PROLYTIC ION EXCHANGES PRODUCED IN HUMAN RED CELLS BY METHANOL, ETHANOL, GUAIACOL, AND RESORCINOL

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When mammalian red cells are suspended in solutions of certain alcohols in isotonic NaCl, the prolytic losses of K are unusually large, while the amount of lysis which follows is exceptionally small (Davson and Danielli, 1938). These systems can accordingly be used to establish points regarding prolytic K loss which cannot be established in systems containing more active lysins. More specifically, it will be shown that human red cells exposed to the action of hypolytic concentrations of a number of alcohols lose up to 70 per cent of their contained K, the loss being rapid at first and then slowing down until an apparently steady state is reached, that Na enters the cells to replace K, that when the cells are in the new steady state some of their cations move freely in and out of them, but that the capacity of such cells for undergoing disk-sphere transformations, as well as their resistance to the hemolytic effects of hypotonic media and lysins such as saponin, differs only in minor respects from that of untreated red cells.

1. The Prolytic Losses of K Produced by Methanol, Ethanol, Guaiacol, and Resorcinol

1. The Losses as Functions of Time and Concentration.—The prolytic losses of K are measured by adding 10 ml. of various concentrations of methanol, ethanol, guaiacol, and resorcinol, dissolved in 172 m. eq./liter NaCl (isotonic saline), to 0.8 ml. of the thrice washed cells of heparinized human blood. After standing for various lengths of time, with occasional mixing by inversion, at 4°C., the cells are gently thrown down and the supernatant fluid is removed. Its K content is found with the Perkin-Elmer flame photometer (see Ponder, 1947 *a*, *b*, for a more detailed description of technique). The K loss occurring from 0.8 ml. of washed red cells into 10 ml. of 172 m. eq./liter NaCl, at the same temperature and after the same lengths of time, is also determined, so that the K losses into the solutions of the alcohols can be compared with it.

Table I gives the results obtained after various intervals of time with 3.1 M methanol, 2.2 M and 0.04 M resorcinol, none of which produces more than 1 to 3 per cent hemolysis at 4°C. even after the longest of the times shown in the table. Plotting values for the K loss, given here as fractions of K_0, the initial K concentration in the cells, against time shows that the rate of K loss decreases with time so that a new steady state characterized by the values of K_∞ given

in the last column is reached in an approximately logarithmic manner. This is the same sort of result as has been obtained already with other lysins (Ponder, 1947 *a*), but the values of K_∞ are much larger than any previously obtained.

Table II gives the average K losses, again as fractions of K_0, obtained with methanol, ethanol, guaiacol, and resorcinol in various hypolytic concentrations and at the end of 20 hours at 4°C. It should be noticed that the variation in

TABLE I

Prolytic Loss of K at 4°C. as a Function of Time

		K losses, fractions of K_0				
		3 hrs.	6 hrs.	9 hrs.	16 hrs.	K_∞
Saline	—	0.04	0.07	0.09	0.10	0.11
Methanol	3.1 M	0.10	0.18	0.23	0.28	0.31
Ethanol	2.2 M	0.11	0.20	0.25	0.33	0.35
Resorcinol	0.004 M	0.14	0.32	0.43	0.60	0.65

TABLE II

Prolytic Loss of K after 20 Hours at 4°C.

		Mean loss as fraction of K_0	Extreme values observed
Saline	—	0.08	0.06, 0.13
Methanol	3.1 M	0.26	0.15, 0.34
Ethanol	1.1 M	0.21	0.16, 0.27
	2.2 M	0.37	0.24, 0.55
	3.3 M*	0.60	0.55, 0.69
Guaiacol	0.01 M	0.17	
	0.02 M	0.33	
Resorcinol	0.02 M	0.35	0.30, 0.43
	0.04 M‡	0.57	0.45, 0.77

* Produces about 3 per cent lysis.

‡ May produce 1 to 3 per cent lysis.

the loss is considerable (last column) even when the experiments are carried out under standarized conditions. The variation does not occur, however, between duplicates, and seems to be due to differences in the ease with which the cells of different individuals lose K in these systems.

