ANOMALOUS FEATURES OF THE LOSS OF K FROM HUMAN RED CELLS: RESULTS OF EXTENDED OBSERVATIONS

By ERIC PONDER

(From the Nassau Hospital, Mineola, Long Island)

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This paper is concerned with a more complete investigation of three unexpected observations made in the course of studies (Ponder, 1948 *a*, *b*, 1949, 1950 *b*) of K-Na exchanges in human red cells in systems containing neither added glucose nor added lysin. The first of these is that the curve which describes the K content of the cell as a function of time at low temperatures proceeds apparently exponentially towards an asymptote corresponding to a greater cell K content than that which would result from the cell K content becoming equal to the K content of the surrounding medium; some of the cell K seems to leave it more slowly than the remainder. The second is that the curves which describe the K content of the cell as a function of time at 37°C. pursue an apparently exponential course for about 24 hours, but again appear to pass towards asymptotes much higher than those which would correspond to a uniform distribution of K throughout the system. The third concerns the volume changes associated with K-Na exchanges occurring in the absence of added hemolysins, both at low temperatures and at 37°C.

Methods

An instance has already been met with (Ponder, 1948 *b*[1]) in which the curves relating the K content of the cells to time follow an anomalous course in that they seem to approach asymptotes much higher than those corresponding to a uniform distribution of K throughout the systems. The measurement of K losses in those systems up to times of 120 hours made it clear that the anomaly is largely due to restricted observation. The course of the curves now under consideration presents the same type of anomaly, and again this may be due to the range of observation being restricted. The method used for determining the K content of the cells in most instances has been to measure the K content by computing $F = (K - p)/(1 - p)$ and $\varphi = (1 - F)$; here K is the K lost, as a fraction of the initial K content of the cells, and is determined by measuring the K content of the supernatant fluid of the system in which the fraction of complete hemolysis is p (Ponder, 1949). This indirect method is satisfactory when p is small, but not when it becomes much greater than 0.1; as a

[1] Many of Davson's (1937) curves relating K loss to time show the same anomaly, for which Davson suggested an explanation based on an initial loss and a subsequent restoration of semipermeability of the red cell to K.

result, the range of experimental observation is restricted. In order to extend it sufficiently, the indirect method has been replaced in this investigation by the direct measurement of φ, the K concentration in the cells, expressed as a fraction of $\varphi_0 = 1.0$, their initial K concentration.

The methods used are essentially the same as those already described (Ponder, 1950 *b*), the systems being composed of 15 ml. of a washed suspension of the red cells from heparinized human blood, added to 75 ml. of 1 per cent NaCl or of NaCl-buffer. The volume concentration of the suspension was always adjusted to 0.4 after the completion of the washing. The cells of about 10 ml. of the diluted suspension are packed immediately and their initial K and H_2O contents are determined; the remaining volume is divided into seven Erlenmeyer flasks, each containing about 10 ml. of the diluted suspension. These flasks are kept at constant temperature; from time to time, one is removed for the determination of the K and H_2O content of its cells, and also for the determination of the amount of lysis and the pH in the system as a whole. The values obtained for each flask provide one point on the curve relating the K content of the cell, etc., to time. To provide for constant temperature with constant shaking, the flasks are kept in large water baths thermostatically controlled to within ± 0.1°C., and are rocked to and fro at a rate of about 30 swings per minute. The experiments at 37°C. last about 48 hours; those at 2–4°C. last about 2 weeks. All manipulations are carried out by a sterile technic which involves the use of sterile solutions, syringes, and vaccine-capped tubes or flasks (Ponder, 1949, 1950 *b*).

1. K Losses at 2–4°C.

The relations found in prolonged experiments at low temperatures are best illustrated by the analysis of a typical curve relating the K content of the cells to time. The experimental values are obtained as m.eq./liter cell H_2O; to simplify the analysis, the initial K concentration of the cells is called $\varphi_0 = 1.0$, and to simplify it still further, the values for the K content φ at various times are replaced by values of $\varphi' = (\varphi - \varphi_e)/(\varphi_0 - \varphi_e)$; this adjustment, based on the uniform distribution of K throughout the system resulting in a concentration of φ_e, gives values of φ' which vary between 1.0 and zero in place of values of φ which vary between 1.0 and φ_e. The numerical value of φ_e can be found either by calculation or by direct measurement of the K concentration in a completely hemolyzed system; in these systems it is about 0.06.

