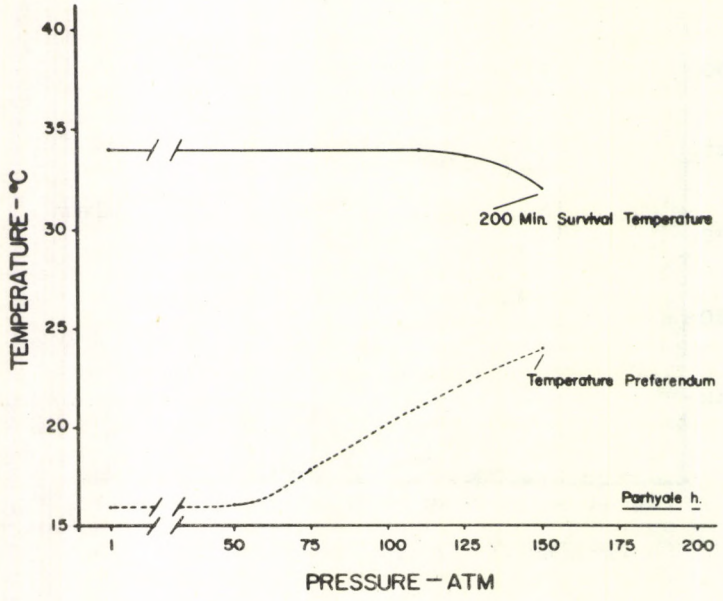


Fig.3B Relations between temperature preferendum, 200 min. survival temperature, and hydrostatic pressure.

crease in temperature preference ((16) and Figure 3A). This reaction does not appear to be a simple one-to-one pressure/temperature interaction, but rather seems to be characterized by a threshold pressure below which temperature preference is not affected, but above which preferred temperature increases rapidly as a function of pressure. It may be of interest to note that the threshold pressure for this effect is very close to what appears to be the threshold pressure for the increase in locomotor activity we discussed above. There are indications that this response pattern is markedly affected by the salinity to which the animals are acclimatized; indeed, in fresh water gammarids the entire pattern is blurred or even inverted. Since the pressures at which these effects occur are sufficiently high to modify sodium and calcium transport mechanisms ((17), and cf. e.g. (18); also R. Roer - unpub.), it seems to us quite possible that in these pressure/temperature interaction phenomena, wa may be coming rather close to the core of the mechanisms giving rise to the HPNS, or at least to parts of the HPNS, in crustaceans.

I would like to make a last point by inquiring how far down on the phylogenetic scale we can trace high pressure--elicited convulsion-like phenomena. As you can see from Table 3, such phenomena are present at least down to the level of nematodes, are absent in most (but not all) flat worms, and are altogether absent from the behavioral repertoire of coelenterates and ctenophores. When subjected to increased pressures, these latter animals go about their business as though nothing had happened until quite high pressures are reached at which point they become progressively more flaccid and undergo a pressure paralysis very similar to that observed as the final stage of the high pressure sequence in crustaceans or mollusks. I suggest to you that these observations furnish an indication of the level of organization required for the elicitation of high pressure convulsions: The data suggest that the simple nerve networks characteristic of coelenterates lack the necessary degree of structural and functional complexity, and that even in flat worms the degree of integrative differentiation (or of numerical complexity) of the central neuropil is still insufficient to elicit this type of reaction. I suggest to you further that the data of Table 3 add support to the inference drawn more obliquely from comparison of whole animals with neurophysiological isolate experiments in more highly developed species (cf.(1)): The HPNS is essentially a phenomenon of complex integrative neuropils. Model systems for the study of its biophysics will have to respect this requirement for complexity either by the choice of a relatively complex neuropil such as the vertebrate spinal cord, ((19), (2o)), or perhaps of central nervous system neurons specialized for integrative function such as the burster neurons of certain mollusks (21).

In this brief sketch, I have tried to survey rapidly a number of areas in which comparative physiological studies are contributing to an understanding of the fascinating effects of high pressure on CNS function, and of ways in application to the study of drug or electroshock induced generalized convulsions until we undertook to apply it to the study of the two types of HPNS convulsions in the mouse (7). It became apparent at once that the two types of seizures are characterized by rather complex but altogether different patterns of distribution of the centers involved in the brain. More to the present point, however, is the observation (Fig. 1) that a complete sequence of radioautographic studies, including the tremor stage, the Type I convulsion, the interval between Type I and Type II convulsions, and Type II convulsions, shows not only that the two seizure events are different in their neuroanatomical manifestations, but that they erupt on relatively quiet backgrounds which suggest that the two seizures represent wholly discrete events with no evidence to suggest that one could be viewed as progressing from or as an exacerbation of the other (8). Thus, the radioautographic data appear to support the interpretation that the convulsion stage of the HPNS in the mouse is not only complex, consisting of two discrete stages,

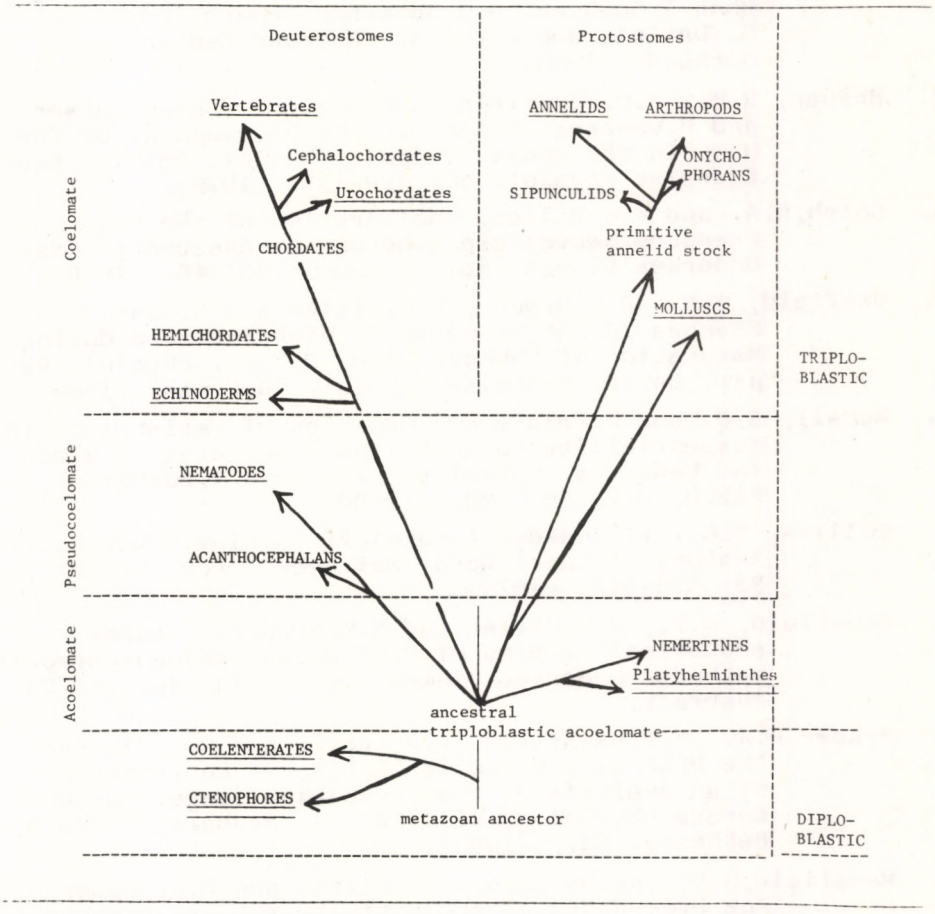
but that the two stages are so sharply discrete as to invite the hypothesis that they represent different neurophysiological mechanisms and hence may well prove to be the consequences of discrete biophysical events.

If one turns from the mouse to other mammals, a second HPNS pattern is sometimes encountered substantially different from that seen in the mouse. This is well developed in the majority of adult rats, where the pattern of two successive HPNS seizure types is not seen but is replaced by a single seizure event which has some of the characteristics of both types of mouse seizures (9, lo). The nature of this event is indicated by the successive HPNS patterns displayed by very young rats while maturing to adulthood: In the young baby rat, HPNS seizures follow in most details the two-seizure type pattern characteristic of the mouse. As the animals mature, the threshold pressures eliciting the two types of seizure change (as, incidentally, they do in the mouse) (4). Unlike the mouse, however, the threshold pressure giving rise to the second type of HPNS seizure does not change or even descreases with age while the pressure giving rise to the first HPNS seizure type increases rapidly so that somewhere in the neighborhood of the twentieth day of age the pressures eliciting the two types of seizures coincide (Fig. 2). At this point, they fuse into the single compound seizure characteristic of the adult rat.

Other mammals seem to fall into one or another of these two HPNS patterns (Table 2 - left side). Amoung the series of mammalian species we have explored (cf. (11)), slightly more than half follow the two-seizure pattern characteristic of the mouse. Elsewhere we have reviewed the data bearing on the situation in a primate, the squirrel monkey (8). While the first HPNS seizure in this species does not fully conform to the pattern for a mouse Type I (i.e., clonic) seizure, the similarities observed, and the subsequent development of the HPNS in the monkey lead us to feel that the most likely working hypothesis at present is that the squirrel monkey shows an HPNS convulsion stage which in general which studies of the neurological effects of high pressures bid fair to contribute to our understanding of the functioning of the CNS of tissue-grade animals.

TABLE 3

DISTRIBUTION OF THE OCCURRANCE OF HIGH PRESSURE CONVULSIONS AMONG DIFFERENT PHYLA



_____ = High Pressure Convulsions Present

_____ = High Pressure Convulsions Absent

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NEUROPHYSIOLOGICAL PROBLEMS WITH NITROGEN AT PRESSURE

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Since the description of the high pressure nervous syndrome (HPNS) (Brauer et al., 1969; Fructus et al., 1969), several authors have tried to show the causes and mechanisms of the HPNS and to reduce the signs of hyperexcitability that they found in the animal by adding more or less narcotic gases to the He-O_2 mixture (Miller et al., 1972; Brauer et al., 1974). They expected to take advantage of the pressure--narcotic agent antagonism (Johnson et al., 1942; Johnson and Miller, 1970; Lever et al., 1971) and of the pressure reversal effect and critical volume hypothesis. The use of a narcotic agent or anesthesic agent to counterbalance the effect of the pressure could thus keep the volume and the flux ionic "constant" and to prevent either the narcosis or the HPNS. (Bennett et al., 1975).

Thus, by using a He-N_2-O_2 mixture during very rapid compressions to 300 meters (33 min) Bennett et al. (1974) report a "suppression of HPNS". These authors concluded that the nitrogen had suppressed the HPNS but that the partial pressure of nitrogen was so high that it caused a narcosis and that it would be necessary to reduce it in the future experiments.

As a result of these experiments, we have decided with COMEX in Marseille to carry out an experimental series in man with the He-N_2-O_2 mixture.

I. Experiments in man

For these experiments we chose a depth of 300 meters and a compression in 4 hours at this depth.

4m/min from 0 to 100 meters
2m/min from 100 to 240 meters
1m/min from 240 to 300 meters

30min stages were made at 100 meters, 180 meters and 240 meters. Four dives were carried out. The quantity of nitrogen was 9% in the first, 4,5% in the second and third and 0% in the last.

Comparison of the results obtained from these four dives allowed us to make the following observations.

- Clinical symptoms
  . The dismetry is more pronounced with the $He-O_2$ mixture
  . Drowsiness and euphoria were found with the $He-N_2-O_2$ mixture.
  . The intensity of tremor is practically the same with 4,5 % and 0% nitrogen; it is less with 9% nitrogen.

The clinical symptoms tend to regress rapidly in $He-N_2-O_2$ while they persits with the $He-O_2$ mixture.

- EEG activity
  . The modifications (increase of theta activity especially) tend to be greater with the $He-N_2-O_2$ mixture (9% and 4,5%) than with the $He-O_2$ mixture (0%).
  . In certain subjects, rapid compression with the $He-N_2-O_2$ mixture containing 9% or 4,5% nitrogen can bring on EEG activities of a paroxistic nature.

Thus as a result of this experimental series in man the helium-nitrogen-oxygen mixture seemed to attenuate certain HPNS symptoms while increasing others.

These divergent results prompted us to carry out a study of the effect of nitrogen during "rapid compressions" in monkey Papio papio. We tried to find the best possible combinations of pressure and nitrogen percentages in order to reduce the disadvantages which nitrogen could have on the EEG and HPNS modifications while profiting from the advantages of this gas at the motor level.

II. Experiments in monkey Papio papio

1°/We have studied the effect of $He-N_2-O_2$ breathing mixture during fast compressions (Corasin I to III).

With a $He-N_2-O_2$ breathing mixture containing 6 bars of nitrogen added at the beginning of compression and a constant compression speed of 200 m/h from surface to 800 meters, the HPNS symptoms appeared in the same conditions that with $He-O_2$ breathing mixture with an epileptic seizure at 790 meters.

A most rapid compression (600m in 2h) and 5 bars of nitrogen added at the beginning of the compression, produced the same HPNS symptoms and a seizure at 600 meters. This seizure in contrast to that seen in $He-O_2$ experiments was confined to the head (clonies of the eyelids, muzzle and face). There was a large increase in power spectra of slow waves (theta: 2000%) and an increase in alpha and beta activities.

Thus, with the quantity of nitrogen used, the compression curve has an important role in the apparition of troubles.

2°/ We have studies different compression curves with $He-N_2-O_2$ breathing mixture (Corasin IV, V, X).

Using an exponential compression to 600 meters in 2 hours

with and without stage and with 4.8 bars of nitrogen added at the beginning of the dive, the HPNS symptoms did not appear systematically and were generally less intense those produced by non exponential compression:

- The tremor appeared at greater depth: 463m 29m (336m±43m with non exponential compression), and was less intense at 600 meters.

- The increase of slow wave EEG activity was less (approximatively 800%).

Consequently, with the same percentage of nitrogen and a total time of compression of 2 hours from 0 meter to 600 meters, an exponential curve induced HPNS symptoms less important than a non exponential curve.

3°/ Using an exponential compression with intermediary stages to 600 meters in 2 hours and 4.8 bars of nitrogen added at various stages different results were obtained (Corasin VI - VII - VIII).

- The effect of nitrogen saturation at 42 meters for the 24 hours preceding the dive was the same as that adding the nitrogen at the beginning of compression.

- Addition of nitrogen at the end of compression diminished all the HPNS symptoms which were appeared during the first part of compression in He-O_2 breathing mixture.

- Progressive addition before each stage produce some different effects:

. Tremors appeared in the extremities at a mean depth of 475 meters; they remained slight and did not spread to the rest of the body.

. The EEG changes were slight: theta activity increase somewhat (100 - 200%) and high frequency activity was slightly depressed.

Consequently, nitrogen injections in the breathing mixture during the compression seem to be a better method to diminish the clinical symptoms without to enhance the EEG modifications.

4°/ These results have permit extrapolation of the method of compression to 1000 meters and beyond (Cornelius series).

Actually the improved method of compression includes:

. An exponential profile;
. Stages of 40 min every 100 meters;
. Introduction of nitrogen into the mixture before each stage.

These latest experiments show that:

- Up to 600 meters, the procedure used in the Cornelius series does not provoke myoclonia or epileptic seizure.

. Tremor occurs at a greater depth (around 600m) than in the rapid compression with the He-O_2 mixture

or during the Corasin series with the $He-N_2-O_2$ mixture. The appearance of tremor at 300 meters and its great intensity in the dives using the same compression curve but without the addition of nitrogen shows that the improvement found in the dives with the $He-N_2-O_2$ breathing mixture at the level of clinical symptoms (tremor, myoclonia tonic muscular spasmes) is related to the injection of nitrogen.

. The EEG modifications are characterized by accentuated theta activities in the anterior region of the skull. This increase (maximum 300%) inferior to those enregistered in the Corasin series; it is similar to that noted in the dive using the same compression curve but without nitrogen. Thus, with the compression curve used, the presence or absence of nitrogen had no influence on the intensity of the EEG modifications.

- Beyond 600 meters, new phenomena appear, beginning at 800 meters:

. Diminution of the amplitude of the whole of the EEG activities;

. Tendency to monorythmicity of EEG activities;

. Periods of "motor perturbations" beyond 1000 meters.

These signs which should be masked by the occurence of epileptic seizures provoked by too rapid compression, remain to be interpreted.

III. General comments

The results obtained in man and the baboon in our experimental series are slightly different from those reported in man (Bennett et al., 1974) as well as in various animals(Brauer et al., 1971, 1974 b; Lever et al.,1971; Miller, 1972; Bennett et al., 1975; Halsey et al., 1975; Belaud et al., 1977).

From the EEG point of view, in our experimental conditions, in man, it is the $He-O_2$ mixture which seems to give better results than the $He-N_2-O_2$ mixture; the latter accentuates the EEG modifications in certain sensitive subjects (Rostain et al., 1980). For Bennett et al.(1974) the $He-N_2-O_2$ mixture would be better than $He-O_2$ mixture. It should be noted the experimental conditions of these authors are different than ours: the depth is the same (300m), but the compression is more rapid (33 min) and the percentage of nitrogen slightly higher (10%).

Moreover, the stay at the bottom is short; it has been reported elsewhere (Rostain et al., 1977 a, 1978) and Coraz experiments have shown that the HPNS and especially the EEG modifications develop with a marked latency and do not appear immediately after a rapid compression (Rostain et al., in preparation).

From a clinical point of view, the He-N_2-O_2 mixture seems much better than the He-O_2 mixture. It lessens the tremor and the dysmetry, and thus concords with the observations made by other authors (Brauer et al., 1971, 1974 b; Lever et al., 1971; Miller et al., 1973; Bennett et al., 1974). It is however, responsible for new behavioral symptoms (euphoria for example) and it increases drowsiness.

With the He-N_2-O_2 mixture, a dissociation appears between the tremor and the EEG modifications, dissociation already reported during experiments in He-O_2 atmosphere (Rostain and Naquet, 1974, 1978). This dissociation between the evolution of certain clinical signs and the EEG modifications can be found in the Papio papio. It could reveal the existence of different mechanisms at the origin of these two symptoms. Thus, tremor would be sensitive to narcotic agents such as nitrogen and would be lessened. This corresponds to observations on the narcotic agent-pressure antagonism (Miller et al., 1973; Bennett et al., 1975; Halsey et al., 1975; Miller, 1975; Belaud et al., 1977). The EEG modifications would not be improved and would be reinforced by the presence of nitrogen in the mixture. This could correspond to a synergic pressure-narcotic agent effect and certain authors have observed this effect on diverse biological or physiological functions (Hsia and Boggs, 1975; Roth, 1975; Kendig and Cohen, 1975; Parmentier et. al, 1979).

The epileptic seizure found with the He-N_2-O_2 mixture is different than that found in the He-O_2 mixture.

The fact that the seizure begins in the fronto-rolandic region with the He-O_2 mixture whereas it appears preferentially in the occipital region with the He-N_2-O_2 mixture also raises yet to be explained problems. Differences of the same order have been observed in the Papio papio either in hyperbaric oxygen (Gosset, 1966; Gosset et al.,1969) or by using drugs causing the decrease of the cerebral GABA level (Meldrum et al., 1970; Meldrum and Horton, 1974; Menini et al., 1977).

An another fact is important; it is in relation with the progressive injection of nitrogen during the compression which makes it possible to exploit antagonistic effects of nitrogen and pressure reported by several authors (Miller et al., 1973; Bennett et al., 1975; Belaud et al., 1977)and to control the synergic effects of nitrogen and pressure on EEG modification demonstrated in man (Naquet et al., 1975; Rostain et al., 1976, 1980) and in monkey Papio papio (Rostain et al., 1977,1978).

The experiments which we have carried in man and in the Papio papio with the He-N_2-O_2 mixture point out the complex role that nitrogen plays in the development of the HPNS; it lessens certain clinical symptoms (tremor) but it can reinforce the EEG modifications (slow waves).

Consequently, the modes of action of pressure and of the nitrogen in the $He-N_2-O_2$ mixture, are much more complex than a simple antagonism; this is probably found at the level of the conformation of the active sites of the proteic or lipoproteic molecules; the effects different, depending on the neuronic circuits and the chemical mediators which come into play.

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AN EXPERIMENTAL PROCEDURE FOR QUANTIFICATION OF INDIVIDUAL HYPERBARIC FACTORS AFFECTING THE PHYSICAL PERFORMANCE OF THE RAT

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INTRODUCTION

The hyperbaric environment is a complex set of physical variables, which includes hydrostatic pressure, gas density, ambient pressure, partial pressure of inspired oxygen, carbon dioxide, inert gases, and possibly interactions between these factors. Conflicting results have been reported on oxygen uptake in humans at rest and during exercise in hyperbaric environments due in part to the inconsistency of these variables at a given pressure level (1, 2, 3). It is equally true of the inconsistency that is found in the symptoms of high pressure nervous syndrome at a given pressure level (4, 5). In order to understand these conflicting results, it is necessary to study the quantitative effect of environmental variables involved. This study was designed to evaluate the separate effects of hydrostatic pressure, gas density, and partial pressure of inspired oxygen on a physiological response. Oxygen uptake of rats at rest and during exercise was used as an example to illustrate the procedure for quantifying the effect of individual hyperbaric factors.

METHODS

Environmental conditions: A total of 10 experimental environments were provided by varying ambient pressure from 1 to 10 ATA, gas density from 0.36 to 10.33 relative to air at 1 ATA, PO_2 from 150 to 1,500 torr, and partial pressure of nitrogen or helium from 0 to 7,400 torr (Table 1).

Gas mixtures used were air, 7% and 2% oxygen in nitrogen, 20%, 7% and 2% oxygen in helium. 7% and 2% oxygen mixtures were used to provide normoxic ambients at 3 and 10 ATA, respectively, whereas 20% oxygen mixtures were used to provide hyperoxic conditions at elevated ambient pressures.

Exercise and oxygen uptake: The details of the hyperbaric swimming chamber with a pneumatic elevator and determination of oxygen uptake for the rat at rest and during exercise have been described elsewhere (6). Briefly, the rat was exposed to hyperbaric conditions in a swimming chamber made of a plexiglas cylinder 58 cm in height and 15 cm in diameter.

The air volume was reduced to approximately 2.5 liters by adding water. A platform, attached to the top of a pneumatic jack, could be raised or lowered by controlling pressure within a telescopic column. The entire chamber was immersed in a water bath regulated at 37°C. The chamber was ventilated with compressed gas mixtures at a rate of 3 liters per min. at 1 ATA. The gas entered the hyperbaric system at the bottom of the cylinder and was bubbled through the water to ensure complete mixing of the gas inside the chamber and complete equilibration of the gas in the water. The exhaust was dehumidified before entering the flowmeter, after which it was analyzed for oxygen with a Beckman OM-11 polarographic oxygen analyzer. All gas analyses were carried out under 1 ATA conditions. Oxygen uptake was obtained by multiplying the flow through the chamber by the difference in oxygen concentration between supply and exhaust gases.

Experimental protocol: An equilibration period of 1 hour was allowed whenever gas mixtures and/or pressures were altered and before resting oxygen uptake was taken. Following the measurement of resting oxygen uptake, the platform was lowered into the water until the animal swam freely. Oxygen uptake for a 30 min. swim period was determined at 3 min. intervals. At the end of this period, the animal was raised above the water and oxygen uptake was measured for 15 min. during recovery, also at 3 min. intervals. Decompression was then carried out in stages over periods ranging from 30 min. to one hour. The peak oxygen uptake was used for analysis of the effect of individual factors.

RESULTS

Resting and peak exercise oxygen uptakes are summarized in Table 2.

Oxygen uptake at rest in 3 and 10 ATA normoxic nitrogen environments was depressed by 24% and 17%, respectively; whereas in hyperoxic nitrogen it was 17% and 31% respectively. The corresponding changes in the helium environments were much less than those in nitrogen environments and a significant depression of oxygen uptake 20% was observed in the 10 ATA normoxic heliox condition.

Peak oxygen uptake during exercise in 3 and 10 ATA hyperoxic nitrogen (compressed air) was depressed 33% and 63% respectively compared to 1 ATA air. Under normoxic nitrogen conditions this decrease was 24% and 49%,respectively at 3 and 10 ATA. The depressive effect on exercise oxygen uptake of hyperbaric helium was 17% and 30% at normoxic 3 and 10 ATA and 7% and 45% at hyperoxic 3 and 10 ATA.

Total effect of compressed air and compressed helium containing 20% oxygen on oxygen uptake is complex, because elevation of inspired P_{O_2}, P_{N_2} or P_{He}, and gas density are all expected concurrent to the elevation of ambient pressure. Utilizing the data presented above, the quantification of the individual effects of hydrostatic pressure, gas density, and hyperoxia on the ability to take up oxygen was made by the following procedures:

Effect of hyperoxia was determined by comparing oxygen

uptakes for varied P_{O_2} at a given ambient pressure, inert gas, and gas density (Fig. 1).

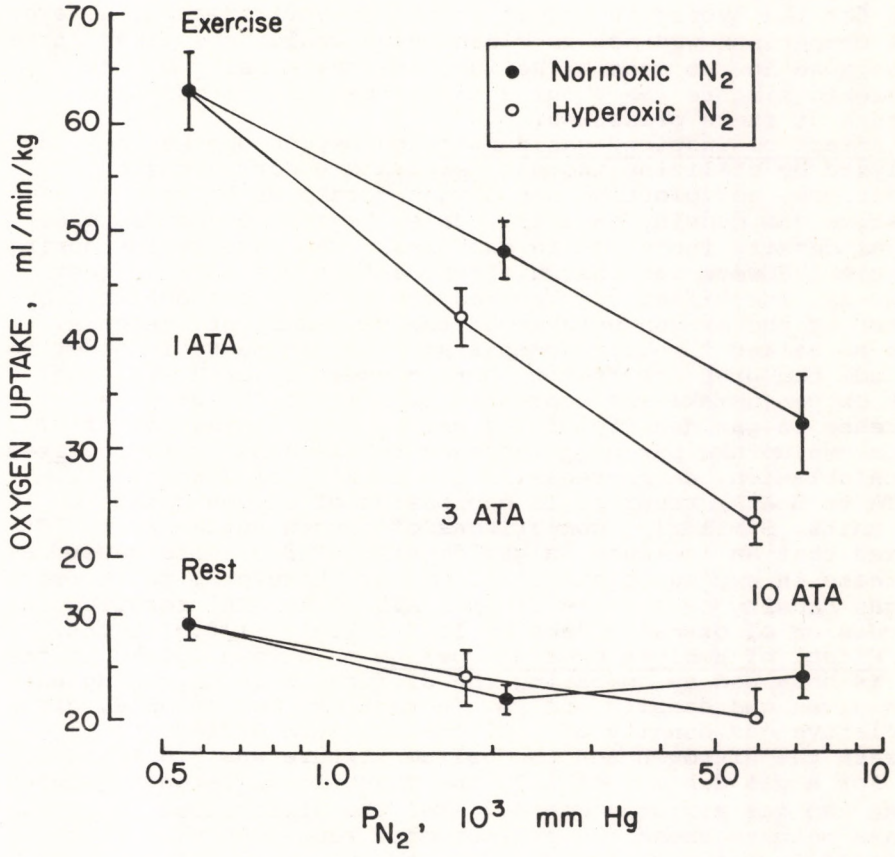

Fig. 1. Oxygen uptake at rest and during peak exercise in normoxic and hyperoxic nitrogen environments. Vertical bars indicate $\pm$ 1. S.E. of mean (6 rats).

In the nitrogen environment, an increase in P_{O_2} from 150 to 450 mm Hg depressed oxygen uptake by 6 units in ml/min/kg of body weight; and an increased in P_{O_2} from 150 to 1500 mm Hg depressed oxygen uptake by 9 ml/min/kg. At rest the effect of hyperoxia was small and was statistically not significant. A similar comparison can be made for the hyperoxic effect in helium environments. However, this comparison was not as clean as we would have liked it to be because in the case of helium, the gas density of the hyperoxic mixture was about 2 times that of the normoxic mixture at these pressures.

Effect of inspired gas density on oxygen uptake was analyzed by utilizing the data obtained under normoxic conditions, and plotting the oxygen uptake as a function of relative gas density on a log scale. Oxygen uptake decreased as gas density increased in each inert gas environment during exercise. However,an insignificant effect was seen at rest (Fig. 2). The effect of elevated gas density was obtained by comparing the oxygen uptakes at the same ambient pressure. This so called "isobaric comparison" is necessary in order to exclude the pressure factor. During exercise at 3 ATA, the peak oxygen uptake was depressed by 2 ml/min/kg with an increase in gas density of 2.4 units, thus depression of 2/2.4 in oxygen uptake per unit increase in gas density is realized. By calculation, an increase in gas density of 2 units (from 1 ATA to 3 ATA) resulted in depression of oxygen uptake by 1.7 units. Similarly, comparisons of oxygen uptake at 10 ATA showed that an increase in gas density of 8.3 units caused a decrease in oxygen uptake of 10 units. Therefore, an increase of gas density of 9 units (from 1 ATA to 10 ATA) resulted in depression of oxygen uptake by 11 (=10(10 - 1)/8.3) units.

Effect of ambient pressure per se on oxygen uptake in the rat is obtained by comparing the difference in oxygen uptake at a given gas density and P_{O_2} (normoxic). For example, given a relative gas density of 1.0, the pressure difference between the nitrogen and the helium mixture was 3.8 ATA (Fig. 2). For a gas density of 1.7, the pressure difference between these two gas mixtures was 8.3 ATA. The differences in oxygen uptake between these two environments represent the effect of hydrostatic pressure per se. With the pressure difference ranging from 3.8 to 8.3 ATA, the depression of oxygen uptake was found to be between 14 to 16 units at peak exersice, and between 6 to 7 units at rest (Fig. 2).

Summation of the effects of elevated ambient pressure, gas density, and P_{O_2} is sufficient to explain the total observed effect on resting oxygen uptake in various hyperbaric nitrogen environments, as well as exercise uptake at 3 ATA in air. As measured experimentally, a statistically insignificant underestimation of the total effect was seen in 10 ATA air (Fig.3). Summation of the effects of these three factors can not be made for hyperoxic heliox conditions due to difficulties in estimation of P_{O_2} effect (see above).

DISCUSSION

"Swim or sink" was the only important drive for the rats to exercise in this experiment.

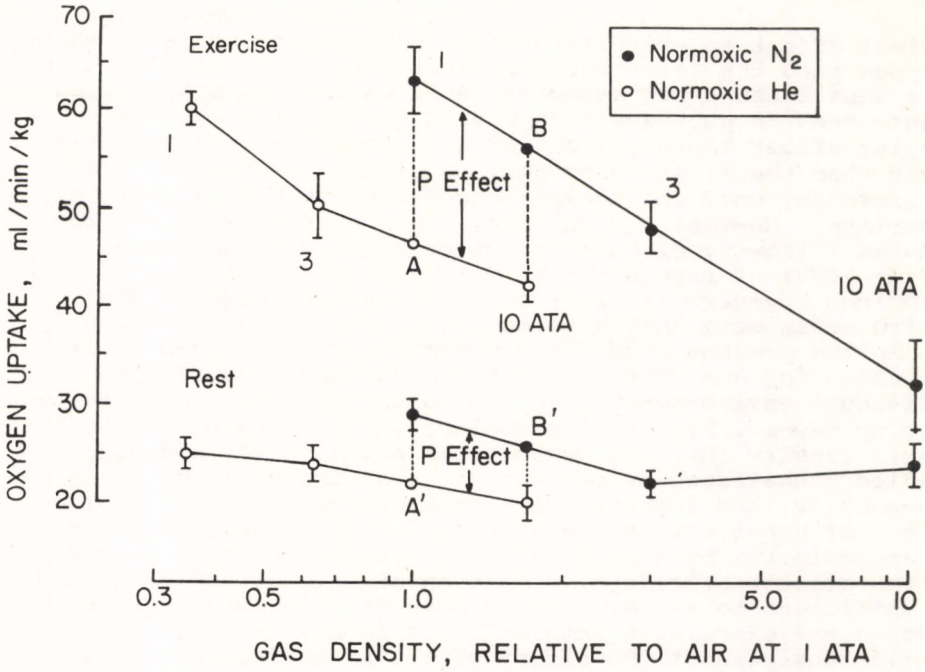

Fig. 2. Oxygen uptake at rest and during peak exercise as a function of gas density. Effect of gas density was determined by comparing oxygen uptake under normoxic and isobaric conditions; and the effect of hydrostatic pressure was determined by comparing oxygen uptake under normoxic and iso-density conditions. Means of 6 rats $\pm$ 1 S.E. are indicated.

The rats invariably exert maximal effort to exercise and to keep afloat initially when dropped into the water for the first time, then settled down to a less intense but steadier level as the swimming period progressed. In subsequent exposures their efficiency in keeping afloat improves, and oxygen uptake was consequently lower than the first exposures. To elicit the maximum effort to exercise, only the unexperienced rats were used in this experiment. Maximal oxygen uptake was observed within a few minutes following exposure to water which declined to a steady level of oxygen uptake within 12 to 15 minutes of swimming. However, all rats exercise in hyperoxic 10 ATA environments were unable to complete the 30 min. exercise (6).

In the present study, the maximal rate of oxygen uptake was taken for analysis of relative contributions of the individual environmental factors to the change in ability to take up oxygen. Stepwise removal of the effects of hyperoxia (P_{O_2}), density (D), and hydrostatic pressure (P) and hence enabled comparisons of oxygen uptake on the basis of normoxia, iso-density, and isobaric at each pressure level. The total effect of hyperbaric air environments on the oxygen uptake can be approximated by summation of the effects of P_{O_2}, D, and P by the present procedure. Summation of P_{O_2}, D, and P effects is sufficient to explain the effects of hyperbaric air on resting and exercising rats at 3 and 10 ATA (Fig. 3). A similar analysis of the effect of helium environments was hampered by the inability to obtain closely matched gas densities of hyperoxic and normoxic heliox mixtures at elevated pressures. At 3 and 10 ATA, the hyperoxic heliox mixture weighs approximately 2 times as much as a normoxic heliox mixture (Table 1). Although qualitatively similar effects of P, P_{O_2}, and D were observed further analysis was not attempted.

The present procedure for separating the effects of P, D, and P_{O_2} by no means a perfect one, and the reasons are:

1) The use of different inert gases in the density study: Gas density can be altered by change in ambient pressure or gas mixture, i.e. nitrogen vs. helium. Since an isobaric comparison is desired, we therefore have no choice but to employ different inert gas mixtures for this purpose. It is possible that the presence of inert gases with substantially different narcotic potencies may introduce an artifact. It is fairly certain that there are no obvious sign of nitrogen narcosis in rats at rest up to a P_{N_2} of 8 ATA (7, 8). However, it is possible that the threshold pressure for nitrogen narcosis is lowered during exercise. In any event, the comparison of the hyperoxic effect was made on the basis of approximately the same P_{N_2} in this study (Fig. 1).

2) Inability to assess interactions between environmental factors: Interactions between several known factors have been studied. The potentiation of hypercapnia on oxygen poisoning (9) and on nitrogen narcosis (10), and of hyperoxia on inert gas narcosis (11), are well documented. Taking into consideration the metabolic rates, increased gas mass in the chamber, elevated ambient pressure, and a constant rate of chamber ventilation, an increase in alveolar P_{CO_2} is predictable.

Thus, it is possible that potentiation of nitrogen narcosis and/or oxygen toxicity may explain why the summation of the partitioned effects of P, D, and P_{O_2}, slightly underestimates the measured effects on oxygen uptake of rats exercising in 10 ATA air. No such underestimation was found in the resting state (Fig. 3).

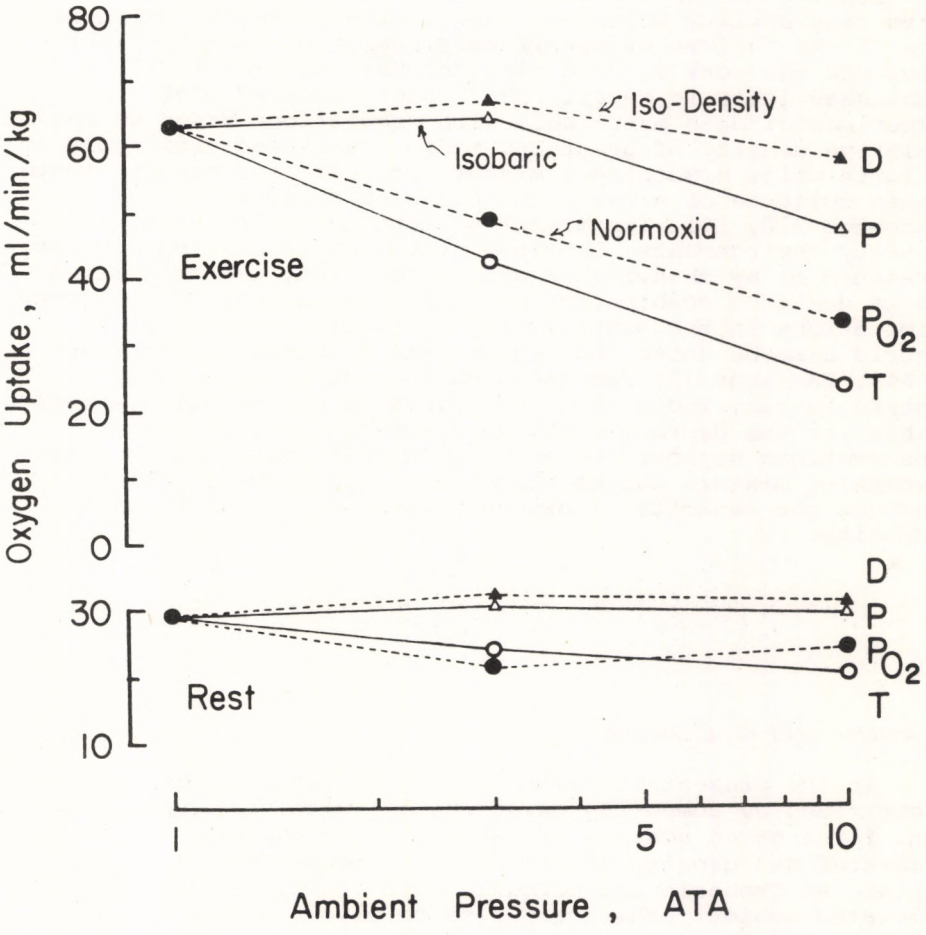

Fig. 3. Comparison of the measured total effects of air under hyperbaric conditions (T) with the estimated individual effects of hyperoxia (P_{O_2}), density (D), and hydrostatic pressure (P). Lines indicated by normoxic, isobaric and iso-density represent stepwise removal of the effects of hyperoxia, hydrostatic pressure, and gas density, respectively.

