THE PROLYTIC LOSS OF K FROM HUMAN RED CELLS

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The prolytic loss of potassium, *i.e.* the loss of K which occurs before the loss of hemoglobin from red cells exposed to hypolytic concentrations of lysin, has been described by Davson and Danielli (1938), and has been shown to vary according to the nature of the hemolysin in the system. The purpose of this paper is to extend these observations, which are limited to the measurement of K loss during a period of 1 hour only, and also to consider the prolytic loss of K in relation to the lytic process which follows it.

General Description of Method

One ml. of freshly obtained heparinized human blood, the volume concentration of which has been adjusted to 0.40, is placed in each of a series of tubes of about 15 ml. capacity. The tubes should be made of heavy glass, to withstand high speed centrifugation. About 10 ml. of 172 m.eq./liter NaCl is added to each, and the cells are washed twice with this medium at moderate rates of spinning. After the second washing, the supernatant fluid is removed as completely as possible without disturbing the cells.

Five ml. of 172 m.eq./liter NaCl is added to the cells in half the total number of tubes, and to the cells in the remaining half is added 5 ml. of sphering agent or lysin dissolved in various concentrations in 172 m.eq./liter NaCl. This results in there being a "standard" system containing NaCl only for each system containing sphering agent or lysin. The contents of each tube are mixed, and all the tubes are allowed to stand at a temperature and for a time which depend on the nature of the experiment. At intervals, the shape of the cells in each system can be ascertained by examining them in uncovered drops on plastic slides. After various lengths of time, one of the tubes containing a system composed of cells and sphering agent or lysin is placed in a centrifuge together with one of the "standard" tubes containing the cells and NaCl only, and the cells of both systems are thrown down simultaneously at a moderate speed (2000 R.P.M. for 5 minutes). The supernatant fluids are removed for the K determinations, the greatest care being taken not to disturb the cells.

The cells of the standard system which has stood for the shortest period of time, and, in some experiments, the cells of other systems, are packed at high speed for 30 minutes, and the upper layer of cells together with the fluid which always remains near the surface are removed with capillary pipettes and blotting paper. If the packing and the removal of fluid are thoroughly done, the mass of cells which remains is at least 98 per cent packed. Micropipettes are used to transfer 0.1 ml. of the packed cell masses to 10 ml. flasks containing water, the technique of transfer being the same as that described for measuring red cell density (Ponder, 1942 a). The contents of the flasks are made up to the 10 ml. mark with water, allowed to stand for an hour or so, and then filtered through a soft filter paper.

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The concentration of K in the supernatant fluids, and the concentration of K, and sometimes of Na, in the hemolyzed packed red cells, are found with the Perkin-Elmer flame photometer. By changing the sensitivity of the scale appropriately so as to give the best results in the case of each concentration to be determined, and by taking a series of readings on each sample, the analyses can be made with a precision of at least ± 2 per cent.

About 1 ml. of a separately prepared sample of packed cells may be weighed before and after drying to constant weight at 80°C. in order to obtain the water content of the cells. Finally, the amount of hemoglobin in the contents of each of the 10 ml. flasks referred to above can be found colorimetrically, and the data thus obtained used to calculate volume changes in the cells (Macleod and Ponder, 1933). In many experiments, these last two procedures can be omitted.

In some experiments the range of hypolytic concentration is accidentally overstepped, and a small amount of lysis occurs in the systems in which the prolytic loss of K is to be determined. The amount of lysis present can be found by a colorimetric comparison of the supernatant fluid of the system with a standard made by adding the cells which have been thrown down from one of the other systems to 100 ml. of water; this standard represents 5 per cent hemolysis. The loss of K which corresponds to the small amount of lysis observed in the system under consideration can then be allowed for, at least approximately.

Davson and Danielli (1938) have pointed out that the loss of K into a medium containing a hypolytic concentration of lysin, referred to here as K_p, should always be compared with the loss which occurs into the same medium with no lysin present, referred to here as K_s, the systems to be compared being treated identically as regards time of standing, duration and speed of centrifuging, and so on. In the experimental procedure used in this investigation, the values of the losses K_p and K_s are first found as fractions of K_0, the initial quantity of K in the red cells of the system under consideration, and these values are given in tables such as Tables I and II. For many purposes, however, the prolytic loss can be expressed more concisely as the ratio K_p/K_s.

