CCXIII. THE CIRCULATION OF PHOSPHORUS IN THE BODY REVEALED BY APPLICATION OF RADIOACTIVE PHOSPHORUS AS INDICATOR

By L. A. HAHN, G. CH. HEVESY and E. C. LUNDSGAARD

From the Institute for Theoretical Physics, and Institute for Medical Physiology, University of Copenhagen

(*Received 15 July 1937*)

The atoms of each element taken up with the food or by the respiration process have an average time which they spend in the body. Hevesy & Hofer [1934] measured the average time a water molecule spends in the human body by using deuterium as indicator and found it to be about a fortnight.

To determine the average time phosphorus spends in the animal body[1] we administered the labelled (radioactive) phosphorus,[2] as sodium phosphate, to a rabbit by subcutaneous injection. The labelled phosphorus was prepared from sulphur by neutron bombardment. Its weight was negligible compared with that of the inorganic phosphorus contained in the plasma, so the normal conditions prevailing in the latter were not influenced by the rapid absorption of the radioactive phosphorus injected. It has been shown recently by Scott & Cook [1937] that administration of radioactive phosphorus (as phosphate) gives rise to some marked effects upon the constituents of the blood of birds. The preparations applied by them were, however, enormously more active than those used by us in this investigation and also in our former researches. In applying radioelements as indicators in biology it is of the greatest importance to use preparations of such limited strength that the effect of the radiation on the organism is negligible. The use of such weak preparations is much facilitated by using very sensitive measuring instruments such as Geiger counters [for experimental procedure, *v.* Chiewitz & Hevesy, 1937].

To administer the phosphorus by injection and not *per os* has the advantage that no account need be taken of the phosphorus which does not get absorbed. To arrive at the figure for the average time which absorbed phosphorus spends in the body, we collected faeces and urine for 27 days and determined the amount of active phosphorus present in these. After the lapse of 27 days we killed the animal and determined the remaining active phosphorus in the different organs of the animal. We found the following figures for the distribution of the active phosphorus.

Table I. *Distribution of the active phosphorus*

	Weight (g.)	% of active phosphorus found	
Urine	2400	45·0	56·5 (Urine + Faeces)
Faeces (dry)	650	11·5	
Skin	327	1·98	43·4 (Skin to Residual body)
Liver	96	1·71	
Blood (calculated)	250	0·74	
Intestinal content	172	0·28	
Kidney	13·2	0·14	
Brain	8·9	0·087	
Residual body	2508	38·5	

[1] The results of similar experiments carried out on human subjects will be published soon.

[2] The half life period of the radioactive phosphorus isotope used was 14·5 days.

As seen from the above figures, after the lapse of 27 days more than half of the phosphorus given has been eliminated. By making use of the data in Table III we can calculate that half of the active phosphorus atoms given, and correspondingly of all phosphorus atoms absorbed, are eliminated after the lapse of 20 days. By dividing the last figure by $\log_e 2$ we get 30 days for the average time a phosphorus atom spends in the body.

The comparatively long average time of a fortnight spent by a water molecule in the body was explained by the fact that the water taken is diluted by the large amount of water present in the body; in consequence the probability of a molecule leaving the body within a few days is much reduced. Not only does the organism of a human subject contain about 60 % of water but in the course of the metabolism new water molecules are produced; furthermore some of the hydrogen atoms present will exchange their places with other hydrogen atoms present in certain organic compounds such as proteins [Krogh & Ussing, 1936] and other compounds [Schönheimer & Rittenberg, 1935], the chief effect of dilution of the individual water molecules taken being, however, due to the water content of the body. The water taken daily becomes diluted roughly 20-fold.

In the case of phosphorus also, the long average life of the atoms taken with food is due to dilution of the phosphorus atoms taken by those present in the organism. We found 14 g. of phosphorus in the body of the rabbit in question, its average daily phosphorus intake, calculated from the daily excretion, being 0·270 g. If all the phosphorus atoms present in the body of the rabbit could enter into exchange processes with those taken with food, i.e. if they could all take part in diluting the latter, we should expect an even longer average life of the atoms of phosphorus than that actually found. We must therefore conclude that an appreciable part of the phosphorus present in the organism of the rabbit is protected from entering into exchange processes with the phosphorus atoms taken with food within 27 days.

