CATION EXCHANGE BETWEEN CELLS AND PLASMA OF MAMMALIAN BLOOD

I. METHODS AND APPLICATION TO POTASSIUM EXCHANGE IN HUMAN BLOOD

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INTRODUCTION

When a small quantity of isotopically labeled potassium is introduced into the plasma of mammalian blood in vitro some of the isotope soon appears in the cellular fraction. It seems well established that in human blood this process is predominantly a penetration of potassium into the erythrocytes. If the labeling material such as the radioactive isotope $^{38}K$ is uniformly mixed with the total plasma potassium the initial percentage rate of decline of radioactivity in the plasma is equal to the rate of penetration of potassium in per cent of plasma potassium per hour. If the system is in a biologically steady state as shown by constant cell and plasma potassium content then the rates of penetration and loss are equal and the process is one of exchange. The historical background of such experiments has been reviewed by Hevesy (1). Early studies in this field such as the investigations of the University of Rochester group (2-4) showed that the erythrocyte is permeable to these cations. Usually semiquantitative estimates of penetration rates were obtained.

Because of our lack of good experimental information we still have no satisfactory picture of the mechanism of electrolyte regulation in erythrocytes. It is felt that the application of isotope techniques to the study of cation transport in mammalian blood in vitro, not only under steady state conditions but also under conditions of controlled disturbance, should provide information useful in understanding these processes so intimately related to the maintenance of cell stability.

In this communication we present the results of a series of determinations in vitro of the rate of potassium exchange in heparinized human blood from normal healthy donors. Individual samples are maintained under a controlled atmosphere and are tagged simultaneously. Cells and plasma of each sample are separated after varying times and analyzed for radioactivity and potassium content. The method of determining the exchange rate from these data is described below.
The average of our determinations at 38°C. is about 1.8 per cent of the cellular potassium per hour. From about 44°C. to about 15°C. the exchange rate follows pseudo first order kinetics with a $Q_{10}$ of 2.35. Below 15°C. the flow from cells to plasma begins to exceed the flow from plasma to cells, the difference being sixfold at 6°C. Studies of sodium and potassium exchange in other species will be reserved for a second communication.

**EXPERIMENTAL**

*Preparation, Tagging, and Maintenance of Samples in Vitro.*—Approximately 50 to 60 cc. of blood (Fig. 1) is taken from the cubital vein of healthy male or female donors. To minimize the effect of alimentary lipemia, venipunctures are usually made 4 or 5 hours after the last meal. The syringe is wet with approximately $\frac{1}{2}$ cc. of heparin.
Approximately 20 cc. of blood is centrifuged and the plasma removed; 10 cc. of this plasma, containing about 200 mg. of added dextrose and 30 to 50 c. mm. of isotonic radioactive potassium chloride, forms the solution with which the blood is tagged. The experiment is performed in a series of paraffin-lined flasks containing 2 compartments (Fig. 2). The flasks are connected in series with rubber tubing and through them is passed a gas saturated with water vapor, and containing 5 to 6 per cent CO$_2$. In most of the experiments, the gas also contains 8 per cent O$_2$, and 86 per cent N$_2$. However, in some instances the blood is “arterialized” by using approximately 14 per cent O$_2$. After carefully flushing the vessels with this gas, each container is removed in turn. Three cc. of blood is pipetted into one compartment, and into the other

\begin{center}
\includegraphics{fig2.png}
\end{center}

Fig. 2. Type of vessel in which blood samples are maintained during the experiment.

\begin{enumerate}
\item \frac{1}{2} cc. of tagging plasma. A cap is used and the pipettes are inserted through a small hole to minimize gas transfer. The flasks are placed in a rocking device and submerged in a water bath. The system is equilibrated for 15 to 30 minutes and is then set into oscillation at a rate of 10 oscillations per minute through an angle of about 120°. The plane which contains the vertical axis of a flask and its partition makes an angle of 45° with the axis of oscillation. Thus at each extreme the blood pours over the end of the partition from one compartment to the other. This method together with the use of paraffin gives gentle mixing but does not cause smearing or foaming of the blood, which rolls like mercury in the bottom of the flasks. When the rocking is started \((t = 0)\), the tagging plasma and the blood are thoroughly mixed and the transfer of K$^{42}$ begins.

\textit{Separation and Analysis; A and B Series.}—At the appropriate time each container is removed, a drop of blood is taken, and its hematocrit determined by the air
CATION EXCHANGE IN MAMMALIAN BLOOD. I

The turbine method of Parpart and Ballentine (5). The remainder of the blood is then centrifuged to separate cells from plasma.

