# ACCUMULATION OF POTASSIUM BY HUMAN RED CELLS

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(Received for publication, March 5, 1950)

It is now established (Danowski, 1941, Harris, 1941, Maisels, 1949 *a*) that there is a tendency for K and Na to be exchanged between the human red cell and the surrounding medium in the same direction as that of the diffusion gradients, *i.e.* for K to be lost and for Na to be gained, and that there is also a process which operates against the diffusion gradients and which results in an accumulation of K. This may be an active process or may be secondary to the excretion of Na (Maisels, 1949 *a*, *b*). These two oppositely directed processes maintain a steady state determined by the difference between their rates (Ponder, 1949 *a*). Provided that there is a permeability of the red cell to K and to Na, there is no difficulty in understanding the movements of the ions in the direction of their diffusion gradients, and in systems containing certain lysins these movements become greatly increased (Davson and Danielli, 1938, Ponder, 1947 *a*, *b*, *c*, 1948 *a*, *b*); active processes directed against the gradients, on the other hand, must involve transport mechanisms, and are less easy to account for as well as being more difficult to investigate.

All the investigators who have studied K accumulation by the red cell have had difficulty in reproducing their results, and all have concluded that quantitative work is impossible unless special care is taken to control a large number of variables. It would be convenient if something equivalent to a "standard system" could be found, in which the accumulation phenomena could be uniformly reproduced as functions of time, and which could be varied in order to bring out the effect of the different factors involved. This paper is concerned with the technical problem of devising such a system, and with a study of some of the more important variables which affect the accumulation process.

## *General Description of Method*

One of the principal causes of difficulty in obtaining consistent results in experiments with systems in which red cells accumulate K is that the accumulation process has a pH optimum and that, being linked to glycolysis, it tends to bring about a pH shift during the course of the experiment (Maisels, 1949 *a*). To reduce this shift, the buffering capacity of the system has to be increased as much as possible, and this can be done (*a*) by making the volume of buffer surrounding the cells as large as is practicable, and (*b*) by taking advantage of the fact that the concentration in which phosphate buffers maintain the volume of human red cells unchanged is about 0.11 M, and not

0.067 M, the concentration usually employed in buffer mixtures.[1] When used in 0.11 M concentration in actively glycolyzing systems in which the volume concentration of the red cells is not greater than 0.1, $Na_2HPO_4$-$NaH_2PO_4$ buffers are able to maintain the pH to within 0.1 for as long as 24 hours. A standard system for the study of accumulation of K by human red cells can accordingly be prepared in the following way.

Using sterile technic throughout (Ponder, 1949 *a*), 10 to 20 ml. of human blood is withdrawn into heparin and allowed to stand, with occasional shaking, at 4°C. for 60 hours (both the temperature and the time of storage can be varied). The cells are then washed twice at refrigerator temperature with a 0.11 M $Na_2HPO_4$-$NaH_2PO_4$ buffer mixture at pH 7.5, and are made up to their original volume concentration with the buffer mixture. The volume concentration $\rho$ is found with a high speed hematocrit.

One ml. of the washed red cell suspension is transferred to each of a series of sterile rubber-capped tubes. The number of tubes used depends on the nature of the experiment, the system in each tube supplying one point on the curves which will finally be obtained. Four ml. of phosphate buffer containing KCl (usually in a concentration of 34.5 m.eq./liter, but this can be varied) and glucose (usually in a concentration of 400 mg./100 ml., but this can be varied) are added to half of the tubes, and 4 ml. of the same phosphate buffer–KCl mixture, without glucose, is added to the remainder. This results in two series of equal numbers of tubes, the first containing systems with added glucose, and the second containing otherwise identical systems with no glucose added. One additional system is prepared by adding 4 ml. of phosphate buffer–KCl mixture, without glucose, to 1 ml. of the washed cell suspension; the cells of this system are packed immediately in the special tubes to be described below, and the K content of the packed cells is found with the flame photometer. The Hb content of the packed cells, in arbitrary units per liter of cells, is found photometrically, and the water content of the packed cells is found by drying to constant weight. These values establish the initial state of the cells, before either K accumulation or K loss has occurred. The tubes of the two series are placed in a water bath at 37°C., in which they are rocked to and fro through a 30° arc. The time during which they are kept at 37°C. can be varied, but when any tube of the first series (glucose added) is removed from the bath, the corresponding tube of the second series (no glucose added) is removed at the same time, and the K concentrations in the packed cells of these two corresponding systems are measured as nearly simultaneously as possible.

