The Entry of Sodium into Human Red Blood Cells in Vivo

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ABSTRACT The kinetics of sodium movement into human red blood cells has been studied in vivo with ^{24}Na. When human serum albumin-^{131}I is used to measure the percentage of plasma trapped in the packed red blood cells after centrifugation, approximately 30% of red blood cell sodium is found to equilibrate immediately with plasma. It is concluded that this immediately exchangeable compartment of red blood cell sodium is an experimental artefact, associated with the use of labeled albumin for measuring plasma trapping. This immediately exchangeable fraction disappears when sucrose-^{14}C is used to measure plasma trapping. The experimental results were examined by compartmental analysis, using an analogue computer. The results obtained, when plasma trapping was measured with sucrose-^{14}C could be simulated by the use of models containing two compartments, arranged in series or in parallel. The errors of the techniques used and the possible physical basis for the results are discussed.

In 1955 Gold and Solomon (1) reported the results of experiments, in which they studied the fall of plasma and the rise of red blood cell specific activity in blood samples obtained serially following intravenous injection of radioactive sodium into normal human subjects. In this paper we describe the results of similar studies and discuss some of the experimental and analytical problems encountered. Outstanding among these is the accurate measurement of sodium concentration in red blood cells.

When blood is centrifuged, some plasma remains trapped in the layer of packed cells. Since the concentration of sodium in plasma is approximately twenty times greater than that in red blood cells, an error of 1% in the estimate of plasma trapped with the cells will lead to an error of 20% in the calculated intracellular sodium concentration. When radioactive sodium is injected in-

travenously, the initial ratio of plasma to red blood cell specific activity is much greater than twenty, and accurate estimation of trapped plasma sodium is still more important. The results of studies of plasma sodium trapping and of the measurement of sodium concentration in human red blood cells are described elsewhere (2). In this paper only those aspects of the problem which have arisen from, or thrown light on, these in vivo experiments are discussed.

A detailed description of the methods used for measuring sodium, ^{24}Na, human serum albumin-^{131}I (^{131}IHSA), and sucrose-^{14}C is given elsewhere (2).

EXPERIMENTAL METHODS

Eight subjects, selected from patients in the wards, were studied. All volunteered to take part in the experiments and to receive radioactive material. None was suffering from a disease, or was receiving treatment, that was considered likely to influence sodium distribution.

Subjects were given a normal ward diet, supplemented with 6 g of salt daily for 5 days before the experiment. After an overnight fast radioactive sodium was injected intravenously at 8 a.m. The subjects remained in bed throughout the experiment, but ate normally after a delayed breakfast at 9:30 a.m. A high fluid intake was maintained throughout the day.

All radioactive material was obtained from the Radiochemical Center, Amersham. ^{24}Na was dispensed in 2 ml sterile isotonic saline by the Department of Physics, King's College Hospital. The dose was 3μc per kg body weight.

Serial blood samples were drawn from the arm not used for injection into plastic syringes containing ammonium heparin (30 units per ml plasma). 9 g samples of blood (5 g in the first experiment) were immediately weighed into cellulose nitrate tubes with an internal diameter of 11 mm which was assumed to be constant; these were centrifuged as soon as possible in a MSE hematocrit centrifuge, governed at 3000 ($\pm$30) RPM for 1 hr. RCF at the base of the tubes was 1460 *g*. The time from sampling to start of centrifugation varied, but was usually 5 to 10 min.

Plasma samples were transferred to beakers for measurement of sodium and ^{24}Na content. The centrifuge tubes containing the packed red blood cells and a layer of plasma were frozen in a CO_2-ethanol mixture and sectioned 0.5 to 1.0 mm below the buffy coat–red blood cell interface by an electric saw, in apparatus specially constructed by the Department of Physics, King's College Hospital. In experiment 5, the frozen red blood cell column was cut into upper and lower halves. ^{24}Na was counted in all samples; plasma and red blood cell sodium were estimated in six to twenty samples and a mean concentration used for all calculations.

