On a high-fat, low-protein diet, these changes were more pronounced. However, the decrease in the weight of the livers and their low fat content was particularly striking when the fat content of the diet was 10%. The content of fat in the diet thus appears to influence the changes brought about by the element.

Tables 2 and 4 show clearly that the development of bones is affected adversely in both the series. The observations of Smith & Larson (1946) that high-zinc diets bring about an anaemia, retarded growth rate and lowered food intake in rats, and the results presented in this paper show that zinc probably affects metabolism in general and assimilation of phosphorus in particular.

SUMMARY

1. The effect of supplements of zinc at levels of 0·5 and 1·0% on the livers and bones of rats has been investigated.

2. Zinc supplements caused a marked lowering of the fat content of livers of rats fed on a diet on which control rats developed fatty liver.

3. Zinc appears to act as a lipotropic factor in the concentrations studied. This element also interferes with development and mineralization of bones.

My thanks are due to Major-General S. S. Sokhey, Director, and Dr K. Ganapathi, Assistant Director, for their interest in this investigation.

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In vitro Measurements of the Turnover Rate of Potassium in Brain and Retina

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(*Received* 16 *October* 1950)

It has been established by the isotope technique (Joseph, Cohn & Greenberg, 1939; Hahn, Hevesy & Rebbe, 1939; Fenn, Noonan, Mullins & Haege, 1941–2; Noonan, Fenn & Haege, 1941) that the potassium ions of animal tissues and of the body fluids continually interchange. The high concentration gradients of potassium between tissues and blood plasma, therefore, cannot be due to permeability barriers, but must be the resultant of two opposing processes: 'leakage' from the tissues, which tends to level out the concentration differences, and 'active', i.e. energy-fed, transport into the tissues, which counteracts the effects of 'leakage'. Under normal conditions the rates of the two opposing processes are usually equal and the problem presents itself of determining their velocities, i.e. the 'turnover rate' of potassium. Data published by Fenn *et al.* (1941–2) and Noonan *et al.* (1941) indicate great variations of the velocities from tissue to tissue. In general the visceral organs exchange potassium much more rapidly than skin, resting muscle or red blood cells. The only material for which accurate quanti-

tative data are available are human red blood cells. On the average 1·6–2·0% of the cellular potassium exchanges in 1 hr., the agreement between the measurements of three independent teams being satisfactory (Raker, Taylor, Weller & Hastings, 1950; Sheppard & Martin, 1950; Solomon, 1950).

Terner, Eggleston & Krebs (1950) reported previously that slices of brain tissue and pieces of retina maintain their normal potassium content when kept in a saline medium containing oxygen, glucose and L-glutamate. If one of these substrates is absent the potassium concentrations in tissue and medium level out, but the loss of potassium to an inadequate medium can be restored by the addition of the missing substrate. Tissues suspended in saline media thus represent a system in which the exchange of ions with the medium can be studied. The present paper is concerned with measurements of the 'turnover rate' of potassium in brain cortex and in retina. ^{42}K was added to the saline medium and the rate of disappearance of the isotope was measured. From these measurements the turnover rate is calculated.

A preliminary account of this work was given to the Biochemical Society on 17 June 1950 (Krebs, Eggleston & Terner, 1950).

METHODS

Incubation of tissues. Guinea pig brain was handled as described by Stern, Eggleston, Hems & Krebs (1949). The technique of Deutsch (1936) was used for slicing brain cortex and the procedure of Terner *et al.* (1950) for preparing pieces of ox retina. The thickness of the brain slices was about 0·4 mm., that of the retina about 0·25 mm. Immediately after weighing on the torsion balance, the tissue, about 100 mg. fresh wt., was put into the medium in a conical Warburg vessel. The medium consisted of 2 ml. of bicarbonate saline supplemented by various substrates. The gas space was filled with 5% CO_2 and 95% O_2. All operations were carried out as quickly as possible and about 2 min. were required for preparing the tissue and the manometer for incubation. The manometers were attached to the water bath at 2 min. intervals, shaken at 40° and removed after varying periods of incubation.