Immediately after the removal of a system from the water bath, its pH is measured with a glass electrode which has a NaCl bridge of about 10 ml. capacity interposed between the system and the KCl bridge of the calomel half-cell. The cells of the system are then packed, at $5 \times 10^3$ *G* for 20 minutes, in special tubes which have a long wide upper part (70 mm. × 10 mm.) and a short narrow lower part (25 mm. × 5 mm.)[2]

[1] The concentration of phosphate buffer which maintains the volume of the human red cell unchanged for about 1 hour at 25°C. is found by comparing the volumes of the cells in systems composed of 0.5 ml. of heparinized blood plus 2 ml. of phosphate buffer mixtures of varying concentration, all in the pH range 7.3 to 7.5, with the volume of the cells in a system composed of 0.5 ml. of heparinized blood plus 2 ml. of 1 per cent NaCl (pH of system, 7.3). The method used is essentially the same as that already described (Ponder, 1949 *b*).

[2] These tubes are made by E. Machlett and Son, New York City.

which, after centrifuging, is occupied by the mass of packed cells. The supernatant fluid is removed with a capillary pipette, a narrow-tipped 0.2 ml. pipette is thrust to the bottom of the packed cell mass, and 0.2 ml. of packed cells is transferred without loss to a 25 ml. volumetric flask. After standing for some hours, the hemolyzed contents of the flask are filtered. The K content in milliequivalents per liter of cells is found with the flame photometer, and the Hb content, in arbitrary units per liter of cells, is found photometrically. The initial cell water being known, volume changes can be calculated from the Hb contents found before and after warming to 37°C., and the

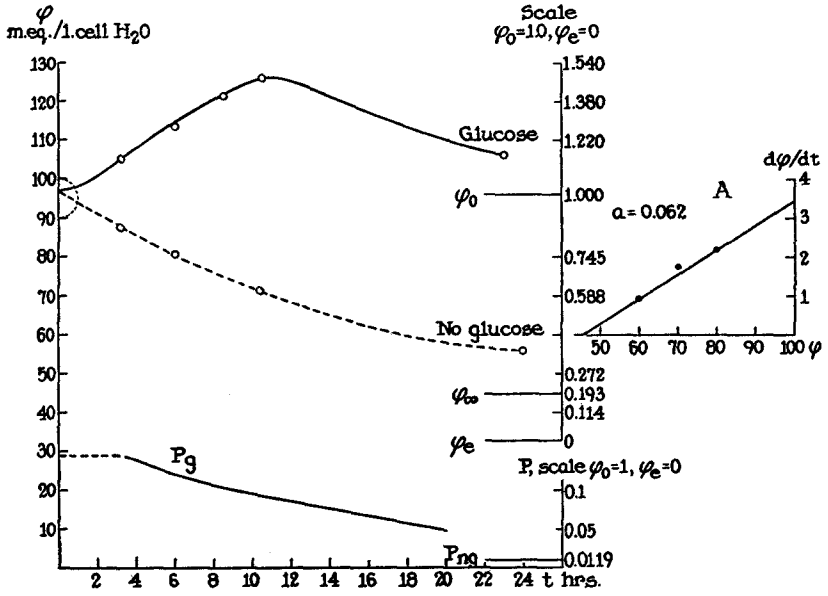


FIG. 1. Accumulation of K in a system with added glucose, and loss of K in a system without added glucose, both as functions of time. To illustrate the analysis described in the text.

volume changes being known, the K concentrations can be computed as concentrations in milliequivalents per liter of cell water.

The effect of factors such as time, pH, glucose concentration, etc., can be studied by employing this basic technic with suitable modifications which will be described in the sections below. The final result is always the expression of the effect of the variable as a curve which covers the experimental range, and which in some cases (*e.g.*, when time is the variable) can be analyzed further.