The effect of layers of plasma trapped among the cells during and after centrifugation has not been stressed by earlier workers. Not only will the cellular fraction be contaminated with this plasma but also exchange can occur between cells and plasma during centrifugation. To avoid these effects two alternatives were employed. In the first series of experiments, called the A series, high speed centrifugation was not possible and the cells were separated at a mean acceleration of about 650 g. Because of the relatively loose packing of the cells, analyses were made of 1 cc. samples of plasma and whole blood, and the cell content determined from the results using the hematocrit data. Because of limited air pressure the acceleration of the hematocrit tubes was only about 3500 g necessitating a correction of about 4 per cent for incomplete packing. With these exceptions the procedure was essentially the same as in the later B series. Here cells are tightly packed at 15,000 g. Hematocrits are spun at 10,000 g. Following centrifugation the plasma is removed and the top layer of cells is washed with 1 cc. of isotonic sodium chloride, the washings being added to the plasma samples. Plasma and cell samples are digested with concentrated nitric acid for about 30 minutes, and excess nitrates are removed with formic acid. After dilution to 25 cc. and removal of 2 cc. for radioactivity determination, the remainder is reserved for potassium analysis. Radioactivities are determined with the usual end-window Geiger-Müller counter in small counting cups. Usually an aliquot of the tagging plasma is also counted.

The radioactive tracer is the isotope K\(^{42}\). This is obtained by exposing Merck's reagent grade K\(_2\)CO\(_3\) in the Oak Ridge National Laboratory nuclear reactor. After activation, the material is dissolved and neutralized with hydrochloric acid. The salt is then evaporated to dryness and enough water added to make the solution isotonic. The radiochemical purity of K\(^{42}\) used in physiological experiments has received considerable discussion (4). In the case of reactor production the thermal neutron activation cross-sections for the parent isotopes K\(^{41}\) and Na\(^{23}\) are 1.0 and 0.63 barn (±20 per cent) respectively (6). Since K\(^{41}\) is 6.61 per cent abundant the activation of sodium is favored by a factor of about 10. The presence of less than 0.02 weight per cent (~0.04 μ per cent) of sodium as claimed by the manufacturer has been confirmed by spectroscopic analysis. Thus, since the counting efficiencies for the two isotopes are roughly the same the resulting contamination should be less than 0.4 per cent. On two separate occasions the absence of any detectable long lived radioactive impurity other than K\(^{42}\) was established by decay studies.

Traces of Rb\(^{88}\) or Cs\(^{137}\) occasionally found are unimportant here because the material is used within a few hours after activation and because of the brief exposure in the reactor (about 16 hours).

Potassium Determinations.—Potassium is determined with the Perkin-Elmer Model 52A flame photometer using 12 p.p.m. lithium as an internal standard. No significant potassium contamination was found in the lithium chloride used. Potassium standards were prepared by weighing quantities of dried potassium chloride, and compared with solutions of potassium acid phthalate standardized both by weighing and by titration against a known base. Comparisons were made in one instance of analyses of plasma.
by the instrument and by the semimicrochemical method of Tenery and Anderson (7). The results of the chemical determinations averaged about 5 per cent lower. Good agreement was obtained between samples of plasma which were diluted 1 to 25 and atomized directly, and those which were first digested with nitric acid and treated with formic acid to remove excess nitrates. Nitric acid up to 1 N was found to have no effect on the results. The digestion procedure was adopted after trichloracetic acid precipitation of proteins performed several ways gave irregular precipitation of potassium up to about 4 per cent.

Since an acetylene flame is used throughout, sodium interference is rather high, increasing the intensity of the potassium light by 12 to 14 per cent in the case of typical plasma. Since the sodium correction may not be as closely controlled as other factors the absolute value of the results may be in doubt within about 5 per cent from one experiment to another; relative values within a given experiment should be more accurate. In the case of analyses of human cells, where subdilutions are made, the already low sodium concentration is reduced to a negligible amount.

