POTASSIUM TRANSPORT IN HUMAN ERYTHROCYTES: EVIDENCE FOR A THREE COMPARTMENT SYSTEM

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The experiments to be reported were undertaken to investigate the efflux of potassium from human erythrocytes using K^{42} as a tracer. During the course of these experiments, it became apparent that the K *efflux* could not be described in terms of the two compartment system, comprising plasma and cells respectively, which had previously proved adequate (1–3) to describe the *influx* of K^{42} from labelled plasma to unlabelled cells. The present experiments show that when labelled cells are incubated with unlabelled plasma, a third compartment located in the cellular phase is required to account for the apparently anomalous time curve showing the appearance of K^{42} in plasma.

I

Experimental Methods

Blood was drawn from normal human beings into a siliconized flask and mixed with heparin (Upjohn, Na: 2 units/ml. blood). A portion of the blood was centrifuged to remove the plasma, which was reserved for subsequent use during the experiment. The remainder of the blood was incubated at 37°C. with relatively large quantities of isotonic K^{42} Cl (of the order of 350 μc.) for periods of ½ to 3 hours according to the method previously described (1). At the close of the first incubation period, which is called pre-incubation, the blood was centrifuged for 30 minutes at 1100 *g*. The radioactive plasma was withdrawn, and the white cells were then removed by swabbing the top of the red cell mass with a small cotton-covered stick. This procedure required about 30 minutes.

A small amount of non-radioactive plasma (1 ml. plasma/10 ml. packed cells) was next added to the cells together with enough buffer to bring the hematocrit reading to the range of 0.40–0.48. The resuspended "blood" was then incubated at 37°C. for a further period of 8 to 10 hours. Following Experiment 11, the buffer was slightly modified from that previously used (1) to make it more nearly isotonic, and to bring the Ca and Mg concentrations closer to those found in human plasma. Its composition is given in Table I. Results with the new buffer were unchanged from those with the old buffer.

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Samples of blood were taken at intervals (usually hourly) for analysis of the K^{42} radioactivity in the plasma during the 8 to 10 hour incubation period. Initial and final K concentrations were measured in a flame photometer using the methods and trapped plasma corrections previously described (1). pH was measured at room temperature in a Cambridge pH meter and usually dropped by 0.25 unit over the course of the experiment.

In the earlier experiments, before Experiment 12, the cells were kept overnight in a refrigerator between pre-incubation and incubation. This procedure was abandoned when it became clear that three compartments were involved in the efflux,

TABLE I

*Composition of Buffer**

Compound	Concentration	Effective milliosmoles
	gm./l.	
NaCl	6.888	235.7
$MgCl_2 \cdot 6H_2O$	0.102	1.5
$CaCl_2$	0.133	3.6
$Na_2HPO_4 \cdot 7H_2O$	0.456	5.1
$NaH_2PO_4 \cdot H_2O$	0.580	1.3
KCl	0.330	8.8
Na_2CO_3‡	1.431	54.0
Glucose	2.50	§
Total		310.0

* Before using the buffer 5 per cent CO_2—95 per cent air is passed through it to bring it to pH 7.4.

‡ Converted to $NaHCO_3$ by passing 100 per cent CO_2 through the solution. Milliosmoles calculated as $NaHCO_3$.

§ Since the cell is quickly permeable to glucose at this concentration level (Lefevre (4)), there is no effective osmolar contribution from glucose.

and that a more detailed investigation of the kinetics of the reaction at 37°C. would be rewarding.

Experiments with Na^{24} were also carried out using similar methods for pre-incubation and incubation. For these experiments, radioactivity was measured in the cells rather than in the plasma using the methods previously described (1).

K^{42} and Na^{24} were received from the Brookhaven National Laboratory. To insure uniformity in preparations of Na^{24} and K^{42}, samples for bombardment are taken by the Brookhaven National Laboratory from very large batches of chemically pure Na_2CO_3 and K_2CO_3. Thus all samples were prepared from the same original batches of Na_2CO_3 and K_2CO_3 respectively and occasional half-life checks were sufficient to indicate that the isotopes were pure. Gamma radioactivity was measured on a Wood well-type scintillation counter (Type SC-2L), thus obviating the need for any self-absorption or dead-time corrections.

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II

RESULTS

Appearance of K^{42} in Plasma.—

In the earlier experiments with cells that had been refrigerated overnight between pre-incubation and incubation, the K^{42} efflux appeared abnormal when calculated according to the equations previously given (1). Furthermore,

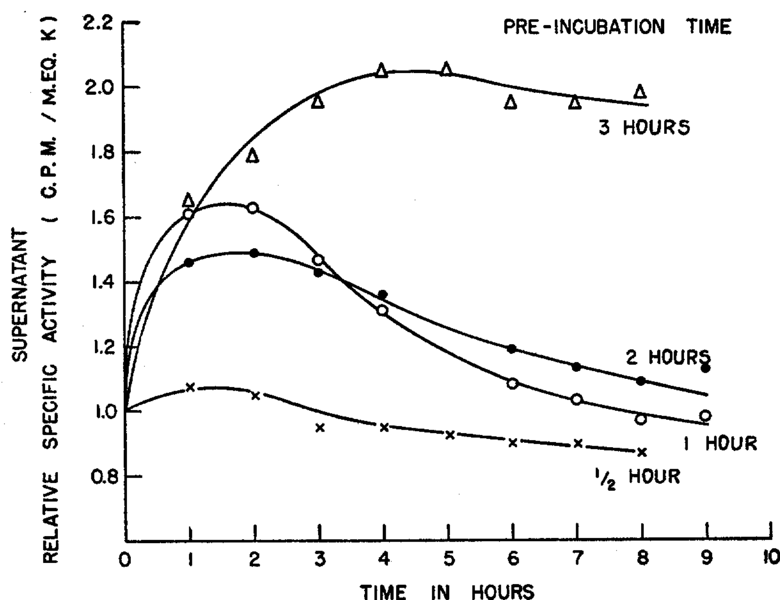

FIG. 1. Time course of the relative specific K activity (relative counts per minute milliequivalents K) of the supernatant in samples of human red cells resuspended in a mixture of plasma and buffer. Average hematocrit reading, 0.435. Following pre-incubation with $K^{42}Cl$ (1 and 2 hours, Experiment 12; ½ and 3 hours, Experiment 13), the plasma and white cells were removed, and the cells were resuspended in a mixture of plasma and buffer for the 8 to 9 hour period shown above. The time scale starts at the beginning of the resuspension period. The curves are smooth lines drawn through the experimental points and have no theoretical basis.

when the cells had come to apparent equilibrium with the plasma, as judged by the fact that the plasma specific activity was approaching a plateau, the specific activity of the plasma was often appreciably higher than that of the cells. In this quasi-equilibrium state the ratio "plasma specific activity/red cell specific activity" reached its maximum, an average of 1.29 ± 0.22 in four experiments, with a pre-incubation time of 1 hour. With longer pre-incuba-

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tion times the ratio was smaller, being 0.97 ± 0.09 (average of four experiments) at 3 hours.

