utilization were observed between the diaphragms of 'cold' and 'warm' rats. Acetoacetate utilization was identical in the two types of rat.

3. Glycogen synthesis and glucose utilization by the abdominal muscle of mice previously exposed to cold were also higher than those of mice maintained at a higher temperature.

4. If Krebs-Ringer-phosphate was substituted for homologous serum as medium, no difference in glucose utilization was observed between the diaphragms of 'cold' and 'warm' rats, and differences in glycogen synthesis were smaller under these conditions.

5. The extent of the temperature variations to which the rats were exposed was not proportional to the metabolic effect in the diaphragm. The effect was still observable when rats were maintained at 15 or 20°, respectively.

6. Glycogen synthesis was much reduced in the diaphragms of rats maintained at 37° compared with that in the diaphragms of rats maintained at 30°, and glycogenolysis was often observed. This effect was demonstrable only when homologous serum and not Krebs-Ringer-phosphate was used as medium. Glycogenolysis was also observed in diaphragms of rats in which fever had been induced by amphetamine sulphate.

7. Rat-liver slices were capable of synthesizing almost as much glycogen from glucose in serum as in Hastings's medium. No differences in oxygen uptake nor in the production of acetoacetate were observed between liver slices from 'cold' and 'warm' rats.

REFERENCES

- Buchanan, J. M., Hastings, A. B. & Nesbett, F. B. (1949). *J. biol. Chem.* **180**, 435.
 Dixon, M. (1951). *Manometric Methods*, 3rd ed. New York: Macmillan.
 Fishgold, J. T., Grant, R., Field, J. & Hall, V. E. (1951). *Amer. J. Physiol.* **166**, 113.
 Gemmill, C. L. (1940). *Johns Hopk. Hosp. Bull.* **66**, 232.
 Good, C. A., Kramer, H. & Somogyi, M. (1933). *J. biol. Chem.* **100**, 485.
 Grant, R. & Hirsch, J. D. (1950). *Amer. J. Physiol.* **161**, 528.
 Hansen, R. G., Rutter, W. R. & Samuels, L. T. (1951). *J. biol. Chem.* **192**, 243.
 Krah, M. E. & Cori, C. F. (1947). *J. biol. Chem.* **170**, 607.
 Krebs, H. A. (1950). *Biochim. biophys. Acta*, **4**, 491.
 Rappaport, F. (1949). *Rapid Microchemical Methods for Blood and C.S.F. Examinations*, 1st ed., p. 45. Springfield: Thomas.
 Somogyi, M. (1937). *J. biol. Chem.* **117**, 771.
 Tuerkischer, E. & Wertheimer, E. (1948). *Biochem. J.* **42**, 603.
 Wertheimer, E. & Bentor, V. (1950). *Exp. Med. Surg.* **8**, 378.

An Investigation into the Turnover Rates of Organophosphates

1. EXTRACELLULAR SPACE AND INTRACELLULAR INORGANIC PHOSPHATE IN SKELETAL MUSCLE

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In experiments involving the determination of the turnover rates of those organophosphates for which inorganic phosphate (inorganic P) is thought to be the immediate source of phosphorus, it is necessary to determine the specific radioactivity (counts/min./ μ g.) of the inorganic P. Furthermore, since these compounds are metabolized within the cell, interest centres upon the intracellular inorganic P. The inorganic P contained in a trichloroacetic acid extract of tissue is the sum of the extracellular and the intracellular inorganic P, and it is necessary to differentiate between these moieties if the turnover rate is to be determined. Considerable emphasis has been placed upon the necessity for such a differentiation by Furchgott & Shorr (1943) and by

Kalckar, Dehlinger & Mehler (1944). The problem has been investigated by these two groups of workers in different ways. Furchgott & Shorr (1943), using slices of heart muscle, have shown that inorganic P may, by a washing technique, be separated into two well defined fractions. One fraction, which was readily diffusible and constituted 30–40% of the total inorganic P of the tissue, was regarded as the extracellular inorganic P. The other fraction was retained in the tissue and was regarded as being of intracellular origin. Kalckar *et al.* (1944), in *in vivo* experiments, employed a perfusion technique and claimed to have achieved a similar separation. Hevesy & Hahn (1940), on the other hand, have calculated the specific radioactivity of the intra-

cellular inorganic P by a method involving the volume of the extracellular space. A similar method was used later by Sacks & Altshuler (1942).

