Application of Isotopes in Biology.

By GEORGE HEVESY.

(The opening contribution to the Discussion held at a joint meeting of the Chemical Society and the Physiological Society on February 9th, 1939.)

THE main use of isotopes in biology is for labelling purposes. To be a suitable indicator for biological research an isotope has to fulfil two conditions: it must be absent or present at a small concentration only in the body or the plant to be investigated; it should be easy to determine. These conditions are fulfilled best by radioactive isotopes. Radioactive isotopes of suitable periods of sodium, potassium, magnesium, calcium, phosphorus, sulphur, chlorine, iodine, iron, copper, and some other elements are available and we can make use of them when faced with the problem of following the path of these elements in the body—the way and extent of their participation in metabolic processes. No radioactive isotope of hydrogen, and none of nitrogen, oxygen and carbon* of suitable period, are known, and therefore we have to make use of rare, stable isotopes such as ^{2}H, ^{18}O, ^{15}N, or ^{13}C as indicators. ^{2}H and ^{18}O are usually determined by measuring the density of water obtained when the compounds containing these isotopes are burnt in normal oxygen and hydrogen, respectively; ^{15}N and ^{13}C are determined with the mass spectrograph. The latter method is less exacting than the density method as to the purity of the samples used, and in the case of nitrogen is reported to require but 1 mg. for the analysis (Schoenheimer and Rittenberg, *J. Biol. Chem.*, 1939, **127**, 285).

The use of isotopic indicators has revealed that many of the atoms present in the organism spend on an average only a short time at a fixed place or in an individual molecule in the body. When using isotopic indicators, the biologist discovers the dynamic aspect of the processes going on in the organism which he otherwise seldom encounters. This will be best understood from a discussion of a few examples of the application of isotopic indicators, as, for example, the radioactive phosphorus isotope (^{32}P). Phosphorus is an important constituent of the body not only in the form of inorganic phosphate but also as phosphorus incorporated in organic compounds. Hence by tracing the course of labelled P we get information not only about several aspects of the mineral metabolism of that element but also as to the place and rate of formation of the important group of organic compounds which contain phosphorus. ^{32}P can be prepared easily by the action of fast neutrons on sulphur (suitably present as carbon disulphide) or by the action of deuterium ions on ^{31}P.† This radioactive indicator decays with a half-life time of 14·5 days and emits β-rays which lose half their intensity after passing an aluminium layer of $\frac{1}{2}$ mm. thickness. Labelled phosphorus is usually administered as sodium phosphate. Active sodium phosphate in minute quantities (10^{-10} g.) can be prepared by the action of neutrons on sulphur. The administration of such a negligible amount has the advantage of leaving the concentration of phosphorus present in the organism unchanged and in all cases permits working under strictly physiological conditions; moreover, the disturbing effect of the radiation emitted by the sample need not be considered if the use of preparations of high activity is avoided.

The animal body contains quite an appreciable amount of potassium which emits β-radiation similar to that emitted by any of the artificially produced radio-elements. If we stress the importance of the noxious effect of the radiation emitted by the radioactive indicator, we arrive at the result that every organism, owing to the presence of potassium, is always under the influence of such an effect; in point of fact, some of the indicator

* The radioactive isotope ^{11}C has a half-life period of 22 minutes. When strong preparations are available, experiments lasting to 3—4 hours can be carried out by means of this isotope.

† According to the equations ${}^{32}_{16}S + {}^{1}_{0}n = {}^{32}_{15}P + {}^{1}_{1}H$ and ${}^{31}_{15}P + {}^{2}_{1}D = {}^{32}_{15}P + {}^{1}_{1}H$, respectively. In many cases we prepared ^{32}P by the first-mentioned method, using a mixture of 600 mg. of radium and of beryllium kindly put at our disposal by Professor Niels Bohr; when strong activities were wanted, samples which were a generous gift of Professor Lawrence in Berkeley were used.

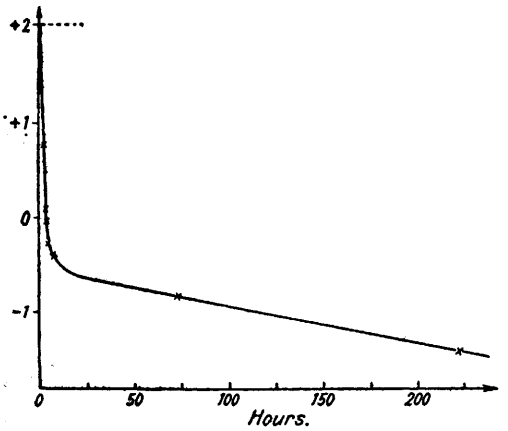
research can be carried out by using activities of the same order of magnitude as that of the potassium normally present in the animal.