As in previous investigations (Ponder, 1949, 1950 *b*), an attempt can be made to represent the curve in Fig. 1 by the equation

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

the solution of which is

$$\varphi' = (1 - P/a)e^{-at} + P/a \tag{2}$$

an expression which becomes

$$\varphi' = e^{-at} \tag{3}$$

when $P = 0$. In order to evaluate the constants in expression 1, the values of $d\varphi'/dt$, obtained by drawing tangents to the experimental curve, are plotted against φ' (inset of Fig. 1). If the only observations used are those corresponding to $t = 144$ hours or less (the points enclosed in circles in the inset), it is possible to pass a fairly good straight line through them; this line gives values of $a = 0.0104$ and of $P/a = 0.027$, so $P = 0.0028$. This is the same result, and with it the same difficulty, as has presented itself before, for it is scarcely conceivable that after 12 days at low temperatures and in the absence of glucose there should be an active process counteracting the effects of outward diffusion

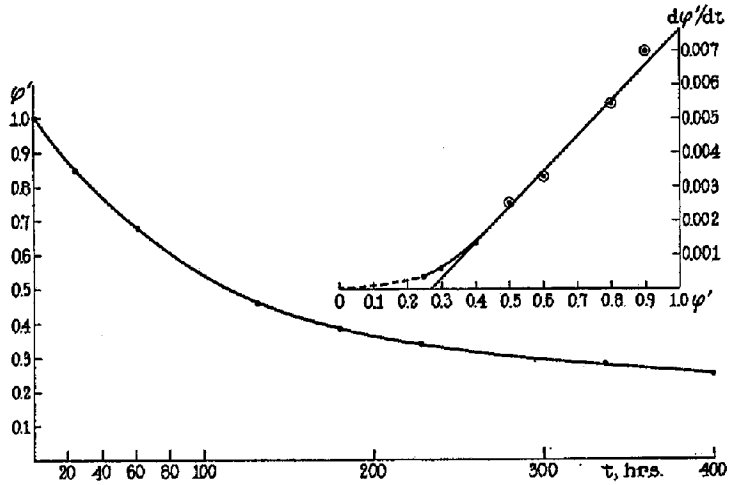

FIG. 1. Loss of K by human red cells at 3°C. Ordinate, K content of cells, expressed as φ' (for definition, see text); abscissa, time in hours. For explanation of the inset, see text.

and of the magnitude corresponding to $P = 0.0028$. An alternative which has already been suggested is that the curve under consideration approaches the value $\varphi' = 0.027$ as an asymptote (the position of the asymptote in the equation which results from the solution of expression 1 being P/a); this would imply that a fraction of the cell K is immobile and not free to diffuse.

When the observations are extended up to times of 400 hours, however, it becomes clear that the relation between $d\varphi'/dt$ and φ' is not really a straight line but a flat curve convex to the φ'-axis. It follows that the curve in its entirety cannot be described as a simple exponential. It is nevertheless highly likely that the outward diffusion of K is essentially an exponential process, and so the possibility ought to be considered that the curve may be the result of exponential processes occurring in a system which has more than one com-

ponent. The curve obtained by plotting log φ' against t (Fig. 2) is certainly not a straight line, but it can be broken without difficulty into two components of approximately equal size, a component I from which the loss of K is relatively rapid ($a_1 = 0.015$), and a component II from which the rate of K loss is about one-sixth that from component I ($a_2 = 0.0023$). The exponential loss of K from both components has $\varphi' = 0$ for its asymptote, so $P = 0$ in expression 1 and the equation for the two-component exponential function

$$\varphi' = N_1 \cdot e^{-a_1 t} + N_2 \cdot e^{-a_2 t} \tag{4}$$

replaces expression 3, N_1 and N_2 being the fractions of the population which make up the first and second components respectively.