In the first five experiments, ^{131}IHSA was used to measure plasma trapping. The material was dispensed by the Department of Physics after twice being passed through an anion-exchange resin (Amberlite IRA-400 Cl) within 12 hr of the start of the experiment; it was then stored at 4°C in a container with free resin, before and throughout the experiment. ^{131}IHSA was added in vitro to blood samples, taken before injection of ^{24}Na and serially afterwards, and mixed. One or two tubes were included in each centrifuge load. These samples were stored for a week after processing to allow ^{24}Na to decay, before counting ^{131}I. A mean value for ^{131}IHSA trapping was obtained

and used to estimate trapped plasma sodium. It was assumed, in the plasma-trapping corrections, that the weights of all red blood cell samples and therefore the heights of the packed cell columns assayed, were constant.

In experiments 6, 7, and 8, sucrose-^{14}C was used to estimate trapped plasma sodium. In experiments 6 and 7, the material was added to samples of blood, which were included in each centrifuge load. In experiment 8, the percentage of plasma trapped was obtained for each sample from a regression equation relating sucrose-^{14}C trapped to the weight of red blood cells sampled (2). The equation was $y = 2.8214 + 0.2398\ x$, where y was the percentage of trapped plasma sodium and x the weight in grams of red cells sampled.

Plasma specific activity and red blood cell specific activity were calculated for each blood sample. In experiments 3 to 8 calculations were performed by digital computer.

ANALYTICAL METHODS

The experimentally observed plasma specific activities were plotted on graph paper against time and the best possible smooth curve was drawn through them with conducting ink. A standard curve follower was used to generate a voltage proportional to plasma specific activity as an input to a PACE analogue computer. Attempts were then made to simulate the experimental red blood cell specific activity data.

Using compartmental analysis, the general model to describe the system is given by

$$R(t) = \sum_{j=1}^{j=N} AjRj(t) \tag{1}$$

where $R(t)$ is the measured red blood cell specific activity at time t, N is the number of compartments, Aj is the fraction of the total number of sodium ions in the jth compartment, and $Rj(t)$ is the specific activity of sodium in the jth compartment.

Equation 1 is analogous to equation 7 of Gold and Solomon (1), except that their term $\beta + \gamma$ has disappeared. In the present model these are by definition fractional parts and their sum is unity, i.e.

$$\sum_{j=1}^{j=N} A_j = 1 \tag{2}$$

Models with compartments in series and in parallel were tested.

For parallel models, the equations for the system are:

$$A_j \frac{dR_j}{dt} = K_j(P - R_j) \tag{3}$$

where P is the plasma specific activity and K_j is the flux constant between the plasma and the jth compartment.

For series models, the equations are:

$$A_1 \frac{dR_1}{dt} = K_1(P - R_1) - K_2(R_1 - R_2) \tag{4}$$

where K_1 represents the flux constant between plasma and the first compartment

$$A_j \frac{dR_j}{dt} = K_j(R_{j-1} - R_j) - K_{j+1}(R_j - R_{j+1}) \quad (5)$$

for $j = 2$ to $(N-1)$

TABLE I

TIME COURSE OF SPECIFIC ACTIVITY OF PLASMA AND RED BLOOD CELLS AFTER INTRAVENOUS INJECTION OF ^{24}Na

Activities* expressed as $\mu c \times 10^{-2}$ ^{24}Na/meq ^{23}Na. Plasma trapping measured with ^{131}IHSA. Additional plasma activities were obtained but are not listed. In experiments 3 and 4 duplicate blood samples were taken at each sampling time. Means and standard deviations are given for all multiple samples. For explanation of A_1 see text.