A surprisingly large influence of hydrostatic pressure on oxygen uptake was found by using the present procedure. The mechanism(s) by which oxygen uptake is suppressed under pressure is not known. Previous observations of suppressed Na-K-Mg ATPase activity (12), reduced $P_{O_2,50}$ (13), lowered relative diffusibility of water vapor (14), hence reduced heat dissipation during exercise, and interactions of some known factors (9, 10, 11) as mentioned above may in some ways relate to pressure affecting the ability of the rats to take up oxygen.

Density of the breathing gases is the main factor affecting maximum voluntary ventilation at depth, which is one of the indices of respiratory capacity. Lanphier (15) reviewed the work of Wood (16) and Maio and Farhi (17, published later in detail, 1967) and concluded that experimental data match well with predictions based on the relative density of breathing media. Increased expiratory efforts while breathing a mixture of increased density could cause collapse of airways and blockage of alveolar gas exchange (18, 19). Impairment of ventilation in high gas density environments, obtained either by increasing ambient pressure or by changing in gas composition, was determined to be due to a combination of airway resistance and inherent limitations in the ventilatory apparatus of man (17). It should also be noted that substituting helium for nitrogen at 3 ATA, Fagraeus (2) demonstrated increased maximum oxygen uptake in man, indicating that elevated gas density is responsible for the depressed oxygen uptake at depth. These observations support the conclusion that increased density of breathing mixture causes elevated respiratory efforts but reduces the capacity of oxygen uptake and hence the work capacity.

SUMMARY AND CONCLUSION

In the present study, the effect of elevated P_{O_2} was determined by comparing oxygen uptake with varying inspired P_{O_2} for a given ambient pressure and gas density; effect of elevated gas density was obtained by comparing the oxygen uptake at isobaric and normoxic conditions; and the effect of elevated ambient pressure per se on oxygen uptake can be closely approximated by the summation of the individual effects of P_{O_2}, density, and hydrostatic pressure according to the procedure established above. The important contribution of this study is the procedure employed by which the individual hyperbaric factors affecting oxygen uptake can be quantified. Although this quantification procedure is established for oxygen uptake, it should also be adoptable for quantification of other physiological variables of importance under hyperbaric conditions.

TABLE 1. SOME PHYSICAL PROPERTIES OF GAS MIXTURES

Pressure (ATA)	Gas[1] Composition	P_{O_2}[2] (mmHg)	P_{N_2}[3] (mmHg)	P_{He}[4] (mmHg)	Density g/L	Density Relative
1	Air	150	563	0	1.108	1.000
	80% He 20% O_2	142	0	570	0.398	0.359
3	Air	469	1764	0	3.393	3.062
	93% N_2 7% O_2	156	2077	0	3.328	3.004
	93% He 7% O_2	156	0	2077	0.713	0.644
	80% He 20% O_2	447	0	1786	1.139	1.028
10	Air	1586	5967	0	11.445	10.329
	98% N_2 2% O_2	151	7402	0	11.147	10.060
	98% He 2% O_2	151	0	7402	1.904	1.718
	80% He 20% O_2	1511	0	6042	3.884	3.505

(1) Approximate composition of oxygen and inert gases is indicated. The gas mixture was obtained from a commercial source.

(2) P_{O_2} listed above are approximated values. The P_{O_2} value of the supply gas was determined for each experiment by using a Beckman OM-11 oxygen analyzer.

(3) P_{N_2} and P_{He} were calculated as the difference between total pressure and P_{O_2}.

TABLE 2. Resting and peak oxygen uptake during exercise in 10 environmental conditions.

Gas compositions	Rest			Exercise		
	1 ATA	3 ATA	10 ATA	1 ATA	3 ATA	10 ATA
Air	29 ± 1.2	24 ± 2.6(a)	20 ± 2.1(b)	63 ± 3.4	42 ± 2.6(c)	23 ± 2.1(c)
20% O_2 in He	25 ± 1.4	23 ± 1.3	24 ± 1.1	60 ± 1.7	56 ± 1.7	33 ± 2.7(c)
7% O_2 in N_2		22 ± 1.2(b)			48 ± 2.4(b)	
7% O_2 in He		24 ± 1.7			50 ± 3.1(b)	
2% O_2 in N_2			24 ± 1.9(a)			32 ± 4.4(c)
2% O_2 in He			20 ± 1.8(a)			42 ± 1.3(c)

Values are X ± 1 S.E. for 6 rats in each experimental condition. The body weight of the rats was 344 ± 2.4 (60 rats) g. and ranged from 305 to 390 g. Oxygen uptake is measured in ml/min/kg (STPD).

(a), (b), and (c) indicates $P < 0.05$, $P < 0.01$, and $P < 0.001$, when compared to their respective inert gas mixtures at 1 ATA, by grouped t-tests.

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CONCLUDING REMARKS ON THE MAMMALIAN NERVOUS SYSTEM AT PRESSURE

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In an introductory presentation, Professor Lambertsen placed the conference in perspective by pointing out that, as applied in the field to problems of health and safety of men engaged in deep diving operations, the phenomena upon which the symposium is focused occur in a framework of multiple stresses. These are likely to complicate and modify the significance of many of the reactions observed by neurophysiologists whose controlled experiments tend to be designed to isolate the effects of a single stress factor such as high pressure.

One outstanding result of presentations at this meeting was the recognition that the so called high pressure neurologic syndrome (HPNS) is not a single, but a compound entity, resulting from the intertwining of several concurrent processes which lead up to different endpoints -- the signs commonly taken as characterizing the syndrome. Since these different components respond differently to variations in compression rate and in the manner of pharmacological treatment, and since their relationships appear to vary independently in different species and among different individuals, wide variations in the clinical appearance of the syndrome become understandable. This theme was further developed in a number of presentations that illustrate specific applications of this concept.

Data were presented to make a case for the view that HPNS-like phenomena are manifestations of nerve network characteristics, and hence not predictable in terms of events in isolated neurons. It was of interest to note that HPNS convulsions and other manifestations are readily elicited in species of animals devoid of a functional neocortex though relations between HPNS manifestations in various parts of the neuraxis of mammals remain only very partially clarified.

While it has long been known that at least some of the HPNS manifestations appear to be transient, and that for others the time course of increase in pressure significantly affects both the severity of the manifestations observed and the pressures at which they occur, data on acclimatization or on adaptation to pressure remain scarce. It was, therefore, welcome to hear of the achievements by Dr. Seki and his colleagues who have succeeded in maintaining fully instrumented laboratory animals at high pressure for long periods of time. Observations in invertebrates once again point up the complexity of the HPNS: while adaptation to high pressure markedly protects the animals against certain manifestations of the invertebrate high pressure syndrome, others remain virtually unaffected.

The same theme of complexity recurred in presentations dealing with the important advances that have been made in recent years in the application of nitrogen as a component of high pressure atmospheres giving some measure of protection against certain HPNS manifestations. Several discussants pointed to the problems imposed by the high density of nitrogen containing diving atmospheres which may lead to carbon dioxide retention, and which, at the pressures now coming in reach, are beginning to impose some limitation on attainable respiratory exchange rates. It was pointed out that this may provide an example of the compounding of hazards: under conditions where convulsions might be elicited at high pressure the enhanced respiratory exchange required to compensate for the muscular effort entailed may be vitiated by the high density of the atmosphere with potentially lethal results. Nitrogen is not uniformly effective in modifying the effects of high pressure environments on CNS function. Thus, for instance, while there is depression of cortical activity, minimal effects seem to have been observed on modification of evoked potentials. Finally, there is the important work of Rostain and his associates pointing out that the time course followed by nitrogen partial pressures during a compression has a very significant effect upon the extent to which this gas additive may ameliorate signs and symptoms of HPNS, and the degree of inert gas narcosis manifested.

Overall, the data presented here provide both, warnings against dangers which had hitherto been less clear, and indications of directions which future work in this field might profitably take. From the point of view of hazard evaluation, Professor Naquet's observations of ominous flattening of the EEG at very high pressures are of special interest. In addition, recognition of the complexity of the syndrome, and of the complexity of the manner in which it responds to pharmacologic intervention, brought into focus the potential danger that procedures designed to ameliorate HPNS signs and symptoms might merely serve to mask warning symptoms while more dangerous developments might proceed unabated and give rise to the unexpected emergence of catastrophic events. Several participants considered the possibility of selecting relatively HPNS resistant personnel and called attention to the difficulties in the way of such selection imposed by the now recognized independent development of different components of the HPNS.

With regard to guide lines for future work, the deliberations showed a gap between investigations at the cellular and at the whole animal levels. In view of the now recognized complexity of HPNS-like events, investigations would seem to be needed at all organizational levels in the hope that within the next several years this gap may be closed. Progress is being made in exploring the effects of high pressure upon release of neurotransmitters and their interaction with receptors. This work has just barely begun, but much more effort in this direction would appear well worthwhile in the future. Some of the contributions raised the possibility that at least some of the components of the HPNS might reflect secondary effects upon internal milieu of excitable cells, or indeed of the CNS as a whole, rather than direct effects at the membrane level, and further attention should be given to this possibility. Data were presented which hold out promise that drugs may be developed which will prove more selective against specific HPNS components than the general anesthetics hitherto most widely employed, and future development of this line of inquiry will be awaited with great interest. Finally, it seems to us that development of noninvasive methods of recording the biochemical and circulatory status of subjects exposed to high pressure environments might contribute greatly both to understanding of the events and to assurance of the safety of the people involved.

PHYSIOLOGY OF STATIC EFFORT

Chairman:

A. R. LIND (USA)

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OPENING REMARKS ON SOME OF THE PHYSIOLOGICAL RESPONSES TO ISOMETRIC CONTRACTIONS AND THE MECHANISMS THAT CONTROL THEM.

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In the last three decades, there has been a rapid accumulation of information on the physiological responses to isometric exercise and the factors that control them. That information enquires into the causes of the rapid fatigue induced by isometric exercise, the recruitment and rate coding of motor units, and the cardiovascular and respiratory consequences of this kind of exercise. For the most part, these laboratory investigations involve "true" isometric contractions where the muscle shortens only by as much as the tendon lengthens and there is no movement around joints. Most commonly the muscles have been required to maintain a steady tension at some known fraction of the maximum voluntary contraction (MVC), but in some experiments the procedure has been to exert a continuous maximum effort where the tension begins with the MVC and rapidly falls because of fatigue. An increasing variety of muscle groups have been examined but the bulk of the existing evidence comes from hand-grip contractions of the forearm muscles. In animal experiments, the common procedure has been to stimulate the appropriate ventral roots synchronously but an interesting recent development has been to stimulate several ventral root bundles in rotation, varying the pattern of recruitment and rate coding, thereby allowing control of the tension generated. We will hear, this afternoon of experiments in which this procedure is used.

Isometric contractions can be held for a very long time at tensions of less than about 15% MVC, and at those tensions they have been considered essentially indefatiguable. But at a tension of 20% MVC fatigue occurs, usually in about 10 minutes. As the tension is increased, fatigue occurs more quickly so that at 50% MVC the endurance time is of the order of one minute and at 70% MVC it is only about 35 seconds. The relationship between tension and endurance is curvilinear and was first described by Monod and Scherrer (1957) and Rohmert (1960). Obvious questions that arise are why do isometric contractions fatigue at all at such low tensions, and so quickly at high tensions? The answer is generally thought to lie in the view put forward first by Gaskell in 1880 that while active muscles release dilator substance(s) to increase the local blood flow, that increase is opposed by a mechanical compression of the vessels by the contracting skeletal muscle. In sustained isometric exercise the mechanical compression is unremitting, resulting in a constant throttling effect on the local blood flow. The matter then depends on the balance between the supply of oxygen and foodstuffs (or the removal of the unwanted

metabolites) and the metabolic demand of the active muscles. Fatigue would then be regarded as a biochemical event, as was proposed by Merton (1954) who produced evidence to exclude failure of neuromuscular transmission as the course of fatigue. The neuromuscular junction has been considered traditionally as the weak link in the chain of continued muscular function although central nervous failure had also been considered as a contributor; however much of that reasoning had come from animal experiments where the frequency of stimulation was high enough to cause no great surprise in the conclusion that transmission failure was the culprit in fatigue. More recently, Stephens and Taylor (1972) concluded that at high tensions, the fault lie in the neuromuscular junction but at lower tensions, failure of the contractile elements shared responsibility. Lind and Petrofsky (1979) found that hard to justify and suggested that the method of electromyography used in such experiments is imperfect if traditional interpretations are applied. However, the probability is that fatigue has different causes depending on the tension exerted and the proportions of different types of motor unit in the active muscles.

It is not possible to deal here with all aspects of the physiological responses to isometric exercise, and the remainder of my contribution addresses itself to the description of cardiovascular responses to isometric exercise and to the mechanisms that may control those responses.

The blood flow through the calf muscles is completely occluded at a tension of 20% MVC (Barcroft and Millen, 1939), and in the quadriceps at about the same comparative tension (Edwards et al, 1972). Barcroft thought that this was most likely to be caused by the "nipping" of a large arterial vessel. But in the forearm, the blood flow is not occluded until the tension reaches about 60% (Humphreys and Lind, 1963; Lind and Williams, 1979). At lower tensions the blood flow can reach quite high values driven in part by a large increase in mean arterial blood pressure (see below). The widely different tensions needed to occlude local blood flows in different muscles presumably reflect variations in anatomical arrangements in the limbs concerned. While there is no evidence to show where the occlusion occurs, the law of Laplace makes it most likely to occur in relatively large vessels. The amount of blood flow through the muscles at any given tension is influenced by the local vasodilatation and the increased perfusion pressure, both of which increase the blood flow, and the compressive force which oppose that increase. In addition, there is evidence to show that in at least some circumstances, there is a sympathetic neural vasoconstriction, induced as a somatomotor reflex (Lind and Williams, 1979). Generally, in an active muscle, that neural vasoconstriction is inhibited by substances released from the active muscle (Shepherd and Vanhoutte, 1979) but in some conditions it appears that the neural control, presumably of larger arterioles upstream, escape the influence of that inhibition (Lind and Williams, 1979).

Given these facts we were left with the following interpretation: 1) the increase in the heart rate was solely responsible for the increase in cardiac output 2) because the peripheral resistance did not change, the increase in cardiac output was at least partly responsible for the increase in mean blood pressure 3) because the blood flow in inactive limbs and the hepatic and renal vascular beds, even in the face of a rapidly increasing perfusion pressure, there must be a widespread sympathetic vasoconstriction, adjusting in intensity to keep the blood flow constant in the inactive tissues, and aiding the blood pressure response.

The dramatic pressor responses demanded, and received, much attention. The next question is, of course, what mechanisms are involved? Three main possibilities had to be considered. First, the cardiovascular

response could be due to a reflex, in which case it had to be evoked from the active muscles or from the active limbs. Second, it could be due to circulating hormones: this was considered less likely because of the rapidity of onset of the response with the contraction and its reduction after the contraction was released. Third, it could be due to "central nervous drive", a difficult factor to investigate.

The first possibility to be examined was that the responses were attributable to a reflex. Performing the contractions in the presence of circulatory arrest to the active muscles showed that the blood pressure and heart rate increased as when the circulation was intact, but more rapidly presumably because fatigue occurred faster in the absence of blood flow. The next step was to do the same experiment but to maintain the circulatory occlusion after the contraction was released. The occlusion was begun 3 minutes before the contraction and was maintained throughout and for 3 minutes after the contraction ended. Occlusion did not affect resting levels of heart rate or blood pressure. During the contraction, which was not held to fatigue, the blood pressure and heart rate increased in the familiar manner. After release of the contraction the blood pressure fell by some 10 mm Hg, and remained steady at that high level until the circulation was restored, when it returned rapidly to its resting value. In contrast, the heart rate returned to its resting level as soon as the contraction was released. So far as the blood pressure is concerned, this seemed clear deductive evidence that the pressor response was not caused by the release into the systemic circulation of a pressor agent from the active muscles, or of the significant effect of circulating hormones from the adrenal glands. Instead it pointed directly to the probability of a reflex originating in the active muscle. The reflex was not related to the tension generated by the muscle. Therefore it is most likely to be a chemically driven reflex. Examination of the venous effluent from the active muscles showed that only one of the factors we measured behaved in the same temporal sequence as the blood pressure, and that was potassium which increased to as much as 7 mequiv.L.min^{-1}. Subsequent measurement by Hnik (1976) of potassium concentrations in the interstitial space of contracting animal muscles show that the value can reach and can probably exceed 12 m . equivalents . liter^{-1}. However, in Hnik's experiments, occlusion of the circulation after 20 sec contractions led to the potassium returning from the interstitial space to the muscle in about one minute, thereby casting real doubt on the possibility that potassium, at least by itself, is the agent responsible for driving the afferent limb of the reflex.

We had the opportunity to study a patient with syringomyelia (Lind et al, 1968). He had complete loss of pain and temperature sensation in one arm while those sensations were almost unaffected in the other arm. The sympathetic nervous system seemed unimpaired in either arm. A sustained hand-grip contraction of his affected arm evoked the normal pattern of response of blood pressure and heart rate. In contrast, the pressor response was absent when the affected arm contracted, but interestingly, at least a portion of the normal heart rate response developed.

These findings reinforced our view that the pressor response was reflex, driven by a chemical stimulus in the muscle and we postulated that the afferent arm may be through some of the many small fibers that Hnik had shown to exist in the interstitial spaces of muscle and to which no function has been allocated. As a rider, we did not think that all the pressor response was necessarily due to a reflex and that the fall of blood pressure, by 10-20 mm Hg, seen on release of a contraction of an occluded arm could well be due to "central nervous drive" or to an increase in

thoracic pressure by chest fixation and breathholding. But the bulk of the response seemed to be due to a reflex.

Shortly thereafter, first Coote and his colleagues (1971) and then McCloskey and Mitchell (1972) showed in animal experiments that a major portion of the pressor response is indeed reflex. After eliciting a pressor response from the intact muscles, the dorsal roots were cut, following which the response was abolished. McCloskey and Mitchell selectively blocked large and small afferent nerves in the dorsal roots and were able to show that the traffic relevant to the pressor response was carried in the small nerves, types III and IV.

We are thereby quite secure that the bulk of the pressor response is reflex and that we can identify the afferent nerves that are responsible. The efferent limb of the arc must be sympathetic. We still do not know what agent or agents are responsible for generating the afferent traffic.

It seems clear that the heart rate is driven by a different mechanism. The results from the occlusion experiments (Lind et al, 1966) and from the patient with syringomyelia (Lind et al, 1968) make that clear. Also, we have been able to show that while the absolute level of blood pressure reached at the point of isometric fatigue is the same irrespective of the tension exerted, the heart rate reaches higher absolute values as the tension increases (Funderburk et al, 1974). The inference is that there may be a peripheral component involved in the increase of the heart rate. If that is so, because it is not affected by occlusion, the heart rate response may depend on some mechanical receptor. This explanation does not satisfy all the circumstances because the heart rate rises throughout the contraction whereas the stimulation of, for example, tendon organs must be presumed to be relatively constant during sustained contractions when the tension exerted is constant.

It is always difficult to prove or disprove the suggestion that "central nervous drive" may play a part in specific physiological responses to a stimulus. It is even more difficult to quantitate it. It seems from our results from the patient with syringomyelia that the absence of a pressor response when his "affected" arm contracted strongly suggested that "central nervous drive" plays no part in the pressor response. But it is always difficult to be certain that results from a patient with disease can be certain to provide normal physiological responses. Other ingenious experiments on man have been performed to investigate the problem. Freyschuss (1970) recorded pressor responses from amputees who made a contraction from an existing limb and were then invited to attempt to contract the missing limb to the same extent. The criticism of this finding is that no steps were taken to ensure that some real muscles did not contract when the amputee envisioned contraction of his absent limb. The matter is not a light criticism because contraction of a very small muscle mass (e.g. adduction of the little finger) induce the pressor response (Lind et al, 1966; McCloskey and Streatfeild, 1975). Furthermore the duration of the contractions, real or imaginary, were very brief and elicited a pressor response which was not dramatic in dimension. More recently, Goodwin and others (1972) have shown that the cardiovascular reponses to isometric exercise can be enhanced or attenuated to some degree by reducing or raising the level of "central command" by vibrating the tendons of the agonist or antagonist muscles of the arm. The alterations in heart rate and blood pressure responses were not large but do indicate the possibility that a central component exists for both.

Obviously, much more remains to be done to determine the mechanisms that control the circulatory responses to isometric exercise. If I had to

provide a brief report of the general state of our present understanding it would be: promising, after a slow start.

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PERIPHERAL NEURAL CONTROL OF CARDIOVASCULAR AND RESPIRATORY RESPONSES TO ISOMETRIC EXERCISE

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It seems to be generally accepted that strong isometric contractions even of small muscle groups (e.g. the hand grip) evoke cardiovascular responses (CR) to muscle work. This has been shown by Lind and his collaborators (Lind et al. 1964, 1966) during volitional isometric contractions in man and by Coote et al. (1971) in response to muscle stimulation in cats. The RR has mostly been studied under conditions of phasic (dynamic) rather than static (isometric) conditions (see e.g. Dejours 1964). Since there is some controversy concerning CR and RR, the two sets of responses are first discussed separately.

1. Cardiovascular responses to muscle activity

   a) The onset of the circulatory pressor response to isometric muscle contractions has been described by several authors as abrupt and has a latency of 1-2 s according to Krogh and Linhard (1913), Asmussen and Nielsen (1951), or even less (Petro, Hollander and Bouman 1970, Smith 1974, Borst et al. 1971).

   b) This short latency of the response is mostly considered to mean that it is neurogenic in origin and that it is triggered by muscle afferents. Furthermore, occlusion of muscle circulation during muscle activity enhances the pressor response, indicating that metabolites released into the circulation are not responsible for this reaction (Alam and Smirk 1938, Humphreys and Lind 1963, Coote et al. 1971).

   c) It has repeatedly been shown that stimulation of the central stump of a muscle nerve does not affect cardiovascular functions until small afferents of group II and III (and possibly of group IV) are electrically stimulated (Laporte, Leitner and Pages 1962, Skoglund 1960 and others).

   d) The main difference between the effect of static and dynamic exercise upon cardiac functions as defined by Lind and McNicol (1967) is that isometric contractions greatly increase blood pressure, while the heart rate

and cardiac output are raised moderately. Rhythmical contractions, on the other hand, tend to enhance cardiac output, blood pressure exhibiting relatively small changes.

2. Respiratory responses to muscle activity

a) One of the first to point out the short latency of the ventilatory response to muscle activity were again Krogh and Linhard (1913). The response to abrupt onset of work appears within 1-2 s, similarly as the cardiovascular response. It was already pointed out by these authors that this early onset of the response may well be associated with irradiation of impulses from the brain into the respiratory centres and with conditioned reflex mechanisms.

b) The short latency of the respiratory response is considered as evidence that the stimulus triggering these responses to work is neurogenic (e.g. Kao 1963, Asmussen and Nielsen 1964, Dejours 1964 and others) and arises in the working muscles. Dejours (1964) differentiates neurogenic control of the fast component and humoral (possibly combined with neural) control of the slow (late) component of the response.

c) There exists considerable controversy concerning the group of afferents mediating the stimulus which triggers the RR during exercise. The arguments speaking in favour of the hypothesis that it is muscle proprioceptors which are involved has brought forth the following evidence : i. stimulation of low threshold muscle afferents is enough to induce respiratory changes in the cat (Bessou,Dejours, Laporte 1959), i.e. group I and II muscle afferents were probably involved. ii. Succinylcholine, considered to stimulate muscle spindles specifically evoked a ventilatory response (Gautier, Lacaisse and Dejours 1969). iii. Passive muscle stretch was found by a number of authors to increase ventilation by a reflex mechanism (Harrison et al. 1932, Comroe and Schmidt 1943, Biscoe and Purves 1967) and this was also the conclusion reached by Asmussen, Johansen, Jørgensen and Nielsen (1963) in man. Passive muscle stretching of limb muscles was also reported to affect the discharge rate of individual phrenic motoneurones (Pleschka et al 1970). These results, however, have not been universally accepted.

Kao (1963) reported, for example, that the reflex respiratory response in dogs could be elicited by muscle contraction and that muscles contain "ergoreceptors" which are responsible for exercise hyperpnoea. Furthermore, Hodgson and Matthews (1968) and McCloskey et al. (1972), employing selective stimulation of Ia spindle terminals by vibration were able to show that respiratory (and cardiovascular) responses could not be evoked by this stimulation. And lastly, Kidd and Kučera (1969) demonstrated that succinylcholine does not

only selectively stimulate spindle afferents, but also non-proprioceptive (NPN) free nerve endings in the muscle.

d) It is apparent from this brief literary survey that while most of the results mentioned so far concerning the cardiovascular responses to muscle activity are in reasonable agreement, the respiratory neurogenic mechanism is still debatable. It is, however, necessary to bear in mind that the majority of experiments concerning the CR was performed under isometric conditions, those concerning RR were mostly contractions performed under dynamic conditions.

3. Increased sensory outflow from contracting muscles.

a) Direct evidence was presented in 1971 by Coote et al. that the CR and RR are caused by muscle contraction evoked by ventral root stimulation. The administration of gallamine, which abolished the contractions, or dorsal root section, eliminated both the responses to stimulation of ventral roots.

b) We were able to show that even a 5-s isometric tetanus leads to increased sensory outflow from contracting muscles both in response to peripheral nerve stimulation or ventral root stimulation in the rat (Hník et al.1969).

c) This was subsequently confirmed by Hutton et al. (1973) in the cat who have designated the phenomenon postcontraction sensory discharge (PCSD). However, the controversy as to the actual type of muscle afferents activated still remains unresolved.

4. Type of muscle afferents involved in PCSD

a) Hutton et al. (1973) argue that PCSD is mainly caused by discharges in spindle afferents since, in their experiments, about 60% of primary afferents exhibited an accelerated rate lasting for several minutes, even after a short tetanus. Furthermore, sudden stretching of the muscle during PCSD abolished this phenomenon.

b) In our experiments we also noted the effect which muscle stretch has on PCSD, but only about 10% of axons from spindles exhibited increased rates of firing after ventral root stimulation. That most of the activity underlying PCSD is due to fusimotor activation of intrafusal muscle fibres seems to have been well established by Perez-Gonzales and Coote (1972). These authors reported that invariably no increase in firing frequency could be obtained in spindle afferents until ventral roots were stimulated at intensities suprathreshold for gamma fibres and is not enhanced by circulatory occlusion. That PCSD is due to persisting contraction of intrafusal muscle fibres thus seems to be obvious. This phenomenon probably has a common (or similar) mechanism to "postexcitatory facilitation" described by Kuffler,

Hunt and Quilliam (1951) and perhaps to Kidd's "persistent excitation" (Kidd 1964). This does not mean, however, that other smaller muscle afferents do not participate in this phenomenon, even though their contribution to the overall sensory activity in dorsal root filaments, in view of their small calibre, would be proportionally smaller as compared with the largest myelinated fibres.

c) We have some more evidence, though indirect, supporting the contention that nonproprioceptive muscle afferents may be involved in PCSD. Firstly, we have shown that blockade of gamma fibres in the stimulated ventral root of the rat does indeed reduce the amplitude of PCSD, but does not eliminate it altogether (Hník, Kučera & Kidd 1970). And secondly, on the model of muscles without spindles, containing only small myelinated (and unmyelinated) afferents terminating as free nerve endings, muscle stimulation via the ventral root showed a transient increase in sensory outflow from contracting muscles (Hník et al. 1970).

It thus seems firmly established that muscle contractions, especially those evoked by electrical stimulation, cause a PCSD involving spindle activity. This might trigger respiratory responses, but from literary data spindle activity seems to be ineffective in mediating cardiovascular responses to work. It is the small myelinated afferents (and perhaps also group IV fibres) which should be stimulated during muscle activity. The evidence for their activation during muscle work is, so far, indirect.

5. Metabolic changes occurring in contracting muscles

It is hardly possible to enumerate all the metabolic changes occurring in the muscle during muscle contractions. Mention will only be made here of the possible role of K^+ in inducing repetitive activity in muscle afferents present in the muscle interstitial space. Loss of K^+ from working muscles has been repeatedly demonstrated by various authors (Fenn 1937, Kjellmer 1965, Lind, McNicol and Donald 1966).

a) Direct measurement of K^+

Using liquid ion-exchanger microelectrodes (Walker 1971) with a side-pore (Vyskočil and Kříž 1972), we were able to measure the time course of changes in extracellular K^+ (K^+_e) concentrations in the gastrocnemius muscle of the rabbit and cat (Hník et al. 1976). It was found that a 20-s isometric tetanus increased K^+_e from 5 mmol/l to 8-10 mmol/l. Furthermore, we employed these ion-selective microelectrodes (ISM) for measuring changes in venous effluent blood (K^+_{ven}) from working muscles. The K^+ lost from cat muscles did not depend upon the fact whether muscles were contracting under isometric or isotonic conditions.

Although there are other changes occurring in the interstitial space of working muscles which could, and probably do contribute, depolarize muscle afferents (especially free nerve endings), besides K^+. But even the K^+ changes probably occur sufficiently abruptly and are of such magnitude to induce repetitive activity in muscle afferents. It should also be stressed that the peak concentrations of K^+ as measured by ISMs are probably somewhat underestimated and a certain time lag of the response due to diffusion is to be expected.

b) Intra-arterial infusions of K^+

It has repeatedly been shown that intra-arterial infusions into the muscle vascular bed cause cardiovascular pressor reflexes (Chernigovsky 1960, Khayutin 1961, Achar 1968, Wildenthal et al. 1968, Chernilovskaya 1969, Liu et al. 1969). Similar results have been reported for respiratory responses (Liu et al. 1969). These results may also be considered as confirmatory evidence that K^+ is capable of stimulating muscle afferents and inducing CRs and RRs.

We obtained direct evidence about the effect of K^+ intra-arterial infusions upon the activity of myelinated muscle afferents belonging to different groups according to their conduction velocity. We found that it is possible to achieve repetitive activity in Ia, Ib and NPN afferents when K^+ is infused in such amounts which do not lead to higher concentrations in venous effluent blood than correspond to those present under physiological conditions during muscle activity (i.e. 8-10 mmol/l). Taking into consideration the "unphysiologically" large diffusion distances, the response from NPN afferents had a latency of about 15-30 s, while encapsulated receptors (spindle primeries and Golgi tendon organs) responded with a latency of 1.2-2.5 min after the onset of infusion. Repetitive activity in group III fibres induced by K^+ infusion is an exceptional example when NPN discharges last for such a long time (Hník et al. 1969).

c) Muscle chemoreceptors

The existence of muscle chemoreceptors has been suggested by a number of authors : Khayutin (1953) designated them as chemoreceptors, Kao (1963) as ergoreceptors, Ramsay (1964) as metaboreceptors, Coote et al. (1971) metabolic receptors, Perez-Gonzalez and Coote (1972) exercise receptors. Our suggestion was to call them non-specific chemoreceptors (Hník et al. 1969) since, they do not behave as central chemoreceptors, although they respond to some metabolic changes in the muscle. However, they are apparently not responsive to low P_{O_2} or high P_{CO_2}.

6. Open questions and conclusions

In spite of the considerable effort devoted to the problem of peripheral neurogenic mechanisms triggering CRs and RRs during muscle exercise, there are a number of questions to be clarified.

a) Are CRs and RRs triggered by the same peripheral neural mechanism ?

b) Why are static (isometric) contractions more effective than phasic (dynamic) contractions (at least as far as CRs are concerned), if both types of contraction induce analogous K^+_e changes in the muscle ?

c) What is the physiological role of spindle activation during and after muscle contractions ? Does this depend upon the gamma-loop and is it of significance for locomotion ? Spindle activation can hardly play a role in triggering CRs, and even their role in RR does not seem to be altogether convincing.

d) What is the basic difference between isometric (static) and isotonic (dynamic) type of contraction ? Do static contractions involve a change in sensitivity of NPN to K^+ ?

e) How do muscle afferents affect the sympathetic output ? Via muscle spindles (and passive muscle stretch, cf. Biscoe and Purves 1967) or via group III and IV (Coote and Perez-Gonzales 1970).

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MECHANICAL, ELECTRICAL AND BIOCHEMICAL CORRELATES OF ISOMETRIC FATIGUE IN CAT SKELETAL MUSCLE

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Introduction

The mechanism of muscle fatigue during isometric contractions has been the subject of intense interest in the last 50 years. However, most studies have examined isometric fatigue resulting from contractions sustained at a variety of tensions and, in animal experiments, often conducted under unphysiological experimental conditions. It is not surprising then, that their results often contradict each other. Therefore, to become more intimate with the neuromuscular system and to be able to control more precisely the experimental conditions, we have utilized a computer to stimulate cat skeletal muscle with similar patterns of recruitment and frequency of motor unit discharge as that which occurs during voluntary activity in man (Bigland and Lippold 1954; Milner-Brown and Stein 1975; Olsen et al. 1958; Petrosfsky 1978; Petrosfsky and Phillips 1979).

The purpose of the present investigation was to extend our previous studies by examining the motor unit action potentials, mechanical properties, and biochemical constituents of a fatigued fast twitch (medial gastrocnemius,MG) and slow twitch (soleus,S) muscle in the cat (Ariano, et al, 1973).

Methods

Female cats were used in these experiments. The animals were anesthetized with α-chloralose (75 mg/kg body weight ip) and maintained with intravenous booster doses as needed.

Surgical Preparation

Each cat was placed in the prone position with one back leg fixed rigidly to the table by two sets of stainless steel pins driven through the knee and lower leg bones. A section of calcaneus with its one remaining tendon attached to the test muscle was tied to a steel chain connected through a stainless steel isometric transducer bar and a position sensor (Linear Variable Displacement Transducer, LVDT) to a load platform. Muscle temperature was monitored with a thermistor and maintained at 38° ± 1.0°C.

A dorsal laminectomy exposed the L_6, L_7 and S_1 ventral roots which were divided surgically and pooled into three groups, each capable of causing the muscle to develop a similar tension following stimulation as described by Petrofsky (1978).

Stimulation

Stimulation was delivered sequentially through three bundles of ventral roots and an anodal block electrode as described by Petrofsky (1978). The anodal block voltage and stimulation frequency were set and maintained by a digital computer (Intel 8080A microprocessor). During fatiguing isometric contractions, the motor unit stimulation frequency was set initially at 10 Hz and the anodal block voltage was reduced until the target tension was achieved. As the muscle began to fatigue, more motor units were recruited to maintain the target tension. Once all the motor units were recruited, the computer increased the stimulation frequency to maintain the target. Once an increase in stimulation frequency was ineffective in maintaining the target, the contraction was terminated. The total length of time the contraction was maintained was called the endurance time.

Blood Gases and Blood Flow

Free flowing arterial and venous blood samples were drawn through indwelling catheters from cats previously administered heperin. Hemoglobin and percent oxygen saturation were determined with a co-oximeter (IL 182) while pH and PCO_2 were determined on an IL Micro 13 blood gas system. The blood oxygen content was determined with a fuel cell analyzer (Lexicon Instruments). Blood flow was measured from a venous catheter with an optical drop counter.

Adenosine Triphosphate, Creatine Phosphate, Lactate and Glycogen

Muscle samples were analyzed for adenosine triphosphate (ATP), creatine phosphate (CP), lactic acid (LA) and glycogen by microanalytical methods (Karlsson et al. 1975). To stop the metabolism at a predetermined point in the contractions, a muscle sample (30 mg) was taken with a pair of rongeurs chilled with liquid nitrogen.

Procedures

Five series of experiments were accomplished as described below:

Series 1. Blood flow, venous LA concentration and potassium concentration were measured in the S and MG before, at the beginning, and the end (first and last 30 sec), and 1, 3, 7 and 12 min (30 sec samples) following isometric contractions at tensions of 10, 25, 50, 75 and 100% of the initial strength of the muscles (tetantic tension of the unfatigued muscle determined at the beginning of each experiment). In previous experiments, we have found that the contractions at 10 and 25% of the initial strength for the S, and 10% of the initial strength for the MG were nonfatiguing (Petrofsky and Lind 1979). Therefore, while all other contractions were carried to fatigue, these nonfatiguing contractions were only sustained for 3 min.

