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CIRCULATION OF PHOSPHORUS IN THE FROG

WITH A NOTE

ON THE CIRCULATION OF POTASSIUM

BY

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This paper contains the description of some experiments on the circulation of phosphorus in the frog using radiophosphorus as an indicator. The experiments described were carried out in the course of the last six years[1].

Experimental procedure.

Most of the radioactive phosphorus (^{32}P) used in these experiments was prepared by irradiating carbon disulphide with neutrons emitted by a radium-beryllium source. The ^{32}P produced was extracted with diluted nitric acid and turned into sodium phosphate. This sodium phosphate of negligible weight (10^{-10} gm. or less) was dissolved in 0.6 per cent sodium chloride solution. A few tenths of a cubic centimetre of the solution were injected into the lymph sack of *Rana esculenta* or *Rana hungarica*. In our earlier experiments, the injection took place at the start of the experiment; in our later work, however, we injected labelled phosphate several times during the experiment in order to keep the activity of the plasma at an approximately constant level. Experiments were carried out both at 0° and at about 20°; their duration varied between 5 minutes and 400 hours. The muscle tissue was put into liquid air immediately after its removal and the acid soluble consti-

[1] O. Rebbe, M. Sc., who has taken the deepest interest in the problems discussed in this paper and worked for several years almost incessantly on their elucidation suffered an untimely death the 5th of December, 1940.

tuents were extracted with cold 10 per cent trichloroacetic acid. The filtrate obtained was at once added to a solution containing ammonia and magnesium citrate. By this procedure, all inorganic P present in the trichloroacetic extract was precipitated. The next step was to hydrolyze the creatinephosphoric acid present in the filtrate. In our early work, we decomposed the creatinephosphoric acid by adding ammonium molybdate and keeping the solution for 1 hour at 40° in 1 N. H_2SO_4. Later, we omitted the addition of ammonium molybdate and split off the phosphate by letting the acidified solution boil for a short time, as proposed by Meyerhof et alia[1]. The phosphate group split off from the creatinephosphoric acid was then precipitated as ammonium magnesium salt. The filtrate thus obtained was acidified and the 1 N. H_2SO_4 solution obtained was boiled for 7 minutes in order to split off the pyrophosphate group of the adenosintriphosphate. In other experiments, the solution was kept at 100° for 100 minutes. By this procedure, the hexosemonophosphate was hydrolyzed. Other fractions were secured by hydrolyzing the filtrate for some hours. The non-hydrolyzed phosphorus compounds remained in the filtrate together with large amounts of neutral salts. From this solution, after wet ashing of the organic compounds present, the phosphorus was precipitated as molybdenum compound. The ammonium phosphomolybdate was dissolved in ammonia, and phosphorus was precipitated as ammonium magnesium phosphate.

The muscle fraction which does not dissolve in trichloroacetic acid contains the phosphatides and the residual

[1] O. Meyerhof, P. Ohlmeyer, W. Gentner and H. Maier-Leibnitz, Biochem. Z. **298**, 400 (1938).

P. To secure the phosphatides, BLOOR's method was used. All fractions were ultimately obtained as ammonium magnesium phosphate which was dissolved in dilute hydrochloric acid. An aliquot part of the solution was used for the colorimetric determination of the phosphorus, according to FISKE and SUBBAROW, another aliquot part was applied in the radioactive measurements. The comparison of the radioactivity of the P fractions is much facilitated if all samples have the same weight. In this case, no correction for the absorption of the β-rays in the sample has to be made. Fractions of equal weight were obtained by adding to the solution of each fraction 80 mgm. of secondary sodium phosphate and by precipitating all P present as ammonium magnesium phosphate. The precipitates obtained were dried at 106°. Corrections for the decay of the activity of the ^{32}P can also be avoided if all samples are measured relatively to the same active phosphorus preparation. As such preparation an aliquot of the solution administered was used, the P content of the preparation being converted into ammonium magnesium phosphate, as described above.

Distribution of phosphorus in the frog.

We determined the phosphorus content of the different parts of the frog by wet ashing of the organs followed by a phosphorus determination by the method of FISKE and SUBBAROW. The results of these determinations are seen in Tables 1a and 1b.

Absorption of phosphate.

In order to get data on the rate of absorption of the labelled phosphate injected into the lymph sack we determined the activity of plasma samples of known weight both of frogs

Table 1 a.

Distribution of phosphorus in *Rana esculenta* weighing 58 gm.

Organ	mgm. P	Per cent of total P
Blood[1]	0.83	0.18
Skin	31.50	6.96
Muscles	59.32	13.20
Bones	335.30	74.16
Liver[2]	4.28	0.94
Remaining part	20.61	4.56
Total	451.84	

[1] Extrapolated value, assuming the blood content to constitute 4 per cent of the weight of the frog.

[2] The P content of the liver phosphatides was found to be 22 per cent of the total P content of the liver.

Table 1 b.

Distribution of phosphorus in the muscle of the frog.

Fraction	Per cent of total P content
Acid soluble P	82.1
Phosphatide P	10.8
Residual P	7.1

kept at 0° and of frogs kept at 18° at different times after injecting the labelled phosphate. The figures obtained (see Figs. 1 and 2) are not a direct measure of the amount of ^{32}P absorbed but indicate the difference between the amount absorbed in the circulation and the amount which left the circulation for the organs. (The amount taken up by the corpuscles in the course of a few hours is very restricted; see p. 27).