Experiment 1 Subject D $A_1 = 0.25$ Mean trapped plasma = 1.427 ± 0.056 %			Experiment 2 Subject Ch. $A_1 = 0.24$ Mean trapped plasma = 1.471 ± 0.073 %			Experiment 3 Subject R $A_1 = 0.25$ Mean trapped plasma = 1.589 ± 0.113 %			Experiment 4 Subject B $A_1 = 0.33$ Mean trapped plasma = 1.450 ± 0.085 %		
	Specific activity			Specific activity			Specific activity			Specific activity	
Time	Plasma	RBC	Time	Plasma	RBC	Time	Plasma	RBC	Time	Plasma	RBC
hr			*hr*			*hr*			*hr*		
0.03	7.57	1.96	0.02	4.02	1.20	0.02	19.57±0.46	4.23±0.32	0.08	7.05±0.29	2.47±0.10
0.13	5.77	2.10	0.10	2.90	1.05	0.08	10.51±0.02	2.58±0.19	0.28	6.25±0.03	2.66±0.11
0.20	5.29	2.20	0.17	2.52	0.99	0.17	8.70±0.01	2.43±0.04	0.48	5.91±0.01	2.76±0.24
0.30	5.73	2.29	0.25	2.39	0.90	0.25	8.04±0.15	2.50±0.28	0.70	5.50±0.17	2.91±0.17
0.37	5.37	2.39	0.35	2.30	0.99	0.33	7.42±0.03	2.56±0.03	0.88	5.30±0.02	3.18±0.37
0.50	5.26	2.39	0.50	2.09	1.14	0.50	6.99±0.06	2.55±0.07	1.08	5.35±0.11	3.38±0.04
0.67	5.15	2.48	0.67	2.07	1.12	0.67	6.50±0.05	2.49±0.01	1.33	5.35±0.01	3.59±0.15
1.00	4.93	2.82	1.00	1.82	1.21	1.00	6.18±0.09	2.75±0.13	1.58	5.35±0.01	3.64±0.20
1.38	4.96	3.10	1.50	1.85	1.34	1.50	6.02±0.36	3.17±0.05	1.70	5.12±0.16	3.56±0.15
1.90	4.78	3.32	1.97	1.83	1.35	2.00	5.86±0.08	3.47	1.88	5.19±0.05	3.74±0.05
2.40	4.73	3.20	2.52	1.80	1.42	2.58	6.06±0.11	3.68±0.05	2.08	5.30±0.01	3.80±0.22
2.88	4.64	3.36	3.00	1.79	1.47	6.15	5.18±0.03	4.25±0.1b	2.28	5.16±0.01	3.73±0.16
4.77	4.51	3.50	4.47	1.76	1.51	7.50	5.17±0.02	4.26±0.11	2.48	5.17±0.11	3.77±0.20
8.08	4.10	3.58	9.30	1.63	1.48	9.00	5.08±0.15	4.28±0.09	2.70	5.18±0.10	4.05±0.0€
11.93	3.87	3.49	11.67	1.56	1.48	11.92	4.89±0.05	4.39±0.18	2.88	5.11±0.21	3.72±0.34
25.63	3.57	3.39	24.12	1.49	1.57	24.42	4.53±0.14	4.44±0.63	24.25	3.94±0.03	3.68±0.24
27.92	3.54	3.54	25.27	1.47	1.46	27.05	4.39±0.01	4.15±0.09			
						29.30	4.28±0.04	4.24±0.14			

* Specific activities were derived from a sample of ^{24}Na of known activity. As the activity of this sample w sometimes in doubt, no weight should be placed on activity differences found between experiments. With each experiment absolute comparisons are valid.

and

$$A_N \frac{dR_N}{dt} = K_N(R_{N-1} - R_N) \quad (6)$$

These equations are the same as those of Gold and Solomon, stated in more general terms.

RESULTS

Plasma and red blood cell specific activities and sampling times from experiments 1 to 4 are listed in Table I, and those from experiment 5 in Table II.

In these experiments in which ^{131}IHSA was used to measure plasma sodium trapped in the packed red blood cell layer, it can be noted that red blood cell specific activity in the first sample taken was appreciable. Furthermore, in experiments 2, 3, and 5, red blood cell specific activity fell for the first 15 or

TABLE II

TIME COURSE OF SPECIFIC ACTIVITY OF PLASMA AND RED BLOOD CELLS AFTER INTRAVENOUS INJECTION OF ^{24}Na

Activities expressed as $\mu c \times 10^{-2}$ ^{24}Na/meq ^{23}Na. Plasma trapping measured with ^{131}IHSA. The packed cell column was sectioned at approximately the midpoint and the two portions analyzed separately.

Time	Plasma	Upper section Plasma trapping: 2.282% ±0.222%	Lower section 1.511 ±0.204%
hr			
0.08	11.59	3.25	2.90
0.32	7.93	2.73	2.40
0.57	7.21	2.74	2.54
1.00	7.03	2.82	2.85
1.25	6.61	2.97	3.16
1.52	6.59	3.28	3.27
2.25	6.63	3.73	3.67
3.25	6.29	3.91	4.01
4.78	5.95	4.44	4.82
8.08	5.70	4.62	4.62
26.30	5.06	3.94	3.87

20 min after isotope injection. The complex nature of the changes in red blood cell specific activity is shown in Figs. 1 and 2, which illustrate graphically the results of experiments 2 and 5.