At higher temperatures the losses of K tend to be larger, but increasing amounts of hemolysis are produced. Thus, in the case of 1.1 M, 2.2 M, and 3.3 M ethanol at 24°C., fractions of K_0 amounting to 0.21, 0.45, and 0.74 are lost at the end of 13 hours, but 3 per cent hemolysis is produced by 2.2 M ethanol, and 13 per cent hemolysis by 3.3 M ethanol.

The final value of K_∞ toward which the curves tend seems to be a function of the concentration of the alcohol, and the losses cannot be made greater by

gently throwing down the cells, removing the supernatant fluid, and replacing it with fresh alcohol in the same concentration as that used originally. Even when this is done several times, the final K concentration in the cells is not more than 5 per cent lower than that in cells which have stood for the same length of time in the same concentration of alcohol with no replacement.[1]

2. The K-Na Exchange.—The loss of K in systems containing hypolytic concentrations of distearyl lecithin or sodium taurocholate has been shown to be accompanied by a nearly equivalent gain of Na, together with a small volume change (Ponder, 1947 *b*). In the case of the alcohols, parallel experiments are carried out by adding 10 ml. of the alcohol, in hypolytic concentration in 172 m. eq./liter NaCl, to 0.8 ml. of washed red cells, allowing the system to stand for from 10 to 20 hours at 4°C. with occasional mixing, separating the cells from the supernatant fluid, and finally determining the K content, K_p, of the supernatant fluid and the K and Na contents of the packed cells. These are compared with the K and Na contents of packed cells which have been washed in, but not allowed to stand in, 172 m. eq./liter NaCl. Changes in volume which persist at the end of the experiment are calculated from the Hb concentrations in the washed packed cells and in the packed cells which have been exposed to the hypolytic concentrations of alcohol.[2]

Table III gives representative values for the K losses, the Na gains, and the volume changes. As in the case of other lysins in hypolytic concentration, the losses of K are associated with gains of Na by the cells; here the latter, like the former, are unusually large. It seems that the excess of Na gained over K lost, observed in hypolytic concentrations of taurocholate and distearyl lecithin which give comparatively small K losses, tends to become less as the K loss

[1] These replacements must be made at the same temperature (usually 4°C.) as that at which the comparison system is kept. If the temperature is allowed to rise, it will appear that the replacement of fresh alcohol results in an increase in the K loss.

[2] No attempt has been made to measure the rapid volume changes which occur in these systems, as, for example, the rapid decrease in volume which may occur immediately after the addition of cells to the hypolytic concentrations of the alcohols. The system of equations developed by Jacobs (1933) probably deals adequately with these. A great deal of the existing treatment of the mammalian red cell as a balloon-like body invested with a semipermeable membrane can no doubt be retained when the time comes to outline a new and more adequate treatment based on the properties of a less artificially simplified red cell structure. So far as rapid volume changes are concerned, it is apparently possible to ignore the slow movement of cations and to treat the changes in ionic composition as being governed by the properties of a surface membrane; the only effect of this simplification of the real situation may turn out to be a change in the values of constants. To fully account for the ionic composition of the cell under all the conditions in which it remains intact, it seems to be necessary to consider new properties which are probably properties of the red cell interior.

increases in magnitude, so that in the case of systems containing methanol, ethanol, or resorcinol the loss of K and the gain of Na are nearly equivalent.

As in the case of previous experiments (Ponder, 1947 *b*), the least satisfactory observations are those of the volume changes. The K-Na exchange is usually accompanied by a 5 to 10 per cent increase in cell volume, but the magnitude of the change is unaccountably variable, and sometimes no volume increase at all can be observed.[3]

3. Demonstration That K and Na Can Enter and Leave Alcohol-Treated Cells.— To ascertain whether the K which is lost from red cells into isotonic NaCl containing alcohol can be made to reenter the cell by washing it with isotonic KCl, and whether, once it has reentered, it leaves the alcohol-treated cell as

TABLE III

K-Na Exchanges after 20 Hours at 4°C.