### *1. K Accumulation as a Function of Time*

Fig. 1 shows a typical relation between time and the K content of the washed red cells of heparinized human blood which has been stored for 60 hours at 4°C. It refers to systems in which the volume concentration of the cells is 0.1, in which the suspension medium is a KCl-buffer-glucose mixture (34.5 m.eq./

liter of KCl, 400 mg./100 ml. glucose) with a pH of 7.6 when measured at 25°C. (the optimum pH, see section 2, below), and in which the buffering capacity is so large as to maintain the pH constant to within 0.1 over the entire 24 hours course of the experiment. The dotted line shows the relation between time and the K content of the same cells suspended in the same medium but without added glucose. The K contents of the cells are given in milliequivalents per liter of cell $H_2O$; *i.e.*, volume changes have been taken into account.

In systems containing glucose and after a short interval during which experimental observations have not been possible, the K content of the cells begins to increase as a result of the activity of an accumulation process. A maximum is reached after some 8 to 12 hours; the K content of the cells then begins to decrease with increasing time, becoming less than the initial value for $t = 0$. In systems containing no added glucose (dotted line), the K content of the cells falls continuously from the initial value. This curve can be represented by the equation

$$-d\varphi/dt = P - a\varphi \tag{1}$$

and can be analyzed in the manner already described (Ponder, 1949 *a*). The units used here, however, are milliequivalents per liter of cell $H_2O$, and in order to give the symbols in expression (1) the same meaning as they have already been given, it is necessary to convert them into units on a scale which measures the initial K content of the cells as $\varphi_0 = 1.0$ and upon which the concentration $\varphi_e$ which exists throughout the system at equilibirum is represented by zero. To convert to this scale, value of $\varphi$ as measured in milliequivalents per liter of cell $H_2O$ require to be given the values $(\varphi - \varphi_e)/(\varphi_0 - \varphi_e)$. The curve for systems containing no added glucose proceeds exponentially towards an asymptote $\varphi_\infty = 45$ m.eq./liter of cell $H_2O$; this becomes $(45 - 32.8)/(96 - 32.8)$, or 0.193, the values of $\varphi_e$ and of $\varphi_0$ being 32.8 and 96 for the system under consideration. Now 0.193 is the position of the asymptote of the curve in the units of a scale on which $P/a = 0.193$; since $a = 0.062$ (see insert A of Fig. 1), $P = 0.0119$.[3] The asymptotic value $\varphi_\infty$ lies above the eqilibrium value $\varphi_e$, and so

[3] This value of $P$ is much smaller than any of those given for the washed cells of freshly drawn human blood in systems at 37°C. prepared with 1 per cent NaCl and of a considerably smaller volume concentration (Ponder, 1949 *a*, Table V, average value of $P$, 0.066). There are two reasons for this. The first is that the value of the asymptote $\varphi_\infty$ depends on the length of time during which the heparinized blood is stored at 4°C. before being introduced into the systems which are warmed to 37°C. The fresher the blood, the greater, in general, is $\varphi_\infty$; the following values, for example, were found for the cells of human blood stored at 4°C. for varying lengths of time:— fresh, 0.50; 60 hours, 0.45; 84 hours, 0.36 (values on the $\varphi_0 = 1$, $\varphi_e = 0$ scale). This steady diminution in the value of $\varphi_\infty$ with time is probably due to a deterioration of the accumulation mechanism during the storage at 4°C. The second is that the experiment illustrated in Fig. 1 has been selected because there is no sign of an accumulation effect in the

there seems to be a process which offsets or prevents the outward diffusion of K, even in the absence of added glucose, when $\varphi$ is as small as 45 m.eq./liter of cell $H_2O$ or 0.193 on the $\varphi_0 = 1$, $\varphi_e = 0$ scale. Were it not for this opposing process, the rate of outward diffusion would be 0.76 m.eq./liter of cell $H_2O$ per hour when $\varphi = 45$ m.eq./liter of cell $H_2O$. In what follows, it will be convenient to distinguish between this value of $P$, found in systems containing no added glucose, and the values of $P$ found in systems to which glucose has been added. Call the former $P_{ng}$ and the latter $P_g$. It will be seen below that $P_g$ almost certainly measures the activity of an ion transfer process dependent on red cell metabolism, but that the significance of $P_{ng}$ is by no means as clear.