The quantities of tissue and saline were so chosen that the initial K content of both was about equal. This favoured accuracy of the measurements.

Handling of ^{42}K. For reasons given later ^{42}K was not mixed with the medium at the start, but after a preliminary incubation period of 45 min. A solution of KCl (0·1 ml.) containing ^{42}K was placed in the side arm of the manometer flask, the quantity of ^{42}K being adjusted so as to give about 4000 counts/min. The amount of total K in the side arm was 0·008–0·032 mg., i.e. about 2–8% of the K in the main compartment. Thus the additional amount of K did not appreciably change the level of K in the medium. At the end of the incubation period the tissue was removed from the medium, drained by rolling on a glass plate, weighed on a torsion balance and dropped into 2·5 ml. of 0·84% NaCl solution. This was stored in the refrigerator for about 4 days to allow for the decay of ^{42}K. The tissue was then ground up in a mortar and 1 ml. of the suspension was diluted with 10 ml. of 0·84% NaCl solution. This diluted tissue suspension was used for the determination of K by flame photometry according to Domingo & Klyne (1949).

The medium in the Warburg cup, after the removal of the tissue, was washed out with several portions of 0·1 N-KCl solution, and medium and washings were made up to a total volume of 10 ml. A KCl solution rather than water was used for washing to prevent retention of ^{42}K by the glass vessel (see Crane & Davies, 1949). The radioactivity of the solution was measured in the liquid Geiger-Müller counting tube of Veall (1948) (Type M-6, 20th Century Electronics Ltd). Corrections were made for isotope decay, dead time and background counts and also for the amount of medium removed from the cup with the tissues (see Terner *et al.* 1950). The error in determination of the radioactivity was of the order of $\pm 3\%$.

The intial K content of the medium and of the tissue was measured on separate samples of the materials; the total K content of tissue plus medium was therefore known. The K content of the medium at the end of the incubation was calculated from the observed K content of the tissue and the total K content of the system. The reliability of this procedure was tested by direct determination of the K content of the medium in special experiments. The value was found to agree within the limits of error with the calculated value.

RESULTS

Changes in the potassium content of brain slices and retina on incubation in vitro

The quantitative analysis of data on the uptake of ^{42}K by tissues is much simplified if the concentration of total potassium ($^{39}K + {}^{42}K$) in the tissue remains constant on incubation. Preliminary tests were therefore carried out to examine whether this holds true. In the previous experiments (Terner *et al.* 1950) the potassium concentration of brain slices found after 40–60 min. incubation was, in fact, of the same order as the initial concentration, but further experiments show that the tissue potassium concentration does not remain constant throughout the period of incubation (Table 1). A substantial loss of potassium occurs during the first 5 min. This is largely recovered within the following 20–30 min. and subsequently the level remains approximately constant for 20–30 min. In some experiments the stabilized potassium level was about the same as the starting value, in others it was up to 20% below the starting value. With ox retina, as already described previously, potassium is lost during transport from the abattoir. During the initial stages of incubation the potassium of the tissue rises to a level similar to that found in fresh tissue and remains constant for the 30–60 min. period. The main experiments with ^{42}K therefore began after a preliminary incubation period of 45 min., when the potassium concentration in brain and retina had become approximately stabilized. The use of homologous blood serum did not prevent the initial loss of potassium by the tissue

Table 1. *Changes in the concentration of potassium in guinea pig-brain cortex and ox retina on incubation* in vitro

(Each vessel contained 2 ml. medium and 80–100 mg. tissue (wet wt.). Glucose (0·02 M) and L-glutamate (0·01 M) were added to both saline and serum.)