		K_p	Cell contents*				
			Initial		Final		
			K	Na	K	Na	Volume change
			m. eq./liter	*m. eq./liter*	*m. eq./liter*	*m. eq./liter*	*per cent*
Methanol	3.1 M	0.17	98	26	80	52	+7
Ethanol	2.2 M	0.46	93	23	50	76	+9
Resorcinol	0.04 M	0.58	110	22	43	94	0

* Values are given to the nearest unit only.

readily as, or more readily than, it did originally, the following type of experiment can be carried out.

Two ml. of heparinized human blood, the volume concentration of which is first adjusted to 0.4 by removing plasma or by adding 172 m. eq./liter NaCl, are added to each of six stout walled glass tubes. The cells are washed three times with 10 ml. of 172 m. eq./liter NaCl, and the supernatant fluids are removed as completely as possible without losing cells. The K and Na contents (K_0 and Na_0) of the cells of one of the tubes (No. 1) are found by packing the cells, transferring 0.5 ml. with water to a 50 ml. flask, filtering after standing, and using the flame photometer for the determinations, as already described (Ponder, 1947 *b*).

To each of the remaining five tubes are added 10 ml. of 2.2 M ethanol in 172 m. eq./liter NaCl. The contents of the tubes are allowed to stand at 4°C., with occasional

[3] In view of the possibility of a reaction between the alcohol and Hb and of a color change which would invalidate the colorimetric volume determinations, the initial cell volume and the volume after standing in the hypolytic concentrations of alcohol were measured in Hamburger hematocrit tubes. The results obtained were substantially the same as those obtained by the colorimetric method.

mixing by inversion. At the end of 20 hours, the cells in one of the tubes (No. 2) are packed, and their K and Na contents (K_1 and Na_1) are found.

The cells of three of the four remaining tubes are thrown down, the supernatant fluids are removed, and 10 ml. of 172 m. eq./liter KCl are added to each tube. After standing for 15 to 30 minutes at 4°C., the cells are thrown down gently and are then washed once with 172 m. eq./liter KCl. After the washing, as much of the supernatant fluid as possible is removed, and the cells of one of the tubes (No. 3) are packed and their K and Na contents (K_2 and Na_2) are found. To the cells of the two remaining tubes containing KCl (Nos. 4 and 5) are now added 10 ml. of 172 m. eq./liter NaCl. The cells are thrown down immediately, and are washed again with 11 ml. of

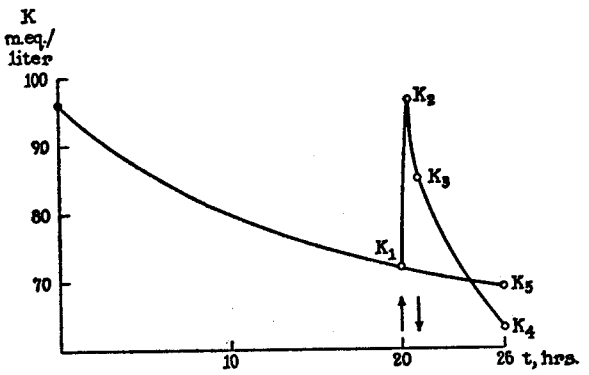

FIG. 1. Red cell K, in milliequivalents per liter of cells, after exposure to 2.2 M ethanol in isotonic NaCl at 4°C. for 20 hours. Washing with isotonic KCl at first arrow; washing with isotonic NaCl at second arrow. For further explanation, see text.

172 m. eq./liter NaCl, without loss of time. The cells in one of the tubes (No. 4) are then packed and their K and Na contents (K_3 and Na_3) are determined. To the cells of the other tube (No. 5) are added 10 ml. of 2.2 M ethanol, and the contents of this tube are allowed to stand for 5 hours at 4°C.

At the end of this time, the cells in tube 5 are packed, and the K and Na contents (K_4 and Na_4) are found. The cells of tube 6, which have been exposed to the action of 2.2 M ethanol at 4°C. from the beginning of the experiment, are packed at the same time; their K and Na contents are K_5 and Na_5.

