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THE EFFECTS OF LEAD, MERCURY, AND GOLD ON THE POTASSIUM TURNOVER OF RABBIT BLOOD CELLS

BY

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Some heavy metals cause a loss of potassium from rabbit erythrocytes (Henriques and Ørskov, 1936; Davson and Danielli, 1938). There is normally a slow exchange of K between rabbit erythrocytes and the surrounding fluid, and this exchange can be demonstrated with radioactive tracer (Mullins, Fenn, Noonan and Haege, 1941). The potassium concentration in the cells is about twenty to twenty-five times that in plasma. This ratio is much greater than that observed with chloride or hydrogen ions; the difference in concentration does not therefore depend on a Donnan equilibrium, and involves the forced inward movement of ions by metabolic activity as well as movement in both directions by diffusion through the more or less permeable cell walls. These points have been fully discussed by Harris and Maizels (1952). Net loss of K from cells is due either to an increased permeability of the cell walls, so that the work done by the cells is insufficient to offset the exaggerated outward leak of K; or to a decrease in the work done by the cells, so that the normal efflux of K is no longer counteracted by adequate pumping of K inwards; or both. With the first change the rate of entry of tracer into cells immersed in a medium containing ^{42}K would be unaltered or accelerated, and with the second the rate of entry would be diminished. In order to examine these possibilities we have examined the movements of K between rabbit erythrocytes and their environment in the presence and absence of the chlorides of lead, mercury and gold, with the results described below.

METHODS

Treatment of Cells.—In order to avoid changes in cells associated with washing and centrifuging (Davson and Danielli, 1938), the inward passage of tracer was followed when fresh rabbit blood was added to a slightly modified Krebs' solution containing ^{42}K and, when appropriate, small quantities of $PbCl_2$, $HgCl_2$, or $AuCl_3$. The solution was composed of NaCl 0.120M, $NaHCO_3$ 0.025M, $CaCl_2$ 0.0025M, $MgSO_4$ 0.0012M, KCl 0.0042M, and dextrose 0.2% (w/v); O_2 + 5% CO_2 was bubbled through it for 20 min. before use to give a *p*H of 7.4. The KCl was made up from irradiated K_2CO_3 which contained ^{42}K (half life 12.4 hr.): the activated K_2CO_3 was converted to KCl by dissolving weighed amounts in N-HCl and diluting appropriately. Blood was obtained by venepuncture or cardiac puncture from apparently healthy rabbits. 5 ml. portions were placed in tared 30 ml. stoppered centrifuge tubes, weighed and left for 15–30 min. in a water bath at 37° C. or in the refrigerator at 7°. 20 ml. portions of salt solution were kept in the same way. To begin each experiment, the Krebs' solutions were poured into the samples of blood. The tubes were shaken gently to mix their contents, weighed rapidly, and put on a rotary mixer turning at 7 rev./min. in an incubator at 37° or in a refrigerator at 7°.

Sampling.—From time to time 2 ml. samples were removed from the mixtures and were centrifuged for 18 min. at 3,000 rev./min. and 15 cm. radius (1,500 *g.*). The tubes used had a bore of 3 mm. for the bottom 40 mm. and 8 mm. for the upper 45 mm., with a tapering junction 10 mm. long. The cells did not completely fill the narrow segment of the tube, and their volume was determined from the length of the cell column measured directly against a millimetre scale. The tubes were calibrated with known volumes of fluid added from a micrometer syringe. The supernatant fluid was separated without disturbing the cell column and the part of the tube above the cells was twice washed with 5% (w/v) dextrose. The washings were added to the supernatant fluid, which was then made up with water or dilute NaCl to a volume (25–100 ml.) suitable for estimating total K and radioactivity. The quantity of NaCl used in diluting was such that the final Na concentration was approximately 0.002M. 1 ml. of 0.05% (w/v) saponin was added to the cells. These were mixed and transferred with washings to 100 ml. volumetric flasks, and made up for estimations like the supernatant fluids. An allowance of 5% of the cell volume was made for trapped supernatant fluid, and the estimated cell concentrations were so corrected. The actual volume trapped may have varied considerably (Jackson and Nutt, 1951), but this average correction is consistent with previous observations under similar conditions (Ponder, 1948; Leeson and Reeve, 1951), and was

supported by experiments with Krebs' solution containing ^{24}NaCl from which the initial uptake of ^{24}Na by the cells is slow.