In view both of the great prevalence of bone phosphorus in the organism and of the structure of bones it seemed almost certain that it is the bone phosphorus which only exchanges to a restricted extent even during a period of a month with the blood phosphorus. That this is actually the case is seen from Table II, which contains the ratio of normal phosphorus to radioactive phosphorus. The former was measured by the colorimetric titration method of Fiske & Subbarow, after bringing the organ in question into solution, while the radioactive phosphorus content was determined by means of a Geiger counter.

Table II

$\frac{\text{Active P}}{\text{Normal P}}$, taking body average

Whole body (average)	Skin	Liver	Blood	Kidney	Brain	Muscles	Femur head	Tibia average
1·0	—	2·20	2·43	2·72	1·58	2·67	0·43	0·26

The ratio of total phosphorus to active phosphorus in an average sample of the rabbit was arbitrarily taken as unity. The mixture was obtained by mincing a vertical half of the whole rabbit after removing the skin and the brain. Table II shows that bones contain less radioactive phosphorus per mg. P than the average mince, while all the other organs, especially the kidneys, the muscles and also the liver, contain appreciably more. As to the bones we investigated both the average tibia and the head of the femur. The fact that the latter, an epiphyseal bone, was found by us, in numerous cases of rabbits, cats, rats and other animals, to contain much more active phosphorus per mg. normal phosphorus than the diaphysis gives us a clue to the understanding of the great difference between the active phosphorus content of the bone phosphorus and that of other organs. An exchange of phosphorus between the atoms present in the blood and those present in the bones can only take place when proper contact is made. This contact is much better in the epiphyseal bones than in the diaphyseal bones, since the former are rich in organic matter, through which the constituents of the blood stream can penetrate comparatively easily. The organic constituents and water amount in the epiphysis to about 50 % and in the diaphysis only to about 30 %. Should the morphological methods for some reason fail, we can easily decide to what extent a bone is diaphysic or epiphysic by determining the relative distribution of active phosphorus in the different parts of the bone in question. Thus the total phosphorus present in the head of the femur still contains only 1/6 of the active phosphorus present in a similar amount of normal muscle phosphorus. If we assume that a full exchange equilibrium is reached between the active and total phosphorus in the latter case it follows that only about 1/10 of the average bone phosphorus actually underwent exchange and that 9/10 of the phosphorus atoms of the bone calcium phosphate were not released within a month by other phosphorus atoms present in the circulation. In other words that 9/10, on an average, of the bones are protected against exchange for at least a month's time. If, as is possible, an exchange equilibrium has not yet been reached between the radioactive and the normal phosphorus atoms in the muscle the above figure of 9/10 would be even larger. To follow up this point is of great interest as it may give new information on the constitution of the bone tissue.

The fact that the ratio of active phosphorus to normal phosphorus is practically the same for the blood phosphorus and for example kidney phosphorus makes it probable that an exchange equilibrium between the active and the normal phosphorus is obtained, but a certain caution in interpreting this result is necessary. We have, it must be remembered, to deal with the distribution of active phosphorus between different phases and a number of different chemical compounds and it is therefore not impossible that the agreement mentioned above may be fortuitous. As to the imperfect equilibrium between normal and radioactive phosphorus in the bone and in the blood, two possible extreme cases have to be envisaged: (*a*) a further interpenetration of the bone tissue would not take place after a longer time, (*b*) interpenetration occurs to an extent proportionate to the time of the experiment. Some information on this question can be obtained in following up the excretion of phosphorus.

Conclusions drawn from excretion data

The active phosphorus travels from the plasma into the different organs; if equipartition of the active phosphorus between the blood plasma and the different organs is obtained a further loss of active phosphorus can only occur through the kidneys and the bowels. If the actual loss in the plasma phosphorus happens to be greater than that found in the excrements, the "missing" active phosphorus must be looked for in the organs. Large blood samples could not be taken from the experimental animals, and small samples contain only small amounts of phosphorus whose activity is correspondingly minute. So instead of following up the change of the activity of the phosphorus of the blood plasma we investigated that of the urine phosphorus. In so doing we made the assumption that the ratio of active to normal phosphorus in the urine cannot differ much from the corresponding ratio for the plasma. As is seen in Table IV, after