Correction of Radioactivity Data for Sample Variations.—It is found that the determinations of radioactivity are considerably more accurate than the precision of delivery of the 1/2 cc. samples of tagging plasma. Thus the radioactivity of the contents of the individual flasks varies, the standard deviation being about 4 per cent. In the A series of experiments these variations are corrected for by dividing each plasma activity by its whole blood activity and multiplying by the mean of the whole blood activities. In the B series, although the delivery error had been reduced, an additional fluctuation occurs from the slight variation in the amount of blood taken for hematocrit determinations and by the occasional loss of 1 or 2 drops of blood in the operation of transfer to the centrifuge tube. Here, instead of the whole blood activities, the sum of the cell and plasma activities is used in the correction procedure. An additional small correction is also made for the small amount of activity in the plasma trapped with the cells.

RESULTS AND DISCUSSION

The experimental results are presented in Tables I to VII. In the A series (Tables I to III) radioactivities per cubic centimeter and potassium concentrations are given. In the B series (Tables IV to VII) total activity and total potassium are shown. In all cases, the raw figures for radioactivity are presented uncorrected for fluctuation in the activity of the total flask contents or the small amount of activity present in the plasma trapped with the cells. The specific activities are obtained by dividing the corrected radioactivity of a given sample of plasma or cells by the corresponding potassium content. Results are then reduced to a common basis by dividing by the initial plasma specific activity.

The results of Experiments 18 B and 19 B are of particular interest since they
TABLE I

Experiment 14 A

Blood drawn............. 1:15 p.m. Venous saturation.. O₂ 5 per cent, CO₂ 5 per cent, N₂ 90 per cent
Experiment started....... 2:35 p.m.
Male donor............ Type O, RH+ Commercial heparin

| Sample No. | Time | Hemolysis | Hematocrit | Counts/min./cc. Plasma | Whole blood | Potassium concentration, mg. per cent Plasma | Whole blood | Relative specific activity Plasma | Cells |
|------------|------|-----------|------------|------------------------|-------------|---------------------------------------------|-------------|-----------------------------------|-------|
| min        |      |           | per cent   |                        |             |                                             |             |                                   |       |
| 1          | 2    | —         | 37.5       | 895                    | 1140        | 14.1                                        | 155         | 1.0                               | 0.009 |
| 2          | 18   | —         | 38.5       | 744                    | 1149        | 10.5                                        | 155         | 1.24                              | 0.014 |
| 3          | 30   | —         | 38.8       | 763                    | 1135        | 14.0                                        | 155         | 0.97                              | 0.010 |
| 4          | 60   | —         | 37.0       | 667                    | 1235        | 14.1                                        | 155         | 0.78                              | 0.027 |
| 5          | 120  | —         | 39.2       | 490                    | 1135        | 12.4                                        | 155         | 0.71                              | 0.033 |
| 6          | 240  | —         | 38.0       | 324                    | 1167        | 12.5                                        | 150         | 0.45                              | 0.046 |
| 7          | 360  | —         | 39.0       | 234                    | 1162        | 13.5                                        | 150         | 0.32                              | 0.054 |
| 8          | 420  | —         | Lost       | 147                    | 1165        | 13.5                                        | 150         | 0.19                              | —      |
| 9          | 540  | —         | 39.5       | 132                    | 1190        | 13.5                                        | 150         | 0.16                              | 0.062 |
| 10         | 1048 | —         | 39.0       | 47                     | 1170        | 14.1                                        | 150         | 0.06                              | 0.068 |

TABLE II

Experiment 15 A

Blood drawn............. 12:30 p.m. Venous saturation.. O₂ 5 per cent, CO₂ 5 per cent, N₂ 90 per cent
Experiment started....... 1:05 p.m.
Male donor............ Type A, RH+ Commercial heparin

| Sample No. | Time | Hemolysis | Hematocrit | Counts/min./cc. Plasma | Whole blood | Potassium concentration, mg. per cent Plasma | Whole blood | Relative specific activity Plasma | Cells |
|------------|------|-----------|------------|------------------------|-------------|---------------------------------------------|-------------|-----------------------------------|-------|
| min        |      |           | per cent   |                        |             |                                             |             |                                   |       |
| 1          | 2    | —         | 40.6       | 590                    | 762         | 16.2                                        | 173         | 0.97                              | 0.005 |
| 2          | 17   | —         | 42.2       | 540                    | 760         | 15.5                                        | 170         | 0.93                              | 0.011 |
| 3          | 31   | —         | 41.3       | Lost                   | 768         | —                                           | 170         | —                                 | —     |
| 4          | 62   | —         | 40.0       | 397                    | 765         | 14.3                                        | 160         | 0.73                              | 0.03  |
| 5          | 120  | —         | 40.7       | 302                    | 768         | 13.4                                        | 165         | 0.59                              | 0.04  |
| 6          | 250  | —         | 41.6       | 163                    | 737         | 12.0                                        | 165         | 0.37                              | 0.05  |
| 7          | 360  | —         | 41.7       | 106                    | 744         | 12.0                                        | 165         | 0.24                              | 0.05  |
| 8          | 480  | —         | 43.5       | 65                     | 780         | 13.0                                        | 168         | 0.129                             | 0.06  |
| 9          | 1062 | —         | 43.6       | 48                     | 762         | 13.3                                        | 170         | 0.097                             | 0.06  |
| 10         | 1303 | ++        | 44.0       | 36                     | 754         | 14.3                                        | 165         | 0.068                             | 0.06  |

