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In a recent letter to Nature[1] we communicated the results of some experiments on the metabolism of phosphorus using a radioactive phosphorus isotope as indicator. What follows is a more detailed description of some of our experiments, carried out chiefly on rats but partly also on human subjects.

Principle of the method used.

Disregarding hydrogen, the only element which is ever met with in a nuclear state (as a proton) in chemical reactions, isotopes do not separate to a measurable extent during chemical or biochemical processes. It follows from this inseparability that when a known amount of radioactive phosphorus is added to, for example, 1 mgm. of phosphorus the presence of the former will always indicate the presence of the latter. We can thus distinguish for example between the phosphorus atoms taken in with the food (to which we add some radioactive phosphorus) and those already present in the system. The use of isotopic indicators is not dependent on an absolute inseparability of isotopes by chemical methods. We know indeed that minute separations almost always occur. It is sufficient that, within the analytical accuracy claimed, no separation takes place.

[1] O. Chievitz and G. Hevesy, Nature, **136**, 754, 1935.

Phosphorus has only one stable isotope ^{31}P but we can prepare unstable radioactive isotopes of phosphorus having atomic weights of 30 and 32; the latter has a half-life of about a fortnight and is very suitable for use as an indicator. It was used by us in many experiments of different kinds.

Preparation of radioactive phosphorus.

Radioactive phosphorus $^{32}_{15}P$ can be prepared from chlorine or from sulphur under the action of fast neutrons, or from ordinary phosphorus under the action of slow neutrons; the nuclear reactions are:

$$^{35}_{17}Cl + ^{1}_{0}n = ^{32}_{15}P + ^{4}_{2}He$$

$$^{32}_{16}S + ^{1}_{0}n = ^{32}_{15}P + ^{1}_{1}H$$

$$^{31}_{15}P + ^{1}_{0}n = ^{32}_{15}P$$

Using neutrons liberated from mixtures of radium and beryllium, ^{32}P can be prepared most conveniently from sulphur. We found it advisable to use carbon disulphide instead of the elementary sulphur used by Fermi and his colleagues in their original experiments. About 10 litres of carbon disulphide were exposed to neutrons from radium-beryllium mixtures and a fortnight later the carbon disulphide was distilled off. The residue contained the radioactive phosphorus formed, along with some of the decomposition products of carbon disulphide. The residue was oxidized and the phosphoric acid obtained converted into the phosphate compound wanted. We used chiefly sodium radiophosphate in our experiments. The weight of the radiophosphorus produced is extremely minute; using a source containing 100 mgm. of radium, less than 10^{-10}gm. of radiophosphorus is obtained. By adding a suitable quantity of sodium phosphate to the sodium radiophosphate solution we obtain the "radioactive" ("labelled") sodium phosphate desired.

To concentrate the radiophosphorus obtained by neutron bombardment of carbon disulphide other methods besides that outlined above were used. A very convenient way to prepare nearly pure radiophosphorus is the following. Under the action of the radiation some decomposition of the carbon disulphide takes place and a partly orange-coloured precipitate is formed which settles on the glass walls. This slight precipitate contains a large part of the radioactive phosphorus formed. The precipitate is possibly identical with the red sulphur described by MAGNUS as far back as 1854, which was found to consist of a mixture of sulphur and organic sulphur compounds. We are engaged on the investigation of this precipitate.

In a third method of preparation the phosphorus formed was removed from the carbon disulphide solution by shaking the latter with diluted (20:1) nitric acid.

Determination of the radioactive sodium phosphate.