Series 2. Oxygen uptake, carbon dioxide production and hydrogen ion concentrations were measured for these two types of muscle during isometric exercise. To accomplish this, 0.35 ml blood samples were taken from veins draining the muscle at rest, just prior to contraction, in the first and last half of the contraction (first and last min), and at 1, 3, 7 and 12 min following the contraction. Arterial blood gases did not change during isometric contractions due to the small muscle mass involved.

Series 3. In this series of experiments we determined the concentration of ATP, PC, glycogen and LA in the muscle at rest and at the end of a fatiguing isometric contraction at a tension of either 10, 25, 50, 75 or 100% of the initial strength. Only one contraction was performed on a given muscle. Just at the point of isometric fatigue, the muscle biopsy was taken across the middle of the S belly and across the portion of the

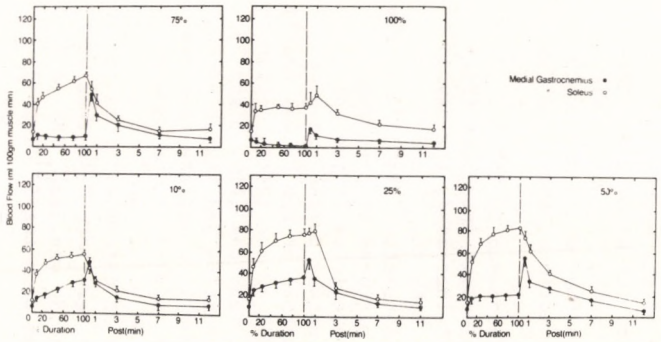

Figure 1

The blood flow in the soleus and medial gastrocnemius muscles at rest (0% duration) during fatiguing and nonfatiguing isometric contractions, and during a 12 min recovery period (post) following the exercise. Each point shows the mean of 4 cats ± the S.D. The blood flow during the contractions has been normalized in terms of the length of the contractions (% duration).

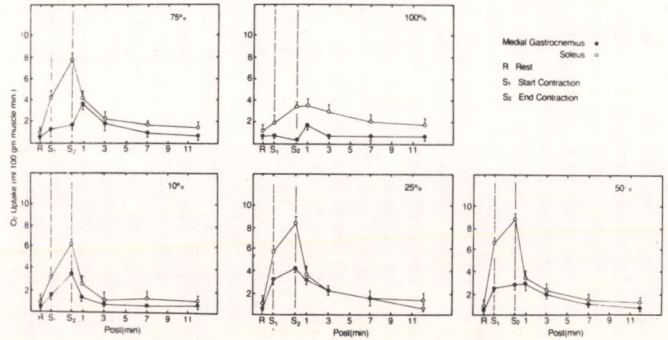

Figure 2

The oxygen uptake (arterio-venous difference in oxygen content x blood flow) of the soleus and medial gastrocnemius muscles of 4 cats ± the S.D. at rest (R), in the first (S_1) and last (S_2) 30 sec of the contraction, and following (post) isometric contractions are illustrated here.

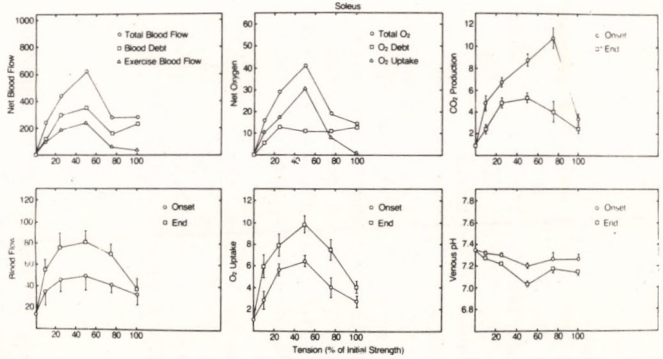

Figure 3

Oxygen uptake (cc/100 g muscle/min), CO_2 production (cc/100 g muscle/min), oxygen debt (cc/100 g muscle), blood debt (ml/100 g muscle) and venous pH, in the soleus muscles of 4 cats ±the S.D. in relation to the tension exerted by the muscles.

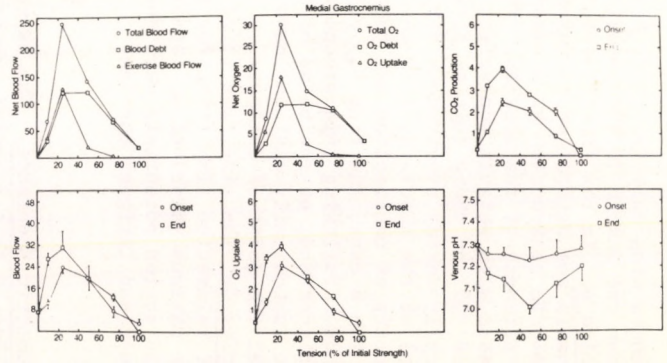

Figure 4

Oxygen uptake (cc/100 g muscle/min), CO_2 production (cc/100 g muscle/min), oxygen debt (cc/100 g muscle), blood debt (ml/100 g muscle) and venous pH in the medial gastrocnemius muscle of 4 cats ± the S.D. in relation to the tension exerted by the muscles.

muscle containing only fast twitch units in the MG. The resting sample was taken from the muscle in the opposite (non-contracting) leg.

Series 4. In the fourth series of experiments, the force-velocity relationship was determined in unfatigued muscle and at the end of a fatiguing isometric contraction at a tension of either 25, 50, 75 or 100% of the initial strength of the muscles. From the initial length, the muscles were afterloaded with weights equivalent to about 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100% of their initial strength and allowed to contract isotonically during synchronous direct square wave stimulation (0.1 ms duration) of the muscle at a frequency of 300 Hertz (tetanizing frequency). The velocity of contraction was then recorded at each load and from these data on the 4 cats, the true V_{mx} and actual velocities of shortening were calculated. Following this procedure, a fatiguing isometric contraction was initiated and, at the point of fatigue, the muscle was allowed to again contract isotonically against loads of up to 100% of the strength of the muscle at the point of fatigue.

Series 5. The rise time (time to 90% of the peak isometric tension), the half relaxation time (time for the tension to fall from the peak to half its maximum value) of an isometric twitch and the height and duration of muscle action potentials were measured for both types of muscle in the unfatigued and fatigued muscles.

Results

Isometric Endurance. The isometric endurance of the S and MG muscles was similar to that reported previously (Petrofsky and Lind 1979).

Blood Flow. For both MG and S, when the muscles contracted at the lower isometric tensions, the blood flow increased continuously throughout the duration of the contractions (Fig. 1). The highest blood flow recorded at the beginning and end of the contractions in the S, occurred after contractions at 50% of the initial strength. The highest blood flow found during contractions in the MG occurred at the end of the contractions at 25% of the initial strength; when contractions were sustained above this tension the blood flow was progressively reduced until, for contractions at 100% of the initial strength, the blood flow to the muscle was occluded by the contraction. Following the isometric contractions, the blood flow recovered to the resting values within 12 min.

Oxygen Uptake. As the tension exerted was increased to 25% for the MG and 50% of the initial strength for the S, the O_2 uptake increased (Fig. 2, 3, 4). Above these tensions, although the load increased, the oxygen uptake was reduced progressively with tension for both the MG and S. No blood flow or oxygen uptake occurred during the contractions at 100% of the initial strength of the MG. These findings were mirrored in the oxygen uptake by the muscles in the hyperemia following exericise (oxygen debt). Unlike the S where the O_2 debt was less than the uptake during exercise except at higher isometric tensions, the oxygen uptake of the MG was nearly equal to oxygen debt for contractions at 10 and 25% of the initial strength and increased markedly for contractions at higher tensions (Fig. 3 and 4).

pH, CO_2, LA. The largest carbon dioxide production and lowest venous PCO_2 was found for the S during contractions at the intermediate tensions (Fig. 5). This resulted in the pH being markedly reduced in the venous effluent (Fig. 6) in the S muscle. Although the pH was reduced to similar levels in the venous blood following contractions at intermediate tensions in the MG, the PCO_2 and carbon dioxide production were both less than was found in the S. However, although the CO_2 production was less, the LA in the venous blood was greater in the MG as compared with the S (Fig. 7),

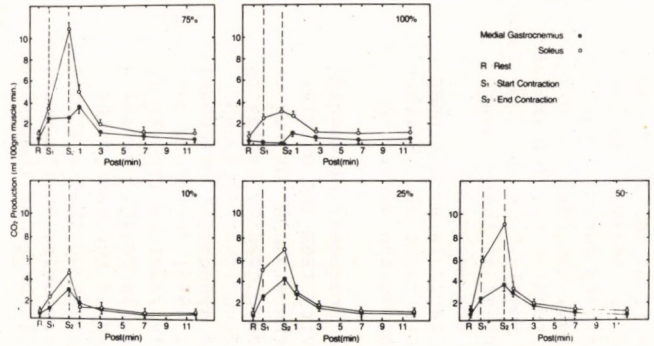

Figure 5

The production of carbon dioxide (arterio-venous difference in CO_2 content x blood flow) in 4 soleus and medial gastrocnemius muscles ± the S.D. with the muscles at rest (R), in the first (S_1) and last (S_2) 30 sec of the isometric contractions, and for 12 min following the contractions (post) at tensions between 10 and 200% of the initial strength. CO_2 efflux has been normalized in terms of the weight of the muscles.

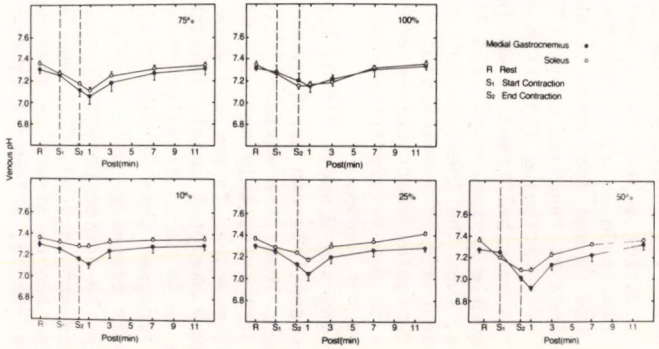

Figure 6

The venous pH of the medial gastrocnemius (●) and soleus (o) muscles of 4 cats ± the S.D. measured at rest (R) at the beginning (S_1) and end (S_2) of isometric contractions and in the 12 min recovery period (post).

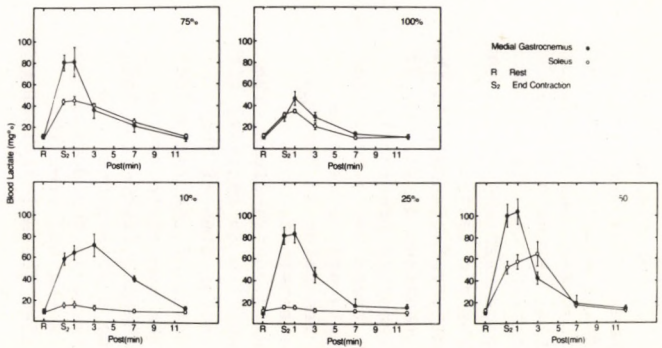

Figure 7

The lactic acid concentrations in the venous blood of the medial gastrocnemius (●) and soleus (o) muscles of 4 cats ± the S.D. measured at rest (R), at the end of a fatiguing contraction (S_2) and in the 12 min recovery period (post).

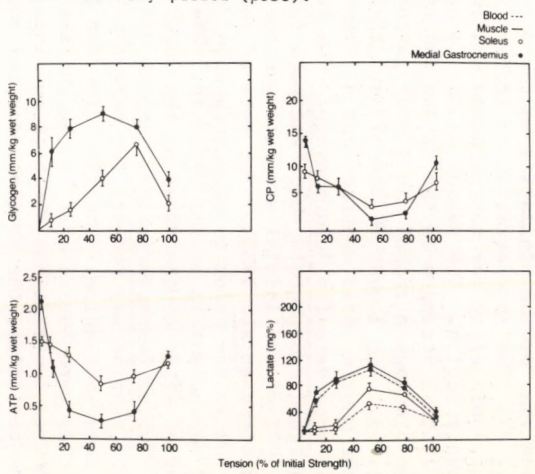

Figure 8

Muscle glycogen, ATP, CP and lactate concentrations in 6 soleus (o) and medial gastrocnemius (●) muscles ± the S.D. at rest (0) and at the end of fatiguing contractions.

accounting for the similarity in the H^+ ion efflux in the two muscles (Fig. 8). The concentration of lactate in the venous blood was largest about 1 min after the contractions and recovered fully after the 12 min recovery period. The venous pH was lower for the contractions at 50% of the initial strength in the MG and 75% of the initial strength in the S. These findings mirrored the tissue glycogen utilization, LA, ATP and PC (Fig. 9). The lowest PC and ATP and highest LA concentrations were found in the muscle after contractions at these same isometric tensions.

Force-Velocity Relationship. The force velocity relationship in the unfatigued, and muscle which had been fatigued at tensions of 25, 50, 75 and 100% of the initial strength was the same as reported previously (Petrofsky et al. 1980). For both of the muscles, there was a reduction in V_{mx} and in the velocity of contraction for the muscle against a given load as the muscle became fatigued. The greatest reduction in the V_{mx} occurred after contractions at the lower isometric tensions.

Twitch Characteristics. At the end of fatiguing contractions, the twitch tension was reduced for both the MG and S muscles in parallel with the tetanic tension. The rise time and half relaxation time of the twitches were both longer at the end than at the onset of the fatiguing isometric contractions for both muscles. Unlike the isometric twitch characteristics, the action potential amplitudes changed little for both the S and MG muscles throughout the duration of the contractions. In contrast, for both muscles there was a marked increase in the duration of the action potentials, increasing an average of 42% and 49% from the control values (fresh muscle) in the S and MG muscles for all fatiguing tensions examined.

Discussion

The small reduction in action potential amplitude and the increase in action potential duration shown here is similar to that reported previously by others during submaximal isometric contractions in normal and ischemic muscle (Lindström et al. 1970; Mortimer et al. 1970). Analysis of the surface EMG in man does seem to show electrical failure during isometric contractions at tensions above 70% MVC. In contrast, the EMG amplitude at the end of a fatiguing isometric contraction at a tension of, for example, 25% MVC is only half that of an MVC in unfatigued muscle. If the amplitude of the muscle action potentials is not reduced further at these lower tensions, then the inescapable conclusion is that the motor unit recruitment and/or frequency of firing has been altered in some manner during fatiguing contractions exerted at these lower tensions. Whether or not this is due to electrical failure at some point in the excitation system is not known.

Grimby and Hannerz (1976) have shown that the frequency of muscle action potentials can be altered by afferent activity from the muscle. This might lower the maximum firing frequency of the alpha motor neuron pool. In itself, this may not alter the tension developed by fatiguing skeletal muscle. In our own work in the cat, we found that although unfatigued skeletal muscle required frequencies of sequential stimulation as high as 40 Hz to tetanize the muscle (Petrofsky 1978; Petrofsky and Lind 1979), fatigued fast or slow twitch skeletal muscle requires frequencies of motor unit discharge only as high as 20 Hz to tetanize fully. Finally, although the action potential amplitude was reduced at the end of the fatiguing contraction, Fink and Luttgau (1976) have shown that a similar reduction has no effect on the contractile characteristics of the muscles. For this reason, a lower frequency of motor unit discharge at the end of

low tension isometric contractions may have little or no effect on the tension developed by fatigued muscle and electrical failure may not be part of the fatigue mechanism in man.

If fatigue in these muscles is not in the excitation, then it must reside in either excitation-contraction coupling or in the contractile mechanism of the muscles. Although the present investigation did not examine the excitation-contraction process, the contractile characteristics and various metabolic indices of muscle performance were evaluated.

Associated with fatigue, we found a sharp reduction in the V_{mx} of these muscles after isotonic contractions. V_{mx}, the maximum velocity of contraction of the unloaded muscle, is often related to the actin and myosin ATPase of the muscle fibers. V_{mx} is not related to the level of activation of the muscle, e.g. number of active muscle fibers. For example, in a homogenius muscle, the soleus, if we recruit 25, 50, 75% or all units, the force velocity curve shows V_{mx} to be uninfluenced by the number of active motor units (Edman 1979; Petrofsky and Phillips 1979). Dawson et al (1979) have shown that the ATPase activity, while being reduced during isometric contraction in isolated frog skeletal muscle, was not related to the concentration of CP, ATP or H^+ in the muscle. The results presented here agree with those of Dawson and his colleagues. Here, however, we have shown that there is an inverse relationship between V_{mx} after the contractions and the tension exerted by the muscles for both fast and slow twitch skeletal muscle. None of the metabolities examined here or the oxygen uptake of the muscles seemed to be correlated with the observed reduction in V_{mx}. Further, the concentration of CP and ATP in the muscle appeared to be different at fatigue for the various tensions examined. Since glycogen was not depleted at the end of contractions at any tension examined here and ATP was still present at fatigue (particularly in the slow twitch muscle S), it would appear that some as yet unidentified metabolite must be involved in the fatigue process.

Acknowledgements

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PHYSIOLOGICAL RESPONSES DURING SUSTAINED ISOMETRIC CONTRACTIONS

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It has been well documented that throughout voluntary sustained isometric contraction the bioelectrical muscle activity and the cardiovascular response are determined by the intensity of the contraction and its duration. A reliable relationship exists between EMG-activity, picked up by means of surface electrodes, and force in defined nonfatiguing contractions (Komi and Buskirk 1970; Kramer et al. 1972; Bräuer et al. 1975). The heart rate is also related to a certain extent only to the force developed during defined contractions. During defined fatiguing contractions the relationship between electrical activity and force and the mean heart rate changes in a typical way: during a sustained constant-force isometric contraction the integrated EMG and the heart rate increase, whereas at a constant level of iEMG the tension development decreases and the heart rate remains almost constant after an initial increase (Kramer et al. 1979) (Fig. 1).

The different reactions of heart rate observable in fatiguing contractions at constant submaximal level of force and at constant level of iEMG respectively, support the assumption that the heart rate seems to be parallel to change in strength of innervation rather than to change in force development. Hence there is no reason to assume that mechanoreceptors are implicated in the regulation of heart activity during static work. The behaviour of the heart rate during sustained isometric contractions results from a reflex response initiated by the local action of humoral agents on sensory endings, and from cortical irradiation of motor impulses which are effective not only at the beginning, but throughout the work. The rapid fall of exaggerate heart rate immediately after termination of the work argues for an absence of this central command.

The postexercise fall of heart rate occurs also, when the blood flow of the working limb is occluded by a cuff whereas recovery of increased blood pressure in this case is delayed or prevented (Rowell et al. 1976). These differences in heart rate and blood pressure responses to muscle ischemia has led several investigators to conclude that these two variables are controlled mainly or partly by seperate mechanisms.

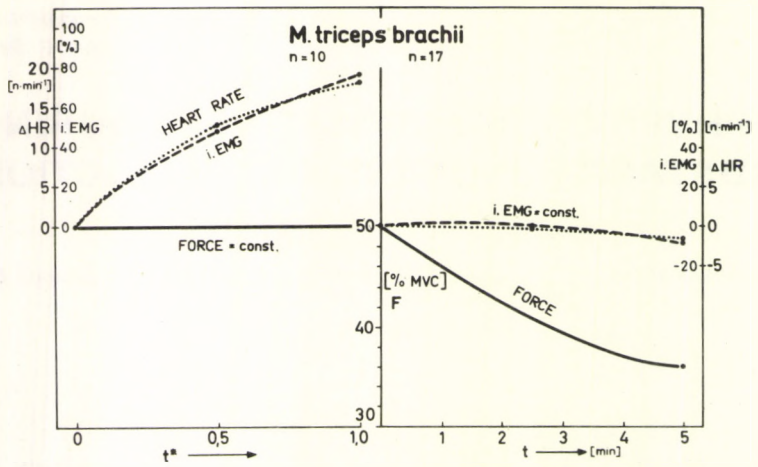

Fig. 1 The relationship between electrical activity and force and the mean heart rate during fatiguing isometric contractions in different conditions: either the force (left) or the myoelectrical activity (right) are constant.

In repeated isometric contractions at a constant level of force (for example 40 % MVC) sustained to fatigue a diminution of isometric endurance occurs and there is an increase in the level of iEMG as well as in the total power of the EMG signal in each of the consecutive contractions if the interspersed pauses last for a few minutes only. From this it can be concluded that recovery is not completed within the allowed periods of rest. Despite this the shift in the EMG power density spectra present in each of the consecutive fatiguing contractions is abandoned in the rest periods (see Fig. 2). Reversible decline in conduction velocity of muscle fibre action potentials has been assumed as the main reason for the increase of duration of action potentials, i.e. the shift of the EMG spectrum towards lower frequencies occuring in fatiguing contraction (Lindström et al. 1977). The transient decrease in propagation velocity may be elicited directly or indirectly by metabolic by-products accumulated in muscles during sustained contractions. It is conceivable that normalization of the propagation velocity follows the return of blood flow.

The assumption has been widely accepted that the increase in ratio of myoelectrical activity to force in sustained contractions is caused by a recruitment of additional motor units in order to compensate for the diminution of the force development of the fatigued fibres (Viitasalo and Komi 1978).

Although the specific origin of fatigue may be obscured by the complexity of the phenomenon it can be concluded that at least two mechanisms with different restitution times play their part in local muscular fatigue: an impaired excitation-

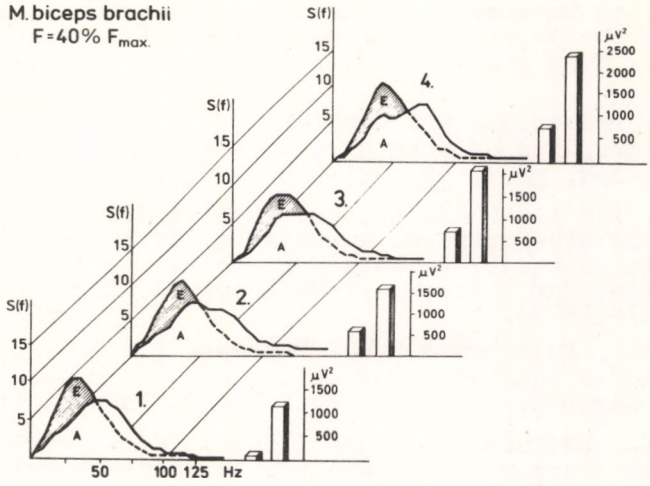

Fig. 2 The change in the power-density distribution of EMG spectra (left) and in the total power of the EMG signal (right) during isometric contractions sustained until the discomfort could no longer be tolerated. Averaged values of 4 repeated contractions; 7 subjects.
Continuous lines: power-density at the beginning; interrupted lines: power-density at the end; first balk: total power at the beginning; second balk: total power at the end.

contraction coupling and a decrease of conduction velocity of muscle action potentials. From a practical point of view it must be pointed out that the EMG spectrum distribution is less suitable for indicating the long lasting element of muscular fatigue, contrary to the iEMG to force ratio which offers some opportunities for investigating the time course in normalization of the exercise-induced depression of electromechanical coupling. Our results, obtained in the course of repeated short and moderate contractions after an exhaustive contraction are consistent with the view that the changed process of excitation-contraction coupling requires several hours for complete restitution (Edwards et al. 1977) (Fig. 3). Its practical importance for muscular performance indicates further studies on this subject.

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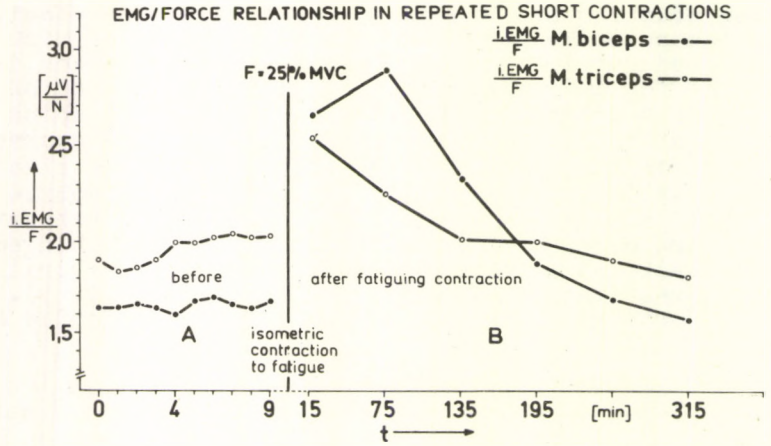

Fig. 3 The ratio between iEMG and force of Mm. biceps and triceps brachii in repeated short and moderate isometric contractions before and after an exhaustive contraction. Mean values in 9 subjects.

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THE INFLUENCE OF ENVIRONMENTAL TEMPERATURE ON HEAT PRODUCTION AND CARDIOVASCULAR SYSTEM IN GROWING PIGS

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It could be shown by theoretical considerations and experimental investigations in turkeys, chickens, some water bird species (Nichelmann et al, 1976 a, 1977 a - c), rabbits (Nichelmann a. Lyhs, 1976) and in piglets (Nichelmann et al., 1975, 1976 b) that

(a) the relationships between ambient temperature and the energy metabolism are to be described by a parabola; obviously there is no wide zone of thermal neutrality as assumed so far;

(b) the relationships between ambient temperature and rectal temperature, influences by acute temperature stress, can be demonstrated by a parabola or by a polynom of higher degree; the extrem value of the parabola is identical with the biological optimum temperature;

(c) the control elements of the temperature regulating system are activated in a typical order. First of all, evaporative heat loss begins to rise as ambient temperature increases, later thermal conductance increases as a measure of cutaneous blood flow. Finally, rectal temperature reaches a minimum and energy metabolism decreases to its lowest degree even in higher ambient temperatures.

Because control systems of the homoiothermic organism are meshed intensively, it is to expect that not only the control elements of the temperature regulating system are influenced continously by the environmental temperature but also the control elements and perhaps the controlled function of other control systems. This assumption has a high degree of probability, since Close (1978), Close and Mount (1978) and Close et al. (1978) have shown that the environmental temperature varies such growth parameters of the pig like the energy and fat retention, the growth rate, the energy content of the body weight increase and other (Fig. 1).

The present investigations shall accomplish two tasks. At first it shall be proved if the regularities mentioned also cover growing pigs and secondly it shall be investigated

which relationships exist between the control elemnets of the temperature regulating system and some factors of the cardiovascular system.

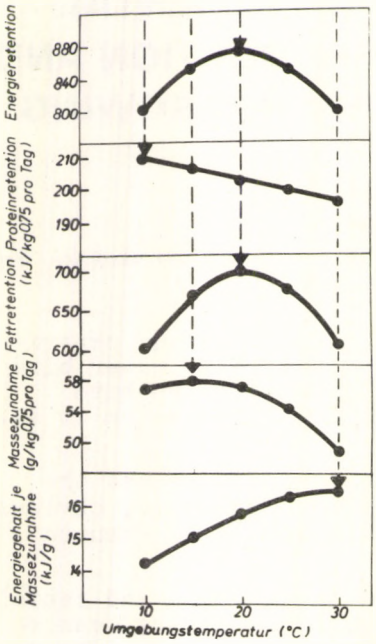

Figure 1
Relationships between the ambient temperature and the energy retention, the fat retention, the growth rate and the energy content of the body weight increase

Material and Methods

The investigations were carried out in castrated growing male hybrid pigs with a body weight of 18...24 kg. Animals were kept for 2 hours in a climatic chamber with a temperature of 5 to 40 °C. The air humidity was 75%, the wind speed was lower than 0.2 m/s.
After the end of the temperature influence heat production was estimated by determination of oxygen consumption and carbon dioxide production, evaporation by using a psychrometer and rectal temperature by the use of a fever thermometer. At the same time the arterial blood pressure and the heart frequency were measured via a cronical catheter implanted in the arta. The hemoglobin concentration and the packed cell volume were estimated in a venous blood sample.

Results and Discussion

The results are summarized in 5 points.

(1) The relationships between the environmental temperature and the rectal temperature are described by a parabola. The extrem value - situated at 18,8 °C - is identical with the biological optimum temperature. The rectal temperature increases above as well as below the extrem value of the function. The increase in decreasing ambient temperature is due to a set

point increase of temperature controller, caused by stimulation of cutaneous cold receptors and due to the following activation of the thermoregulatory heat production and heat conservation mechanisms.
The increase of the rectal temperature in high ambient temperature is the result of the low efficiency of the heat loss mechanisms in pigs (Fig. 2)

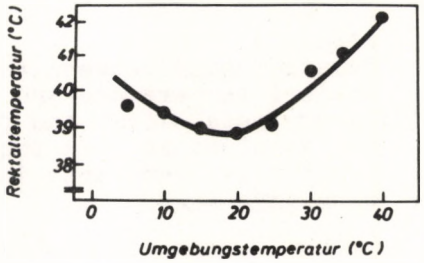

Figure 2
Influence of ambient temperature on the rectal temperature in growing pigs

(2) Similarly as in other species of mammals and in birds there is no thermical neutral zone in growing pigs; the relationships between the environmental temperature and the energy metabolism can be described by a parabola (Fig. 3)

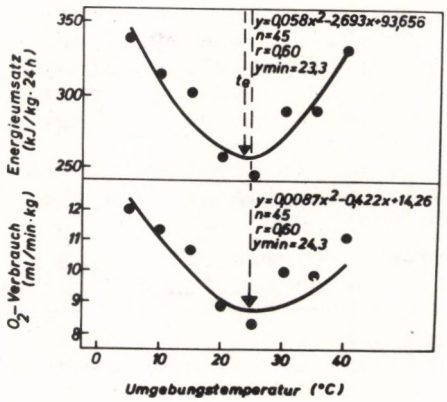

Figure 3
Influence of ambient temperature on the energy metabolism and the oxygen consumption in growing pigs

(3) The investigated control elements of the temperature regulating system are activated in the typical order found already in other species of mammals. The evaporative heat loss begins to increase above an ambient temperature of 14.0 °C, heat production, however, reaches its minimum at 23.4 °C. That is an environmental temperature in which the evaporation is

increased and in which the rectal temperature begins to increase. Therefore a temperature field exists in a medium range of environmental temperature in which heat loss as well as heat conservation mechanisms are activated simultaneously (Fig. 4).

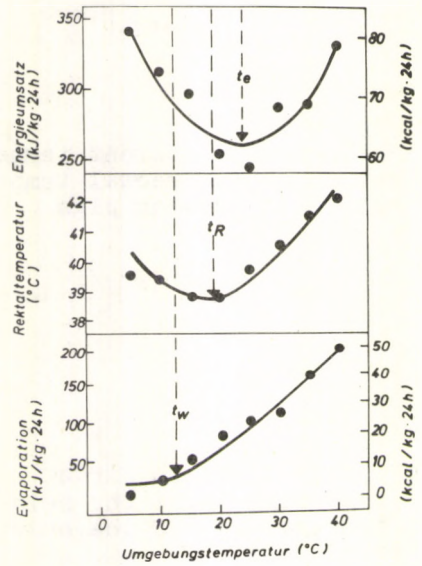

Figure 4
Relationships between the ambient temperature and the energy metabolism, the rectal temperature, and the evaporative heat loss in growing pigs

(4) Like energy metabolism relationships between ambient temperature and the heart frequency as well as the systolic and diastolic blood pressure can be described by a parabola-shaped function. The control elements of the cardiovascular system are stressed minimally in environmental temperatures near the thermic neutral temperature (Fig. 5).
It is very interesting that the optimum temperature of the blood pressure and the heart frequency is higher than the biological optimum temperature in every case (Fig. 6).

(5) The functions which described the interrelationships of the environmental temperature with the hemoglobin concentration and with the packed cell volume have the character of a polynom. The physiological interesting extrem value of the functions are situated near the biological optimum temperature (Fig. 6).

It can be summarized:

Firstly
The control elements of the temperature regulating system are activated in a typical order in growing pigs. This order can be deviated from Blighs neuron model of temperature regulation (Bligh, 1972).

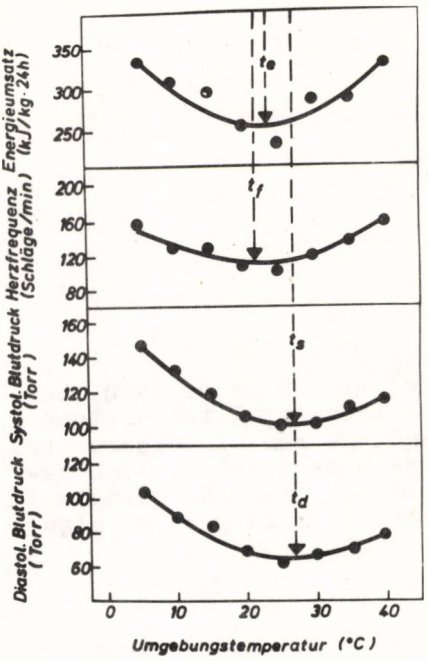

Figure 5
Relationships between the environmental temperature and the energy metabolism, the heart frequency and the systolic and diastolic blood pressure

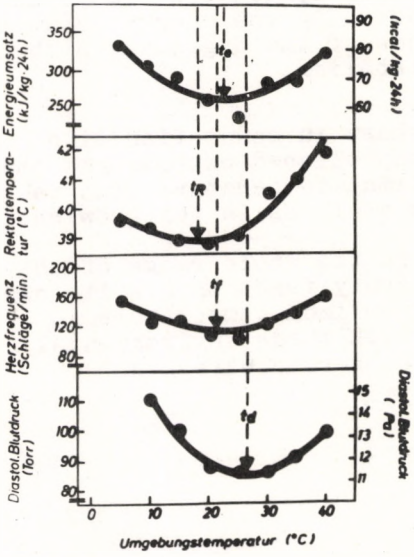

Figure 6
Relationships between the environmental temperature and the rectal temperature, the heart frequency, the energy metabolism, and the diastolic blood pressure in growing pigs

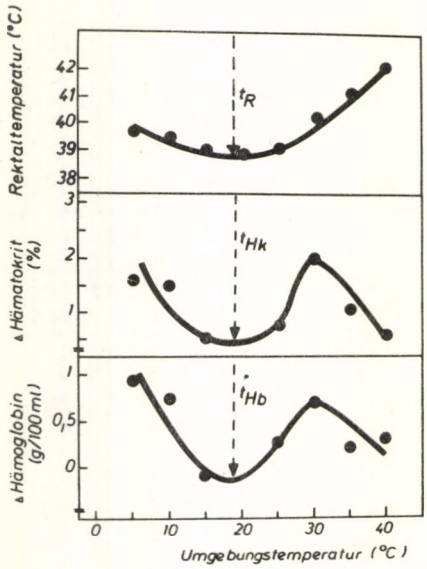

Figure 7
Relationships between the environmental temperature and the rectal temperature, the packed cell volume variation, and the hemoglobin variation in growing pigs

Secondly
The relationships between the environmental temperature and various functions of the cardiovascular system are described by parabolas. That indicates that not only the energy metabolism but also some functional systems of the organism which have service functions in the energy metabolism.

Thirdly
The results of the investigations shows in connection with various literature datas and some unpublished experiments that every body function has his own optimum temperature. The tabula shows some of such optimum temperatures in the growing pigs.
The organism is stressed thermally in the whole range of the environmental temperature, because every function has its own optimum temperature. The biological optimum temperature is an integrating value und identical with those environmental temperature in which the organism is thermic stressed minimally.

Table

Optimum temperatures of various body functions and growth parameters in the pig (datas of growth parameters from Close, 1978, Close and Mount, 1978, Close et al., 1978)

Body function	Optimum temperature (° C) (Extrem value)
Temperature regulating system	
Heat production	23.4
Evaporation	14.0
Rectal temperature	18.8
Cardiovascular system	
Blood pressure, systolic	27.2
Blood pressure, diastolic	27.1
Heart frequency	21.9
Hemoglobin concentration	18.7
Packed cell volume	19.0
Metabolic system	
Free fatty acid	-
Plasma glucose	20.6
Glucocorticosteroide	20.1
Growth parameters	
Energy retention	20.0
Protein retention	10.0
Fat retention	20.0
Growth rate	15.0
Energy content of weight gain	30.0

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CONCLUDING REMARKS ON THE PHYSIOLOGY OF STATIC EFFORT

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Following Dr.Hnik's presentation, the main thrust of the discussion was related to the role that K^+ may play as a generator of the reflex cardiovascular responses. Dr.Hudlicka particularly wanted to know what happened to the cardiovascular responses when the soleus was stimulated. Dr.Lind reported that he and Dr.Petrofsky had found that stimulation of the motor nerves of the soleus elicited no increase in arterial blood pressure. Dr.Coote considered that metabolites other than K^+ might be considered and Dr. Hnik agreed that the interaction of several metabolites might be very powerful.

Dr.Edwards was asked by Dr.Hultman how he explained the results of his hypothyroid patients. He replied that those patients have a much higher proportion of slow twitch motor units, whereas the hyperthyroid patients were much weaker than normal individuals because of loss of muscle mass.

Dr.Hnik asked Dr.Petrofsky whether it was possible to indulge in rotary stimulation of ventral rootes without such a complicated and sophisticated apparatus. Dr.Petrofsky replied that he did not presently see how it could be done but that to avoid gross tremor involved in synchronous and confused contractions, it was worth the extra trouble to control the muscular function.