Exp. no.	Tissue	Medium	Period of incubation (min.)	Amount of K found in tissue (m-equiv./kg.): Before incubation	After incubation: Total	After incubation: Change	Amount of K found in medium (m-equiv./l.): Before incubation	After incubation
1	Brain cortex	Saline	5	99·0	52·9	−46·1	5·20	6·96
			10	99·0	69·0	−30·0	5·20	6·50
			20	99·0	76·0	−23·0	5·20	6·17
			40	99·0	79·4	−19·6	5·20	5·85
2	Brain cortex	Guinea pig serum	5	93·6	57·5	−36·1	7·32	9·04
			10	93·6	61·7	−31·9	7·32	8·97
			20	93·6	74·2	−19·4	7·32	7·95
			40	93·6	73·5	−20·1	7·32	8·15
3	Brain cortex	Saline	30	98·4	77·5	−20·9	5·28	5·81
			40	98·4	94·5	− 3·9	5·28	5·28
			50	98·4	88·8	− 9·6	5·28	5·62
			60	98·4	82·9	−15·5	5·28	5·73
4	Brain cortex	Guinea pig serum	30	106·0	96·4	− 9·6	7·46	8·03
			40	106·0	98·4	− 7·6	7·46	7·70
			50	106·0	98·6	− 7·4	7·46	7·72
			60	106·0	86·2	−19·8	7·46	8·36
5	Brain cortex	Ox serum	30	101·5	33·4	−68·1	6·50	9·06
			40	101·5	30·9	−70·6	6·50	9·35
			50	101·5	28·2	−73·3	6·50	9·70
			60	101·5	24·7	−76·8	6·50	9·60
6	Retina	Saline	30	39·8	65·6	+25·8	5·32	3·68
			40	39·8	73·7	+33·9	5·32	3·51
			50	39·8	76·7	+36·9	5·32	3·04
			60	39·8	72·8	+33·0	5·32	3·03
7	Retina	Ox serum	30	40·2	59·8	+19·6	6·46	5·58
			40	40·2	67·6	+27·4	6·46	4·95
			50	40·2	71·6	+31·4	6·46	4·65
			60	40·2	82·2	+42·0	6·46	4·20

(Table 1); saline medium was therefore used in the main experiments. In heterologous serum brain slices lost more potassium than controls suspended in saline and the loss was not reversed on prolonged incubation.

Uptake of ^{42}K *by tissues*. A series of parallel manometer vessels containing the tissue suspended in the saline medium was incubated as described, and at graded intervals vessels were removed from the bath for analysis. One sample of tissue was put aside for the determination of the initial potassium content. Preliminary experiments showed that a measurable uptake of ^{42}K occurred within a few minutes and intervals of 2–4 min. were therefore chosen. The results are recorded in Table 2. The tenth column of the table shows that the radioactivity of the medium rapidly decreased whilst the total potassium content of the medium (eighth column) showed no major changes. The data confirm that a rapid exchange of potassium between tissue and medium occurs, the rate being somewhat greater in retina than in brain.

Calculation of the rate of potassium exchange. The following quantitative treatment of the data is based on three simplifying assumptions, namely: (i) That the total potassium content (i.e. ^{39}K + ^{42}K) in tissue and in medium remains constant; in other words that the quantities of potassium ions moving into, and out of, the tissue per unit time are equal. The data in Table 2, columns 4 and 6, show that this supposition is approximately correct. (ii) That all tissue potassium is 'free'; i.e. that there is only one form of potassium in the tissue. That this assumption is approximately correct for brain and retina is borne out by the observation that 80–90% of the tissue potassium rapidly diffuse into the medium when the energy supply is cut off by the withdrawal of oxygen and substrate. (iii) That the rate of potassium exchange under the experimental conditions (i.e. in thin layers of tissue) is a function of the whole tissue and not of the surface layer only. The basis for this assumption is the fact that the maintenance of the steady state depends on the supply of energy which

Table 2. *Migration of* ^{42}K *into brain slices and retina*

(Tissue suspended in bicarbonate saline containing 0·02 M-glucose and 0·01 M-L-glutamate. ^{42}K added as ^{42}KCl after 45 min. preliminary incubation.)