When these results were examined with the analogue computer, it was found that the experimental results could only be simulated (with both parallel and series models) by the inclusion of a red blood cell compartment of appreciable size with a time constant approximating zero. In other words an appreciable amount of red blood cell sodium appeared to be in immediate equilibrium with plasma sodium. The fractional volume of this immediately exchangeable compartment, A_1, could be determined approximately because

$$A_1 = t \xrightarrow{\text{Limit}} 0 \frac{R(t)}{P(t)}$$

since $R_1(0) = P(0)$ and $R_j(0) = 0$ for $J \neq 1$.

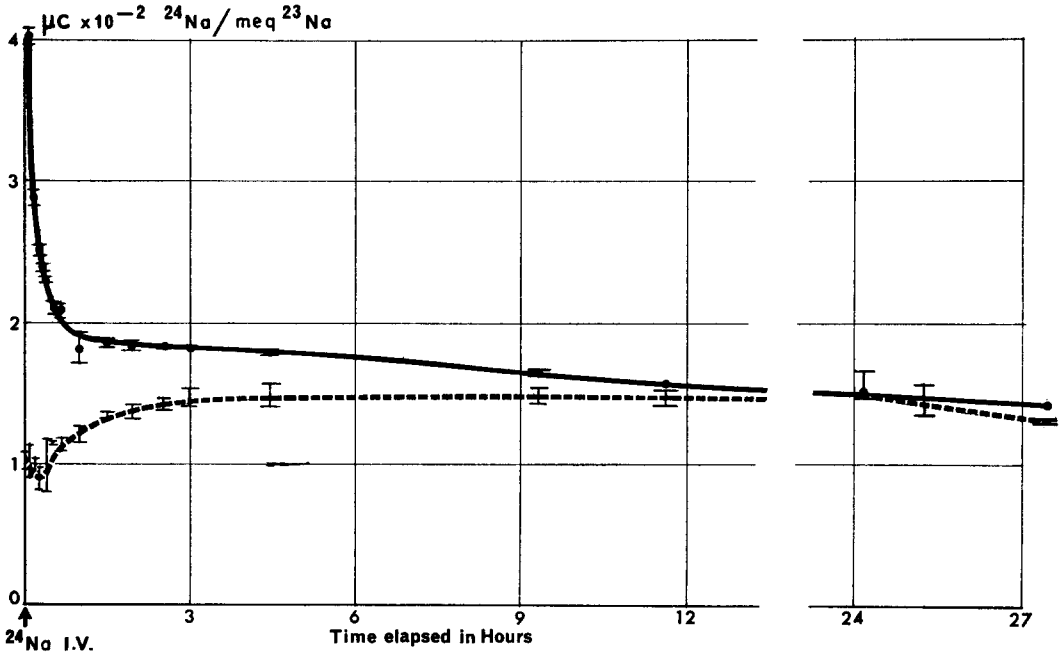

FIGURE 1. Specific activity of plasma (continuous line) and red blood cells (interrupted line) at intervals after injection of ^{24}Na intravenously. Experiment 2, subject Ch. The means and standard deviations of the paired observations are shown and the best curves fitted by eye.

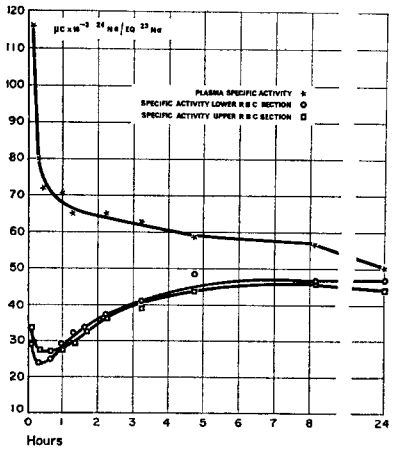

FIGURE 2. Specific activities of plasma and upper and lower portions of the red cell column at intervals after injection of ^{24}Na intravenously. Experiment 5, from results listed in Table II.