I. The Prolytic Loss of K in Systems Containing Various Lysins

1. *The Loss as a Function of Time.*—In systems containing human red cells suspended in 172 m.eq./liter NaCl, and in the same medium to which hypolytic quantities of lysin are added, K_s and K_p increase with time in a characteristic way. Table I and Fig. 1, A give the results observed with the sphering agent, distearyl lecithin, and with the sphering agent and lysin sodium tetradecyl sulfate (C-14), both in concentrations too small to produce commencing hemolysis in 20 hours at 25°C. In the former case, the prolytic loss is much greater than in the standard system after the same interval of time, so that K_p/K_s has values of from 3.2 to 4.5. In the latter case, the prolytic losses are very little greater than they are in the standard systems, so that the largest value of K_p/K_s is 1.1.

In any one of these systems, a new steady state seems to be approached, in which the concentration of K in the cells is less than it was initially, although still being much greater than that in the surrounding medium. Call this new concentration $(1 - K_\infty)$; then the expression

$$dK/dt = k(K_\infty - K)$$ (1)

TABLE I

Prolytic Loss of K as a Function of Time

		1 hr.	3 hrs.	5 hrs.	20 hrs.
Lecithin	2,500 γ per 0.4 ml. cells	0.051	0.100	0.131	0.230
	Standard	0.012	0.025	0.036	0.065
C-14	40 γ per 0.4 ml. cells	0.010	0.020	0.030	0.059
	Standard	0.009	0.020	0.029	0.055

TABLE II

Prolytic Loss of K as a Function of Lysin Concentration

C-14		Taurocholate		Saponin		Digitonin	
γ/0.4 ml. cells	K lost	γ/0.4 ml. cells	K lost	γ/0.4 ml. cells	K lost	γ/0.4 ml. cells	K lost
80*	0.064	400*	0.216	20*	0.011	8*	0.072
40	0.057	200	0.140	10	0.086	4	0.071
20	0.055	100	0.129	5	0.081	2	0.070
Standard	0.053	Standard	0.121	Standard	0.078	Standard	0.070

* Just commencing hemolysis.

expresses the rate of K (K_s or K_p) loss, as can be seen from Fig. 1, B, in which t is plotted against $\log K_\infty /(K_\infty - K)$. For the purposes of a first approximation,[1] the points lie on a straight line with $k = 0.06$. The principal char-

[1] For the four curves shown in Fig. 1, A, the values of K_∞ used are 7.0, 24.5, 6.0, and 6.4, in that order from above down. A better selection of values might result in a better fit to the data for the individual curves and in different values of k being assigned to each. Considering the experimental errors and the variability encountered even in apparently identical systems, this would be carrying the analysis too far. There is also the question as to whether 20 hours is a long enough time upon which to base an estimate of the position of the asymptotes; if this time of observation is exceeded, however, spontaneous hemolysis becomes troublesome. It should be emphasized that the concentrations of distearyl lecithin and of sodium tetradecyl sulfate are so chosen that they do not produce lysis at the end of 20 hours. If they were somewhat greater, the prolytic loss of K would be followed by lysis and a further loss of K (see Fig. 3).