In another set of experiments, we injected large amounts of phosphate (corresponding to 6.1 mgm. P) into the lymph

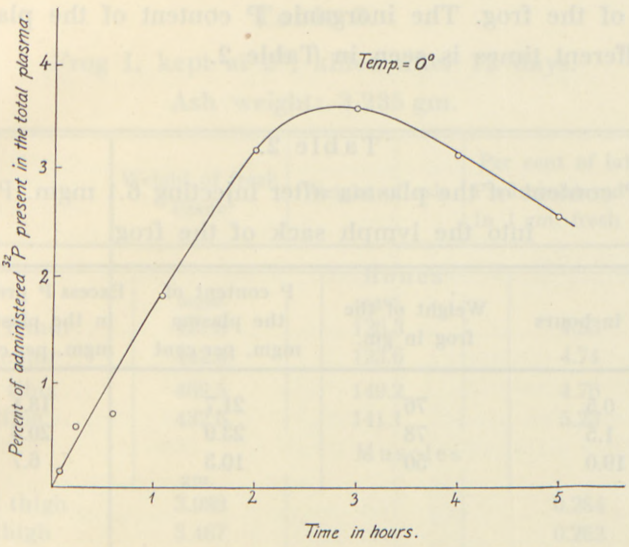

Fig. 1. ^{32}P content of the frog's plasma after injecting ^{32}P into the lymph sack. T = 0°.

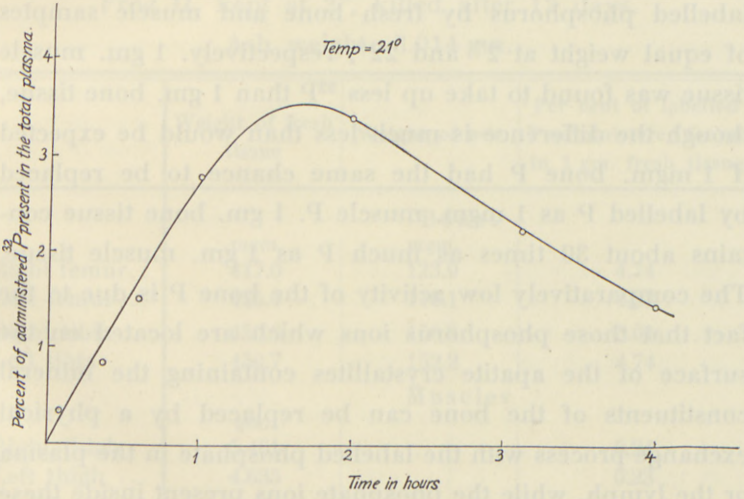

Fig. 2. ^{32}P content of the frog's plasma after injecting ^{32}P into the lymph sack. T = 21°.

sack of the frog. The inorganic P content of the plasma at different times is seen in Table 2.

Table 2.

P content of the plasma after injecting 6.1 mgm. P into the lymph sack of the frog.

Time in hours	Weight of the frog in gm.	P content of the plasma mgm. per cent	Excess P present in the plasma mgm. per cent
0.5	76	21.7	18.1
1.5	78	23.9	20.3
19.0	50	10.3	6.7

Relative rate of uptake of labelled phosphorus by the bone and the muscle.

In our preliminary experiments, the results of which are reported in Tables 3—10, we compared the uptake of labelled phosphorus by fresh bone and muscle samples of equal weight at 2° and 22°, respectively. 1 gm. muscle tissue was found to take up less ^{32}P than 1 gm. bone tissue, though the difference is much less than would be expected if 1 mgm. bone P had the same chance to be replaced by labelled P as 1 mgm. muscle P. 1 gm. bone tissue contains about 30 times as much P as 1 gm. muscle tissue. The comparatively low activity of the bone P is due to the fact that those phosphorus ions which are located on the surface of the apatite crystallites containing the mineral constituents of the bone can be replaced by a physical exchange process with the labelled phosphate in the plasma or the lymph, while the phosphate ions present inside these crystals cannot be replaced. Labelled phosphate ions can

Table 3.

Frog I, kept at 2°, killed after 12 days.

Ash weight: 3.235 gm.

	Weight of fresh tissue	Weight of ash	Per cent of labelled P administered found in 1 gm. fresh tissue
		Bones	
	mgm.	mgm.	
Right femur..	427.8	126.3	4.33
Left femur...	442.8	123.6	4.74
Right tibia...	469.5	149.2	4.76
Left tibia	432.6	141.1	5.23
		Muscles	
	gm.		
Right thigh ..	3.989	...	0.284
Left thigh ...	3.467	...	0.262
Right calf....	1.401	...	0.237
Left calf	1.366	...	0.199

Table 4.

Frog II, kept at 2°, killed after 12 days.

Ash weight: 3.014 gm.

	Weight of fresh tissue	Weight of ash	Per cent of labelled P administered found in 1 gm. fresh tissue
		Bones	
	mgm.	mgm.	
Right femur..	417.0	123.9	4.74
Left femur...	425.4	116.1	4.38
Right tibia...	450.6	150.8	5.01
Left tibia	450.7	152.2	4.74
		Muscles	
	gm.		
Right thigh ..	5.461	...	0.24
Left thigh ...	4.635	...	0.23
Right calf....	1.670	...	0.28
Left calf	1.679	...	0.31

Table 5.