In order to examine this point more closely, experiments were carried out in which the pre-incubated red cells, after removal of the very "hot" plasma, were resuspended at once in buffer and plasma for the incubation period of about 8 hours at 37°C. The results of two of these experiments (Experiments 12 and 13) are given in Fig. 1. The hump in the plasma specific activity which is particularly evident in the cases with 1 and 2 hour pre-incubation, cannot

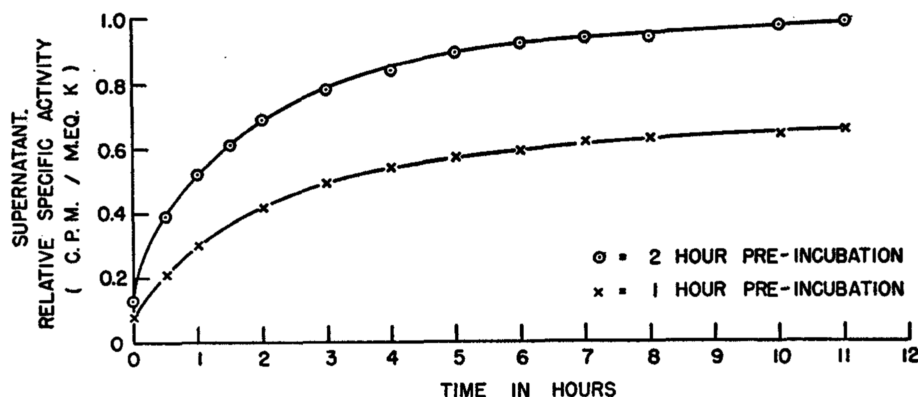

FIG. 2. Time course of the relative specific K activity (relative counts per minute milliequivalents K) of the supernatant in samples of human red cells resuspended in a mixture of plasma and buffer. (Experiment 16 *a* hematocrit reading 0.415, 2 hour pre-incubation; Experiment 16 *b* hematocrit reading 0.349, 1 hour pre-incubation.) The initial conditions differ from those in Fig. 1 in that the cells were washed once with buffer before resuspension. The time scale starts at the beginning of the resuspension period. The curves are smooth lines drawn through the experimental points and have no theoretical basis.

occur in a simple two compartment system. In both cases the initial specific activity is very close to the 8 hour quasi-equilibrium specific activity. Consequently it is necessary to assume that part of the cellular component was initially more radioactive than the remainder. If this were not the case, there would be no reservoir of high specific activity to drive the plasma specific activity up transiently before its return to the quasi-equilibrium position.

The presence of these characteristic humps has been confirmed in five other cases in subsequent experiments. Humps have also been observed in three experiments with cells refrigerated overnight between pre-incubation and incubation.

Another type of experiment was carried out in which the cells were washed once with buffer before the incubation period. This washing required approxi-

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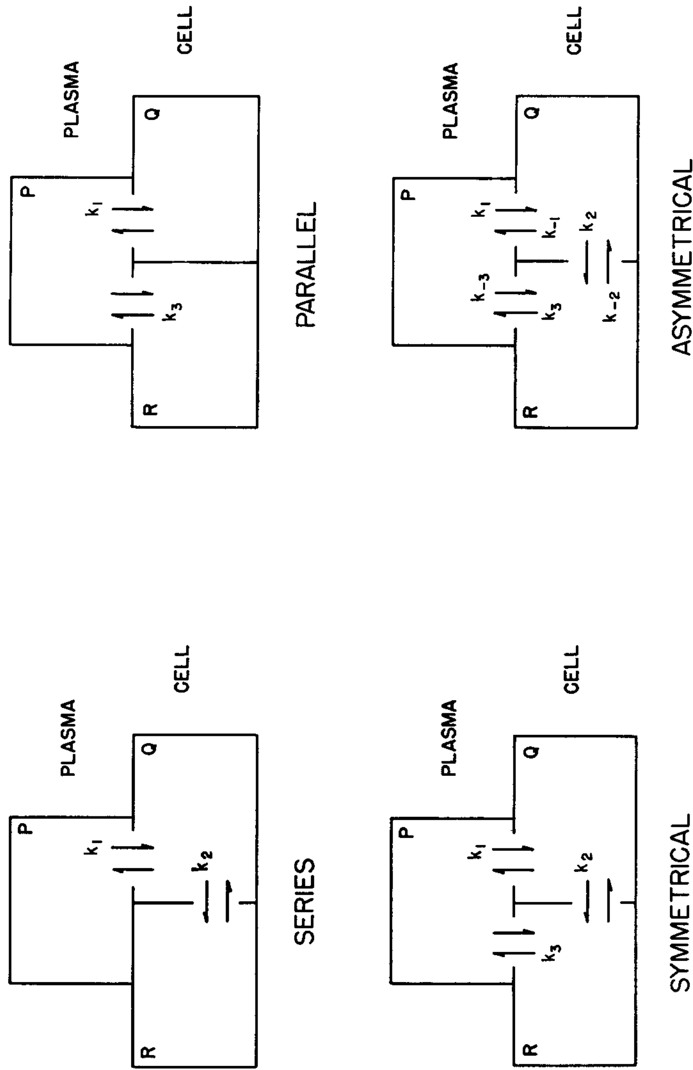

FIG. 3. Arrangements of compartments for theoretical analysis of data.