When discussing the application of isotopes as indicators, we must remember that isotopes are not strictly chemically identical atomic species. The difference can best be seen in the case of the isotopes of hydrogen. The equilibrium constants of reactions in which H on the one hand and D on the other are involved can differ quite materially, and the same is true of the velocity constants. The difference in the chemical properties of all the other elements is much less marked than that between hydrogen and deuterium, hydrogen having in many respects an unique position, since it is the only element which even temporarily splits off atomic nuclei in the course of chemical reactions. In spite of its shortcomings, deuterium has been used in numerous cases with great success as an indicator. Some of these applications will be reviewed by Dr. van Heyningen. We may expect that the lack of strict chemical identity of the isotopes of elements which are heavier than hydrogen will still less hamper their use in indicator work. The difference in the chemical properties of isotopes, such as ^{31}P and ^{32}P, may lead, for example, to a difference in the distribution coefficient of the phosphate radical between inorganic phosphate and lecithin phosphate in the body, but the difference will be a very minute one and will influence the results obtained much less than the errors incidental to the experiment. If we find that in the liver ^{32}P which was originally present as inorganic phosphate becomes incorporated at a definite rate into lecithin-phosphate, we can without hesitation extrapolate this result as to the behaviour of ^{31}P. Should one of the enzymes, the presence of which is indispensable to the reactions studied, fail to act on one of the two isotopes, this would seriously hamper the applicability of the isotopic indicators in such a case. However, no example of such behaviour is known, and it is very improbable that enzymes which act in the presence of ^{31}P would fail to do so in the presence of ^{32}P. In what follows we shall discuss some applications of ^{32}P as an indicator.

Disappearance of the Labelled Phosphorus from the Circulation.

We inject labelled sodium phosphate into the veins of, for example, a rabbit, take at intervals small blood samples (0·3 c.c.) from the ear veins, and determine their activity or that of their plasma content. By this procedure we can easily follow the rate of disappearance of the labelled phosphate from the blood or the plasma. An appreciable part of the labelled phosphate injected into the blood plasma will almost instantly go over into the extracellular fluid present in the body. This process will be responsible for the disappearance of up to $\frac{6}{7}$ of the labelled phosphate introduced into the plasma. As seen from Fig. 1, however, in the course of 2 hours the labelled P content of the plasma decreases to 2% of its initial value. Most of the ^{32}P which has left the plasma can be located in the bone tissue, in the cells of the muscles, the liver, and other organs, and also in the blood corpuscles, in which it replaces an equal number of non-labelled P atoms, which move into the plasma. A keen competition is thus going on between the plasma, the bone tissue, and the cells for the possession of each individual phosphorus atom. To this competition is due the fact, illustrated in Fig. 2, that, when the labelled phosphate is administered by subcutaneous injection or by mouth, only a fraction of the labelled P atoms will be found *simultaneously* in the circulation.

FIG. 1.



Change of the logarithm of the labelled* P *content of the plasma of a goat with time after administration of labelled sodium phosphate by intravenous injection. (Aten, Dissert., Utrecht, 1939.)

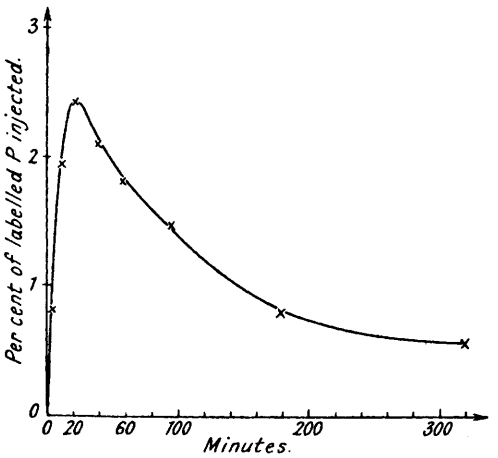
Owing to increased absorption the labelled P content will first increase with time, but after about 25 minutes a maximum content of only about 3% of that administered by subcutaneous injection will be reached, which will be followed by a decrease due to the interaction described above. ^{32}P will also be removed from the plasma by excretion through the kidneys and the bowels, but, in the course of 24 hours, the amount is less than 15% of the amount administered, as discussed in more detail on p. 1218.

Elements which do not form organic compounds and are not main constituents of the bone tissue show a very different behaviour, as seen in Fig. 3, which shows the change of the plasma activity with time after intravenous injection of labelled sodium as sodium chloride.

Distribution of Labelled P between Different Organs in Rats.

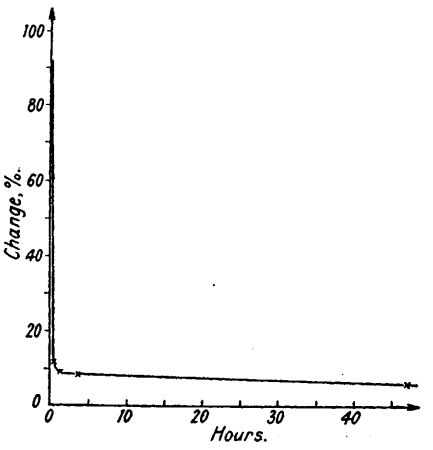
The distribution of ^{32}P between different organs in a rat, 4 hours after subcutaneous injection of labelled sodium phosphate of negligible weight, is seen in Table I, which contains also data on the total P content of the organs. The total P was determined by the colori-

FIG. 2.