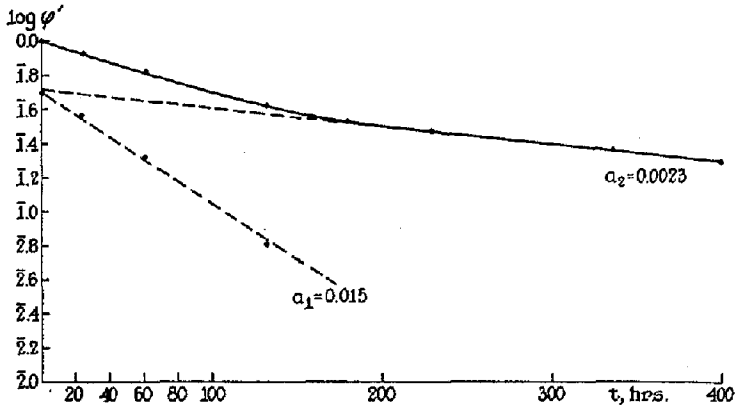

FIG. 2. Log φ' plotted against time. The two straight lines (dotted) refer to the two exponential components the sum of which gives the experimental curve in solid line.

This type of analysis must be used with great caution, for it is well known that it tends to give a greater number of components than really exist. The most that can be claimed is that the experimental results can be described as the sum of at least two[2] exponential processes with asymptotes $\varphi' = 0$, but not in terms of one such process only, that the two components are approxi-

[2] It has been recognized since the beginning of these investigations that the loss of K may be either a loss of all the K from some o the cells, a loss of some of the K from all the cells, or any combination of these two extreme possibilities. The present data point to there being at least two kinds of cell in the population, but there may be more, and a more thorough analysis of better data may show that the cells are distributed about a mean value as regards the rate at which they lose K. In problems of this kind, indeed, a multicompartment system of a mammillary type tends to appear as a two-compartment system (Sheppard, private communication). Variations in the K content of the individual cells of a population have been observed directly by microincineration followed by phase contrast microscopy (Kruszinski, 1950).

mately equal in size,[3] and that the velocity constant of the more rapid component is about six times that of the slower.

This type of result has been obtained in five out of seven experiments. In the remaining two, the experimental curve turned out to be of the "intermediate" variety (Ponder, 1949, and section 2, below), the values of φ' corresponding to times greater than about 200 hours being unexpectedly small. These "intermediate" curves are regularly found in systems in which there is extensive lysis, large changes in pH,[4] or both; they seem to be due to the system undergoing radical and uncontrollable changes which make it impossible to compare its initial and its ultimate behavior in any simple way.

2. K Losses at 37°C.

The curves relating φ' and t at 37°C., when analyzed in terms of expression 1, *i.e.* when considered as the result of processes occurring in a system with a single component, behave very like the curves relating φ' and t at low temperatures in that they seem to approach asymptotes higher than those which would correspond to a uniform distribution of K throughout the system. The similarity of behavior suggests that the anomalously large values of φ' at the apparent asymptotes are again due to a system with two or more components being treated as if it contained only one component. An extension of the range of observation would seem to be a likely way of clarifying the situation, just as it was found to be in the case of the curves at 2–4°C.

Attempts to extend the observations at 37°C. in unbuffered NaCl systems with small volume concentrations of cells almost always result in curves such as that shown in Fig. 3. For the first 18 to 24 hours the curve follows a course which, superficially at least, is exponential, but at the end of that time it turns downwards, changes curvature again, and passes towards an asymptote at $\varphi' = 0$. This "intermediate" type of curve has already been described as occurring both at 25°C. and at other temperatures (Ponder, 1949); the sudden change in the course of the curve appears to be the result of the accumulation of autolytic substances, metabolites, etc., as shown by an increase of as much as a pH unit in unbuffered systems, or by the appearance of considerable lysis in systems containing phosphate buffers. Conditions no doubt exist under which the change in the course of the curve is longer delayed, or occurs less abruptly, than under others, but these investigations have not given much indication of what these conditions are.

Considering only the part of the φ', t relation within the first 18 to 24 hours, an attempt to treat it as a single exponential function by plotting $d\varphi'/dt$ against φ' results in the inset of Fig. 3. As in the inset of Fig. 1, a fairly good

[3] There is a tendency for the slower component to be the larger.

[4] The pH in systems from which curves like that shown in Fig. 1 are derived remains constant to within 0.3 pH unit, the pH usually rising slowly.

straight line can be drawn through the points (enclosed in circles) on the early portion of the curve; this line gives $a = 0.135$ and $P/a = 0.67$, whence $P = 0.09$. This is the same anomalous situation which has occurred so often before.