Although the perfusion technique of Kalckar *et al.* (1944) has been criticized in the second paper of this series (Ennor & Rosenberg, 1954), on the grounds that marked changes occur in the specific radioactivities of organophosphates on perfusion, it was, nevertheless, thought worthwhile to subject the method to further examination. This communication is concerned with an investigation into the validity of the approaches to the problem of differentiating between intra- and extra-cellular inorganic P made by Kalckar *et al.* (1944) and Hevesy & Hahn (1940).

The subject-matter of this paper was presented to the Victorian Society of Experimental Pathology on 10 December 1951.

EXPERIMENTAL

Animals. Well-fed, adult, hutch rabbits of about 2.4 kg. body weight were used throughout.

Isotopes. ^{32}P and ^{24}Na were obtained from the Atomic Energy Research Establishment, Harwell, and were used in the form of $\text{NaH}_2^{32}\text{PO}_4$ and $^{24}\text{NaCl}$, respectively. All samples of ^{32}P were heated at 100° for 1–2 hr. in N-HCl to convert any pyro-P present into ortho-P. The samples were then adjusted to pH 7.0 by the addition of 2N-NaOH and about 1 mg. of carrier P was added. A 1.0 ml. sample of the solution was injected intravenously and contained in all cases about 1 mc of ^{32}P and approx. 0.3 mg. of ^{31}P .

The amounts of ^{24}Na injected varied and are specifically referred to in the text.

Preparation of tissue extracts. For those experiments involving only ^{32}P , the thigh muscles either before or after perfusion were treated as described by Ennor & Rosenberg (1954). In the experiments in which both isotopes were administered, the procedure described by Ennor & Rosenberg (1952*a*) was followed. Some experiments have been carried out using ^{24}Na only and in these instances portions (about 5 g.) of the thigh muscles, after removal, were dropped into a tared vessel containing 5N-NaOH . The vessel was then weighed, placed in a water bath at 60° , and shaken at intervals and the solution, when homogeneous, was quantitatively transferred to a measuring flask and made up to known volume with water.

^{31}P and ^{32}P determinations. The concentration and radioactivity of inorganic P only was determined in these experiments, and the methods used were those described by Ennor & Rosenberg (1952*b*), using the isobutanol extraction procedure. Appropriate decay corrections and variations in counter sensitivity were made by reference to a ^{32}P standard.

^{24}Na determinations. In some experiments both ^{32}P and ^{24}Na were injected into the same animal. The separation of the ^{24}Na from the ^{32}P and the subsequent determination of its radioactivity was carried out as described by Ennor & Rosenberg (1952*a*).

In those experiments in which ^{24}Na alone was employed, measurements of radioactivity were made directly on the alkaline digest of the muscle using a technique similar to that described above.

Perfusion. Perfusion of the hind limb was carried out as described in the following paper (Ennor & Rosenberg, 1954).

RESULTS

The initial experiments were designed to determine the specific radioactivity of the intracellular inorganic P. The approach to this problem followed the lines suggested by Hevesy (1948), who has pointed out that the specific radioactivity of the intracellular inorganic P may be derived indirectly by calculating the contribution which the extracellular phase makes to the total inorganic P. Such a calculation is possible if the specific radioactivity and concentration of the inorganic P in the muscle extract and plasma and the volume of the extracellular space in the muscle are known. This calculation involves certain assumptions which Hevesy (1948) believed to be justified.

From the results of a series of experiments, the contribution which the extracellular inorganic P makes to the radioactivity (counts/min.) of the total inorganic P has been calculated (Table 1), assuming that the extracellular space occupies 12% of the muscle volume and that both the concentration and specific radioactivity of the inorganic P have identical values in that space and in plasma (Hevesy, 1948).

