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DETERMINATION OF SODIUM AND POTASSIUM BY NEUTRON ACTIVATION ANALYSIS FOR THE INVESTIGATION OF THE ELECTROLYTE-BALANCE OF THE INNER EAR

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BUDAPEST

DETERMINATION OF SODIUM AND POTASSIUM BY NEUTRON ACTIVATION ANALYSIS FOR THE INVESTIGATION OF THE ELECTROLYTE-BALANCE OF THE INNER EAR

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I. Introduction

The microchemical structural changes which take place in the sense organs of higher class living organisms, like the human body, as a consequence of physiological and pathological alterations, or pharmaceutical medical intervention, play an important part in the proper diagnosis of normal and pathological processes.

Even the few experimental data available to date on the electrolyte balance of the inner ear are considered to be a valuable contribution to our better knowledge of the biochemsitry of the sense organs. On the other hand, the discrepancy of the results in the reported biochemical investigations suggests the necessity of further, primarily comparative studies and the critical evaluation of the experimental findings in this field.

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It can be already inferred from the compiled data [1,2] that the difference between the perilymphatic and endolymphatic electrolytes is due essentially to different relative concentrations of the sodium and potassium ions. This seems to correspond to the known data on the composition of the extracellular and intracellular electrolytes. In fact, recent investigations have shown the electrolyte of the perilymph to be of the extracellular type i.e. having a large fraction of sodium and few potassium ions, while the endolymph contains, like the intracellular type, a high ratio of potassium to sodium ions.

Recent pathophysiological data have revealed, in addition, that this difference in the composition of the extra tra- and intracellular electrolytes is also a result of dynamic equilibrium. The dynamic balance depends on many factors varying with the functional state of the organ in question. Even a very slight damage may upset the equilibrium between the sodium and potassium ions and this alteration may, in turn, affect various physiological functions. That is why, it was thought of interest to analyse these two most important electrolytes of the living organism in connection with the investigations of the inner ear.

Activation analysis has been attempted already by HÜRLIMAN [2] in order to determine the sodium and potassium ion content of the inner ear fluids. His experiments, performed on guinea pigs, showed that the non-destructive technique based on gamma spectrometry cannot be applied in this case. The analysis of beta radiation complemented with absorption measurements was found to yield more accurate results. Taking the sodium activity for unity, the relative concentrations were found to be

	Na	K
Perilymph	1	< 0,2
Endolymph	1	7,02

Disturbing Nuclear Reactions

The neutron activation analysis of sodium and potassium might be interferred with, in principle, by the following reactions

$$^{24}Mg/n,p/^{24}Na \qquad 1.$$

$$^{27}Al/n,\alpha/^{24}Na \qquad 2.$$

$$^{42}Ca/n,p/^{42}K \qquad 3.$$

BOWEN and CAWSE [3] give for whole blood 40 /ug/g of Mg, 0,37 /ug/g of Al and about 60 /ug/g of Ca. CITRON et al. [4] reported on the concentration of these elements in the perilymph, as 3.0 MEQ /about 60 /ug/ of Ca and 2.0 MEQ /about 24 /ug/ of Mg and in the endolymph 3.0 MEQ of Ca and 0,9 MEQ of Mg. Our calculations show that no interference with the activation analysis is to be expected from these elements at the given low concentrations.

II. Experimental Method

The neutron activation analytical method for the determination of sodium and potassium reported here, was developed partly on model experiments partly on small amounts of human serum. Sample quantities of about 20 mg were used. Since, as known from the literature the sodium ion content of the pure serum is about 140 MEQ, while the potassium content varies from 4,5 to 5,5 MEQ, the amounts to be detected in a 20 mg sample are about 60 /ug and 4 /ug, respectively. Consequently it is impossible to identify nondestructively the potassium and sodium activities in the presence of each other.

Three different methods have been tried for the determination of potassium, a/ single precipitation of potassium, b/ double precipitation of potassium and c/ separation of potassium by isotopic exchange. Sodium could be determined nondestructively from aliquots of the filtrate obtained on filtering off the potassium precipitate.