The radioactivity of the samples of blood, bones, etc. to be analysed is in most cases too feeble to be measured even by means of a very sensitive electroscope. GEIGER-MÜLLER counters, much more sensitive instruments, are therefore utilised for measuring purposes. We use for the most part tubes having an available surface of about 1.5 cm.^{2}. The sample to be measured must accordingly be spread over about the same area. The β-rays emitted by the radio phosphorus are fairly penetrating and are not appreciably weakened when an aluminium dish of 1.5 cm.^{2} surface is filled to a depth of a few millimeters with a bone sample weighing 100 mgms. We want to know what percentage of the radioactive phosphorus taken is to be found after a certain time in, for example, the bones. The procedure is as follows. We take a solution of active sodium phosphate, use 99 per cent. of it for feeding the animal and keep 1 per cent. as a "standard". We kill the animal, separate a bone sample, ignite it, and measure its activity. Should the latter be, for instance, half as large as that of the standard which is measured simultaneously, then we can conclude that 0.99×0.5 per cent. of the active phosphorus atoms eaten are actually present in the bone sample investigated. Although the β-radiation from radioactive phosphorus atoms is not appreciably weakened in penetrating through 100 mgms. of bone ash, we can entirely eliminate the possible error due to this absorption by adding 100 mgms. of calcium phosphate to the standard solution; this has the same absorbing power as the bone sample. It is advisable to make the standard as similar to the sample to be measured as possible. In dealing with urine, faeces, muscles, liver etc. we first destroyed the organic matter by one of the usual methods; in several cases, however, these were replaced by treatment with fuming nitric acid. Then calcium phosphate and calcium oxide were added if necessary to make the sample more similar in its composition to our standard preparation and finally the sample was ignited.

To demonstrate the utility of the isotopic indicator method we will first consider the problem of the origin of the phosphorus in the faeces.

Origin of the phosphorus in the faeces.

Chemical analysis enables us to determine the phosphorus content of the excreta but not to decide to what extent the phosphorus found in the faeces is undigested material and what fraction of it is phosphorus having its origin in the organism. The investigations described in this paper have revealed that a fairly rapid interchange takes place between the phosphorus present in the different bodily organs and that present in the blood. A part of the latter finds its way, when the digestive fluids are formed, into the intestinal tract and is thus added to the faeces. The following experiment permits us to distinguish between food phosphorus and that originating from the blood. We add a known amount of radioactive phosphorus to the diet and determine what percentage of the latter is to be found in the faeces. In a separate experiment we inject a known amount of radioactive phosphorus (sodium phosphate) into the blood and determine what part of this phosphorus appears in the faeces. The combination of the two results enables us to determine what part of the phosphorus found in the faeces is due to incomplete digestion of the food eaten.

In Table I the amount of radioactive phosphorus eliminated through the kidneys and the gut is given for the case of a patient fed on a normal hospital diet to which 0.5 mgm. of labelled sodium phosphate was added. Within 5 days 21.7 per cent. of the phosphorus was eliminated in the urine and 15.5 per cent. in the faeces. Similar results were obtained in other cases. Table II shows the results obtained when the radioactive phosphorus was injected into the blood of the same patient. Within 5 days 20.5 per cent. was lost through the kidneys and 2.5 per cent. through the gut.

Table I.

Radioactive phosphorus given to human subject per os.

Number of days after taking P	Diuresis in gm.	Percentage of original radioactive P: in 1 gm. of the urine ash	Percentage of original radioactive P: in total urine
0—1	1880	1.23	11
1—2	1800	0.31	2.8
2—3	1620	0.31	2.8
3—4	1670	0.26	2.4
4—5	1540	0.29	2.7
5—6	1860	0.25	1.8
			in total faeces
0—1	..	..	0
1—2	..	..	7.0
2—3	..	..	5.6
3—4	..	..	1.8
4—5	..	..	1.1
5—6	..	..	0

Table II.

Radioactive phosphorus injected into the blood of a patient.

Number of days after injection	Diuresis in gms.	Percentage of original radioactive P: in 1 gms. of the ash	Percentage of original radioactive P: in total urine
0—1	1650	0.78	12.5
1—2	1510	0.20	3.1
2—3, 3—4	1850	0.15	2.9
4—5	850	0.16	1.6
5—6	1450	0.13	2.2
7—8	800	0.09	0.6
8—9	2000	0.10	1.8
		in 1 gms. of the ash	in total faeces
0—1	..	0.085	0.24
1—2	..	0.11	1.37
2—3	..	0.072	0.37
3—4	..	0.072	0.56
4—5	..	0	0

Thus about $^1/_8$ of the phosphorus atoms eliminated from the blood pass through the gut. By combining the above results it follows that of the phosphorus found in the faeces about 20 per cent. was not undigested material but was phosphorus which had already had a share in building up the organism and had left it by entering the digestive liquids and thus getting into the faeces.

In the case above, 22.3 per cent. of the rad. P left through the kidneys within 6 days and in other cases values varying between 20 and 25 per cent. were obtained.