It is apparent, in those experiments wherein the muscle was removed from the hind limb and dropped into liquid oxygen 45 min. or less after the injection of ^{32}P (with the exception of the 30 min. experiment), that the calculated contribution which the extracellular inorganic P makes to the total number of counts/min. in the inorganic P fraction actually exceeds this total. Such a result must be indicative of either an incorrect value for the extracellular space and/or the falsity of the assumptions upon which the calculations were based, provided that the extraction technique and the methods used for determination of ^{31}P and ^{32}P were reliable. That these can be relied upon is indicated by the results of previous investigations (Ennor & Rosenberg, 1952*a*, 1954). Attention was at first directed to the generally accepted value of 12% for the volume of the extracellular space in skeletal muscle. This value was obtained by Manery & Bale (1941), who worked on muscles which were carefully dissected and thus contained no large blood vessels, minimal amounts of fibrous tissue and were free from tendons. In our experiments a block of thigh muscle was rapidly removed and dropped into liquid oxygen. The frozen material was ground in a chilled mortar so that the sample taken for extraction would include a greater proportion of blood vessels, fibrous tissue, etc., than was the case in Manery & Bale's (1941) material. The possibility, therefore, existed that a figure of 12% for the extracellular space could not

Table 1. *Inorganic phosphate concentrations and specific radioactivities in plasma and muscle relevant to the calculation of the contribution which the extracellular inorganic phosphate makes to the total radioactivity of the inorganic phosphate in muscle*

Period after ³² P injection (hr. min.)	Muscle		Plasma		Radioactivity of inorganic P (counts/min./g. muscle)	
	(mg./100 g.)	(counts/ min./μg.)	(mg./100 g.)	(counts/ min./μg.)	Total observed (B × E × 10)	Calculated extracellular contribution (0.12 × A × D × 10)
	B	E	A	D		
5	18.4	71.7	5.6	3 860	13 200	26 000
5	18.8	33.6	4.6	3 070	6 310	16 920
10	18.1	38.0	4.4	1 760	6 875	9 290
15	19.6	33.5	4.5	1 630	6 560	8 800
15	16.3	55.5	3.3	3 770	9 050	14 920
15	23.4	33.6	4.7	3 220	7 860	18 150
15	21.0	36.2	5.5	1 665	7 600	9 990
30	23.2	34.0	3.9	1 201	7 890	5 635
45	22.0	32.9	4.4	2 320	7 240	12 250
20 00	26.7	20.0	4.3	42.2	5 340	218
24 00	24.8	19.3	7.0	31.8	4 710	267
90 00	16.8	16.5	4.9	19.2	2 860	113

Table 2. *The simultaneous determination of Na space and the contribution which the extracellular inorganic phosphate makes to the total inorganic phosphate in muscle*

(²⁴Na injected at zero time. ³²P injected at zero plus 20 min. Animal killed at zero plus 30 min.)

Tissue	²⁴ Na found (counts/ min./g.)	Na space as % muscle volume	Inorganic phosphate		Radioactivity of inorganic phosphate (counts/min./g. muscle)	
			(mg./100 g.)	(counts/min./g.)	Total observed (B × E × 10)	Calculated extracellular contribution (0.111 × A × D × 10)
Plasma	8690	—	4.4 (A)	1760 (D)	—	—
Perfused muscle	374	—	—	—	—	—
Non-perfused muscle	962	11.1	18.1 (B)	38.0 (E)	6875	9290

be applied to the present experimental material, and it was decided to determine the volume of the space on the same sample of muscle as was used for ³¹P and ³²P analyses.

For this purpose a rabbit, under Nembutal anaesthesia, (45 mg./kg. intravenously) was given an intravenous injection of ²⁴Na at zero time. The ²⁴Na was in the form of ²⁴NaCl and the amount of the stock solution (1.3 g. ²⁴NaCl ≡ approx. 300 μc/16 ml. water) injected was such that it contained approximately 30 μc of ²⁴Na. It will be noted that the stock solution of ²⁴NaCl was hypertonic and thus that the actual amount of NaCl injected was greater than is physiologically desirable. This, however, was unavoidable because of the decay in radioactivity of the ²⁴NaCl which occurred during transit from Harwell to Australia. The effect of this amount of NaCl on the validity of the result was checked in subsequent experiments described below.

After the injection of ²⁴NaCl, the abdomen was opened, the aorta, inferior vena cava and left iliac vessels were exposed, and loose ligatures were placed in position. The abdomen was then closed and at zero plus 20 min. 1 mC ³²P approximately was injected intravenously. At zero plus 30 min. a sample of blood was taken from the aorta, heparinized and the plasma separated by centrifugation. At the

same time the aorta and left iliac vessels were ligated and the thigh muscles from the left hind limb rapidly removed and placed in liquid O₂. The aorta and inferior vena cava were then cannulated and the right hind limb perfused as described in the following paper (Ennor & Rosenberg, 1954). At the end of the 30 min. perfusion, when about 1 l. of Ringer's solution had passed through the limb, the muscles were removed and dropped into liquid O₂. Trichloroacetic acid extracts of both sets of muscles were then prepared, as described by Ennor & Rosenberg (1954). Determinations of ²⁴Na and ³²P were carried out on these extracts without further treatment.