The samples were irradiated in sealed quartz ampoules for 10 hours with the 2.10^{13} n/cm^{2}sec neutron flux of the VVR-S type reactor. The sodium and potassium standards were exposed simultaneously with the samples. After irradiation the samples were dissolved from the quartz ampoules by nitric acid, then destroyed by a mixture of concentrated nitric acid and hydrogen peroxide. The nitric acid was removed from the destroyed sample by boiling with concentrated hydrochloric acid and finally the sample was evaporated to dryness.

a/ Single precipitation of potassium

Potassium was precipitated with the aqueous solution of 3% sodium-tetraphenyl borate / $Na[B/C_6H_5/_4]$/ reagent at pH = 4-6, in the presence of 10 mg of potassium and 3 mg of sodium as inactive carrier. The reagent reacts with potassium according to the formula

$$[B/C_6H_5/_4]^- + K^+ = K\,[B/C_6H_5/_4] \,.$$

The resulting white precipitate was left to stand for a short time, then filtered. The filtrate containing the sodium activity was diluted to 100 ml from which 2 ml was evaporated to dryness and used for the activity measurement by gamma spectrometry. For the activity measurement Nuclear Enterprises mark, 3 in x 3 in NaI/Tl/ crystal and EMI 9531A mark photomultiplier tube were used in combination with a 256 channel analyzer /System KFKI, NTA-256 type/.

The potassium-tetraphenyl-borate precipitates proved always to be contaminated to varying extent by sodium activity as apparent from the gamma spectrum of a potassium-tetraphenyl-borate precipitate with sodium activity, shown for illustration in Fig. 1.

b/ Double precipitation of potassium

To obtain the potassium-tetraphenyl-borate precipitate in a pure form, more convenient for evaluation, double precipitation was performed. The sample was first precipitated

in the presence of 10 mg potassium carrier in the manner described above, then the filtered precipitate was dissolved in acetone. On removing the acetone by heating on water-bath the potassium was precipitated and filtered again before the activity measurement. The twice precipitated potassium tetraphenyl-borate was found to be in each case free from any sodium activity and the gamma spectra could be easily evaluated. The only drawback of this method is its lengthiness.

c/ Separation of Potassium by Isotopic Exchange

In spite of its simplicity and rapidity, isotopic exchange has been seldom applied in combination with activation analysis for the separation of the components in irradiated samples. It was first used by SUNDERMAN and MEINKE [5] for studying the isotopic exchange between radioactive silver ions and inactive silver chloride. Recently QUERESHI and SHABBIR [6, 7] performed experiments on the radiochemical recovery of cobalt and antimony by means of isotopic exchange.

Investigations about separation by isotopic exchange are now in progress at our Laboratory, too. CSAJKA [8] applies this technique for the quick separation of tin contaminant from cathode nickel. The method permits to recover tin to more than 99 % in 7 to 8 minutes. This result suggested to try the separation of potassium by isotopic exchange. For the tentative experiments potassium-tetraphenyl-borate was chosen as inactive precipitate.

In order to develop the optimum technique following experiments were performed.

The necessary amount of the inactive potassium-tetraphenyl-borate precipitate for the maximum possible binding of the radioactive potassium ions was established by adding about 10^4 cpm radioactive potassium to successively 50, 100, 200, 300, 400 and 450 mg of precipitate.

The acidity was adjusted to pH = 5 knowing that the solubility of potassium-tetraphenyl-borate is the lowest at

pH = 4-6 [9]. The mixture was shaken mechanically each time for 15 minutes, then filtered through No.G4 glass filter and washed with 50-60 ml of distilled water. The measure of the isotopic exchange in percents can be seen in Table I.

Varying the pH and shaking time to observe the effect of these parameters on the binding of potassium ions, for pH values from 2 to 7 the isotopic exchange was found to be left unaltered. Because of the possible binding of other ions it is, however, advisable to use rather slightly acidic /pH = 3-5/ system. The isotopic exchange remained the same also on shaking the mixture for 5, 10 or 30 minutes. Because of the strong foaming of the mixture on shaking, an agitation for 5 minutes was tried instead and since the isotopic exchange was found to reach in this case also 99,5 to 100 % for 400 mg of precipitate, subsequently only 5 minute agitation was applied that did not cause the mixture to foam.

The most important aim of the separation was to eliminate the interference from radioactive sodium. Experiments were performed therefore to observe the isotopic exchange in the presence of sodium activities of 2.10^4, 5.10^4 and 2.10^5 cpm. It was found that the precipitate did not bind any sodium at all. Nor did the presence of phosphate ions interfere with the isotopic exchange of potassium ions.

Finally a calibration curve was plotted for potassium concentrations from about 2 to 10 µg that is shown in Fig. 2.

As a result of above investigations the serum and perilymph samples used for the experiments were treated as follows.