The results of a typical experiment of this kind are shown in Fig. 1. The exposure of the cells, with their initial K content of 96 m. eq./liter, to 2.2 M ethanol in isotonic NaCl at 4°C. results in a loss of 24 m. eq./liter of K at the end of 20 hours. Washing with 172 m. eq./liter KCl (first arrow) raises the K content of the cells from 72 m. eq. /liter (K_1) to 98 m. eq./liter (K_2), but washing with 172 m. eq./liter NaCl (second arrow) results in a fall to the value K_3. This value is considerably higher than K_1 and takes about 15 minutes to

be reached; it can therefore be concluded that the K which has entered the cells from isotonic KCl does not leave them immediately when they are immersed in isotonic NaCl. It leaves considerably more rapidly, however, than the cell's own K leaves it at any stage of the experiment. The final value of 63 m. eq./liter (K_4) is reached after a further 5 hours' exposure to 2.2 M ethanol in isotonic NaCl. This is somewhat less than the value reached after 26 hours in 2.2 M ethanol in isotonic NaCl at 4°C. (K_5 = 69 m. eq./liter); the difference is probably associated with the washing of the cells, twice with KCl and twice with NaCl, operations which tend to increase K loss.

The Na gains correspond approximately with the K losses. When K reenters the cell from isotonic KCl (at K_2), an equivalent quantity of Na is replaced, and when the losses of K occur again into isotonic NaCl (at K_3 and K_4), Na reenters in approximately equivalent amounts. The volume exchange observed in this experiment was +6 per cent; *i.e.*, the final volume of the cells (at K_4 or K_5) was 6 per cent greater than the initial volume.

The conclusion to be drawn from these results is that the equivalent quantity of Na which replaces the K lost by the cell into a hypolytic concentration of alcohol in isotonic NaCl is relatively mobile, and is itself quickly replaced by an equivalent quantity of K when the cell is placed in isotonic KCl. The K which replaces it is relatively mobile also, and is lost and replaced by Na, although not immediately, when the cell is again placed in isotonic NaCl.

The situation may be described by saying that the action of a hypolytic concentration of ethanol or of alcohols acting similarly is to render mobile a fraction of the cell K, the fraction made mobile being a function of the concentration of alcohol, the duration of its action, the temperature, and other factors. This mobile fraction is lost and is replaced by an approximately equivalent amount of mobile Na when the cell is immersed in isotonic NaCl; similarly this mobile Na is replaced by mobile K when the cell is immersed in isotonic KCl. The remainder of the cell K remains non-mobile; *i.e.*, in the same non-diffusible state as that in which it exists initially in the untreated red cell.[4]

2. The Resistance of Alcohol-Treated Cells to Lysins

The effect of the alcohols on the resistance of red cells to lysins can be ascertained by preparing suspensions of washed red cells which are allowed to stand at 4°C. in 172 m. eq./liter NaCl, and of washed red cells which stand for various lengths of time at the same temperature in various hypolytic concentrations of methanol, ethanol, guaiacol, or resorcinol. The latter are prepared by

[4] A similar situation may exist with respect to the cell Na, for it will be noticed that the Na which is replaced by K when the alcohol-treated cell is placed in isotonic KCl is not the entire cell Na, but only the Na which has entered to replace the lost K.

adding 10 ml. of the alcohol in 172 m. eq./liter NaCl to 0.8 ml. of washed red cells; after the system has stood for the desired length of time, the supernatant fluid is removed for a K determination, and the cells, after two washings with 10 ml. of 172 m. eq./liter NaCl, are suspended in 20 ml. of 172 m. eq./liter NaCl. The suspension with which this is to be compared is prepared similarly, except that the cells stand in 172 m. eq./liter NaCl instead of in the hypolytic concentration of alcohol.

The resistance of the cells of these suspensions to lysins such as saponin and digitonin can be determined by plotting time-dilution curves for complete

TABLE IV

Resistance to Saponin and to Hypotonicity after Exposure to Ethanol and to Resorcinol at 4°C.