In carrying out experiments like those described above, the most satisfactory procedure would be to replace by radioactive labelled phosphorus atoms the normal phosphorus present in all the foodstuffs administered. By bombarding the material in question with a strong source of slow neutrons we could turn some of the phosphorus atoms into radioactive phosphorus; but such a process always leads to a disruption of the molecular bonds of the phosphorus atoms which become activated and so to a destruction of the chemical compound. We must therefore content ourselves with adding inorganic radioactive phosphate to the food consumed and try to obtain a mixture of radioactive inorganic phosphate and food as uniform as possible. In our experiments carried out with human subjects the sodium radiophosphate was administered in a large volume of milk. Milk contains 0.0795 per cent. of inorganic phosphorus and about half that amount (0.036 per cent.) of phosphorus in organic form. Although the latter does not exchange with the atoms of the inorganic radioactive phosphate, the bulk of the phosphorus (0.0795 per cent.) reaches a state of kinetic equilibrium with the radioactive phosphate added and becomes radioactively indicated. During the digestion process the 0.036 per cent. will be set free from its molecular binding and only at this stage will it have an opportunity to become thoroughly mixed (in an atomic sense) with the radioactive phosphate atoms. While, as has already been mentioned, it would be preferable in investigating phosphorus metabolism to utilize food in which all the phosphorus atoms are labelled, it is not probable that the information obtained with such material would be noticeably different from that obtained in the experiments described in this paper. The blood plasma, where the phosphorus eaten first arrives, contains mostly inorganic phosphorus; we must therefore assume that it is primarily phosphate ions that are involved. Furthermore experience shows that the retention of phosphorus does not depend on the form in which the phosphorus is present[1] in the food, on whether it is present as inorganic and thus exchangeable phosphate or as non-exchangeable. Ducks reared on diets containing phosphate only in inorganic form matured normally and laid 85 to 795 eggs during the first summer.[2] About 15 per cent. of the phosphorus present in meat, more than half that present in milk, and the greater part of that present in vegetables, i. e. the bulk of the phosphorus eaten, is present in inorganic and thus exchangeable form.

Rats are inclined to eat their offspring and they could easily be fed on young rats born by a mother fed on radioactive phosphorus, but the chief source of phosphorus would in this case, too, be inorganic phosphorus, namely that present in the skeleton.

[1] M. Speirs and H. C. Sherman. J. Nutrit. **11**, 216, 1936.
[2] G. Fingerling, Biochem. Z. **38**, 448, 1911.

Elimination of phosphorus by rats.

We carried out numerous experiments with rats which were fed on a normal diet to which radioactive phosphorus was added. In some cases we added 0.1 mgm. or less in the form of sodium phosphate dissolved in a few drops of water which was then soaked up by a small piece of bread given to the animal. The average of several experiments gave a total excretion of 26 per cent. through the kidneys and of 32 per cent. through the gut. In some other experiments calcium phosphate was administered, mixed with butter, which was given to the rat on a small piece of white bread. The result of such an experiment is seen in Table III, which contains the results of the analysis of the urine and the faeces collected during 19 days. The urine was concentrated by evaporation, treated with fuming nitric acid, and ignited; a known fraction of the ash obtained was then introduced under the Geiger counter. 19 days later the rat, which weighed 256 gms., was killed, the corpse was treated with fuming nitric acid to destroy organic compounds, the fatty residue was treated with conc. sulphuric acid, and then ignited in an electric oven. 50.2 per cent. of the

Table III.

1.5 mgm. radioactive calcium phosphate added to normal diet of adult rat.

Number of days after taking rad. P	Percentage of original rad. P	
	in the urine	in the faeces
0— 3	11.4	13.1
3— 7	3.9	4.7
7—10	2.7	2.4
10—13	1.8	0.93
13—16	1.3	1.1
16—19	1.2	1.8[1]
Total	22.3	24.0

[1] Faeces contaminated by urine.

phosphorus given was found in the ashes, which were to a large extent composed of calcium phosphate, and had a total weight of 5.84 gm.