Perilymph samples were taken from living animals narcotized by ether. After chiselling the bulla ossea, a sterile and chemically pure glass capillary was introduced through the round window /fenestra rotunda/ to collect from the perilymphatic vessels sufficient sample material.

The samples were irradiated under the conditions described in the foregoing, dissolved from the quartz ampoules by hot cc. nitric acid, then destroyed by boiling with a mixture of cc. nitric acid and hydrogen peroxide. Nitric acid was removed by adding three times cc. hydrochloric acid before evaporating the sample to dryness on water bath. The dry residues were taken up with distilled water and the pH adjusted to 3-5 before pouring the already prepared inactive precipitate to each sample solution. The mixtures were agitated for 5 minutes and filtered through No. G4 glass filters of precisely identical sizes. After washing with 50-60 ml of distilled water the gamma spectra were taken and compared with the potassium standard treated in the same way as the sample.

The precipitates were usually free from sodium activity. The small sodium contamination found in some spectra, seeing that sodium ions had never been bound by the precipitate in preliminary experiments, is thought to be due to the sodium in non-ionic bondage present in some of the samples that could not be destroyed and adhered to the precipitate at the pH applied. In some perilymph samples photopeaks at 0,41 and 0,51 MeV were observed which by half-life measurement turned out to be due to ^{198}Au and ^{64}Cu /Fig. 3./

III. Experimental Results and Discussion

Extremely small volumes of fluid are available in the inner ear for electrolyte analysis. By a single intervention /punction/ the perilymphatic liquid obtainable from living animal /guinea pig/ varied from 2 to 5, occasionally to 10 µl. Upon regeneration of the inner ear fluid it is possible to obtain periodically by repeated interventions further experimental material. The technique of activation analysis developed here permitted to study some features of the regenerated perilymph.

As known, also from the literature, even the most careful intervention is due to lead to microscopic haemorrhage which alters the biochemical relations of the inner ear

fluids. For this reason human serum was investigated in the experiments along with the "live" samples, first in pure state, then upon addition of a given number of erythrocites. As regards the two principal electrolytes of the organism, sodium and potassium, the results obtained from the serum and the perilymph are essentially the same. For the neutron activation analysis two methods were used.

Twice separated potassium /II.b/ gives for pure human serum 5,75 ± 0,48 MEQ by activation analysis and 4,76 ± 0,38 MEQ by flame photometry. For sodium the two methods yield 135,4 ± 10,3 MEQ and 148,2 ± 1,8 MEQ, respectively. The difference between activation analytical and flame-photometric results is seen to be + 20 % for potassium and - 8,5 % for sodium.

By isotope exchange technique /II.c/ for pure human serum the result was 4,99 ± 0,60 MEQ of potassium and 130,7 ± 8,2 of sodium. The flame photometry in this case gives 4,68 ± 0,06 MEQ of K and 141,2 ± 1,6 MEQ of Na, thus the difference is + 6,6 % for K and - 7,5 % for Na.

In the next set of experiments a given number of erythrocites on the electrolyte equilibrium is seen from the values listed in Table II. A definite increase in the potassium concentration is observable if the number of erythrocites per mm^3 of sample is above 80.000. This proves that on the biological death of the cells caused by the intervention or even earlier due to the deterioration of metabolic processes, the rapid diffusion of the intracellular potassium ions into the anyhow small volume of the experimental material will alter its biochemical composition within a few minutes. These experimental findings are confirmed by RAUCH 's experiments [2] in which the perilymph of the experimental animals was found to change in five minutes after being killed. Since the presence of erythrocites in a higher number than 80.000/mm^3 is manifested by rosa coloring, samples with this coloring cannot be used for finer analysis.

For information about the electrolyte balance of the inner ear, thorough perilymph studies were performed on guinea pigs. The results are listed in Table III. The perilymph samples taken in fractions from the experimental animal show that in the different phases of the intervention the electrolyte balance reported in the literature [1] reflects only the averages of the larger quantities. In the neutron activation analysis combined with isotope exchange, the K-ion content of some samples increased /10-14 MEQ/ because of microscopic haemorrhage in the first intervention, while the Na-content remained normal /150-170 MEQ, Column I in Table III/. As the haemorrhage ceased, the regenerated perilymph yielded again the values known from the literature /Column II/. For protracted intervention, with repeated punctions also the endolymph system of the inner ear may suffer some damage. In this case a higher K-content was found while the Na-concentration was normal or somewhat lower. In the case of intentional or accidental opening of the endolymph system the measured values of Na were low, those of K generally about 20 MEQ or higher /Column IV/.