			Resistence to saponin, R	Resistance to hypotonicity	K loss, fraction of K_0
		hrs.			
Ethanol	2.2 M	4	0.9	0.00	0.16
		20	0.8	0.02	0.35
Resorcinol	0.04 M	4	0.8	0.01	0.18
		20	0.7	0.06	0.60

hemolysis, and their resistance to hypotonicity (fragility) can be found in the usual way. The resistance to lysins such as saponin and digitonin can also be found by a method which is analogous to that for determining resistance to hypotonicity, in which the percentage of complete hemolysis produced by various quantities of lysin after 7 to 12 hours, *i.e.* at the asymptotes of time-dilution curves, is found (Ponder, 1930).

The results which are obtained are summarized in Table IV, which shows the resistance to saponin, determined as an R-value at 37°C., and the decrease in resistance to hypotonicity, expressed in tonicity units.[5] Short exposures (up to 4 hours) to hypolytic concentrations of ethanol or resorcinol produce only small changes in the resistance of the cells to hypotonicity; cells which have been exposed to 0.04 M resorcinol at 4°C. for 4 hours, for example, begin to hemolyze at a tonicity of 0.57 instead of at a tonicity of 0.58. These cells, however, have exchanged about 18 per cent of their K for Na. At the same time the resistance to saponin decreases, so that the average value of R is 0.8 to 0.9.

Longer exposures (20 hours) produce greater changes in the resistance to

[5] A 172 m. eq./liter NaCl solution has a tonicity of 1.0.

hypotonicity (0.02 to 0.06 tonicity units) and to saponin (R = 0.7 to 0.8[6]), but the K losses now amount to from 35 to 65 per cent of K_0. In spite of these large losses and their replacement with roughly equivalent quantities of Na, the behavior of the cells towards lysins such as saponin, digitonin, and

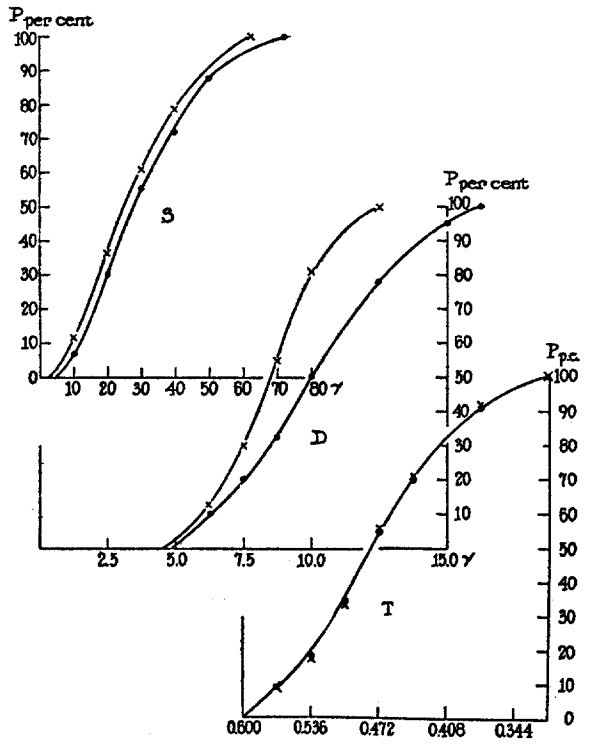

FIG. 2. Integrals of frequency distributions of resistances of red cells at 25°C. to saponin (S), digitonin (D), and hypotonicity (T). Curves through points are for suspensions of cells standing 4 hours in isotonic NaCl at 4°C; curves through crosses are for suspensions of cells standing 4 hours at 4°C. in 2.2 M ethanol in isotonic NaCl, and then washed.

taurocholate, and also towards the reduction of tonicity, is so like that of untreated cells that it would be difficult to detect the effect of exposure to the alcohols unless the two kinds of suspension were compared side by side.

