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NOTE ON THE PERMEABILITY OF RED BLOOD CORPUSCLES TO POTASSIUM

BY

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The change in permeability of red blood corpuscles under the action of certain substances which affect the cell surface has been the subject of a number of investigations described earlier (1). It was found that very dilute solutions of bee venom cause a swelling of the cells or even hemolysis, and it was assumed that these changes in the cell surface are accompanied by a change in permeability both to anions and cations.

It was the aim of the above mentioned studies and of those to be described in the following to measure these changes in permeability with the aid of radioactive indicators. Radio-phosphorus and radio-potassium were applied as indicators, and the measurements were performed mainly on human blood. The experimental technique when using potassium as an indicator is somewhat different from the usual technique developed for phosphorus, mainly in view of the short half-life period of potassium (12.4 h) and, furthermore, owing to the fact that it involves some difficulty to precipitate potassium quantitatively within a reasonable time.

Since a comparative evaluation of permeability measurements claims a detailed knowledge of the prevailing experimental conditions, the following account will include a rather detailed description of the experimental procedure.

Radio-potassium.

The preparations of artificially radioactive potassium applied here were most kindly put at our disposal by the Nobel Institute for Physics, Stockholm. The author takes the opportunity to express her most sincere thanks to the director of this Institute, Prof. M. Siegbahn, and to Dr. H. Atterling for their great

readiness and the trouble in providing us with especially strong preparations.

Due to the experimental conditions during bombardment of the potassium sample the latter always contained some radio-phosphorus (c. 5 % of the initial activity) and mostly even radio-sodium. It was therefore necessary to subject the preparation to a chemical purification which had to be quantitative and—in view of the 12.4 h period—had to be carried through as quickly as possible. Moreover, when developing a suitable method of purification it had to be taken into consideration that neither the precipitate nor possible excess precipitants contained substances which might be injurious to living cells[1].

The radioactive preparation of KCl (20—40 mgm.) in aqueous solution obtained by deuteron bombardment of KOH in the Stockholm cyclotron was in the first approximation freed from ^{32}P by adding a given quantity of ordinary sodium phosphate which was precipitated with $CaCl_2$ at pH 8 as hydroxyl apatite. Although this precipitation occurred in 50 % alcohol, only 98 % of the phosphate were removed. This proved to be insufficient in view of the strong P-activity present and its comparatively slow decay. For a further purification which moreover aimed at a separation of the radio-potassium from traces of radio-sodium, the filtrate after the phosphate precipitation was evaporated on the water bath to c. 1 ml. and transferred to a platinum crucible. After addition of a slight excess of (10 %) perchloric acid plus an equal quantity of 96 % alcohol and evaporation, the crystalline potassium perchlorate was washed repeatedly with a few ml. of 1 % perchloric acid in alcohol and the supernatant liquid was removed with a pipette. The precipitate was then dried in the crucible, a c. twentyfold quantity of ammonium chloride was added, and the mixture was evaporated over a Bunsen burner. Generally, the latter manipulation was repeated once. The resulting KCl was dissolved in distilled water.

Decay measurements over 6—8 half-life periods on samples of this initial labelled KCl showed no residual phosphorus activity worth mentioning. The strength of the preparations available for our experiments was of the order of magnitude

[1] The author wishes to express her best thanks to Magister Th. Rosenberg for valuable advice concerning the purification of the potassium samples.

of $50 \cdot 10^6$ impulses per minute, measured on a Geiger counter of the Copenhagen type (2), corresponding to c. 0.5 milliCurie. The ^{32}P impurities still present were at least below 0.1 $^0/_0$. Control measurements and experiments which will be described elsewhere (3) indicated that the KCl preparations even were practically free from radio-sodium.

Measurement of the Distribution Coefficient.

Experimental. When labelled potassium is added to blood *in vivo*, a fairly rapid exchange with the potassium present in the organs and the muscles takes place. Hevesy (4) has shown that labelled K injected intravenously into a rabbit disappears from the plasma at a high rate, entering mainly the tissue cells. A marked activation of the corpuscles can only be obtained after repeatedly introducing labelled K into the organism.

The present experiments on human blood corpuscles *in vitro* showed that the rate of penetration of potassium ions through the membrane of red cells is of the same order of magnitude as that of phosphate ions (cf. also (5)). The experimental procedure for the determination of the distribution coefficient was as follows.

The blood was drawn from healthy persons by venous puncture (without special regard to the preceding diet). Either citrate or heparin was employed as anticoagulant (cf. below). Immediately after drawing, the blood was cooled in ice-water. A quantity of c. 30 ml. was then transferred to an Erlenmeyer flask containing labelled KCl and was shaken in a water thermostat at 37° C. At intervals, c. 3 ml. blood were removed with a pipette and centrifuged in ice-cooled centrifuge tubes of known weight. After separation from the plasma the corpuscles were washed twice with physiological NaCl-solution and centrifuged sharply. After weighing, the tubes containing the washed corpuscles or the plasma were placed into a glycerine bath at 110° C until most of the water had boiled off, and the samples were subsequently dried to constant weight at 120° C. Their wet weight and their dry weight were thus determined. Finally, the dried substance was ground in a mortar and a quantity of each sample was weighed into a small aluminium dish for activity measurements.