The result of this experiment (Table 2) indicates that the volume of the extracellular space is 11.1 %, which is in good agreement with the values of Manery & Hastings (1939) and of Manery & Bale (1941). When this value is used to calculate the contribution which the extracellular inorganic P makes to the radioactivity of the total inorganic P in the muscle, the figure obtained still exceeds the observed total. Of interest, also, is the fact that about one-third of the ²⁴Na remains in the muscle even after perfusion.

Table 3. *Sodium space in skeletal muscle and the effect of perfusion on the washing out of ^{24}Na*

Animal	Tissue	^{24}Na found (counts/min./g.)	Na space as % of muscle volume	^{24}Na remaining after perfusion (%)
1	Plasma	15 800	—	—
	Non-perfused	{ Thigh	1 725	10.9
		{ Thigh	1 820	11.5
		{ Gastrocnemius	1 965	12.4
	Perfused	{ Thigh	775	—
		{ Thigh	1 460	45.0
		{ Gastrocnemius	460	80.2
2	Plasma	13 940	—	—
	Non-perfused	{ Thigh	1 420	10.2
		{ Thigh	1 270	9.1
	Perfused	{ Thigh	160	11.3
		{ Thigh	340	26.6
3	Plasma	11 670	—	—
	Non-perfused	{ Thigh	1 680	14.4
		{ Gastrocnemius	1 700	14.6
	Perfused	{ Thigh	305	18.3
		{ Gastrocnemius	490	28.7
4	Plasma	8 760	—	—
	Non-perfused	{ Thigh	1 100	12.6
		{ Thigh	1 150	13.2
		{ Gastrocnemius	1 260	14.4
	Perfused	{ Thigh	155	13.9
		{ Thigh	220	19.2
		{ Gastrocnemius	200	15.7

As previously noted, the above experiment was open to criticism because of the unphysiological amounts of sodium chloride which were injected. Several other experiments have been carried out to check this point. In these, rabbits of about the same age and weight were used as in the previous experiment and each was injected, 30 min. before killing, with approximately $30\ \mu\text{C } ^{24}\text{Na}$. This was contained in 0.5 ml. and the solution was isotonic with respect to sodium chloride. In these experiments three muscle samples were taken from each of the two hind limbs (one of which was perfused), two from the thigh muscles and one from the gastrocnemius. It will be seen (Table 3) that the 'sodium space', as measured under these conditions, agrees with the previous value and that, as before, the effect of perfusion is to reduce the amount of ^{24}Na to only about one-third of its initial value as judged from the results obtained with the non-perfused limb. Perfusion in all experiments was carried out for 30 min., during which time 1–1.5 l. of perfusate was collected.

The evidence then suggests, if the 'sodium space' as determined represents the true extracellular space, that the premises on which Hevesy (1948) based his method of calculating the specific radioactivity of the intracellular inorganic P are incorrect.

On the basis of the relevant results of other experiments which have been carried out, a different approach has been made. It should be possible to calculate the volume of the extracellular space if the specific radioactivity of the intracellular inorganic P is known and if it is assumed (Hevesy, 1948) that (1) the inorganic P in the extracellular space is present in the same concentration as in the plasma, and (2) that the specific radioactivity of the extracellular inorganic P is the same as that of the plasma. For the purpose of this calculation one further assumption is made, i.e. that the inorganic P which remains in a muscle after perfusion represents the true intracellular inorganic P both with respect to concentration and radioactivity. The validity of each of these assumptions will be discussed below, but on the basis of them a formula for the extracellular space can be derived.