Exp. no.	Tissue: Organ	Tissue: Fresh weight (mg.)	Period of incubation after addition of ^{42}KCl (min.)	Amount of K found: Tissue: Before incubation (m-equiv./kg.)	Amount of K found: Tissue: After incubation (m-equiv./kg.)	Amount of K found: Medium: Before incubation (m-equiv./l.)	Amount of K found: Medium: After incubation (m-equiv./l.)	Radioactivity of medium (counts/min.): Before incubation	Radioactivity of medium (counts/min.): After incubation	Rate of exchange: (m-equiv. K exchanged/min.) ($\times 10^{-4}$)	Rate of exchange: $\frac{\text{m-equiv. K exchanged/min.}}{\text{total tissue K}} \times 100$: Individual slice	Rate of exchange: $\frac{\text{m-equiv. K exchanged/min.}}{\text{total tissue K}} \times 100$: Average
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
1	Brain	84	2	100·0	102·9	5·65	5·53	3470	3275	3·40	4·0	**4·0**
		84	4	100·0	95·8	5·65	5·81	3470	3130	3·31	4·1	
		77	6	100·0	89·0	5·65	6·06	3470	3050	2·96	4·3	
		84	8	100·0	91·0	5·65	6·03	3470	2960	2·80	3·7	
2	Brain	103	4	101·0	95·0	5·95	6·25	880	795	3·50	3·6	**3·4**
		83	8	101·0	81·5	5·95	6·75	880	800	1·91	2·8	
		80	12	101·0	91·0	5·95	6·35	880	725	2·64	3·6	
		97	16	101·0	96·1	5·95	6·18	880	640	3·31	3·6	
3	Brain	80	4	102·5	96·5	5·65	5·88	3410	3060	3·45	4·5	**4·5**
		87	6	102·5	86·6	5·65	6·33	3410	3005	3·03	4·0	
		90	8	102·5	96·0	5·65	5·94	3410	2700	4·20	4·9	
4	Brain	86	8	102·5	107·1	5·95	5·75	3275	2690	3·30	3·6	**3·5**
		105	12	102·5	93·8	5·95	6·41	3275	2550	3·28	3·3	
		88	16	102·5	100·9	5·95	6·03	3275	2380	3·28	3·7	
5	Retina	127	2	58·5	63·2	5·95	5·65	3140	2930	4·21	5·3	**7·4**
		125	4	58·5	76·7	5·95	4·82	3140	2510	6·14	6·4	
		102	6	58·5	86·0	5·95	4·55	3140	2240	6·60	7·5	
		93	8	58·5	87·1	5·95	4·63	3140	1980	8·49	10·5	
6	Retina	109	2	68·1	73·1	5·95	5·68	4090	3570	8·54	10·7	**10·7**
		132	4	68·1	73·5	5·95	5·60	4090	3160	8·69	9·0	
		119	6	68·1	64·0	5·95	6·19	4090	2890	11·46	15·0	
		86	8	68·1	93·9	5·95	4·84	4090	2785	6·62	8·2	

is produced evenly throughout the tissues. It is difficult to assess the extent to which this assumption is valid. It is obvious that in a very thick piece of tissue loss of constituents from the surface layer must be more rapid than from the central layers. The critical thickness below which central and surface layers do not behave differently remains to be ascertained. Let a = quantity of total potassium in medium, b = quantity of total potassium in tissue, x = quantity of ^{42}K in medium at time t, y = quantity of ^{42}K in tissue at time t, v = quantity of total potassium passing from tissue to medium, and in the opposite direction, per unit time. A known amount (x_0) of ^{42}K is added to the medium at $t=0$. The total quantity of ^{42}K in the whole system is assumed to remain constant

$$x+y=x_0.$$

In the time dt the quantity of ^{42}K passing into the tissue is

$$\frac{x}{a}v\,dt,$$

and the quantity of ^{42}K travelling in the opposite direction is

$$\frac{y}{b}v\,dt.$$

Therefore $$\frac{dx}{dt}=v\left(\frac{y}{b}-\frac{x}{a}\right)=v\left(\frac{x_0-x}{b}-\frac{x}{a}\right).$$