In some cases we added large amounts of calcium phosphate containing active phosphorus to the diet. When for example 18 mgm. of phosphorus as calcium phosphate were given — this corresponds to about four times the phosphorus present in the normal diet — 41 per cent. of the active phosphorus was eliminated through the gut in the course of 19 days and only about 10 per cent. through the kidneys. Furthermore an analysis of the active phosphorus content of the corpse and the excreta revealed that when large amounts of phosphorus were added to the diet the animals would eat only part of it, however, carefully it was administered. We decided thereforet to study the effect of the intake of large amounts of phosphorus on dogs.

The phosphorus atoms absorbed have ample opportunity to enter into kinetic exchange with the phosphate ions present in muscles, bones, and other organs and also to a certain extent to enter organic molecules and replace the phosphorus atoms present there. Many of the last mentioned processes are dependent on enzymatic action. The rate at which the active phosphorus enters the blood corpuscles, the particulars of this process, and the distribution of the radioactive phosphorus between the blood and the different organs were investigated by Professor Lundsgaard and one of us and the results will be published shortly.

Phosphorus exchange in adult rats.

A preliminary investigation revealed the following distribution in adult rats killed three weeks after eating the radioactive phosphate administered in the form of 0.5 mgm. sodium phosphate added to the normal diet.

Table IV.

Distribution of rad. P in adult rats killed 3 weeks after eating it.

	p. c. rad. P
Urine	26.3
Faeces	31.8
Skeleton	24.8
Muscles and fat	17.4
Liver	1.7
Brain and Medulla	0.1
Kidneys and Pancreas	0.1

In interpreting the results obtained it is convenient to compare the radioactivity of equal weights (say 100 mgms.) of the ashes, of the bones, the teeth, the liver, and so on. These all contain about the same percentage of phosphorus (17 per cent., 17 per cent., 16 per cent.); the phosphorus content of the ash of the blood is rather different, but as was stated above the behaviour of the active phosphorus in the blood was not investigated to any great extent in the course of this work.

In a series of experiments we gave the same amount of radioactive phosphorus to 6 rats. One pair of rats was killed after one week, a second pair after two weeks, and a third pair after three weeks. The results are seen in the following table.

Table V.

Animal killed weeks after eating rad. P	p. c. of rad. P found	
	in the skeleton	in the incisors
1	34.2	2.1
1	35.3	2.1
2	32.2	2.8
2	27.2	2.1
3	24.6	2.8
3	25.4	2.7

The weights of the different skeletons vary to an appreciable extent; the weights of the animals were 225, 210, 200, 215, 235, and 220 gms. before, and 220, 205, 200, 205, 235, and 220 gms. resp. after the experiment. In comparing the rad. P content of different organs of the same rat we are independent of the assumption that all the rad. P given was actually eaten by the animal, though we are not, when we compare the rad. P content of organs from different rats. The greater rad. P content of the bones of the animals killed after the lapse of only a week cannot, however, be due chiefly to such a reason as this, because in that case the rad. P content of the incisors would also be appreciably higher in the case of rats killed after the lapse of one week. This is not the case, as can be seen from the figures in Table V. We must therefore conclude that the rad. P taken up by the bones, and in exactly the same way all the phosphorus taken up by the bones, has a certain chance of being lost again. Indeed a uptake of phosphorus atoms by the bones of an adult rat can only be explained by a corresponding

Table VI.

	p. c. of rad. P taken, present in 100 mgms. of ashes	weight of ashes of the organ in mgms.	p. c. of rad. P taken, present in the total ashes
a) rat killed after 1 week.			
Bones	0.8	4300	34.3
Molars	0.2	100	0.2
Incisors	1.3	253	3.3
Liver	3.2	103[1]	—
b) rat killed after 2 weeks.			
Bones	0.7	4200	29.5
Molars	0.2	100	0.2
Incisors	1.9	215	4.1
Liver	2.0	210	4.2

[1] The weight of the ashes of the liver was found to be very variable.

process in the opposite direction. Another example of the decrease in the active phosphorus content of the bones with time is seen in Table VI.

While the bones show a decrease in their rad. P content with time and the molars no change to within the accuracy of experiments, the incisors show a marked increase. The incisors of adult rats show a very pronounced growth. The discussion of their behaviour is therefore better postponed and will be dealt with in the next chapter, where experiments on young rats are described.

The results of an experiment carried out with two rats both killed after 5 days time are seen in Table VII.