Figs. 2 and 3 show the results obtained by a method which is particularly well adapted to bringing out differences between untreated cells and those

[6] $R = c_2/c_1$, where c_2 is the concentration of lysin which produces complete hemolysis of a suspension of alcohol-treated cells in the same time as that in which another concentration c_1 produces complete hemolysis of a standard suspension of untreated cells. An R-value of 0.8 represents a small but appreciable acceleration.

which have been exposed to hypolytic concentrations of lysins.[7] The procedure results in the integrals of the distributions of resistances of the cells to saponin and to digitonin (or other similar lysins) being obtained together with the integral of the distribution of resistances to hypotonicity. The

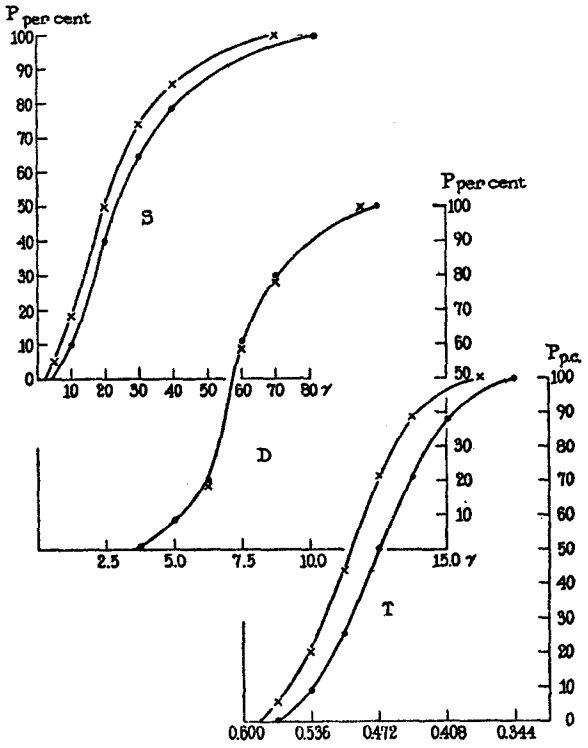

FIG. 3. Similar to Fig. 2, but curves through points are for suspensions standing for 20 hours at 4°C. in isotonic NaCl, while curves through crosses are for suspensions of cells standing for 20 hours at 4°C. in 0.04 M resorcinol in isotonic NaCl, and then washed.

curves through points in Fig. 2 are for untreated red cells, and the curves through crosses are for cells exposed to 2.2 M ethanol in isotonic NaCl for 4 hours at 4°C. (K loss = 0.18 K_0), and then washed twice with isotonic NaCl. The exposure of the cells to this hypolytic concentration of ethanol results in a considerable decrease in the resistance to digitonin, a much smaller decrease in resistance to saponin, and in scarcely any change in the resistance

[7] The details of the method will be published shortly in the hematological journal, *Blood*. The measurements of the resistances to lysins and to hypotonicity are carried out at 25°C.

to hypotonicity. The corresponding curves in Fig. 3 are those for untreated red cells and for cells exposed to 0.04 M resorcinol in isotonic NaCl for 16 hours at 4°C. (K loss = 0.48 K_0), and then washed twice in isotonic NaCl. The resistance to digitonin is scarcely affected, although the resistance to saponin is considerably reduced. The resistance to hypotonicity is decreased, but only by about 0.02 tonicity units.[8]

These changes in resistance are no doubt due to permanent changes, produced by the exposure to the alcohols, in parts of the red cell structure which undergo change during the lytic processes. The point which calls for comment is that the changes in resistance are so small under conditions in which the ionic changes are so great.

3. Shape and Shape Transformations of Alcohol-Treated Cells

The discoidal shape and the ability to undergo disk-sphere transformations are retained to a remarkable extent by human red cells which have been exposed to alcohols and which have lost large fractions of their K as a result.