Let A be the concentration of inorganic P in plasma and hence in the extracellular fluid; D , the specific radioactivity of inorganic P in the extracellular fluid; B , the concentration (mg./100 g.) of inorganic P in the non-perfused muscle; E , the specific radioactivity of the inorganic P in the non-perfused muscle; and F , the specific radioactivity of the inorganic P in the perfused muscle (assumed to equal that of the intracellular inorganic P). Then in 100 g. muscle we have B (mg. inorganic P); this

includes Ax of extracellular P where x is a percentage. Hence counts/min. in intracellular space = $BE - DAx$ and the intracellular $P = B - Ax$. Hence

$$F = \frac{BE - DAx}{B - Ax}.$$

Therefore
$$x = \frac{B(E - F)}{A(D - F)}. \quad (1)$$

In short-term experiments, the specific radioactivity of the plasma inorganic P (D in the above equation) is many times greater than that of the intracellular inorganic P (F in the above equation), so that $(D - F)$ approximates to D , and the equation may be written

$$x = \frac{B(E - F)}{AD}. \quad (2)$$

When these equations are applied to those experiments for which measured values exist for the various terms, the apparent volume of the extracellular space can be calculated. The results of such calculations (Table 4) indicate a much greater variation in the volume of the extracellular space than is the case when this is measured by ^{24}Na . In experiments up to 45 min. there is little difference between the figures obtained in any one case for the apparent volume of the extracellular space, when either equation (1) or (2) is used as a basis for calculation. The situation is different when the time interval between the injection of ^{32}P and the death of the animal is increased to 90 hr. Here the value of the specific radioactivity of the intracellular inorganic P is no longer negligible compared with that of the plasma inorganic P and, as is apparent from the results of this experiment (Table 4), the approximation which gave rise to equation (2) is no longer valid. It will be noted that the calculated values (regardless of the equation used) for the volumes of the extracellular space in the case of this

longer time interval greatly exceed those for the shorter time intervals. This follows from the fact that in long-term experiments the specific radioactivities of the inorganic P of the perfused muscles are relatively higher than is the case in the short-term experiments. This, however, is not due to incorporation of ^{32}P but to a phenomenon occurring as a result of perfusion. This phenomenon is fully discussed in the following paper (Ennor & Rosenberg, 1954).

In assessing the validity of these results it must be remembered that equation (1) is based upon the assumption that F is the true value of the intracellular inorganic P, as was asserted by Kalckar *et al.* (1944). This, however, is not the case as is shown in the following paper (Ennor & Rosenberg, 1954). Thus it was proved that F , as determined on the perfused limb, must be lower than the true value because of the extensive breakdown of weakly labelled organophosphates with the liberation of inorganic P of low specific radioactivity and the consequent dilution of the intracellular inorganic P.

Of the terms in equation (2), B and E may be determined with certainty, and the value for F as already discussed must be lower than the true value. The calculated magnitude of the numerator must then be greater than the true value and (if the correctness of the denominator be assumed) the true value of the extracellular space (x) must be less than the calculated value. On the basis of this argument the values for extracellular space seen in Table 4 must be greater than the true value. Such a conclusion is difficult to reconcile with the average value of 12% which is derived from experiments with Na^+ , Cl^- (Manery & Hastings, 1939; Manery & Bale, 1941; Winter, 1934), and CNS^- (Corper, 1915). In histological studies involving geometrical calculation of the extracellular spaces from quickly fixed tissue slices, Hermann (1888) and Fenn (1936) have arrived at slightly higher, but similar, values,

Table 4. *The volume of the extracellular space in skeletal muscle, as calculated on the basis of ^{31}P and ^{32}P determinations*

(For details of calculation and meaning of symbols see text.)

Interval between injection of ^{32}P and death of animal (hr. min.)	Non-perfused muscle		Plasma		Perfused muscle ^{32}P	Extracellular space (calculated)	
	^{31}P (mg. %) B	^{32}P (counts/ min./ $\mu\text{g.}$) E	^{31}P (mg. %) A	^{32}P (counts/ min./ $\mu\text{g.}$) D	(counts/ min./ $\mu\text{g.}$) F	$x = B(E - F)/$ $A(D - F) \times 100$	
						$x = B(E - F)/$ $AD \times 100$	
5	18.4	71.7	5.6	3860	9.2	5.34	5.32
5	18.8	33.6	4.6	3070	9.3	3.25	3.24
10	18.1	38.0	4.4	1760	10.3	6.50	6.46
15	19.6	33.5	4.5	1630	14.3	5.19	5.14
15	23.4	55.5	3.3	3770	15.7	5.25	5.21
15	21.0	36.2	5.5	1665	8.0	6.47	6.50
30	23.2	34.0	3.9	1201	22.2	5.94	5.82
45	22.0	32.9	4.4	2320	18.2	3.20	3.17
90 00	16.8	16.5	4.9	19.2	14.8	132	58.2