On the assumption that the addition of glucose does not change the value of $a$, the curve for the system containing added glucose can be analyzed in a similar manner, $P$ being found from $(a - d\varphi/dt)$. The result is shown in the curve marked $P_g$ at the bottom of Fig. 1, the ordinate being the small scale at the right. Large at first, $P_g$ falls with increasing time, tending to reach a final value which is the same as that of $P_{ng}$, 0.0119. This steady fall is probably due to the accumulation mechanism undergoing progressive deterioration with time. The deterioration does not seem to be due to either a lack of glucose or a failure on the part of the cells to utilize it; it is possible that it is the result of the using up of some other substance; *e.g.*, a component of an enzyme system essential to the ion transfer mechanism.

The way in which the K content of the cells varies with time is not very accurately established by this technic when the times are short (less than 1 to 2 hours) or very long (greater than 24 hours). For the first hour or two after the cells have been warmed to 37°C., the course of the curve in the case of systems with no added glucose is probably similar to that shown in Ponder, 1949 *a*, Fig. 2, inset D, *i.e.* concave to the $t$-axis at first, but becoming convex and apparently exponential later on. Further, a small accumulation effect sometimes occurs during the first few hours of warming to 37°C. even in the absence of added glucose; this distorts the course of the curve and makes the K loss less than it otherwise would be. I have observed very definite effects of this kind, accumulation amounting to as much as 8 m.eq./liter of cell $H_2O$ occurring in the first 3 hours of warming in systems containing no added glucose. Harris (1941) has made the same observation. It is true that the systems in which the effect occurs may have contained some glucose although none was added, and in a few cases in which it has been estimated it has been found to be about 5 mg./100 ml. (presumably derived from the cells). Since the cells of an individual system use up only about 0.16 mg. per hour when utilizing glucose at the rate at which it is used up in whole blood, this quantity is not altogether negligible. Even a transient accumulation of K can affect the course of the initial part of the curve considerably, and greatly increases the number of observations required to outline it.

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absence of added glucose (see below); it happens that the curve for the system containing no added glucose passes, in this instance, to an asymptote unusually close to $\varphi_e$.

The initial part of the curve relating the K concentration to time in the case of systems containing added glucose and showing accumulation is probably convex to the $t$-axis at first, becoming concave as the rate of K accumulation rises towards its maximum. This uncertainty as to the initial part of the curves for the system containing added glucose, together with the uncertainty as to the initial part of the curve for the system with no added glucose, makes it impossible to say how $P_g$ behaves during the first hour of warming to 37°C. When the system is raised to this temperature, $P_g$ may either assume a high value almost immediately and then start to fall, or it may take some time to reach a maximum value from which it steadily declines.

When there is no accumulation effect, and if the course during the first few hours is disregarded, the curve in the case of systems containing no added glucose appears to fall exponentially towards an asymptote corresponding to a value of $\varphi$ considerably greater than that at which the K concentrations inside and outside the cell would be equal. After some 24 to 36 hours, however, the curve turns downwards, as in the "intermediate" type of curve already described (Ponder, 1949 *a*, Figs. 2 and 3). This change in its course is accompanied by considerable hemolysis, less marked in systems containing added glucose, in which the cells seem to deteriorate less rapidly. The assumption is made, for analytical purposes, that the curve for a system containing added glucose proceeds towards the same asymptote $\varphi_\infty$ as that for an otherwise comparable system containing no added glucose, but the systems begin to hemolyze and the curves become "intermediate" in type long before any such convergence can be demonstrated conclusively.

The most puzzling feature of these curves is that they appear to approach asymptotes situated at values of $\varphi$ so much greater than those, denoted by $\varphi_e$, which correspond to a uniform distribution of K throughout the systems. This can be accounted for in terms of expression 1 if one can suppose that there is a value of $P = a\varphi$, but in a system containing no added glucose it is difficult to find a source of energy which will supply the hypothetical pumping mechanism $P$. Alternatively, the course of the curve and its approach to an asymptote at $\varphi_\infty$ might be the result of some of the K in the cell being relatively "immobile," so that $d\varphi/dt = a(\varphi - \varphi_\infty)$. A preferential binding of K to the Hb or other material in the red cell interior (Stratman and Wright, 1948, and *cf.* Cowie, Roberts, and Roberts, 1949, Roberts, Roberts, and Cowie, 1949) might lead to this result, but the matter requires much further investigation and certainly cannot be settled on the basis of existing data.