Table VII.

	p. c. rad. P taken found in 100 mgms. of ashes	
	I	II
Bones	1.3	1.4
Molars	0.24	0.34
Incisors	2.4	2.3
Liver	2.7	1.7
Muscles	1.7	1.8
Brain	0.46	0.58

As is seen from the above figures the muscles show a somewhat large content of rad. P than the bones. The active P content of the brain ash is decidedly lower. To ascertain if the phosphorus atoms present in the brain phosphatides are also replaced by active P atoms, the brain was treated with 6 per cent. trichloracetic acid solution. By this means all the acid soluble phosphorus was removed. The operation was carried out with great care. After igniting the filtrate and residue, the activity of both fractions was measured. We found both fractions to be active, the activity of the phosphatide fraction being about $^1/_3$ of that of the trichloracetic acid extract. We are engaged in following up this point in greater detail, using more trustworthy methods of separation.

Exchange of phosphorus by growing rats.

The uptake of phosphorus shown by different organs of rats about 2 weeks old is seen in Table VIII. The rats were killed three days after being fed with radioactive phosphorus added to their normal diet.

Table VIII.

	Rat I (weight 27 gms.)		Rat II (weight 24 gms.)	
	weight of ashes in mgms.	p. c. of rad. P taken present in 100 mgms. of ashes	weight of ashes in mgms.	p. c. of rad. P taken present in 100 mgms. of ashes
Bones (Leg)...	65.4	10.5	59	10.9
Incisors.......	—	5.8	—	5.8
Molars........	39.4	2.9	33.8	2.6
Muscles.......	—	11.0	—	—
Blood.........	—	2.8	—	2.6

Focussing our attention first on the bones we notice that 100 mgms. of ash contain more than ten times as much radioactive phosphorus as was found in the case of adult rats. The high radioactivities of the bones are due to the fact that in this case an appreciable part of the bones are actually grown from blood of high radioactive phosphorus content; a rapid formation of new cells takes place, in whose building up radioactive phosphorus participates.

A very conspicuous difference is found between the active phosphorus content of the molars of rapidly growing and of adult rats, the great difference being due primarily to the low exchange values in the latter. The brain as a whole was found to contain 0.5 per cent. of the active phosphorus taken by the animal.

The ratio between the rad. P content of the muscles and the bones is nearly unity in the case of the young rats, while in adult rats the muscles show a higher rad. P content.

When we compare the radioactive phosphorus content of the bones of growing rats, we find for example more activity in 100 mgms. of the ashes of the bones of animals killed after one week and than in those killed after two weeks. This is due chiefly to the fact that the phosphorus atoms present in the bone at a certain time will soon be found in an entirely different part of the growing skeleton, and will also have a certain chance of leaving the skeleton entirely. If we want to obtain information on the latter point we must compare the "radioactive" phosphorus contents of whole skeletons. We carried out such experiments, comparing the whole of the leg material. Five very young rats having a total weight of only 25 gms. were fed on their normal diet plus some radioactive phosphorus (0.50 mgms. each). Two were killed 2 days later and three 65 days later. 10 mgms. of the ashes of the leg bones of animals killed after 2 days contained 8.4 times as much radioactive phosphorus as that of rats killed after 65 days. The active phosphorus atoms were in fact distributed all through the greatly increased amount of bone tissue; the leg bones increased in the course of 63 days to about ten times their original weight, as can be seen from Table IX. When we compare the radioactive phosphorus content of the total bone material of the legs the difference between the rats killed after 2 days and after 65 days is much less; the difference still present is due to the loss of phosphorus atoms by the bone material. The phosphorus atoms which were present in the bone for a while and left it again will be found chiefly in the excrements but to a small extent also in some of the organic compounds building up the organism, which are formed slowly. In the course of two months about one-third of the phosphorus atoms originally present left the skeleton entirely.

A comparison of the behaviour of the active phosphorus present in the incisors with that in the bones is difficult in view of the rapid using up and replacement of the incisors. Prof. Holst, Prof. Krogh and one of the writers of this paper are at present engaged on an investigation of the exchange of phosphorus in the incisors on different lines.

Table IX.

Period between taking of radioactive P and killing	Weight of bone ash (legs) in mgms.	p. c. of radioactive P present
2 days	65.4	7.4
2 ,,	59.0	7.5
65 ,,	440	4.1
65 ,,	514	5.1
65 ,,	613	5.5

Uptake of phosphorus in pregnant rats and in human placenta.