as have these authors, for the extracellular volume in muscle. Attention may therefore be directed to the two terms in the denominator of equation (2). Both A and D may be measured with accuracy, so that the values assigned these terms may be accepted. Doubt, however, arises at the identification of these terms with the extracellular medium, i.e. the validity of the assumption that there is a rapid equilibration between the inorganic P of the plasma and the extracellular medium. The fact that there are theoretical grounds for the belief that the value of the numerator should be smaller than calculated on the basis of the determinations made, and that x , as calculated, is smaller than the generally accepted value, suggests that the assumption that the plasma inorganic P is identical with the extracellular inorganic P with respect to specific radioactivity and/or concentration is invalid. It may thus be inferred that these two phases, i.e. plasma and extracellular fluid, do not come into rapid equilibrium and that any calculation of the specific radioactivity of the intracellular inorganic P based on such assumptions is therefore erroneous.

DISCUSSION

Hevesy (1948) and Sacks & Altshuler (1942) have used the generally accepted value of 12 % for the volume of the extracellular space in skeletal muscle and have calculated the specific radioactivity of the intracellular inorganic P and from this the turnover rate of certain organophosphates. The calculation was based upon the assumption that the specific radioactivities of the inorganic P in the plasma and in the extracellular fluid are equal, i.e. that there is a rapid equilibration of inorganic P between these two media. Hevesy (1948) has, however, pointed out that this assumption may not be justified in short-term experiments, although this author has not specified the interval necessary for equilibration. Sacks & Altshuler (1942), quoting values for the contribution (counts/min.) which the extracellular inorganic P makes to the sum of the extracellular and intracellular inorganic P, have noted that in many cases the calculated contribution often exceeds the whole. These authors have disregarded these results, but it is doubtful if such results can be disregarded, since they cast doubt upon the validity of the assumption on which they are based. The mere fact that it is possible, on occasion, to arrive at a value for the extracellular inorganic P contribution which falls below the total of the extracellular and intracellular inorganic P does not permit of the acceptance of the results as being correct.

The results of the present experiments indicate that, even as long as 45 min. after the injection of ^{32}P , calculation of the contribution which intracellular inorganic P makes to the total radioactivity

of inorganic P leads to an impossible result. The calculation involves only two factors about which doubt may be entertained, namely the volume of the extracellular space and the assumption that there is a rapid equilibration of inorganic P between plasma and extracellular fluid.

The volume of the extracellular space in skeletal muscle is accepted as being about 12 % of the muscle volume and this figure has been obtained, in the case of ^{24}Na , as a result of experiments based on the assumption that Na^+ is exclusively extracellular. There is evidence to indicate that this is not necessarily the case (cf. Griffiths & Maegraith, 1939; Hodgkin, 1951). In the present experiments it was shown that perfusion was able to remove only about 70 % of the ^{24}Na present in skeletal muscle, but this cannot be accepted as indicating that the remainder of the ^{24}Na is of intracellular distribution. There is the definite possibility that occlusion of small areas of the vascular bed may have occurred as a result of vasoconstriction and/or clot formation, and thus ^{24}Na would have remained. In addition, perfusion has been shown to lead to marked alterations in the concentration and nature of the compounds in the intracellular, and therefore presumably in the extracellular, phase (Ennor & Rosenberg, 1954). There is, however, no sufficient body of evidence available to invalidate the conclusion that 12 % represents the proportion of skeletal muscle which is present as extracellular space.

It has been argued, as a result of calculations based on equations (1) and (2), that the volume of the extracellular space cannot exceed 3–6 % of the muscle volume, but this argument becomes invalid if the assumption on which the equations are based is invalid. Since there is good reason to accept the value of 12 %, the indication is that the assumption is incorrect, i.e. that inorganic P is not rapidly equilibrated between the extracellular and intravascular phases.

From the results reported in the following paper (Ennor & Rosenberg, 1954), it may be inferred that ^{32}P passes rapidly from the plasma into the cell so that this latter 'compartment' must be taken into account in any consideration of equilibria which exist as between the extracellular and intravascular 'compartments'.