were obtained after considerable experience was achieved. The variation of the activity of cells and plasma with time in Experiment 19 B is shown in Fig. 3. Although in the other experiments similar smooth radioactivity curves are
TABLE III

Experiment 16 A

| Sample No. | Time  | Hemolysis | Hematocrit | Counts/min./cc. | Potassium concentration, mg. per cent | Relative specific activity |
|------------|-------|-----------|------------|-----------------|--------------------------------------|--------------------------|
|            | min.  | per cent  |            | Plasma Whole blood | Plasma Whole blood | Plasma Cells |
| 1          | 2     | 34.8      |            | 810 565         | 15.1 173       | 1.0 0.0047   |
| 2          | 17    | 33.4      |            | 784 565         | 15.5 170       | 0.98 0.0048  |
| 3          | 31    | 33.3      |            | 716 495         | 16.2 150       | 0.97 0.0031  |
| 4          | 60    | 33.3      |            | 661 567         | 17.7 150       | 0.71 0.018   |
| 5          | 120   | 34.0      |            | 544 595         | 17.7 150       | 0.56 0.032   |
| 6          | 240   | 33.8      |            | 428 537         | 17.5 150       | 0.50 0.037   |
| 7          | 382   | 32.0      |            | 254 605         | 16.2 155       | 0.28 0.054   |
| 8          | 471   | 34.7      |            | 214 614         | 17.2 175       | 0.226 0.050  |

TABLE IV

Experiment 7 B

| Sample No. | Time  | Hemolysis | Hematocrit | Counts/min. | Total potassium, mg. | Relative specific activity |
|------------|-------|-----------|------------|-------------|----------------------|--------------------------|
|            | min.  | per cent  |            | Plasma Cells | Plasma Cells | Plasma Cells |
| 1          | 0     | 42.3      |            | 2130 93     | 0.265 4.80     | 1.0 0.00      |
| 2          | 26    | 41.0      |            | 2000 358    | 0.290 4.70     | 0.82 0.008    |
| 3          | 60    | 40.5      |            | 1610 705    | 0.250 4.80     | 0.68 0.020    |
| 4          | 114   | 40.7      |            | 1670 583    | 0.324 4.45     | 0.65 0.018    |
| 5          | 184   | 38.4      |            | 1045 1325   | 0.378 4.45     | 0.34 0.045    |
| 6          | 353   | 29.5      |            | 600 1700    | 0.282 4.70     | 0.28 0.056    |
| 7          | 479   | 38.1      |            | 410 2010    | 0.282 4.30     | 0.21 0.075    |
| 8          | 612   | 37.0      |            | 322 2180    | 0.282 Lost     | 0.16 Lost     |

obtained, the specific activity curves are usually subject to more fluctuation. In four cases, the data are obtained over a period of about 20 hours at which time approximately equal specific activity of cells and plasma is reached.

In three of these experiments there is a slight persistent tendency for the
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TABLE V

Experiment 8B

| Sample No. | Time | Hemolysis | Hematocrit | Counts/min. | Total potassium, mg. | Relative specific activity |
|------------|------|-----------|-------------|--------------|----------------------|--------------------------|
|             |      |           |             | Plasma | Cells | Plasma | Cells | Plasma | Cells |
| 1          | 0    | —         | 41.3        | 3400   | 228   | 0.310  | 5.07  | 1.0    | 0.00  |
| 2          | 22   | —         | 39.5        | 2840   | 552   | 0.328  | 5.15  | 0.865  | 0.002 |
| 3          | 50   | —         | 42.8        | 2560   | 885   | 0.320  | 4.60  | 0.805  | 0.018 |
| 4          | 131  | —         | 41.6        | 1920   | 1770  | 0.260  | 5.50  | 0.655  | 0.033 |
| 5          | 169  | —         | 40.0        | 1420   | 2110  | 0.320  | 5.30  | 0.458  | 0.045 |
| 6          | 316  | —         | 45.2        | 890    | 2700  | 0.312  | 4.80  | 0.312  | 0.063 |
| 7          | 406  | —         | 39.9        | 635    | 3120  | 0.312  | 5.00  | 0.260  | 0.068 |
| 8          | 467  | —         | 42.5        | 520    | 3060  | 0.300  | 4.80  | 0.219  | 0.074 |