The question of K^+ as a stimulating metabolite for either hyperemia or for blood pressure responses was raised and discussed by Dr. Shepherd, Hnik,Lind, Saltin and Edwards. The discussion was prolonged and full agreement was not attained. Dr.Edwards commented that the intramuscular pressure in his own experiments was substantially greater than was in the case in Dr.Saltin's experiments. Concerning these findings, their differences may perhaps be attributed to individual variation, species differences and differences in responses of different kinds of muscles.

SATELLITE SYMPOSIUM

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INTRODUCTION TO HORMONAL AND PHARMACOLOGICAL ASPECTS IN PRESENT-DAY EXERCISE PHYSIOLOGY

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Our days tend to direct attention both in research and in the clinical practice of sports medicine as well as sports physiology - in addition to the classic themes of circulation, respiration and muscular adaptation - toward the problems of hormonal and pharmacological relations in sports physiology. These two topics are not random associates. Beside the need to clarify the part/s/ played by hormonal regulation in sports activity, we are burdened with the application of hormones as drugs, with their screening procedures, with the efforts to protect the purity of sports by medical means. On the other hand, the legitimate ways of supporting the athletes in reaching the necessary conditions of top level performance have to be found and employed too.

All these points were considered in defining the main theme for this Satellite Symposium as well as in deciding about the subtopics to be included which were as follows.

1. The Hypothalamic-Pituitary-Adrenal System
2. Anabolic Preparates
3. Catecholamines /with special emphasis on the use of beta receptor blocking agents in sports/
4. Free Papers

It was hoped that the contributors would touch upon really important points in this field. The Symposium fulfilled the best hopes of the organizers who are glad that the proceedings of this interesting meeting can reach also those who were unable to attend it and all others who might be interested in this fastly expanding territory.

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EXERCISE, NUTRITION AND GROWTH

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It is generally accepted that inactivity leads to atrophy of musculoskeletal system [1], and that prolonged fast or undernutrition block somatic growth [2]. However, the converse interactions between physical activity,nutrition, and growth are not well understood and have been subject to the following series of investigations. The starting point of this research has been the observation [3] that voluntary exercise in ad-libitum fed adult hamsters accelerated ponderal growth (rate of weight gain per day). Our research since then has centered around the following four questions:

(1) Does exercise-induced increase in weight gain represent true somatic growth?

(2) How does nutrition affect exercise-induced growth?

(3) What characterizes growth-inducing exercise?

(4) What are the neuroendocrine implications of acceleration of growth by exercise? A summary of these research issues, methodologies involved,and results obtained, follows.

(1) DOES EXERCISE-INDUCED INCREASE IN WEIGHT GAIN REPRESENT TRUE SOMATIC GROWTH ?

The prevailing notion, based on human and general mammalian condition, is that skeletal growth stops within a finite period of time upon attainment of sexual maturity.A seldom acknowledged fact is that growth in well-nourished rodents proceeds throughout most of their lifetime [4,5]. It was, therefore, of importance to clarify the following points concerning the increased weight gain in exercising hamsters:

(a) Does this weight gain represent accumulation of excess fat rather than proportional somatic growth?

(b) Is increased weight gain associated with increased skeletal growth?

(c) Is there nuclear or cellular proliferation in the musculoskeletal system during exercise-induced weight gain?

(a) Exercise-induced weight gain is not a consequence of excess fat.

In two studies we measured body fat content with an indirect method: carcasses were dried to constant weight to determine body water content, which constitutes 73% of lean body mass in a variety of mammals [6]. In two other studies body fat was determined by direct method: by petroleum-ether extraction of lipids from a dehydrated sample of homogenized carcass.Body fat was determined during exercise, when animals expend energy and gain weight rapidly, during the first few days of retirement, when the rapid

weight gain continues in the absence of exercise, and several weeks after the termination of exercise, when the rate of weight gain has returned to the slow sedentary level. Table 1 summarizes the results of these studies.

TABLE 1
Body fatness of exercising hamsters expressed as percent of sedentary hamster body fatness.

Condition	Method	No.of animals	%	Reference
day 42 of exercise	direct	6	41	[7]
day 12 of retirement	direct	6	70	[7]
day 28 "	indirect	6	77	[4]
day 42 "	direct	6	83	[7]
day 45 "	direct	6	94	[9]
day 93 "	indirect	6	103	[8]

Table 1 shows that voluntary exercise in hamsters leads to a severe depletion of body fat after 6 weeks of exercise. During the retirement from exercise body lipids are replenished.At no time is body fat of exercising hamsters significantly higher than that of sedentary hamsters. Therefore, increases in ponderal growth observed during, and immediately following, voluntary exercise are not due to accumulation of body fat but due to proportional increase in the major body constituents.

(b) Exercise induces increased skeletal growth.

Direct assessments of skeletal growth were done from the whole-body radiographs [10] of exercised and sedentary hamsters at the start of the experiment,on day 34 of exercise, and on day 29 of retirement. Results are shown in Table 2.

TABLE 2
Bone lengths of exercising hamsters expressed as percent of sedentary bone lengths.

Condition	Bone	Percent
day 34 of exercise	skull	102
"	vert.column	106
"	tail	103
"	humerus	102
"	femur	102
day 29 of retirement	skull	102
	vert.column	107
	tail	104
	humerus	104
	femur	103

All of the changes presented in Table 2 were statistically significant. It follows,therefore, that voluntary exercise in ad-libitum fed hamsters stimulates bone elongation. This process extends into the period of retirement.

(c) Exercise induces increased cellular proliferation in bone and nuclear proliferation in muscle.

We looked for incidence of cellular proliferation in the distal femoral epiphyseal growth zone and of nuclear proliferation in quadriceps of exercising ad-libitum fed hamsters and of their sedentary controls. On the 30th day of exercise hamsters were injected intraperitoneally with 2 μCi/g of ^{3}H-thymidine (New England Nuclear,Boston,Mas,40-60 mCi/mM) three hours prior to tissue removal. Incorporation of label into the DNA of proliferating cell nuclei was determined from autoradiographs of 10 μ sections

of femur and from the radioactivity in the DNA fraction of muscle homogenates(dpm/g tissue or mg protein). There was a 55% increase in the proportion of labelled nuclei in the femoral epiphyseal growth plate, and a three-fold increase in the labelled DNA in the quadriceps of exercising relative to sedentary hamsters. Thus, exercise and adequate food intake promote cellular proliferation in the bone and nuclear proliferation in the muscle in the adult hamster [11].

(2) HOW DOES NUTRITION AFFECT EXERCISE-INDUCED GROWTH ?

Exercising hamsters given unlimited access to food increase their food intake [3] in proportion to their increasing body size [9]. If they are prevented from increasing their food intake during exercise, they lose weight and show no evidence of linear growth [12] or cellular proliferation in the bone [11] until such time when unlimited access to food is again allowed. When they are reintroduced to ad libitum food, such retired undernourished hamsters display intense ponderal and linear catch-up growth [12], indicating that exercise exerted its stimulatory effect on growth but that inadequate nutrition blocked it.

We investigated the mechanism by which nutrition modulates exercise induced growth by studying the effects of acute fast, of refeeding following an acute fast, of chronic undernutrition, and of ad libitum realimentation of undernourished animals,in exercising and sedentary hamsters on serum concentrations of the two anabolic hormones which are likely to play a central role in the control of growth and catch-up growth: growth hormone (GH) and insulin. Three experiments were performed. All blood collections were done at the same time of day. In the first experiment, serum concentrations of GH and insulin were determined after 0, 4, 8, 12, 16, 20, and 24 hr of fast in exercising and sedentary hamsters maintained up to that point on ad libitum food. In the second experiment, ad-libitum fed and food restricted exercising and sedentary hamsters were subjected to an acute fast of 24 or 12 hr, respectively, and then allowed to refeed for 1, 2, 4, 8, or 12 hr. In the third experiment, undernourished exercised and sedentary hamsters were retired and given 1, 3, 5, or 7 days of ad libitum realimentation before determining the effects of either ad libitum feeding, 12 hr of fast, or 4 hr of refeeding after 12 hr of fast, on their serum GH and insulin concentrations.

The results were as follows (13,14). Concentrations of both GH and insulin were increased in rapidly-growing well-nourished hamsters with uninterrupted access to food. The high concentrations of both these anabolic hormones in rapidly growing hamsters were suppressed by several hours of fast,16 hr for GH and 4 hr for insulin. High concentrations of GH were fully reinstated by 4 hr of refeeding in well-nourished as well as in undernourished exercising hamsters. High concentrations of insulin were partially restored by 1 to 2 hr of refeeding in well-nourished hamsters and were marginally increased by 1 hr of refeeding in undernourished exercising hamsters.These experiments indicate that intake and deprivation of food rapidly influence changes in serum GH and insulin concentrations in hamsters stimulated by exercise to grow. Unlike the GH response, the secretion of insulin in response to feeding is greatly attenuated in proportion to the severity of food deprivation.

We have also investigated the dependence of exercise-induced growth on the availability of dietary protein. In this study [9], we wanted to determine whether the increase in somatic growth of exercising hamsters results from incidental increases in protein consumption which accompany in-

creases in food consumption. To resolve this question, we gave sedentary and exercising hamsters identical quantity of dietary protein and supplemented the diet of exercising hamsters with carbohydrate to accomodate the energy cost of their physical activity and growth. In addition we have done this study at two levels of dietary protein, 18% and 39% which bracket the protein content (25%) of the standard laboratory diet. Results [9] are summarized in Table 3.

TABLE 3

Dependent variable	Diet	Exercising hamsters	Sedentary hamsters
Weight increments (g)	High protein	69.4 ± 5.9	43.4 ± 4.3
	Low protein	60.1 ± 3.5	42.6 ± 7.9
Length increments	High protein	18.4 ± 1.1	12.4 ± 1.2
	Low protein	18.0 ± 0.7	13.1 ± 1.2

It is evident from Table 3 that dietary protein is not a limiting factor in the growth of sedentary hamsters which displayed equivalent ponderal and linear growth on 18% and 39% protein diets. It is also evident that exercise-induced growth does not result from the incidental increase in protein consumption by exercising hamsters as a consequence of their increased consumption of food. Exercising hamsters grew significantly longer and heavier on identical quantities of dietary protein consumed by sedentary hamsters.

(3) WHAT CHARACTERIZES GROWTH-INDUCING EXERCISE ?

We have tried to identify those characteristics of exercise that are responsible for acceleration of growth in hamsters. We have approached this question in two ways. In one experiment [15], we studied the changes in total running activity and in the net somatic growth as a function of age and body weight to determine the age span of maximal activity and of maximal exercise-induced acceleration of growth. In the second study [16], we have asked whether acceleration of growth by exercise is specific to golden hamster and the horizontal activity disc used in our studies [3], by measuring the effects of voluntary exercise in rotating wheels and on horizontal discs in rats, gerbils and ground-squirrels. In the same study, we have also looked at the effects of age and body weight on the several parameters of running activity: speed, duration of individual runs, number and duration of pauses, total time spent running, and total distance run.

The results were as follows. Hamsters started freely running around the 15th day of life, increased their activity levels to 30 000 RPD by day 35, and ran progressively less as their weight increased above 65 g. Exercise accelerated growth only in hamsters which have entered the slow phase of growth and which weighed between 60 and 180 g and generated over 15 000 RPD. Exercise-induced growth was found not to be species-specific, since wheel exercise stimulated increased weight gain in gerbils, nor device-specific, since exercise accelerated growth in hamsters running on either the horizontal discs or in rotating wheels [15,16].

Finally, greatest acceleration of growth is seen in exercising hamsters within the 50 to 150 g weight range, and their running pattern has the following additional characteristics: they run at moderate speeds of between 35 and 51 cm/sec for up to one hour without a pause; they break their running for an average of 2 to 3 minutes; they run a total of over 15 000 RPD (5.9 to 8.5 km/day) and spend between 5 and 10 hours actually running [16]. These two studies suggest that the long time spent running at relatively low speeds, a combination of factors characterizing hamsters

during their optimal part of life span,and gerbils, may constitute a stimulus for acceleration of growth by exercise.

(4) WHAT ARE THE NEUROENDOCRINE IMPLICATIONS OF ACCELERATION OF GROWTH BY EXERCISE ?

Somatic growth depends on an intact pituitary source of GH [17], and GH secretion is under neuroendocrine control [18]. In animals like rodents, which retain the capacity for rapid somatic growth throughout their adult lifespan, such growth can be reinstated through damage to the GH-inhibitory circuit. In a series of experiments involving brain lesions [19] or knife cuts [20,21], we have determined that in the adult hamster limbic forebrain inhibits GH secretion and somatic growth.Lesions of rostromedial septum [19], knife-cuts across fibers interconnecting brainstem and septum via hypothalamus [21] or across fibers interconnecting septum and hippocampus [20], all bring about dramatic acceleration of ponderal and skeletal growth in adult hamsters and show evidence of damage to serotonergic and noradrenergic innervation to hippocampus and forebrain.

It is possible that voluntary exercise in hamsters blocks the normal operation of the GH-inhibitory circuit in adulthood, although we have been unable to secure direct evidence for that to date. In addition, if inhibition of growth is viewed as a maturational phase in the sequential neuroendocrine changes during aging, then exercise in hamsters may represent a means of interfering with this neuroendocrine clock.The full implications of the neuroendocrine effects of exercise on growth and aging, remain to be explored.

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THE REGULATION OF SERUM LH AFTER DIFFERENT LONG-DISTANCE RACES

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With the increasing interest in the anabolic action of endogenous sexual hormone testosterone in sport's medical research also increased investigations of luteinizing hormone (LH), which participates in the regulation of testosterone production. In the past we found only a few reports in the period between 1969 and 1974 (Sutton et al. 1969/74 and Alford et al. 1973). But since 1976 the number of papers, concerning the regulation of LH is increasing (Lignieres et al. 1976; Dessypris et al. 1976; Kuoppasalmi et al. 1976; Bonen et al. 1979; Brisson et al. 1979; Dole et al. 1979 and others). The results of these papers show, that it is found an increase of serum LH after long works and no variation or a decrease of LH concentration after short loads. There is no information on the influence of duration or intensity of work on the regulation of LH after distance races. The aim of our work was therefore, to investigate the dependence of serum LH concentration after load on duration of work.

Experimental conditions, materials and methods

Measurement of serum LH-concentration was performed with RIA-kits of Sächsisches Serumwerk Dresden (GDR). The coefficient of variation was 9,6% over all values. Volunteers were athletes, aged 18 to 42 years, in a good health without endocrine disease, who were not taking drugs. All athlets were training three to five times a week in the last years.

We investigated long distance races of following kinds:
- running until individual breakoff at a velocity around 5 m/s (medium distance: about 10 km)
- running until individual breakoff at a velocity around 3 m/s (medium distance: about 34 km)
- classical marathon race
- a race over a distance of 100 km

Sampling of blood was done before the start and after the end of the load and during recovery each hour until 6 hours after the end of the work. Investigating the marathon race we took the blood after a recovery of 3,20 and 48 hours. After the 100 km race blood was taken after 5 and 48 hours of recovery. Samples were taken in all cases from the v. cubitalis.

Results

Regulation of LH concentrations was found in the individual volunteers at very different hormone levels, but mostly regulation pattern was similar.
We found LH variations after load depending on duration of work. It might be, that intensity (training or race) has also a certain influence on LH regulation, but our investigations don't allow to answer this question exactly (Fig. 1)

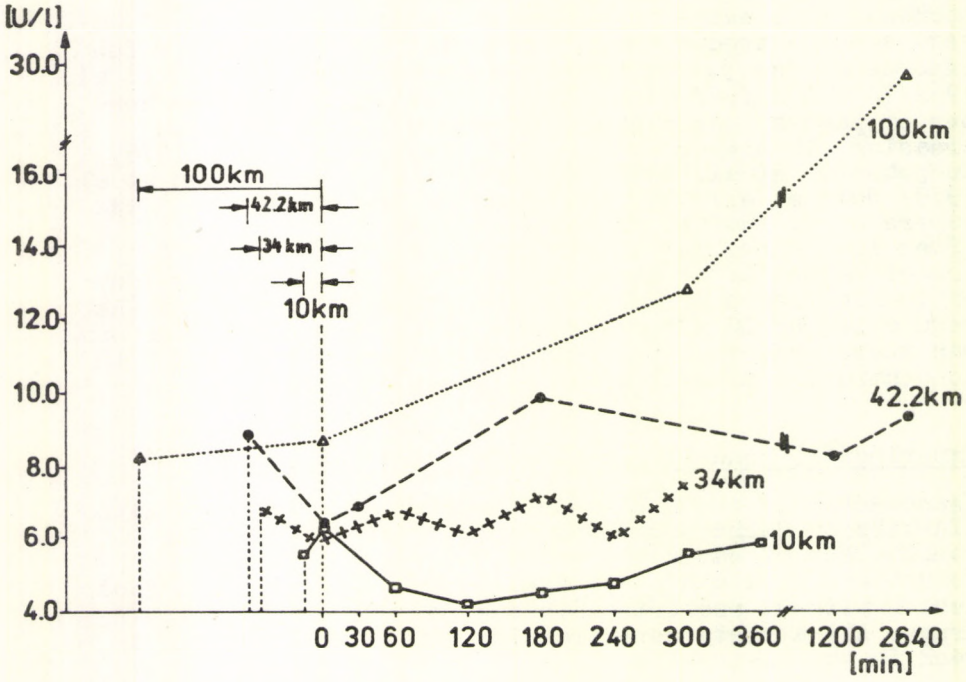

Fig.1 LH-concentration of serum before and after long distance races

After the 10 km run we only found a little peak at the end of load, followed by a decrease of LH concentration on serum for several hours of recovery.
The run over a medium distance of 34 km practically did not cause variations of serum LH concentration. We found clearly increased LH concentrations of serum during athletes recovery after marathon or 100 km races. Three hours after finishing a classical marathon race LH concentration reached the maximum, afterwards falling down nearly to normal values. After the 100 km race the maximum hormone concentration was reached not before 48 hours after the end of the work. LH concentration absolutly increases with duration of race.

Discussion

We couldn't find an exact explaination of the regulation of LH level after work by other scientists. It seems to be clear, that there exists a difference between increases of hormone concentration shortly after load and variations of LH levels some hours or days after finishing work. The letter case of increasing hormone concentration we only found after races of a very long distance, linked with exhaustions of substrates and an increase of catabolic processes. Fore these reasons it seems, that both cases of increasing hormone concentration are of principally different origin. Considering variations of LH level after long distance races and their dependence on duration of race, it may be, that theorigin of increasing LH concentration under these conditions is connected with an increased turnover rate of protein in the body. Lignieres et al. (1976) stated in an investigation of cyclists and Aakvaag et al. (1978) in an examination of soldiers, that hard work for some days decreases the serum concentration of endogenous anabolic hormones (for example testosterone and DHT). During the same investigation Lignieres et al. observed increasing LH levels after some cycling series. It is supposed, that the increase of LH in serum is a counteraction of the body to prevent a further decrease of testosterone levels to still lower values. It is kown, that testosterone produced by testes is inhibiting the system of hypothalamis and hypophysis with a negative feedback control. In the same manner, we believe, that the rising LH concentration in our investigation, depending on the duration of work, is probably a negative feed back of testosterone. Feedback mechanisms will be induced by increasingprotein metabolism as a consequence of load. Loss of proteins firstly will be realized at the transport proteins as globulins. It is known from patients with excessive protein loss (for example with a nephrosis, Nieschlag 1979) to have a decreased capacity of binding globulins for testosterone. In the consequence testosterone has increased metabolism, what counteractscatabolism of

proteins. Increased metabolism of testosterone will lead to low values of this hormone, and consequently the productions of hormone must be increased. High levels of LH are the signal for this.
An other explaination of the observed regulation theoretically would be an exhaustion of steroid hormones in the suprarenal glandula (especially glucocorticoids), which also could diminish precursor pool of testosterone. Such an action also would make necessary an increased LH signal for producing the same or an increased quantity of testosterone. But we must say, that we never found an exhaustion of glucocorticoids in our research. What of both mechanisms will regulate LH concentration after long distance race must show the investigation of future.

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OXYTOCIN INFLUENCES ON THE CNS OF HEALTHY YOUNG MEN

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The aim of the present study was to determine the influence of oxytocin on the central nervous system (CNS) of male students. Our previous results (Schäker et al.1966) suggested a dose-dependent effect of this neurohormone on the EEG, the startle reaction and the motoric activity in both rats and cats.

Peripheral administration of oxytocin (60 IU, parenterally) in young men caused an increase of the CNS activity. This effect is illustrated in Fig.1. The lines represent the mean values of occipitally recorded alpha-potentials of 15 students. After oxytocin administration the following changes occur:
Firstly, the presence of alpha-rhythm during recording time is prolonged, i.e.the time in which alpha-waves may be recorded was longer than after placebo.
Secondly, it is evidently that the frequency of alpha-waves is also increased in response to neurohormone administration. Fig.2 that illustrates the effects in one representative man, confirms this result: The frequency of occipitally recorded potentials is increased whereas the frequency of precentrally recorded potentials is decreased following oxytocin administration.
These both effects together with the prolonged presence of alpha-rhythm are interpreted as expression of an increased functional activity of the CNS, since the same alterations may be demonstrated under defined physiological conditions,

as for instance during effort. So we draw the conclusion that oxytocin provokes an enhanced activity of the CNS.

Furthermore, the oxytocin administration influenced the functional lability of the visual and the sensomotoric system. As compared to placebo, the fusion frequency of light signals was increased and the reaction times were improved by the neurohormone. The results of these studies correspond to the presented electrophysiological data.

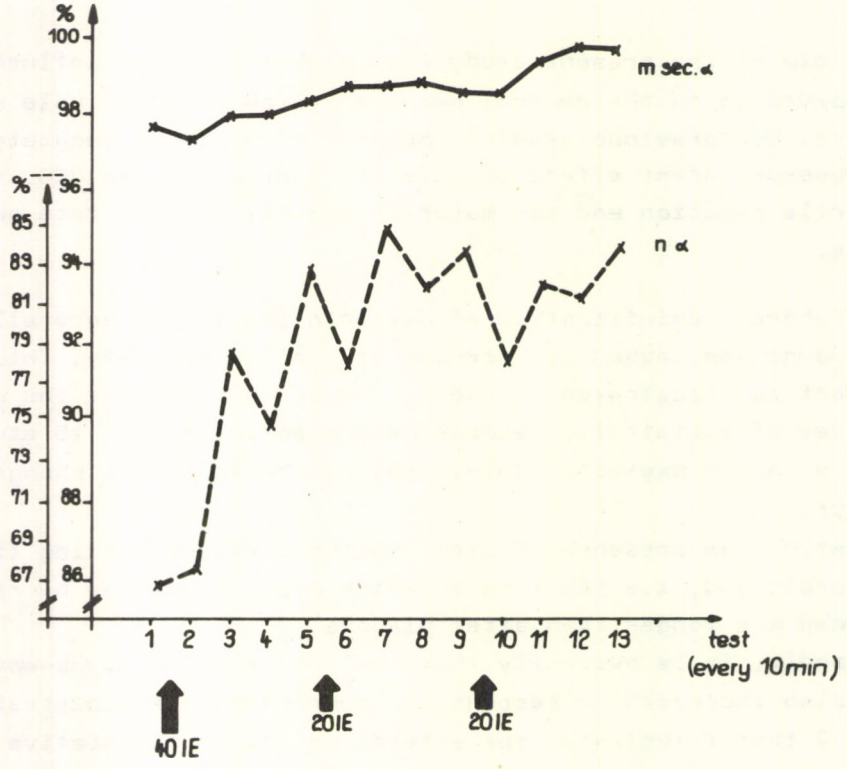

Fig.1. Influence of oxytocin (administered parenterally by tablets; arrows indicate administration of 40 IU and twice 20 IU) on the functional activity of the CNS of 15 male students. The presence of alpha-rhythm (msec α) during recording time is prolonged and the frequency of alpha-waves (nα occipitally) is increased.

In the most cases, the first effects could be demonstrated 8 to 12 min after parenteral administration; maximal changes of about 15 % occured from 15 to 35 min. However, contrary to rats and cats, in some men reverse effects (e.g.,prolonged reaction time) were observed. These striking differences between individuals after oxytocin administration may be caused by several reasons. So we cannot exclude,that among other things differences in resorption, in distribution of hormone molecules following administration and/or in the psychological state of the test persons contribute to this phenomenon.

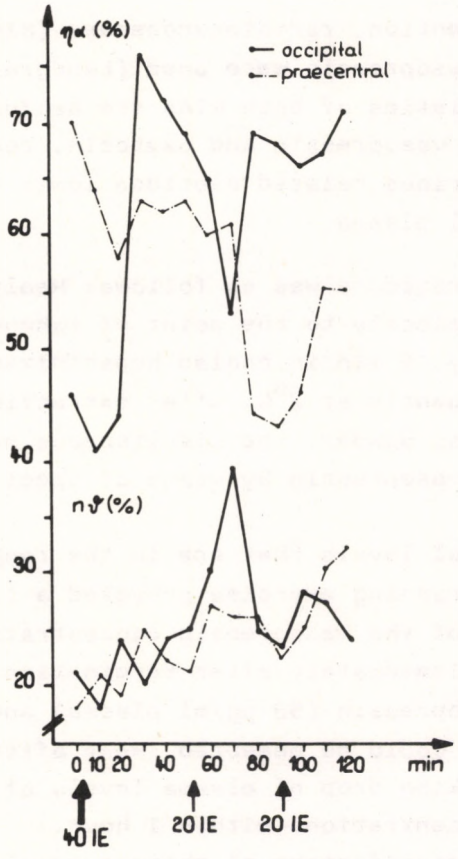

Fig.2. Effect of oxytocin application (40 IU and twice 20 IU as indicated by the arrows) on the frequency (%change) of both alpha-potentials (nα) and theta-potentials (nϑ) in one representative man.

Summarizing, the effects after systemic administration of oxytocin suggest that this neurohormone is able to increase the activity of the CNS under the conditions described.

The question arose whether or not this action of oxytocin on the CNS might be of physiological significance. For that reason we are interested in proving a relationship between plasma concentration of endogenous neurohormones and effort, i.e., are the levels of both oxytocin and arginine-vasopressin changed in dependence on e.g. a running exercise.

To realize our intention, radioimmunoassays (RIAs) for oxytocin and arginine-vasopressin were used (Landgraf, submitted). The mean characteristics of both RIAs are as follows: cross-reactivity against vasopressin and oxytocin, respectively, as well as against various related peptides lower than 1%; sensitivity 1 to 2 pg/ml plasma.

The experimental procedure was as follows: Healthy young men ran at a defined velocity to the point of exhaustion. Blood was collected every 15 min. in cooled heparinized tubes and centrifuged subsequently at $2^{o}C$. After extraction of 2 ml plasma using Vycor glass powder, the simultaneous measurement of both oxytocin and vasopressin by means of specific RIAs was performed.
As compared to basal levels that are in the range of 1 to 3 pg/ml plasma, the running exercise provoked a significant increase especially of the vasopressin concentration in one representative man. Immediately after termination of the effort, peak values of vasopressin (58 pg/ml plasma) and oxytocin (4,8 pg/ml plasma) could be measured. Rest after running exercise caused a striking drop of plasma levels of both neurohormones to basal concentrations within 1 hour.
To illustrate the significance of changes in plasma levels during effort, the diurnal variation of both plasma oxytocin and vasopressin was investigated in the same person. Besides a nocturnal increase of vasopressin concentration it should be emphasized that peak values of only 4 pg/ml plasma could be de-

termined.

What about the role of the enhanced plasma level of these endogenous neurohormones during effort? Peripheral effects must be taken into consideration, but in relation to the former mentioned actions of exogenous oxytocin and to data presented in the literature (De Wied 1979, Landgraf et al.1979, Legros and Gilot 1979) also an influence of both endogenous oxytocin and vasopressin on the function of the CNS might be suggested. Further studies in this direction will be performed.

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INCREASED AEROBIC METABOLIC RATE DURING PHYSICAL EXERCISE AFTER ALDOSTERONE APPLICATION

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Investigations with animals demonstrated that aldosterone increases sodium transport by enhancing activities of several cytoplasmic and mitochondrial enzymes (4,6). Furthermore, it has been demonstrated that application of aldosterone induces increased levels of oxygen consumption in the toad bladder (2). In the present study on human subjects it was investigated whether an increased level of aldosterone would elevate the aerobic metabolism during muscular work.

MATERIALS and METHODS

Ten male subjects were assigned to two sets of treatment. The control experiments (CE) employed a placebo, whilst the aldosterone experiments (AE) involved injection of aldosterone twice with a two hour interval in between. Aldosterone injection consisted of 0.5 mg Aldocorten®, a synthetic steroid, which was given subcutaneously; for the placebo, isotonic solution of NaCl was given in the same way.
Three hours after the second injection, exercise commenced on a bicycle ergometer. Work load was increased stepwise by 40 W every 3 min until the level of exhaustion was reached. Ventilatory parameters were determined by a computerized breath by breath analysis (10). Half of the subjects received treatment CE first and the others AE. After seven days the subjects were switched to the other treatment condition.

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RESULTS and DISCUSSION

In ventilation and respiratory frequency no significant differences between AE and CE existed. At the beginning of the work load oxygen uptake (Fig. 1A) increased significantly more in the experiments with aldosterone injections ($P < 0.05$). Differences were in the order of 100 ml/min on commencement of work load and increased during the final steps. Maximal oxygen uptake was increased significantly in the AE by about 400 ml/min or 10 $\pm$ 12 % ($P < 0.02$).

In addition to the increase in oxygen uptake, the level of carbon dioxide output (Fig. 1B) is also shown to exhibit a similar trend. It can be seen from Fig. 1A and B that the difference between the two treatments are not as marked

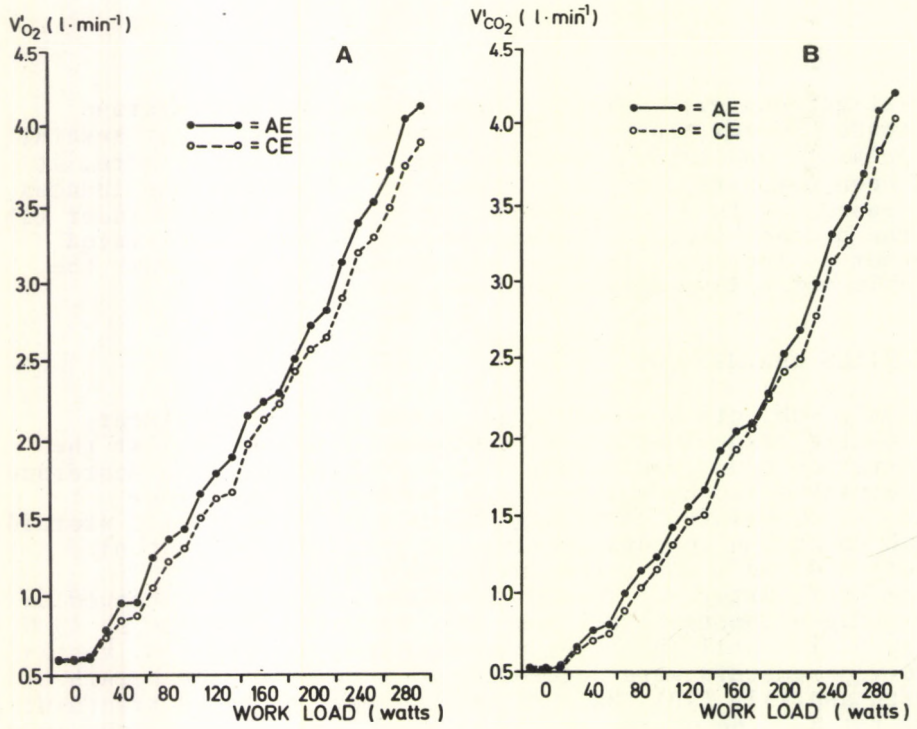

Fig. 1: Behavior of oxygen uptake (A) and carbon dioxide output (B) during bicycle ergometer work load after aldosterone application (AE) and during control experiments (CE). Work load of 280 W was the level which could be performed by all subjects.

during the lower levels of work load in the case of carbon dioxide output. Due to the small discrepancy of oxygen uptake and carbon dioxide output a more marked decrease of respiratory quotient (Fig. 2) in AE was found in the range below endurance capacity.

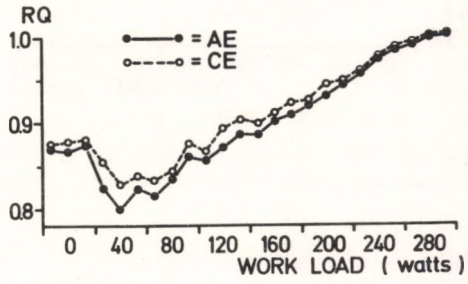

Fig. 2: Respiratory quotient during bicycle ergometer work load in aldosterone (AE) and control experiments (CE).

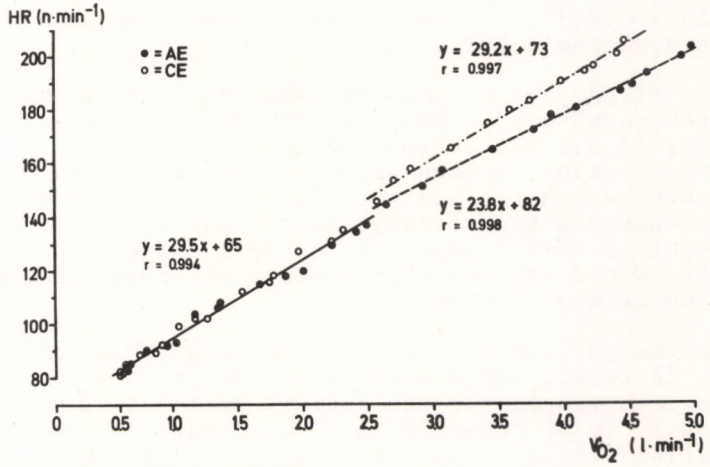

Fig. 3: Heart rate of different steps of work load in relation to oxygen uptake. AE: aldosterone experiments; CE: control experiments

Increase of heart rate did not differ significantly between the experimental conditions. In figure 3 heart rates of different steps of work load are shown as a function of oxygen uptake. The especially augmented oxygen uptake induced by aldosterone during submaximal and maximal work loads led to a significant increase of oxygen pulse in this range. This indicates that during heavy work load

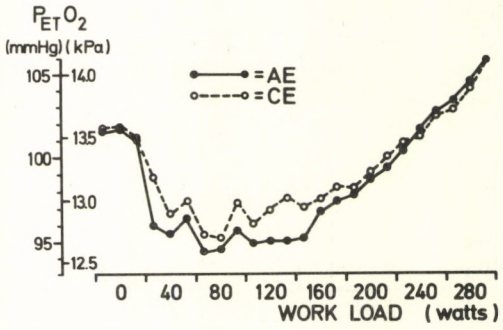

Fig. 4: End-tidal oxygen pressure during bicycle ergometer work load in aldosterone (AE) and control experiments (CE).

aldosterone increased the oxygen consumption at the same level of heart rate. Consequently an augmented oxygen extraction of blood would be expected. Indications for such an increase are presented for the range below endurance capacity (Fig. 4). The end-tidal oxygen pressure was reduced significantly more in AE than in CE. But no differences existed in the upper range, in which an especially enhanced oxygen uptake by aldosterone was to be seen. Perhaps this can be explained by results of other authors (9,11) indicating an inotropic action of aldosterone on the heart.

Activities of enzymes of the tricarboxylic acid cycle as well as the respiratory chain was found to be enhanced by aldosterone (4,5,6,8). Dalton and Snart (2) stated an increased respiration through aldosterone. It is open to question whether these findings all obtained in toad bladder and rat kidney are transferable to the muscular system (1). In the event of such findings being applicable to the muscular system this would explain the increase in oxygen uptake during application of aldosterone.

There are indications that part of the augmented oxygen uptake is due to increased lipid metabolism. During work load in the submaximal range, the respiratory quotient was reduced significantly in AE compared to CE (see Fig. 2). In other investigations where aldosterone had been used on the toad bladder this had facilitated the conversion of pyruvate into fatty acids and increased the content of unsaturated fatty acids (3), whilst in rats, plasma level of free fatty acids has also been shown to increase (7).

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EXERCISE DURATION, INTENSITY AND PLASMA CORTISOL LEVEL IN ATHLETIC AND UNTRAINED CHILDREN, ADOLESCENTS AND ADULTS

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Relations between muscular work and the ACTH - cortisol system have already been the subject of many studies. In these the main objective has been to clarify the nature of physiological changes, but attempts were made from the very beginnings to use hormonal response also as a means for checking physical fitness or condition. To keep this point on the agenda also such efforts have helped that tried to increase physical performance by steroid administration. Nevertheless, even some of the fundamental problems have all the while escaped a definitive answer, and the reported results continued to be discordant /1, 2/. In addition to the methodological differences in hormone estimation a large part of the discord has arisen we believe by using a diversity of test exercises in subjects of unidentified, but variable levels of fitness.

In this paper we wish to summarize the results of a multifaceted series of experiments. The purpose in it was to get ahead systematically concerning the effects of submaximum and maximum intensity laboratory and event specific exercise in competitors of different age, level of fitness and favourite sport as well as in comparable control subjects. In all experiments cortisol level was estimated by using a modification of Mattingly's method /3/.