Estimations of Potassium and Radioactivity.—Potassium was estimated on a flame photometer, similar to that described by Holiday and Preedy (1953) as modified by Neil (1953), in the range $0.5 - 4 \times 10^{-4}$M by comparison with appropriate standard solutions of KCl in 0.002M-NaCl. The accuracy of the estimations was $\pm 0.04 \times 10^{-4}$M (S.E. of duplicate determinations). Radioactivity of the solution was determined from duplicate counts with an M.R.C. type M.6 liquid counter and a Dynatron type 1009B scaling unit, with suitable corrections for background activity and decay and resolution time. Usually at least 10,000 impulses were counted, giving a theoretical standard error of ± 1%, and the agreement between duplicate counts was of this order.

Calculation of the Minimum Quantities of an Ion Species Entering and Leaving Cells in a Given Period.—For unit quantity of cells, let the quantity of ions of a particular species moving through the cell walls be Q_i inwards and Q_o outwards in a given period. Let the amount of ion in the cells at the beginning and end of the period be b_1 and b_2 moles and the radioactivity y_1, y_2. Let the specific activity of the medium be A_p and that of the cells A_c at the beginning of the period. Then the quantity of ions of the particular species moving inwards cannot be less than the increment in cell radioactivity divided by the original specific activity of the medium; and the quantity moving outwards cannot be less than the difference between the amount of ion in the cells at the beginning and end of the period (with due regard to sign). Thus,

$$Q_i \geqslant \frac{y_2 - y_1}{A_p} \quad (1)$$

and

$$Q_o \geqslant b_1 - b_2 \quad (2)$$

The estimated inward movement is too small because it does not allow for any loss of radioactive ions from the cells during the period, and it does not allow for the decrease in the specific activity of the medium due to the efflux of inactive ions from the cells. Some allowance can be made for the loss of radioactive ions by using expression (2) and the initial specific activity of the cells, giving

$$Q_i \geqslant \frac{y_2 - y_1}{A_p} + (b_1 - b_2).\frac{A_c}{A_p} \quad (3)$$

Also, the outward movement must be large enough to balance the inward movement as well as the net change in cell ionic content. So

$$Q_o \geqslant b_1 - b_2 + \frac{y_2 - y_1}{A_p} + (b_1 - b_2).\frac{A_c}{A_p} \quad (4)$$

Progressive approximations can be obtained by continuing to adjust the inward and outward estimates alternately, and the series obtained converges more or less rapidly according to the magnitude of the ratio A_c/A_p. The result obtained is still an underestimate because it does not take into account changes in specific activity of the medium and cells during the period, and in practice it has not been worth proceeding further. The estimated minimum movements given in Tables II and III are obtained from the expressions (3) and (4).

Results

Untreated Cells.—Typical results are shown in Table I and in Figs. 1 and 2. As we have been concerned with the changes in poisoned cells which usually began to haemolyse after a few hours at 37°, we have studied normal cells at this temperature only for comparably short periods, in which time the amount of potassium which exchanged was rather small for a satisfactory estimate of the rate to be made. In the first four to six hours it makes very little difference whether the uptake of ^{42}K by the cells is regarded as linear or as the beginning of an exponential approach to equili-

TABLE I

THE EFFECT OF LEAD CHLORIDE ON THE EXCHANGE OF POTASSIUM BY RABBIT BLOOD CELLS AT 37° C.

Time: Min.	Cell Vol.: % Suspension	K^+ μ.equiv. in Cells of 1 ml. Suspension	K^+ μ.equiv. in 1 ml. Cells	Specific Activity Counts/ μ.equiv.
A. *Normal Cells*				
0	—	—	101	0·0
X	6·3	6·4	102	1·5
30	6·2	6·3	102	4·4
90	5·8	6·3	109	9·6
120	5·9	6·4	109	11·6
With complete exchange	—	—	—	112·0
B. *Cells in Medium containing* $0{\cdot}8 \times 10^{-5}$M-$PbCl_2$				
0	—	—	101	0·0
X	6·3	6·3	101	2·4
30	5·7	5·2	92	10·8
60	5·5	5·0	92	11·7
90	5·5	4·9	90	16·2
120	5·6	5·1	91	17·8
With complete exchange	—	—	—	112·0
C. *Cells in Medium containing* $1{\cdot}6 \times 10^{-5}$M-$PbCl_2$				
0	—	—	101	0·0
X	6·2	6·2	101	7·3
30	4·9	3·8	78	44·6
60	4·8	2·9	61	51·0
90	4·9	2·8	57	51·9
120	4·9	2·6	53	57·0
With complete exchange	—	—	—	112·0