Table III

Time in days	% of active phosphorus given to the rabbit present in: Urine	Faeces	Total excretion
3	22·62	2·58	25·20
4	2·91	0·75	3·66
8	4·97	1·84	6·81
9	2·47	0·46	2·93
13	4·31	1·47	5·78
14	0·87	0·37	1·24
18	3·55	1·61	5·16
19	0·79	0·40	1·19
24	2·18	1·70	3·88
25	0·33	0·34	0·67

Table IV

Time in days	% of active phosphorus present at the time in question in the body found in 1 mg. urine phosphorus
4	0·0217
9	0·0186
14	0·0143
19	0·0102
21	0·0076

4 days we found, per mg. urine phosphorus, 0·0217 % of the total activity present at the time in the rabbit. If, for example, between the fourth and the ninth day, the blood had only lost active phosphorus by excretion, the urine should have contained on the ninth day again 0·0217 % of the activity present at the time in the rabbit, which latter can be computed from the excretion data.

It can, however, be seen in Table IV that the latter figure is appreciably less, namely 0·0186 %. The difference between the value calculated and that found can only be due to a further uptake of active phosphorus atoms from the blood by the bone phosphate and by a corresponding uptake by the blood of inactive phosphorus atoms from the bone phosphate. In view of the great preponderance of the bone phosphorus in the body no conclusions can be drawn, if a corresponding further uptake takes place between blood phosphorus and e.g. muscle phosphorus simultaneously with the above-mentioned process. It can, however, be concluded from the figures in Table IV that an intense uptake (exchange) of active phosphorus atoms by those parts of the bone tissue, which have not till then taken part in such a process, still goes on after the lapse of 21 days.

Some information on exchange processes in the muscle can be obtained from the following experiment, carried out 12 days after administering the active phosphorus. A muscle sample was taken and the inorganic phosphorus was precipitated from one portion and the inorganic phosphorus + the phosphagen phosphorus from the other. Both the phosphorus contents and the activities of the two samples were then determined. The ratio of the normal phosphorus content of the two samples determined by the colorimetric method was 4, while the ratio of the active phosphorus contents was only 2, showing that in the phosphagen sample the equilibrium between the active inorganic and the active phosphagen phosphorus had not yet been reached. Equipartition of the active phosphorus between plasma phosphorus and the inorganic muscle phosphorus was, however, already reached after 12 days. As explained already we determined the ratio of active phosphorus to normal phosphorus in the urine and assumed that a similar value can be used for the corresponding ratio in the blood plasma. The figures obtained were 0·013 and 0·012 % respectively of the active phosphorus administered per mg. of normal phosphorus present (as inorganic phosphorus) in the muscle and the urine (plasma) respectively.

SUMMARY

The distribution of radioactive phosphorus administered to a rabbit was investigated. It was found that within 27 days 45 % of the phosphorus given was excreted through the kidneys and 11·5 % through the bowels. The average time a phosphorus atom spends in the body was found to be 30 days. The ratio between active and normal phosphorus was found to be highest in the kidney, in the muscles and in the liver, and lowest in the bones, especially in the diaphysis.

It is probable that in the course of 27 days an equipartition of the active phosphorus between the muscles and the blood takes place while about 9/10 of the bone phosphate is protected against the influx of the active phosphorus. It could, however, be shown by following up the ratio of the active phosphorus to normal phosphorus in the urine that an exchange of the phosphorus atoms present in the bones and in the blood, and thus an atomic rejuvenation of the bones to a further extent, is still going on after the lapse of 21 days. Since the active phosphorus shows the same chemical behaviour as normal phosphorus, the conclusions arrived at apply to all the phosphorus taken up with the food.

The radioactive phosphorus used in our experiments was prepared from sulphur by the action of neutrons emitted by a radium-beryllium mixture, kindly put at our disposal by Prof. Niels Bohr. We would like to express our best thanks to Prof. Niels Bohr for his kind interest in this work and to Miss Hilde Levi and to Mr Rebbe for their assistance.

REFERENCES

Chiewitz & Hevesy (1937). *Proc. Copenhagen Acad. Sci.* **13**, 1.
Hevesy & Hofer (1934). *Nature, Lond.*, **135**, 879.
Krogh & Ussing (1936). *Skand. Arch. Physiol.* **75**, 90.
Schönheimer & Rittenberg (1935). *J. biol. Chem.* **111**, 163.
Scott & Cook (1937). *Proc. nat. Acad. Sci., Wash.*, **23**, 265.