Two diagrams of our former rat experiments are first recalled /4, 5/. In Fig. 1 plasma corticosterone concentrations of trained and control rats are shown after an exhaustive bout of swimming. Though significantly higher after than before the exercise, the steroid level of the animals subjected to previous regular training was much lower than that of the controls. Figure 2 illustrates the effects of a single ACTH injection. The response to ACTH of both groups is a maximum, ruling out suggestions that would explain the difference seen in Fig.1. by attributing it to an exhaustion of the adrenal glands in the trained animals. Quite on the contrary, the reduced response can be fully accounted for by the development of an adaptation process in the exercise-trained rats.

All the other figures to be shown refer to human subjects. In the first series submaximum ergometric exercise was studied in awimmers, handball players and nonathletic

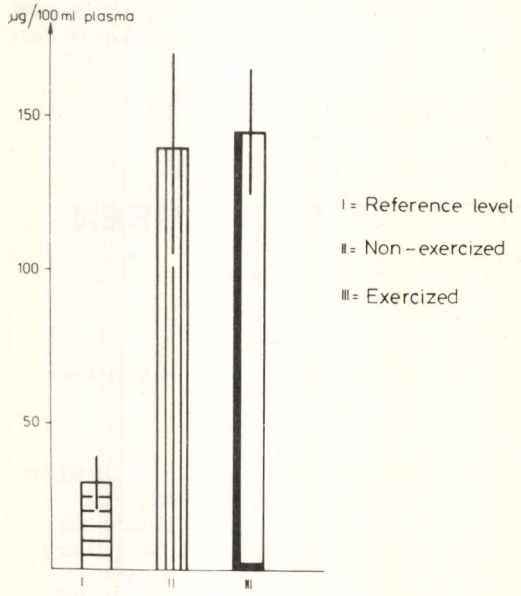


Fig. 1. The effect swimming on plasma corticosterone level in previously exercised and non-exercised rats /$\bar{x} \pm$ sd/.

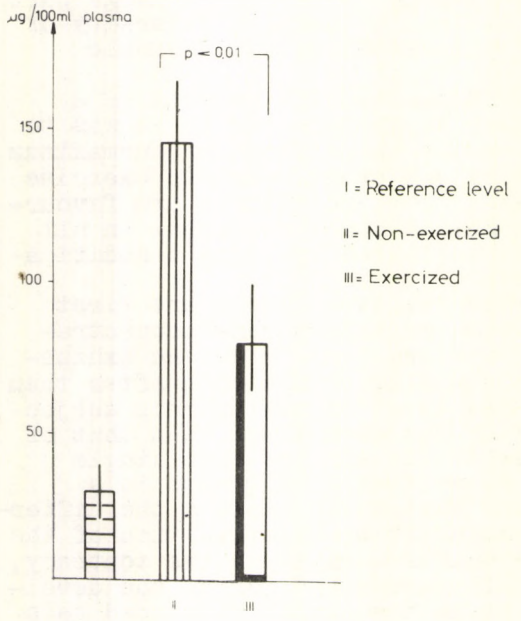


Fig. 2. ACTH effect on plasma corticosterone in previously exercised and non-exercised rats /$\bar{x} \pm$ sd/.

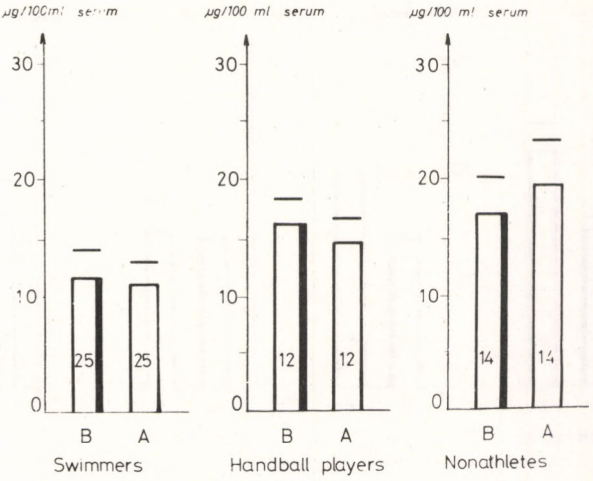


Fig. 3. The effect of a submaximum laboratory exercise on serum hydrocortisone level. B: pre-exercise values. A: post-exercise values /$\bar{x} \pm$ sd/.

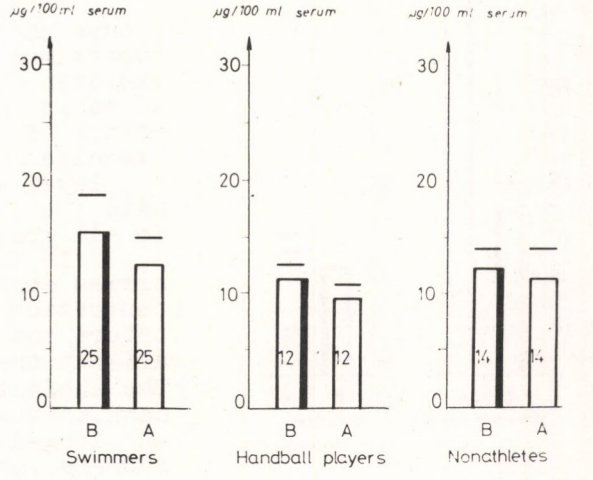


Fig. 4. Submaximum sport specific activity and serum hydrocortisone level /$\bar{x} \pm$ sd/.

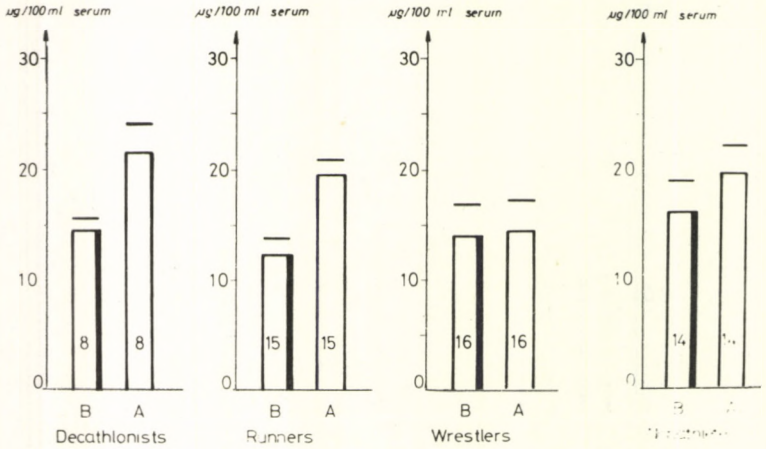


Fig. 5. Serum hydrocortisone level in all-out exercise /$\bar{x} \pm$ sd/.

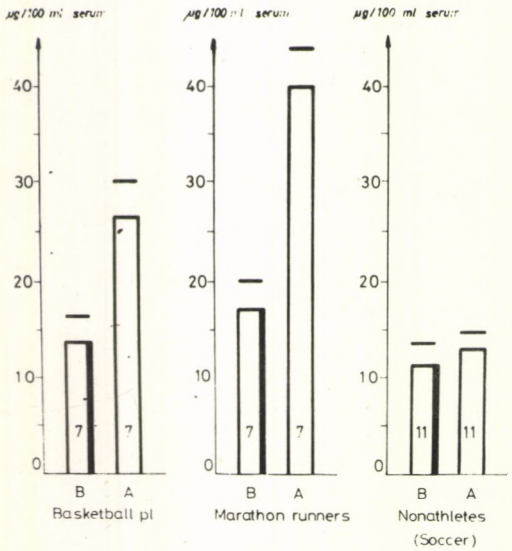


Fig. 6. Serum hydrocortisone level and event-specific sport competition /$\bar{x} \pm$ sd/.

technology students aged 18 to 25. This work load failed to affect serum cortisol in all of the subject groups /Fig. 3/.

When the same groups underwent a submaximum intensity exercise conforming to their favourite branch of sport, post-exercise serum cortisol level again agreed with the initial one in all groups /Fig.4/.

In the next series cortisol concentration was studied before and after an all-out treadmill run. The subjects were decathlonists, medium and long-distance runners, wrestlers and nonathletic adults. Decathlonists and runners displayed a significant rise while

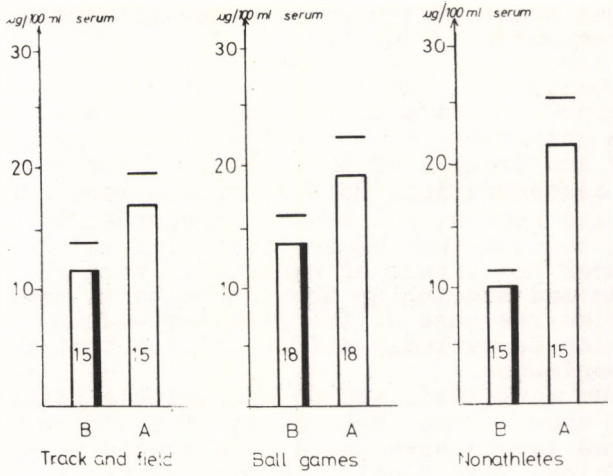
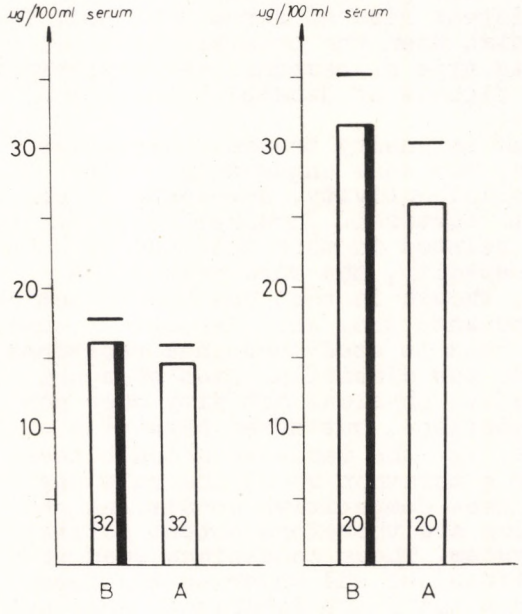


Fig. 7. The effect of an all-out exercise on serum hydrocortisone level in juniors /$\bar{x} \pm$ sd/.



in the wrestlers no appreciable change was found. The slight rise seen in the nonathletic subjects was not significant statistically /Fig. 5/. Competitive activity, on the other hand, could double the serum concentration of cortisol in both first class basketball players and marathon runners, as shown in Fig. 6, whereas a friendly no-risk match of soccer did not produce any change in the nonathletes.

The role of age was studied in junior athletes of 15 to 18, in players of some ball games and in nonathletic subjects. The all-out

Fig. 8. All-out exercise and serum hydrocortisone in child-age swimmers grouped according to pre-exercise serum hormone level /$\bar{x} \pm$ sd/.

exercise brought about a significant rise of cortisol level in all the three groups /Fig. 7/.

All-out laboratory exercise was studied also in child swimmers aged between 11 and 13 years. Of this group two subgroups were formed by using initial cortisol level as a classification criterion. Group 1 consisted of children with low initial levels and Group 2 of those having a relatively high initial serum concentration. Both subgroups were subjected to the same exercise regime, yet neither responded by a rise. It is interesting to note that higher initial levels even tended to decrease under the effect of exhaustive work /Fig. 8/.

Analysing and summing up these results we have stated that the exercise response of the pituitary-adrenal system in healthy humans is markedly different from that found in the animal experiments.

Submaximum work loads had no appreciable effect on serum cortisol under either laboratory or field conditions. High preexercise levels have usually decreased to normal after the exercise. This finding is consistent at all ages studied and does not depend on the event of sport used as the test exercise in the case of the competitive athletes. Divergent results were, however, obtained with all-out exercises on the treadmill. Factors such as age, level of fitness and event of sport may all have contributed to the differences.

In well-trained adults being engaged in dynamic events of sport and having high aerobic power all-out running elicited a slight, but consistent rise of serum cortisol. The same response was present also when the preexercise level had been relatively higher. This type of response was characteristic of the extraordinary fitness of decathlonists and distance runners, for instance.

Despite of the maximum intensity treadmill exercise subjects, such as wrestlers, who were engaged in a more static kind of regular physical activity, developed no change in the concentration of serum cortisol. Physical activity and cortisol response are thus related in more than one way. The higher the subject's work capacity, the more marked the pituitary-adrenal response, though in that one has to notice a dependence on the event pursued too. When laboratory tests of ergometric exercise are used to study hormonal reactions, it is advisable to take also the dissimilarities of sport events into account. Recreative physical activity does not change the level of serum cortisol, but after high-risk matches or competitions, as e.g. the decisive match between Class I basketball teams or a marathon race, the rule has been a marked adrenal response. Competitive conditions and maximum intensity performance are therefore potent activators of the pituitary-adrenal system. These conditions seem to mobilize such additional mechanisms and reserves that are inaccessible for even maximum intensity laboratory workloads. Accordingly, emotional factors and motivation may play a prime part in man in the development of a marked adrenal response. Naturally, the respective effects of emotion and severity of physical effort cannot be sharply discerned, if for nothing else then for the high level of motivation required by really exhaustive activity. The importance of

the emotional factors has been evidenced also by the values obtained in the juniors and children, such as the higher levels of cortisol found before the all-out exercise. The intensity of the adrenocortical response is therefore a function of work capacity under laboratory conditions and one of the level of motivation and exercise intensity under competitive conditions.

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COMPARISON OF URINARY EXCRETION OF METHANDROSTENOLONE AND ITS METABOLITES IN RATS AND HUMANS

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An important group of anabolic steroids comprises 17α-methyl-testosterone and related compounds, among them methandrostenolone (17α-methylandrosta-1,4-diene-17β-ol-3-one; other trivial name is methandienone, Hungarian trade name: Nerobol®). Our laboratory has been involved in analytical work concerning methandienone measurements for spot control since 1975. Since that time urine samples were measured by radioimmunoassay in more than 8,000 cases and in order to improve the efficiency of the drug detection, technical and theoretical developments were necessary. The main technical achievements during this time involved a twenty-five fold sensitivity increase of the methandienone RIA over our original method (1), antisera and partially also tracer production for RIA of testosterone, 19-nor-testosterone and epi-methyl-testosterone (2) as well as combined application of high pressure liquid chromatography (HPLC) and RIA (3). Theoretical work concerned on one hand collection of experimental data on the methandienone metabolism, on the other hand several problems compelled us to investigate also radioimmunoassay method itself. This paper presents an outline of our data obtained on the metabolism of methandrostenolone; our theoretical considerations which aim the use of cross-reactions in RIA determinations, will be discussed in the next one (4).

Radioimmunoassay determination was made by two procedures, one described before (1,3), the modified procedure is as described by Brooks et al. (5) with minor alterations; the range of the measurement extends to 6 pg - 1 ng. High pressure liquid chromatography was performed as it is shown on Fig. 1. The chromatograms of the injected urine extracts showed several peaks but, without added standard, peaks corresponding to methandienone, testosterone or metabolites could not be detected by UV light, so fractions were collected according to the retention data obtained with standard mixtures. Fractions were later measured by RIA. Since for chromatography urine extracts were needed while in RIA measurements usually unpretreated samples are applied in our practice, it was necessary first to control, whether the immunoreactivity contained in the urine samples, can be ex-

tracted quantitatively as authors in the literature generally suppose though do not state. It was found that ethyl ether extracts contain about one third of the immunoreactivity of the original samples and the more polar dichlormethane-ethanol (9:1) mixture extracts also not more than 60%. For practical reasons, however, ether extracts were used throughout the chromatographic experiments. Another problem was encountered when rat urine specimens were subjected to RIA measurements. A -still unidentified- substance contained in the urine of rats suppressed RIA response to such an extent that in most cases nonsense negativ numerical values were obtained for methandienone concentrations. A substitution of the diluting buffer with control rat urine in standard solutions helped to overcome this problem. Human urine usually does not alter significantly the binding of the steroid to the antibodies except several observed cases. The explanation of this phenomenon is uncertain at present.

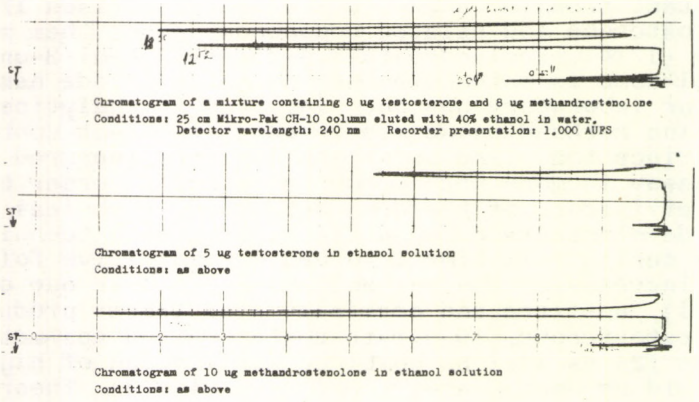

Fig.1 HPLC chromatograms of testosterone, methandrostenolone and their mixture

Experiments on rats were conducted in order to obtain metabolism data with radioactively labelled substance; to control, whether these data can be compared to results in humans, and to investigate, if our experience on human material can be transferred to mammals. Latter aspect has become rather important as anabolic steroid control is expected to be obligatory not only for race-horses but in commercial meat products, as well.
Experimental conditions are tabulated in Table 1.
Figure 2 shows excretion profile of methandienone equivalent immunoreactivity in the human subject during and after the administration, ng/ml concentration values as well as ng/mg creatinine ratios are plotted. Here RIA was made applying unextracted samples. Figure 3 shows the results of RIA measurements from extracted and fractionated samples. It can be seen, that the highest portion of the extractable immunoreactivity is contained in the fraction eluting before methan-

dienone (fraction 1) and testosterone (fraction 2) thus showing its more polar nature. Being extractable in ether, this material (fraction O) cannot comprise possible conjugates of the steroids, but 6β-OH-methandienone, one of the main metabolites (6) is certainly included. (Later we also could detect this compound in urine extracts by gas-chromatography). It has to be noted that the other class of main metabolites, 17-epi-configurated products (7,8) are not included in our present measurements as this substance will not be measured by the RIY for methandienone. An appropriate RIA method for measuring epi-configurated products has been developed in our laboratory recently (2). It can also be observed that a small but measurable fraction of the immunoreactivity coincides also with the methandienone and testosterone fractions.

Table 1. Experimental conditions

	MAN	RAT
Methandrostenolone unlabeled	0.2 mg/kg per day p.o.	0.2 mg/kg per day i.p.
tritiated	-	925 kBq/day
amount given		0.2 mg/kg per day i.p.
Period of administration	6 days	5 days
Collection of urine samples	first daily excretes content related to creatinine conc.	24-hr collection in metabolic cages
Determination procedure used	RIA + HPLC RIA	RIA + scintillation counting

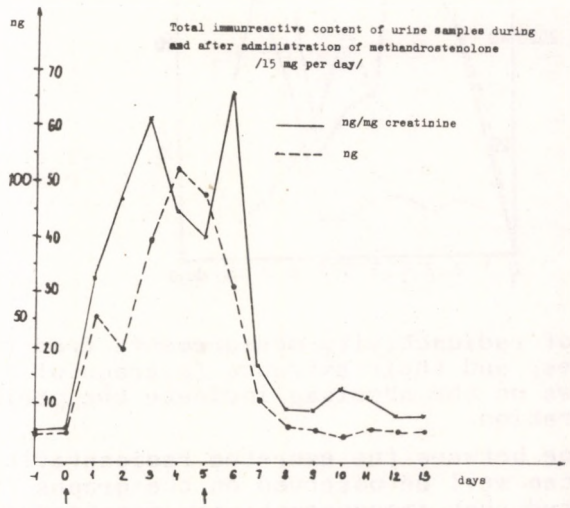

Fig.2 Total immunreactive content in unextracted urine samples. Arrows indicate period of administration.

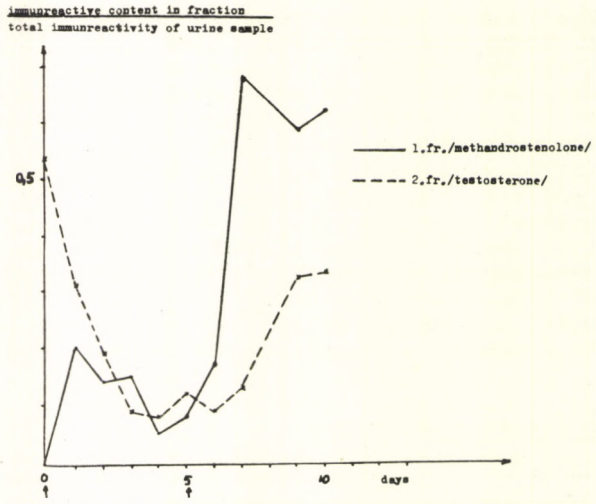

Fig.4. Immunreactive content of the fractions 1 and 2 related to the total immunoreactivity of the samples.

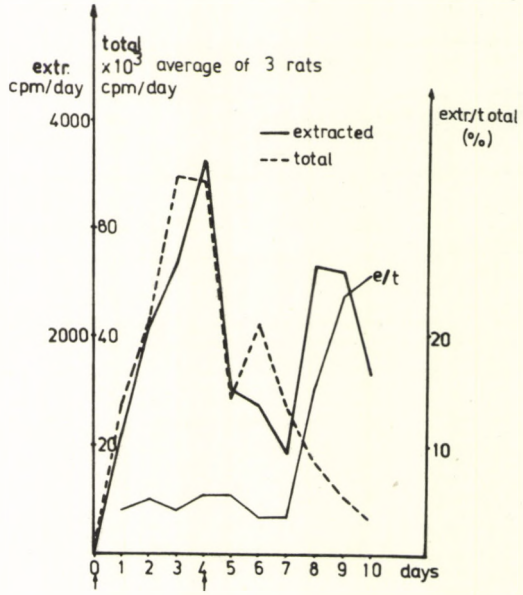

Fig.5. Results of radioactivity measurements from total urine samples, and their extracts (average of 3 rats). The arrows on the abscissa indicate the period of the administration.

The difference between the excreted radioactivity and immunoreactivity can well be observed on the graphs of Fig.6. It has to be noted that immunoreactivity measurements were done on samples collected from another three animals thus some observed differences can be explained by the individual

Further in the methandienone fraction a non-endogenous product reacts, which fact is proven by the very low reactivity of this fraction in the sample taken before the administration. The multiple excretion peaks of methandienone and metabolites are known in the literature and they were observed also, when measurements were performed by gas chromatography-mass-spectrometry thus following only amounts of individual, identified substances (9,10). This alternation in excreted amounts suggests that the ration of the different excreted metabolites varies during the administration. A proof of this theory can be seen in Fig.4 where immunoreactivity measured in fractions 1 and 2 divided by the total immunreactive content of the extracted sample is presented. It is apparent from the curves that during the administration the ratio of the more polar metabolites is much higher than that after the end of the administration when the extractable material eluting with methandienone represents about the half of the total immunoreactivity extracted.

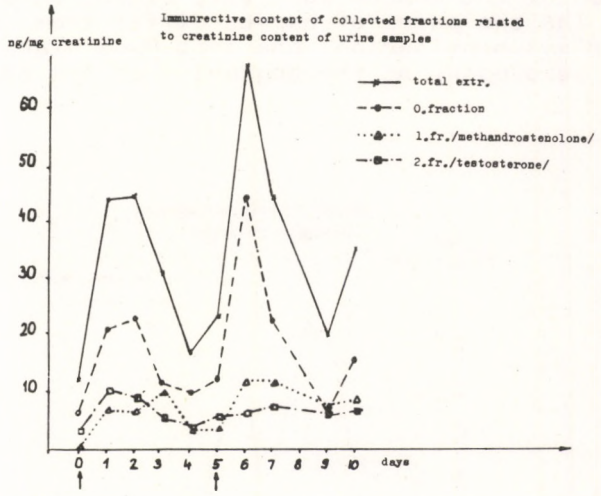

Fig.3 Excreted immunoreactivity during and after metandienone administration (O.fraction eluting before methandienone in HPLC).

The same phenomenon can be observed in rats. Figure 5 presents results of experiments performed with tritiated methandienone. Measurements in these cases were done solely by counting radioactivity as RIA would have been interfered by the tritium content of the samples. The curves show the average of the extractable radioactivity and the ratio of the extracted and the total excreted radioactivity. The extractability of the metabolites significantly increases after the administration. Here, of course, one cannot be certain if the steroid-like metabolites really do contain the label excreted or not. The extractable radioactivity, however, is most probably excreted in form of some steroid-type molecule.

variations. The main differences, however, are too characteristic to disregard them. It is evident that the predominant products of the first and second days are either metabolites with a very low reactivity in our assay or the measured radioactivity can be assigned to some very simple product/s/ of a primary metabolic attack on the carbon atoms holding the tritium labels (positions 1,2 and 4). As at the end of the examined period a substantial amount of immunoreactivity was excreted without significant radioactivity, it is more probable that the metabolism of at least a part of the exogeneous steroid commences with substitution reactions at the mentioned positions in the rat. An important characteristic in methandienone metabolism common to rat and man is that according to literature data (7) and our experience no significant amounts of sulphatase and/or glucuronidase sensitive metabolites could be detected. This fact supports the idea that the mentioned non-extractable fraction of metabolites are not easily hydrolysable conjugates. Figure 7 shows the comparison of the immunoreactivity excretion profiles in rat and man. The two pairs of curves differ from each other only in their synchronisation: the left hand side curves have their common zero point at the beginning of the administration.

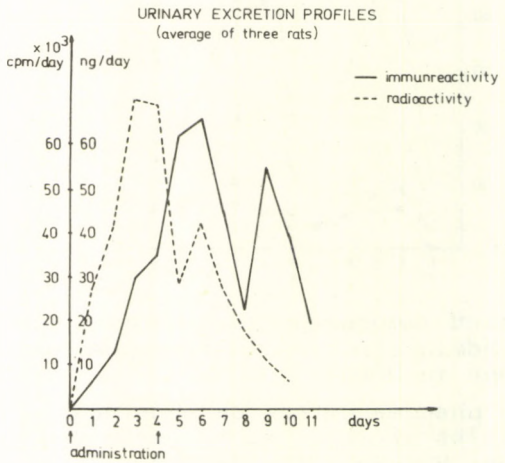

Fig.6 Excreted immunoreactivity and radioactivity in urine samples of rats administered unlabelled and tritiated methandrostenolone.

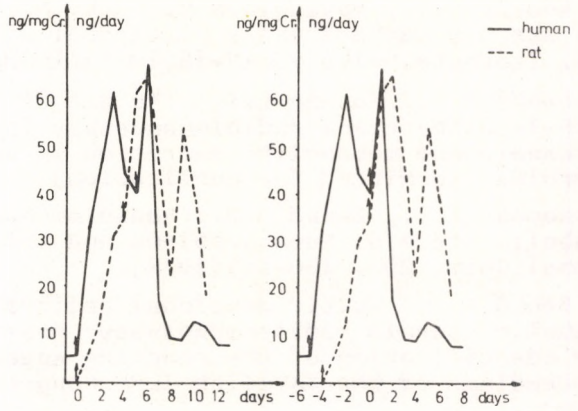

Fig.7 Comparison of immunoreactivity excretion profiles in man and rat. Left: curves are synchronised at day 0, right: pair of curves have their common day at the end of the drug administration.

Here the resemblance of the peaks in the excretion during the administration can be observed. The other pair is, in turn, synchronised at the end of the treatment. For sports control purposes, that latter is the more significant comparison as we have to keep in mind the problem of drug detection after the cessation of the intake. The peak at the 5th-6th post administration days is common to rat and man, showing a general phenomenon observed in many other cases, too. This can probably be the result of the mobilisation of the amounts of the steroid, stored during the treatment. Our preliminary investigations on distribution of methandienone injected to rats showed high concentrations in the kidneys and the fat. Latter can well serve as a depo of the substance. In our earlier experience, similar post-administration excretion peaks were observed even after a longer period when higher amounts of the steroids were applied.
Summarising the presented results we can state, that:
1. the extractability of the immunoreactive products is quite poor, and it changes during and after the administration;
2. the metabolism of the methandienone is only partially similar in rat and man, but the similarity involves a very important post-treatment excretion of immunoreactive material;
3. the excretion curves of the tritium label and the immunoreactivity are not parallel and
4. methandienone is being excreted in forms of unconjugated metabolites in both species examined.

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MULTIDIMENSIONAL CALIBRATION OF ANABOLIC STEROID RADIOIMMUNOASSAYS: POSSIBILITY FOR THE IDENTIFICATION OF THE REACTING SPECIES

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Investigations on similarities and dissimilarities in methandienone (17α-methyl-androsta-1,4-diene-17β-ol-3-one) metabolism of man and rat were discussed in previous paper (1). Another important and interesting conclusion which could be drawn from our experiences with human samples was, that in general, radioimmunoassay (RIA) can be regarded to have a higher effective sensitivity in detecting excreted anabolic steroids or their metabolites than gas chromatography-mass spectroscopy has. The problem with RIA has always been the comparatively high possibility of false positive results, due to cross-reactions from either endogenous substances or other "permissible" steroids as for example contraceptives. Comparison of samples obtained after an experimental administration of anabolics, however, clearly showed that the often observed discrepancy between RIA and GC-MS results can sometimes also be due to false negative decisions made by GC-MS, the cause of which can be found in the insufficient knowledge of steroid metabolism and the too high specificity of the GC-MS method. The mentioned facts gave us an impulse for at first theoretical, later experimental investigations to make use cross-reactivity of RIAs in detecting and possibly identifying low amounts of steroids and their metabolic products.

It has been long known that according to their similarity in sterical and chemical characteristics, closely related compounds can cross-react in each other's assay. This observation was especially significant in case of hapten antigens, among others steroids. Cross-reactivity has been usually regarded as a disadvantage since artificial or metabolic products, impurities can and do interfere with the determination of the substance in question. Nevertheless, cross-reactions can be useful, too, e.g. in measuring a substance with a high cross-reaction in another assay or in determining an unknown metabolite of very similar structure to the original antigen, etc., as it is in the case with some anabolic steroid.

To illustrate the cross-reactions of different extent, Fig. 1 and 2 show standard curves and several cross-reaction curves in the assays for methandienone (methandrostenolone) and testosterone resp..

The main objective of our investigations at first was to obtain a simple method to separate reaction of testosterone (T) and its metabolites from that of methandione (M) and metabolites which mutually-though not equally-cross-react in each other's assays.

It is evident that to solve this problem, samples have to be measured by two assays, one for T and the other for M. The evaluation of these "twin"-results, however, is far from being simple as neither of the presented reaction curves is linear; additivity therefore cannot be supposed. Another paper dealing with cross-reactivity of T and 5αDHT led to

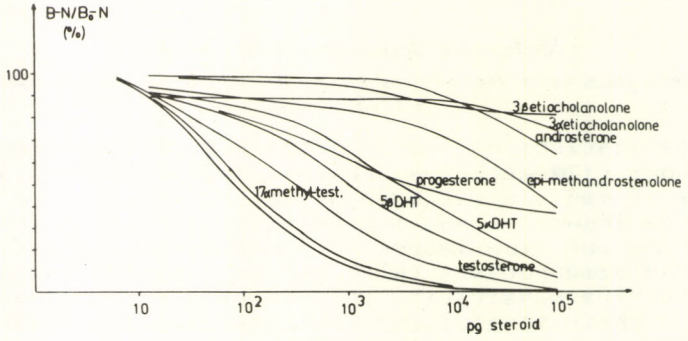

Fig. 1 Standard curve for methandrostenolone (unmarked) and several cross-reaction curves in methandrostenolone radioimmunoassay

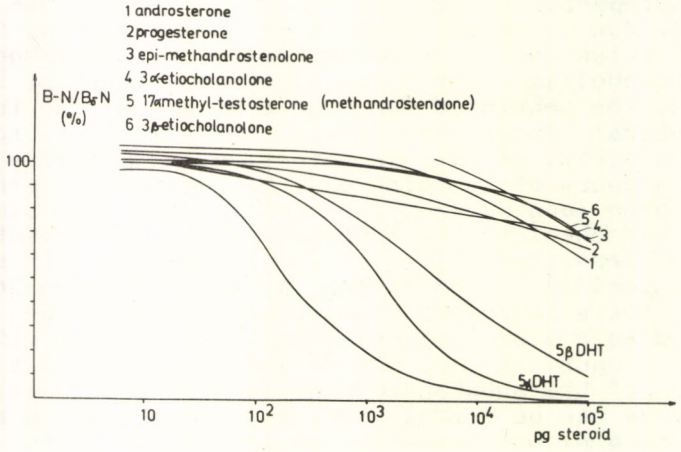

Fig. 2 Standard curve for testosterone (unmarked) and several cross-reaction curves measured in the RIA for testosterone

conclusions somewhat resembling to ours (2).

Let us introduce some -to a certain extent unorthodox- symbols. Be A and B the two cross-reactive substances (here A will stand for methandienone, B for testosterone). Be further the concentration of A represented by x, that of B by y. Let R stand for the response measured, thus being the response dependent on the assay used, we shall have R_A and R_B, i.e. the responses measured in the A and B assays, resp.. Finally, a mixture which contain x_1 pg of A and y_1 pg of B will be represented by a pair of numers in brackets (x_1,y_1), the corresponding responses measured can thus be expressed as $R_A(x_1,y_1)$ and $R_B(x_1,y_1)$. Naturally, the function $R_A(x,0)$ will then mean all the response values which can be obtain by varying the amount of A in the A assay, with other words this symbol is that of the standard of A. $R_A(0,y)$, $R_B(x,0)$ and $R_B(0,y)$ will be similarly interpreted as cross-reaction curve of B in the A assay, cross-reaction curve of A in the B assay and the standard curve of B, respectively. It can be seen from the cross-reaction curves, that -within a reasonable range- to each amount of A or B a quantity of the other substance can be found, which gives the identical response, and these "equireactant" amounts depend on the assay concerned. Let us denote the equireactant amounts in the following way: $y'_{1,A}$ is equireactant to x_1 in the A assay, if $R_A(x_1,0) = R_A(0,y'_{1,A})$, $y'_{1,B}$, $x'_{1,A}$ and $x_{1,B}$ can be interpreted similarly. If for the graphic presentation, inhibition % versus concentration is chosen, the two pairs of standard and cross-reaction curves look like in Fig.3.

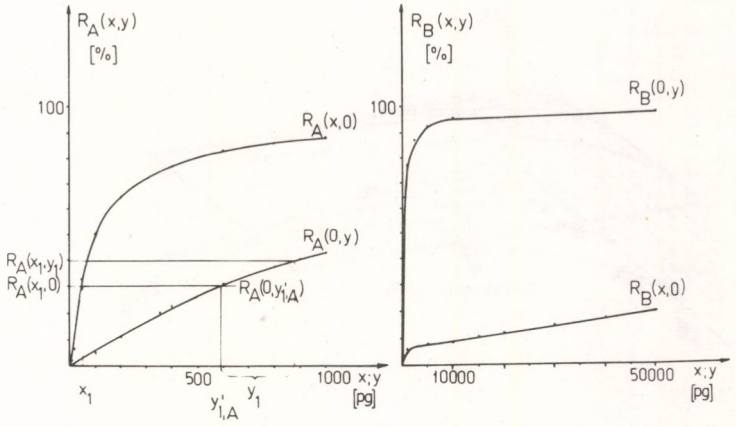

Fig.3 Standard and cross-reaction curves of methandione and testosterone measured in RIA for methandienone (left) and for testosterone (right), presentation: inhibition vs concentration

It can be observed, that methandienone cross-reactivity in the testosterone assay is much less than vice versa.

When, as usual, a single substance is measured in one RIA, the calibration curve is visualised in a plane, the two axes of which are the concentration of the substance and the response measured. Working with mixtures, however, calibration has to be made in a three-dimensional space, in which two axes -they determine the concentration plane- represent the concentrations of the two substances, the third again the response measured in one of the assays.

Let us regard at first the RIA for A. The conventional standard curve for A, which was denoted in our symbols $R_A(x,0)$, will be obtained on the plane determined by the axes $x\text{-}R_A$ like in the simple calibration procedure. Cross-reaction curve of B, in turn, will have to appear on the plane $y\text{-}R_A$, since the y axis represents all the "mixtures" in which the concentration of A, that is x equals to zero. As every point on the plane concentrations corresponds to a mixture, theoretically to each of these points a response, R_A can be measured, and the points determined by both concentrations and the respective response will form a surface in the three-dimensional space.

This surface is shown in Fig. 4. Similar problems were discussed by Feldman et al. (3) who regarded cross-reactivity in fact to be avoided or eliminated.