In Table X the result of the investigation of adult normal and pregnant rats is seen. Those designated I were killed after a lapse of one week, those marked II after two weeks.

As can be seen from the above figures the different organs of the pregnant rats took up less rad. P than normal rats the difference being found at least partly in the foetus and placenta. In the first rat, which was in an advanced stage of pregnancy, the foetus and still more the placenta had a high content of rad. P, higher than any organ of the mother. We find here again a very conspicuous illustration of the difference between the taking up of P through an exchange

Table X.

	Normal rat p. c. of rad. P taken present in 100 mgms. ashes	Pregnant rat p. c. of rad. P taken present in 100 mgms. ashes
I Bones	0.78	0.49
II Bones	0.74	0.52
I Incisors	1.3	1.2
II Incisors	1.9	1.7
I Molars	0.21	0.12
II Molars	0.23	0.16
I Liver	2.0	1.6
II Liver	1.94	1.0
I Foeta	—	2.7
II Foeta	—	0.54
I Placenta	—	4.0
II Placenta	—	2.3

process and through actual growth, the latter being much more effective in introducing rad. P into the tissue. An appreciable part of the foetus has actually been built up by utilising blood of rad. P content and has correspondingly a high content of the latter. This is still more the case for the rapidly growing placenta, the latter also being subject to a very thorough blood circulation. In the case of the second animal pregnancy occurred at a much later date than the intake of rad. P. The foetus was nourished by blood poor in rad. P, and correspondingly the rad. P content of the foeta was much less. Whereas in the first case the weight of the ash of all foeta was 345 mgms., in the second case it was only 52 mgms., the weight of the placenta ash being 43 and 12 mgms. respectively.

We also had an opportunity to witness what was a comparatively very high rad. P content for the placenta of a human subject; as much as 0.095 per cent. was found in the ash of the placenta, which weighed 133.8 mgms. We can estimate the total ash which the patient in question should give on ignition as 2800 gms. The weight of the placenta ash thus amounted to less than $^1/_{20\,000}$ of the total ash, while the rad. P content was as much as $^1/_{1000}$ of the total amount of rad. P given, showing a concentration of rad. P in the placenta ash more than twenty times as great as that in the average ash of the body. One might try to explain the high rad. P content of the placenta by its high blood content. That this explanation fails is seen, however, from the following. The ash of the placenta was found to weigh 133.8 mgms. and the ash of about 5 ccs. of blood would weigh the same. But as early as 8 hours after the injection of rad. P such a volume of blood was found to contain less than $^1/_{10\,000}$ of the latter[1], and after the lapse of a few days — when the placenta were removed — still less. The high rad. P content of the placenta cannot therefore be due to their blood content. No activity could be detected in the ash of the few weeks old foetus removed in the course of an operation, but the weight of this amounted to only a few mgms.

Uptake of phosphorus by rachitic rats.

We carried out a set of experiments on two months old rachitic rats, which had been used by Fredericia and Gudjonson in their experiments on the effect of vitamin A and D deficiency on rickets. The rats were fed before and during the experiments on a diet free from or poor in vitamins A and D. The weights of the animals before the

[1] In the case of another subject we found 1 cc. of blood to contain 0.0027 per cent. of the phosphorus injected after the lapse of 12 hours, the blood particles containing 11 times as much active phosphorus as the plasma.

Table XI.

Killed	p. c. from the rad. P taken found in 100 mgms. ashes				Weight in mgms.			
	Bones	Incisors	Molars	Liver	Bones[1] (legs)	Incisors	Molars	Liver
1 week....	4.2	3.8	0.7	3.2	358	100	72	135
1 „	4.2	3.8	1.1	5.9	329	105	76	103
2 weeks...	3.0	4.1	0.9	5.0	403	113	55	86
2 „ ...	3.5	3.7	0.9	5.0	361	96	64	84
3 „ ...	2.7	5.0	1.4	1.8	313	115	69	168
3 „ ...	2.2	3.6	1.1	1.2	419	109	57	145
3 „ ...	2.9	4.3	0.9	1.8	422	115	81	205

experiment were 89, 83, 85, 93, 90, 95, and 103 gms. The results are seen in Table XI.

The above figures show no outstanding difference as compared with normal rats of the same age. We are engaged in carrying out further experiments on rats with rickets.