At beginning of experiment, all media contained 6·0 μ.equiv./ml. K^+ at 229 counts/μ.equiv.

At end of experiment, medium A contained 5·8 μ.equiv./ml. K^+ at 226 counts/μ.equiv.

At end of experiment, medium B contained 7·0 μ.equiv./ml. K^+ at 184 counts/μ.equiv.

At end of experiment, medium C contained 9·8 μ.equiv./ml. K^+ at 127 counts/μ.equiv.

(X: samples taken as soon as possible after mixing; centrifugation begun within 7 min. of mixing.)

brium with the suspending medium (Sheppard and Martin, 1950). Moreover, a small net gain of K, similar to that described by Danowski (1941), often occurred during this period in the untreated cells (though not in the experiment shown in Table I), and much K was usually lost from the poisoned cells, so that movements in opposite directions through the cell membranes were unequal and calculations of the "exchange rate" were not usually applicable to our conditions. Instead, minimum values for the entry and exit of K in the cells have been calculated and are shown in Table II. It will be observed that the rates of movement, at least inward, were greater at first than later. Similar observations have been made before (Sheppard and Martin, 1950; Raker, Taylor, Weller and Hastings, 1950), and their interpretation is uncertain. At 7° the rates of movement were of the order of one-tenth of those observed at 37° (Table III); there was a slow loss of K from the cells, barely detectable in the first 24 hr. and varying from 4 to 15% of the total cell K in 72 hr.

Cells Treated with Lead Chloride.— In all the experiments, concentrations of heavy metals are recorded in terms of the quantity of metal per unit volume of entire suspension. In fact erythrocytes take up lead rapidly from saline suspensions (Mortensen and Kellogg, 1944), and the final concentration of lead per unit volume of cells may have been up to twenty times greater than the concentrations which are given for the entire suspension. In some experiments, not otherwise reported here, in which a larger proportion of blood to saline was used, the same total concentrations of lead had much less effect, as would be expected if the lead was distributed over a greater number of cells. As the cell suspensions discussed here all contained about the same proportion of cells, and as the amount of lead fixed is uncertain, the total concentration is the most convenient one to use. Comparisons between the concentration of different metals producing similar effects are not significant, as the amounts of mercury or gold fixed by the cells are probably not even approximately equal to the amounts of lead which are fixed.

Lead chloride in sufficient concentrations caused a loss of K from erythrocytes (Table I and Figs. 1 and 2). The curves relating the quantity of K in the cells to time were approximately exponential in shape and are conveniently described by the new level which the cell K approached and by the half-time of the approach, as has been done in