The biochemical analysis of the inner ear fluids seems to be a promising method of investigation in both experimental and practical medicine that will prove as helpful as some other already extensively used laboratory methods for proper diagnosis. The pathophysiology of hearing and body balance is closely related to the electrolyte equilibrium of the inner ear fluids [1] [2]. The results of present experiments seem to point out some of the problems and possible errors involved in these investigations and offer a methodical base and new data for the continued investigations.

Acknowledgment

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Table I.

Variation of the isotopic exchange with the weight of the inactive precipitate

Amount of precipitate mg	Extent of isotopic exchange %
50	15
100	39,3
200	87,5
300	96,0
350	99,2
400	99,5
450	~ 100,0

Table II.

Concentrations of Na^+ and K^+ in human serum determined by different methods and their variation with the number of erythrocites added

Serial No	Activation analysis MEQ Na^+	K^+	Flame photometry MEQ Na^+	K^+	Percentual difference % Na^+	K^+	Erythrocytes added per mm³ of serum
27/19	135,4	4,59	142	4,7	-4,7	-2,3	-
44/13	133,2	4,30	142	4,6	-6,2	-6,5	20.000
38/14	138,9	4,9	142	4,6	-2,2	+6,5	40.000
7/19	147,0	6,86	141	6,0	+4,2	+14,3	80.000
3/17	133,7	9,15	146	8,2	-8,5	+11,5	100.000
205/19	133,5	9,26	143	8,6	-6,7	+7,6	200.000

Table III.

Variation of the Na^+ and K^+ concentrations in the different phases of experimental intervention. Samples for activation analysis were taken in fractions

	Serial No.	Sample	Weight g	Na^+ MEQ	K^+	Erythrocites per mm³	Remark
I.	29/64 74/22 67/22 51/23	peri- lymphe + erythro- cites	0,0025 0,0031 0,0019 0,0045	150 156 174 167	14 10,1 13 12,6	20.000- -250.000	hardly any haemorrhage in the initial stage of the intervention
II.	82/21 88/22 75/21 58/23	peri- lymph	0,0048 0,0092 0,0048 0,0034	147 124 172 162	4,8 5,74 5,04 5,49	5.000- -20.000	pure sample
III.	80/18 59/24	peri- lymph + endo- lymph	0,0029 0,0030	129,8 174,8	12,5 12,1	20.000 -60.000	several, protracted interventions
IV.	68/25 73/24 81	endo- lymph+ peri- lymph	0,0051 0,0029 0,0030	104,5 114,0 99	27,9 24,43 17,0	100.000- -300.000	appreciable haemorrhage after chisselling of the cochlea bone