In other words the ratio of red blood cell specific activity to plasma specific activity at zero time equals the fractional volume of this compartment. The values for A_1 obtained in experiments 1 to 4 are given in Table I.

There are a number of possible explanations for the finding of an immediately exchangeable compartment which we discuss elsewhere (2). One

hypothesis was, however, tested in these experiments. If red blood cells are heterogeneous in respect of penetration by the sodium ion, and if this heterogeneity is a function of the age of the cells, the rise of specific activity of cells in the upper part of the packed layer should differ from that in the lower part of the layer, for Borun, Figueroa, and Perry (3) with centrifugal conditions similar to those used in these experiments, have shown that older cells are present at the bottom of the tube and younger cells at the top. Fig. 2 illustrates the results obtained in experiment 5, in which the packed cell column was cut

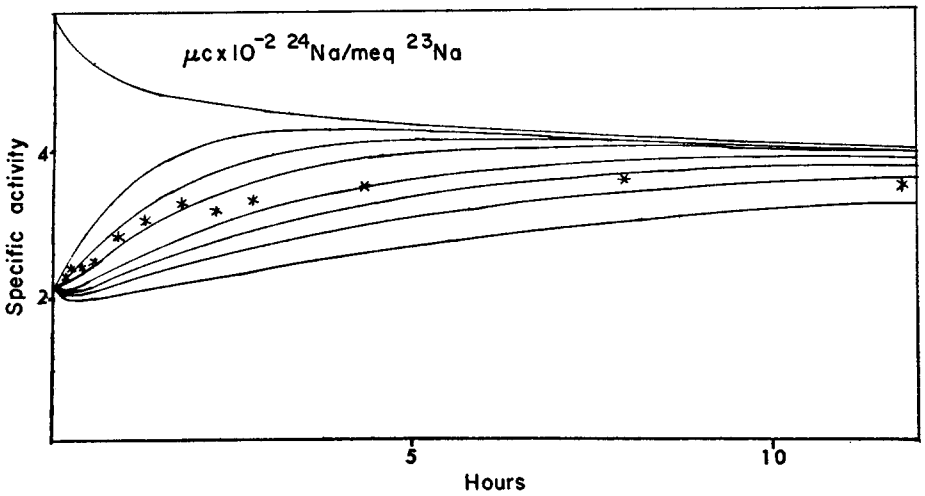

FIGURE 3. Tracing of the analogue computer plot for a simple single red blood cell compartment of fractional volume $A_2 = 1 - A_1$. Observed specific activities of red blood cells at intervals following injection of ^{24}Na are shown by stars. The top curve is the input plasma specific activity. The remaining curves show the solutions generated for $R(t)$ by the computer when K_2 was varied; none fits the experimental points. See text for explanation.

into two portions and the specific activity of each was calculated separately. Although there are differences between the curves for younger and older cells, these differences are small and are inadequate to explain the existence of the immediately exchangeable compartment in terms of this hypothesis.

It was concluded (2) that the immediately exchangeable compartment was in fact trapped plasma sodium, which was erroneously being estimated as red blood cell sodium, and that ^{131}IHSA is not a suitable indicator for plasma sodium trapping.

Further analysis of the results of these experiments with the analogue computer showed that no simple single compartment of fractional volume $A_2 = (1 - A_1)$, in series or in parallel with the immediately exchangeable compartment, could account satisfactorily for the experimental curves. This conclusion was proved by setting up on the computer the equations:—

$$R_1(t) = P(t)$$

$$A_2 \frac{dR_2}{dt} = K_2(P - R_2)$$

and

$$R(t) = A_1R_1(t) + (1-A_1)R_2(t)$$

TABLE III

TIME COURSE OF SPECIFIC ACTIVITY OF PLASMA AND RED BLOOD CELLS AFTER INTRAVENOUS INJECTION OF ^{24}Na

Activities expressed as $\mu c \times 10^{-2}$ $^{24}Na/meq$ ^{23}Na. Plasma trapping measured with sucrose-^{14}C. In experiment 7 duplicate blood samples were taken at each sampling time; for plasma, time is that of blood sampling; for RBC, time is that of starting centrifuge.