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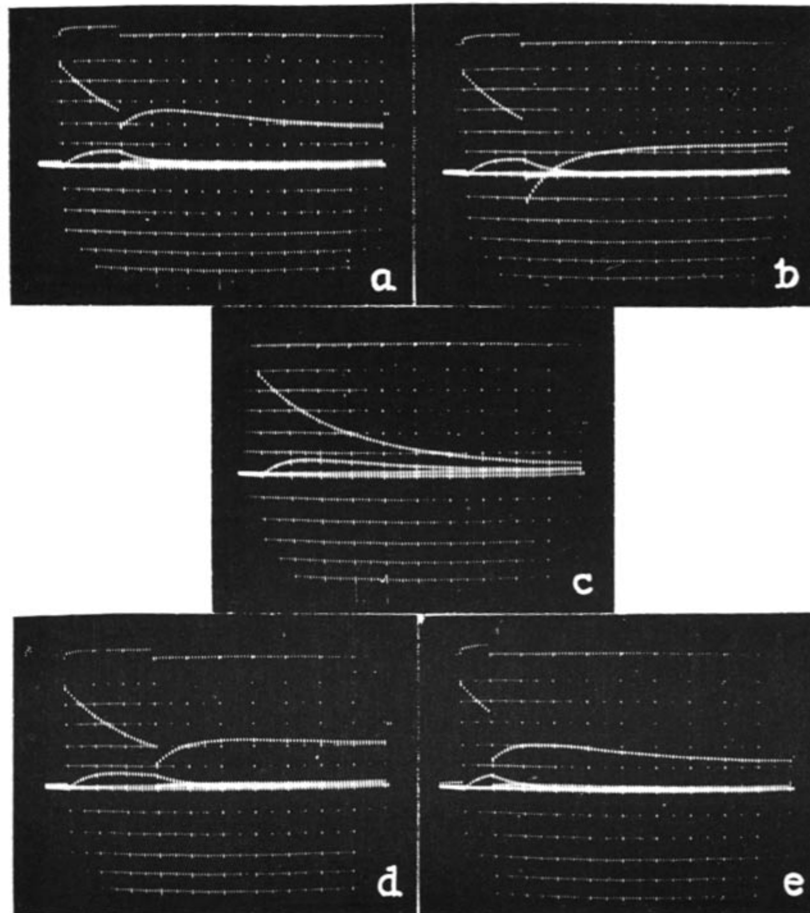

FIG. 4. Analogue computer curves showing the specific activity ($\bar{p}$, $\bar{q}$, and $\bar{r}$) as the ordinate, against time in hours as the abscissa. The scale for the ordinate is arbitrary, a uniform value having been chosen to represent the initial plasma specific activity, $\bar{p}$, in each case. The scale for the abscissa covers a space of 10 hours, each large division representing 1 hour. Time is measured from the beginning of the pre-incubation period.

The central figure (4 *c*) is an analogue to the normal situation when K^{42} is added to the plasma at zero time and the incubation is allowed to proceed without interruption. The top curve represents $\bar{p}$, (plasma specific activity) which is seen to fall off slowly much as it would in a two compartment system. The middle curve representing $\bar{q}$ (the small intracellular compartment specific activity) is seen to reach a maximum at 1 to 2 hours and then to fall off until it joins r (the barely discernible curve representing the major part of the intracellular K) at 6 to 7 hours.

FIG. 4 *a* represents the case in which the plasma is removed from the cells following

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mately 30 minutes in addition to the 30 minutes that usually elapsed between pre-incubation and incubation. The results of this experiment are given in Fig. 2. In this case too, it was not possible to obtain characteristic K^{42} fluxes when these were calculated according to the equations previously given (1).

Theoretical Analysis of Data.—

Three separate arrangements of a three compartment system were studied in trying to obtain a satisfactory theory. They are shown schematically in Fig. 3 in which they are described as series, parallel, and symmetrical. Initially the greatest attention was focussed on the series case, because this seemed the simplest system that the cell might use to transport K. It is possible to obtain a solution to the equations for this case using methods similar to those that have already been described (5). The equation giving the time course of the plasma radioactivity is of the form:

$$p = A_1 e^{\lambda_1 t} + A_2 e^{\lambda_2 t} + A_3 \quad (1)$$

in which p represents the concentration of radioactivity (K^{42}) of the plasma in counts/ml., t represents time, and the λ's and the A's are constants. The λ's, which are independent of boundary conditions, are complicated but soluble functions of the compartment sizes and of the fluxes between the compartments. A_3 is the equilibrium concentration of radioactivity in the plasma. A_1 and A_2, however, are dependent upon the boundary conditions, and hence no general relationship exists between them and the fluxes. If the initial conditions are such that either all or none of the radioactivity is in the plasma at zero time, expressions relating A_1 and A_2 to the fluxes and compartment sizes may be found. However, in the most interesting case, that shown in Fig. 1,

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2 hours of pre-incubation. As a consequence the plasma specific activity, $\bar{p}$, drops sharply to a very low value at 2 hours and cannot be distinguished thereafter from the abscissa. For display purposes, $\bar{p}$ has been multiplied by ten, and 10 $\bar{p}$ is displayed as the top curve in Fig. 4 *a* in the period between 2 and 10 hours. The curve for 10 $\bar{p}$ is not visible during the period 0 to 2 hours, as it is off the oscilloscope scale. Since the removal of K^{42} from the plasma at 2 hours does not affect the amount of intracellular K^{42}, there is no shift in the *level* of $\bar{q}$, the middle curve showing the small intracellular compartment specific activity; the abrupt change in *slope* in $\bar{q}$ is occasioned by the disappearance of the reservoir of high specific activity, $\bar{p}$, which had previously supplied K^{42} to $\bar{q}$. Since $\bar{q}$ now has the highest specific activity of the three compartments, it decays by loss of K^{42} to both $\bar{p}$ and $\bar{r}$, thus occasioning the characteristic hump observed in the plasma specific activity ($\bar{p}$). The curve for $\bar{r}$ lies so close to the abscissa that it cannot be seen.

An identical procedure has been followed for displaying $\bar{p}$ in Figs. 4 *b*, *d*, and *e*, which represent: *b*, cells with 2 hour pre-incubation washed before incubation; *d*, cells with 3 hour pre-incubation; *e*, cells with 1 hour pre-incubation.