Integrating

$$x=x_0\frac{a}{a+b}+A\exp\left[-vt\left(\frac{1}{a}+\frac{1}{b}\right)\right].$$

For $t=0$, $x=x_0$, so that

$$A=\frac{x_0 b}{a+b}.$$

Hence $$x=\frac{ax_0}{a+b}+\frac{bx_0}{a+b}\exp\left[-vt\left(\frac{1}{a}+\frac{1}{b}\right)\right]$$

or $$v=\frac{ab}{t(a+b)}\ln\frac{bx_0}{x(a+b)-ax_0}. \qquad (1)$$

This equation may be used to calculate the rate of potassium exchange (v) from sets of data for t and x. It is noteworthy that the value of v is independent of the units in which x and x_0 are expressed. The absolute values of x and x_0 are therefore not required for the calculation of v, the relative values, as measured by the Geiger counter, being sufficient. The turnover rate, expressed as percentage of tissue potassium turned over per unit time equals $v/a\times 100$. The accuracy of the evaluation of v is evidently low when the value of x is either near x_0 or near its maximum, $ax_0/(a+b)$; x should be between 30 and 70 % of its maximum.

The problem of calculating the turnover rate in the type of system under consideration has been treated before by Cohn & Brues (1945), Sheppard (1948), Raker *et al.* (1950) and Sheppard & Martin (1950). The present treatment is similar to that of the previous workers, especially that of Sheppard & Martin. It is somewhat simpler and more suited to the particular experimental data. It should be noted that the theory is independent of, and supplies no information on, the mechanism by which the ion exchange is effected.

On applying equation (1) to the measurements recorded in Table 2 the figures given in the last two columns of this table are obtained. For the purpose of the calculation it is ignored as unimportant that the potassium concentration in the tissue and in the medium were for practical reasons measured in different units (mg./kg. and mg./l. respectively). The figures for the turnover rates can be regarded as reasonably consistent, considering that each figure is based on a considerable number of independent measurements, which are all liable to error, and that the premises on which the theory rests are only approximately true. The data for brain show less scattering than those for retina, although the handling of retina, which did not involve slicing, might be expected to be less injurious. Possibly differences in the histological structure of the various parts of the retina are responsible for some of the variations.

The average turnover rates for brain were between 3·5 and 4·0 % and of retina 7 and 10 %/min. Compared with the turnover rate of red blood cells (0·03 %/min. according to Raker *et al.* 1950; Sheppard & Martin, 1950; and Solomon, 1950) these rates are very high. In brain potassium exchanges on the average about 120 times and in retina about 250 times more rapidly than in human red cells.

Experiments on other tissues

The potassium levels of slices of guinea pig liver, pigeon pancreas and pigeon gizzard (smooth muscle) on incubation in saline media and serum were investigated in the same way as with brain and retina, but it proved impossible to maintain the normal potassium level of the tissue *in vitro*. Slices of these tissues are thus not satisfactory material for turnover rate studies. Guinea pig-kidney cortex, on the other hand, was found to behave similarly to brain.

Liver. Guinea pig-liver slices, containing initially 85–90 m-equiv. potassium/kg., lost 40–60 % of their potassium content on aerobic incubation in saline media or guinea pig serum. The loss occurred within the first 10 min. of incubation and was not prevented by the following additions to the saline media (0·01 M, unless otherwise stated): glucose (0·02 M), glucose plus L-glutamate, pyruvate, succinate, fumarate, citrate, α-ketoglutarate, oxaloacetate, adenosine triphosphate (0·002 M) in combination with various substrates. Addition of hormones known to play a role in the distribution of electrolytes, namely adrenaline (10^{-3}–10^{-7} M), ephedrine (10^{-3}–10^{-5} M) plus adrenaline, extract of adrenal cortex ('eschatin', Parke Davis and Co., 1–10 dog units/ml.) was like-

Table 3. *Effect of various substrates on the loss of potassium by slices of guinea pig-kidney cortex*

(About 100 mg. slices (wet wt.) incubated in 2 ml. bicarbonate saline; 40°; 5% CO_2 in O_2.)