Experiment 6, Subject St. Mean trapped plasma 3.372 ± 0.160%			Experiment 7, Subject K Mean trapped plasma 3.092 ± 0.203%			
	Specific activity					
Time	Plasma	RBC	Time	Plasma activity	Time	RBC activity
hr			*hr*		*hr*	
0.03	5.01	0	0.08	21.32±0.92	0.18	1.52±0.08
0.08	5.02	0	0.27	15.57±0.51	0.37	2.10±0.61
0.25	5.00	0.23	0.60	13.17±0.09	0.68	3.34±0.10
0.58	4.23	0.69	1.00	11.89±0.17	1.13	3.71±0.80
0.85	3.83	1.08	1.67	11.74±0.50	1.78	5.03±0.35
1.33	3.67	1.25	2.17	11.62±0.17	2.25	5.47±0.43
1.58	3.66	1.32	2.83	11.03±0.25	3.02	6.22±0.03
1.92	3.65	1.63	3.78	10.68±0.15	3.88	6.59±0.08
2.30	3.53	1.75	4.88	10.53±0.36	5.00	6.96±0.02
2.63	3.44	1.90	8.08	10.09±0.08	8.18	7.38±0.24
3.18	3.42	2.07				
3.73	3.54	2.02				
4.12	3.45	2.13				
4.70	3.35	2.41				
7.42	3.20	2.48				

Solutions were generated for $R(t)$ for a range of values of K_2 sufficiently wide to embrace all the experimental points. Since K_2 was the only parameter which could be varied in the equation, the lack of agreement between any individual curve and all the experimental points showed conclusively that a simple single compartment cell theory is untenable. Fig. 3 illustrates this with the results of experiment 1.

When two compartment cell models were set up, either in series or in parallel with the immediately exchangeable compartment, good agreement with the experimental results was obtained. The fractional compartment sizes A_2 and A_3 were approximately equal to each other, and to A_1, the immediately exchangeable compartment.

In experiments 6, 7, and 8, a figure derived from sucrose-^{14}C trapping was used to estimate plasma sodium trapped with the packed red blood cells. The results of these experiments are listed in Tables III and IV. It can be noted that red blood cell specific activities approximate zero at zero time. The disappearance of the immediately exchangeable red cell compartment was confirmed when the experimental curves were simulated on the analogue computer, but the results were still inconsistent with a simple single compartment model for the red blood cell.

TABLE IV

TIME COURSE OF SPECIFIC ACTIVITY OF PLASMA AND RED BLOOD CELLS

Activities expressed as $\mu c \times 10^{-2}$ ^{24}Na/meq ^{23}Na.

Experiment 8. Subject Cr. Plasma trapping for each sample calculated from regression equation relating percentage sucrose-^{14}C trapped to RBC sample weight. For plasmas time given is that of blood sampling, for red blood cells that of starting centrifuge. The initial rise of plasma specific activity was due to some extravenous injection of ^{24}Na. Two samples taken at each time.

Time	Plasma activity	Time	RBC activity
hr		*hr*	
0.08	2.72±0.01	0.13	0.07±0.12
0.57	6.30±0.04	0.63	0.75±0.30
1.08	6.74±0.09	1.13	1.82±0.19
1.57	6.69±0.02	1.66	2.60±0.16
2.13	6.60±0.01	2.18	3.23±0.54
2.73	6.42±0.07	2.78	3.33±0.05
3.48	6.28±0.06	3.50	3.67±0.22
4.47	6.13±0.01	4.52	3.89±0.12
5.77	5.89±0.08	5.84	4.56±0.18
6.83	5.82±0.01	6.88	4.11±0.12
7.95	5.78±0.11	8.13	4.25±0.22
10.08	5.69±0.08	10.27	4.14±0.01
12.33	5.36±0.05	12.45	4.24±0.04
24.17	5.41±0.10	24.33	4.25±0.04
24.17	5.36±0.10	24.33	4.44±0.26

Two compartment models for the red blood cell were set up, with compartments both in series and in parallel, and good agreement was obtained with the experimental results. The subscripts f and s are used to describe these two compartments.