acteristic of the individual curves accordingly is the value of K_∞ for the new steady state which tends to be established in the system, and this depends on the nature of the surrounding medium, *i.e.* on whether it is NaCl alone, on whether it contains distearyl lecithin or sodium tetradecyl sulfate, and so on. It should be emphasized that expression (1) says nothing but that the quantity of K in the cells tends to pass from an initial value denoted by unity to a new value $(1 - K_\infty)$ at a rate which depends on the excess of K in the cells above
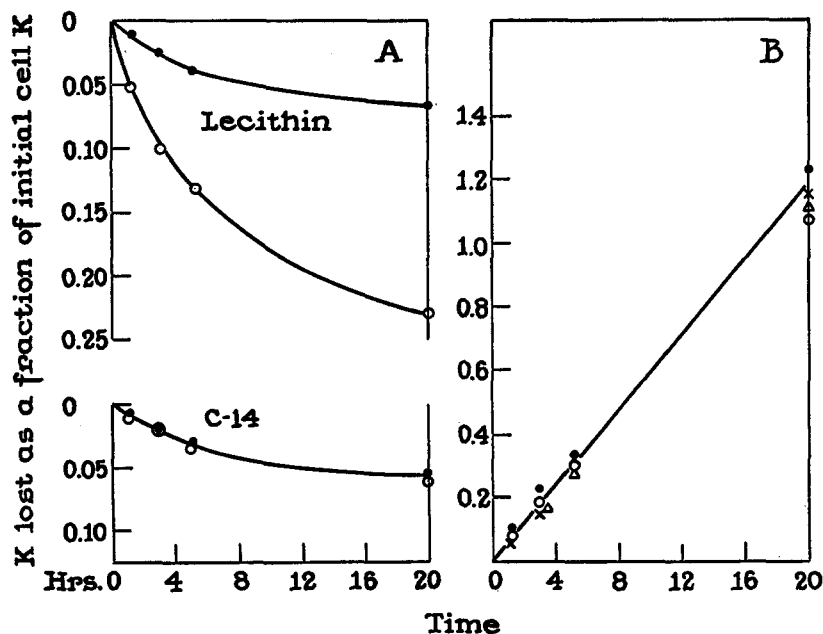

FIG. 1. *A*, loss of K from red cells as a function of time. Ordinate, K lost as a fraction of initial cell K; abscissa, time in hours. Points indicate K losses into standard systems containing NaCl only, circles the losses into systems containing lecithin or C-14. *B*, log $K_\infty/(K_\infty - K)$ plotted against time; crosses, standard system for lecithin, dots, lecithin system, circles, standard system for C-14, triangles, C-14 system.

that which characterizes the new steady state; nothing is implied as to the mechanism by means of which this comes about.

2. The Loss as a Function of Lysin Concentration.—Table II and Fig. 2 show the loss of K at the end of 20 hours at 4°C. as a function of the concentration of lysin present in the system. To make values for various lysins comparable, the concentrations are expressed in Fig. 2 as fractions of $c_{\infty,0}$, the quantity of lysin, in γ per 0.4 ml. of cells, which gives just commencing lysis after 20 hours at 4°C. The values of $c_{\infty,0}$ have to be found in preliminary experiments.

For comparable concentrations, the values of K_p/K_s vary greatly for different lysins. On the basis of experiments of 1 hour duration in which rabbit red cells were used, Davson and Danielli came to the conclusion that the stronger the hemolysin, the smaller the prolytic loss of K. If the asymptotic concentration is used as a measure of the "strength" of a lysin, this rule holds for the lysins used in these experiments, except for sodium tetradecyl sulfate, which alls out of order. The prolytic loss of K in systems containing saponin, how-
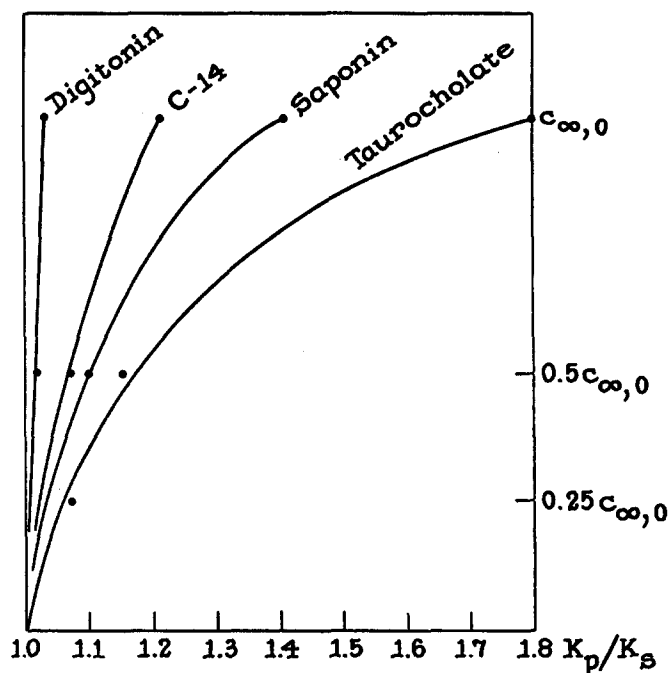

FIG. 2. The loss of K as a function of the concentration of lysin. Ordinate, lysin concentration in terms of $c_{\infty,0}$, the quantity required for just commencing hemolysis. Abscissa, K_p/K_s.

ever, is much greater than in Davson and Danielli's systems, in which it seems to be negligible. This may be due in part to their observations being made at the end of 1 hour only.

