appreciably affected by the presence of magnesium or manganese ions.

2. The monophosphates of oestradiol are much weaker inhibitors than oestradiol 3:17-diphosphate, while oestrone glucoside does not exert any inhibitory effect in the concentrations investigated.

3. The inhibitory effect of oestradiol 3:17-diphosphate on alkaline phosphatases is greatest in

the region of pH 7.5-9.3, and is not demonstrable on the alkaline side of the pH optimum.

4. The inhibition is independent of the nature of the substrate; using phenyl phosphate, β -glycerophosphate, or glucose-6-phosphate, essentially the same degree of inhibition is observed.

5. The inhibition of alkaline phosphatase by oestradiol 3:17-diphosphate is reversible and non-competitive.

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The Fate of Radioactive Potassium Chloride Administered Intraperitoneally or Intravenously to Rats

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It is known that when potassium salts are administered intravenously to animals the small increase found in the concentration of potassium in the serum can only be explained by assuming that the injected potassium is distributed in a volume of body water greater than that of the extracellular fluid. At least some of the cells of the body, therefore, are readily permeable to potassium. Hahn, Hevesy & Rebbe (1939), Joseph, Cohn & Greenberg (1939), Noonan, Fenn & Haege (1941), Fenn, Noonan, Mullins & Haege (1941) and Hevesy (1942) have studied the distribution of injected radioactive potassium salts. Noonan *et al.* (1941) administered radioactive potassium chloride intraperitoneally to rats and found that equilibrium between the extracellular and intracellular potassium of liver, heart, lung, kidney, gut and skeletal muscle was reached in 4-6 hr. The doses of potassium chloride varied from 6 to 59 mg. (0.3-4.1 m-equiv. potassium/kg.). Within 0.5-1 hr. after the injection, the liver, heart,

lungs, kidneys and gut were so highly radioactive as to suggest that radioactive potassium had, at least, in part entered the cells as a result of a process other than exchange. They remarked that it was not possible, on the basis of their experiments, to separate the two processes.

In the experiments described below, the distribution of large doses of ^{42}KCl after a number of hours and of doses of 0.01 and 1 mg. of ^{42}KCl 5 min. after injection was studied.

METHODS

Female albino rats weighing between 192 and 230 g. were used.

The potassium salts used were irradiated KCl or K_2CO_3 . The latter was neutralized to pH 8 by N-HCl and used in some experiments since the irradiation of KCl in the pile of the Atomic Energy Research Establishment, Harwell, yields ^{42}K and traces of ^{36}S and ^{32}P . We are grateful for information from Harwell that the activity due to NaCl present

as an impurity in the irradiated KCl accounted for about 0.1 % of the total radioactivity.

The decay curves of samples of ^{42}KCl followed the theoretical curve for pure ^{42}K during the 3 or 4 days following irradiation. This was the period during which the experiments were carried out, so that in calculating results, the half-life period of the radioactive material was taken to be 12.4 hr. The decay curve of $^{42}\text{K}_2\text{CO}_3$ followed the theoretical curve for 7–8 days, after which the two curves were different, an effect probably due to traces of rubidium present as an impurity in the K_2CO_3 .

Four solutions of ^{42}KCl or neutralized $^{42}\text{K}_2\text{CO}_3$ in distilled water were prepared: A, 2 %; B, 0.2 %; C, 0.002 %; and D, 0.00002 %. B, C and D were obtained by diluting A with distilled water. D was used as a standard of ^{42}K activity and A, B and C were used for injections. The results to be described later in this paper were the same whichever solution was injected.

Two groups of experiments were performed. *Series 1.* Rats were taken from stock and injected intraperitoneally with radioactive material. After the appropriate time the rat was anaesthetized with sodium amytal (10 mg./100 g. rat, intraperitoneally), the carotid artery was dissected out and the animal bled for a period of about 1 min. into a centrifuge tube containing a trace of heparin. By this means about half the total amount of blood of the rat was usually obtained easily and without loss. The organs were quickly removed, weighed, and dissolved in hot concentrated HNO_3 . Foaming was minimized by the use of an antifoam silicone grease smeared on the stirring rods. The digests were made up to a known volume using dilute HNO_3 . *Series 2.* Rats were starved for 24 hr. but were allowed water. Each rat was then fed 2 ml. of milk by stomach tube and kept at 25°, and quiet. 1–2 hr. later, the animal was anaesthetized with sodium amytal and then injected either intraperitoneally or intravenously (the intravenous injection occupying 2–3 min.) with radioactive material. After 5 min. the animal was bled and the organs were analysed.