TABLE VI

Experiment 18 B

| Sample No. | Time | Hemolysis | Hematocrit | Counts/min. | Total potassium, mg. | Relative specific activity |
|------------|------|-----------|-------------|--------------|----------------------|--------------------------|
|             |      |           |             | Plasma | Cells | Plasma | Cells | Plasma | Cells |
| 1          | 0    | —         | 34.7        | 686    | 25    | 0.486  | 4.50  | 1.0    | 0.00  |
| 2          | 40   | —         | 34.0        | 615    | 130   | 0.390  | 4.50  | 0.855  | 0.0118 |
| 3          | 60   | —         | 34.1        | 537    | 197   | 0.390  | 4.50  | 0.760  | 0.0199 |
| 4          | 120  | —         | 34.2        | 469    | 282   | 0.389  | 4.70  | 0.655  | 0.0278 |
| 5          | 183  | —         | 34.5        | 351    | 414   | 0.389  | 4.70  | 0.490  | 0.0415 |
| 6          | 238  | —         | 34.8        | 306    | 394   | 0.362  | 4.50  | 0.51   | 0.044 |
| 7          | 360  | —         | 34.7        | 221    | 493   | 0.336  | 4.50  | 0.392  | 0.0575 |
| 8          | 475  | —         | 34.8        | 151    | 560   | 0.350  | 4.50  | 0.272  | 0.0645 |
| 9          | 1184 | —         | 37.0        | 58     | 629   | 0.358  | 4.50  | 0.152  | 0.074 |
| 10         | 1231 | —         | 36.2        | 57     | 627   | 0.384  | 4.40  | 0.118  | 0.077 |

specific activity of the plasma to exceed that of the cells. This suggests either a small radioactive sodium contamination or an overestimation of the correction for plasma trapped with the cells.
TABLE VII

**Experiment 10 B**

Blood drawn.............10:00 a.m. Venous saturation...O$_2$ 6 per cent, CO$_2$ 6 per cent, N$_2$ 88 per cent
Experiment started........11:00 a.m. Donor hematocrit..45.6 per cent
Male donor..............Type A, RH+ Sodium heparin salt

| Sample No. | Time | Hemolysis | Hematocrit | Counts/min. | Total potassium, mg. | Relative specific activity |
|------------|------|-----------|------------|-------------|-----------------------|----------------------------|
|            | min. |           | per cent   | Plasma Cells | Plasma Cells Plasma Cells | Plasma Cells |
| 1          | 0    | -         | 38.9       | 798 28   | 0.284 4.87            | 1.0 0.002                  |
| 2          | 60   | -         | 39.0       | 689 282 | 0.284 4.87            | 0.73 0.017                 |
| 3          | 124  | -         | 39.3       | 416 388 | 0.284 4.87            | 0.52 0.029                 |
| 4          | 181  | -         | 39.6       | 316 504 | 0.275 4.95            | 0.41 0.036                 |
| 5          | 300  | -         | 39.7       | 192 628 | 0.275 4.95            | 0.25 0.045                 |
| 6          | 420  | ++        | 37.8       | 138 692 | 0.279 5.13            | 0.175 0.048                |
| 7          | 540  | ++        | 40.2       | 100 768 | 0.275 4.95            | 0.123 0.052                |
| 8          | 1270 | ++        | 42.9       | 60 858  | 0.292 4.95            | 0.065 0.055                |

*Calculation of Exchange Rates—The Two-Compartment System.*—It is of considerable interest that human cells and plasma *in vitro* behave very much like an ideal two-compartment system. This is a special case of a system of
In compartments previously discussed by one of us (8). The mathematical analysis of the two-compartment case has been discussed several times in the literature (9) and thus requires only a brief summary here. We define the following terms:

- $S_1$ = substance in compartment 1 (plasma potassium).
- $S_2$ = substance in compartment 2 (cellular potassium).
- $a_1$ = specific activity in compartment 1 (counts per minute per millimol).
- $a_2$ = specific activity in compartment 2 (counts per minute per millimol).
- $dS_{12}$ = amount of $S$ leaving compartment 1 and entering compartment 2.
- $dS_{21}$ = amount of $S$ leaving compartment 2 and entering compartment 1.
- $dS_2$ = net change in compartment 2 due to inequality of opposing transfers.
- $dR_2$ = change in total radioactivity in compartment 2.
- $\rho$ = steady state exchange rate (millimols per hour).
- $a_0$ = initial specific activity in compartment 1.