Exp. no.	Substrate (final concentration)	Period of incubation (min.)	Amount of K in tissue: Initially (m-equiv./kg.)	After incubation: Found (m-equiv./kg.)	After incubation: Change (%)
1	None	5	70·2	44·4	−27
	Glucose (0·02 M); L-glutamate (0·01 M)	5	70·2	54·0	−23
	None	10	70·2	44·2	−27
	Glucose (0·02 M); L-glutamate (0·01 M)	10	70·2	60·0	−15
	None	20	70·2	49·7	−29
	Glucose (0·02 M); L-glutamate (0·01 M)	20	70·2	64·5	− 8
	None	40	70·2	45·6	−35
	Glucose (0·02 M); L-glutamate (0·01 M)	40	70·2	51·8	−26
2	None	30	72·5	51·6	−29
	Glucose (0·02 M)	30	72·5	46·2	−36
	L-Glutamate (0·01 M)	30	72·5	61·3	−15
	Glucose (0·02 M); L-glutamate (0·01 M)	30	72·5	61·3	−15
3	None	30	66·3	44·0	−34
	Fumarate (0·01 M)	30	66·3	44·5	−33
	α-Ketoglutarate (0·01 M)	30	66·3	68·3	+ 3
	DL-Aspartate (0·02 M)	30	66·3	55·2	−17
	L-Glutamate (0·01 M)	30	66·3	53·2	−19
4	None	30	73·4	40·3	−45
	α-Ketoglutarate (0·01 M)	30	73·4	77·7	+ 6
	NH_4Cl (0·01 M)	30	73·4	26·3	−64
	NH_4Cl (0·01 M); α-ketoglutarate (0·01 M)	30	73·4	45·7	−38
	Citrate (0·01 M)	30	73·4	36·0	−51
5	None	30	75·5	51·5	−32
	Pyruvate (0·01 M)	30	75·5	47·6	−37
	Succinate (0·01 M)	30	75·5	51·6	−32
	Citrate (0·01 M)	30	75·5	34·2	−55
6	None	30	76·2	51·6	−32
	α-Ketoglutarate (0·01 M)	30	76·2	72·3	− 5
	α-Ketoglutarate (0·005 M)	30	76·2	63·7	−17
	α-Ketoglutarate (0·0025 M)	30	76·2	56·7	−26
	α-Ketoglutarate (0·00125 M)	30	76·2	52·3	−31

wise ineffective, as was an increased potassium concentration up to five times the normal level, i.e. 25 m-equiv./l. Anaerobically the potassium loss was of the order of 70%. After the initial loss the potassium level in the tissue became stabilized at concentrations still four to ten times higher than that of the medium. Similar results were obtained with pigeon liver.

Pancreas. The initial potassium concentration of pigeon pancreas was found to be 100–120 m-equiv./kg. On incubation in saline media 20–30% of the tissue potassium were lost, irrespective of the presence of substrates. Apart from glucose and L-glutamate and the intermediates of the tricarboxylic cycle, glutamine, asparagine, casein hydrolysate and α-ketoglutarate plus ammonium chloride were tested.

Pigeon gizzard. The muscular layer was examined as an example of smooth muscle. The initial potassium concentration was 115–120 m-equiv./kg., 50–80% of which were lost on incubation. Again addition of substrates was ineffective.