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in which K^{42} is present in each compartment at zero time, we have been unable to find an explicit relationship between A_1 and A_2 and the fluxes and the compartment sizes.

Under these conditions it is possible to adopt graphical methods of solution and fit the exponentials by hand. The process is extremely tedious; furthermore, it is difficult to have much confidence in the results, since the labor involved in determining whether the fit is unique is prohibitive. Consequently we have had recourse to an analogue computer[1] to study the problem. In the analogue computer used in these studies (George A. Philbrick Researches, Inc., computer), it is possible to display the radioactivity in each compartment simultaneously on a single cathode ray tube together with an electronic grid showing the coordinates. Furthermore, a modification was made in the computer so that in the analogue system, as in the real one, some or all of the radioactivity could be withdrawn from the plasma at the end of the pre-incubation time. Before this modification was made it had been necessary to treat the specific activities of the two intracellular compartments at the start of incubation as variables, adjusting them with no exact reference to the fluxes and compartment sizes. With the modification, however, the initial incubation conditions were determined quantitatively and automatically by the fluxes and compartment sizes. Thus two further variables were effectively removed. Fig. 4 *a* shows the computer solution for the case of 2 hour pre-incubation.

The equations which follow have been developed for the symmetrical three compartment case. Since this is a general case, it is possible to derive the equations for either the series or parallel case from it by setting either k_3 or k_2 equal to zero, as can be seen from Fig. 3.

$$\alpha d\bar{p}/dt = k_3(\bar{r} - \bar{p}) - k_1(\bar{p} - \bar{q}) \quad (2\ a)$$

$$\beta d\bar{q}/dt = k_1(\bar{p} - \bar{q}) - k_2(\bar{q} - \bar{r}) \quad (2\ b)$$

$$\gamma d\bar{r}/dt = k_2(\bar{q} - \bar{r}) - k_3(\bar{r} - \bar{p}) \quad (2\ c)$$

$\bar{p}$, $\bar{q}$, and $\bar{r}$ represent the specific activities (counts per milliequivalent of K) in compartments P, Q, and R respectively. α, β, and γ represent the K content

[1] A direct analogue computer forms an electrical analogue to the three compartments in the blood. Each of the three compartments is represented by a separate capacitance and the flux between compartments is represented by variable resistances. The initial introduction of K^{42} is simulated by putting a charge on one or more of the capacitances; the subsequent history of the system is followed by observation of the potential of each of the three capacitances. Modern analogue computers such as we have used form indirect analogues rather than direct ones, but the principles are similar. A general discussion of analogue computers and other calculating machines may be found in Calculating Instruments and Machines by D. R. Hartree, Urbana, University of Illinois Press, 1949.

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(milliequivalents per liter "blood") in compartments P, Q, and R respectively. k_1 represents the flux (milliequivalents of K per liter "blood" hour) between compartments P and Q; k_2 the flux between Q and R; and k_3 the flux between R and P. Since α, the K content in the plasma, is considered a constant, it is possible to divide the set of equations by α, thus removing this constant from further consideration in the system, as follows:

$$d\bar{p}/dt = k_3'(\bar{r} - \bar{p}) - k_1'(\bar{p} - \bar{q}) \quad (3\ a)$$

$$\beta' d\bar{q}/dt = k_1'(\bar{p} - \bar{q}) - k_2'(\bar{q} - \bar{r}) \quad (3\ b)$$

$$\gamma' d\bar{r}/dt = k_2'(\bar{q} - \bar{r}) - k_3'(\bar{r} - \bar{p}) \quad (3\ c)$$

In Equations 3, the primed symbol connotes division by α, thus $k_3' = k_3/\alpha$, etc. Since α, β' and γ' are treated as constants, we are assuming that the system is in the steady state, and that the K content of each compartment is invariant with time. Although it has been shown by Streeten and Solomon (6) that there is a small net gain of K by the cells during incubation, this effect is small and has been neglected. The other usual assumptions inherent in compartmental analysis of this kind have been previously discussed (1) and are made here. Thus deviations from ideal behavior, as for example mixing which is not instantaneously complete on our time scale, are not considered in the equations above.

There are two important advantages in setting up the set of equations using specific activities ($\bar{p}$, $\bar{q}$, and $\bar{r}$) as variables rather than counts per milliliter (p, q, and r). First, the equilibrium condition for K^{42} distribution is represented on the cathode ray screen as the common asymptote of the curves for, $\bar{p}$, $\bar{q}$, and $\bar{r}$. Thus it is possible to check that the analogue is behaving in a steady state manner merely by observing that all three curves approach a common constant asymptote. Second, influx and efflux between any pair of compartments in the series and parallel cases are given by a single constant, minimizing the number of constants and thus insuring that the steady state condition obtains. The restriction imposed on the fluxes in the symmetrical state by these equations will be discussed later.

There are five constants in the set of Equations 3. However, a reciprocal relationship exists between β' and γ'.

$$\beta' + \gamma' = (\text{cellular K content})/\alpha \quad (4)$$

Consequently there remain four independently adjustable constants in the symmetrical case.

In addition to the two initial conditions represented by Figs. 1 and 2, it is also necessary that the solution fit the case when all the radioactivity is in the plasma at the start of the experiment since the system must respond in the predicted fashion regardless of the compartment in which the tracer is initially placed. Therefore, after the computer was set up to serve as an analogue to

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Equations 3, the constants representing k_1', k_2', and k_3', and the β', γ' relationship were adjusted to match the three sets of initial conditions simultaneously (a: $\bar{p}_0$, $\bar{q}_0$ and $\bar{r}_0$ unknown; b: $\bar{p}_0 \cong 0$, $\bar{q}_0$ and $\bar{r}_0$ unknown; c: $\bar{p}_0 = 1$, $\bar{q}_0 = \bar{r}_0 = 0$). Initially, a good deal of effort was spent trying to match the curves for the series case with three adjustable constants (k_3 equal to zero). However, the solutions obtained in this case were not satisfactory, for sets of constants which gave a moderate fit for the case of 2 hour pre-incubation gave unsatisfactory results when the pre-incubation time was increased or decreased. Although less time was spent on the parallel case with three adjustable constants (k_2 equal to zero), it also appeared that no satisfactory solution could be found in this case.