A particularly unsatisfactory feature of the situation is that the exponential nature of the curves relating $\varphi$ and $t$ at 37°C. in systems without added glucose is none too securely established. If the effects which are observed at small values of $t$ (the small transitory accumulation effects) and at large values of $t$ (hemolysis and the change in the course of the curve to that of an "intermediate" type) are left out of consideration, the remainder of the curve appears to be exponential and the asymptote $\varphi_\infty$, greater than $\varphi_e$, is approached as $t$ becomes very large. But since the extreme parts of the experimental relation have been left out of consideration, it is possible that $\varphi_\infty$ is, to some extent at least, a creation of the analysis.[4]

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[4] *Note Added to Proof.*—The anomalously high position of the asymptotes of these and similar curves obtained at lower temperatures has now been shown to be due to part of the population of red cells losing K more readily than the remainder. This

Were it practicable to do so, it would certainly be desirable to study the effect of variations in pH, etc., on the entire course of the curve relating the K content of the cells to time, both in the presence and in the absence of added glucose. The difficulties which would be met with, however, would be very great for purely technical reasons, and so the investigation has to be restricted to the study of the effect of variations in pH, etc., on some selected parameter of the curve. Of the many parameters which might be chosen, the one which seems to be most likely to convey information of a preliminary character is the *maximum rate of accumulation of K* at 37°C. A good approximation to this is the difference between the increase in red cell K in a system containing added glucose and the decrease in red cell K in a similar system with no added glucose, both quantities being measured during the first 3 hours of warming to 37°C, The following sections contain a description of the effect of variations in pH, duration of storage at 4°C., sugar concentration, and temperature on these rates and on their difference, and the maximum rate of accumulation.[5]

### *2. K Accumulation as a Function of pH*

The effect of variations in pH can be studied by using systems containing the washed red cells of 1 ml. of heparinized blood which has been stored at 4°C. for 60 hours. The cells are washed in a phosphate buffer at pH 7.5, and the system is completed by adding 4 ml. of KCl-buffer-glucose mixture (34.5 m.eq./liter of KCl, 400 mg./100 ml. of glucose) at various pH's. Convenient pH's for the KCl-buffer-glucose mixtures used to complete the systems are 6.0, 6.6, 7.6, and 8.9; after the addition of these to the washed red cells, the pH's of the completed systems are measured, and in the experiment to be described below they were 6.3, 6.8, 7.6, and 8.0.[6] The buffering power of the systems is sufficient to keep the pH constant to within 0.1 pH unit for the 3 hour period of warming to 37°C. A series of systems, identical with the foregoing but containing no added glucose, is prepared and warmed to 37°C. along with the glucose-containing systems.

The curve marked *a* in Fig. 2 shows the change in the K content of the red cells in glucose-containing systems after 3 hours' warming to 37°C.; the values shown on this curve are the differences between the K content of the cells and the K content before warming (the "cold storage value"). A loss of K takes

results in a curvilinear relation between $d\varphi/dt$ and $\varphi$, and anomalously high values of $\varphi\infty$ result from the treatment of the upper part of this curve as a straight line.

[5] Accumulation should be measured as this difference, and not merely in reference to a "cold storage value." The type of error introduced by using the "cold storage value" as the only point of reference is illustrated in the discussion of Fig. 2.

[6] All pH's are measured at 25°C. To correct them for 37°C., it is necessary to subtract a small factor, which has a value in the neighborhood of 0.1. The pH maximum observed is quite a flat one and so the pH optimum for the accumulation process may lie even nearer to the pH of the circulating blood.

place when the pH is less than 7.15; when the pH is higher, the loss is replaced by a gain which passes through a maximum at pH 7.6, with an uncertainty of about 0.2 pH unit. The curve marked *b* shows the change in the K content of the cells in systems containing no added glucose but treated similarly; again the values on this curve are referred to the "cold storage value" as zero. A loss of K occurs at all pH's, the loss being smallest at pH 7.6. The gain attributable to a metabolic process which depends on the presence of the added