The purpose of Fig. 3 is to show the prolytic loss in relation to the hemolytic process as a whole. The curve marked P is obtained by plotting the percentage hemolysis P found after 2 hours at 25°C., in systems containing 0.4 ml. of human red cells, against the amount of sodium taurocholate added to the system. Hemolysis begins at $c_0 = 1.6$ mg./0.4 ml. cells, and is complete at $c_{100} = 9$ mg./0.4 ml. cells. The curve marked K_p shows the loss of K ex-

pressed as a percentage of the total amount lost into the suspension medium in a completely hemolyzed system.[2] The part below the concentration c_0 represents the prolytic loss; the intercept on the abscissa represents K_s, the loss into the standard system. The part of the curve between c_0 and c_{100} shows the loss of K during the hemolytic process; the upper portion of this
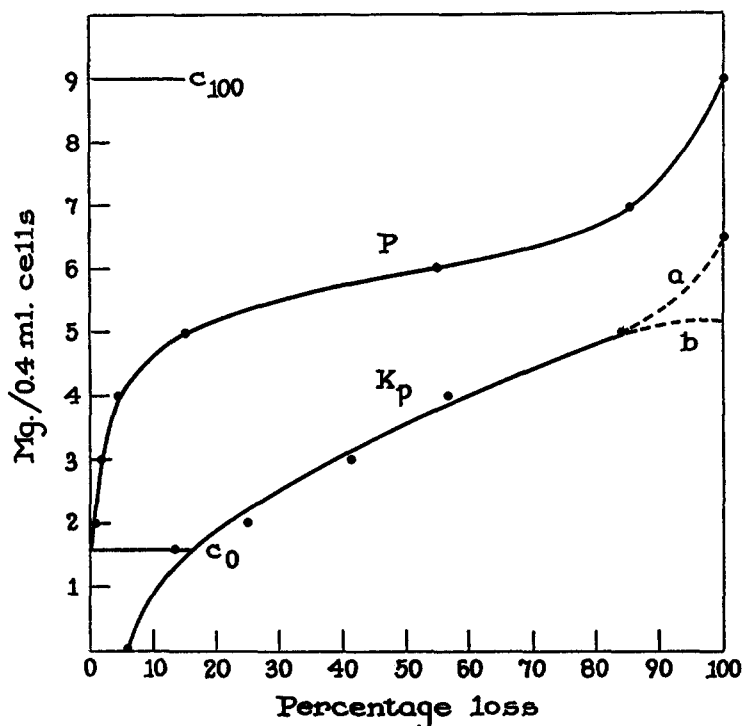

FIG. 3. Hemolysis and K loss as functions of lysin concentration. Ordinate quantity of lysin in system; abscissa, percentage loss of Hb (curve marked P) and of K (curve marked K_p). Lysis begins at the concentration c_0 and is complete at the concentration c_{100}. The upper part of the curve K_p is doubtful, but lies between the branches marked a and b.

curve cannot be decided with certainty in this experiment, and may be either as represented in the dotted part a (in which case the curve would be sigmoid),

[2] Even in a completely hemolyzed system, the concentration of Hb in the ghosts remains somewhat in excess of the concentration in the suspension medium (Ponder, 1942 b). It is possible that the concentrations of K are also unequal. This would make the concentration of K found in the suspension medium of a completely hemolyzed system a little less than that calculated by dividing the total cell potassium by the volume of the system. The discrepancy, if it exists at all, is not large.

or as in the dotted part *b*. In either case, the loss increases very rapidly as the concentration of lysin and the amount of hemolysis increase, about 80 per cent of the total K being lost from the cells when there is only about a 10 per cent loss of hemoglobin.