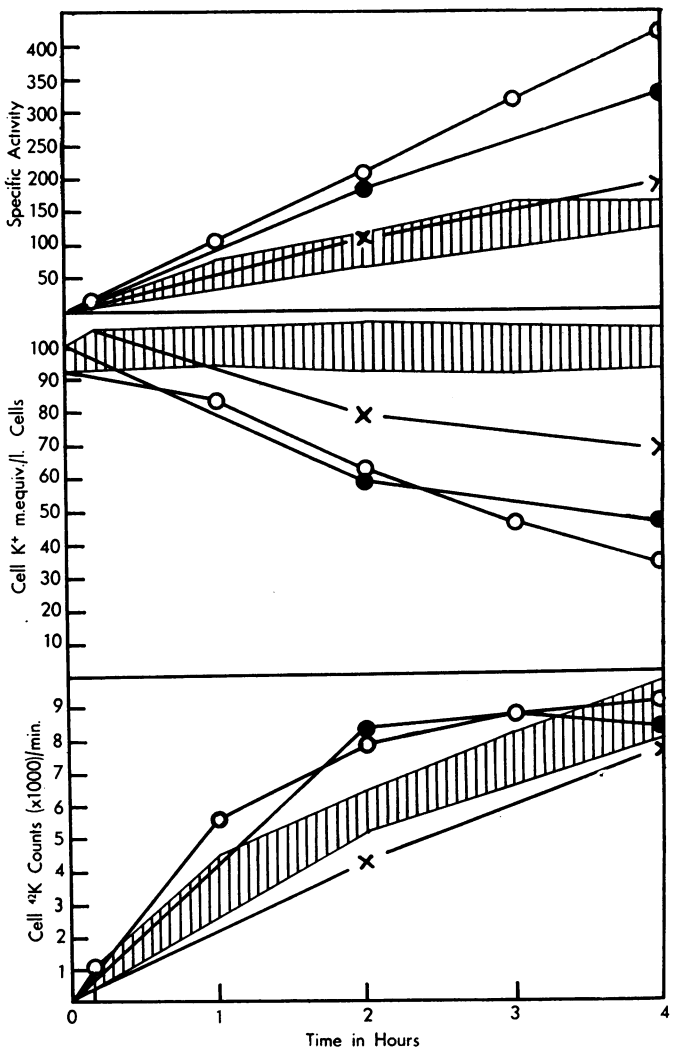

FIG. 1.—The effect of low concentrations of $HgCl_2$, $PbCl_2$, $AuCl_3$ on rabbit blood cells at 37°. Top ordinate: Specific activity, counts/min./m.equiv. cell potassium. Middle ordinate: Potassium, m.equiv./l. of cells. Bottom ordinate: Radioactive potassium, counts/min. Abscissae: Time, hours. ×——× $HgCl_2$ $3{\cdot}2\times10^{-5}$M. ●——● $PbCl_2$ $0{\cdot}8\times10^{-5}$M. ○——○ $AuCl_3$ $2{\cdot}7\times10^{-5}$M. Range of control observations shaded.

Table II. The limiting level S_∞ has been obtained either graphically or by the expression

$$S_\infty = \frac{S_0S_3 - S_1S_2}{S_0 + S_3 - S_1 - S_2}$$

where S_0, S_1, S_2 and S_3 are successive estimates of the quantity of K in the cells either at constant time intervals or such that the interval between S_0 and S_1 is the same as that between S_2 and S_3 (Johnson, 1952). The half-time has been obtained by fitting straight lines by eye to values of $\ln\frac{S-S_\infty}{S_0-S_\infty}$ plotted against time (Fig. 3). Except when small errors in the original measurements had a large effect on the magnitude of the transformed values, most of the results were quite well fitted in this way.

The K concentration approached in the cells appears to depend somewhat on the concentration of Pb. Small changes were observed in 0.8×10^{-5}M-$PbCl_2$, rather variable ones in 1.6×10^{-5}M and in this or any higher concentrations up to 6.4×10^{-5}M the largest losses of K, about 75% of the total contained in the cells, were observed. With still higher concentrations of Pb, haemolysis of some of the cells occurred within an hour, and the changes were not studied in detail. The rate at which the cell K fell was not clearly related to the concentration of Pb.

The cells also lost water in the first half hour after exposure to lead, but not subsequently. As a result, changes in the K concentration in the cells follow a somewhat different course from changes in the total quantity of K, and, on the whole, the changes in concentration were less well fitted by an exponential relationship. A few observations on the cell Na showed that Na was gained as K was lost; but the molecules of Na gained were fewer than those of K lost, and the total (Na+K) in the cells was at least approximately proportional to the cell volume.