Change of labelled P content of the plasma with time after subcutaneous injection of labelled sodium phosphate to a rabbit.

FIG. 3.



Change of the labelled sodium content of the plasma of a rabbit with time after administration of labelled sodium as $^{24}NaCl$ by intravenous injection.

metric method of Fiske and Subbarow and the radioactive measurements, mostly made by Miss Hilde Levi, were carried out with a Geiger counter.

As the Table shows, most of the ^{32}P absorbed into the circulation is found in the bones, the muscles, the liver, and the digestive tract. In many cases it is of importance to consider the ratio of labelled P to total P present in an organ. This quantity—the specific activity (the percentage of the activity administered shown by 1 mg. of P)—tells us to what extent the P atoms present in the organ participated in the exchange process in which were involved the labelled P atoms present in the circulation. From the fact that the specific activity of the muscle P is about nine times larger than that of the bone P it follows, for example, that, during the time in which about 9P atoms present in the muscle tissue were replaced by labelled P atoms, only 1P atom of the bone tissue was involved in an exchange process. At the start of the experiment all labelled P atoms are present in the plasma, but during the experiment a stage will be reached at which the labelled P atoms will be equally distributed between the plasma P and the muscle P. At this stage, probability considerations alone determine how many labelled P atoms will be present in the plasma and how many in the muscle tissue; an equipartition of the labelled P atoms between plasma and muscle is reached, the P atoms present in the muscle all having become " renewed." The extent to which equipartition is reached is a convenient measure of the

rate of replacement of the P atoms in question. In this connection it may be permissible to mention that considerations which apply to a labelled P atom apply to every P atom. Each individual P atom present in the plasma will have a chance to be incorporated into the muscle tissue which can be determined by ascertaining the rate of incorporation of ^{32}P atoms in the latter. In experiments of short duration, as seen in Table I, only about $\frac{1}{5}$ of the activity administered is to be found in the bones, but with longer time the active P accumulates to an increasing extent in the skeleton and, after 98 days, 92% of all labelled P atoms present in the body are in the skeleton (Table II). On the other hand, the share of the muscles in the uptake of labelled P present in the body (compare Table II) increases at first, but decreases again after about 20 days. The rats in question excreted

TABLE I.

Distribution of labelled phosphorus between different organs in a rat 4 hours after subcutaneous injection. Weight of the rat, 188 g.

Organ.	Weight, g.	Total P content, mg.	% Labelled P present.	Specific activity.
Bones	21·6	931	18·6	0·020
Muscles	75·2	118	15·4	0·131
Liver	7·5	29·5	14·0	0·475
Digestive tract + content	16·2	34·8	12·7	0·365
Skin	29·7	45·8	8·8	0·192
Lungs + heart	9·1	15·8	5·0	0·317
Blood	9·0	3·58	2·00	0·558
Kidneys	1·9	5·05	1·87	0·370
Spleen	1·2	3·91	1·00	0·256
Brain	1·9	5·07	0·16	0·032
Place of injection	3·2	3·01	20·5	—

TABLE II.

% Total labelled P present in the body found in some organs of rats.

	Time after which the rat was sacrificed:						
Organ.	½ hour.	4 hours.	10 days.	20 days.	30 days.	50 days.	98 days.
Muscles	18·3	19·4	25·8	28·8	25·2	12·1	3·6
Carcase after removal of bones, muscles, blood, and skin	37·0	43·6	21·0	16·4	13·1	6·7	2·9
Total skeleton	19·1	23·4	43·1	43·1	51·8	76·5	92·0

daily about 12 mg. of P through the kidneys. As the inorganic P present in the circulation amounts to only about 1 mg. and the total acid-soluble P to about 6 mg., most of the labelled P atoms present in the circulation at any moment will be swept out by excretion within a day. This will have the result that the labelled P atoms present in the organs will migrate into the plasma. In fact, such a movement takes place in all stages of the experiment, but its effect is now overcome by a larger movement taking place in the opposite direction. Different organs will participate in this movement to very different extents. Those P compounds, such as most of the acid-soluble ones (see p. 1218), which are easily formed will also readily decompose and the labelled P incorporated in the latter will reach the impoverished plasma quickly. On the other hand, the labelled P atoms which find their way with difficulty into the bone tissue will also be given off at a slow rate. This consideration applies especially to the diaphysial (hard) bone, the P atoms of which are exchanged at a slower rate than those of the epiphysial (soft) bone (Hevesy, Holst, and Krogh, *Kgl. Dansk. Vidensk. Selsk. Medd.*, 1937, *B*, 1, **13**; Dols *et al.*, *Nature*, 1938, **142**, 953). The epiphysis of a rabbit exchanges, within the first 3 hours of the experiment, 1/400 part of its P atoms, presumably those which are in contact with the circulating lymph containing labelled phosphate, but replacement of all the P atoms present in the diaphysis is not effected even after several months. After 50 days (see Table III) the specific activity of the diaphysial P amounts to only one half of that of the urine P and thus also of the inorganic P in the plasma. In contrast to the urine, the plasma contains only small amounts of inorganic P; it is therefore advisable in experiments of long duration, when