The general transport process is indicated schematically in Fig. 4. The radioactivity is represented for simplicity by the shaded portion in each case, although strictly it is a quantity proportional to it. From the figure the total change in the radioactivity of compartment 2 is

$$dR_2 = a_0 dS_{21} - a_0 dS_{21} + a_0 dS_2$$

and the rate of transport is

$$\frac{dS_2}{dt} = \frac{a_2 dS_2/dt - dR_2/dt}{a_2 - a_1} \quad (1)$$
The return rate $\frac{dS_t}{dt}$ is obtained by interchanging subscripts. If the specific activity in each compartment, the change in radioactivity of one compartment, and the change in total amount of $S$ in this compartment are known, then the two opposing transport rates can be found provided that the contents of each compartment are uniformly mixed and the tagged and untagged species behave identically.

![Graph showing theoretical specific activity changes in a two-compartment system.](image)

**Fig. 5.** Theoretical specific activity changes in a two-compartment system.

When, as in the present experiments, the system is in a steady state

$$\frac{dS_t}{dt} = \frac{dS_u}{dt} = \rho \quad \text{and also} \quad dS_t = dS_u = 0.$$

Under these conditions

$$\frac{d\rho}{dt} = \frac{\rho(a_2 - a_2)}{S_1} \quad \text{and} \quad \frac{d\rho}{dt} = \frac{\rho(a_1 - a_2)}{S_2}$$

(2)

If at the beginning of the experiment the tagged material is all in one compartment, the rate at which it leaves one compartment and appears in the other is constant and directly proportional to the exchange rate. As mixing of radioactive and non-radioactive material progresses, conditions alter with time and the variations of radioactivity and specific activity depart from linearity.
Ultimately the specific activities are the same in both compartments and no further change occurs in the system. Fig. 5 shows the theoretical curve of plasma specific activity as a function of time. It declines uniformly at first but, as time progresses, it flattens out to an asymptotic base line. As shown, the curve for cellular specific activity is obtained by reflection about the base line with a change in vertical scale. Integration of Equations 2 shows that the curves are exponential in character (9, 10), thus

\[ \frac{a_1}{a_0} = \frac{S_1 + S_2 e^{-\mu t(1/\gamma_1 + 1/\gamma_2)}}{S_1 + S_2} \]

\[ \frac{a_2}{a_0} = \frac{S_1 (1 - e^{-\mu t(1/\gamma_1 + 1/\gamma_2)}}{S_1 + S_2} \]

(3)

If the differences between the value of \( a_1 \) or \( a_2 \) at any time and the base line value (without regard to sign) are plotted on semilogarithmic coordinates the result will be a straight line. From the slope the "half-value time" \( T_1 \) is obtained. Since

\[ \rho = \frac{0.693 S_0}{T \sqrt{(S_1 + S_2)}} \]

the exchange rate may be determined. This method is best adapted to cases where the exchange is fairly rapid. Where exchange is proceeding slowly the rate is determined from the initial slope, i.e.

\[ \rho = \frac{-1}{a_1} \frac{dR_1}{dt} \]

(4)

if all the activity is initially in compartment 1. The exchange rates obtained for the seven experiments are shown in Table VIII.

Statistical Distribution of Exchange Rates.—Fig. 6 shows the variation of the specific activity of the cells and plasma in experiment 19 B. The semilog plot of the plasma specific activity (minus base line) is shown in Fig. 7. Since the results follow the theoretical predictions so closely we have analyzed this experiment for evidence of inhomogeneity in the exchange rates caused by possible variability among the individual cells. In a separate communication (10) it is shown that if the exchange rates of the cells vary the semilogarithmic plot will become convex to the origin. The initial slope of the curve gives the arithmetic mean of the exchange rates. Although the degree of convexity is determined by the inhomogeneity of the system it is rather insensitive to a relatively large spread in the exchange rates. By an approximation method the degree of initial curvature can be quantitatively related to the standard deviation of the exchange rates. The data in Fig. 7 have been analyzed for curvature by the least squares method. The results indicate a most probable value for the
standard deviation of about 8 per cent. However, the uncertainties in the data and the insensitivity of the effect make this result barely significant.