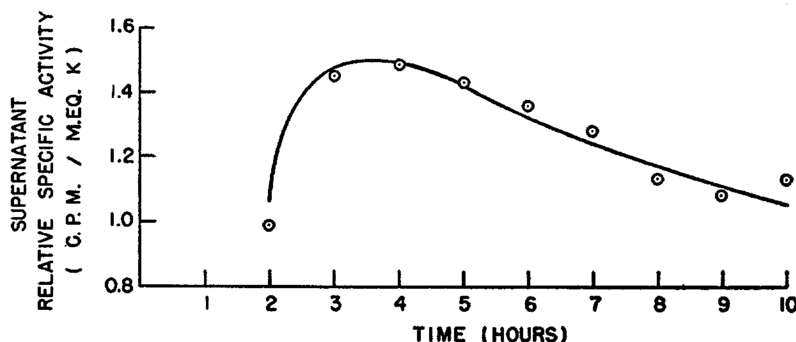

FIG. 5. Fit of the analogue computer curve to the data in the case in which the cells were not washed between pre-incubation and incubation. The points are experimental data from the 2 hour case shown in Fig. 1. The curve has been scaled from the analogue computer solution shown in Fig. 4 a, and has been normalized to the data at 4.0 hours. The time scale starts at the beginning of the pre-incubation period.

Fig. 5 shows the fit obtained for the two hour pre-incubation (Experiment 12 a, Fig. 1) using the full set of constants required for the symmetrical case. The analogue computer solution is given in Fig. 4 a. In Fig. 5 the computer curve has been normalized to the experimental data at 4.0 hours.

Fig. 6 shows the fit obtained for Experiment 16 a (2 hour pre-incubation, Fig. 2), with the analogue computer solution shown in Fig. 4 b. This is perhaps the least satisfactory fit in the set, which may be due to the loss of radioactivity from the cells during the additional 30 minute period of washing. As can be seen from Fig. 4 b, an additional delay of 0.6 hour was simulated in the analogue by washing out slightly more radioactivity than remained in the plasma, so that the curve for $\bar{p}$ drops below the axis for about 36 minutes. Such "negative" radioactivity cannot exist, and consequently the model may be inaccurate during the early part of the incubation. To obtain the fit shown in Fig. 6, the analogue computer curve has been shifted so that the origin (where $\bar{p}$ crosses

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the axis) is at 2.35 hours, and the asymptote is normalized to a relative specific activity of 1.0. When the constants are adjusted so that the fit is best for the other two initial conditions, it becomes difficult to obtain a good fit for this initial condition. Consequently the difference between the experimental points and the computer curve given in Fig. 6 may well represent a real difference between the model and the true experimental solution. Such a difference could probably be removed in a system containing another adjustable constant; however, at the present state of our knowledge it does not seem that the addition of another constant can be justified.

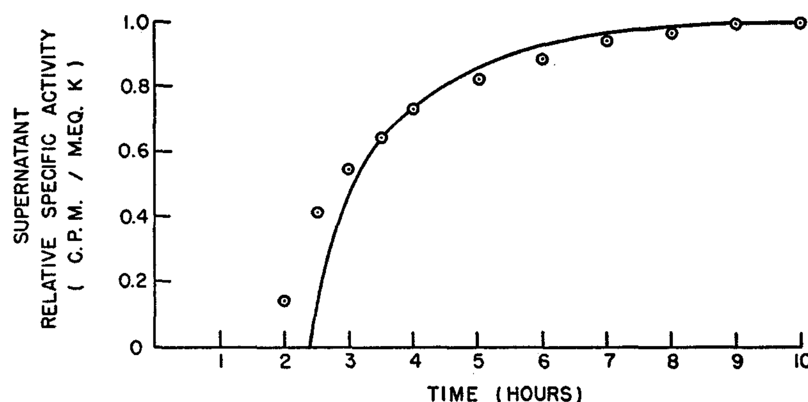

FIG. 6. Fit of the analogue computer curve to the data in the case in which the cells were washed between pre-incubation and incubation. The points are experimental data from the 2 hour case shown in Fig. 2. The curve which has been scaled from the analogue computer solution shown in Fig. 4 *b* has been shifted so that the origin is at 2.35 hours and has been normalized to the data at 10.0 hours. The time scale starts at the beginning of the pre-incubation period.

Fig. 7 shows the fit obtained when all the radioactivity is initially in the plasma, which is not subsequently removed from the cells. The analogue computer solution is shown in Fig. 4 *c*. The points in Fig. 7 are not derived from an experiment, but represent the theoretical behavior to be expected in a model two compartment system (6). The model system chosen is one with an influx and an efflux of 1.95 m.eq./liter cells hour, in a system with a hematocrit reading of 0.473, a plasma K concentration of 5.0 m.eq./liter plasma (the mean of Experiments 12 through 16 at 37°C), and a cellular K concentration of 98.0 m.eq./liter cells. The analogue computer curve has been normalized to the theoretical points at 2.0 hours.

The computer solutions for cases with 1 and 3 hour pre-incubation are shown in Fig. 4, *d* and *e*. They may be compared with the experimental data shown in Fig. 1 for these cases.

The constants obtained from the computer solution are given in Fig. 8. The

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hematocrit reading is taken as 0.473. The K content of the various compartments is given in milliequivalents of K per liter blood, and it can be seen that the third compartment, which comprises 2.35 m.eq. K/liter blood, is almost

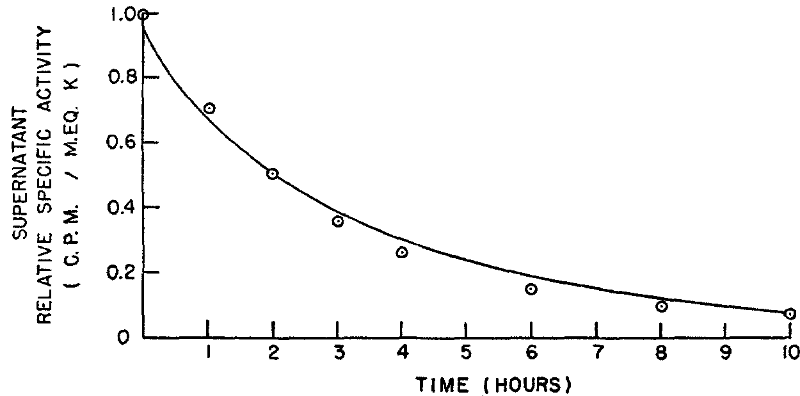

FIG. 7. Fit of the analogue computer to theoretical points for the normal influx case. The points have been calculated for a representative experiment as described in the text. The curve has been scaled from the analogue computer solution shown in Fig. 4 *c*, and has been normalized to the data at 2.0 hours.