samples of sufficient activity are desired, to investigate the urine P. We found that the specific activity of urine P is equal to that of the plasma inorganic P. The urine data are those obtained by an investigation of the urine collected on the day preceding the sacrifice of the rabbit. As a part of the labelled P was incorporated at a stage at which the plasma P was much more active than it was on the last day of the experiment, the extent of equipartition of labelled P between plasma and diaphysis in the course of 50 days is, in fact, less than 50%.

TABLE III.

Specific activity of femur diaphysis P *and of urine* P *of rats.*

Time after which the rat was sacrificed.	A. Specific activity. Of femur diaphysis P.	B. Specific activity. Of urine P.	Ratio A/B.
10 days	0·0045	0·0528	0·085
20 ,,	0·0036	0·0190	0·190
30 ,,	0·0057	0·0174	0·328
50 ,,	0·0059	0·0126	0·468

The extreme case of a hard, highly mineralised bone is the enamel. This contains about 5% of non-mineral constituents and therefore there is only a very slight lymph circulation in it, which suffices, however, to enable it to take up a minute amount of ^{32}P.

Study of Excretion.

Chemical analysis of the food taken and of the excreta permits the determination of the extent to which the organism is in balance. Chemical methods, however, cannot determine to what extent the substances found in the fæces originated from non-digested food and to what extent they were carried into the digestive tract, coming from the body proper in the form of digestive juices. This problem can be solved under strictly physiological conditions by making use of isotopic indicators.

The simplest procedure is the following: at a suitable time after the administration of labelled sodium phosphate, we determine the specific activity of the urine P and that of the fæces P. Both originate from the blood plasma and, if only we wait for a sufficient time, the specific activity of the P compounds carried into the digestive tract from the body will be about equal to that of the urine P. If the fæces P were entirely of endogenous origin, it should show a specific activity equal to that of the urine P. If we find the fæces P to be less active than the urine P, the active fæces P of endogenous origin must have been diluted by non-active P. Since the sole source of non-active P is the diet, the ratio of the specific activities of the fæces P and the urine P tells us to what extent the endogenous fæces P was diluted by food P.

The ratio 100 × specific activity of fæces P/specific activity of urine P gives the percentage of P present in the fæces which originates from the body proper. The amount of non-digested P found in the fæces of rats is seen in Table IV. The samples were collected 10, 20, 30, and 98 days respectively after administration of the labelled P.

TABLE IV.

Food P *and endogenous* P *in the fæces of rats.*

Rat.	$\frac{\text{Specific activity of fæces P}}{\text{Specific activity of urine P}}$	% Non-absorbed food P in total fæces P.
I	2·47	60
II	3·10	68
III	2·29	56
IV	2·44	59

In the case of human subjects, $\frac{3}{4}$ to $\frac{4}{5}$ of the P present in the fæces was found to originate from non-absorbed food P. Many other problems of food absorption, having not only physiological but also clinical interest, can be investigated by making use of

isotopic indicators. Vegetables raised on labelled soil, meat, eggs, or milk produced by a labelled organism can be fed, and the rate of resorption tested by measuring the activity of the urine and fæces.

Formation and Turnover of Organic Phosphorus Compounds.

So far we have discussed some aspects of the mineral metabolism of phosphorus. We will now consider the application of labelled P for the purpose of obtaining information as to the place and rate of formation of organic compounds containing phosphorus.

As the organic P compounds are built up inside the cells, the formation of labelled P compounds must be preceded by penetration of labelled phosphate from the plasma, or lymph, into the cells. The rate at which this process takes place can be estimated by comparing the specific activities of the inorganic P present in the cells and that present in the plasma. In experiments on rabbits, in which the labelled phosphate was injected drop by drop (see p. 1220), we found that after 3 hours the specific activity of the inorganic P extracted from the muscles amounted to about 8% of that found for the inorganic P present in the plasma. The exchange of the plasma phosphate and muscle phosphate is therefore a comparatively slow process. The rate of penetration of the phosphate ion into the cells was found, in an investigation carried out in collaboration with Dr. Hahn and Mr. Rebbe, to differ markedly for different tissues, as seen in Table V. In view of the comparatively low inorganic P content of the extracellular fluid * the above figures represent to a first approximation the relative rates of penetration of labelled phosphate into the cells of the different organs.