Variation of Exchange Rate with Temperature—Low Temperature Leakage.—We have determined exchange rates by the initial slope method (Equation 4) at different temperatures ranging from 6–44°C. The experiment at 44°C yielded acceptable results only during the first 90 minutes. Beyond this time extensive potassium leakage occurred from the cells into the plasma. Fig. 8 shows the initial values for the specific activities of the cells (in per cent of the initial plasma specific activity) at the various temperatures. A representative experiment from the series at 38°C is included.

The slopes of the various curves in Fig. 8 yield the exchange rates in per cent of cellular potassium per hour directly thus:

$$\frac{1}{a_0} \frac{dR}{dt} = \frac{S_1 d (R/S_2)}{R_0 dt} = \frac{p}{S_2},$$

since $\frac{dR}{dt}$ is the same for cells and plasma except for sign. Fig. 9 shows a plot of the log of the exchange rate as ordinates against the reciprocal of the absolute temperature as abscissas. The function is linear with a $Q_10$ of 2.35 down to about
15°C, indicating pseudo first order kinetics. At the two lower temperatures the total plasma potassium concentration shows a progressive increase with time and the rate of loss of potassium exceeds the rate of uptake. The two rates are indicated separately in the figure.

Physiological Behavior in Vitro, Initial Disturbances.—The simplification which occurs when blood is studied in vitro is especially well shown in the present experiments in which we have shown the close correspondence between the
behavior of the system of cells and plasma and that of an idealized two-compartment system. Nevertheless some analysis must be made of the nature of the disturbances which may occur under these artificial conditions. In this respect we attempt here to improve on the earlier observations in which such discussions were notably incomplete, and the results were justified more from the rough similarity between occasional exchange rates determined both in vitro and in vivo.

![Graph](image)

**Fig. 8.** Initial penetration of radioactive plasma potassium at different temperatures.

We first consider the disturbances produced by the addition of potassium, anticoagulant, and sugar to the blood. We can rule out the disturbing effect of the potassium since the amount added is never more than 5 per cent of the total plasma potassium in the samples, and usually considerably less. In the next communication we will show that no significant alteration of the exchange rate occurs if the plasma potassium concentration is raised fourfold. More attention must be given to the selection of the anticoagulant since the recent observations of Levi (11) which indicate that the exchange rate is affected by the removal of calcium from the blood. Even in the use of heparin caution is
warranted in view of the difficulties experienced by some investigators with impure material (12). The commercial heparin which was first used contains chlorobutanol which appears in the blood in a final concentration of about $2 \times 10^{-4}$ molar. This is about one-third the threshold concentration for narcotic action (13) but we nevertheless used the purified sodium salt\(^1\) dissolved in

\(^1\) Samples were provided by the courtesy of the Connaught Laboratories and the Abbott Laboratories.
isotonic saline in the later work. No significant difference appears in the results (Table VIII). Since the addition of sugar was found to retard the deterioration of canine erythrocytes this practice was adopted routinely. The amount added raises the blood sugar level to 350 to 400 mg. per cent. The absence of any detectable decrease in cell volume in human blood is in agreement with earlier observations that human cells are permeable to dextrose (14).

Progressive Deterioration of Cells.—The extensive literature on the preservation of human blood describes some of the changes which occur in vitro. Among these are the accumulation of products of glycolysis, deterioration of organic phosphorus compounds, osmotic disturbances, loss of glycolytic ability, and potassium leakage (15). Some of the changes such as potassium loss

| Experiment | Donor* | Heparin | Atmosphere | Radiation | Half-value time | Ratio cellular to plasma potassium | Exchange rate |
|------------|--------|---------|------------|-----------|----------------|-----------------------------------|--------------|
| 14 A       | A      | Commercial | Venous     | 0         | 2.8            | 15                                | 1.5          |
| 15 A       | B      | Commercial | Venous     | 0         | 2.2            | 18                                | 1.6          |
| 16 A       | C      | Commercial | Arterial   | 0         | 2.8            | 12                                | 1.9          |
| 7 B        | A      | Sodium salt | Venous    | 1200 r    | 2.7            | 12                                | 1.7          |
| 8 B        | B      | Commercial | Arterial   | 0         | 2.5            | 14                                | 1.6          |
| 18 B       | D      | Sodium salt | Venous     | 0         | 2.5            | 12                                | 1.1          |
| 19 B       | B      | Sodium salt | Venous     | 0         | 1.9            | 17                                | 2.0          |