These changes were accompanied by a surprising and large increase in the rate of entry of ^{42}K to the cells, so that the total radioactivity of the cells was commonly ten or twenty times that of the untreated controls half an hour after the beginning of the experiment. After about this time, long before the net efflux of potassium was complete, the radioactivity diminished again, and there was usually little further increase in the specific activity of the cells from this time onwards. The changes in the early part of the experiment were rapid, and in view of the difficulty of determining the exact time of separation of cells and supernatant fluid, the course of the uptake and loss of tracer was

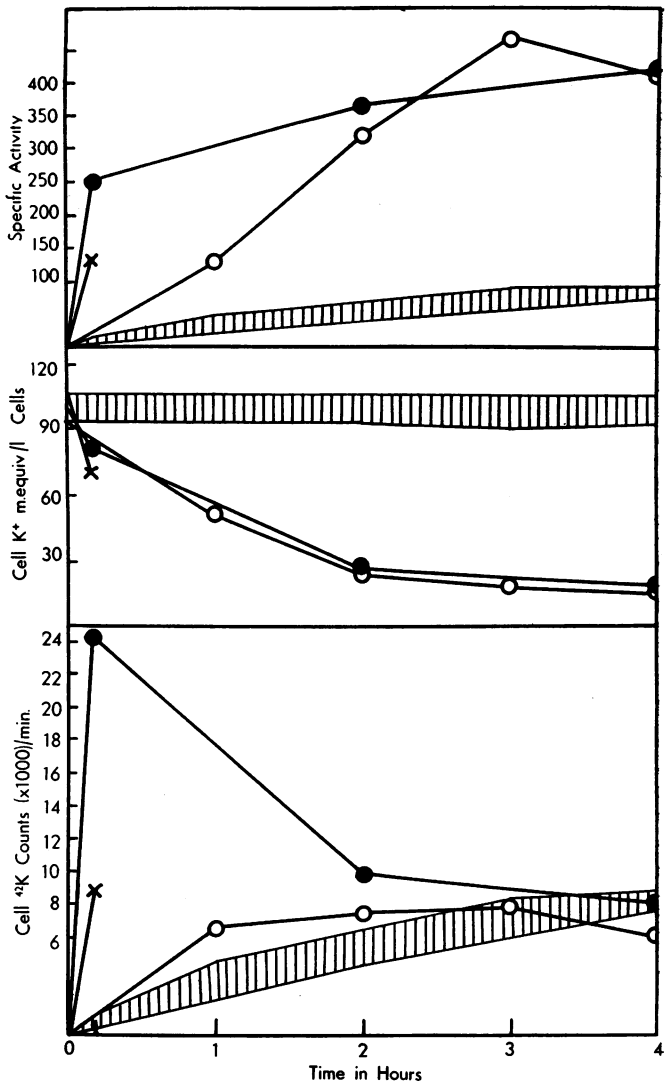

FIG. 2.—The effect of high concentrations of $HgCl_2$, $PbCl_2$, $AuCl_3$ on rabbit blood cells at 37°. Ordinates and abscissae as in Fig. 1. ×——× $HgCl_2$ $6{\cdot}4\times10^{-5}$M. ●——● $PbCl_2$ $1{\cdot}6\times10^{-5}$M. ○——○ $AuCl_3$ $5{\cdot}4\times10^{-5}$M. Range of control observations shaded.

TABLE II

MOVEMENT OF POTASSIUM INTO AND OUT OF RABBIT BLOOD CELLS AT 37° C.
(All values as % of original K^+ concentration)

Condition	Net Change in Cell K^+		Minimum Inward Movement/hr.				Minimum Outward Movement/hr.		
	Equilibrium Level	Half-time of Approach to Equilibrium (min.)	Mode of Calculation	0–½ hr.	0–2 hr.	2–4 hr.	0–½ hr.	0–2 hr.	2–4 hr.
Normal ..	99·4–103·6	—	A B	2·6– 4·8 —	1·3–4·1 1·3–2·7	1·3–1·7 1·1–1·6	0–7·4	0–2·9	0–2·1
$PbCl_2$ $0·8 \times 10^{-5}$M	*ca.* 87·4	*ca.* 18	B	6·6–19·0	3·3–6·0	—	37·8–44·0	15·3	5·7
$1·6 \times 10^{-5}$M	18·9–47·3	31–38	B	19·8–33·4	6·8	0·6	101·2–124·6	34·8–42·3	2·9
$3·2 \times 10^{-5}$M	27·3	16	B	26·8	9·6*	—	151·8	35·7	1·7
$6·4 \times 10^{-5}$M	27·9	17	B	25·8	11·9*	—	142·4	61·9	—
$HgCl_2$ $3·2 \times 10^{-5}$M	*ca.* 100·0	—	A or B	—	2·0	2·0	—	2·0	0
$6·4 \times 10^{-5}$M	59·0	88	B	—	2·2	2·0	—	12·1	7·4
$AuCl_3$ $0·5 \times 10^{-5}$M	*ca.* 100·0	—	A or B	—	3·1	1·6	—	0–1·7	1·7–2·8
$2·7 \times 10^{-5}$M	< 36·0	—	B	—	5·6	2·0–3·0	—	39·6	3·5
$5·4 \times 10^{-5}$M	15·1–16·8	—	B	—	2·5–3·5	1·8	—	10·2–39·2	6·5–20·5