The activity of the samples was measured by means of a Geiger counter arrangement as described earlier (2), and the activities determined repeatedly at any time were calculated back to a given time on the basis of the exact formula for the decay with a period of 12.4 h. In this way, samples could be remeasured and accuracy considerations could be taken into account for each step involved in the whole procedure.

Results. The distribution coefficient for potassium, viz. the quotient

$$\frac{\text{activity per g corpuscles}}{\text{activity per g plasma}}$$

in human blood has been measured over a period of 3 h. The curves obtained are represented in Fig. 1. Curve A was found on freshly drawn citrate blood and with a small quantity of labelled K of a very high specific activity. It appears from the curve that a distribution coefficient 1 is reached already after 100 min. of shaking at 37°, and after 3 h of shaking the quotient assumes a value 2.5. Obviously, this does not mean equilibrium or complete exchange between the potassium of the plasma and that of the corpuscles, since the potassium content of the corpuscles is almost 20 times that of the plasma. However, it appears impossible to obtain complete exchange during *in vitro* experiments as the state of the blood after some hours of shaking by no means can be regarded as physiological. When radio-phosphorus is applied in an analogous way a distribution coefficient 1 is reached in the course of c. 2 h. (cf. 1.).

The experiment exhibited in curve A was repeated with the same potassium preparation 48 h later, however, in view of the decay of the potassium activity c. 15 times the quantity of KCl had to be employed. Moreover, the preparation contained some excess $CaCl_2$ after a repeated phosphate precipitation. The distribution coefficient measured is represented in curve B, and the difference between curves A and B is very marked.

In view of these results it is very tempting to assume that either the presence of Ca or the increased amount of K present, or a combination of both factors might strongly affect the permeability of blood corpuscles to potassium ions under the conditions prevailing in these experiments. An attempt was therefore made

to clear up this question by measuring the distribution coefficient in the presence of various amounts of Ca. Experiments with varying concentrations of potassium had to be delayed to a later date (cf. the conclusive remark on p. 8). As the application of sodium citrate as an anticoagulant involved the removal of Ca

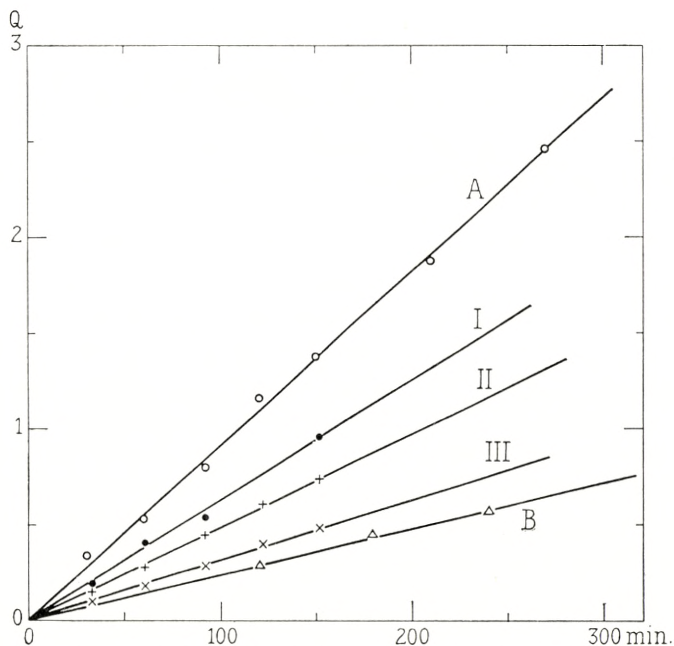

Fig. 1. Distribution coefficient of labelled potassium in human blood corpuscles.

ordinates: $\frac{\text{activity per g corpuscles}}{\text{activity per g plasma}}$

abscissae: time in min.

from the blood, citrate blood was considered to represent the Ca-content zero, while heparin blood was taken to represent blood of the ordinary calcium content. Finally, to 25 ml. heparin blood of the same subject 11 mgm. $CaCl_2$ were added and all three samples were shaken with the same quantity of radio-potassium in the manner described above. The curves I, II, and III illustrate the dependence of the permeability to K upon the amount of Ca present in the blood. The permeability of red cells to potassium ions *in vitro* is markedly reduced in the presence of increasing amounts of calcium.

It was moreover studied at what rate the labelled K atoms

which entered the blood corpuscles leave the cell and re-enter the plasma. For this purpose total blood was shaken for 2 h with labelled KCl just as described above, the corpuscles were centrifuged off, washed twice with physiological NaCl-solution and then resuspended in inactive plasma. Subsequently, the blood was shaken in a thermostat at 37° and samples were taken at intervals during a period of 2 h. The activity then found in the plasma originates from labelled K which left the activated corpuscles. In the course of 2 h, 5 % of the corpuscle activity had entered the inactive plasma. Corresponding measurements with radio-phosphorus showed that 2—3 % of the corpuscle activity had permeated into the plasma in the course of 2 h.

The present investigations were carried out at the Wenner-Gren Institute for Experimental Biology, Stockholm. Due to the end of the war in Europe my work at this institute had to be discontinued; however, it is planned to resume these problems and to complete the experiments in Copenhagen as soon as possible.

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