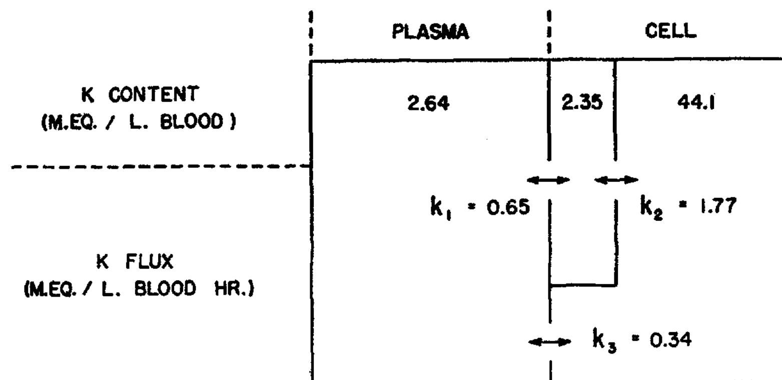

FIG. 8. Red cell compartment system using constants derived from the analogue computer solution.

as large as the plasma compartment (2.64 m.eq./liter blood or 5.0 m.eq./liter plasma) though it represents only about 5 per cent of the total red cell K (46.5 m.eq./liter blood or 98.0 m.eq./liter cells). The influx in milliequivalents of K per liter blood hour is 0.65 between the plasma and the fast third compartment, 1.77 between the fast compartment and the rest of the red cell, and 0.34 be-

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tween the inner slow compartment and the plasma. The total efflux from the plasma to both other compartments as shown in Fig. 8 is 0.99 m.eq./liter blood hour which would correspond to a flux of 2.09 m.eq./liter cells hour, in good agreement with the typical fluxes observed (6) (normal influx at 5.0 m.eq./liter plasma = 2.13 m.eq./liter cells hour; normal efflux = 1.95 m.eq./liter cells hour).

The Effect of White Cells.—

The swabbing procedure, carried out between pre-incubation and incubation removed the majority of the white cells, leaving behind less than 2000 white cells/mm.^{3} blood. In order to make sure that these white cells did not constitute the third compartment, measurements were made of white cell K content and K influx on samples of separated white cells obtained from Dr. J. L. Tullis of the Harvard University Laboratory of Biophysical Chemistry. Fig. 9 shows the K influx to a sample of separated white cells at 37°C. In this experiment the initial cell population was 14,000 white cells/mm.^{3} blood (92 per cent viable as measured by ameboid motility) and 36,000 red cells/mm.^{3} blood. At the close of the experiment, which lasted for 3 hours, 89 per cent of the white cells were still viable. Since the white cells occupied only about 0.5 per cent of the total volume, and the cellular K^{42} counts/minute were obtained by difference, the scatter of the points is not unexpected. All samples were determined in triplicate.

The K influx may be estimated as 89 m.eq./liter white cells hour using the usual equations given in reference (1). When normalized to a cell population of 7400 white cells/mm.^{3} blood (Standard Values in Blood (7)), this flux becomes 0.26 m.eq./liter blood hour and drops further to 0.07 m.eq./liter blood hour when the cell population is reduced to 2000 cells/mm.^{3} blood. It should be pointed out that these values were obtained on white cells that were not refrigerated during collection and were pre-incubated at 37°C. for 33 minutes before addition of tracer. White cells lose K very rapidly during refrigeration, and regain it equally rapidly upon incubation at 37°C.

The K concentration was measured in two other preparations of separated white cells. Since these values were obtained by difference between measurements of the white cell suspension, which was very dilute, and the supernatant, their accuracy is not great. Further, it is necessary in the calculations to make an estimate of white cell volume which may itself be in error. The estimate, based on a population of 62 per cent granulocytes, 4 per cent monocytes, and 34 per cent lymphocytes (7), and using the figures of Tivey, Li, and Osgood (8) for the volumes of leukemic cells (466 μ^3, granulocytes; 472 μ^3, monocytes; and 245 μ^3, lymphocytes) leads to a mean white cell volume of 391 μ^3. This may be compared with the red cell volume of 84 μ^3 given by Ponder (9). Using these values the white cell (K) in these two preparations was found to be

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138 and 153 m.eq. K/liter white cells, giving an average of 146 m.eq. K/liter white cells. This value is in disagreement with the results obtained from micro-incineration studies of human leucocytes by Kruszynski (10) who suggests that the Na and K content of these cells is "slight." The contribution of the white cell K to that of the blood is quite small, using the value of 146 m.eq. K/liter white cell, since it amounts to only 0.77 m.eq. K/liter blood