A = Calculated by the method of Sheppard and Martin (1950). B = Calculated by linear approximation. * = 0–90 min. figure.

not followed in detail, and the time and the magnitude of the peak of the curve showing the uptake of ^{42}K by the cells have not been determined accurately. The linear interpolations in Figs. 1 and 2 are a convenient way of connecting related points rather than an attempt to represent the true course of the reaction. The mechanism of these changes is discussed later, but it seems worth remarking that this greatly increased flow of K into the cells occurred against a substantial concentration gradient, and decreased rapidly and almost completely before the cell K finished falling. It is also notable that the specific activity showed little subsequent change, although it was not identical in cells and plasma; usually about half to three-quarters of the total possible exchange had taken place at this time. With the higher concentrations of lead, haemolysis began to occur after one or two hours, and with the lower concentrations after about four to twenty hours, and observations were not continued when this began to happen.

Minimum rates of entry and exit of K are shown in Table II: their calculation is subject to much larger errors than the calculation for normal cells; but all the errors tend to make the figures too small, and the actual rates may well be two or more times the estimated minima. Nevertheless, the estimated rates of entry in the first two hours vary from two to six times those normally occurring, whereas the minimal rates of exit are up to sixteen times the normal, and are therefore undoubtedly very much increased.

At 7° similar changes occurred more slowly, so that the cell K did not reach its minimum until 24–48 hr. or longer, and the peak in the cell radioactivity was reached after two or three hours. One difference was very striking; this was the

TABLE III

MOVEMENT OF POTASSIUM INTO AND OUT OF RABBIT BLOOD CELLS AT 7° C.
(All values as % of original K^+ concentration)

Condition	Net Change in Cell K^+		Minimum Inward Movement/hr.				Minimum Outward Movement/hr.		
	Equilibrium Level	Half-time of Approach to Equilibrium (min.)	Mode of Calculation	0–2 hr.	0–24 hr.	24–48 hr.	0–½ hr.	0–24 hr.	24–48 hr.
Normal ..	92·0–100·0	—	B	0·9	0·10–0·17	0·06–0·11	0	0·11–0·20	0·18–0·35
$PbCl_2$ $0·8 \times 10^{-5}$M	28·3	1,560	B	6·9	0·60	0·13	14·6	2·7	1·20
$1·6 \times 10^{-5}$M	16·9–18·8	448–620	B	22·9*	0·20–1·00	0·09–0·10	16·3–32·4	3·0–4·3	0·13–0·30
$HgCl_2$ $1·6 \times 10^{-5}$M	> 80	—	B	—	0·13	0·05	—	0·20	0·30
$6·4 \times 10^{-5}$M	> 80	—	B	0·20	0·13	0·08	0·20	0·35–3·6	0·50
$12·8 \times 10^{-5}$M	Not calculable		B	3·4	—	—	43·6	—	—
$AuCl_3$ $5·4 \times 10^{-5}$M	4·1	1,860	B	—	0·20	0·33	0	1·23	1·65
$10·8 \times 10^{-5}$M	Not calculable		B	0·6	1·10	—	2·0–2·9	3·4–4·5	0·05

B = Calculated by linear approximation. * = 0–60 min. figure.

absence of haemolysis, in concentrations up to 3.2×10^{-5}M, even when the cells were maintained on the rotor for up to 72 hr.; at 37° the same concentration of lead was sufficient to cause gross haemolysis within four to eight hours.