Frog III, kept at 2°, killed after 21 days.
Ash weight: 3.104 gm.

	Weight of fresh tissue	Weight of ash	Per cent of labelled P administered found in 1 gm. fresh tissue
		Bones	
	mgm.	mgm.	
Right tibia epiphysis ...	155.1	29.2	6.03
Left tibia epiphysis	142.9	29.1	5.90
Right tibia diaphysis ...	205.7	77.0	6.34
Left tibia diaphysis	200.4	76.1	6.50
		Muscles	
	gm.		
Right calf	1.269	...	0.51
Left calf.....	1.252	...	0.42

Table 6.

Frog IV, kept at 2°, killed after 22 days.
Ash weight: 3.347 gm.

	Weight of fresh tissue	Weight of ash	Per cent of labelled P administered found in 1 gm. fresh tissue
		Bones	
	mgm.	mgm.	
Right tibia epiphysis ...	144.2	33.1	3.68
Left tibia epiphysis	158.7	39.6	4.50
Right tibia diaphysis ...	205.8	86.6	5.07
Left tibia diaphysis	215.2	80.8	4.52
		Muscles	
	gm.		
Right thigh.....	4.116	...	0.26
Left thigh	4.109	...	0.29
Right calf	1.477	...	0.34
Left calf.....	1.548	...	0.36

Table 7.

Frog V, kept at 20—24°, killed after 8 days.

Ash weight: 3.65 gm.

	Weight of fresh tissue	Weight of ash	Per cent of labelled P administered found in 1 gm. fresh tissue
		Bones	
	mgm.	mgm.	
Right femur epiphysis..	172.9	33.5	3.30
Left femur epiphysis...	183.9	36.9	3.22
Right femur diaphysis..	412.7	123.5	2.99
Left femur diaphysis...	436.4	123.9	2.84
Right tibia epiphysis...	265.9	59.6	2.92
Left tibia epiphysis	237.1	62.4	3.16
Right tibia diaphysis...	374.4	141.9	3.02
Left tibia diaphysis	358.7	130.2	2.91
		Muscles	
	gm.		
Right thigh.....	5.372	...	1.04
Left thigh.....	5.632	...	0.92
Right calf	1.925	...	1.12
Left calf.....	1.980	...	1.11

only be incorporated into the inside of the apatite crystallites during the formation of such crystallites from a plasma containing labelled phosphate. The dissolution of "old" apatite crystals and the formation of "new" ones is, however, a slow process and, correspondingly, the incorporation of labelled phosphate into the bone apatite is also a slow process[1]. In contrast to the bones, the greatest part of the

[1] Comp. G. Hevesy, H. Levi and O. Rebbe, J. Biochemistry, April 6 (1940).

Table 8.

Frog VI, kept at 22°, killed after 8 days.

Ash weight: 3.46 gm.

	Weight of fresh tissue	Weight of ash	Per cent of labelled P administered found in 1 gm. fresh tissue
		Bones	
	mgm.	mgm.	
Right femur epiphysis..	150.6	37.1	4.44
Left femur epiphysis ...	169.8	37.6	2.66
Right femur diaphysis..	398.9	130.4	3.26
Left femur diaphysis ...	382.0	122.3	3.20
Right tibia epiphysis ...	263.9	60.2	2.97
Left tibia epiphysis	252.4	60.7	3.12
Right tibia diaphysis ...	304.3	122.6	3.66
Left tibia diaphysis	282.5	120.9	3.42
		Muscles	
	gm.		
Right calf	1.520	...	1.1
Left calf.....	1.584	...	1.2

phosphorus in the muscles is present as a constituent of organic compounds, as seen in Table 1b. The bulk (about 80 per cent) of the organic P is present in the muscle of the frog in the form of acid soluble phosphorus compounds and, in a corresponding manner, the rate of activation of the muscle P depends mainly upon the rate of formation of active acid soluble P compounds. This process is much faster than the formation of the apatite crystals of the bone tissue and this fact explains why the replacement of phosphorus in the muscle tissue takes place at a much more rapid rate than the replacement of phosphorus in

Table 9.

Frog VII, kept at 20—24°, killed after 12 days.
Weight: at the start 56.5 gm.; at the end 49.5 gm.
Ash weight: 2.359 gm.

	Weight of fresh tissue	Weight of ash	Per cent of labelled P administered found in 1 gm. fresh tissue
		Bones	
	mgm.	mgm.	
Right femur epiphysis..	79 8	17.1	4.27
Left femur epiphysis...	77.6	16.7	4.35
Right femur diaphysis..	97.9	49.1	5.02
Left femur diaphysis...	103.1	48.3	4.70
		Muscles	
	gm.		
Right thigh.....	2.608	...	1.1
Left thigh	2.139	...	1.1
Right calf	1.067	...	0.9
Left calf.....	1.630	...	1.1

the bone tissue. From the fact that the ratio of the uptake of ^{32}P by 1 gm. muscle tissue and 1 gm. bone tissue much decreases with increasing temperature (see Table 10) we can conclude that the temperature coefficient of the inter-diffusion and subsequent incorporation of labelled phosphate into the organic compounds of the muscle of the frog is much greater than the temperature coefficient of the formation of apatite crystals.