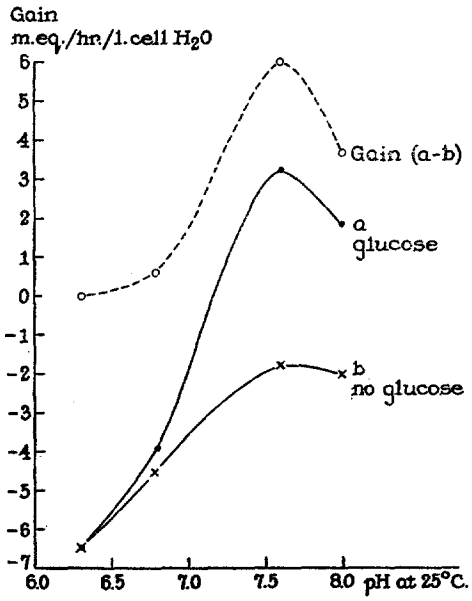


FIG. 2. The maximum rate of accumulation of K as a function of pH. Curve *a*, gains or losses of K during first 3 hours at 37°C. in systems containing added glucose; curve *b*, the same for systems containing no added glucose. In the case of both curves, the values are referred to the "cold storage value" as zero. Dotted curve, the maximum rate of accumulation derived from curves *a* and *b*.

glucose is given by the differences between the K content of the cells in the glucose-containing systems and in the corresponding systems to which no glucose has been added, and is shown by the dotted curve passing through circles. This gain, in milliequivalents per hour per liter of cell $H_2O$, increases from zero at pH 6.3 to a maximum of 6.0 at pH 7.6; when the pH is increased still further, the gain diminishes.

These observations are in agreement with Maisel's conclusion that the accumulation process has an optimum pH lying between 7.3 and 7.8. It will also be noticed that the rate of loss of K into a medium containing no added glucose is at a minimum at the same pH as that at which the accumulation is at a maximum.

It will be clear that a serious underestimate of the gain would be made if we were to use the initial K content of the cells ("cold storage value") as the only point of reference and were to restrict the term accumulation by requiring that the K content of the cells should exceed this value. This restriction would lead to the conclusion that there is no accumulation below pH 7.15, whereas there is measurable accumulation down to pH 6.6 at least; it would also lead to an underestimate of the amount of accumulation in 3 hours, *i.e.* during the time at which the rate of accumulation is at a maximum, by some 50 per cent.

*3. The Effect of the Duration of Storage at 4°C.*

The effect of the duration of storage at low temperatures can be studied by using heparinized blood kept at 4°C. for varying lengths of time, washing the

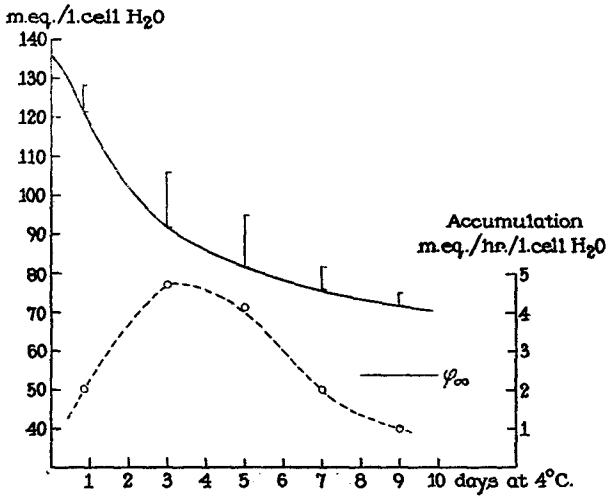


FIG. 3. The maximum rate of accumulation of K as affected by the length of time of storage of cells at 4°C. For description, see text. The scale at the right refers to the dotted curve.

cells in phosphate buffer at pH 7.5, and preparing systems in KCl-buffer-glucose (34.5 m.eq./liter of KCl, 400 mg./100 ml. of glucose, pH 7.6) and in KCl-buffer without added glucose. The systems are warmed to 37°C. for 3 hours, and the difference between the K contents of the cells in systems with and without glucose is expressed in milliequivalents per liter of cell $H_2O$.

Fig. 3 shows the fall in the K content of the cells as they are stored at 4°C. for various lengths of time; equality of K concentration inside and outside the cells would be reached in this particular case at a level of 54 m.eq./liter of cell $H_2O$. The gain in K content resulting from warming to 37°C. for 3 hours is shown by the short vertical lines, and the rates of accumulation per hour are shown as circles joined by the dotted line and referred to the ordinate on the right.