Information on the rate of formation of organic P compounds, for example, of creatine-phosphoric acid, can be obtained by comparing the specific activity of the creatine-phosphoric acid P with that of the inorganic P present in the muscle cells. As seen in Tables VI and VII, the formation of several of the acid-soluble P compounds is a compar-

TABLE V.

Relative rates of penetration of labelled inorganic phosphate into the tissue of different organs.

Organ	Brain	Muscle	Corpuscles	Spleen	Heart
Relative rate of penetration of labelled phosphate	1	4	6	17	20
Organ	Lungs	Kidney (medulla)	Liver	Intestinal mucose	Kidney (cortex)
Relative rate of penetration of labelled phosphate	25	26	28	30	50

TABLE VI.

Specific activity of P fractions extracted from rabbits 4 hours after the start of the experiment. The labelled sodium phosphate was injected into the veins drop by drop during the experiment.

Fraction.	Relative specific activity.
Plasma inorganic P	100
Muscle inorganic P	7·3
,, creatine P	1·0
,, ester P	0·8
,, lecithin P isolated as chloro-cadmium compound	0·07
Tibia epiphysis P	0·32
,, diaphysis P	0·17

TABLE VII.

Specific activity of the acid-soluble phosphorus fractions extracted from the liver and kidneys of rabbits 4 hours after the start of the experiment.

P fraction.	Relative specific activity.
Plasma inorganic P	100
Liver inorganic P	55
,, pyrophosphate P	38
,, ester P	18
Kidney inorganic P	100
,, pyrophosphate P	36
,, hexose P	50
,, non-hydrolysed P	25

atively rapid process. In the case of the kidneys, within about 4 hours, a perfect exchange equilibrium is reached between the phosphate ions of the kidney cells and those of

* From the distribution of labelled sodium in the organs of a rabbit Griffiths and Macgraith (*Nature*, **1939, 143**, 159) calculated the volume of the extracellular fluid in these organs.

the plasma. We see also the rejuvenation of a large part of the acid-soluble organic P compounds present in the liver, especially that of the adenosinetriphosphate molecules.

That of the three P atoms of the adenyltriphosphate molecule the two labile ones are replaced at a very fast rate, was shown also in experiments *in vitro* by Meyerhof and his collaborators (*Biochem. Z.*, 1938, **298**, 396), who found that when active sodium phosphate was added to a solution containing adenyltriphosphate, cozymase, muscle extract and other ingredients, about 40% of the labile P atoms of the adenyltriphosphate molecules were replaced by active ones within 20 seconds. As to the replacement of the third P atom of the last-mentioned compound, Parnas and Korzybski (*Z. physiol. Chem.*, 1938, **255**, 195), by measuring the activity of inosinephosphate P, into which the non-labile P atom of the adenosinetriphosphate molecule was converted, showed that it took place at a very slow rate.

Parnas and his colleagues, in collaboration with us (*Acta Biol. Exp.*, 1938, **12**, 34), prepared various labelled phosphorus compounds and determined in mixtures containing partly labelled and partly non-labelled P compounds, which of these compounds transferred its phosphate group to phosphorus acceptors. For example, when adenyltriphosphate was formed from adenylic acid in the presence of phosphoglycerate and inorganic P, it was found that the phosphate group of the latter, without an inorganic intermediary, was incorporated into the adenyltriphosphate molecule. Although the investigation of the formation of labelled acid-soluble P compounds, both by *in vivo* and by *in vitro* experiments, supplies us with valuable information as to the formation of organic compounds of this type, the very appreciable speed with which some of them are resynthesised in the organism somewhat restricts the applicability of isotopic indicators in the study of their formation. This is not the case for the other large groups of organic P compounds which include the phosphatides, nucleoproteins and phosphoproteins, which we shall discuss in what follows.

Phosphatides.

In one of our earliest researches we investigated the extent to which the phosphatides present in the brain tissue of fully grown animals were rejuvenated. We administered labelled sodium phosphate to aged mice, rats, rabbits, and cats; in each case the formation of some labelled phosphatide could be demonstrated. Detailed work was carried out on the relative turnover of phosphatides in different organs. Artom and his colleagues (*Arch. Intern. Physiol.*, 1937, **45**, 32; 1938, **47**, 245), Chaikoff and his collaborators (*J. Biol. Chem.*, 1937, **122**, 169; 1938, **123**, 587; 1938, **127**, 1795; 1939, **126**, 493; Dols and his colleagues, *Nature*, 1937, **141**, 77), and also our group (Hahn and Hevesy, *Skand. Arch. Physiol.*, 1937, **77**, 148; Hevesy and Lundsgaard, *Nature*, 1937, **140**, 275; Hevesy and Hahn, *Kgl. Dansk. Vidensk. Selsk. Medd.*, 1938, *B*, 2, XIV; Hevesy and Aten, *ibid.*, 1939, *B*, 5, XIV) compared the relative activities of the phosphatides extracted from different organs.