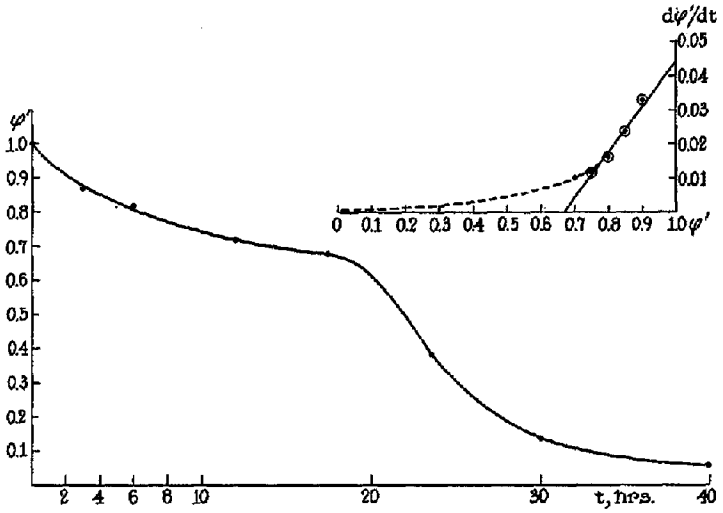

FIG. 3. Similar to Fig. 1, but for human red cells at 37°C.

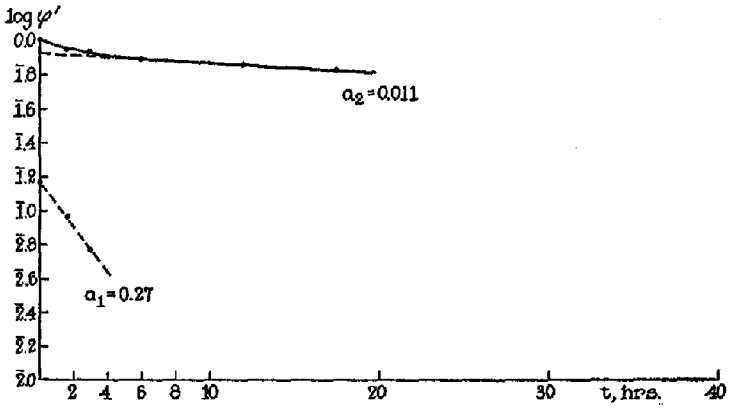

FIG. 4. Similar to Fig. 2, but for human red cells at 37°C.

and again it is unlikely that an inwardly directed active process of the magnitude corresponding to $P = 0.09$ would exist in the absence of added glucose. Guided by experience with the analogous case at low temperatures, we may plot $\log \varphi'$ against t (Fig. 4); this gives a curve instead of a straight line, but the curve can again be represented as the sum of two exponential functions

with $\varphi' = 0$ as their asymptotes. The more rapid process affects only 15 to 20 per cent of the population, has an appreciable influence on the φ', t relation only during the first 6 to 8 hours, and has a velocity constant about 20 times that of the slower process. Neither the experimental data nor the analysis is sufficiently reliable to justify attempts at the identification of these components with those found at lower temperatures or in other systems (*cf.* Sheppard and Martin, 1948). The most that can be concluded is that there is evidence of there being more than one component present, and of the anomalously high asymptote which appears when the first part of the curve is treated as a simple exponential being due to an oversimplification of the situation. As in the case of the curves at low temperatures, the relation between $d\varphi'/dt$ and φ' is not really a straight line, but part of a curve (inset of Fig. 3).

TABLE I

Medium	Temperature	Duration	φ'	p	V/V_0
	°C.	*hrs.*			
NaCl, pH 7.0 to 7.2	4	247	0.23	0.26	1.08
NaCl, pH 6.8 to 7.1	3	336	0.19	0.20	1.10
NaCl, pH 6.8 to 6.4	37	34	0.20	0.04	1.05
NaCl, pH 6.8 to 6.3	37	42	0.13	0.06	1.19

As regards the part of the curve corresponding to times longer than 18 to 24 hours, the assumption is that if autolytic processes had not brought about the relatively sudden change in its course, the curve would have continued to the asymptote $\varphi' = 0$ as the exponential function $\varphi' = N_2 \cdot e^{-a_2 t}$, with the velocity constant a_2 of the slower component. On these assumptions, the change in the course is attributable to the velocity constant having undergone a relatively sudden change, after about 20 hours, to a value much greater than a_2.