Average .......................................................... 1.8

* Donors were: A, male, type O; B, male, type A; C, female, type O; D, female, type O. All were Rh+.

may be partly reversible (16–18) but as deterioration proceeds the processes become irreversible and progressive hemolysis is observed. Although a large number of characteristics of stored blood have been used for determining the extent of deterioration in vitro (19) practical considerations have prevented the measurement of all these in every experiment. Those which we have observed are hemolysis cell volume and potassium leakage, the results being given in Tables I to VII. Observations of plasma hemoglobin were made visually. In canine blood the hemolysis of about 0.1 per cent of the cells could be detected. In the more highly colored human plasma the threshold concentration of hemoglobin (+ in the tables) was about 5 times higher. The maximum level (++) which was accepted corresponded to the hemolysis of about 1 per cent of the cells. As might be expected the increase in hematocrit was usually somewhat more sensitive than the observation of hemolysis. Often as in Experiments
15 A and 19 B this occurred perceptibly before any hemoglobin was observed. A progressive decrease as in Experiment 7 B was later traced to sedimentation of the blood during the initial pipetting of the samples. Probably the most sensitive index of cell stability is the plasma potassium content. In the earlier studies some of the fluctuations such as in Experiments 15 A and 7 B may have been due in part to analytical difficulties. However, the progressive increase in Experiment 16 A seems well established. The results in Experiments 18 B and 19 B are of particular interest since the determinations were made with considerable care and had the benefit of considerable prior experience. Hereafter about the first 2 hours the ratio of cellular to plasma potassium increases systematically with time. Since this occurs in two different experiments it must be accepted as real. The effect has been previously observed by Danowski (17) and may be due to an increased penetration rate or a decreased rate of loss. A selection between the two alternatives is scarcely warranted without further investigation.

CONCLUSION

The first few experiments of the B series were done during the same period as the investigation of the temperature effect and the results all seemed to show a remarkable uniformity. However, since the rates determined in the two best cases (Experiments 18 and 19 B) were definitely higher, and the over-all results show a maximum deviation from the mean of about 15 per cent it is evident that a small but significant biological variation still exists which is less well controlled than the analytical variables. This is particularly true in the A series experiments. The mean exchange rate of 1.8 per cent of the cellular potassium per hour compares favorably with 1.6 per cent per hour found by Taylor and his associates (20). It is higher than the mean value 1.4 per cent per hour obtained in two experiments on human cells in Ringer solution by Dean et al. (3). Our Q_{10} of 2.35 is close to the value 2.4 given by them for rabbit cells. The value of 4.4 per cent per hour cited by Mullins and his associates (4) is definitely outside of our limit of variability.

Since the exposure of the blood in Experiment 7 B to 1200 roentgens was without any significant effect, the amount of radiation from the isotope (<1 roentgen equivalent) is without importance. Similarly, variations in oxygen content of the gas have little effect. Although no large differences are observed as the CO₂ content is varied, small variations may have occurred which escaped observation. Because of the large temperature coefficient, a change in bath temperature of 1°C. will produce a change in exchange rate of 10 per cent, and close regulation is required for precise results.

In the next communication we will present determinations of potassium and sodium exchange rates in different species, and an investigation of the effect of increased plasma potassium concentration on the exchange rate of potassium in human blood.
We wish to thank several members of the Biology Division for donating blood for these experiments. Some of the data were obtained with the technical assistance of Maryann Huddleston.

SUMMARY

The exchange of potassium between cells and plasma of heparinized human blood has been studied in vitro using the radioactive isotope $^{40}$K. The changes in cell and plasma specific activity are characteristic of a simple two-compartment system. The mean of seven determinations of the exchange rate at 38°C is 1.8 per cent of the cellular potassium per hour. The results indicate that at 38°C the rate is relatively insensitive to oxygenation or reduction of the hemoglobin, and to 1200 r of gamma radiation. With varying temperature the rate follows pseudo first order kinetics with a $Q_{10}$ of 2.35. Below 15°C the rate of loss of potassium exceeds the rate of uptake.

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