For both series and parallel models, the procedure used for determining the best fitting values for K_f, K_s, A_f, and A_s was the same. As it was impossible to solve the problem in an economically feasible time using standard fixed scaling techniques, a technique of variable time scaling was applied, so that time varied slowly in the initial stages when the rate of change of specific activity was high, and continuously more quickly as the rate of change of specific activity decreased.

In this group of subjects $K_f/K_s > 8/1$ and, by placing K_s at zero while determining A_f and K_f, it was possible to converge relatively quickly on the solution, only small adjustments then being necessary to A_f and K_f, when A_s and K_s were being determined.

TABLE V

VALUES FOR A_f, K_f, A_s, AND K_s ± THE "VISUAL" LIMIT OF ERROR OBTAINED FROM THE ANALOGUE COMPUTER

For description see text.

Experiment	Total RBC Na	Model	A_f	K_f	A_s	K_s
	meq/kg		*meq Na/kg cells*	*meq Na/kg cells/hr*	*meq/kg cells*	*meq Na/kg cells/hr*
6	7.79	Series	5.03±0.20	2.62±0.21	2.76±0.19	0.167±0.022
		Parallel	4.28±0.04	2.41±0.05	3.51±0.14	0.167±0.008
7	7.77	Series	5.75±0.23	2.39±0.20	2.02±0.23	0.147±0.015
		Parallel	5.52±0.22	2.21±0.18	2.25±0.23	0.090±0.014
8	5.90	Series	4.04±0.04	2.53±0.13	1.86±0.04	0.036±0.005
		Parallel	3.94±0.04	2.36±0.12	1.96±0.04	0.037±0.005

TABLE VI

TIME COURSE OF ACTIVITY OF PLASMA AND RED BLOOD CELLS AFTER INTRAVENOUS INJECTION OF ^{24}Na

The results of experiment 5 recomputed using a regression equation (derived from sucrose-^{14}C trapping) to estimate plasma trapping.

Time	Plasma activity	RBC activity	
		Upper section	Lower section
hr			
0.08	11.59	0.23	−0.21
0.32	7.93	0.91	0.38
0.57	7.21	1.31	0.88
1.00	7.03	1.62	1.29
1.25	6.61	2.07	1.82
1.52	6.59	2.46	1.93
2.25	6.63	3.03	2.40
3.25	6.29	3.55	2.95
4.78	5.95	4.59	4.07
8.08	5.70	3.55	3.90
26.30	5.06	3.48	3.73

An attempt was made to determine what might be called the visual limit of error. When a plot had been obtained which gave the best visual fit to the experimental results, the parameters could still be varied within a limited range, and with compensation to the other parameters, still produce a curve which appeared to give as good a fit.

Values for A_f, A_s, K_f, and K_s with the visual limits of error as described, are listed in Table V, and a typical curve (from experiment 6) illustrated in Fig. 4.

DISCUSSION

Reasons are given elsewhere (2) for concluding that the finding of an immediately exchangeable compartment of red blood cell sodium is an experimental artefact, and is due to the use of ^{131}IHSA to measure plasma trapping. Even when the immediately exchangeable compartment is excluded, model systems

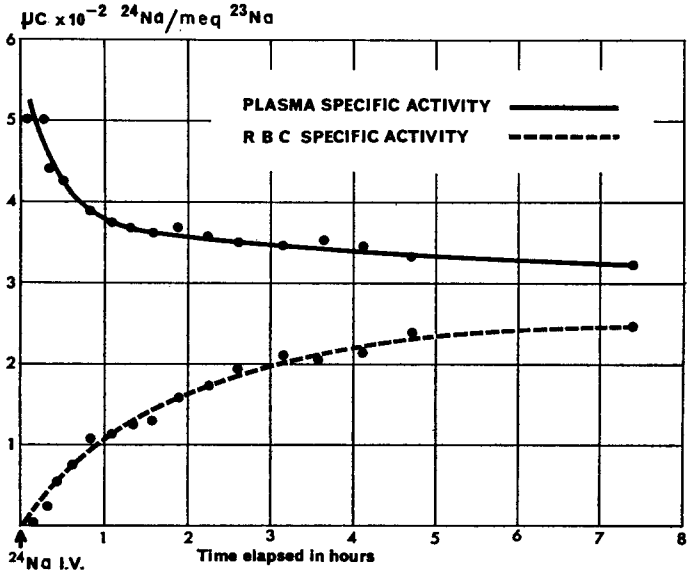

FIGURE 4. Specific activities of plasma and red blood cells at intervals after injection of ^{24}Na intravenously. Subject St., experiment 6. See text for explanation.

containing at least two compartments are required to account for sodium exchange between plasma and cells, a finding in agreement with previous studies, both in vivo and in vitro (4–6).