The radioactivity of the digests was determined by means of an M. 6 liquid counter (Veall, 1948). These and the counts obtained from a standard solution were corrected for decay

since the time of injection. Usually, the counts on a sample were recorded to give a total of 4500 or more to reduce the error of counting. The specific gravity of the solution did not influence the rate of counting in the specific gravity range 1.00–1.30 met with in the solutions used.

Recovery experiments were carried out on a number of rats in both series. The carcasses, after removal of a number of organs which were 'counted' separately, were dissolved in HNO_3 . The fat which separated was extracted with ether, the extracts were washed with dil. HNO_3 and the combined aqueous phases were diluted to a known volume and then 'counted'.

Table 1 is a record of a typical experiment giving details of the experimental observations and of the method of calculation.

RESULTS

Series 1. Ten rats were given equiactive doses of 2 % potassium chloride in distilled water intraperitoneally. The amount of potassium chloride injected varied from 1 to 80 mg. (0.06–4.83 m-equiv. potassium/kg.). The animals were killed after 6, 24 and 48 hr. and the tissues analysed for radioactivity as described above.

Noonan *et al.* (1941) calculated, for each tissue, the ratio of $^{42}\text{K}/^{39}\text{K}$ in the tissue to that in the plasma, and called this the relative potassium activity (R.P.A.) of the tissue. Therefore, when the radioactive potassium in the extracellular fluid has reached equilibrium with the intracellular potassium, the R.P.A. is 1. The R.P.A. of the tissues of the rats in Series 1 were calculated, assuming values (obtained from other experiments) for the potassium content of the tissues studied. The findings of Noonan *et al.* (1941) were, in general, confirmed in that after 6–48 hr. the R.P.A. of the tissues examined was about 1 (Table 2), except that skeletal muscle had not quite reached equilibrium in 6 hr. The recovery of ^{42}K varied from 90 to 96 %.

Table 1. Record of a typical experiment showing the radioactivities of various tissues after intravenous injection of ^{42}KCl

(Rat no. 33 (body wt. 204 g.) starved for 24 hr. prior to being given 2 ml. of milk at 8.55 a.m. on 3 May 1950. At 10.45 a.m. 0.25 ml. of 10 % sodium amytal solution was injected intraperitoneally. At 11.00 a.m. 0.5 ml. radioactive KCl solution containing 1.0 mg. KCl was injected intravenously over 3 min. At 11.05 a.m. the rat was bled and the tissues removed, weighed, dissolved in HNO_3 , and counted. Amount injected equivalent to 120,000 counts/min.; background count, 13/min. Volume counted, 8 ml.)

Organ	Wt. of organ (g.)	Vol. of diluted digest (ml.)	Counts/min. (uncorr.)	Counts/min. (corr.) to time of injection*	Counts/min.		a/g. tissue as percentage of injected counts/min.
					Total in organ (a)	a as percentage of injected counts	
Liver	5.73	25	2,640	3,316	10,363	8.6	1.5
Kidney	0.78	25	1,850	2,271	7,096	5.9	7.6
Heart	0.69	25	860	1,038	3,243	2.7	3.9
Lung	1.08	25	644	781	2,440	2.0	1.9
Plasma	2.92	25	464	544	1,699	1.4	0.48
Residue	—	500	1,130	1,496	93,520	77.9	—
					Recovery	98.5 %	

* Allowance made for resolving time of counter, the background count, and radioactive decay.

Table 2. *Mean values of relative potassium activity (R.P.A.) 6, 24 and 48 hr. after injection of radioactive potassium into rats*

(R.P.A. is the ratio of $^{42}\text{K}/^{39}\text{K}$ in the tissue to that in the plasma.)

Time (hr.)	Liver	Kidney	Heart	Muscle
6	0.85 (1.46)	0.96 (1.23)	1.04 (1.33)	0.63 (1.02)
24	1.05	0.87	1.04	0.89
48	0.92	0.91	0.89	0.78

Figures in brackets are mean R.P.A. values for the 5 and 6.5 hr. experiments of Noonan *et al.* (1941).

The results, however, give no indication of the initial distribution of the potassium injected or of the mechanisms by which the final distribution is achieved. Noonan *et al.* (1941) determined the dis-

tribution of ^{42}K injected 30 min. previously, but they used quantities of potassium comparable with those normally present in all the extracellular fluid. Under these conditions transport of potassium ions into cells was probable. This was avoided in Series 2 in which very small quantities of potassium chloride, containing amounts of potassium approximately equal to 0.05 and 5% of the total extracellular potassium, were injected intraperitoneally or intravenously.