Cells Treated with Mercuric Chloride.—Superficially, Hg had similar effects to Pb, but there were several differences of detail. Haemolysis appeared to depend more on the concentration of mercury than on the temperature; at either 7° or 37° lysis occurred within two hours after 12.8×10^{-5}M, and between 6 and 24 hr. after 6.4×10^{-5}M. Any lysis that was observed was always extensive, whereas after lead the appearance of haemoglobin in the supernatant fluid was often very gradual and not necessarily the harbinger of prompt generalized destruction of the cells. Potassium loss from Hg-poisoned cells was either rapid and followed quickly by haemolysis, or much slower than from Pb-poisoned cells and towards a level not less than half the original cell concentration. There was no appreciable change in cell volume, and the entry of tracer was accelerated only slightly (when the net changes were not extensive) or considerably when the cells were about to haemolyse. No striking flow of tracer back from the cells to the medium occurred, but the exchange rate of the poisoned cells probably fell after a time to levels below those of the untreated controls. At 7° the changes were similar though slower, and, as at 37° the range between inactive and haemolytic concentrations was not large, detailed studies were not made.

Cells Treated with Gold Chloride.—Again there was a broad resemblance to the effects of Pb, and differences of detail. In most respects the effects of Au lay between those of Pb and of Hg. Haemolysis occurred less quickly and less extensively than after Hg; at 37° concentrations of Au sufficient to cause appreciable loss of K began to cause lysis after about 6 hr. The K losses themselves were usually greater in the second and third hours than in the first hour at 37°, or about the sixth to tenth hours at 7°. Correspondingly, the exponential transformation of the results did not give a straight line, and the nearest approach to such a line is shown in Fig. 3. Accelerated uptake of ^{42}K was greater than after Hg (taking as comparable concentrations those which produced an approximately equal loss of cell K) but less than after Pb, and the time of the maximum uptake corresponded to the time of the maximum loss of K from the cells. Slight swelling of the cells occurred rather than shrinkage, and it remains to be seen how this relates to changes in the cell Na content.

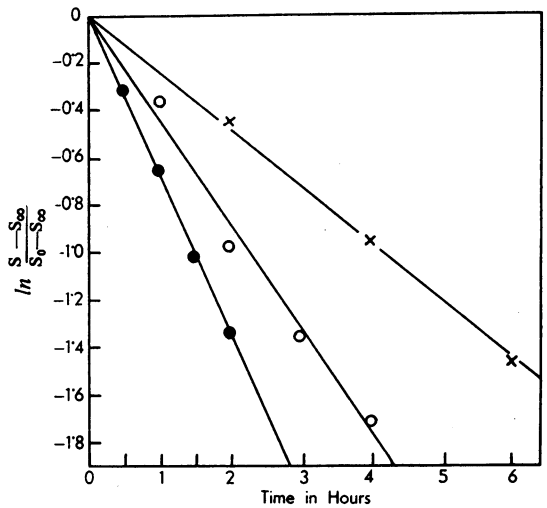

FIG. 3.—The rate of loss of potassium from poisoned rabbit blood cells at 37°. Ordinate: $\ln \frac{S-S_\infty}{S_0-S_\infty}$ (see text). Abscissa: Time, hours. ×—×—× $HgCl_2$ $3{\cdot}2 \times 10^{-5}$M. ●——● $PbCl_2$ $1{\cdot}6 \times 10^{-5}$M. ○—○—○ $AuCl_3$ $5{\cdot}4 \times 10^{-5}$M.

DISCUSSION

The most striking finding in these experiments was that the loss of potassium from poisoned cells was generally, and particularly after lead, accompanied by an accelerated entry of potassium into the cells. If the metal affected the cell potassium simply by increasing the permeability of the cells to the ion, the influx as well as the efflux would be accelerated. The absolute acceleration of the influx would be much smaller because of the much lower external concentration of potassium. The increments which we have observed appear to be too large to be accounted for in this way; also, the rate of inflow is much greater in the first half hour or so after poisoning than it is later, so a simple increase in permeability is not sufficient to account for the findings.