Because of differences in experimental and analytical methods, it is not possible to compare flux rates and compartment sizes in these experiments with those obtained by Gold and Solomon (1). Thus values for total red blood cell sodium obtained by Gold and Solomon, using ^{131}IHSA to measure trapped plasma sodium were higher (mean 12.1 meq/liter cells) than values obtained in these studies (means of 9.00 and 7.15 meq/kg cells for experiments 1 to 4 and 6 to 8 respectively). Furthermore Gold and Solomon assigned to K_f a value derived from previous in vitro experiments (4); finally their model curve did not fit the experimental data satisfactorily.

If it is accepted that the exchange of sodium between plasma and red blood

cells can only be described by a multicompartment cell system, containing at least two compartments, in series or in parallel, two important questions must be posed. First, how accurate are the figures for compartment sizes and flux constants for an individual? Second, do these values derived from mathematical models have any physical meaning?

To obtain an intelligible estimate of the error of the compartment sizes and flux values, it will be necessary to translate the subjective and visual results of the analogue computer to the objective output of a digital computer, and then to obtain a measurement of error in terms of probability. The three main sources of error are: first, errors in the measurement of plasma trapping, which are relatively more important during the early stages of an experiment, when plasma specific activity is high; second, errors in timing which are constant and are attributable to continued equilibration between plasma and cells after centrifugation is started; and third, errors based on the assumption that plasma and cells are in a steady state throughout the course of an experiment. While no significant differences in total red blood cell sodium concentration have been demonstrated between day and night (7), it is possible that shifts in compartment sizes and flux rates may occur. To fix the slow compartment parameters with any degree of assurance, it is at present necessary to continue sampling for 24 hr.

The second question posed earlier is related to the physical significance of compartmental models. Two main hypotheses have been advanced to account for the relatively complex nature of sodium flux into red blood cells.

The first hypothesis provides a parallel solution and the second a series solution.

Joyce (8) suggested that sodium flux between plasma and an individual cell may be described by a simple first-order process, and that inability to describe the sodium flux of a cell population in terms of a first-order process may be due to heterogeneity of the red cell population, some cells being more permeable and some cells less permeable to sodium ions. His experimental results appeared to lend some support to this hypothesis. In his experiments, however, a constant value, 4.65%, for trapped extracellular fluid was applied to the top, middle, and bottom portions of the packed red cell column. Since the radius of centrifugation varied from 10 cm at the top to 15 cm at the bottom, the mean relative centrifugal force applied to each portion of the column must have varied, and the results therefore should be treated with reserve.

The results of experiment 5 showed that the immediately exchangeable fraction of red blood cell sodium (present when ^{131}IHSA was used to measure plasma trapping) was not a function of red blood cell age. The data were recomputed using a value derived from sucrose-^{14}C trapping to estimate trapped plasma sodium. The revised values for plasma and red blood cell specific activity of younger and older cells, listed in Table VI, indicate that heterogeneity in respect of permeability to sodium exists as a function of cell

age. It is, however, inadequate to account for the complex nature of sodium flux into an unselected population of red blood cells. Furthermore, the difference between the values of K_f and K_s in Table V for parallel models does not suggest the existence of a red blood cell population continuously distributed as regards permeability to sodium, about a single mode. It is more suggestive of a population distributed about two modes.

An alternative hypothesis, providing a series solution (6), is that sodium flux into individual cells depends on two processes, first, exchange through the membrane and second, diffusion into the convection-free interior of the cell. The results of these experiments are consistent with this hypothesis. Although possible, it is more difficult to conceive of a parallel system affecting individual cells.

It is concluded that the most plausible working hypothesis that would account for the observed results is that sodium flux into red blood cells depends on a single process of exchange across the membrane coupled with slow diffusion into a convection-free interior. Both these processes are probably affected by increasing age of the cell.

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