Excretion of labelled P.

In a few cases, we determined the percentage ^{32}P which was excreted by the kidneys of the frog. In one experiment,

Table 10.

Comparison of the labelled P content of bones and muscles.

Frog	Duration of the experiment days	Ratio of the labelled P content of bone and muscle of equal weight
	Temperature: 2°	
I	12	19.4
II	12	17.7
III	21	12.8 (epiphysis)
III	21	13.9 (diaphysis)
IV	22	13.2 (epiphysis)
IV	22	15.5 (diaphysis)
	Average value ...	15.4
	Temperature: 22°	
V	8	3.0 (epiphysis)
V	8	2.9 (diaphysis)
VI	8	2.9 (epiphysis)
VI	8	2.9 (diaphysis)
VII	12	4.1 (epiphysis)
VII	12	4.6 (diaphysis)
	Average value ...	3.4

1.5 cc. 0.6 per cent sodium chloride solution containing 0.008 mgm. P as phosphate was injected into the lymph sack of the frog weighing 55 gm. and kept at 18°. Urine was collected during 14 hours and the ^{32}P content of the urine was determined. It was found to make out 10.6 per cent of the ^{32}P injected while, in other experiments, 7.1 and 5.8 per cent, respectively, was found.

Uptake by the frog of ^{32}P from a solution containing labelled sodium phosphate.

A frog weighing 88 gm. was kept at 18° in 100 cc. 0.6 per cent sodium chloride solution containing 4 mgm.

labelled P as sodium phosphate. The solution was renewed every day. After the lapse of $2^1/_2$ days, the frog was washed, killed and its P content extracted. It was found to be 695 mgm. or 7.9 mgm. per gram of fresh weight of the frog. The specific activity of this P was found to constitute 1/450 of the specific activity of the P of the solution in which the frog was kept. Thus, in the course of $2^1/_2$ days, 1/450 of the total P of the frog was replaced by solution P. We investigated, furthermore, the activity of the inorganic P extracted from the liver of the frog which was found to show a specific activity amounting to 0.99 per cent of the specific activity of the solution P. It was, thus, 4.5 times more active than the average P of the frog.

Rate of renewal of the phosphorus compounds in the muscle.

In the preceding sections, experiments were described in which the percentage of the administered ^{32}P present in the skeleton and the muscles was determined. In the following, we wish to discuss the rate at which the organic phosphorus compounds present in the muscles of the frog are renewed. We shall consider those cases of renewal[1]) in which phosphate is split off and consecutively reincorporated into the organic molecules. For example, creatinephosphoric acid is degraded under splitting off of phosphate and resynthesized under uptake of phosphate radicals. If labelled phosphate ions are present, they will have the same chance to be incorporated as have non-labelled ones. Let us assume 10^6 free phosphate ions present in the muscle cells to contain 10 $^{32}PO_4$ ions while from 10^6 P atoms

[1] It is conceivable that molecules get renewed without the splitting off and reincorporation of phosphate group.

isolated from hexosemonophosphate of the muscle tissue only 1 is ^{32}P, then we have to conclude that 10 per cent of the hexosemonophosphate molecules got renewed during the experiment under incorporation of free phosphate. The ratios of the specific activities of the inorganic P and the organic P are, thus, a measure of the extent of renewal of the organic P compound which took place during the experiment. When trying to arrive at quantitative data we encounter the following difficulties: (a) The free phosphate extracted from the muscle tissue is partly cellular and partly extracellular phosphate; it is, however, the specific activity of the cellular phosphate only which is to be considered when calculating the rate of renewal. (b) The specific activity of cellular phosphate changes during the experiment, the change being due, for example, to an increasing influx of labelled phosphate into the muscle cells. In this connection it should be also mentioned that the method permits to distinguish between renewed and non-renewed, between "old" and "new" molecules; but no information is supplied on the point whether the molecules are repeatedly renewed in the course of the experiment or not.

As to point (a), to account for the share of the extracellular phosphate in the total phosphate of the muscle tissue, we must know the specific activity of the plasma phosphate which we assume to be identical with the specific activity of the extracellular phosphate. We must also know the phosphate content of the plasma and that of the muscle tissue and, finally, the size of the extracellular space. The last mentioned magnitude can be determined in each case by administering simultaneously with the labelled phosphate labelled sodium[1], or it can be assumed that the

[1] Comp. G. Hevesy and O. Rebbe, Acta Physiol. Scand. 2, 171 (1940).

extracellular space makes out 14 per cent of the weight of the muscles. Another procedure which we used repeatedly is the following. We remove one leg of the frog 1 hour after the start of the experiment and determine the specific activity of the free muscle phosphate P. After further 3 hours, we extract the phosphate of the other gastrocnemius and determine the specific activity of the free phosphate P. If, within 1 hour, a proportional partition of ^{32}P between plasma phosphate and the extracellular phosphate took place, then the increment of the specific activity of the tissue phosphate between 1 hour and 4 hours is solely due to an increase in the specific activity of the cellular inorganic P. By this method, we can determine the percentage of cellular P which was replaced in the muscle by plasma P between 1 hour and 4 hours after the start of the experiment.