Kidney cortex. Data on the changes of the tissue potassium level of sliced guinea pig-kidney cortex on incubation in saline media are shown in Table 3. When no substrate was added about one-third of the tissue potassium was lost on aerobic incubation and about two-thirds on anaerobic. Addition of L-glutamate, L-aspartate and α-ketoglutarate to the medium reduced the potassium loss, the latter being the most effective substrate. At a concentration of 0·01 M, α-ketoglutarate always prevented the loss of potassium, whilst pyruvate, succinate, citrate, fumarate and glucose had no effect. As in brain cortex and retina there was a rapid initial loss even in the presence of α-ketoglutarate or glutamate. This was reversed on further incubation.

DISCUSSION

Turnover rates in vitro *and* in vivo. The question may be raised whether measurements of turnover rates on isolated tissues reflect the behaviour of the tissue *in situ*. No definite answer can be given, but we see no decisive arguments against the assumption

that slices of brain and pieces of retina maintain the potassium gradients between tissue and environment in the same way as the intact organ, and that the turnover rates found *in vitro* are of the same order as those *in vivo*. However, the behaviour *in vivo* remains to be tested by independent methods of investigation. The rapid and irreversible loss of potassium by some tissues, e.g. liver, is no doubt an indication of tissue damage, but even *in vivo* losses of potassium may readily occur as an early, perhaps the earliest, sign of damage, as a result of injury (Cuthbertson, 1936), of surgical operations (Wilkinson, Billing, Nagy & Stewart, 1950; Blixenkrone-Møller, 1949), of asphyxia (Cattell & Civin, 1938) or shock (Holmes, 1947).

Mechanism of potassium discharge by tissues. The rapid initial loss of potassium from freshly cut tissue slices bears on the mechanism by which potassium is discharged by the tissue. In two experiments on brain cortex recorded in Table 1, 47 and 39 % of the tissue potassium were lost within 5 min. The average rate of loss was thus 9 and 8 %/min. Other experiments, not recorded in the table, showed that most of the loss found after 5 min. occurred within the first 3 min. and that the rate of loss during the first minute was over 20 % of the tissue potassium. This is a much higher rate than the turnover rate of potassium in the steady state and it follows that the 'leakage' rate of potassium is not constant, the discharge being less in the steady state than in freshly prepared slices. It cannot be assumed, therefore, that the migration of potassium from the tissue to the medium is solely due to passive leakage. Actively controlling factors must also be operative, a conclusion which has already been drawn by other workers from observations on the increased rate of discharge following the stimulation of muscle or nerve.

The time course of the loss of and recovery of potassium by brain tissue recalls the rapid onset and gradual disappearance of the injury potentials of animal tissues. Changes in the tissue potassium concentration have long been assumed to be the main cause of these potentials (see Cowan, 1934).

Effect of heterologous serum. The fact that in heterologous serum brain tissue fails to maintain normal potassium levels may be ascribed either to an increased rate of potassium discharge or to a reduced rate of potassium uptake. As the respiration, and thus the energy supply, is not adversely affected by serum, it is more likely that the point of attack of serum is the mechanism controlling the discharge rather than the uptake of potassium. The loss of tissue potassium caused by heterologous serum may possibly have the same basis as the losses due to injuries (Cuthbertson, 1936), surgical operations (Wilkinson *et al.* 1950; Blixenkrone-Møller, 1949) or the action of hormones.

Potassium and glycogen synthesis. Buchanan, Hastings & Nesbett (1949) have found that the synthesis of glycogen from glucose and pyruvate in liver slices is optimal if the potassium concentration of the medium approximates to that of the tissues. This observation may be explained on the basis of the present results. A high potassium concentration in the tissue appears to be essential for the synthesis of glycogen. The synthesis is therefore bound to be adversely affected by the inability of liver slices to maintain their normal potassium level in the usual media. In rat diaphragm, which on incubation in a saline medium does not lose potassium ions (Kamminga, Willebrands, Groen & Blickman, 1950), an increased potassium concentration of the medium does not stimulate the rate of glycogen synthesis (Stadie & Zapp, 1947).