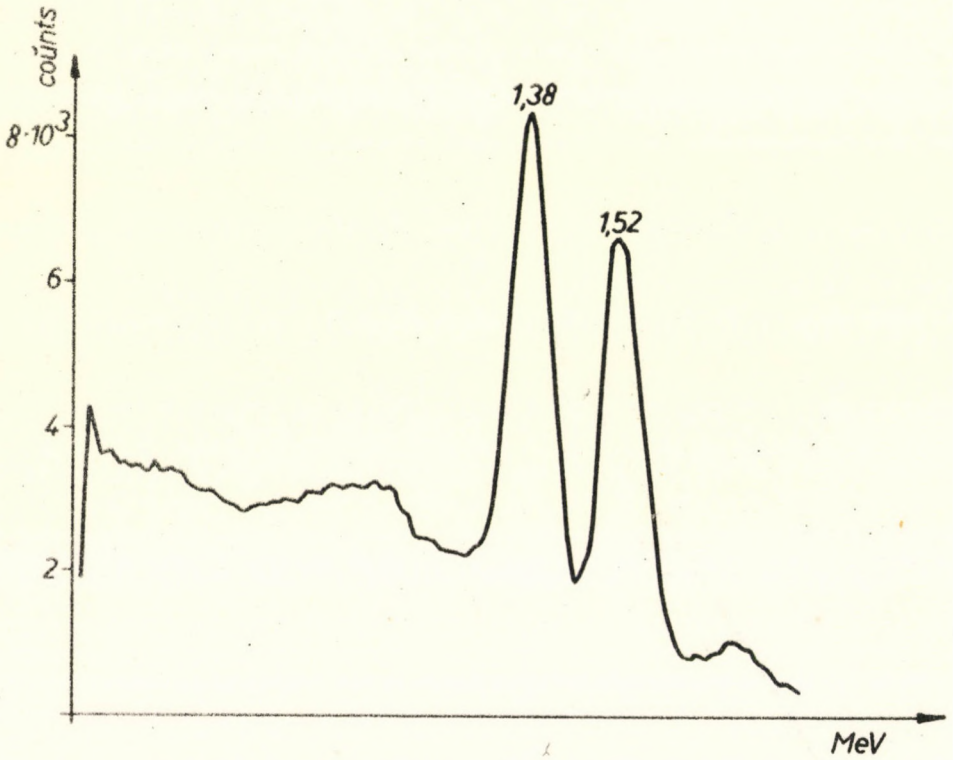

Fig. 1. Separation of potassium by single precipitation

µgk	Peak area counts/100sec
2,1	7,332
3,9	13,613
6,0	19,396
8,1	25,892
9,9	31,847