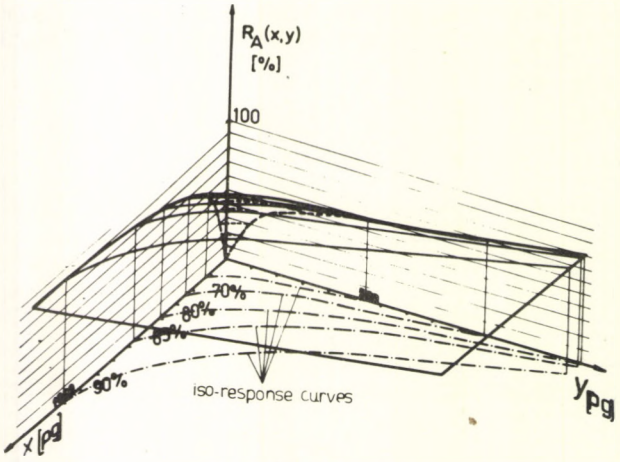

Fig.4 Surface formed from points representing mixtures and their response (calculated on data of RIA for methandienone) in the three dimensional space determined by concentration of methandienone, testosterone and the measured response.

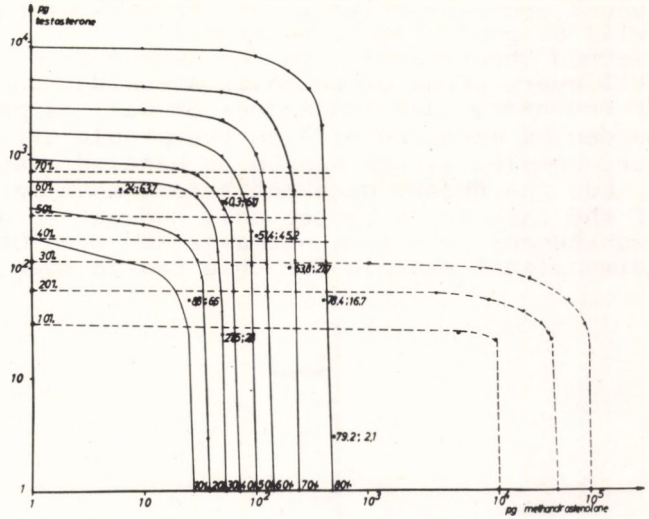

Fig.5 Calculated iso-response curves on the concentration plane i.e. curves joining points of mixtures with identical responses in one of the assays. Solid line: iso-response curves in methandienone assay, dashed line: those in testosterone assay.

The graphic illustration of the above formulae will make the concept more clear (Fig.3). Response of mixtures is calculated taking first the response of the more reactive component, by projection of the response obtained, the corresponding equireactant amount of the less reactive substance can be found, adding to this amount the amount of the less reactive substance in the mixture, the calculated response of the mixture can be read from the graph. This method allowed the estimation of responses of different mixtures within the working range of the two RIAs with an acceptable deviation, as the points in Fig. 5 show. The points here with assigned data pairs are measured mixtures, the constitution of which can be read from the two coordinate axes. The first numbers in the brackets represent response in the A assay, the second ones those in the B assay.

It can be seen, that the range of the method is limited by the response obtainable with the less reactive substance. That is, for the too high specificity of our testosterone antiserum has become a disadvantage. Far better results could have been obtained with a less-specific serum. The proof of this was obtained applying the described calculation method to literature data obtained with substances and antisera of

higher mutual cross-reactivity (3). Results of these calculations will be published elsewhere (4).

The method theoretically can be extended to many dimensions until every group of anabolic steroids, their metabolites (if necessary) and endogenous as well as permissible compounds can be measured with an acceptable efficiency. Such a very complex system has to be handled, naturally, by computer, but the theory does not become much more complicated. If the task is to detect only and not to quantitate certain substances, the response axis can even be omitted. A three-dimensional example for this can be seen on the Fig. 6.

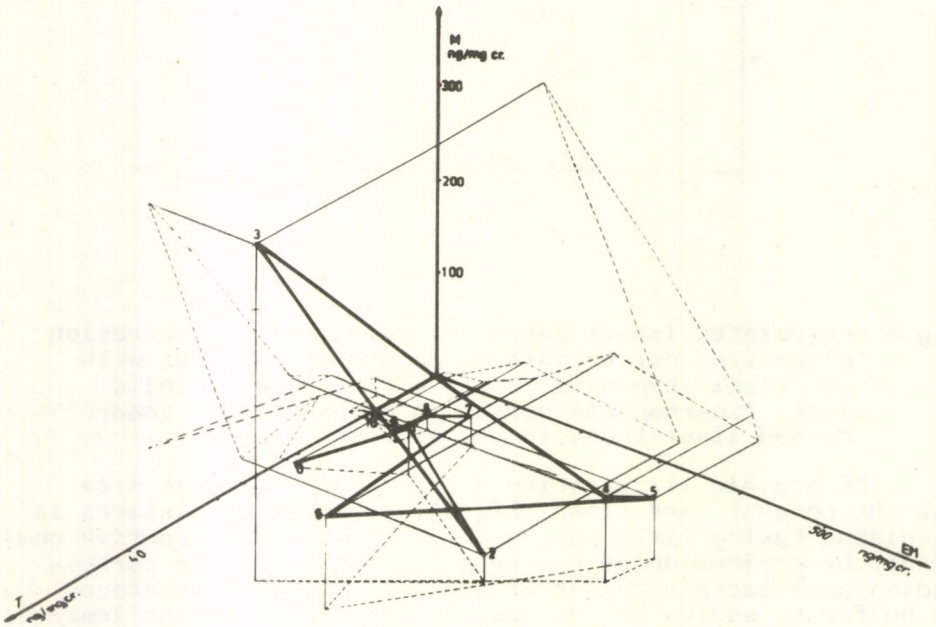

Fig.6. Points representing triple concentrations of human urine samples collected during and after methandienone administration. Concentration axes are: M--methandienone, EM-17-epi-methandienone, T-testosterone. Numbers represent days of the experiment. Solid thick line: curve joining the points in the space, thin lines and dashed lines: projector lines and projected images.

Here the human samples of the experiment discussed in the previous paper (1) were measured by the assays for testosterone, methandienone and 17-epi-methandienone. The latter assay was developed in our laboratory this year (5).

The solid, three-dimensional line joins the points corresponding the urine samples collected on consecutive days during and after the administration. Thinner and dashed lines are projector lines and projected images which are shown only to help spectators to imagine the line in the

space. What is to be observed here is, that on the control day (day 0) the point determined by the measured triple concentrations is quite near to the T axis which represents here the endogeneous compounds. As the excretion of methandienone metabolites starts on the first day, the point leaves the vicinity of this axis and wanders in the direction of epi-methandienone, later also in the direction of methandienone, thus showing the changes in the ratio of the different types of metabolites. Some days after the administration the respective point will return again to the testosterone axis.

By the use of this method not only sports control may become more strong but just, endocrinologists can also gain information on the changing metabolic processes affecting steroid hormones (and probably other substances too). The theory, which will be published this year (6), supposingly can find its use in other radioimmunoassays as well.

Supported by the Scientific Research Council, Ministry of Health, and the National Sports and Physical Education Board, Hungary.

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EFFECT OF BETA-BLOCKING DRUGS ON CORONARY BLOOD FLOW DURING EXERCISE IN DOGS

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Recent experiments have shown that coronary blood flow is under autonomic nervous as well as metabolic control (Ross, 1976).

The purpose of the present investigation was to evaluate the effect of loss of beta-adrenergic activity upon myocardial blood flow and upon the oxygen balance of the heart during exercise in conscious dogs.

METHODS

Experiments were performed on 8 mongrel dogs, chronically instrumented with a Doppler ultrasonic flow probe around the left circumflex coronary artery to measure coronary blood flow, with a pressure gauge in the left ventricle to measure left ventricular pressures, and with catheters in the aorta and in the coronary sinus for measuring of arterial and coronary sinus oxygen content. Three weeks after surgery, hemodynamic measurements were made at rest and at the third minute of a standard submaximal exercise on the treadmill. Each dog was subjected to several experiments on separate days, either in "control" conditions or 15 minutes after the administration of propranolol (1-1.5 mg/kg i.v.).

RESULTS

A control exercise run elicited an increase in heart rate (from 108 ± 7 to 206 ± 11 beats/min), peak left ventricular dP/dt (from 3222 ± 103 to 7062 ± 366 mmHg/s), LV dP/dt/P (from 61 ± 3 to 114 ± 8 sec^{-1}), LVEDP (from 4.5 ± 0.5 to 7.9 ± 0.6 mmHg), and mean aortic blood pressure (from 94 ± 3 to 118 ± 6 mmHg). Mean coronary blood flow rose from 39 ± 5 to 77 ± 9 ml/min, myocardial oxygen consumption shifted from 4.32 ± 0.38 to 10.51 ± 1.22 ml/min, while coronary sinus oxygen content decreased from 4.97 ± 0.35 to 3.95 ± 0.24 vol %. The oxygen delivery - to - oxygen consumption ratio decreased from 1.42 ± 0.05 to 1.29 ± 0.03.

During an identical exercise after propranolol, heart rate (160 ± 8 beats/min), peak LV dP/dt (3107 ± 188 mmHg/s,

and LV dP/dt/P (73 ± 6 sec^{-1}) were markedly lower than during the control run. LVEDP (13.2 ± 1.2 mmHg) was higher, and mean aortic blood pressure was not significantly different. Mean coronary blood flow (51 ± 5 ml/min) and myocardial oxygen consumption (7.94 ± 0.89 ml/min) were significantly lower. The coronary sinus oxygen content (2.58 ± 0.33 vol %) and the oxygen delivery - to - oxygen consumption ratio (1.16 ± 0.02) were also significantly lower than during the control exercise.

DISCUSSION

Propranolol has a depressant effect on myocardial function and coronary blood flow during exercise in conscious dogs, as has been shown by others as well (Bassenge et al. 1972; Horwitz et al. 1974). The lower myocardial oxygen consumption may be caused by the reduction in heart rate and in myocardial contractile force, two important determinants of myocardial oxygen consumption.

The lower coronary blood flow during exercise after propranolol does not necessarily imply that the oxygen demand was not adequately covered since myocardial oxygen consumption was also decreased. In order to evaluate the adequacy of coronary blood flow, myocardial oxygen supply should be related to its demand. Therefore changes in the myocardial oxygen balance expressed as the ratio between oxygen delivery and oxygen consumption were analysed and related to the changes in the coronary sinus oxygen content.

During exercise after propranolol, the oxygen delivery to oxygen consumption ratio as well as the coronary sinus oxygen content were significantly lower than during the control run. These results indicate that propranolol alters the balance between myocardial oxygen supply and demand during exercise, so that a given level of myocardial oxygen consumption is achieved with a proportionally lower myocardial blood flow and a higher oxygen extraction.

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ON THE ADRENERGIC REGULATION OF THE INTRAMUSCULAR GLYCOGEN BREAKDOWN DURING EXERCISE

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The available data on the role of the adrenergic system in activation of glycogenolysis in skeletal muscles during exercise are controversial. In the rat, adrenomedullectomy as well adrenomedullectomy combined with 6-hydroxydopamine treatment were found to prevent fully (4,11) and to have no effect (12,14) on the exercise-induced intramuscular glycogen breakdown. Also the beta-adrenergic receptor blockade gave contradictory results both in the dog (7,9) and in the rat (5,8). A reason of these discrepancies could be different work loads applied by the authors. In the present work, the possibility that the involvement of the adrenergic system in skeletal muscle glycogenolysis during exercise could depend on a work load was examined in rats performing different muscular efforts.

Methods

The experiments were carried out on male Wistar rats, 230-250 grams of the body weight, fed on commercial pellet diet for rodents. They were subjected either to running or to swimming exercise. The level of glycogen was determined by the method of Carroll et al. (1956) in samples of the following muscles: 1-the most superficial layer of the left vastus lateralis (FGmuscle), 2-the deepest layer of the same muscle (FOG muscle), and 3-the soleus (SO muscle). Blood glucose level was determined by the method of Hultman (1959). The obtained results were evaluated statistically using the Student-t test for unpaired data. Mean values were calculated from the data obtained from ten rats.

Results

Muscle glycogen (table 1).

Running 30min, 12m/min, 0^{o}incline. In the control rats, the exercise resulted in significant reduction of the glycogen level in the examined muscle ($P<0.001$ for each muscle). Treatment with propranolol fully prevented the reduction of the glycogen level in FG and FOG muscles. The glycogen level in SO muscle of the propranolol-treated rats was also reduced

($P<0.001$ vs. the resting level) but it remained higher ($P<0.001$) than the respective level in the control rats.

Running till exhaustion, 12m/min, 0^{o} incline. The time of running till exhaustion in the control group was 239 ± 32 min and in the propranolol-treated group 186± 44 min ($P<0.01$ vs. the control). The exercise induced significant reduction of the glycogen level in the muscles of the control group ($P< 0.001$ for each muscle) and treatment with propranolol had no effect on the process.

Running till exhaustion, 30m/min, 5^{o} incline. The time of running till exhaustion was 19.1± 8.1 min in the control group and 14.5± 4.9 min in the propranolol-treated group (the difference was insignificant). The post-exercise glycogen levels in the muscles were significantly lower than the r resting levels both in the control and in the propranolol--treated rats (P 0.001 at each case). However, the glycogen levels in FOG and SO muscles of the propranolol-treated rats were higher than in the control rats ($P<0.001$ for each muscle).

Table 1. The effect of propranolol (6mg/kg, intraperitoneally, 20min before the exercise) on the exercise-induced intramuscular glycogen level (μmol of glucose/g of tissue). C-control group; P-propranolol-treated group.

	Group	Vastus superficial (FG muscle)	Vastus deepest (FOG muscle)	Soleus (SO muscle)
Resting control		31.7± 2.9	27.8± 3.3	29.1± 5.6
Running 3o min 12m/min 0^{o} incline	C	27.3± 2.5	19.7± 3.2	12.5± 2.9
	P	34.2± 4.1	25.5± 3.5	22.6± 2.2
Running till exhaustion 12m/min 0^{o} incline	C	23.6± 5.3	16.4± 3.7	14.0± 2.5
	P	26.8± 5.0	14.1± 6.4	14.3± 3.1
Running till exhaustion 30m/min 5^{o} incline	C	12.8± 7.6	6.1± 1.5	7.3± 1.7
	P	14.5± 6.9	10.2± 1.9	16.2± 2.4
Swimming 75min	C	31.6± 4.5	16.0± 2.3	16.0± 3.8
	P	35.1± 6.3	29.6± 4.8	27.7± 4.5

Swimming 75min. The swimming reduced glycogen level in FOG and SO muscles ($P < 0.001$) in the control group and it was fully prevented in the propranolol-treated group.

Blood glucose level.

The blood glucose level was sifnificantly reduced only in the rats running till exhaustion with the speed 12m/min (the resting level-6.24± 0.89, the control group -3.79±0.86, the propranolol-treated group-4.21±0.43 mmol/l; for the both groups $P < 0.001$ vs. the resting level).

Discussion

The obtained results clearly show that the requirement for the adrenergic system to activate the skeletal muscle glycogenolysis during exercise depends both on intensity and duration of exercise and on the type of examined muscle as well. Thus this finding seems to elucidate the reason of the discrepancies in results obtained by the other authors.

A factor regulating of the adrenergic involvement in the mobilization of the muscle glycogen during exercise remains unclear. Only the rats running till exhaustion with the low speed developed hypoglycemia. In consequence, the working muscles were inadequately supplied with the blood glucose. It is very likely that the marked shortage of glucose could activate the nonadrenergic mechanism stimulating glycogenolysis (i.e. mainly that mediated by ionized calcium-ref.3) so strongly that it could overcome the inhibitory effect of the beta-receptor blockade. The same mechanism could activate strongly the nonadrenergic mechanism also in FG muscle of rats running till exhaustion with the speed 30m/min. This muscle is known to be particularly involved during vigorous exercise (1) and because of the low activity of hexokinase (10,13) the supply of the muscle with glucose was probably far below the requirement, despite normoglycemia. Basing on this hypothesis one might speculate that during the short lasting efforts of low intensities in which the glucose supply was sufficient, the factor activating the nonadrenergic system did not appear. On the other hand, in those cases in which the beta-receptor blockade prevented partly the glycogen mobilization, the supply of the muscles with glucose was probably only slightly lower than the requirement of the particular muscle. It triggered the nonadrenergic mechanism but its activation was to low to overcome fully the beta-receptor blockade.

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A STUDY OF AUTONOMOUS INFLUENCES ON ADRENERGIC RESPONSIVITY OF PHYSICALLY TRAINED HUMANS AND ANIMALS

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Several reports have shown that regular physical training modifies the cardiovascular responsivity to exogenous catecholamines, but as regards the direction of this shift, the data are rather contradictory /1,2,3,4,10,11,12,13,14,15/. Previously we have made several studies in this respect /6,8/ according to which in physically trained humans and albino rats the elevation produced by alpha adrenergic stimulation was smaller, while reduction in blood pressure level after alpha blockade was larger. In regard of the heart rate response, beta stimulation was more effective, and beta blockade decreased cardiac frequency only moderately.

These results indicate that an altered sensitivity of the adrenergic receptors is not the most important cause of this modified responses. It seems to be a more likely assumption that it is a shift in the resting autonomous equilibrium, i.e. the modified level of some resting cardiovascular parameters, such as bradycardia or reduced cardiac output of the physically trained subjects, which would bring about these altered responses /5,7,9/.

The purpose of the present work was a further study of this assumption. To this end top-athletes of various branches of sports, swim-trained albino rats and the respective controls were subjected to artificial or pathological conditions. In these cases there was no longer any difference between the trained and non-trained groups in the resting heart rate and blood pressure. It was examined further whether the inter-group differences in the catecholamine-induced responses still existed or not. As such models, bilateral vagotomy and the ulcer-provoking restraint stress were used in albino rats, and duodenal acidification was made in humans.

Animal experiments were made in Wistar female albino rats. Physical training was a regular daily swimming of 60 to 120 minutes with a ballast of 4 g/100 g body weight. The training program lasted 10 to 12 weeks. Non-trained animals of the same age were the control group. In humans the physic-

ally trained group consited of top-athletes of endurance sports. Non-athletic university students were the controls.

In the first experiments the effect of bilateral vagotomy was examined in albino rats, under i.p. urethane or chloralose anaesthesia. The right common carotid and the left jugular vein were cannulated to record blood pressure and to administer the test substances. ECG, heart rate and blood pressure were recorded continuously by a four-channel Hellige hot-pen recorder.

After vagotomy there was no difference between the trained and non-trained groups either in resting blood pressure or in resting heart rate, and there was no intergroup difference in the norepinephrine-induced pressor responses, which had been established before vagotomy /Fig.1/.

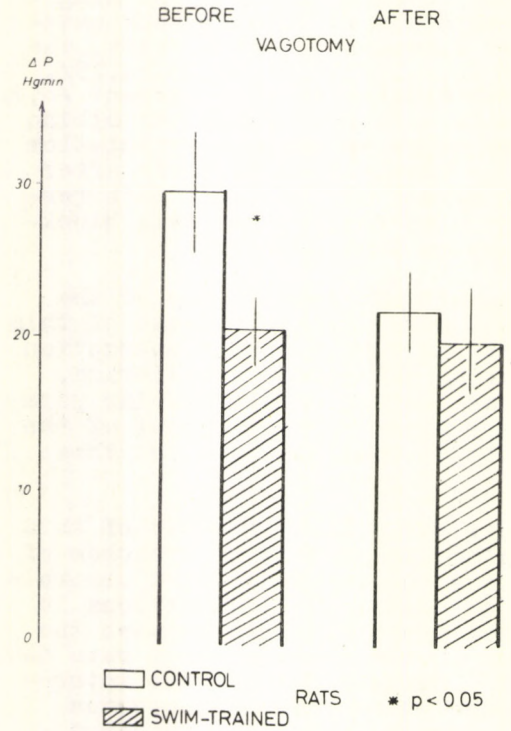

Fig.1. Blood pressure responses to 0.5 /ug/kg of norepinephrine $/\bar{x} \pm s_{\bar{x}}/$

The 2nd Figure indicates the heart rate responses to isoproterenol before and after bilateral vagotomy. Both before and after it tachycardia responses of the swim-trained animals were more marked.

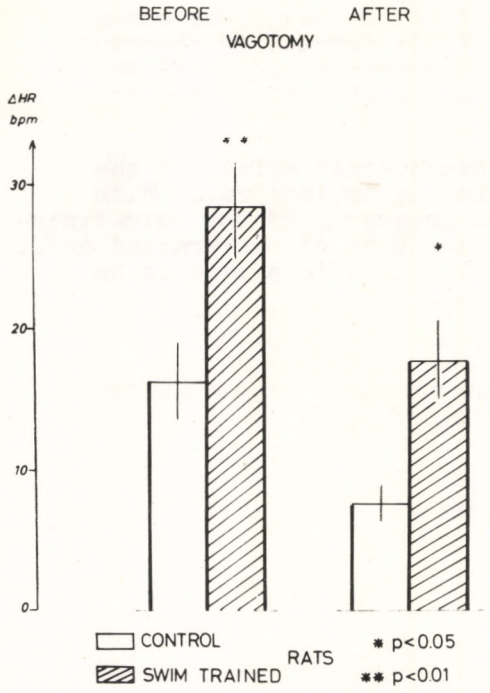

Fig. 2. Heart rate responses to 0.5 ug/kg of isoproterenol / $\bar{x} \pm S_{\bar{x}}$ /

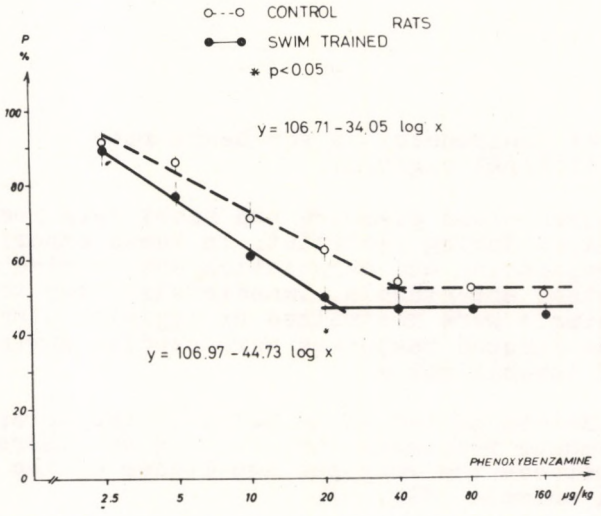

Fig.3. Effect of phenoxybenzamine on the blood pressure after bilateral vagotomy / $\bar{x} \pm S_{\bar{x}}$ /

In respect of the vasodepressor effect of the alpha antagonistic phenoxybenzamine only a small difference is observed between the swim-trained and the non-trained animals: the decrease of blood pressure is slightly steeper in the trained rats /Fig. 3/.

The 4th Figure demonstrates the bradycardia effect of the beta blocking agent propranolol in vagotomized rats. With 500 ug/kg of propranolol cardiac frequency of the swim-trained rats was significantly lower than that of non-trained ones. This difference indicates that beta blockade proved to be more effective in the trained group.

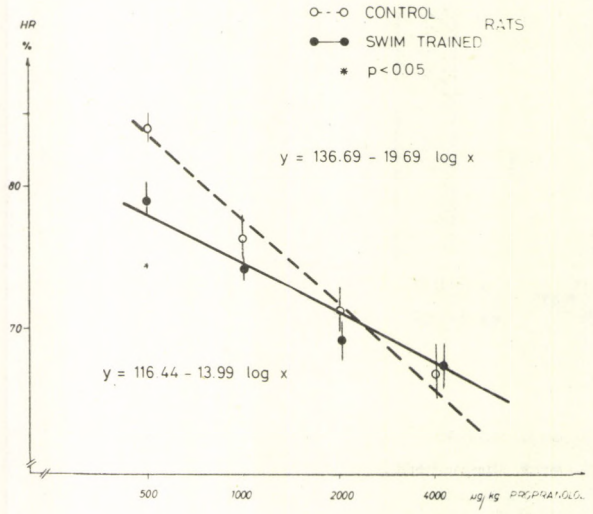

Fig. 4. Effect of propranolol on the heart rate after bilateral vagotomy

In the next two figures blood pressure and heart rate responses are demonstrated during restraint. In these experiments the vessel preparation and cannulation was carried out under superficial ether anaesthesia. Immediately after the intervention the animals were restrained by applying plaster casts. Catecholamine-induced responses were studied during a four hour period of immobilization.

During the immobilization period an increase of the norepinephrine-induced pressor responses was observed, but there was no difference between the response amplitudes of the trained and control animals /Fig. 5/.

As there was no time-related change in the tachycardia response, the 6th Figure indicated only the average values of the isoproterenol-induced heart rate responses. With every dose the swim-trained rats showed a more marked elevation,

with 2 /ug/kg of isoproterenol the difference was significant.

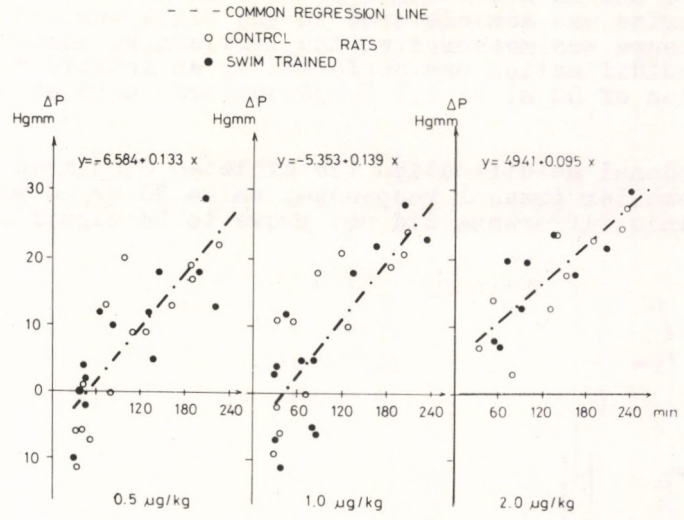

Fig. 5. Blood pressure responses to norepinephrine during immobilisation

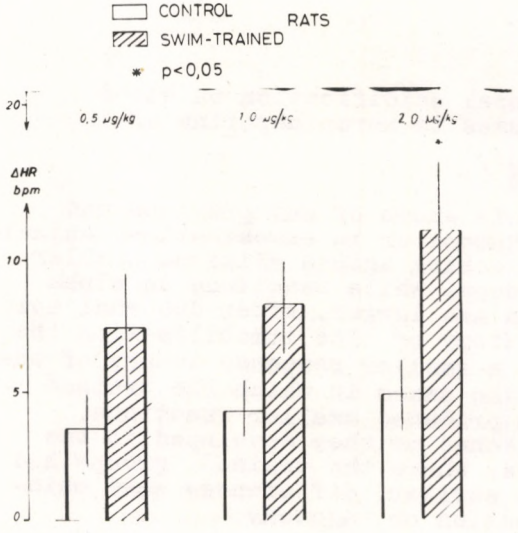

Fig. 6. Heart rate responses to isoproterenol in immobilized rats
/ $\bar{x} \pm S_{\bar{x}}$ /

The 7th Figure demonstrates the norepinephrine-induced pressor responses before and after duodenal acidification in physically trained and in non-trained humans. In these examinations norepinephrine was administered in the right cubital vein, blood pressure was measured on the left arm by auscultation. Duodenal acidification was performed by an intraduodenal administration of 50 ml of 0.1 N hydrochloric acid at body temperature.

Before duodenal acidification the athletes displayed significantly smaller pressor responses, while 30 or 60 minutes after it this difference did not prove to be significant.

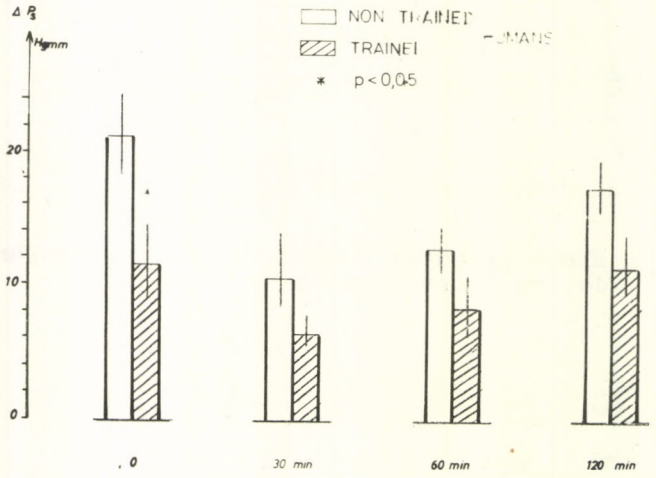

Fig. 7. Effect of duodenal acidification on blood pressure responses to norepinephrine of 5 /µg / $\bar{x} \pm s_{\bar{x}}$ /

In the 8th Figure a summary is shown of our previous and present results. In intact humans or in anaesthetized animals alpha stimulants and beta blocking agents elicited smaller responses in the trained groups, while reactions to alpha blockade or beta stimulation are larger. After duodenal acidification or bilateral vagotomy or else immobilisation the trained groups did not give a smaller response to any of the studied substances. In all the cases in which the trained - groups of intact regulation produced smaller reactions, these differences were abolished or they developed in the opposite direction. In cases, where the trained groups had shown the greater responses earlier, differences were maintained also after immobilisation or vagotomy.

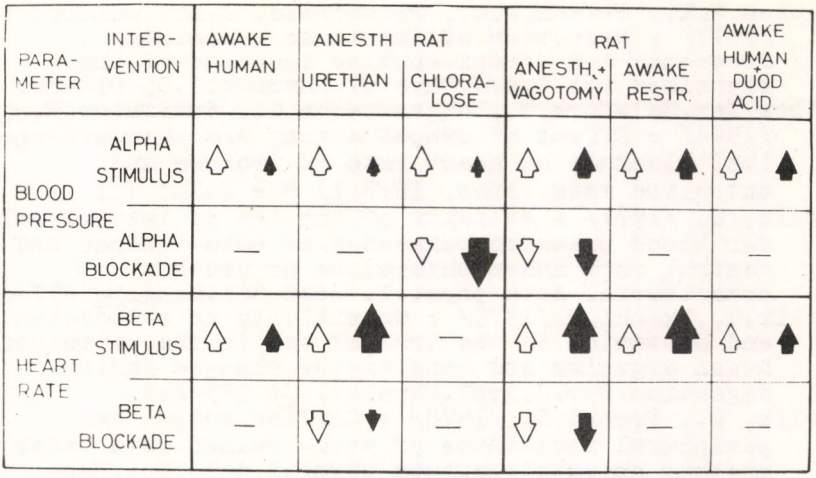

Fig. 8. Cardiovascular responses to exogenous catecholamines in physically trained /black arrows/ and in non-trained /white arrows/ subjects.

According to these data the assumption is untenable that regular physical training would decrease the sensitivity of the adrenergic receptors. If it does have any direct modifying effect upon receptor sensitivity, that might be rather an upgraded responsivity, in particular with the beta receptors. Naturally, in an in vivo experiment responses given to different adrenergic stimulants or blocking agents depend on several other factors as well, so the responses may be even smaller in the trained group. Among these other factors the autonomous equilibrium of the physically trained subjects seems to have a special importance.

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THE CHANGE OF DOPAMINE HYDROXYLASE ACTIVITY IN RESPONSE TO MUSCULAR WORK IN HUMANS AND IN ALBINO RATS

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INTRODUCTION

Dopamine beta hydroxylase /DBH/ is the enzyme catalyzing the last step in the biosynthesis of norepinephrine. The presence of DBH has been established in the organs under sympathetic nervous control, so for instance in the vessels, heart, spleen, adrenal medulla and also in the brain. DBH liberation in the sympathetic nerve endings, in the neurons of the CNS and in the adrenal medulla was reported to take place under nervous stimulation together with that of norepinephrine and in amounts proportionate to the latter. Later DBH was found also in the serum of a number of species each species having a particular serum concentration of it.

The existence of a parallelism between adrenergic nervous activity and the level of DBH in the adrenergic neurons and in the adrenal medulla has been supported by many data. Effects, such as psychological stress, cold or immobilization stress, which enhance sympathetic activity, have been found to increase also DBH activity in the heart the sympathetic ganglia the adrenal gland and the brain as well as in the serum of animals. It is reasonable to suggest that on the basis of these observations DBH activity is an acceptably good indicator of sympathetic activity in animal tissues and serum.

Observations in humans are, however, more controversial in that the serum level shows large interindividual variation. This makes the evaluation of DBH activity in human sera more difficult.

The point in the present study was the question whether DBH activity may be used to assess or specify the level of physical fitness in rats and in human subjects /1, 2, 3/.

METHODS

DBH level was weekly estimated in the serum and adrenal gland of albino Wistar rats, both at rest and after an all-out bout of swimming, in the course of a swim training of 12 weeks and for four weeks after cessation of the exercise regime.

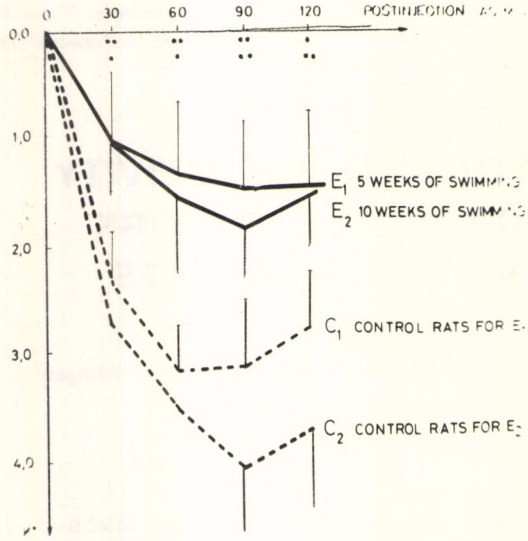

Fig. 1. Histamin-induced decrease in rectal temperature in relation to regular swimming exercise in the rat. Lag time in minutes.

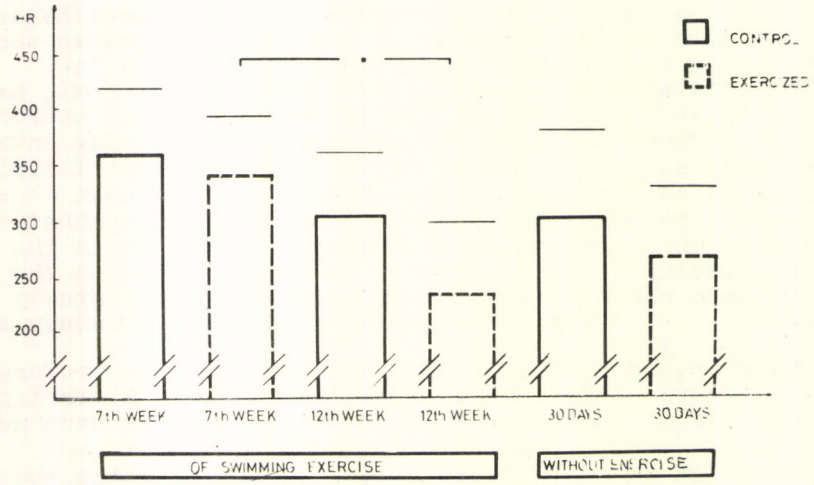

Fig. 2. Heart rate at rest in relation to duration of regular swimming in the rat /$\bar{x} \pm sd$/.

The level of fitness achieved by the animals was assessed by measuring the decrease in rectal temperature under the effect of histamine as well as by recording heart rate.

In the human studies serum DBH level was estimated in samples obtained before and after bouts of bicycle or treadmill exercise. The subjects were Class I athletes and nonathletic university students.

In the several methods reported for the estimation of DBH activity in tissues and body fluids the basic principle is the nonspecific nature of DBH in that it can catalyze also the hydrolysis of thyramine into octopamine in the presence of suitable cofactors. The amount of octopamine produced was used in the present study too to estimate DBH activity, octopamine being determined chemically as para-hydroxy-benzaldehyde following oxidation by sodium periodate /4, 5, 6/.

RESULTS

Histamine had a significantly reduced effect on the rectal temperature of swim-trained rats, the difference from controls being the most marked by the tenth week of regular exercise /Fig. 1/.

The rats subjected to regular exercise developed progressive bradycardia so after the seventh week a significant intergroup difference arose /Fig. 2/.

Both of these experiments served actually the verification of the training effect. This decrease in histamine sensitivity was described first by Frenkl and associates /7/ while the development of training bradycardia is a fundamental phenomenon in exercise physiology.

Figure 3 refers to the change in DBH level in the course of an exercise regime. The curves of the respective groups run actually in parallel during the 12 weeks of training, but they fail to demonstrate such changes that would correspond to those found in the two other parameters observed.

Figure 4 presents the DBH levels obtained before and after an ergometric exercise on the bicycle in P.E. majors and in nonathletic technology students. While DBH activity was significantly higher in the technology students after the exercise, P.E. majors showed no appreciable change.

Postexercise means of wrestlers and runners are shown in Fig. 5. The treadmill exercise elicited a significant rise in the DBH activity of both groups, but it has to be noted that there had been a considerable difference between the levels of rest too.

DISCUSSION

These experiments studied the effect of physical exercise on DBH activity in the serum of rats and human subjects. Progressive improvement of physical fitness in the animal experiments was ascertained by assessing the development of histamine resistance and exercise bradycardia. In the evolution of DBH activity a trend similar to the signs of increasing fitness could not be observed.