Energy relations. If the rate of exchange of potassium between tissue and environment is known, it is possible to calculate the energy required to maintain the concentration gradient using the formula

$$-\Delta F = RT \ln \frac{C_1}{C_2},$$

where $-\Delta F$ is the change of free energy, or work of concentration, required to bring 1 g.mol. of potassium from the concentration C_1 to the concentration C_2, R the gas constant (1·987 cal./degree) and T the absolute temperature (see Borsook & Winegarden, 1931). In brain cortex C_1/C_2 is about 18 and 1 kg. of tissue contains about 0·1 g.mol. of potassium. The energy required for potassium transport by brain cortex is therefore 365 cal./hr./kg. Assuming the Q_{O_2} value of -18 µl./mg. dry wt./hr. (Krebs, 1950) and wet wt./dry wt. ratio for guinea pig-brain cortex of 6 (based on measurements in this laboratory; see also Palladin & Bjeljaewa, 1924) the energy available for respiration is about 15,000 cal./hr./kg. Thus about 2·5 % of the available energy is required for the maintenance of the normal potassium level. Considering that potassium is only one of many diffusible cell constituents and that there may be concentration differences within the cells (see Opie, 1948, 1949), it would seem that a substantial proportion of the energy provided by brain respiration serves to maintain the unstable environment of the tissue.

SUMMARY

1. Slices of guinea pig-brain cortex lose about 40 % of their potassium content by discharge into the medium within a few minutes on being suspended in a saline medium or in homologous serum. The normal potassium content is restored in about 30 min. on aerobic incubation if glucose and L-glutamate are present; thereafter it remains approximately constant for 20–30 min.

2. After a preliminary incubation period of 45 min., i.e. after a steady state had been established, ^{42}K in the form of ^{42}KCl was added to slices of guinea pig-brain cortex and to pieces of ox retina, and the rate of migration of ^{42}K into the tissue was measured. From these measurements the rate of potassium exchange in the steady state was calculated. The turnover rate $\left(\frac{\text{potassium exchanged/min.}}{\text{potassium present in tissue}} \times 100\right)$, was 3·5–4 in brain cortex and 7–10 in retina.

3. With liver (guinea pig, pigeon), pancreas (pigeon) and smooth muscle (pigeon gizzard) the normal potassium gradient between tissue and environment was not maintained when the tissues were suspended in saline media or homologous serum. The bearing of this observation on the finding of Buchanan *et al.* (1949) that the rate of synthesis of glycogen in liver slices is optimal when the potassium concentration of the medium resembles that of the tissue is discussed.

4. The potassium level of sliced guinea pig-kidney cortex behaved similarly to that of brain and retina except that α-ketoglutarate was more effective in maintaining the normal level than L-glutamate.

The authors are indebted to Dr C. A. Stewart, Department of Mathematics, University of Sheffield, for contributing the mathematical part of this paper.

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Studies in Detoxication

35. SPECTROPHOTOMETRIC DETERMINATION OF β-GLUCURONIDASE USING *p*-CHLOROPHENYLGLUCURONIDE AS SUBSTRATE

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(*Received* 8 *July* 1950)

In recent years, there has been a considerable interest in the enzyme β-glucuronidase. Its only proved activity is the hydrolysis of conjugated β-glucuronides, but the work of Levvy and his co-workers (Levvy, Kerr & Campbell, 1948; Karunairatnam, Kerr & Levvy, 1949*a*; Kerr, Campbell & Levvy, 1949, 1950; see also Odell & Fishman, 1950) shows that when there is an increase in proliferative activity in certain tissues there is also an increase in the β-glucuronidase activity. The enzyme has been found to occur in increased amounts in cancer tissue (Fishman & Anlyan, 1947; Karunairatnam, Kerr & Levvy, 1949*b*; Odell & Burt, 1949) and in tissues undergoing repair after damage (Levvy *et al.* 1948). The use of β-glucuronidase as an agent for the smooth hydrolysis of β-glucuronides is likely to increase,