As far as inorganic ^{32}P is concerned there are two reactions involved: (1) the passage from the intravascular to the extracellular space, and (2) the passage from the latter to the intracellular space. It is known that the ^{32}P concentration is higher in the plasma than within the cell, but whether or not it is equal to that in the extracellular medium will be dependent upon the relative speeds of (1) and (2). It is difficult to conceive of an experimental approach towards a resolution of this problem, but the present

results suggest that the speed of reaction (2) is greater than that of reaction (1).

The problem of the differentiation between intra- and extra-cellular inorganic P in tissue extracts therefore remains unsolved and, as a consequence, it is not possible to determine the absolute turnover rate of any organophosphorus compound which has inorganic P as its immediate source of phosphorus.

SUMMARY

1. Two methods of determining the specific radioactivity of intracellular inorganic phosphate have been investigated.

2. Experimental evidence and arguments have been brought forward to suggest that either (a) the volume of the extracellular space in skeletal muscle is much less than 12 %, or (b) the speed with which inorganic phosphate is equilibrated between the two phases, plasma and extracellular fluid, is slower than has been supposed.

3. It is concluded that with the present state of knowledge it is not possible to determine the specific radioactivity of intracellular inorganic phosphate and hence the *in vivo* turnover rate of organophosphates for which inorganic phosphate is the source of phosphorus.

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REFERENCES

- Corper, H. J. (1915). *J. infect. Dis.* **16**, 38.
 Ennor, A. H. & Rosenberg, H. (1952a). *Biochem. J.* **52**, 591.
 Ennor, A. H. & Rosenberg, H. (1952b). *Biochem. J.* **50**, 524.
 Ennor, A. H. & Rosenberg, H. (1954). *Biochem. J.* **56**, 308.
 Fenn, W. O. (1936). *Physiol. Rev.* **16**, 450.
 Furchgott, F. R. & Shorr, E. (1943). *J. biol. Chem.* **151**, 65.
 Griffiths, J. H. E. & Maegraith, B. G. (1939). *Nature, Lond.*, **143**, 159.
 Hermann, L. (1888). *Pflüg. Arch. ges. Physiol.* **42**, 1.
 Hevesy, G. (1948). *Radioactive Indicators*. New York: Interscience Publishers.
 Hevesy, G. & Hahn, L. (1940). *Biol. Medd., Kbh.*, **15**, 5.
 Hodgkin, A. L. (1951). *Biol. Rev.* **26**, 339.
 Kalckar, H. M., Dehlinger, J. & Mehler, A. (1944). *J. biol. Chem.* **154**, 275.
 Manery, J. F. & Bale, W. F. (1941). *Amer. J. Physiol.* **132**, 215.
 Manery, J. F. & Hastings, A. B. (1939). *J. biol. Chem.* **127**, 657.
 Sacks, J. & Altschuler, C. H. (1942). *Amer. J. Physiol.* **137**, 750.
 Winter, K. A. (1934). *Z. ges. exp. Med.* **94**, 663.

An Investigation into the Turnover Rates of Organophosphates

2. THE RATE OF INCORPORATION OF ^{32}P INTO ADENOSINE TRIPHOSPHATE AND PHOSPHOCREATINE IN SKELETAL MUSCLE

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The previous paper in this series (Ennor & Rosenberg, 1954) dealt with the determination of the specific radioactivity (counts/min./ $\mu\text{g.}$) of intracellular inorganic phosphorus, and it was concluded that, with the techniques at present available, it was not possible to determine this value. It is generally assumed that the intracellular inorganic phosphorus is the precursor of the 'energy-rich' phosphorus of phosphocreatine (PC) and of adenosine triphosphate (ATP), and it follows that in the absence of a value for the specific radioactivity of this fraction it is not possible to determine the turnover rate of these compounds.

In the work reported here we have therefore determined the turnover rates of each of the phosphate groups of ATP and the phosphate of PC relative to one another. Whilst such a procedure does not permit the assignment of a definite value to the amount of, for example, ATP which is formed in unit time, it does give a qualitative idea of the speed with which each of the phosphorus atoms is replaced.

This paper is also concerned with the reliability of the results achieved by the perfusion technique of Kalckar, Dehlinger & Mehler (1944) which is essentially similar to the slice-washing methods