The fact that the inorganic P of the tissue is partly of extracellular origin will lead to an overestimation of the activity of the cellular inorganic P and, thus, to an underestimation of the renewal figures of the organic P compounds. This source of error is mainly to be considered in experiments of short duration carried out at low temperature. On the other hand, even if the greatest precautions are observed, we risk a decomposition of some of the creatine phosphate present in the tissue prior to the separation of the inorganic P. Such a decomposition will lead to a decrease in the specific activity of the inorganic P, the inorganic P originating from creatine P being on the whole less active than the "free" phosphate P. We shall, thus, underestimate the specific activity of the inorganic P and, correspondingly, overestimate the rate of renewal of the organic P compounds. This error will also be larger in experiments of short duration carried out at low tem-

perature. We wish to mention a further possible experimental error. If the free phosphate is not precipitated quantitatively, we risk to find some strongly active phosphate in the creatine phosphate fraction. A non-negligible amount of phosphate may remain in solution in cases in which the amount of P to be precipitated is very small.

The following objection can be put forward regarding the calculation of renewal rates from the ratio of the specific activities of the inorganic P and the P split off from organic compounds. The P secured as inorganic phosphate might even after the most careful handling of the tissue have been largely present not as free phosphate in the tissue cells but incorporated in very labile compounds which were decomposed in the course of the extraction process. It is possible that this is the case, it is even quite possible that a large part of the inorganic P extracted as such from the muscle cells was originally present incorporated in very labile compounds and was decomposed during the extraction process. General experience indicates, however, that very labile phosphorus compounds are renewed at a fast rate and we can, therefore, expect the P of such labile phosphorus compounds to obtain within a short time a similar specific activity as shown by the inorganic P present in the cells. Should that not be the case, then the comparison of the specific activity of the "inorganic" P with that of the P split off from the organic compound in question would obviously lead to an overestimation of the rate of renewal.

The specific activity of different P fractions is seen in Tables 11—18.

Though all precautions were taken to prevent decomposition of creatinephosphoric acid it is difficult to state

whether the variations in the values obtained for the rate of renewal of creatinephosphoric acid molecules in some of the experiments are genuine or are due to a more or less successful prevention of the decomposition of the creatine-phosphoric acid prior to the removal of the inorganic phosphate of the muscle tissue.

The results of an experiment, in which the frog was

Table 11.

Specific activity of phosphorus fractions extracted from the gastrocnemius of a frog, 4 hours after injecting labelled sodium phosphate into the lymph sack. Temp.: 2°.

Fraction	P content in mgm.	Activity in per cent of the standard preparation	Per cent of total activity administered per mgm. P	Relative specific activity
I. Inorganic P .	0.313	69.5	0.888	100
II. Inorganic + creatine P...	0 935	100	0.428	48.2
III. Creatine P calculated as II—I	0.622	30.5	0.20	22.1
IV. Creatine P (isolated)....	0.362	17.1	0.19	21.4
V. Pyrophos-phate + hexose P....	0.215	8.3	0.16	17.4
VI. Acid soluble residual P...	0.113	3.2	0.11	12.7
VII. Non acid soluble P....	0.500	1.6	0.013	1.4

We denote as pyrophosphate + hexose P the inorganic phosphorus obtained after the hydrolysis of a fraction for one hour at 100° in 1 N. H_2SO_4 after the removal of the inorganic and creatine P.

Table 12.

Specific activity of phosphorus fractions extracted from the gastrocnemius of frogs. Temp.: 2°.

Time of the experiment hours	Fraction	Specific activity
4	Inorganic P	100
4	Creatine P	14.8
4	Inorganic P	100
4	Creatine P	21.4
3	Inorganic P	100
3	Creatine P	8.8

Table 13.

Specific activity of phosphorus fractions extracted from the gastrocnemius of a frog 40 hours after injecting labelled sodium phosphate. Temp.: 2°.

Fraction	Relative specific activity
I. Inorganic P	100
II. Inorganic + creatine P	62.7
III. Creatine P (calculated as II—I)	33.7
IV. Creatine P (determined)	34.4
V. Pyrophosphate + hexose P	22.8
VI. Acid soluble residual P	11.3
VII. Non acid soluble P	2.1

kept at 20° for 4 hours and then for 1 hour at 0°, is seen in Table 18. The muscles were immersed in liquid air and treated with cold 5 per cent trichloroacetic acid. The extract was sucked through a glass filter into cooled Fiske's reagent. These operations took 2 minutes. In this experiment, we tested to what extent the inorganic P gets precipitated by

Table 14.

Specific activity of phosphorus fractions extracted from the gastrocnemius of a frog 24 hours after injecting labelled sodium phosphate. Temp.: 20°.

Fraction	Relative specific activity
Inorganic P	100
Creatine P	95
P hydrolyzed in the course of 1 hour	91
Acid soluble residual P	36

Table 15.

Specific activity of phosphorus fractions extracted from the gastrocnemius of a frog 400 hours after injecting labelled sodium phosphate. Temp.: 20°.

Fraction	Relative specific activity
Inorganic P	100
Creatine P	99
P hydrolyzed in the course of 1 hour	87
Non acid soluble P	16

FISKE's reagent. After precipitation of the "free" phosphate, 60 mgm. sodium phosphate were dissolved in the filtrate, the phosphate was then precipitated and its activity tested. If the first precipitation was strictly quantitative, this second precipitate should be inactive. The counter registered 228 counts while, in the case of the first precipitate, 2500 counts were registered. When the 228 non-precipitated counts are considered, the specific activity of the creatine P fraction works out to be 14.1 instead of 15.6.