3. Volume Changes

In systems containing added lysins, large and sometimes almost complete exchange of K for Na may occur without much increase in the volume of the red cells (Ponder, 1948 *a*, *b*).[5] The volume increases found in the experiments

[5] The volumes of the cells remaining intact in these systems were measured by a

described above were also small (Table I). It seems to be possible for the cells which remain intact in these systems, a number equal to from 76 to 96 per cent of the whole, to lose from 73 to 87 per cent of their K, largely in exchange for Na (Ponder, 1947 *b*), without undergoing more than a 5 to 19 per cent increase in volume. The statement (Davson, 1936, Jacobs and Stewart, 1947, Maisels, 1949) that a physical or a "physiological impermeability" to cations is necessary for the continued existence of the red cell in a salt solution is apparently incomplete in some important respect, for in these systems there is neither a physical impermeability nor a "physiological impermeability" in the sense of an active ion transfer process directed against the diffusion gradients and dependent on metabolism. The red cells nevertheless remain intact and only slightly swollen. This weakness of the "dual mechanism of hemolysis" hypothesis (Davson, 1936, Davson and Danielli, 1938, Davson and Ponder, 1938, 1940) and of the "colloid-osmotic hemolysis" hypothesis (Wibrandt, 1941) of hemolysis, as at present expressed, has already been commented on in relation to systems containing hemolysins (Ponder, 1948 *a*, *b*).

Restatement of the "Dual Mechanism of Hemolysis" Hypothesis.—A red cell in isotonic NaCl constitutes a system which has peculiar properties in both its initial and final states, for while there is an osmotic equilibrium between its K-rich interior and the Na-rich environment, there is a large initial difference in ionic composition, and the colloidal osmotic pressure of the Hb and other non-diffusible anions is offset by an anion and cation distribution in accordance with the requirements of a Donnan equilibrium. If the steady state in the blood stream is due to the operation of transfer mechanisms, there is no reason to think of the value of P for the inward transfer of K as being necessarily the same as the value of P' for the outward transfer of Na, nor need the diffusion constant a for the outward escape of K from a cell in which $P = 0$ be the same as the diffusion constant for the inward passage of Na when $P' = 0$. The final state has the peculiarity that the colloidal osmotic pressure inside the cell tends to move water, and slowly penetrating cations along with it, from the surrounding medium into the cell.

Consider a simplified one-component situation in which there is no active ion transfer, and in which the diffusion constant for the outward movement of K is a while that for the inward movement of Na is b. The cell volume V, which depends on the sum of K and the Na concentrations in the cell, is given by

$$e^{-at} + \frac{be^{bt} - b}{be^{bt}} \tag{5}$$

When $t = 0$, $V = 1.0$, and similarly when $t = \infty$, $V = 1.0$, the initially high K concentration inside the cell having fallen to become equal to that outside, and the initially

high-speed hematocrit, and it is probable that the columns of packed cells contained rigid or semirigid ghosts (Ponder, 1950 *a*). This would lead to the cell volumes being overestimated to a small but unknown extent. Note: An error appears in the composition of the buffer of pH 6.6 (Ponder, 1948 *b*, footnote 1). It should be 12.5 ml. of M/15 NaH_2PO_4 and 7.5 ml. of M/15 Na_2HPO_4.

low Na concentration inside the cell having risen to equal that outside. For intermediate values of t, V may be greater ($b > a$), equal to ($b = a$), or less ($b < a$) than unity.