3. The Loss as a Function of Temperature.—Table III shows K_s, K_p, and the ratio K_p/K_s for systems containing sodium taurocholate in hypolytic concentration, and also for systems containing distearyl lecithin, at three temperatures and after various times. The results are not very satisfactory in a quantitative sense, for there are relatively great variations in the numerical values from one experiment to another, but two points can be regarded as demonstrated. The first is that the loss of K into the standard system, and also the losses into the systems containing taurocholate and lecithin, tend to be smaller at 25°C. than at 4°C. or 37°C. This recalls the observation of Harris (1941) that the loss of K (in systems of a different composition,

TABLE III

Prolytic Loss of K as a Function of Temperature

	Taurocholate 0.8 $c_{\infty,s}$ = 360 γ/0.4 ml. cells			Distearyl lecithin 750 γ/0.4 ml. cells					
	12 hrs.			5 hrs.			12 hrs.		
°C.	K_s	K_p	K_p/K_s	K_s	K_p	K_p/K_s	K_s	K_p	K_p/K_s
4	0.118	0.161	1.37	0.049	0.059	1.21	0.105	0.130	1.24
25	0.080	0.125	1.56	0.042	0.069	1.65	0.070	0.119	1.70
37	0.224	0.470	2.10	0.062	0.110	1.78	0.122	0.260	2.13

however) tends to be larger at low temperatures than in the neighborhood of 20°C. The second point is that the temperature coefficient of K_p/K_s is positive, from 3 to 5 times as much loss taking place at 37°C. as at 4°C.

4. The Relation of the K Loss to Shape Changes.—Throughout this investigation I have looked for evidence which might support the possibility that the loss of K is related to the shape changes which the red cell undergoes as a preliminary to hemolysis. No such evidence has been found. On the contrary the observations show that the shape changes and the K loss are probably unrelated. Very little loss of K, for example, occurs in systems containing sodium tetradecyl sulfate, a substance which turns discoidal red cells into spheres long before the stage of hemolysis is reached, and a considerable loss of K occurs in systems containing saponin, in which the disk-sphere transformation does not occur until just before the lysis of the cell. Even the subsidiary hypothesis that the loss of K is related to the formation of the *prolytic* sphere, *i.e.* the spherical form which takes the place of the ordinary spherical form immediately before the cell hemolyzes, can be set aside on the basis of

the large loss of K in systems containing distearyl lecithin, which produces spheres, but not prolytic spheres in appreciable numbers.

II. Inhibition of K Loss by Plasma Components

Small quantities of plasma, added to systems containing lecithin or lysins which produce a prolytic loss of K, inhibit the prolytic loss. This can be shown by setting up systems containing 0.4 ml. of washed red cells and 5 ml. of diluted lysin on the one hand, and 0.4 ml. of washed red cells and 5 ml. of diluted lysin plus diluted plasma on the other. After various lengths of time at 4°C., the K losses are compared with each other and with the losses occurring into systems containing NaCl only; the losses into the systems containing plasma will be found to be much smaller than those into systems containing lysin only.

TABLE IV

Inhibition of K Loss by Plasma Components

	Taurocholate	Distearyl lecithin
	400 γ/0.4 ml. cells	1500 γ/0.4 ml. cells
Saline K,	0.064	0.070
Lysin only	0.160	0.240
Lysin plus cholesterol 1000 γ	0.112	0.186
Lysin plus serum albumin 2000 γ	0.070	0.096

The hypothesis which at once suggests itself is that the reduction in the loss of K is due to the plasma components combining with the lysin and reducing its concentration. The results given in Table IV, however, show that this is not the entire explanation. In this experiment, quantities of cholesterol (1000 γ, in the form of a sol) and of serum albumin (2000 γ) were selected on the basis of their being found to give approximately equal inhibition of hemolysis of sodium taurocholate. These quantities were added to systems containing 400 γ of sodium taurocholate and also to systems containing 1500 γ of distearyl lecithin, and the K losses occurring after 15 hours at 4°C. were determined.