Table 16.

Specific activity of the phosphorus fractions of the muscles of a frog kept at 20° for 4 hours and subsequently at 0° for 1 hour.

Fraction	P content in mgm.	Counts per min.	Specific activity	Relative specific activity
	Left gastrocnemius			
Inorganic P	0.055	427	7770	100
Creatine P	0.240	290	1210	15.6
Hexose P	0.143	98	686	8.7
	Right gastrocnemius + sartorius			
Inorganic P	0.350	2500	7150	100
Creatine P	0.908	1010	1110	15.6
Residue after 17 hours hydrolysis	0.372	45	120	1.68

The same technique was used in the following experiments.

The lowest value found for the percentage renewal of creatinephosphoric acid molecules in the course of 4 hours at 0° is 9 and in the course of 17.5 hours 10 while, in most experiments, appreciably larger figures were found. The rate of renewal of the creatinephosphoric acid molecules of the resting frog is, thus, quite appreciable even at 0° though not as high as stated in a preliminary note[1]. At 20° the lowest figure found after 4 hours is 16 per cent.

Rate of interaction of the plasma phosphate and the cellular phosphate of the muscle tissue.

In the preceding section, we calculated the rate of renewal of the organic P compounds present in the muscle tissue

[1] G. Hevesy and O. Rebbe, Nature **141**, 1097 (1938).

Table 17.

Specific activity of the phosphorus fractions of the muscles of 2 frogs kept at 0° for 17.5 hours.

Frog	Fraction	P content	Specific activity
I Gastrocnemius	Inorganic P	0.330	100
	Creatine P	0.474	29.8
	Product of 100 min. hydrolysis	0.226	27.6
	Residue after 120 hours hydrolysis	0.070	2.0
I Sartorius	Inorganic P	0.799	100
	Product of 7 min. hydrolysis	1.056	25.1
	Product of 17 hours hydrolysis	0.758	19.5
	Residue	0.246	6.8
II Gastrocnemius	Inorganic P	0.398	100
	Creatine P	0.452	11.6
II Sartorius	Inorganic P	0.475	100
	Creatine P	0.694	10.1

Table 18.

Specific activity of phosphorus fractions isolated from different organs of a frog after administration of labelled phosphate during 45 hours at 20°.

Fraction	Specific activity
Plasma P	100
Corpuscle P	3.6
Gastrocnemius inorganic P	4.9
Gastrocnemius creatine + pyrophosphate P	5.3
Liver P	10.1
Epiphysis P	0.35
Diaphysis P	0.20

Table 19.

Specific activity of phosphorus fractions isolated from different organs of a frog after administration of labelled phosphate during 4 days at 22°.

Fraction	Specific activity
Plasma P	100
Gastrocnemius inorganic P	8.3
Gastrocnemius creatine P	7.4
Sartorius total acid soluble P	8.5
Gastrocnemius phosphatide P	1.5

by comparing the ^{32}P content of the tissue inorganic P with the ^{32}P content of the phosphorus extracted from the compound in question. In the following, we shall discuss the interaction of the plasma phosphate with the cellular phosphate. This is clearly a very different problem, the rate of interaction between the plasma phosphate and the cellular phosphate being determined by the permeability of the cell membrane.

The low rate at which phosphate ions migrate through the membrane of the gastrocnemius is seen in Tables 18 and 19. In the course of 4 days at 22° only somewhat less than 1/10 of the P atoms present in the labile P compounds got replaced by plasma P. The molecules of the labile P compounds were repeatedly renewed during this interval (see p. 19) and many P atoms present in the muscle cells interchanged lively; however, the interchange between cellular and extracellular P took only place on a restricted scale.

The results of further experiments in which the activity of the plasma was compared with the activity of the muscle is seen in Table 20.

To keep the plasma activity at an approximately constant level throughout the experiment, 0.4 cc. solution was injected at the start of the experiment and further 0.08 cc. every hour. As seen in Table 20, within 1 hour and 4 hours the activity of the plasma changes only slightly, the

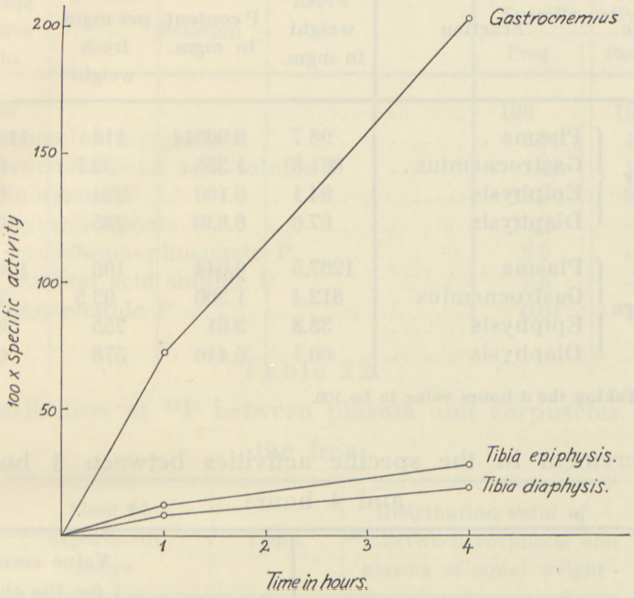

Fig. 3. Specific activity of tissue P.

average being 108, taking the end value to be 100. The values obtained for the specific activity of the tissue P are seen in Table 20 and Fig. 3.