The effect of the colloidal osmotic pressure is something almost entirely distinct from what occurs during this ion exchange.[6] At any time during the exchange, the colloidal osmotic pressure has still to be compensated for by a Donnan equilibrium, since the only result of the ion exchange considered in expression 5 is a substitution of Na for K intracellularly and of K for Na extracellularly. The osmotic pressure due to the indiffusible intracellular anions produces an inward movement of water at a rate proportional to the osmotic gradient p. Let V' be the volume of water entering under this gradient, which decreases as V' increases; then $dV'/dt = cp/(1 + V')$, and $V' = cpt$ if terms in V'^2 are ignored. Since isotonicity is to be maintained, the rate of the movement of water will be determined by the rate at which cations, with their accompanying anions and in the proportions in which they exist in the medium, can enter the cell under the gradient p. This determines the magnitude of the pro-

TABLE II

$a = 0.04$, $b = 0.032$, $c = 0.04$

t	0	25	50	100	150	250	∞
φ	1.0	0.61	0.37	0.14	0.05	0.01	0.0
V, $p = 0.02$	1.0	0.96	0.95	1.02	1.08	1.19	∞
V, $p = 0.03$	1.0	0.97	0.97	1.06	1.14	1.29	∞

portionality constant c, which must be about that of a and of b; the value of p is probably between 0.02 and 0.03.

The volume of the cell at any time t accordingly is

$$e^{-at} + \frac{be^{bt} - b}{be^{bt}} + cpt \tag{6}$$

Table II shows the relation between t, φ, and the volume calculated from expression 6 in which reasonable values for the constants are inserted. At the smaller values of t, the initial small shrinkage in cell volume resulting from a K-Na exchange with $a > b$ tends to be counterbalanced by the small swelling due to the effect of the colloidal osmotic pressure term; towards the end of the ion exchange, the swelling due to the colloidal osmotic pressure term is more in evidence, and this becomes indefinitely great with further increase in t, as required by case III of Jacobs and Stewart (1947).

[6] It could be considered as altogether distinct were it not for the fact that the fluid which enters under the colloidal osmotic pressure gradient always has the composition of the medium surrounding the cell; at the beginning of the K-Na exchange, this is NaCl, but when the exchange is completed it is a NaCl-KCl mixture of the same ionic composition as that of the cell interior. The effect of this progressive change in the composition of the surrounding medium is sufficiently small to be left out of consideration in the calculations which follow.

In systems containing hypolytic concentrations of lysin, it is understandable that the values of the diffusion constants *a*, *b*, and *c* would be much larger than those in lysin-free systems. This would result in both the K-Na exchange and the "colloid-osmotic hemolysis" occurring much more rapidly in the former systems than in the latter; expression 6, indeed, contains nothing which restricts the rapidity of these events. The only experimental feature which remains to be accounted for is the dependence of the critical volume at which the cell hemolyzes on the nature and concentration of the lysin in the system (Ponder, 1948 *a*). An independent statement has to be made about this. Reactions between the lysin and the cell ultrastructure may be thought of as altering the stability of the latter, and as determining the critical volume in this way. Finally, the magnitude of the colloidal osmotic pressure gradient would be affected by changes in the degree of molecular association in the red cell interior, *e.g.* the gradient might become steeper in the presence of lysins (*cf.* Lindemann's (1949) "dissociation theory of osmotic hemolysis"), or less steep if the cell interior were to become paracrystalline.

4. Résumé of the Kinetics of K Loss from Human Red Cells

These investigations (Ponder, 1947 *a*, *b*, *c*, 1948 *a*, *b*, 1949, 1950 *b*) into the kinetics of the exchange of K and Na between the human red cell and surrounding fluids may be concluded by summarizing the results in an order different from that in which they have been obtained. Two kinds of processes have to be considered. The first is observed in its simplest form in systems, at any temperature, in which red cells are suspended in isotonic NaCl or isotonic NaCl-buffer to which no glucose is added. Here the movements of K and of Na are in the direction of their diffusion gradients, K leaving the cell and Na entering. A simple example of the second is found in systems at temperatures in the neighborhood of 37°C., in which red cells are suspended in mixtures of isotonic NaCl and KCl to which glucose has beeen added. Such a system is usually set up by taking a system of the first kind, *i.e.* one of cells in isotonic NaCl, and allowing K loss to occur at low temperatures; this results in the medium surrounding the cells becoming an isotonic NaCl-KCl mixture, to which glucose is added before the system is brought to the temperatures at which accumulation of the lost K will take place. Here the movements are against the gradients, K accumulating in the cell and Na leaving it or being excreted by it. In certain systems, as in the blood stream where steady states as regards the K and Na contents of the cell are maintained, both types of process occur simultaneously.