General Considerations.

The rapid entrance of the labelled phosphorus into the bone is in no way puzzling. If solid calcium phosphate, one of the chief constituents of the bone, is in contact with the solution containing labelled phosphate ions a rapid distribution of the latter takes place between the surface of the solid phase and the liquid phase, as was seen from the following experiment. 3.950 gms. freshly precipitated $Ca_3(PO_4)_2$ were shaken with 5 ccm. of water saturated with $Ca_3(PO_4)_2$ at room temperature and containing an infinitely small amount of labelled sodium phosphate. After lapse of four hours 84.1 per cent. of the labelled phosphate ions were found in the solid phase and only 15.9 per cent. in the solution. The calcium phosphate of the bone tissue

[1] The weight of the total skeleton is obtained by dividing the figures obtained for the legs by 0.26.

being in a very intimate contact with the blood stream, i.e. with cells containing labelled phosphate, a similar exchange to that described above will take place between the unlabelled phosphate of the bone and the labelled phosphate present in the liquid phase.

Beside the above mechanism we have to consider two others yust as important. During growth, the bone tissue formed will be built up from labelled phosphorus as long as the blood stream contains the latter.

Finally we have to envisage a third possibility, namely the entrance of labelled phosphorus into the bone through a constant break-down of the bone tissue already formed and the formation of new tissue in the case of adult animals as well.

The following examples may help to make the three ways of entrance of the labelled atoms into the bone easier to understand.

1) When solid salts are in contact with labelled ions of the solution within a short time a distribution equilibrium of the labelled ions between the surface layer of the solid and the solution will take place, as is seen for example in the experiment described above. This phenomenon was studied extensively by Paneth and his collaborators[1] in the case of lead salt which were shaken with solutions containing labelled (radio- active) lead ions.

2) If we deposit for example lead electrolytically from a solution containing labelled lead ions, the metallic deposit will be a labelled one, just as the bone grown from blood containing labelled phosphorus will contain labelled phosphorus.

3) In investigating the exchange between metallic lead

[1] F. Paneth and W. Vorwerk, Zs. phys. Chem. **101**, 445, 480, 1922.

and a solution of labelled lead ions, or vice versa, we find[1] a different behaviour to that described above in the case of lead salts. The exchange in the case of metal is not restricted to the uppermost atomic layer of the lead surface; many atomic layers are involved in the exchange process. This is due to the fact that the lead actually goes into solution from certain parts of the surface, while lead ions are discharged, at other parts. This is a much more effective process in bringing about an exchange between the lead atoms in the solid and in the liquid phase than that observed in the case of solid salts where only the uppermost atomic layer is involved (within any reasonable time) in the exchange process. The entrance of labelled phosphorus into the bone will also be much facilitated if it is not only the uppermost phosphate layer that is involved in the exchange process; if in fact the bone is destroyed at certain places and rebuilt at others. In view of the important enzymatic actions[2] going on in the bone tissue such a reversible breakdown process will easily occur.

Summary.

By adding radioactive phosphorus (phosphate) to the diet of rats, the metabolism of the phosphorus atoms taken in with the diet can be followed up in the animal body. An appreciable part of the phosphorus taken finds its way not only in growing but also in adult animals into the bones, teeth, muscles, and different bodily organs.

In growing animals it was found that the atoms already present at an early stage of the formation of the skeleton

[1] G. Hevesy, Phys. Zs. **16**, 52, 1915.

[2] R. Robison: The significance of phosphoric esters in metabolism, New York 1932.

become equally distributed in the course of time over the different parts of the skeleton and other organs demonstrating thus the dynamical nature of the building up of bone tissue. Some of the phosphorus atoms present in the bones leave the skeleton for good, being eliminated through the kidneys or the bones or becoming located in other organs of the body.

The replacement of individual phosphorus atoms by other phosphorus atoms also takes place in the bone tissue of adult animals including that of the teeth.

It was ascertained that about one-seventh of the phosphorus found in the faeces of a human subjects is due to material which has entered the intestines through the digestive juices after being located in the blood stream or in the organs of the body for a shorter or longer time.

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The Finsen Institute and
the Institute of Theoretical Physics, Copenhagen.

Færdig fra Trykkeriet den 22. Marts 1937.

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