This comparison led to the result that in the liver, in the kidneys, in the intestinal mucose, and also in the milk gland (Aten and Hevesy, *Nature*, 1938, **142**, 111) a much larger turnover takes place than, for example, in the muscles or the brain. Similar results were obtained by Sinclair (*J. Biochem.*, 1936, **115**, 211; 1937, **118**, 122; 1937, **121**, 361), using elaidic acid as an indicator. The presence of this easily traceable substance in the phosphatide molecules extracted from the organs enabled us to conclude that the latter were formed following the administration of the elaidic acid in the same way as the phosphatides containing active P were formed following the administration of labelled sodium phosphate. Elaidic acid proved to be a very useful nonisotopic indicator, but is inferior to the isotopic indicators in its applicability for quantitative purposes, as shown by the following example: In rats provided with large amounts of elaidic acid throughout the entire period of prenatal and postnatal development, the elaidic acid content of the fatty acids in the phosphatide of the brain was found by Sinclair to be only $\frac{1}{4}$ of that of the liver and the muscles. There is clearly a greater degree of selection in the building up of the brain phosphatides than those of the liver and muscles, which vitiates the application of this indicator in quantitative research. In the case of the P isotopes such a disturbing selectivity is absent.

The very marked rate of the turnover of the phosphatides in the liver compared with the muscles and the brain is seen in Table VIII, which contains also data on the phosphatide turnover in tumour tissue. The percentage of the phosphatides which are actually

TABLE VIII.

Specific activity of the phosphatide P *extracted from different fractions of a mouse* 4 *hours after administration of labelled sodium phosphate.*

Fraction.		Relative specific activity.
Liver		100
Muscle		2·9
Spontaneous breast tumour	fresh tissue	11·8
	necrotic tissue	10·3

renewed during the experiment cannot be determined from these figures, or from those given by experimenters who state the percentage of the activity administered present in the phosphatides extracted from different organs—a magnitude of restricted interest. To determine to what extent the muscle phosphatides are renewed, for example, after the administration of labelled phosphate, we must determine the extent of equipartition of the labelled P atoms between the inorganic P and the phosphatide P extracted from the muscle: if we find that the former is fifty times more active than the latter per mg. of P, we have to conclude that active P was incorporated in 2% of the phosphatides and was therefore formed in the course of the experiment. The comparison of the specific activities of the muscle inorganic P and phosphatide P which is carried out at the end of the experiment is vitiated by an error due to the appreciable change in the specific activity of the plasma inorganic P during the experiment. This error can be to a large extent eliminated by different devices. One is to inject the labelled phosphate into the veins of the animal drop by drop so as to cause the specific activity of the plasma inorganic P to remain constant or to change in a way which can be simply accounted for: we thus found that about 14% of the residual ("nucleo-protein") P present in the liver of rabbits were newly formed in the course of 3 hours; for the residual P present in the kidneys, almost the same value was found.

Place of Formation of the Phosphatides.—A daily-laying hen deposits daily about 60 mg. of phosphatide P in the yolks present in the ovary. Whence does this phosphatide originate, in which organ is it formed?

The phosphatides extracted from the ovary of a hen, after the administration of labelled sodium phosphate, are only slightly active (see Table IX). From this fact we can conclude that the phosphatide is not formed in the ovary. Neither is it formed in the corpuscles, as the corpuscle phosphatides are less active, having a smaller specific activity than the plasma phosphatides. Table IX* shows that the bulk of the phosphatides incorporated in the yolk has been formed in the liver of the hen and conveyed by the plasma to the ovary. We can follow the flow of the labelled phosphatides, *i.e.*, of the phosphatides formed during the experiment, from the liver through the plasma to the ovary. As the daily incorporation of phosphatides into the yolk is much larger than the total phosphatide content of the plasma (the amounts of phosphatide P being about 60 mg. and 20 mg., respectively), the phosphatide content of the plasma is poured into the yolk and renewed several times in the course of 24 hours. To lessen the strain of the phosphatide transport by the plasma, Nature has endowed the hen with an unusually high plasma phosphatide content (corresponding to about 20 mg. % of phosphatide P), that of a mature hen being much higher than that of other animals (Lorenz *et al.*, *J. Biol. Chem.*, 1938, **123**, 577).

The sequence of decreasing percentage of labelled phosphatide in the total phosphatides extracted from the different organs indicates the place of phosphatide formation and the path of its flow. No similar conclusions can be drawn from the change in the total concentration of the phosphatides extracted from different organs. The phosphatide

* The figures recorded are those found for the average of the phosphatide mixture present in the organs; as seen in Table VI, very different values are obtained when lecithin, cephalin, etc., are separately investigated.