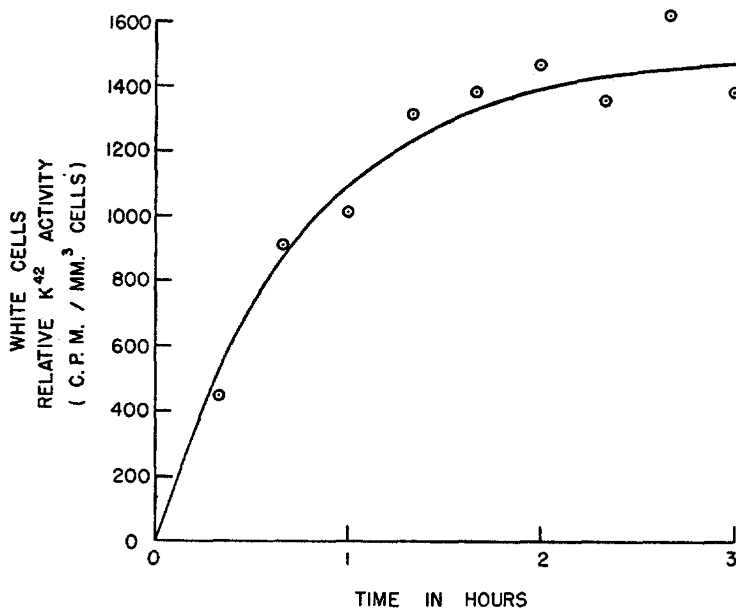

FIG. 9. Uptake of radioactive K by separated white cells. The points represent experimental results (relative K counts per minute cubic millimeters cells) and the curve is the theoretical one for which the figures on flux have been calculated according to the usual two compartment equation as described in the text. The initial cell population consisted of 14,000 white cells/mm.³ blood (92 per cent viable) and 36,000 red cells/mm.³ blood.

for a white cell population of 7400 white cells/mm.³, and 0.21 m.eq. K/liter blood for a population of 2000.

From the values shown in Fig. 8, it can be seen that the white cell K flux and K content are too small by almost an order of magnitude to be responsible for the third compartment. Furthermore, under our conditions the white cells that remain after swabbing form clumps which do not, in most cases, break up in the subsequent shaking operations. These cells are probably no longer viable, since as Tullis (11) has pointed out, agglutinated cells are dead. (There was no evidence of any red cell clumping.) Further confirmation of the unimportance of white cells in these studies is afforded by an experiment in which

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the white cells were removed twice, once before pre-incubation, and later between pre-incubation and incubation in our usual manner. Only 600 white cells/mm.[3] remained after this double scrubbing, yet the plasma radioactivity still showed its characteristic hump.

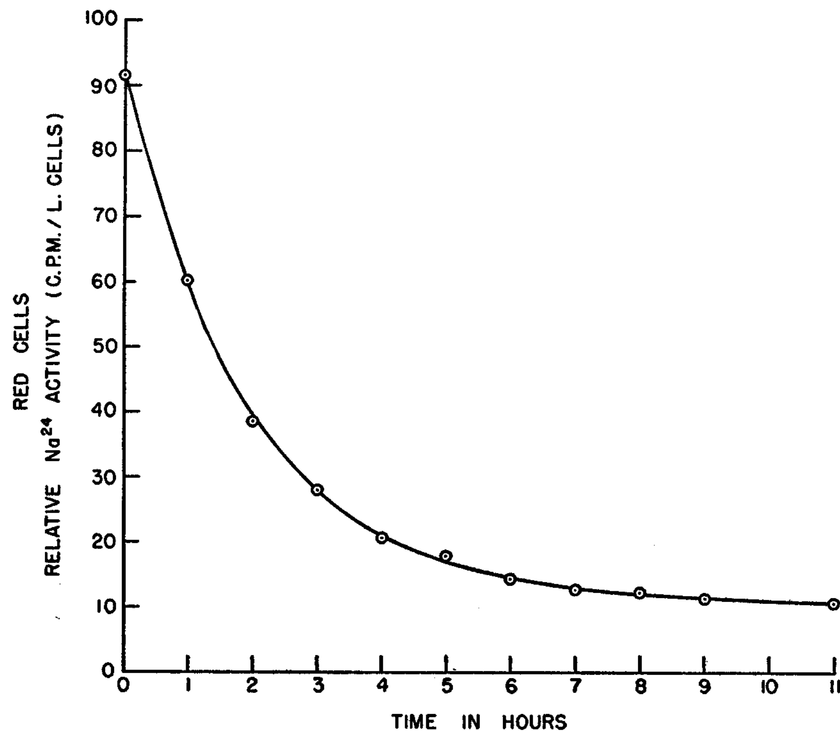

FIG. 10. Efflux of Na^{24} from human red cells suspended in a mixture of plasma and buffer. Following pre-incubation with Na^{24} Cl for 2 hours, the cells were resuspended in a mixture of plasma and buffer for an 11 hour period. The points are the measured cell Na^{24} radioactivities (relative Na^{24} counts per minute liter cells) and the curve is the theoretical curve according to the usual two compartment equation given in the text (Equation 5).

Na Efflux from Red Cells.—

In an effort to determine whether the efflux of Na from red cells also involved a third compartment of the same dimensions and with the same time scale as K, an experiment was carried out with 1 and 2 hour pre-incubations using radioactive Na instead of K. In this case the efflux was studied in the cellular fraction rather than in the plasma since, as has been shown (1), the cells provide a more sensitive index for Na flux than plasma due to the low cellular Na content. No evidence of a third compartment appeared; indeed the

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relative specific activity curves of cellular Na were superposable for the 1 hour and 2 hour pre-incubations. Fig. 10 shows the experimental points (relative counts per minute milliliter of cells) for the case of the 2 hour incubation, together with the theoretical curve derived from the data on the basis of a 2 compartment system, according to the equation below derived from Equation 9 of reference (1) to fit the specific case considered.

$$q = (q_0 - q_\infty)e^{Tt} + q_\infty \tag{5}$$

in which q = cell counts (relative counts per minute milliliter of cells). The subscripts 0 and ∞ refer to q at initial time and infinite time respectively. T is the slope of the function $(q/q_\infty - 1)$ when plotted on logarithmic scale against time t. [2]

III

DISCUSSION

Two important questions must be raised concerning the model of K transport in human erythrocytes given in Fig. 8. First, is the model unique; second, is the model complete? Since there are four smoothly adjustable constants involved in the set of Equations 3, there are obviously an infinite number of possible combinations of these constants, making it imposible to examine all combinations. However, the conditions which the solution had to meet are relatively rigorous, and a good deal of computing time was spent in trying to adjust the constants so that the conditions were met. Though it was relatively easy to satisfy the case with initial conditions shown in Fig. 5, it was quite difficult to satisfy these conditions together with the initial conditions shown in Figs. 6 and 7. It would seem therefore that the number of possible solutions with this model is limited.