In some cases, very low values were obtained for the distribution ratio of labelled phosphate between plasma and muscle tissue. The fact that in these experiments frogs kept through the winter were used, the experiment being carried out in the spring, suggests the explanation that poor circulation may be responsible for the low values obtained.

Table 20.

Activity of different fractions of the frog 1 hour and 4 hours, respectively, after the start of the experiment. Temp.: 22°.

Time	Fraction	Fresh weight in mgm.	P content in mgm.	Activity per mgm. fresh weight	Specific activity of P
1 hour	Plasma	98.7	0.00344	116[1]	116
	Gastrocnemius	861.8	1.355	32.7	0.72
	Epiphysis	94.1	6.100	224	0.12
	Diaphysis	67.6	6.830	235	0.08
4 hours	Plasma	1267.5	0.044	100	100
	Gastrocnemius	812.4	1.300	93.5	2.03
	Epiphysis	38.8	3.61	755	0.28
	Diaphysis	60.1	6.410	578	0.19

[1] Taking the 4 hours value to be 100.

Increment in the specific activities between 1 hour and 4 hours.

Fraction	Experimental value	Value corrected for the change in the specific activity of the plasma
Gastrocnemius	1.31	1.21
Epiphysis	0.16	0.148
Diaphysis	0.11	0.102

^{32}P content of the liver fractions.

As in the case of mammalia, in the frog the liver phosphate interacts at a much faster rate with the plasma phosphate than does the muscle phosphate. The fast rate of renewal of the acid soluble P compounds and the very

Table 21.

Specific activity of the phosphorus fractions extracted from the organs of the frog kept at 15° and the organs of the rabbit 10 hours after administration of labelled phosphate.

Fraction	Specific activity	
	Frog	Rabbit
Plasma	100	100
Gastrocnemius inorganic P	2.11	15.5
Gastrocnemius total acid soluble P	1.49	11.0
Liver inorganic P	12.9	85
Liver pyrophosphate P	15.2	—
Liver hexosemonophosphate P	8.9	—
Liver residual acid soluble P	3.5	—
Liver phosphatide P	0.04	12.8

Table 22.

Distribution of ^{32}P between plasma and corpuscles of the frog.

Time of experiment hours	Temp.	Distribution ratio of ^{32}P between corpuscle and plasma of equal weight
10	15°	0.28
14	20°	1.1
45	20°	3.6

slow renewal of the phosphatides of the liver of the frog are seen in Table 21. This table contains also corresponding data for the P fractions of the rabbit.

^{32}P content of the red corpuscles.

As seen in Table 22, a very slow interaction was found to take place between the plasma P and the corpuscle P present in the nucleated corpuscles of the frog.

In this connection, it is of interest to recall that the non-nucleated erythrocytes of the rabbit were found to contain after the lapse of 10 hours at 37° 5 times as much ^{32}P as plasma of equal weight, while the corresponding figure for the nucleated corpuscles of the hen was found to be about 0.5, only.

Summary.

The rate of absorption of phosphate injected into the lymph sack of the frog was studied using radiophosphorus as an indicator. The maximum amount of labelled phosphate present in the circulation at any moment was found to be 3 to 4 per cent of the amount administered, thus a similar value as found in the case of mammalia.

While at 2° after the lapse of 1 to 3 weeks 1 gm. bone tissue contained about 15 times as many labelled P atoms as 1 gm. muscle tissue, the corresponding ratio was found to be but 3 at 22°, showing that the temperature coefficient of the penetration of labelled phosphate into the muscle cells followed by incorporation of labelled P into the phosphorus compounds of the muscle tissue is much larger than the temperature coefficient of the formation of labelled bone apatite crystals.

The amount of labelled phosphate excreted by the kidneys and the amount of labelled phosphate taken up by the frog kept in physiological sodium chloride solution containing labelled phosphate were investigated.

The rate of renewal of various acid soluble P compounds extracted from the gastrocnemius of the frog was determined by comparing the specific activity of the inorganic P extracted from the muscle with the specific activity of the

phosphorus split off from various organic compounds of the muscle tissue. Creatinephosphoric acid molecules, adenosintriphosphoric acid molecules, and also hexose-monophosphate molecules were found to be renewed at an appreciable rate even at 0°. The rate of renewal was found to increase with decreasing chemical stability of the compound and with increasing temperature.

The rate of interaction of the plasma phosphate with the phosphate of the muscle cells was found to be very much lower than the rate of interaction of the free cellular phosphate with the phosphate of several organic phosphorus compounds.

The rate of penetration of labelled phosphate into the liver cells is much faster than the rate of penetration into the muscle cells. The rate of interaction of plasma phosphate and the phosphate of the corpuscles was found to be very low.

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Institute of Theoretical Physics and Laboratory of Zoophysiology, University of Copenhagen. June, 1941.

On the Circulation of Potassium.