1. K Losses in the Direction of the Diffusion Gradient.—Red cells in saline media slowly exchange K for Na. The loss can be considered as an exponential function of time, as might be expected on the basis of its being the result of diffusion, but not as an exponential loss from cells which are uniform in their properties. From an analytical point of view, the population has to be treated as consisting of *at least* two components, one of which loses K more rapidly

than the other. Restricted observations and failure to recognize the complex nature of the population have led, at various stages of these (and of other) investigations, to a wrong estimate of the position of the asymptotes which the curves seem to approach, and to the idea that the loss of K is restricted in some way, *e.g.* by the cell becoming impermeable to it after having passed through a stage of permeability (Davson), by its being opposed by an active accumulation process, or by some of the K being relatively immobile or bound. In a population represented by two components, the curves relating the cell K content to time are exponentials with asymptotes corresponding to the uniform distribution of K throughout the system, but the components lose K at different rates as measured by the values of their velocity constants. The factors upon which the differences depend are still unknown.

The rate of loss of K from the components of the red cell population increases with increase of temperature.[7] Addition of lysins to the systems also increases it. In systems containing lysins, the curves relating the cell K to time are again exponentials with their asymptotes corresponding to the uniform distribution of K throughout the system, apparent asymptotes corresponding to higher values being again the result of restricted observation and of the cell population containing more than one component. The velocity constants in these lysin-containing systems are functions of the lysin concentration, becoming those of the simple system of saline-suspended cells when the lysin concentration is zero. The effects of substances such as NaF and iodoacetic acid on the K content of red cells at low temperatures or in systems which do not contain glucose are best considered as similar to the effects of lysins, *i.e.* as effects on the velocity constants of the exponentials which apply to the systems, and the effect of pH on systems of this type can probably be treated similarly. There does not seem to be any objection to thinking of these modifications of the values of the velocity constants as resulting from the creation of new pathways, through the surface ultrastructure, along which K and Na diffusion can take place.[8]

[7] The temperature coefficient of the loss of K is low (about 1.4), as would be expected for a diffusion process.

[8] If diffusion involves the movement of ions in association with carrier molecules which are free to move in either direction, in contrast to accumulation which involves the movement of ions in association with carrier molecules which are created at the surface and which diffuse inward along *their* concentration gradient, the diffusion of K and Na along the K and Na gradients in a cell treated with a lysin, *e.g.* resorcinol, may differ in nature from that in an untreated cell because the normally occurring carrier molecules are replaced by resorcinol, the chemical nature of which suggests that it may be a good ion exchanger. Some indication that such a difference really exists may be derived from the course of the curves relating φ to t in systems containing normal and resorcinol-treated red cells respectively; the former seem to behave

The K-Na exchanges which occur in the lysin-containing systems in which the phenomena can best be observed are not associated with changes in shape or in shape transformations, and are accompanied by remarkably little change in resistance to hemolysis by hypotonicity or by lysins such as saponin and digitonin. The exchanges may also be accompanied by remarkably small volume changes; *e.g.*, an exchange of 90 per cent of the cell K for Na may involve a 10 to 20 per cent volume increase only. This observation requires that the "dual mechanism of hemolysis" and the "colloid-osmotic hemolysis" hypotheses be restated in such a way as to allow for the slow movement of cations (see above).

2. K Gain against the Diffusion Gradient.—This requires energy derived from metabolism and occurs at temperatures in the neighborhood of 37°C. and in the presence of glucose or some other material which can undergo glycolysis. Small gains of K may occur over short periods in systems at 37°C. containing washed red cells in isotonic NaCl, but these depend primarily on the glucose or other glycolyzable substances which the cells still contain.

The accumulation process has a temperature coefficient of 2.4 in the range 27–37°C., and has a pH optimum lying between pH 7.3 and pH 7.6. There is also an optimum concentration of glucose (50 to 150 mg. per cent). Under optimum conditions, the rate of accumulation of K is about 6 m.eq./liter cell H_2O/hour, but the accumulation mechanism is liable to deteriorate both with the length of time during which the cells are allowed to lose K at low temperatures (so as to enable accumulation to be observed when the temperature is raised to 37°C.) and as a function of time at 37°C. This deterioration, which is observed even when there is ample glucose and when glycolysis is active, is probably due to an exhaustion of the enzyme systems involved in the accumulation mechanism. The accumulation process as observed *in vitro* accordingly results in only a temporary increase in the cell K content. While this may rise at a rate of about 6 m.eq./liter cell water/hour from the low values characteristic of red cells which have lost K by standing in the cold, and may continue to rise until values are reached which are not much less than those originally present in the cells when they were in the blood stream, the steady state in which the rate of accumulation is equal to the rate of loss is difficult to maintain *in vitro*. After 6 to 12 hours, the rate of K loss begins to exceed the rate of K accumulation, and from that time onward φ, the K content of the cell, declines. The kinetics of the process throughout its entire course can be ex-