If the cells are regarded as consisting of a membrane which offers a fairly high but not complete resistance to the passage of potassium, and a pump which injects potassium (or ejects sodium, or both) against the concentration gradient, it is necessary to postulate also some homeostatic mechanism which controls the pump, since at 37° the cell potassium and sodium concentrations normally remain fairly constant even when the environmental concentrations are altered considerably (Raker *et al.*, 1950; Solomon, 1952; Streeten and Solomon, 1954). On these assumptions lead may be regarded mainly as damaging the cell

membrane, so that its resistance to the passage of potassium is greatly reduced. As soon as potassium leaks out of the cells, the activity of the pump is increased and the rate of entry of potassium is raised five- to ten-fold, though not sufficiently to make up for all that is leaking out. Later, possibly because there are insufficient metabolites to maintain this high rate of working for a long time, the pump decreases in activity and the cell potassium concentration settles down at a new level; possibly also at this stage the initial increase in the permeability of the cell membrane is followed by a decrease as it becomes further disorganized. With mercury and gold there is less injury to the cell membrane and more interference with the pump, either directly or by obstructing the metabolism which supplies the necessary energy, and so the cells lose potassium both because they are more leaky and because the pump fails to respond adequately to the decline in the cell potassium concentration.

This hypothesis may be correct, but it is perhaps not very useful in the present state of knowledge about permeability of membranes and about homeostatic pumps at the cellular level. Also, the rates of movement of potassium are evidently changing too rapidly and in too complicated a fashion for the present experiments in themselves to throw much light on the details of either process.

Such a pump-and-permeability hypothesis also presupposes that the cells are behaving uniformly and that their internal ionic concentration may be treated as constant throughout the population. This appears to be true of normal human erythrocytes (Raker *et al.*, 1950; Sheppard and Martin, 1950), but it seems quite likely that some cells will be more affected than others by poisons: this is certainly so after a time, when some cells haemolyse while others do not, and, *a priori*, it is probably better to assume that the early changes in the cells are not uniformly distributed over the whole population. In this case, measurements of the average rate at which the population of cells are admitting or extruding potassium have a very questionable significance, and, to take an extreme position, some of the present results can be explained if a proportion of the cells become so permeable to potassium and sodium that they equilibrate completely with the external medium during the experiments, while other cells remain entirely or almost entirely unaffected. (A somewhat similar argument has been advanced by Ponder (1951) in connexion with the potassium loss from human erythrocytes suspended in an isotonic K-free medium.) The extreme case of an all-or-none response is insufficient to account for the observed efflux of ^{42}K from lead-poisoned cells, though it may be applicable in mercury poisoning. But the examination of intermediate situations is complicated and does not appear profitable at present.

That all the observed changes under the influence of heavy metals show much the same dependence on a change of temperature from 37° to 7° suggests that they are acting on a single process rather than partly on permeability and partly on a pump; it is unlikely that these two processes are equally sensitive to temperature changes, or that the extent to which they are upset by poisoning is dependent on temperature just sufficiently to offset their initial difference. The delay in the onset of changes after exposure to gold also requires explanation; it may indicate that some metabolic process is inhibited for some time before its effects become apparent, or it may simply indicate that a longer period is necessary for the gold itself to react with its receptors.

Numerous other substances, probably including all haemolytic agents, cause loss of potassium and gain of sodium in erythrocytes (Davson and Danielli, 1938; Ponder, 1948; Maizels, 1951), but we have failed to find observations from which estimates of the actual rates of movement of ions into and out of the cells can be made. The present observations show that estimates of permeability based simply on net gains or losses of ion species (e.g. Davson and Reiner, 1942) can give more or less identical values with greatly differing rates of movement. Accurate treatment of the situation when the rates are changing rapidly is difficult, but is evidently necessary for any pharmacological examination of the control of cell electrolytes to be useful.

Summary

1. Changes have been observed in the K content and in the entry of ^{42}K into rabbit blood cells poisoned with lead chloride, mercuric chloride and gold chloride at 37° and 7°.

2. All three metals caused loss of K from the cells, and later (except lead chloride at 7°) haemolysis.

3. Lead chloride greatly accelerated the entry of ^{42}K into the cells, at the same time as net losses of K were occurring. These changes occurred both at 37° and 7°, but were followed by haemolysis only at 37°.

4. Mercuric chloride mainly caused loss of K from the cells, but with large losses and incipient haemolysis the ^{42}K entry was accelerated.

5. Gold chloride acted less rapidly than the other two substances, and then produced changes intermediate between their effects.

6. The interpretation of these changes is discussed.

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