Series 2. The results reported in Table 3 were obtained from rats prepared as described earlier. This seemed to lessen the scatter of the results. The animals were anaesthetized with sodium amytal and then given 1.0 or 0.01 mg. of radioactive potassium chloride in 0.5 ml. of water intraperitoneally or intravenously into the jugular vein. After 5 min. the rats were bled and the organs removed and

Table 3. *Distribution of ^{42}K 5 min. after intraperitoneal or intravenous injection of radioactive potassium into rats*

(Results are expressed as percentage (x) of total radioactivity. x is defined by the expression

$$x = \frac{\text{counts/min./g. tissue}}{\text{total injected counts/min.}} \times 100 D,$$

where D is a correction factor allowing for decay. The irradiated material for Exps. 25-33 was K_2CO_3 , neutralized as described above.)

Wt. of KCl injected in 0.5 ml. water (mg.)	Rat no.	Liver	Kidney	Heart	Lung	Plasma	Recovery (%)
Intraperitoneal							
0.01	25 }	2.22	2.72	0.85	0.63	1.11	92.3
	26 }	2.45	2.00	1.14	0.52	1.27	92.0
	34 }	2.40	2.10	—	—	0.29	—
	35 }	1.22	1.40	—	—	0.25	—
1.0	27	2.32	3.58	1.74	0.82	0.35	98.4
	28 }	3.37	3.03	1.16	0.76	0.47	97.3
	29 }	3.46	3.55	1.84	1.08	0.52	102
	38 }	2.48	3.16	—	—	0.28	—
	39 }	2.92	2.35	—	—	0.24	—
	Means	2.54	2.65	1.35	0.76	0.53	—
Intravenous							
0.01	30	1.53	7.65	4.79	2.57	0.29	99.6
	36 }	1.50	8.45	—	—	0.34	—
	37 }	1.72	7.91	—	—	0.36	—
	(23)†	1.41	7.33	4.63	2.11	0.35	93.6
1.0	31	1.66	8.16	5.53	2.22	0.58	103
	32 }	1.27	6.67	3.26	1.77	0.44	95.3
	33 }	1.51	7.62	3.89	1.89	0.49	98.4
	40 }	2.34	6.86	—	—	0.45	—
	41 }	1.44	7.54	—	—	0.42	—
	57 }	1.82	7.21	—	—	0.19	—
	58 }	1.79	10.95	—	—	0.32	—
	59 }	1.69	7.95	—	—	0.22	—
	60 }	2.25	9.28	—	—	0.27	—
	Means	1.69	7.95	4.42	2.11	0.36	—
	85* }	1.63	7.95	—	—	0.52	—
	86* }	2.53	10.10	—	—	0.65	—

* KCl solution injected made up in normal saline.

† Rat 23 had no preparation prior to injection.

analysed. When the injection of ^{42}KCl was given intraperitoneally, the abdominal organs were dipped in saline before analysis. Activities were calculated as percentage of injected radioactivity/g. of tissue, allowance having been made for radioactive decay.

Observations common to experiments in which intraperitoneal or intravenous injections were administered (Table 3)

Although the doses given were 0.01 and 1.0 mg. KCl , for a given route of injection, the uptake of ^{42}K in 5 min. by the tissues was not widely different.

The activities of the tissues were high 5 min. after injection, far higher than can be accounted for by the extracellular fluid present.

The radioactivity of the plasma showed wide variations. The experiments which have been bracketed were carried out on the same day. It will be observed that the plasma activities of these pairs of experiments agree closely but we are unable to account for the fact that the plasma activities varied widely on different days.

Recovery of injected ^{42}K ranged from 92 to 103 %.

Results of intraperitoneal injections (Table 3)

The activities per g. of liver and kidney were high and of the same order.

The plasma activities varied from 0.24 to 1.27 %.

Results of intravenous injections (Table 3)

The lung, heart and kidney showed very high uptakes of radioactive potassium in comparison with the corresponding uptakes observed after intraperitoneal injection.

The activity of the plasma varied from 0.19 to 0.58 %, but did not show as large a variation as in the corresponding series in which the dose was given intraperitoneally, although the orders of activity were similar.

The activity of the liver was about half, and that of lung, heart and kidney about three times that observed after intraperitoneal injection.

The very high activity of the lungs, heart and kidneys was not due to the hypotonicity of the injected fluid. The results obtained from rats 85 and 86 in which the injected fluid was ^{42}KCl in 0.9 % sodium chloride were identical with those obtained when aqueous solutions of ^{42}KCl were injected.

DISCUSSION

Five minutes after intraperitoneal injection of ^{42}KCl the tissues of the intestine contained 4–5 % of the injected $^{42}\text{K/g.}$ After intravenous injection, this figure was 2.5–3 %, a change comparable with that

which occurred in liver. In similar experiments, the gastrocnemius muscle contained less than 0.3 % of the injected $^{42}\text{K/g.}$ whether the injection was given intraperitoneally or intravenously.