As measured in this way,[7] accumulation does not take place in the red cells of freshly drawn blood, presumably because there has been no K loss. As the K loss increases with time, however, the maximum rate of accumulation increases to a maximum value which is found after some 3 to 5 days of storage at low temperatures; thereafter the maximum rate of accumulation decreases to become almost negligible when the cells have been stored for 10 days or more. Since red cells which have been stored for 10 days at low temperatures still show rates of glycolysis as great as 50 per cent of the initial rate (*cf.* Rapaport 1947), the failure to accumulate K must be attributed to a deterioration of the accumulation mechanism rather than to an inability of the cells to utilize glucose.

### *4. Other Properties of the Accumulation Mechanism*

These can be summarized briefly, as they have been studied by essentially the same methods as described above, but with obvious modifications of the standard system.

*1. Sugar Concentration.*—The maximum rate of accumulation at pH 7.6 occurs in systems which contain an initial concentration of glucose of between 50 and 200 mg /100 ml. When the concentration is smaller, the rate is less, and larger concentrations apparently depress the activity of the accumulation mechanism. The maximum is not a very sharp one, however, and the depressing effect of concentrations in the neighborhood of 400 mg./100 ml. is only such as to reduce the maximum rate of accumulation by some 30 per cent. This effect has a counterpart in the depressing effect of high concentrations of glucose on glycolysis (Rona and Wilenko, 1914). In concentrations less than about 50 mg./100 ml., on the other hand, the rate of accumulation and the total amount of K accumulated fall quite sharply. This dependence of the accumulation of the glucose concentration makes it impossible to maintain optimum conditions for accumulation in these systems over a long period of time, for an initial concentration of glucose which would not be exhausted by a 24 hour utilization at the rate of 400 mg./liter of cells/hr. would itself depress the maximum rate of accumulation in the earlier hours of the experiment.

*2. Temperature Coefficient.*—The temperature coefficient of the accumulation process, derived from a comparison of the maximum rate of accumulation at

[7]Accumulation of K at a rate $P_g$ is to be thought of as continually going on in the red cells of the circulating blood, offsetting the tendency for K to be lost at a rate $a\varphi_0$ and so maintaining the steady state. As a result of cooling and storage, $P_g$ falls to a very small value, and $\varphi$ falls because K is lost. The accumulation measured here is that which takes place because the original value of $P_g$ is restored, at least partially, by raising the temperature; if the accumulation process had undergone no deterioration, it would be able to restore $\varphi$ to its original value of $\varphi_0$ and then to maintain the original steady state.

37°C. with that at 27°C., is 2.4. This is only a little greater than the temperature coefficient for glycolysis over the same range (Irvin, 1926), and is the same as Sheppard and Martin's (1949) temperature coefficient for the exchange of radioactive K. Below 20 — 22°C., the movement of K is in the direction of a loss by the cells.

*3. Quantity of Glucose Utilized.*—The value of the ratio

$$\frac{\text{Milliequivalents of K accumulated/hr./liter of cell } H_2O}{\text{Grams of glucose used up/hr./liter of cell } H_2O}$$

in systems at pH 7.6 during the first 3 hours of warming to 37°C. varied in different experiments from 4 3 to 8.1 with an average value of 6.2. Accumulation of K at the rate of 6 m.eq./liter of cell $H_2O$/hr. with an initial K concentration of 86 m.eq./liter of cell $H_2O$ would require $RT \log c_2/c_1$ calories, or about 42 calories/liter of cell $H_2O$/hr., when calculated as if it were taking place between two dilute solutions, and so ratios lying between 4.3 and 8.1 would imply that the glucose is providing between 5,500 and 10,300 calorie/mol to the ion transfer system. This value, rough though it is, is so large as to suggest that there cannot be more than a few such ion transfer mechanisms operating in the red cell at the same time, or, alternatively, that it is altogether wrong to substitute ionic concentrations for ionic activities.