For observations of shape and of the shape transformations produced by lecithin, saponin, rose bengal, and other lysins, the cells should be suspended in a mixture of equal parts of 3 per cent sodium citrate and M/15 phosphate buffer (NaH_2PO_4 and Na_2HPO_4) at pH 7.0, since shape is so well preserved in this medium (Ponder, 1947 *c*). The alcohols in hypolytic concentration can be added to the citrate-buffer, and typical disks are observed after the cells have been exposed to 3.1 M methanol, 2.2 M ethanol, or 0.04 M resorcinol for as long as 20 hours at 4°C.[9] The sphering effect of added lecithin sol is

[8] Measurement of the increase in volume of untreated cells which have stood in isotonic NaCl for 20 hours at 4°C. and of the increase in volume of cells which have stood for the same length of time at the same temperature in hypolytic concentrations of ethanol, etc., shows that both behave as imperfect osmometers when added to NaCl of a tonicity of about 0.7. The value of R in the equation for osmotic swelling (Ponder, 1943) is from 0.65 to 0.75 in both cases, with no constant difference between them.

[9] When suspended in the plasma from which they were originally derived, the capacity of alcohol-treated red cells for forming rouleaux is not changed except in so far as shape changes result from the exposure to the hypolytic alcohols. The sedimentation rates in suspensions in plasma of cells which have stood for periods up to 5 hours in 172 m. eq./liter NaCl, in 2.2 M ethanol in NaCl, or in 0.04 M resorcinol in NaCl are substantially the same. As the length of the exposure to the alcohols is increased, the sedimentation rates in these reconstructed plasma systems tend to become less because some of the cells are crenated and cannot form rouleaux. Even in systems containing cells which have been exposed to the hypolytic concentrations of the alcohols for less than 5 hours, some of the cells sediment much more slowly than the remainder and can be seen as a stratum of very low optical density situated above the upper margin of the sedimenting column; these are crenated cells which cannot form rouleaux.

quantitatively the same for cells in citrate-buffer and for cells in 2.2 M ethanol in citrate-buffer after standing for 20 hours at 4°C. Both alcohol-treated cells and untreated cells are converted into spheres by saponin and by rose bengal, and the formation of spheres is followed by the appearance of prolytic spheres and by hemolysis.

For observation of shape changes between glass slide and coverglass, the cells should be suspended in unbuffered isotonic NaCl. The sphering seems to be substantially the same in preparations of cells which have been exposed to 3.1 M methanol, 2.2 M ethanol, or 0.04 M resorcinol for 10 hours at 4°C. as it is in preparations of cells which have stood for the same time at the same temperature in isotonic NaCl; this shape transformation, however, is difficult to observe quantitatively. The familiar reversal of the shape change on the addition of plasma occurs in an apparently identical manner in preparations of cells which have been exposed to the alcohols in hypolytic concentration and in preparations of cells which have stood in isotonic NaCl at 4°C.

DISCUSSION

Attempts to account for the results described in this paper on the basis of the existing views as to the structure of the red cell and the permeability of a surface membrane meet with two principal difficulties.

The first difficulty is one which has already arisen in connection with the results obtained with hypolytic concentrations of other hemolysins (Ponder, 1947 *a*, *b*), and is that the loss of K increases with time in such a way that what appears to be a new steady state is approached logarithmically. No further quantity of K leaves the cell thereafter, but the Na which has entered to replace the lost K can pass in and out of the cell, and can be replaced by K which can also pass in and out although the rest of the K cannot. Some restriction to the movement of K between the cell and its environment is apparently modified, in an irreversible manner, when the cell is exposed to the action of the hypolytic concentrations of lysin. Unless the loss in the case of the individual red cell is all or none (a possibility which cannot be excluded on the basis of existing evidence), the modification must be such that not all the K, but only a fraction of it, is affected, the fraction being a function of the lysin concentration, the duration of its action, and other factors. What is involved is no doubt a modification of some part of the cell structure and of the properties which depend on its integrity. One possibility is that some parts of the cell, *e.g.* the more superficial parts, lose K more readily than other parts do. Another possibility is that the bonds between K and molecules in the cell interior (possibly Hb, possibly stromatin, and possibly a Hb-stromatin complex) are more readily broken in the case of some molecules than in the case of others, and still another posssibility is that the difference between the K content of the red cell and that of the surrounding medium is due to a metabolic process which is interfered with to a greater or lesser extent in greater

or lesser lysin concentrations. There does not seem to be any way in the meantime of deciding between these possibilities. On the other hand it is scarcely a tenable idea that the results are due solely to the lysins producing changes in the extent to which a paucimolecular membrane restricts the movement of ions; *i.e.*, to their producing changes in the permeability of the red cell membrane in the sense in which these terms are generally used.