content of the liver is higher than that of the plasma; nevertheless it is conceivable that the phosphatides found in the liver were carried into it by the plasma from another organ, as the organs have the ability to concentrate substances. If we find, however, that the percentage of the phosphatides renewed in the course of the experiment is larger in the liver than in the plasma, we can conclude with certainty that the bulk of the newly formed phosphatides was formed in the liver and not carried into it from other organs. The only channel through which the phosphatides formed in another organ can reach the liver is the plasma. If the latter had carried strongly active phosphatides, its phosphatide content would have become correspondingly active: this, however, was not the case. The fact that the specific activity of the phosphatide P of the liver is higher than that of the plasma is incompatible with the assumption that the phosphatide P of the liver originated in another organ and was carried by the plasma into the liver. This example illustrates the great importance of investigating the specific activities of the different fractions isolated from the plasma simultaneously with those extracted from the different organs. The conclusion that the phosphatides present in the yolk were carried into it while located in the ovary is supported by experiments in which labelled sodium phosphate was administered to hens at a time when the yolk investigated had left the ovary and was located in the oviduct. While from the strongly active plasma a strongly active shell was formed, and the white and yolk were also found to be active, the latter did not contain active phosphatides.

TABLE IX.

Specific activity of the phosphatide P *extracted from the organs of a hen* 5 *hours after administration of labelled sodium phosphate.*

Organ.	Specific activity.
Liver	1
Plasma	0·8
Ovary	0·07
Yolk	0·065
Intestinal mucose	0·18

TABLE X.

Activity per mg. of phosphatide P *extracted from milk and organs of a goat* 4½ *hours after administration of labelled sodium phosphate.*

Fraction.	Relative specific activity
Milk	1
Plasma	0·02
Milk gland	1·4
Liver	1
Kidneys	1·2

Milk Phosphatides.—In contrast to the phosphatides present in the yolk, which are carried into it by the plasma, the phosphatides present in the milk are built up to a large extent in the milk gland. The phosphatides extracted from the milk of goats were found to be more active than those extracted from the plasma (see Table X) and less active than those present in the milk gland.*

The Origin of the Phosphorus Compounds in the Embryo of the Chicken.—One more application of labelled P for locating the formation of phosphatides may be discussed, namely, the question whether the phosphorus compounds present in the embryo are formed in it or are taken up from the egg. We can answer this point by introducing labelled (radioactive) sodium phosphate into the egg before incubation and determining to what extent the phosphatides of the yolk and of the embryo become labelled. If none becomes labelled, we can conclude that the phosphatide molecules in the embryo are not newly synthesised by incorporation of labelled inorganic phosphate. If, however, the yolk phosphatide remains unlabelled while that of the embryo becomes radioactive, we can conclude that the phosphatide molecules present in the embryo have not come from the yolk but have been built up in the embryo with the participation of labelled inorganic P. Similar considerations apply to certain other compounds occurring in the embryo. Physiological sodium chloride solution (0·1 c.c.) containing a trace of labelled P was injected into the white of the eggs and the latter were incubated for several days; different phosphorus compounds of the chick's embryo and also of the remains of the yolk were then extracted, and their P content determined both by chemical and by radioactive methods. Some

* The formation of labelled casein P and labelled ester P and phosphatide P present in milk was investigated in detail by Aten (Dissert., Utrecht, 1939).

of the results obtained (Hevesy, Levi, and Rebbe, *Biochem. J.*, 1938, **32**, 2147) are in Table XI.

TABLE XI.

Specific activity of P *extracted from different fractions of an egg incubated for* 11 *days. (Specific activity of* P *extracted from the white taken as* 100.)

Fraction.	Specific activity.
Embryo:	
Average acid-soluble P	100
Inorganic P	86
Adenosine P + inorganic P ...	107
Creatine P	102
Phosphatide P	113
Residual non acid-soluble P...	122
Yolk: Phosphatide P	0·11

TABLE XII.

Specific activity of acid-soluble P *fractions extracted from rabbit's blood* 3 *hours after the start of the experiment.*

Fraction.	Specific activity.
Plasma inorganic P	100
Corpuscle inorganic P	14·8
Corpuscle pyrophosphate P ...	15·1
"Hexose" P	14·8
"Diphosphoglycerate" P	12·6

As the figures show, the phosphatides extracted from the yolk are only slightly active, whereas those extracted from the embryo show strong activity; 1 mg. of embryo phosphatide P is nearly 1000 times as active as 1 mg. of yolk phosphatide P. Furthermore, the specific activity of the embryo phosphatide P is about as high as that of the embryo inorganic P, showing that an inorganic P atom reaching the embryo has about the same chance of entering the skeleton as of being incorporated in a phosphatide molecule by an enzymic process—which of the two systems it enters being governed solely by probability considerations. From this it follows that the phosphatide molecules in the embryo are not identical with those derived from the yolk, but are synthesised in the embryo.

Permeability of Blood Corpuscles.

As mentioned on p. 1215, by comparing the specific activities of plasma phosphate and muscle phosphate, information is obtained on the rate at which phosphate ions penetrate into the muscle cells. Similar considerations have been applied in the study of permeability of blood corpuscles.