Previous workers have established the general distribution of injected potassium in rats after periods varying up to 22 hr., and reached the conclusion that the element was then distributed according to the distribution of body potassium. In our experiments, the doses of potassium salts varied more widely than in the experiments of Noonan *et al.* (1941), but the final distribution of the injected material appeared to be the same; that is, according to the distribution of body potassium.

The phrase 'transport of potassium' is used in this paper to mean the entry of potassium ions with anions into the cells because of osmotic requirements and as a mechanism for the removal of an excess of potassium ions from the extracellular fluid. Exchange operates as a result of a dynamic equilibrium between intra- and extra-cellular potassium ions. Hence, if a quantity of radioactive potassium, small in amount (say, less than 5 %), compared with the total potassium in the extracellular fluid were injected, any entry of ^{42}K into the tissues should be the result of ionic exchange, and not of transport.

On the assumption that the total extracellular fluid in a rat of 200 g. is 35–40 ml., the extracellular potassium would amount to about 7–8 mg. Thus, the amount of ^{42}K injected in the experiments referred to in Table 3 could not increase the total extracellular potassium by more than 0.06 and 6 %. Neither increase is likely to lead to transport of potassium into the cells. In spite of this, liver, heart, lung and kidney were highly radioactive 5 min. after the injection. This must be ascribed to a process of rapid exchange between intra- and extra-cellular potassium ions and indicates a considerable degree of permeability of these cells to potassium. Within 5 min. of injection the gastrocnemius muscle, on the other hand, showed scarcely any activity, so that the surmise of Noonan *et al.* (1941, p. 487) 'that it is possible that exchange is no more rapid in liver than in muscle' is not supported.

The rate at which the injected solution leaves the peritoneal cavity is proportional to the hypotonicity of the solution (Cunningham, 1926; Orlow, 1895). The solutions of 0.2 and 0.002 % potassium chloride used in our experiments were very rapidly absorbed, about 50 % of the active material leaving the peritoneal cavity in the first minute or so. In 5 min. 90 % was absorbed. The absorption of active potassium from the somewhat hypertonic solutions used by Noonan *et al.* (1941) would depend partly upon exchange and partly upon the rate at which the fluid injected could be converted into a plasma ultrafiltrate. This would involve transport of potassium into the portal blood. That these pro-

cesses of transport and of exchange are fairly rapid is shown by the statement by these workers that only about 15 % of active material remained in the peritoneal cavity after 10 min.

Since the order of radioactivity of the plasma following intravenous and intraperitoneal injection is practically the same, the two sets of experiments are comparable. The notable differences concern the activities of the liver, kidney, heart and lung. The increase in activity of the kidney, heart and lung when the solution was injected intravenously over that found after intraperitoneal injection was roughly in the same proportion for each organ. It can be calculated that because of their favourable anatomical positions when the intraperitoneal route is used for injection the liver and gut remove, by exchange, some 40–50 % of the active potassium injected during the passage of the highly radioactive blood through these tissues for the first time. This must indicate therefore a very high exchange rate.

After intraperitoneal injection it is probable that absorption of potassium occurs mainly into the portal blood, so that the liver cells are perfused with blood rich in ^{42}K and, being readily permeable to potassium ions, a large proportion of ^{42}K from the perfusing blood exchanges with potassium of the liver cells. The blood in the hepatic vein would have, therefore, a lower radioactivity than the portal blood. The cells of the lungs, heart and kidney, though readily permeable to potassium ions, are perfused with blood of lower activity than the liver and so their opportunity for acquiring ^{42}K by exchange is less. The liver is therefore anatomically the most favourably situated of the organs mentioned and this would account for its high radioactivity. When the injection is given intravenously, however, the liver is least favourably situated, since only a part of its

blood supply is derived from arterial blood rich in ^{42}K . The portal venous blood has already traversed the gut, the cells of which are readily permeable to potassium ions. This interpretation accords with the differences shown in Table 3. No attempt has been made to calculate the extent of the exchange which has occurred in the experiments lasting 5 min., because the activity of the plasma leaving the different organs was not known. The values of plasma activity given in Table 2 cannot be assumed to represent the activity of the venous outflow from any particular organ. In experiments of short duration, therefore, R.P.A. values are misleading.

SUMMARY

1. The distribution of 1–80 mg. of potassium chloride injected intraperitoneally after 6, 24 and 48 hr. has been studied using radioactive potassium (^{42}K) as a tracer. The distribution at equilibrium is according to the location of the body potassium for the tissues analysed.

2. The distributions of 1.0 and 0.01 mg. of radioactive potassium chloride administered intraperitoneally or intravenously 5 min. previously are given and the differences discussed.

3. Evidence is presented that there is a rapid exchange between the extra- and intra-cellular potassium of the liver, gut, kidney, heart and lung, but it is slow for the gastrocnemius muscle.

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