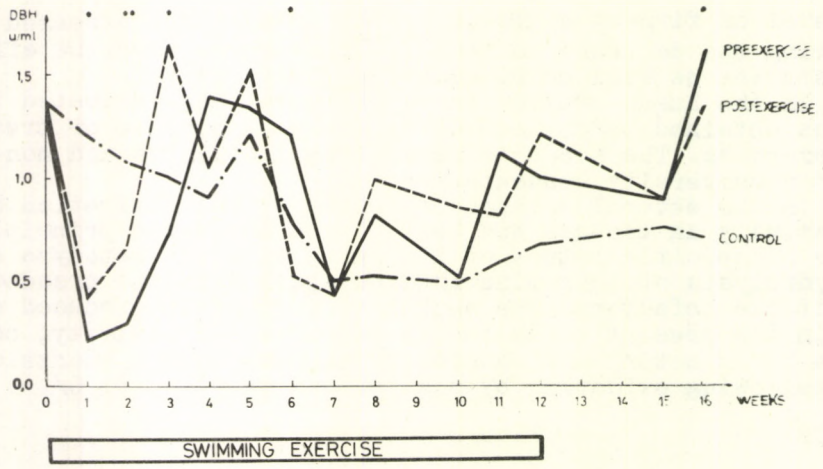

Fig. 3. Serum DBH activity before and after exhaustive swimming in the rat.

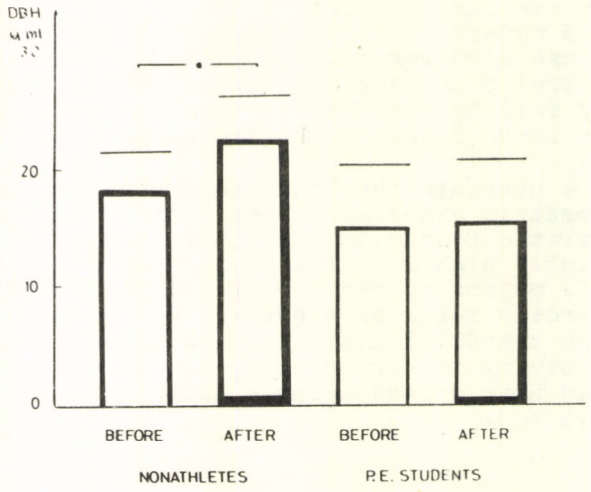

Fig. 4. Serum DBH before and after exhaustive ergometric work in groups of different activity /$\bar{x} \pm$ sd, asterisk denotes significant difference/.

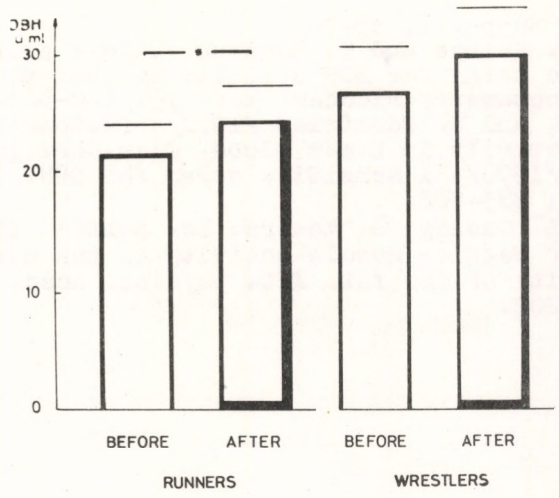

Fig. 5. Serum DBH before and after exhaustive ergometric work in top level athletes /symbols as in Fig. 4/.

The human studies were not of a prospective nature, that is, it was not the improving fitness that was monitored. Here the postexercise response was observed in human subjects which were grouped according to their athletic history. The obtained findings resemble the ones observed in the course of previous cortisol studies in which the extent of the response depended on the intensity and volume of the work load imposed under laboratory conditions, and on the emotional factors under competitive circumstances.

The role of emotions cannot, naturally, be ruled out under laboratory conditions either. The response or its absence seen in the present studies may also be related to the mentioned two factors. As a conclusion, it may be suggested that the estimation of DBH activity in human serum can be used to complement exercise studies involving nervous stress, but at least in view of the present results, without the promise of more specific issues.

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HUMAN EXERCISE AND ENZYME INDUCTION

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INTRODUCTION

Regular muscular activity has been shown by our previous experiments to influence the biotransformation rate of certain drugs in the same manner as the administration of such substances that induce the microsomal enzyme system of the liver. To mention some of these results, hexobarbital effect was shorter in rats subjected to regular swimming or running, the activity of hepatic monooxygenase enzymes of the microsomal system was higher, and the content of cytochrome P_{450} of the liver rose /1, 2/.

This enzyme-inducing effect of muscular exercise could be established in man as well. Active athletes showed a faster excretion rate of spironolactone and also the half life of intravenous Aldactone was shorter /3/.

The present paper reports on our observations made in three respects in athletic and nonathletic university students.

1. In clinical practice the question of whether or not the system of drug metabolising enzymes in the hepatic microsomes has been induced can be settled also by estimating the biological half life of antipyrine. So first antipyrine elimination was studied in the further course of our experiments.

2. In the subsequent experiments the disappearance rate of intravenously administered bromophenolphtalein /BSP/, another substance widely used in clinical practice, was examined.

3. Substances which induce the microsomal monooxygenase system enhance the urinary excretion of D-glucaric acid /DGA/ as well. The amount of excreted DGA is an accepted though indirect indicator of enzyme induction. This third approach also warranted closer examination. /4/

MATERIAL AND METHODS

All experimental groups consisted of male healthy nonsmoker students of the same age /2o to 22 yrs/.

Antipyrine half life was calculated in 2o physical

education majors /8 females and 12 males/ engaged in regular competitive sportactivity as well as in 12 athletic students without regular competitive engagement. The results were compared to a nonathletic group of 12 students. Plasma antipyrine was estimated by Brodie's method /5/ in samples taken 4, 8, 12 and 24 hours after oral administration of 18 mg per kg body weight.

BSP was determined in 8 athletic and 8 nonathletic subjects by using Bromthaleine® /Merck, Darmstadt/ and the photometric method suggested by the producer.

The method of Marsh /6/ was used to estimate DGA in samples of urine pooled for 24 hours. Both athletic and nonathletic groups consisted of 30 subjects each.

RESULTS

Antipyrine halving times are shown in Fig. 1. Noncompetitive students had a faster elimination than nonathletic controls, and the half life of antipyrine was the shortest in the P.E. majors who were engaged in regular competitive activity and involved in the most intense training work, being top athletes.

Figure 2 illustrates antipyrine elimination in the female handball players of the Hungarian University of Physical Education. They were studied four times in a year, in different periods of preparation. Mean half lives were rather similar at all points of time, revealing that elimination rate is fundamentally related to the level of general physical fitness. Of the 32 studies performed, there were three cases in which elimination rate was markedly slower for a time, both in comparison with the group mean and the subject's usual individual level. In all the three cases an intercurrent health impairment was disclosed to have occurred preceding the study.

BSP elimination rate was significantly faster in the physical education students than in the nonathletic ones /Fig. 3/.

The amount of excreted DGA did not differ between the examined groups. When, however, RGH-3332, an enzyme inducing agent manufactured by Richter Ltd., Budapest, was administered to the same subjects in a daily dose of 300 mg over a week, the excreted amount of DGA was larger in the athletic group /Fig. 4/.

DISCUSSION

Regular exercise acts as an inducer of the hepatic microsomal entyme system also in man. Antipyrine half life time becomes shorter in proportion to the intensity of habitual muscular exercise. The serial examination of the female handball players has shown that, in addition to general fitness, the rate of drug elimination is also related to the actual sports form or health status.

The mechanism by which endogenous induction develops has not yet been cleared. It is reasonable to assume that besides a changing activity of the microsomal system of en-

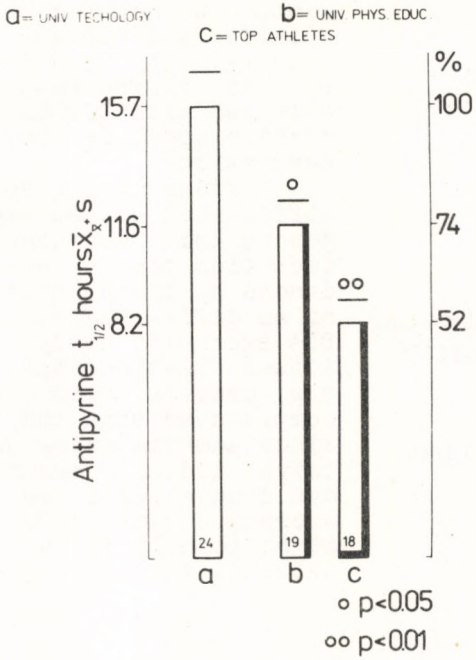

Fig. 1. Elimination rate of antipyrine in three groups of different habitual activity.

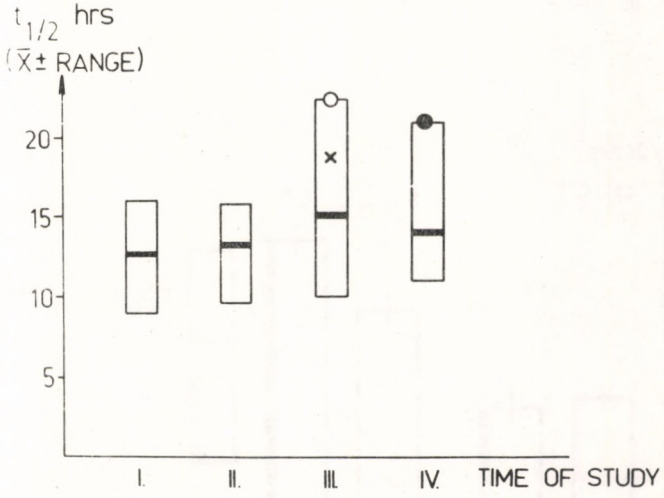

Fig. 2. Antipyrine half life in female handball players at different times of the training year /$\bar{x}$ ± range/.

zymes also hepatic circulation and biliary secretion may have some role in it. The results obtained with BSP excretion speak for this assumption.

There may be some difference between exogenous and endogenous induction too, as evidenced by the verdict of no difference in the DGA excretion study. Actual relationships are, however, even more complicated than that since administering an enzyme inducing agent could accelerate the excretion rate of DGA significantly in the athletic group.

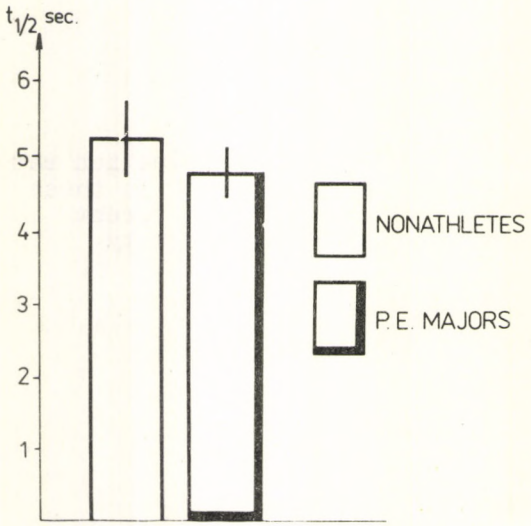

Fig. 3. Bromthalein elimination rate in male students /$\bar{x} \pm$ sd/.

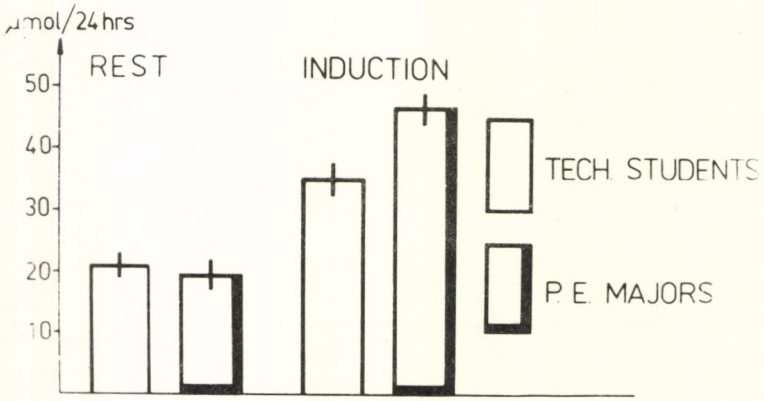

Fig. 4. Urinary DGA excretion before and after pharmacological enzyme induction /$\bar{x} \pm$ sd/.

The clarification of the possible mechanisms and the probable changes in the drug sensitivity of athletic subjects is the objective of our further studies.

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A STUDY OF DRUG METABOLISM IN ATHLETES

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The microsomal enzyme system has been previously shown to be more active in rats exercized by regular swimming or running than in untrained ones. This phenomenon seemed to be very similar to pharmacological enzyme induction: it could be demonstrated by a reduction in hexobarbital sleeping time, by an increase of the cytochrome P450 content and NADPH-cytochrome reductase activity of the liver as well as by a rise in canrenone elimination rate /1, 2/.

The phenomenon has been observed also in human subjects. In athletes antipyrine half life was markedly shorter than in nonathletic subjects /3/. Execise induced changes have been reported concerning the kinetics of tetracyclines, sulfonamides and also of diazepam / 4 /. The obvious inference was that among the factors influencing the pharmacokinetics and metabolism of drugs the level of general physical fitness might be one of greater importance.

The aim of our present work was to study the pharmacokinetics and metabolism of metronidazol in athletic and nonathletic groups of young adults and to gain more accurate knowledge about the mechanism of the exercise induced changes. This chemotherapeutic agent is metabolized mainly by the microsomal enzyme system and is excreted via the kidney / 5 /.

MATERIAL AND METHODS

Our observations were carried out on volunteers, seven of whom were physical education students involved in regular physical training while other seven were technology students who did not report of any regular physical activity or exercise. The volunteers underwent a series of laboratory studies and endurance fitness tests /Table 1/. The two groups had a different mean body weight, but no difference could be demonstrated between them in regard of aerobic power.

After an overnight fasting 500 mg of metronidazol /Klion®/ was administered orally. Blood was sampled at 0, 50, 90 minutes and 2, 3, 4, 8, and 24 hours after ingestion of the drug. Urine samples were pooled in three fractions /one for between 0 and 8, 8 and 16, respectively 16 and 24 hours each/. Estimation of plasma metronidazol was performed by

Table 1. *CHARACTERISTICS OF THE GROUPS UNDER STUDY*

* $p < 0,05$

	AGE	WEIGHT kg	VO_2 max ml/kg min	CREATININE mg%
TRAINED	21,3 ±1,9	74,0 ± 6,0	53,0 ±6,0	0,99 ± 0,1
UNTRAINED	22,2 ± 2,1	* 68,0 ± 7,0	47,0 ±4,0	0,92 ± 0,1
	CLEARANCE ml/min	ALBUMINE g%	GLOBULINE g%	HAEMATOCRIT %
TRAINED	132,6 ± 10,6	4,1 ±0,2	3,3 ± 0,5	50,2 ± 4,0
UNTRAINED	126,2 ±13,7	4,2±0,2	3,3 ±0,4	49,6 ± 6,0

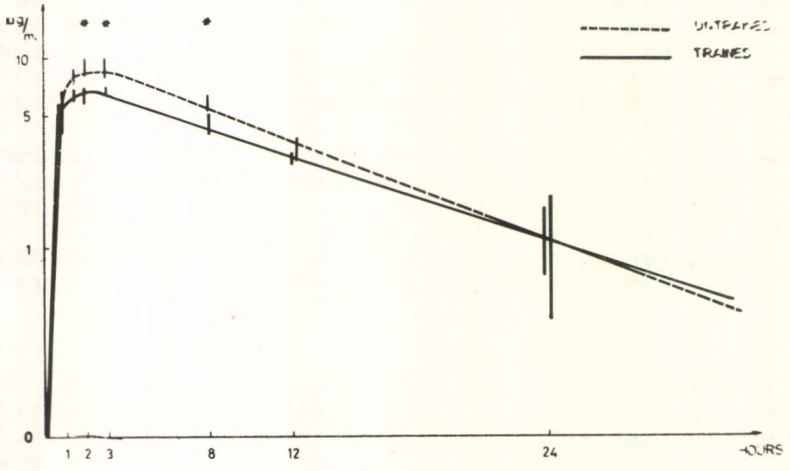

Fig. 1. Metronidazol concentration curves in the plasma of athletic and nonathletic subject groups /n= 7 each/.

Table 2. CHARACTERISTIC PHARMACOKINETIC PARAMETERS

* $p<0,05$

	C_{max} µg/ml	T_{max} min	$T_{1/2}$ h	K_{el} h	V_d l	Pl CLEARANCE l/h
TRAINED	6,7 ± 0,7	180	8,5 ± 3,9	0,10±0,07	*81,0±27	8,1 + 2,4
UNTRAINED	8,6 ±0,5	120	6,7± 3,0	0,12±0,09	50,5±13	6,0 ± 1,9

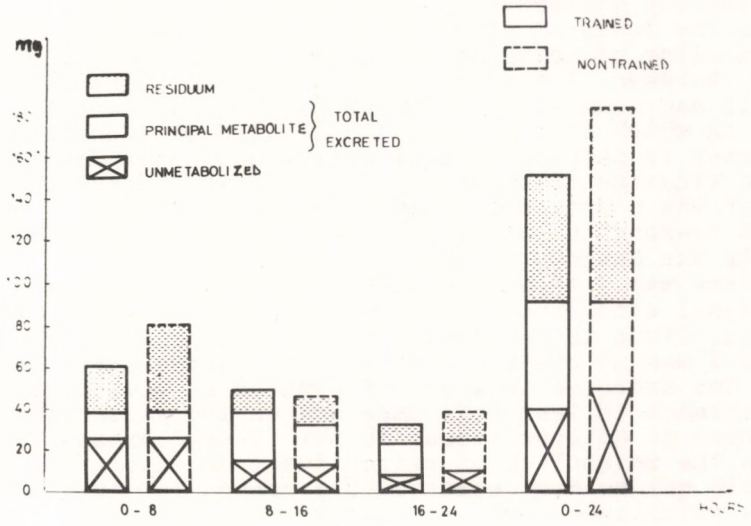

Fig. 2. Urinary excretion of metronidazol and metronidazol metabolites in the various fractions of pooled urine in athletic and nonathletic subjects.

photometry /6/. In the urine samples the concentrations of metronidazol, of 1-/hydroxyethyl/-2-hydroxymethyl-5-nitroimidazol and total excreted metabolites were determined by a photometric method newly developed for this purpose /7/.

In order to correct for the differences existing in the body weight of the subjects the obtained plasma concentrations were multiplied by the individual's body weight. Elimination rate constants were estimated after logarithmic transformation by regression analysis. The calculated pharmacokinetic parameters were V_D, beta weights, half life, plasma clearance and maximum concentration Cmax. Statistical analysis was performed by using the t test.

RESULTS AND DISCUSSION

The plasma concentration curves are represented in Fig. 1. The values at 90, 120 and 180 minutes, respectively at 8 hours after ingestion were significantly lower in the athletic group. The most important kinetic parameters are summarized in Table 2. The volume of distribution was significantly larger in the athletic group.

The obtained data on the urinary excretion of metronidazol and its metabolites are demonstrated in Fig. 2. Neither the successive fractions, nor the total amount of excreted metronidazol, nor its main metabolite did show any significant intergroup difference.

On the basis of previously reported results concerning the metabolism of metronidazol a significant difference was expected between the trained and untrained groups. This expectation was founded on former human observations and animal studies in which the biological half life of a number of drugs was shorter in physically more active subjects. Considering that the kinetics of these test substances depends on the activity of the microsomal enzyme system, this kind of adaptation may reasonably be called a state of "trained liver" so termed by the analogy to cardiac adaptation.

There was a significant intergroup difference in peak metronidazol concentration as well as in its volume of distribution. Since these parameters are independent from the microsomal enzyme system, the kinetic changes may be attributed to the exercise induced differences in body composition: to an increase in lean body mass and an increased ratio of muscle mass to adipose tissue in the trained group.

In the metabolism of metronidazol an intergroup difference could not be demonstrated. One reason for this might be that the volunteers could not be categorized in two distinctly separate groups by the standard endurance fitness test. Another factor might be that metronidazol behaved differently from the substances studied before, especially from antipyrin.

Our results can be summarized by concluding that the reported changes in the kinetics found in the athletes are due, in addition to an increased metabolization rate, to the differences in body composition.

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CHANGES OF BLOOD VISCOSITY IN ADOLESCENT SWIMMERS AND ADULT WEIGHT-LIFTERS

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In 1974 Barclay and Stainsby demonstrated that the degree of blood flow is the main limiting factor of muscle work. In the literature there are very few data about the alteration of the blood viscosity as a consequence of physical loading. In 1936 Sato found that muscle work lasting for one minute increased the serum viscosity by 1 %, whereas Pavey /cited by Harkness/ could not observe an unidirectional and characteristic change in the plasma viscosities of two swimmers after swimming for 1 hour. The reason for these unsatisfactory and contradictory data lays on the well-known difficulties of measuring blood and plasma viscosity.
Our investigations were carried out with an earlier-developed capillary viscometer /Matrai et al, 1977/, which is suitable for serial measurements. The aim of our work was to study the different effects of training upon sportamen of different kinds and untrained volunteers, with special regards to the blood viscosity. Searching for correlation between the sports-results and the measured parameters, blood viscosity was checked in the competitiv periods.

Methods

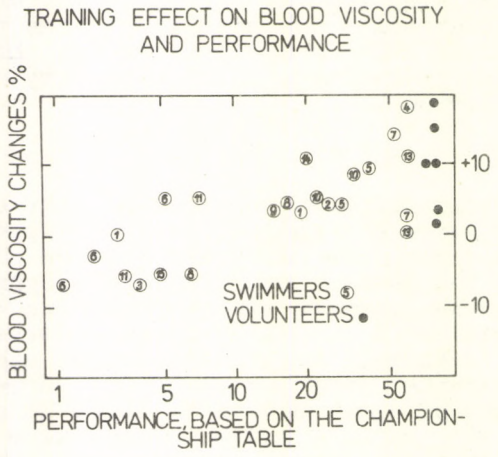
Thirteen 11-16 year old swimmers, nine 18-24 year old weightlifters and six 20 years old medical students were investigated, the sportsmen during the different periods of an one year training program. The medical students and weight-lifters were males, while the swimmers were represented by both sexes though the tendencies in the alterations observed were independent of sex differences. The tested sports, athletic weight-lifting and endurance demanding swimming, represent two of the utmost points of sport activity. There is also a difference in the training record, since the swimmers begin to learn and train at an early age /5-6 years old/, whereas the weight-lifters are loaded with a serious physical exertion after a relatively untrained childhood, at the end of puberty. Furthermore, the swimmers train twice a day in the morning and in the evening, while the weight-lifters train only once daily the afternoon.

The blood samples from the medical students were obtained before and after a 2-hour running period in the afternoon, and from the weight-lifters and swimmers before and after the afternoon or evening training session. The following parameters were determined: blood viscosity, plasma viscosity, blood fibrinogen concentration, fibrinogen degradation products D and E, microhaematocrit, prothrombin concentration, platelet count, erythrocyte count, haemoglobin concentration, peripheral blood smear and acid-base parameters /Astrup/. The present paper discusses only the alterations in the blood viscosity and blood fibrinogen level.

For viscosity measurements the blood was collected in 4 ml plastic tubes, closed until assay, which was carried out within one hour at 38 °C. 12.5 IU/ml heparin was used to prevent blood coagulation /for further details see Matrai et al. 1977/.

Results

Figure 1. shows the changes in the blood viscosities of swimmers and medical students after training. For some swimmers more than one training result is included. Horizontal axis is a logarithmic presentation of the sports success attained during the testing period and that estimated on the basis of the national championship table. Regression analysis of the data proves that a strong correlation exists between the two variables /$p < 0.001$/. The blood viscosity values were converted to values relating to a standard haematocrit level /45 %/: this is possible since the logarithm of the viscosity is proportional to the haematocrit level.



The data obtained from the weight-lifters did not show a similar correlation with the blood viscosity values. However, as compared to the medical students, the increase in the blood viscosity after training was of a smaller degree, see Table I.

As previously reported /Fendler et al. 1977/, the blood fibrinogen level is generally increased by severe physical exertion, and this increase is inversely proportional to the degree of the performance. However, in juvenile swimmers, even if they begin their training with a higher fibrinogen level as a result of the previous training, a strongly decreased fibrinogen concentration can be observed at the end of the training, see second column of Table II. The characteristic changes in the fibrinogen levels are presented in Table II.

Table I.

The changes of blood viscosity by the effect of training means and SE in centipoise

control group		swimmers		weight-lifters	
before	after	before	after	before	after
$3,6\pm o,1$	$4,o1\pm o,o6$	$3,6\pm o,1$	$3,8\pm o,2$	$4,2\pm o,2$	$4,4\pm o,2$
converted on 45% haematocrit level					
$3,7\pm o,1$	$4,o\pm o,1$	$4,2\pm o,1$	$4,3\pm o,1$	$4,2\pm o,1$	$4,4\pm o,2$

$\longleftrightarrow$ $p < o,o5$

Table II.

The changes of blood fibrinogen level in g/l

control group		swimmers		weight-lifters	
before	after	before	after	before	after
$3,4\pm o,1$	$5,o\pm o,1$	$5,1\pm o,3$	$3,8\pm o,2$	$3,8\pm o,2$	$3,9\pm o,1$

Discussion

The experiments show that the regulation of the blood viscosity may possible play a role in the physical fitness and the performance of the active sportsmen. This ability is

apparently very strong in childhood and is able to survive as a consequence of constant training. If a person has not followed such a way of life in early childhood this ability is lost /i.e. weight-lifters and volunteers/. A possible mechanism of this phenomenon operates via the fibrinogenolysis. It is well known from the literature /Ferguson et al. 1979/ and from our earlier experiments on the effect of strenuous exercise the fibrinolytic activity markedly accelerated. In these experiments this phenomenon was very strong in swimmers group II. /euglobulin lysis time markedly decreased/. The increased fibrinogenolysis after strenuous exercise is a widely discussed question in the literature /see Ferguson et al. 1979/. There are a possibility that the occurence of this depends from the type of exercise, the performance, the frequency of training, the starting level of fibrinogen and so on. Since the report Marder et al. /1969/, and later Rampling and Gaffney /1969/, it is known that fibrinogen degradation products y,E and D act against platelet aggregation, preventing blood clotting. This phenomenon may play a role in the changes of the blood viscosity.
To draw nearer to understanding of the problem, we subsequently initiated a method of measuring the fibrinogen degradation products.

Besides the above mentioned facts, some additional factors may affect the viscosity changes observed after training. The alterations in the haemotocrit level are eliminated in our study by conversion of the values to a standard reference basis, but the flexibility changes in the red cells should also be taken into account.

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THE NATURE OF THE REDOX SYSTEM OF ARTERIALIZED CAPILLARY BLOOD AFTER PHYSICAL EXERCISE

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As it is well known, human physical work capacity and physical fitness depend on numerous physiological biochemical components. Their measuring is a very difficult problem. Nowadays it is possible to measure many physiological factors. Their collective analysis makes possible the better evaluation of physical fitness. But, to reveal the processes taking place on a molecular level is not yet possible. Consequently, we had to look for such a substance, which is easily accessible and provides a lot of information. This substance is the blood. Numerous essential and measurable changes take place in the blood during physical exercise. Its capacity for transport of inorganic and organic substances is nearly inexhaustible. The transport of organic substances is realised by the higher ordred structures of proteins.

As a method we have chosen the polarographic analysis of the serum according to Bridicka. The blood was drown from the cubital vein before and after exercise. We have found, that the double waves of the Bridicka filtrate became higher after intense physical exertion. The results were reported several times in previous papers. The next question was, what substance cases the increase of the second wave. Based on literary data we assumed, the sulfur containing substances are responsible for the increase. In the next experiments attempts were made to reproduce exercise effects in vitro by chemical means. Such compounds were chosen, that normally occur

in human serum.

No. of sample	mmole added	1st wave Filtr. + methion.	2nd wave	1st wave Filtr. + gluthath.	2nd wave	1st wave Filtr. + cysteine	2nd wave
1.	0.0	Absolute height of 1st wave: 48; of 2nd wave: 71					
	0.2	−12	−16	+ 4	+ 6	−12	+ 41
	0.4	−12	−18	+ 2	+ 6	−10	+ 85
	0.6	−12	−17	+ 2	+ 7	−11	+129
2.	0.0	Absolute height of 1st wave: 26, of 2nd wave: 32					
	0.2	0	+ 1	−10	− 9	− 2	+ 82
	0.4	+ 2	+ 1	− 5	− 2	+ 2	+ 96
	0.6	+ 1	+ 3	− 2	+ 2	+ 5	+123
3.	0.0	Absolute height of 1st wave: 29, of 2nd wave: 43					
	0.2	−11	−16	−11	− 6	− 9	+ 26
	0.4	−11	−16	− 8	+11	− 9	+ 42
	0.6	−10	−16	− 8	+18	− 9	+ 63

Table 1. - Polarography of the sulfosalicylic serum filtrate with the in vitro addition of methionine, glutathione and cysteine; intrawave differences, in mm.

These substances were methionine, gluthathione and cysteine. After a polarographic record of the filtrate sample was repeated in the same sample with the addition of 0.2 then 0.4 and 0.6 mmol-s of all three aminoacids. The changes in double waves are tabulated as a function of added concentration in the 1.table.

Cysteine may be stated to have had the must active influence of the substances investigated by polarography. The reproduction, however, of the changes seen after physical exertion could be achieved only partially since cysteine addition gave rise only to an elevation of the second wave. In view of these results the observed phenomena connected with physical exercise may be regarded as being brought about by several polarographically active compounds, for example by various sulfhydryl group carriers.

Based on these observations we thought, that the concentration of reduced proteins increased during intence muscular work. It was further supposed, that the increase of postexercise sulfhydril content in blood is connected with aerobic capacity.

Materials and method

The subjects, the athletes and the nontrained medical students, accomplished the same work in every case. The acute physical exercise was performed on a motor-driven treadmill, according to the principles of the so colled "all out" exercise. After a warming up period the subjects ran to exhaustion uphill on the treadmill at speeds of 8, 10 and 12 km/hour. The first slope was 5%, and every third minute it was increased by 3%. The cardiorespiratoric functions were measured during exercises. The capillary blood was drown from the hyperemised ear lobe, before and 3 minutes after physical exercise.

The adopted method for measuring the sulfhydryl content was potentiometric titration with 10^{-4}mol silvernitrate. The blood was hemolised and diluted to twenty fold volume. The titration mixture was the same by amperometric titration according to J.R. Carter /1959/. The oxigen was driven out of the mixture using nitrogen. The increase of voltage was registered by an universal pH-meter. The ionselective electrode was made by the Analitical Department of the Technical University of Budapest.

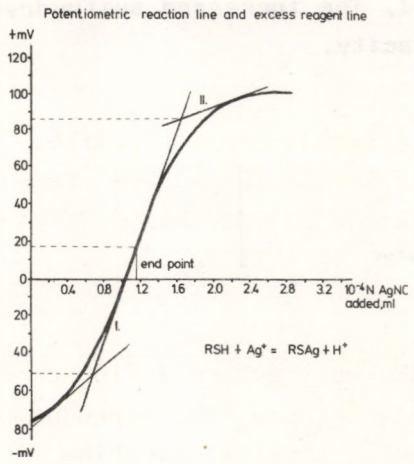

Fig.1.-Potentiometric titration of sulfhydryl groups with 10^{-4}mol silvernitrate; I reaction line II excess reagent line.

Results and discussion

	Boxers		Wrestlers		Footballers		Orienteerers		Swimmers (females)		Medical students (untrained)	
	at rest	after exercise	at rest	after exercise	at rest	after exercise	at rest	after exercise	at rest	after exercise	at rest	after exercise
n	6		9		7		4		8		6	
$\bar{x}$	1,02	1,29	0,95	1,33	0,90	1,16	1,27	1,64	1,20	1,36	1,13	1,31
±S.D.	0,13	0,14	0,57	0,28	0,20	0,28	0,18	0,13	0,26	0,27	0,26	0,12
$\pm S.D._{\bar{x}}$	0,05	0,06	0,19	0,09	0,08	0,11	0,08	0,06	0,09	0,09	0,11	0,05
P<	0,001		0,001		0,05		0,001		0,001		0,001	

Table 2. - Sulfhydryl concentration calculated for 100 ml of blood, at rest and after acute physical exercise.

The increase of post-exercise sulfhydryl level is significant on a five per cent significant level. The increased sulfhydryl level is connected with aerobic capacity.

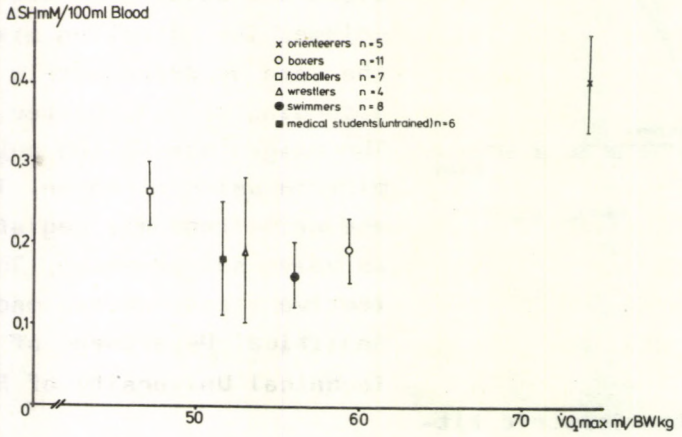

Fig.2. - The means of the differences of sulfhydryl groups, before and after exercise, against the aerobic capacity.

It was supposed, that the differences of sulfhydryl groups, before and after exercise, decrease linearly with increasing aerobic capacity, or the inspired oxigen during physical work is sufficient to oxidize the reduced components of the respiratory chain. The data was analized statistically by means of exponential regression, because the connection did not seem to be linear.

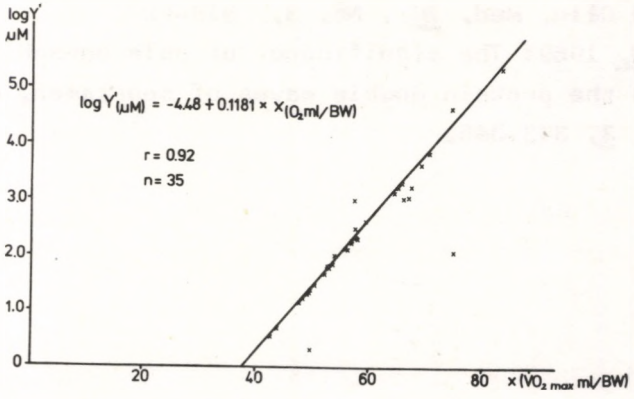

Fig.3. - Exponential regression line of sulfhydryl differences, before and after exercise, against aerobic capacity.

The correlation coefficient was 0.92, the assumed exponential connection proved to be closed.

We assume, that the oxigen intake of the athletes with low aerobic capacity is not sufficient to oxidize the hydrogen ions coming out from the respiratory chain. The sulfhydryl changes of athletes with high aerobic capacity is probably due to the inhibition of the oxidative enzimatic system.

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ESTIMATION OF PLASMA LIPIDS IN HUMAN SUBJECTS AFTER PHYSICAL EXERCISE

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Sedentary and intellectual workers are mostly effected by the noxious consequences of the lack of movement. Among the several risk factors of atherosclerosis the most important are the rise of the level of cholesterol and triglyceride in the blood lack of movement, the calorific overfeed and obesity. Between 1973 and 1979 preventive measures were taken all over the world in order to diminish the frequent occurrence of myocardial infarction. The mortality of the ischemic heart diseases decreased by 13,5% in the United States, in Australia and in the Scandinavian countries, but the number of re-infarction continued to increase. In the other countries of the world -- as a consequence of less efficacious prevention - the number of ischemic heart diseases further increased. The reduction of risk factors needs further significant modifications in the life style.

The importance of the so called beta-lipoprotein in the sclerosis of coronaries is well known. The first transversal epidemiological studies were published between 1975-77 on the lower level of high density lipoprotein /HDL/ and HDL-C cholesterol of patients who suffered ischemic diseases as compared to the healthy population. At present the correlation between these factors and myocardial infarction seems more significant than the increased level of cholesterol and triglyceride in the serum.

The changes of HDL cholesterol of a healthy population and those of patients recovered from myocardial infarction was

studied in Hungary for two years. Colleagues László Romics and Gyula Pados compared the HDL cholesterol levels of patients' sera to those of the same aged normal population. Colleague Romics found significant changes in the level of HDL cholesterol in some hereditary familial, hyperlipemias which might explain the frequent comlication of the disease - the sclerosis of coronaries.

American authors had studied the effect of regular training on the level of HDL cholesterol in cross country skiers and long distance runners. It had been shown, that regular training increases the concentration of HDL cholesterol in the blood.

This paper is part of longitudinal series on the lipid fractions of the blood serum, namely on the levels of cholesterol, triglyceride FFA and HDL cholesterol. The above mentioned parameters will be studied on a large number of athletes of varying age, from sport school students to retired former athletes.

Subjects

Healthy, non-trained female and male medical students served as controls in this study. Boxers - trained for short, exhaustive work - and cyclists and rowers - trained for endurance work - were chosen for well-trained groups. In each group 10 persons' lipid parameters were studied.

Standardization of the acute physical exercise

The subjects accomplished the same work; so the changes of the metabolic parameters could be compared. The acute physical exercise was performed on a motor-driven treadmill, according to the principles of the so called "all out" exercise. After a warming up period the subjects ran to exhaustion uphill on the treadmill at speeds of 8, 10 and 12 km/hour. The first slope was 5 %, and every third minute it was increased by 3 %.