as two-component systems, but it is possible to represent the latter as one-component systems. This result must not be accepted until more reliable experimental values have been obtained, but it suggests that the pathways through which prolytic cation exchange occurs may have properties which are experimentally distinguishable from those of the pathways involved in the K-Na exchanges in systems to which lysins have not been added.

pressed in a semiquantitative fashion by the expression $d\varphi/dt = P - a\varphi$, in which P is the rate of accumulation and $a\varphi$ the rate of loss by diffusion. Closer inquiry, however, would probably show that it is necessary to treat the system as being made up of more than one component, at least as regards the rate of K loss. Under *in vivo* conditions, in which the deterioration of the K-Na exchange system is presumably very much slower, the K content of the cell φ can be maintained in a variety of steady states each defined by $P - a\varphi$ with a variety of values of P and of a, and so can pass from one steady state to another.

Osterhout's picture (1949) of the accumulation mechanism, in which K enters the cell by combining with carrier molecules at the cell surface, the combination of carrier molecules and ions diffusing inwards and the ions being split off in regions of the ultrastructure remote from the surface or in even more deeply seated regions, seems entirely satisfactory in its general aspects. This process requires energy derived from metabolism, both for the building up of new carrier molecules and for the destruction of those already engaged in carrying ions. In this picture the carrier molecules behave essentially as mobile cation carriers which select K in preference to Na, and the metabolic aspect of the picture involves the formation of new carriers. In the absence of metabolism there is no inwardly diffusing carrier or exchanger capable of binding K preferentially; under these circumstances, the only directions along which ions move are those of their concentration gradients. Very little is known about the way in which energy is made available and utilized in the process, except that glycolysis and possibly the synthesis of phosphoric esters (Maisels) are involved. The accumulation process is inhibited by fluoride and by iodoacetate, although a good deal of confusion has been introduced through a failure to distinguish between the inhibitory effect of these substances on the metabolic processes underlying accumulation and their effect in increasing the rate of K-Na exchanges in the direction of the diffusion gradient. The fraction of the cell surface involved may be small,[9] as in the case of the Cu-inhibited transport of glycerol (Jacobs and Corson, 1934, LeFevre, 1948) and it has been suggested (Maisels) that the enzyme system is situated on the inside of the surface ultrastructure. Maisels (Maisels, 1949, Flynn and Maisels, 1950) also considers Na excretion to be the primary active process, K accumulation being secondary to it and perhaps "passive." Although the results of some disputed observations concerning the failure of cells suspended in LiCl to accumulate K have been claimed to support this view, it rests principally on the apparent necessity of a cation-permeable red cell swelling and hemolyzing in a Na-rich medium unless there is some mechanism for expelling the Na which diffuses into it from outside. An outwardly directed Na ion transfer mechanism, in addition to an

[9] If the time during which a pathway is occupied with an ion exchange is r seconds, an accumulation rate of 6 m.eq./liter/hour requires one pathway per $25/r$ A^2 of cell surface.

inwardly directed K transfer mechanism, seems to provide a satisfactory, if somewhat non-committal, explanation for the various experimental observations.

SUMMARY

1. The anomalous course of the curves relating K loss to time in systems containing human red cells in isotonic NaCl, and particularly the high positions of the asymptotes to which the curves apparently proceed, are due to the population of red cells consisting of at least two components, one of which loses K more readily than the other.

2. Since large K-Na exchanges can occur between red cells and an isotonic suspension medium without there being large volume changes, a restatement of the "dual mechanism of hemolysis" hypothesis, which takes account of the cell's being slowly permeable to cations, is required. If some approximations of minor consequence are allowed, the hypothesis can be restated in a quantitatively satisfactory way.

3. The general features of K-Na exchanges, including prolytic exchanges are summarized.

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