We saw that phosphate present in the plasma (extracellular fluid) penetrates at a fairly slow rate into the muscle cells of the frog and it seemed of interest to compare the rate of penetration of phosphate with the rate of penetration of potassium into the muscle cells. The results of experiments in which labelled potassium (^{42}K) was used as an indicator are communicated in this note.

Experimental procedure.

About 100 mgm. potassium chloride were bombarded with a stream of deuterons in the Copenhagen cyclotron. A solution containing 8 mgm. potassium chloride per cc. was prepared and 0.5 cc. or less of this solution was injected

Table 1.

Interchange of potassium between plasma and gastrocnemius of a frog weighing 36 gm., at 18°.

Time in min.	Sample	Weight in mgm.	Per cent of ^{42}K administered, present in the sample	Per cent of ^{42}K administered, present in 1 gm.	Ratio of ^{42}K content of 1 gm. gastrocnemius and 1 gm. plasma
5	Plasma	50	0.085	1.70	0.31
5	Gastrocnemius .	440	0.234	0.53	
20	Plasma	247	0.393	1.59	1.20
20	Gastrocnemius .	549	1.05	1.91	

Table 2.

Interchange of potassium between plasma and gastrocnemius of a frog weighing 35 gm., at 18°.

Time	Sample	Weight in mgm.	Per cent of ^{42}K administered, present in the sample	Per cent of ^{42}K administered, present in 1 gm.	Ratio of ^{42}K content of 1 gm. gastrocnemius and 1 gm. plasma
10 min.	Plasma	102	0.269	2.64	0.57
10 —	Gastrocnemius .	342	0.517	1.51	
3 hours	Plasma	343	0.173	0.50	6.48
3 —	Gastrocnemius .	294	0.950	3.24	

Table 3.

Interchange of potassium between plasma and gastrocnemius of a frog weighing 62 gm., at 18°.

Time in hours	Sample	Weight in mgm.	Per cent of ^{42}K administered, present in the sample	Per cent of ^{42}K administered, present in 1 gm.	Ratio of ^{42}K content of 1 gm. gastrocnemius and 1 gm. plasma
1	Plasma	268	0.319	1.19	1.24
1	Gastrocnemius .	828	1.21	1.47	
24	Plasma	783	0.198	0.25	8.1
24	Gastrocnemius .	727	1.55	2.3	

into the lymph sack of the frog. After the experiment, a known weight of the muscle tissue and of the plasma was ashed below 360°. To the ash of the plasma non-active tissue ash and to the ash of the tissue non-active plasma

Table 4.

Interchange of potassium between plasma and gastrocnemius of a frog weighing 82 gm., at 18°.

Time in hours	Sample	Weight in mgm.	Per cent of ^{42}K administered, present in the sample	Per cent of ^{42}K administered, present in 1 gm.	Ratio of ^{42}K content of 1 gm. gastrocnemius and 1 gm. plasma
24	Plasma	547	0.144	0.25	4.9
24	Gastrocnemius .	1177	1.43	1.22	
64	Plasma	1010	0.15	0.15	9.3
64	Gastrocnemius .	1051	1.46	1.39	

Table 5.

Interchange of potassium between plasma and gastrocnemius of a frog weighing 65 gm., at 18°.

Time in hours	Sample	Weight in mgm.	Per cent of ^{42}K administered, present in the sample	Per cent of ^{42}K administered, present in 1 gm.	Ratio of ^{42}K content of 1 gm. gastrocnemius and 1 gm. plasma
24	Plasma	675	0.294	0.38	4.6
24	Gastrocnemius .	985	1.72	1.75	
68	Plasma	991	0.21	0.21	8.4
68	Gastrocnemius .	1015	1.79	1.77	

Excreted between 0 and 24 hours, 12.3 per cent of the ^{42}K administered;

Excreted between 24 and 68 hours, 1.8 per cent of the ^{42}K administered.

ash was added. By this procedure, samples of about identical weight and identical chemical composition were obtained. The activity of the samples weighing about 300 mgm. was compared using a Geiger counter.

Results.

The results obtained at 18° are seen in Tables 1—5. In experiments carried out at 0° which lasted 1 hour, 1 gm. of gastrocnemius was found to contain 0.51 and 0.63 times, respectively, as much ^{42}K as 1 gm. of plasma, a value about half as large as that found at 18°.

The labelled potassium found in the muscle tissue is located partly in the cells and partly in the extracellular space. If we assume the extracellular space of the gastrocnemius of the frog to make out 14 per cent of the tissue weight, about $^1/_4$ of the ^{42}K found in the muscle tissue after the lapse of 10 min. (see Table 2), $^1/_9$ after 20 min. (see Table 1), and $^1/_{46}$ of the value found after 3 hours (see Table 2) is located in the extracellular space. The amount of labelled potassium which penetrated into the muscle cells is very much larger than the amount of labelled phosphate which passed the cell membrane during the same time. The interchange of potassium between plasma and muscle cells is thus very much faster than the interchange of phosphorus between plasma and muscle cells. While it requires 10 hours or more that 1 gm. gastrocnemius contains as much labelled P as 1 gm. plasma, it takes only $^1/_2$ hour or less that the ^{42}K content of 1 gm. gastrocnemius becomes equal to the ^{42}K content of 1 gm. plasma.

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