The table shows that the effect of serum albumin in inhibiting the prolytic loss of K is distinctly greater than that of the cholesterol,[3] although the two

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[3] An increase in the viscosity of the medium in which K is determined by the flame photometer itself produces spuriously low values. This is an important technical point to be borne in mind when working with media containing serum albumin, hemoglobin, etc.

substances in these concentrations have approximately the same inhibitory effect on hemolysis. This is not the only case in which serum albumin is much more effective in inhibiting a prolytic phenomenon than are the other inhibitory components of plasma. Serum albumin occupies a unique position among the agents which produce reversal of the disk-sphere transformation produced by lecithin and by a number of lytic agents (Ponder, 1946), its properties in this respect being possibly related to its being the "anti-sphering substance" for the shape transformation which occurs, as the result of a pH change, between glass slides and coverglasses (Furchgott, 1940 a, b; Furchgott and Ponder, 1940).
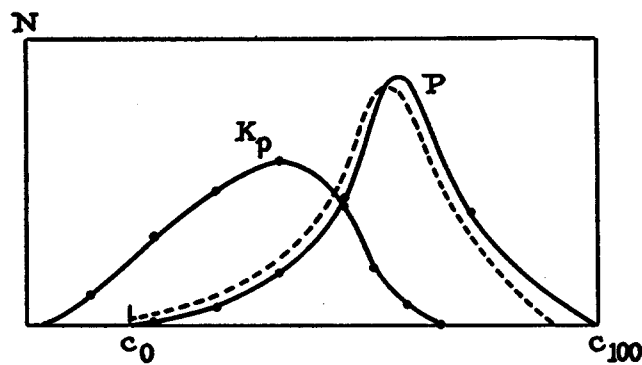

FIG. 4. Differentiation of the curves P and K_p of Fig. 3. The dotted curve shows the general form of a K_p curve for a system containing digitonin. For further explanation, see text.

DISCUSSION

The principal point requiring discussion is the relation of the K losses to the losses of Hb from the cells; *i.e.*, to the hemolytic process as ordinarily recognized.

The loss of Hb is all-or-none in the case of the individual red cell (Saslow, 1929), but the loss of K may be either a loss of all of the K from some of the cells, a loss of some of the K from all of the cells, or any combination of these two extreme situations. Until the question is decided experimentally, we can consider the consequences of first the one possibility and then of the other.

If all the loss of K observed is due to the loss of all of the K from some of the cells, K_p can be treated as a function of N, the number of red cells in the system, just as we relate the percentage of Hb lost to the percentage of the total number of cells which are hemolyzed. Differentiation of the curves marked P and K_p in Fig. 3 then gives two frequency distributions which show the number of cells which lose Hb or K in systems containing different concentrations of lysin (Fig. 4). No simple relation between the two dis-

tributions suggests itself, but it should be noticed that the position of the K_p distribution with respect to that P distribution is determined by the extent to which the loss of K precedes the loss of Hb. In the case of a system containing a lysin such as digitonin, where the prolytic loss is very small, the K_p dis-
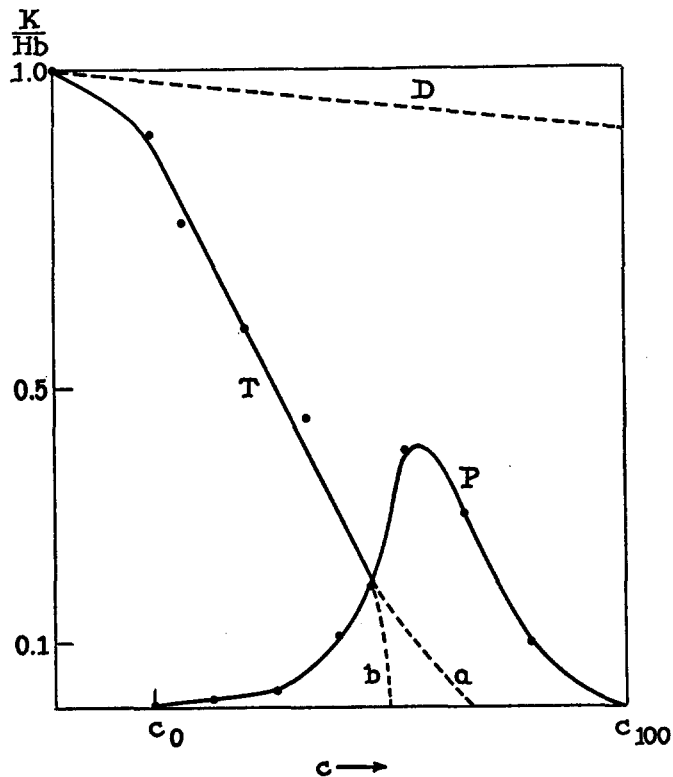