It is more difficult to state whether the model is complete. In both the series case and the parallel case, the steady state condition can only be met if the influx and efflux into each compartment are equal. In other words if k_1 represents the flux from compartment P to compartment Q, and k_{-1} the flux from Q to P as shown in the asymmetrical case in Fig. 3, the steady state condition for the series and parallel cases requires that $k_n = k_{-n}$ for all values of n. In the asymmetrical case, this restriction does not hold and we could maintain the steady state, if the following relations among the constants held:

$$\begin{aligned} k_1 - k_{-1} &= k_3 - k_{-3} \\ k_2 - k_{-2} &= k_3 - k_{-3} \end{aligned} \tag{6}$$

[2] In agreement with previous observations (1) the exchange at 11 hours was incomplete, representing the exchange of 80 per cent of the intracellular Na. This previously observed "slow" compartment for Na is so slow that it is probably not related to the "fast" K compartment discussed here.

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Although this would provide six adjustable constants among the k's, the two restrictions imposed by Equation 6 would leave only four degrees of freedom, and thus provide an increase of one over the number of independent adjustable constants in the symmetrical system. We can conclude that the symmetrical model is not necessarily complete, but that it represents the system in a compact fashion with a minimum of constants.

The presence of more than one intracellular K compartment is supported by Ponder's (12) observation of a fast component amounting to 15 to 20 per cent of the red cell K, in studies of the efflux of K from red cells suspended in isotonic NaCl at 37°C. Other workers (Mudge (13); Cowie, Roberts, and Roberts (14); Stanbury and Mudge (15)) have also reported the presence of "bound" K in a variety of other living systems.

Nonetheless, the nature of the third compartment is completely unspecified. It may be a region of space, as for example the red cell plasma membrane itself, or it may be a chemical combination of the K with another molecule. In this connection, it is interesting to note that no similar third compartment has been found for Na which supports the observation (1) that different mechanisms are involved in Na and K transport. It should be pointed out that the more complex asymmetrical model system in which k_1 is unequal to k_{-1} is adapted to the unidirectional round trip transport of a single ion, and is indeed a close analogue to the working hypothesis previously put forward by us (1). It would be tempting to link the third compartment with the lipid-soluble lipid–non-dissociable carrier which has been proposed for K transport (1). However, the evidence at present is insufficient, and this must be regarded as a speculative association only.

From what has been said above it is clear that the model proposed represents a working hypothesis only, though a more detailed one than has hitherto been available. As such, it must be tested by further experiment and modified and expanded as experimental data accrue.

This work has been supported in part by the Atomic Energy Commission. It is a pleasure to acknowledge the instruction and advice in the use of the analogue computer provided by Mr. George A. Philbrick, Dr. Henry M. Paynter, and Mr. Daniel H. Sheingold, and to express our thanks to Dr. J. L. Tullis for the white cell preparations we have used, and to Miss Jean McCormick and Miss Dorothy Driscoll for their help in counting and preparing the isotopes.

SUMMARY

Whole human blood is incubated for periods of ½ to 3 hours with K^{42} at 37°C. At the close of this period, called pre-incubation, the plasma is removed from the cells and the cells, now become radioactive, are again incubated in a mixture of plasma and buffer for periods of up to 10 additional hours. The time course of the K^{42} activity of the incubating medium is followed. Char-

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acteristically, after 2 hours of pre-incubation, the activity in the medium rises to a peak about 1 and ½ hours after resuspension, and then falls slowly until at 10 hours it is very close to its initial value at the beginning of the resuspension interval. This transient rise in K^{42} activity in the medium is taken to indicate that the red cell does not consist of a single uniform K compartment, but contains at least two compartments. Thus one cellular compartment contains a reservoir of high specific activity K which provides the specific activity gradient necessary to drive the K^{42} content of the medium to its transient peak. Experiments with Na indicate that its behavior in this respect is unlike that of K.

The experimental data are matched to a simple model system which is capable of theoretical analysis with the aid of an analogue computer. The model system, whose characteristics agree fairly well with those observed experimentally on red cell suspensions, comprises two intracellular compartments, one containing 2.35 m.eq. K/liter blood, and the other 44.1 m.eq. K/liter blood. The plasma K content is 2.64 m.eq./liter blood. The flux between plasma and the smaller intracellular compartment is 0.65 m.eq. K/liter blood hour; that between the smaller and the larger intracellular compartment, 1.77 m.eq. K/liter blood hour; and that between the larger intracellular compartment and the plasma is 0.34 m.eq. K/liter blood hour.

BIBLIOGRAPHY

1. Solomon, A. K., *J. Gen. Physiol.*, 1952, **36,** 57.
2. Raker, J. W., Taylor, I. M., Weller, J. M., and Hastings, A. B., *J. Gen. Physiol.*, 1950, **33,** 691.
3. Sheppard, C. W., and Martin, W. R., *J. Gen. Physiol.*, 1950, **33,** 703.
4. Lefevre, P. G., *J. Gen. Physiol.*, 1948, **31,** 505.
5. Solomon, A. K., *in* Advances in Medical and Biological Physics, (J. H. Lawrence and C. A. Tobias, editors), New York, Academic Press, Inc., 1953, **3,** 65.
6. Streeten, D. H. P., and Solomon, A. K., *J. Gen. Physiol.*, 1954, **37,** 643.
7. Standard Values in Blood, (E. C. Albritton, editor) AF Technical Report No. 6039, United States Air Force, 1951.
8. Tivey, H., Li, J. G., and Osgood, E. E., *Blood*, 1951, **6,** 1013.
9. Ponder, E., Hemolysis and Related Phenomena, New York, Grune and Stratton, 1948, 14.
10. Kruszynski, J., *J. Physiol.*, 1950, **111,** 89.
11. Tullis, J. L., *in* Blood Cells and Plasma Proteins, (J. L. Tullis, editor), New York, Academic Press, 1953, 266.
12. Ponder, E., *J. Gen. Physiol.*, 1951, **34,** 359.
13. Mudge, G. H., *Am. J. Physiol.*, 1953, **173,** 511.
14. Cowie, D. B., Roberts, R. B., and Roberts, I. Z., *J. Cell. and Comp. Physiol.*, 1949, **34,** 243.
15. Stanbury, S. W., and Mudge, G. H., *Proc. Soc. Exp. Biol. and Med.*, 1953, **82,** 675.

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