*4. Effect of Lithium and Cesium Ions.*—To investigate the effect of LiCl and CsCl on the accumulation of K, the standard technic was modified by adding 3 ml. of blood to 7 ml. of isotonic solutions of NaCl, LiCl, or CsCl in flasks which contained a few drops of heparin to prevent clotting. The mixtures were stored at 4°C. for 60 hours. At the end of this time, 3.3 ml. samples were transferred to tubes (3 for the NaCl systems, 3 for the LiCl systems, and 3 for the CsCl systems); the cells were centrifuged down, the supernatant fluids were removed, and 4 ml. of KCl-buffer or of KCl-buffer-glucose at pH 7.6 was added, as in the standard technic. By this procedure, washing in phosphate buffer and the exposure of the cells to large volumes of solutions containing Na were avoided. Accumulation was allowed to take place at 37°C. for 7 to 10 hours.

The result usually obtained was that accumulation occurred in all three types of system, that in the LiCl and CsCl systems usually being about half to three-quarters that in the NaCl system.[8] The glucose present in the unwashed cells of these systems is apparently sufficient to enable some accumulation to occur, for a small accumulation of K has been observed in a number of experiments with LiCl and CsCl systems to which no glucose was added. Although

[8] In the absence of glucose and both at 4°C. and 37°C., the loss of K into isotonic LiCl is a little smaller than that into isotonic NaCl (measurements of loss over several days at 4°C. and over 24 hours at 37°C.; pH 6.6 to 7.6, $\rho = 0.067$). The losses into isotonic CsCl have not been investigated in detail, but they are about the same as those which occur into isotonic NaCl.

it is true that about 20 per cent of the cations in the suspension medium of the LiCl and CsCl systems is Na, the active accumulation of K which occurs, under optimum conditions, in the LiCl and CsCl systems does not support, while it does not absolutely invalidate, Maisels' suggestion that the K accumulation is secondary to the excretion of Na. The K accumulation in these systems, indeed, does not seem to depend greatly on the nature of the other cations; whether the somewhat decreased activity of the accumulation is an effect of Li ion on glycolysis (*cf.* MacLeod, Swan, and Aitken, 1949) or on another step in the accumulation process remains to be investigated.[9] This does not mean, of course, that there may not be an active mechanism for the excretion of Na as well.

## SUMMARY

1. A method is described for measuring the accumulation of K at 37°C. by washed human red cells in glucose-containing systems in which the pH is kept constant, the K content of the cells being compared with that of the cells of systems which contain no added glucose but which are otherwise treated similarly.

2. In systems containing added glucose, the accumulation of K begins shortly after the cells have been warmed to 37°C., proceeds to a maximum which is reached after about 10 hours, and then falls exponentially. The maximum rate of accumulation is found during the first 3 hours. In systems which contain no added glucose, the K content of the cells appears to decrease exponentially with time for about 18 to 24 hours; thereafter the K content of the cells may decrease rapidly and the systems may show considerable hemolysis. Sometimes a small accumulation effect is observed during the first 2 to 3 hours; this may be the result of the washed cells not having been completely freed of glucose.

3. The accumulation process proceeds at its maximum rate at pH 7.4 to 7.6, which is also the pH at which the K loss from the red cells is at a minimum in systems containing no added glucose.

4. When red cells are stored at 4°C. for increasing lengths of time, the storage is accompanied by increasing K loss and the maximum rate of accumulation observed when the cells are warmed to 37°C. at first becomes greater. If the storage at 4°C. is continued for more than 3 to 4 days, the rate of the accumulation which occurs at 37°C decreases again, the accumulation mechanism showing progressive deterioration with time even at low temperatures. This deterioration has a counterpart in the progressive deterioration (deduced from the analysis of the curves relating K content and time) of the accumulation mechanism with time at 37°C.

[9] Attention should be paid to the purity of the LiCl used. Some preparations are badly contaminated. The samples of LiCl and CsCl used in these experiments were prepared by Dr. Theodore Shedlovsky for conductivity work.

5. The accumulation of K occurs at a maximum rate when the concentration of glucose in the system is between 50 and 200 mg./100 ml. Its temperature coefficient over the range 27–37°C. is 2.4. In the presence of glucose and at pH 7.6, accumulation of K takes place from isotonic mixtures of KCl and LiCl or of KCl and CsCl only a little less actively than from mixtures of KCl and NaCl; *i.e.*, the accumulation of K under optimum conditions seems to be an active process which is at least partly independent of the excretion of Na.

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