The second difficulty is associated with the smallness of the changes in resistance to hypotonicity, but this is not so surprising as it may appear at first sight. Supposing lysis to depend on the entrance of water under an osmotic pressure difference, with swelling of the cell and with deformation of its structural components, the essential feature of the mechanism would be the rapid entrance of the water. This would not be substantially affected by a very slow movement of cations, and the proportion of Na and K inside the cell would make little difference to the result always provided that the K + Na content were constant so that the same osmotic pressure difference resulted between the cell interior and the surrounding hypotonic NaCl. It is important to notice, however, that on this hypothesis the rate of the swelling of the cell, and the tonicity in which its critical volume is attained and in which it hemolyzes, would be determined almost exclusively by the factors in the situation which determine that water shall enter rapidly, and that slow ionic exchanges could occur without measurements of osmotic resistance giving any indication of their occurrence. The present views regarding red cell structure and the permeability of its membrane may accordingly be adequate so far as the prediction of rapidly occurring changes in volume and in ionic composition is concerned, but they do not always allow us to draw valid conclusions as to other changes which occur more slowly; *e.g.*, as to the slow movement of cations. The prediction that a red cell permeable to both anions and cations will undergo a Donnan swelling to the point of hemolysis is a case in point, the real situation being, at least sometimes, such as to allow of a slow cation exchange without the predicted volume increase.

The observation that exposure to hypolytic concentrations of the alcohols results in comparatively small changes in resistance to other forms of lysis is one which does not give rise to difficulty, because lysis by saponin and related lysins has never been thought of as being intimately associated with the ionic composition of the red cell. It is remarkable, nevertheless, that such great changes in ionic composition as are observed after exposure of red cells to hypolytic concentrations of the alcohols should be accompanied by so little change in the stability of the Hb in the cell interior.

SUMMARY

When the washed red cells of heparinized human blood are exposed at 4°C. to methanol, ethanol, guaiacol, or resorcinol in hypolytic concentrations in

isotonic NaCl, the prolytic loss of K at the end of 20 hours varies from about 25 per cent of the initial K content of the cells in the case of 3.1 M methanol to about 55 per cent of the initial K in the case of 0.04 M resorcinol. As in the case of the prolytic losses observed with other lysins, the K loss is rapid at first and then slows down so that what appears to be a new steady state is reached logarithmically.

The K lost from the cells during the period of the prolytic loss is replaced by an approximately equivalent amount of Na, derived from the isotonic NaCl in which the cells are suspended. The Na which enters can be replaced by K by washing the cells in isotonic KCl, and this K can again be replaced by Na by washing the cells in isotonic NaCl. The remainder of the cell K, *i.e.* the K which was not lost during the period of the prolytic loss, is retained in the cell unaffected by these washing procedures.

The capacity of red cells for undergoing disk-sphere transformations is scarcely affected by their having been exposed to hypolytic concentrations of methanol, ethanol, guaiacol, or resorcinol in isotonic NaCl, and their resistance to osmotic hemolysis and to lysis by saponin and digitonin is altered only in minor respects even when as much as 50 per cent of the cell K has been exchanged for Na.

Some restriction to the movement of K between the cell and its environment is apparently modified irreversibly when the cell is exposed to hypolytic concentrations of lysins, and the modification is such that only a fraction of the cell K is affected, the fraction being a function of the lysin concentration, the duration of its action, and other factors. A modification of some part of the cell structure and of the properties dependent on its integrity is probably involved: K may be lost more readily from some cells than from others, from some parts of the cell more readily than from other parts, or the explanation may lie in changes in the extent to which Hb binds ions or in modifications of metabolic processes.

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