We administered labelled sodium phosphate drop by drop to rabbits and examined blood samples taken at intervals. In the course of 3 hours about $\frac{1}{3}$ of the labelled P atoms originally present in the plasma were found in the corpuscles, where they were mostly present as a constituent of acid-soluble organic phosphorus compounds. The first step was the fairly fast penetration of labelled phosphate ions into the corpuscles and the simultaneous migration of an equal number of non-labelled phosphate ions from the corpuscles into the plasma. The labelled phosphate, after its intrusion into the corpuscles, reached almost at once an exchange equilibrium with the adenosinetriphosphate present, labelled phosphate being used in the building up of the new adenosinetriphosphate molecules (see p. 1219) and non-labelled phosphate being released by the breaking up of old ones. As seen in Table XII, labelled hexosephosphate, glycerophosphate, and other acid-soluble compounds were also formed at an appreciable rate.

The study of the interaction between plasma P and corpuscles, which can be carried out *in vitro* as well, suffices to show to what extent an individual P atom is buffeted about in the circulation. Of 1000 individual P atoms present at any moment in the plasma of a rabbit as inorganic P, all but about 20 remained * in the plasma after 3 hours (compare p. 1220), the rest having invaded the tissues, which, in turn, gave off an equal number of P atoms to the plasma. The individual inorganic P atom thus spends on the average only a short time in the plasma and therefore most of the inorganic P atoms diffusing from the plasma into the corpuscles were, shortly before, located in the tissues. As stated above, they will soon be incorporated into organic molecules, but within a few hours these will break up and a large percentage of the P atoms will find their way back into the plasma and the tissues.

* A large percentage of these remaining 20 atoms also left the plasma in the course of 3 hours, but returned again.

Non-acid-soluble P *Compounds.*

In contrast to the fast turnover of the acid-soluble P compounds present in the corpuscles, the phosphatide and nucleo-protein molecules present in the corpuscles show only a minute or no turnover. No labelled phosphatides are formed in the blood; they are formed in the liver and other organs (see p. 1220) and carried into the circulation. However, an exchange of the phosphatide molecules between plasma and corpuscles takes place to a restricted extent. By shaking plasma containing labelled phosphatides with corpuscles we find, as recorded in Table XIII (Hahn and Hevesy, *Nature*, **1939**, **141**, 72), the corpuscles taking up labelled phosphatides and vice versa. Even after several days,

TABLE XIII.

Extent of equipartition of labelled phosphatide between the phosphatide of the corpuscles and the plasma in experiments in vitro. (*Plasma containing labelled phosphatide shaken with corpuscles of another rabbit.*)

Time, hours.	Extent of equipartition, %.
0·5	1·8
1·5	3·6
3	4
4·5	5·0

however, the exchange is restricted to a minor part of the total phosphatides present in the corpuscles. The phosphatide molecules present in the outermost layers of the walls of the corpuscles are presumably those which can be replaced by phosphatide molecules present in the surrounding plasma, whereas molecules situated in deeper layers are presumably prevented from taking part in such an exchange process. The plasma containing labelled phosphatides was produced by administering labelled sodium phosphate to a rabbit. After a day the plasma contained an appreciable amount of labelled phosphatide. The plasma, recovered by centrifuging the blood of the rabbit, was shaken with the corpuscles or with the total blood of another rabbit; after few hours corpuscles and plasma were separated and analysed. An alternative procedure was to compare the specific activities of the plasma phosphatide and corpuscle phosphatide after different intervals in experiments *in vivo*.

At the present time we are engaged in a study of the permeability of corpuscles to sodium- and potassium-ions on similar lines to those mentioned above, using ^{24}Na and ^{32}K as indicators.

Application of Isotopic Indicators in Botanical Research.

A few examples of the application of isotopic indicators in the study of processes going on in the animal body have been given above. Time does not permit me to discuss in detail the application of such indicators in botanical research, and I will therefore restrict myself to the following problem (Hevesy, Linderström-Lang, and Olsen, *Nature*, **1937**, **139**, 149): Do the atoms or molecules incorporated into the leaves, the stem, etc., of a plant migrate, for example, from one leaf to another, or are they fixed in their places? This question can be answered by growing maize or a sunflower in a culture solution until some leaves are formed and then shifting it to a second culture solution in which ordinary phosphorus has been replaced by labelled phosphorus. While the plant is in the second solution, new leaves are formed. The P content of both the old and the new leaves and also their activities are then determined. If no migration took place between the P atoms of the old and the new leaves, the P extracted from the old leaves should remain inactive. Actually, after 9 days it was found to be nearly as active (ratio of the specific activities = 0·99) as that extracted from the new leaves.

When carrying out similar experiments with nitrogen, using heavy nitrogen (nitrogen containing 2·5% of ^{15}N) kindly presented to us by Professor Urey, we found a migration of the nitrogen atoms as well; after a fortnight, however, more than one half of the nitrogen atoms originally present in the old leaves of maize was found to be still located there.*

* The analysis of the nitrogen was kindly carried out by Professor Schoenheimer by use of the mass-spectrograph.