Fig. 5. The ratio K/Hb plotted against lysin concentration for a system containing sodium taurocholate (curve T) using the data from Fig. 3. The frequency distribution is a replica of that in Fig. 4, marked P. The dotted curve marked D shows the way in which the ratio K/Hb would behave in a system containing digitonin. For further explanation, see text.

tribution would be like that shown by the dotted curve, and almost superimposable on the P distribution.

If the loss of K observed is due to the loss of some of the K from all of the cells in the system, K_p is not a function of N and the behavior of the K/Hb ratio in the unhemolyzed cells becomes of special interest.[4] Fig. 5 shows the

[4] The values of Hb and of K considered in this ratio are those in excess of the values which the red cell would contain as a result of its coming into complete

way in which this ratio varies in the experiment upon which Fig. 3 is based, the lysin in this case being sodium taurocholate (curve marked T). The prolytic loss of K, occurring before the concentration c_0 for just commencing hemolysis is reached, results in a decrease in the K/Hb ratio which continues, after lysis has begun, as a roughly linear function of the lysin concentration. The line cuts the abscissa, $i.e.$ all the K is lost and K/Hb = 0 at a concentration of lysin which produces between 20 and 75 per cent loss of Hb; the course of the lower part of the curve is doubtful, and is marked as a and b corresponding to the doubtful part of the curve in Fig. 3. The slope of the line is an expression of the extent to which the loss of K precedes the loss of Hb; in the case of a system containing a lysin such as digitonin, where the prolytic loss is small, the slope would be very much less, as in the dotted line marked D.

The mechanisms which can be suggested to account for K escaping while Hb is retained depend so much on what the structure of the red cell is conceived to be that a discussion of them is better postponed until more is known about the volume changes and the movement of other ions which accompany the K loss.

SUMMARY

The prolytic loss of K, $i.e.$ the loss of K which takes place from red cells exposed to hypolytic concentrations of lysins, has been measured in systems containing distearyl lecithin, sodium taurocholate, sodium tetradecyl sulfate, saponin, and digitonin, by means of the flame photometer. The lysins are added in various concentrations to washed red cells from heparinized human blood, and the K in the supernatant fluids is determined after various intervals of time and at various temperatures. The prolytic loss K_p is compared in every experiment with the loss K_s into standard systems containing isotonic NaCl alone, with no lysin.

The losses K_s and K_p increase with time, so that new steady states are approached logarithmically. The values of K_p which correspond to the new steady states depend on the lysin used, being greatest with taurocholate and smallest with digitonin. The temperature coefficient of the loss is positive, and the extent and course of the losses have no apparent relation to the prolytic shape changes.

In systems in which the loss of K is appreciable, it can be inhibited by the addition of plasma or of either cholesterol or serum albumin. Of these two

equilibrium with the medium surrounding it, and correspond to the "surplus Hb" (Ponder, 1942b) and to an analogous "surplus K." Since the values for the "equilibrium Hb" and the "equilibrium K" are not exactly known (see footnote 2), calculations of the lower values of the ratio are even more doubtful than they would otherwise have been. There does not seem any escape, however, from the conclusion that a red cell, situated towards the upper end of the resistacne distribution, may lose virtually all of its K without losing any of its Hb.

substances, even when used in quantities which have an approximately equal effect in inhibiting hemolysis, serum albumin is much the more effective.

Just as the prolytic loss of K occurs without the loss of any Hb, so in concentrations of lysin sufficient to produce hemolysis the loss of K, expressed as a percentage of the total red cell K, increases much more rapidly with lysin concentration than does the loss of Hb expressed as a percentage of the total Hb. The explanation of these relations depends on whether the loss of K is treated as being all-or-none in the case of the individual cell or as being the result of the loss of part of the K from all of the cells. This point has still to be decided.

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