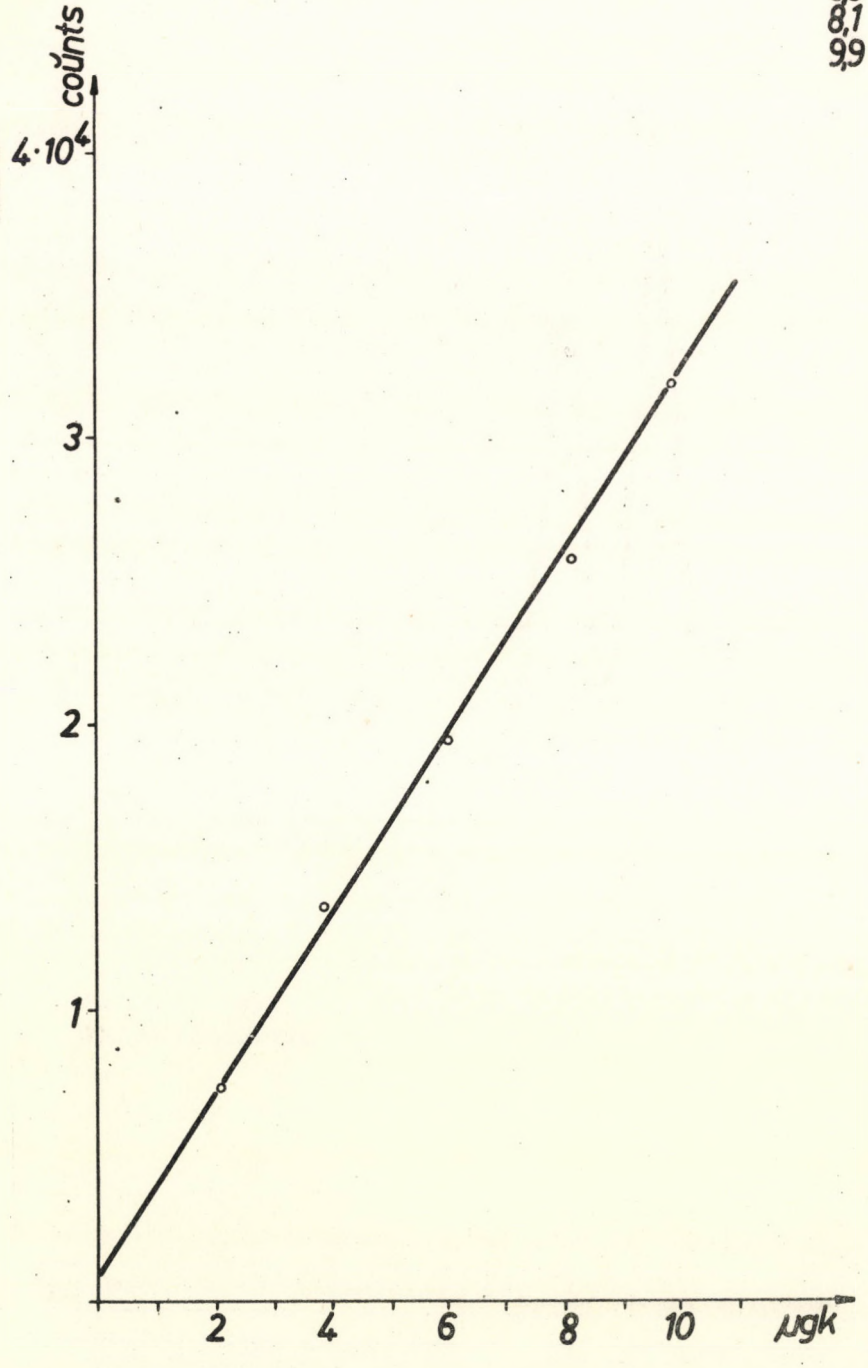

Fig. 2. Calibration curve for potassium

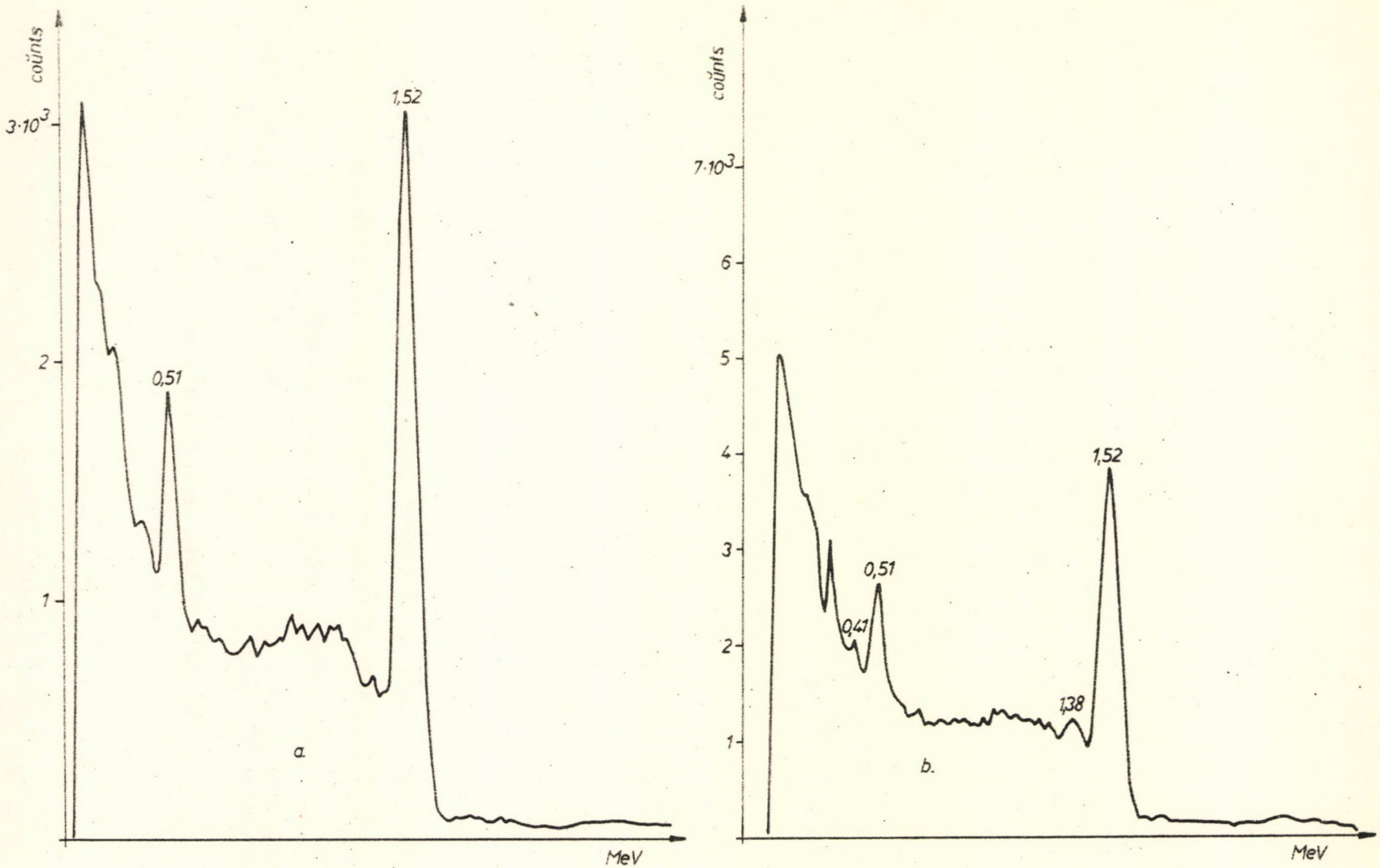

Fig. 3. Separation of potassium by isotopic exchange

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