The principles of the "all out" exercise are the following:

1, The oxygen intake after reaching a peak value levels off;
2, The RQ value of the expired air rises above 1,0
3, The base excess exceeds -12 mmol/l
4, The H^+ value is less than 40,7 nmol/l
5, The pulse rate reaches the maximal value what suits to the age;
6, The time of exercise is at least 4 minutes and does not exceed 8 minutes;
7, There is a general exhaustion, the efficacy of the respiration decreases.

Methods

Modern lipid analytical methods were applied. The total lipids were analysed by Folch, the triglyceride and cholesterol were measured by means of Boehringer test. The fractions were separated by thin layer chromatography. The HDL cholesterol was measured by Janet's enzymatic precipitation method, by means of Boehringer cholesterol standards.

Results

The 1. table shows the concentration of cholesterol, triglyceride and total lipids in the plasma. At rest, in the cyclist group - well trained for endurance work - the concentration of triglyceride was significantly lower than in the control group. In the rower group - very similar to the cyclist from the point of view of endurance work capacity - the triglyceride level was lower by 38% as compared to the non-trained group. The concentration of cholesterol in the serum was within the physiological range in all the five groups. There was no statistically significant difference between the groups. The values of the female group were slightly higher. The higher concentration of boxers was probably due to the neglection of the 12 hours starvation before the exercise. The concentration of total lipids had not differed signifi-

cantly. The effect of hemoconcentration on the lipid parameters was studied and found statistically non significant, so further it was neglected. After exercise the concentration of cholesterol in serum increased significantly in the boxer, cyclist, male and female control groups. The rowers' cholesterol level decreased.

	Cholesterol		Triglyeride		Total lipid	
	B.E.	A.E.	B.E.	A.E.	B.E.	A.E.
C. men	185 ± 9,3	197 ± 8,9	65 ± 11,4	76 ± 12,9	660 ± 25	693 ± 23
C.women	197 ± 11,9	206 ± 11,5	37 ± 9,0	45 ± 9,1	652 ± 35	650 ± 29
Boxers	211 ± 15,5	228 ± 18,2	70 ± 18,5	127 ± 27,0	716 ± 64	793 ± 69
Cyclings	195 ± 10,9	206 ± 13,6	36 ± 4,6	38 ± 3,7	604 ± 24	626 ± 37
Rowings	188 ± 5,3	188 ± 5,1	40 ± 7,2	35 ± 12,0	680 ± 37	758 ± 24

Table 1. - Effect of acute physical exercise on the serum lipids fractions in Man /mg/100ml/ ± S.D., n=10 in all groups. B.E; before exercise, A.E: after exercise.

Comparing the values before and after exercise a significant difference was shown in the level of triglyceride between the cyclists and control group before exercise; although the level of triglyceride of rowers was also lower than that of the control group.

After exercise similar changes were observed, namely significantly lower concentration of triglyceride in cyclists and a lower - but not significantly lower - level of triglyceride in the rower group. Moreover, after exercise the concentration of cholesterol of rowers proved to be significantly lower as compared to the normal group. The value of HDL cholesterol was studied in an inactive control group as well as in boxers, wrestlers, rowers and cyclists before and after exercise. Before exercise HDL cholesterol level of boxers and non-trained did not differ; the resting values of the other groups were significantly higher /at a 5 percent level/.

After exercise HDL cholesterol level increased in all groups /Fig.1./. The values of boxers and inactive subjects differ significantly from those of wrestlers, rowers and cyclists. After exercise HDL cholesterol level of cyclists was as high as 1.86 mmol/l. The values of HDL cholesterol of other athletic groups is still being studied.

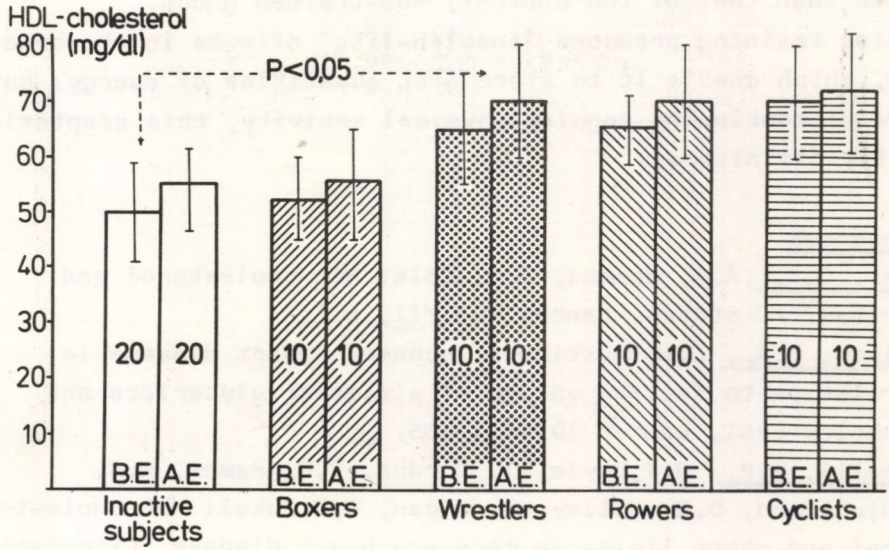

Fig.1. - Effect of acute physical exercise on the HDL - cholesterol concentration in Blood serum. B.E.:before exercise, A.E.:after exercise.

Summary

According to statistical data the number of myocardial infarctions decreases among physical workers and active athletes. When regular training is discontinued, however, the number of myocardial infarction exceeds even that of the non-trained. Presently physical exercise is not only a preventive measure but also one of the most important curative factors in the therapy of myocardial infarction.

The effect of acute physical exercise on the metabolism of cholesterol and triglyceride was studied. The concentration of triglyceride and cholesterol of athletes, well trained for endurance work /cyclists, rowers/ was lower compared to non-trained. The lipid concentration of athletes, well trained for short, exhaustive work /boxers/ did not change. The concentration of HDL cholesterol of endurance-trained athletes were higher than that of the control, non-trained group.
Regular training produces "insulin-like" effects in the organism, which enable it to store grat quantities of energy; but after discontinuing regular physical activity, this adaptation quickly diminishes.

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AEROBIC METABOLISM AND PHYSICAL EFFORT IN YOUNG ATHLETIC CHILDREN

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The metabolic cost of running in healthy school children has been studied in detail by Astrand (1952). He found that they have high values of maximal aerobic power output ($\dot{V}o_{2\ max}$) when expressed in relation to body-weight (ml kg^{-1} Min^{-1}) but relatively low levels of efficiency during running, under standardised conditions on a motor-driven treadmill. This latter result is of interest, particularly in the light of our finding (Godfrey et al 1971) that children can pedal a stationary bicycle ergometer with the same "intrinsic" muscle efficiency as adults. Of course, in cycling body weight is supported and not carried as in running and one possible solution to the problem may be in the relationship between applied forces and velocity of (leg) movement in the two activities. In man it has been shown (Wilkie, 1950) that the force a muscle develops is inversely proportional to its speed of shortening and for the optimal conversion of energy into mechanical work these two factors must be kinetically balanced. In lightweight athletic children with highly developed aerobic power outputs this condition may not be fulfilled and, during running as opposed to cycling, economy of effort is possibly sacrificed for speed. In the present investigation observations were made of the aerobic cost of running on a treadmill and outdoor performance times on 23 children. In 15 of the children the treadmill experiments were repeated with the addition of external loading corresponding to 5% and 10% of their body weight.

The 23 children were subdivided into 9 active and 9 athletic boys and 5 athletic girls. The athletic children regularly competed in club and county 800m - 3000m athletic events and the active boys in normal school athletics. The mean age, weight, height and $\dot{V}o_{2\ max}$ for the athletic, active boys and athletic girls respectively, were as follows:-

12.6 yr., 36.43 kg., 147.3 cm., 2.54 L Min^{-1} (70.0 ml kg^{-1} Min^{-1}) 12.2 yr., 37.84 kg., 148.4 cm., 2.36 Lmm^{-1} (62.0 ml kg^{-1} Min^{-1}) and 13.8 yr 38.62 kg., 152.0 2.45 L Min^{-1} (64.0 ml kg^{-1} Min^{-1}).

Oxygen intake ($\dot{V}o_2$) was measured at various speeds (8-16 km h^{-1}) on the treadmill using an open circuit technique. External loading was applied using sand which was evenly distributed over the upper body in small pockets of a specially designed weight jacket. Maximal aerobic power output ($\dot{V}o_{2\ max}$) was measured (Davies 1968) in separate experiments and performance times on the track were obtained by questionnaire and from official

records.

The results are summarised in Fig.1. The $\dot{V}o_2$ data for given speeds (V) were not significantly different in three groups studied and therefore

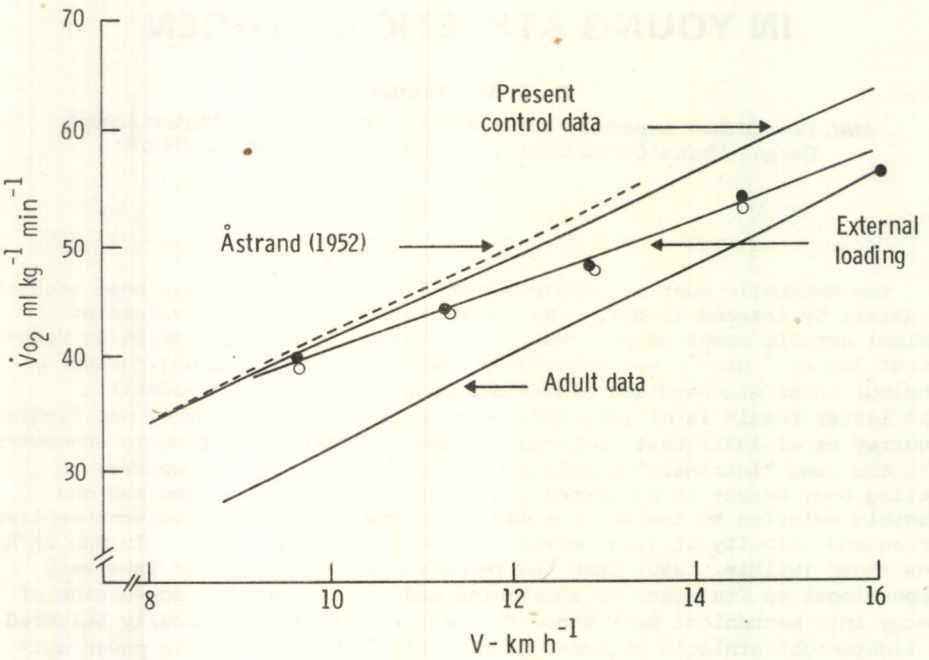

Fig. 1. The effect of external loading on the metabolic cost of running in children. External loading equivalent to 5% (●) and 10% (○) of body weight. Regression lines for the childrens' control data and from the study of Åstrand (1952) are shown. The regression line for adults is taken from Davies and Thompson (1979).

could be combined; $\dot{V}o_2$ (ml kg^{-1}Min^{-1}) = 5.714 + 3.541 V (km h^{-1}) r=+0.95. At a set speed of 15 km h^{-1}, $\dot{V}o_2$ was not closely related to neither stride length (r = -0.39) nor stride frequency (r = +0.27) but external loading equivalent to 5% body weight significantly ($P<0.05$) decreased the slope and increased the intercept of the $\dot{V}o_2$/V relationship. However, additional loading (≡ 10% body weight) did not change the $\dot{V}o_2$/V relationship further (fig. 1).

The highest recorded values of the $\dot{V}o_2$ max with athletic boys and girls were 73.7 ml kg^{-1} Min^{-1} and 73.5 ml kg^{-1} Min^{-1} respectively. The best guide to $\dot{V}o_{2max}$ in the boys was given by their 1500m track time: $\dot{V}o_{2\ max}$ (ml kg^{-1}Min^{-1}) = 113.33 - 8.894 (1.5km-Min) r =-0.748.

It would seem that in young active and athletic children their light body weights and resultant forces are not optimally matched to the required frequency of leg movement to produce the most economic conversion of aerobic energy into mechanical work, particularly higher speeds of running. Thus, though their performance times are associated with $\dot{V}O_{2\ max}$ they are nevertheless inferior to those one might have expected from previous aerobic power weight data collected on adult male and female athletes (Davies and Thompson, 1979).

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SPIROTONOMETRY AND FLACK TEST

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30 regulary trained university students were subjects of different step and positive-pressure breath holding /apnea/ tests. Spirotonometry and Flack test belong to the positive-pressure breath holding tests. In analizing the correlations, Spearman's rank correlation coefficient was used. 17% of the examined persons belong to the excellent /1/ 20% to the good /2/ 33% to the medium /3/ 10% to the sufficient /4/ and 20% to the unfavourable condition category /5/ by spirotonometry. If Flack test was used 66% of the athletes belong to the good /I. type/ 27% to the medium /II. type/ and 7% to the unfavourable condition type /III. type/. Spearman's rank correlation coefficient of the two tests is quite high /r_s= 0.906/; only Martinet /r_s= 0.974/ and Kereszty test /r_s= 0.967/ have more close correlations with spirotonometry. The correlation coefficient of Cureton /shortened Schneider/ test and spirotonometry is almost equal /r_s= 0.905/ while that of Harvard test is also high /r_s= 0.843/. The correlation coefficients of Master, Schneider and shortened Harvard test are lower /r_s= 0.654; r_s= 0.646 respectively r_s= 0.434/. 5 second's heart rate graphs are used in Flack test. On the figure 1 the three types of heart rate graphs of Flack test are seen /the arrow shows the mean apnea time/. 90% of the athletes, who belong to Flack I. type pulse category have excellent, good and sufficient form by spirotonometry, 10% of this group in the unfavourable category by spirotonometry. The athletes of Flack's II. type have worse condition spirotonometrically,

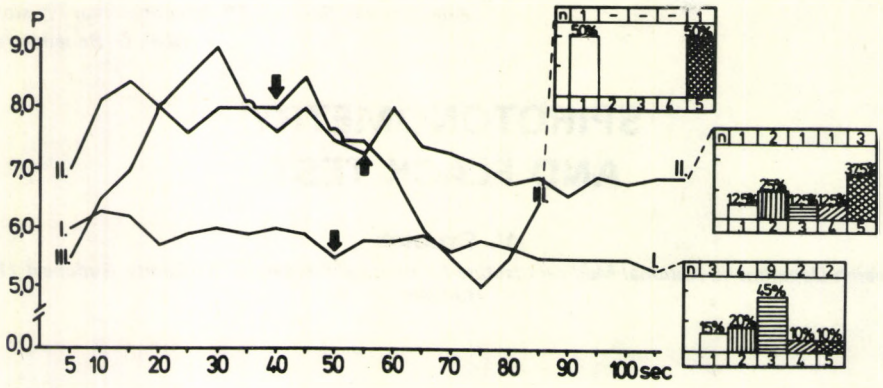

Fig. 1 The three types of 5 second's heart rate graphs of Flack test /mean values/. The arrows show the mean apnea times, the column-diagrams the spirotonometric category-distributions of the cases in these three types /n= number of cases/.

nevertheless excellent form also occured among them. One of the athletes of Flack's III. type has a good form while the other has an unfavourable condition by spirotonometry. Comparing the mean apnea time of athletes of II. and I. types it is interesting to observe that the value of the former group exceeds that of the later. This indicates the important role of subjective factors in the duration of apnea time. There is a close correlation between spirotonometry and the apnea time of Flack test, /r_s= 0.928/ i.e. the longer is the apnea time the better condition is to be expected. If the athlete is interested in some form in a better result he should produce such an apnea time what far exceeds his capacity. In such cases his cardiovascular parameters and other connected functions became worse. Very typically the longer is the apnea time of Flack test the worse are the cardiovascular functions. Namely, the improvement of one factor /i.e. apnea time/ should automatically produce the worsening of the other

/heart rate reaction/. If the test was for some reason of no importance for the athlete the apnea time should became shorter, nevertheless it would not indicate an insufficient condition. There should be no uniformity even in heart rate reactions of Flack test if the apnea time was favourable long. On the figure 2 heart rate graphs of three athletes are seen. Each of them had an eighty second's apnea time

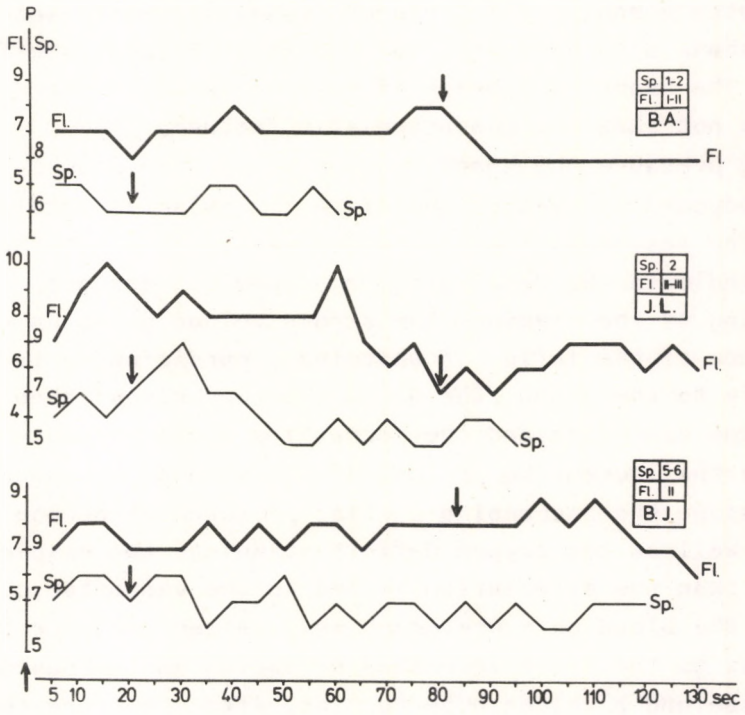

Fig. 2 5 second's heart rate graphs of three sportsmen with 80 second's apnea time. Thick line= Flack test /Fl./, thin line= spirotonometry /Sp./. The arrow upwards shows the start, the downwards the end of apnea. On the right side are seen the condition-evaluations after spirotonometry and Flack test.

/the arrow downwards shows the end of apnea time/. The heart rate graph of athlete B.A. is quite uniform; according to Flack test it is between 1. and 2. types and should also be ranged in 1. and 2. category by spirotonometry. The heart rate graph of athlete J.L. should be ranged to Flack's third type although the return of his pulse graph to normal at the end of the pressure may indicate an emotional tachycardia /Flack's V. type/; consequently he should be classified between II. and III. type. Flack's heart rate graph of athlete B.J. indicates a medium /II./ Flack's condition while spirotonometry shows a form between unfavourable /5/ and insufficient /6/. The heart rate graph of spirotonometry on this figure does not show any characteristic feature.

During pressure the heart rate grows up then a postpressor bradycardia develops therefore the pulse is hardly valuable. The measurement of the blood pressure is a better method to indicate the developing hemodynamical changes. At the beginning of the pressure the stroke volume grows up as a consequence of the influx of abdominal, cardic and pulmonal blood supply to the heart /the blood pressure rises/ then the stroke volume decreases and the increasing blood pressure is provided by the increasing peripheral resistance. At the end of the pressure the increasing partial pressure of carbon dioxide as well as the oxygen deficit stimulate the respiratory center, then the stimulation is led to the vasomotor center making the blood pressure graph wavy. After pressure the blood influx to the heart increases producing an increased stroke volume and a volume hypertension. After pressure the cardiovascular changes are based on reflex mechanismes of pressoreceptors. At the beginning of the pressure hemodynamics lead the changes then regulating functions became more and more pronounced. On basis of blood pressure measurements this regulating mechanism is better observable. The blood pressure graphs of the previously mentioned three athletes also indicate it /figure 3/. Spirotonometric graph of athlete B.A. should be ranged to the best category but his systolic blood pressure of 140 mm Hg at rest and the diastolic blood

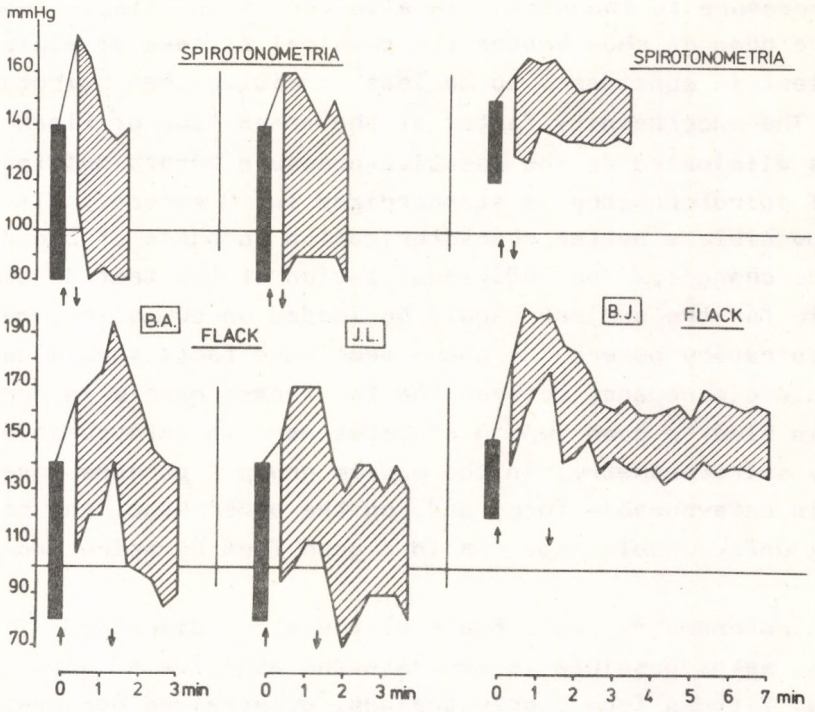

Fig. 3 Blood pressure during spirotonometry and Flack test. The arrows show the start and end of apnea.

pressure of 110 mm mercury at the end of pressure worsen it between 1. and 2. category. A longer apnea time /Flack/ increases either the systolic or the diastolic values. Although the heart rate graph of athlete J.L. is the worst by Flack test, spirotonometry indicates a good form /2/; this makes probable an emotional tachycardia /V. type/. Flack 5 second's heart rate graph is hardly suitable to make it certain. As for athlete B.J. Flack's heart rate graph could not find out his physical fitness. A blood pressure of 150/120 mm Hg at rest should indicate a serious overload so Flack's medium conditon should be refused. The restitution of his

blood pressure to the normal is also very slow. Since blood pressure changes show better the physical fitness of athletes, Flack test is considered to be less sensitive than spirotonometry. The uncertainity factor of the apnea time of Flack test is eliminated as the positive-pressure breath holding time of spirotonometry is standardized in 20 seconds. /It makes possible a better characterization on basis of blood pressure changes./ The individualization of the test is also possible for the subject should be loaded on 60% of his maximal exspiratory power. The above mentioned facts should cause a certain discrepancy between the two tests; namely in our study in Flack's good type 4 athletes were in unfavourable form by spirotonometry; in the medium group 4 persons were found in unfavourable form, and, on the other hand, one of Flack's unfavourable type was in a good form by spirotonometry.

Spirotonometry - on basis of the above discussed facts - makes possible to separate the athletes of good physical fitness from poorly trained, overtrained persons, furthermore to reveal fatigue, and exhaustion.

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LEVEL AND INCLINE RUNNING ON THE TREADMILL; SOME THOUGHTS ON THE RELATIONS OF ITS METABOLIC COSTS IN YOUNG BOYS

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Exercise tests using a treadmill have recently been introduced for trainees aged between 10 and 18 years and attending programmed training in the Central School of Sports. Former experience with bicycle ergometry had to be updated, because of treadmill power output only vertical work can be directly expressed in physical units while horizontal work cannot be neglected either.

Table 1. Correlations of $\dot{V}O_2$ and body parameters

Parameter	Stature	Weight	Body surface area	O_2 uptake
Age	.70	.75	.75	.58
Stature		.91	.96	.79
Weight			.99	.89
BSA				.87

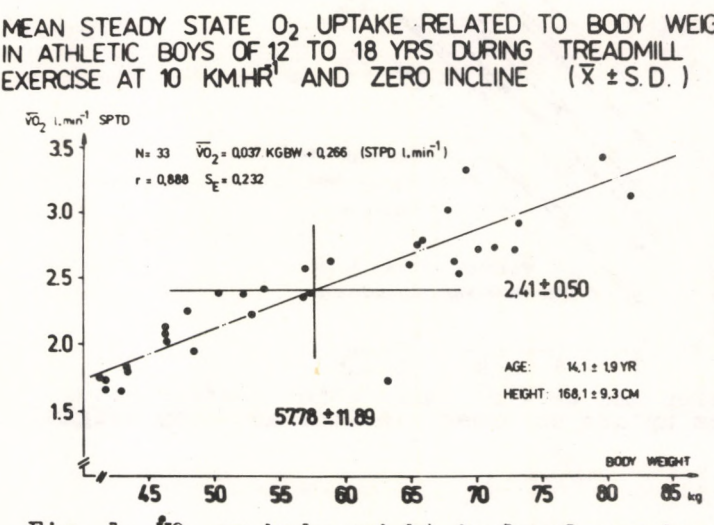
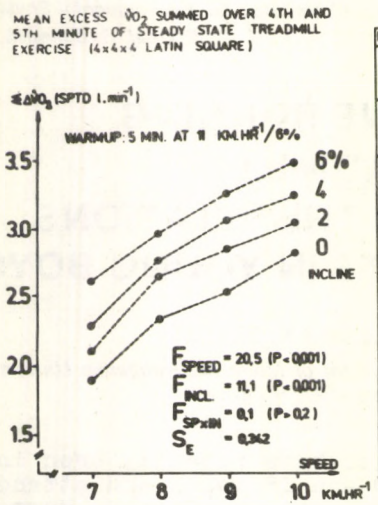


Fig. 1. $\dot{V}O_2$ vs body weight in level running



EXPERIMENTAL DESIGN

DAY	ORDER AND SEQUENCE	
	SUBJECT A: 11.46yrs 35.4kg	SUBJECT B: 11.27yrs 31.3kg
1	FIRST RUNNER 7 KM.HR^{-1} 2-4-6-0 %	SECOND RUNNER 8 KM.HR^{-1} 6-0-2-4 %
	32.3kg	31.0kg
2	FOURTH RUNNER 9 KM.HR^{-1} 6-0-2-4 %	FIRST RUNNER 10 KM.HR^{-1} 2-4-6-0 %
	35.5kg	31.5kg
3	THIRD RUNNER 8 KM.HR^{-1} 4-6-0-2 %	FOURTH RUNNER 9 KM.HR^{-1} 0-2-4-6 %
	35.8kg	31.5kg
4	SECOND RUNNER 10 KM.HR^{-1} 0-2-4-6 %	THIRD RUNNER 7 KM.HR^{-1} 4-6-0-2 %
	SUBJECT C: 11.73yrs 32.9kg	SUBJECT D: 12.14yrs 45.2kg
1	THIRD RUNNER 9 KM.HR^{-1} 2-4-6-0 %	FOURTH RUNNER 10 KM.HR^{-1} 6-0-2-4 %
	32.7kg	45.4kg
2	SECOND RUNNER 7 KM.HR^{-1} 6-0-2-4 %	THIRD RUNNER 8 KM.HR^{-1} 2-4-6-0 %
	33.4kg	45.5kg
3	FIRST RUNNER 10 KM.HR^{-1} 4-6-0-2 %	SECOND RUNNER 7 KM.HR^{-1} 0-2-4-6 %
	32.5kg	45.3kg
4	FOURTH RUNNER 8 KM.HR^{-1} 0-2-4-6 %	FIRST RUNNER 9 KM.HR^{-1} 4-6-0-2 %

Fig. 2. Experimental design and plot of the obtained means

MEAN SUMMED EXCESS $\dot{V}O_2$ PREDICTION BY BODY WEIGHT, SPEED AND INCLINE ON THE TREADMILL IN BOYS AGED 11-12 AND WEIGHING 30-45 KG

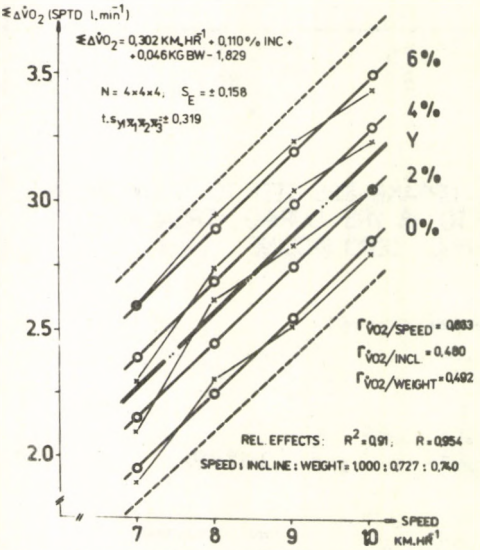


Fig. 3. Regression and partial correlation coefficients of excess oxygen uptake on speed, incline and body weight

Relation between $\dot{V}O_2$ and work rate, the latter containing belt speed and incline as independent variables, was taken to be linear. In trying to disclose potential covariates age, weight, height and body surface area were correlated with O_2 uptake averaged over the four last minutes of a six min run at 10 km h^{-1} on the level in 33 males. These subjects were aged between 12 and 18 years and had a training history in track and field, modern pentathlon, paddling or water polo. The obtained correlations ranged from .58 to .89 with $\dot{V}O_2$, body weight having the highest coefficient /table 1/.

Since the other parameters were rather closely related to weight, this was taken as the main covariate. Gross oxygen uptake in level running at this speed was rather high and linearly related to body weight, the cost for every kg being 37 ml per min. /Fig. 1/

Though this finding seemed to cover 80% of total variability in $\dot{V}O_2$ in this experiment, we still could not account for the shares of incline and other belt speeds. Energy consumption in level running on the treadmill has been reported to be a linear function of speed since nonlinearities introduced by outdoor air resistance and acceleration are absent /Margaria et al. 1963, Lloyd 1967/. Four young subjects, four speeds /7, 8, 9, 10 km h^{-1}/ and four treadmill inclines were arranged /0, 2, 4, 6%/ according to a Latin square design /Fig. 2, right/ to establish quantitative effects. The subjects were males, age 11 to 12 years, and were engaged in programmed training in track and field since one year. They ran another schedule every day in order to compensate for interactions of environmental and sequential factors.

These speeds and inclines were chosen to ensure steady rate submaximum workloads and represented 45 to 85% of the subjects $\dot{V}O_2$ max. Oxygen uptake was determined by an Ergoanalyser /Mijnhardt, Holland/ every minute before, during and after the runs. Of every 5 min bout gross and baseline corrected oxygen uptakes of the fourth and fifth minutes were treated as dependent variables. Differences in speed and incline effects were separated by a two-way anova with repeated measures. F tests evidenced excess $\dot{V}O_2$ to be preferable to uncorrected uptake, because F ratios became higher when mean preexercise uptake was subtracted from gross O_2 consumption. The respective means for the speeds and inclines used are shown in Fig. 2, left.

Despite that the subjects were carefully familiarized with the protocol, we first thought of a continued learning effect when the plot of the means was found to be slightly deviating from linearity. In this respect the influence of successive days on preexercise and exercise oxygen uptakes as well as on body weights were also analyzed, but the obtained F ratios favoured a "no difference" verdict. The sequence of inclines as another factor possibly not sufficiently controlled , because whereever it began it always followed the same order, could also be ruled out from among the potential causes of nonlinearity.

Then under the hypothesis of additive relations body weight was introduced as another independent variable in the $\dot{V}O_2$ prediction. The analysis of the trivariate formula:

$$Y = 0.15\ x_1 + 0.06\ x_2 + 0.25\ x_3 - 0.92 \pm 0.16;\ R = 0.95,$$

where Y = excess $\dot{V}O_2$ STPD l min^{-1},
x_1 = belt speed km h^{-1},
x_2 = treadmill incline %,
x_3 = body weight kg,

evidenced variable independence and additivity with non-significant interactions and gave an estimate also for the relative share of each predictor variable /speed being dominant was taken as unity and related to it incline and body weight had an equal contribution of .75, Fig. 3/.

Even the trivariate model is but an estimate, and we were of course interested if it represented a special case because of the relatively low case number and the restricted variability associated with that or else it was also workable with an independent sample. To this end estimates were made by it for the subjects of the first zero incline study of broader age and weight range. It turned out that this formula would underestimate oxygen uptake the more the older the subjects were.

When, however, weight was not entered as an independent variable, instead it was used to obtain relative $\dot{V}O_2$, this predictive formula yielded much closer estimates /i.e. all estimates lying within s_E, the square root of the residual mean square/. This formula is:

$$Y = 3.85\ x_1 + 1.49\ x_2 + 0.75 \pm 3.23;\ \ R = 0.86,$$

where Y = excess $\dot{V}O_2$ STPD ml min^{-1} kg^{-1},
x_1 = belt speed km h^{-1},
x_2 = treadmill incline %.

The summary conclusions are:

1. Oxygen consumption in steady rate treadmill exercise is linearly related to belt speed and treadmill incline in young subjects too so these parameters may be used to predict it.
2. Body weight must be taken into account as well. While it may be used as an additional independent predictor, this appears to underestimate real oxygen demands. It is preferable to use it instead to calculate relative oxygen uptake estimates which give sufficiently close values to real demands. For both approaches formulae are given, they refer to excess uptake elicited by the workload above baseline /resting/ level.
3. Appropriate formulae need be evolved also for females and if warranted, also for endurance and non-endurance events of sports in which young subjects are engaged.

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CLOSING REMARKS ON HORMONAL AND PHARMACOLOGICAL ASPECTS IN PRESENT-DAY EXERCISE PHYSIOLOGY

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Hopefully I am not alone with the conviction that this one and a half day we have spent together has been rewarding to all of us. I am greatly indebted, and I am speaking also on behalf of the Organizing Committee, to all the speakers and to every active participant if our hopes concerning this Satellite have come true. Any effort of the organizers is in vain if the ultimate goal is not attained, if scientific content fails to get the upper hand. And this latter depends on the participants in the first place. I believe you agree that the presentations were up to the mark, also when compared to international standards. The vigour of the Symposium could be felt everywhere, in and out of doors; thoughts were exchanged in lively discussion both in this hall and outside it, in the corridors and during the receptions too. We may take it for certain that the end of this meeting is also a beginning for a number of connexions between scientists that will last long. And that is what really counts.

Without any attempt to recite them in full I reckon there are some points worth being mentioned here, in particular as regards the main theme. We have got nice evidence that the relationships between hormonal regulation and sports, respectively exercise, deserve a treatment of their own. No matter from which direction we may approach these relations, be it the aspect of physiological research or sport medicine or sport practice, every way seems promising. The unprecedented rate of methodological development of the past decade has immensely contributed to it. The series of experiments conducted by Dr. Borer - which commanded respect by its impressive volume too - established among other factors that physical activity has an impact on the growth of the body as a whole and is indispensable for the development of muscle tissue. Beyond developmental physiology this is a point worth considering for the physiology of nutrition and sports alike. In a broader sense it is a challenge for further studies in human biology, to people interested in the research of secular trends and developmental acceleration as well.

The participation of the Leipzig group, the papers presented by Doctors Langer and Landgraf have been regarded as important contributions to the session. In addition to the

valuable and concrete results reported by them, they helped us recognize the vistas of research by launching studies on hormones hitherto regarded by exercise physiology as lying outside of immediate interest. We are looking forward to hearing of their results again.

Unknown regions of the hormonal system were opened up through the studies of Dr. Skipka in respect of the role of aldosterone in promoting performing capacity. The question of whether we have to do here with a physiological action of aldosterone or probably with a pharmacological influence upon some point along the chain of mitochondrial oxidation is a rather exciting one.

We were glad to have here the team of radiobiologists from the Frédéric Joliot Curie Institute. Their work points much beyond the problem of doping, considering both the practical and the theoretical implications of their methodology.

When we stood up after listening to the papers of Drs. Pannier, Górski and Pavlik dealing with the part of beta blocking agents and adrenergic receptors in physical activity, we regretted not being told more about this field awaiting further discoveries.

While looking at the diversity of data and methodical approaches we had to realize the fact that hormonal balance developed by regular training would be different both in respect of exercise responses and conditions at rest. We must take care not to lose sight of the integrated whole as a system when we are trying to find our way through the necessarily isolated studies.

The interactions between the hormonal system and the biologically active endogenous or exogenous substances have not got as much direct coverage as they deserved. Hopefully nobody feels hurt by this critical remark.

The sessions devoted to the free papers had a merit of their own. They conveyed a lot of information of which I wish to illuminate one facet only: that of young age being as well important for its human aspects as for its implications for animal experiments. Several of the papers alluded to this point today and yesterday too, demonstrating the sensitivity of research people to practical questions as well.

Hormonal regulation recognized as an integrated system in connexion with physical exercise, the problem of interactions, and the age-related questions getting nearer to the focus of attention were the three lines of thought that appeared important to me in recollecting my impressions of our work.

I want to thank you once more for the keen spirit of this Symposium. Thanks are due also to those who have helped me to get along: to my colleagues, the members of the Organizing Committee, and to the ladies of the Conference Bureau.

I wish you good luck, further successful work, and a really pleasant stay during the World Congress in Budapest.

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