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Some applications of isotopic indicators

Nobel Lecture, December 12, 1944

The method of isotopic indicators had its ultimate origin in the Institute of Physics at the University of Manchester, which then was under the inspiring leadership of that great physicist the late Lord (then Professor) Ernest Rutherford.

The cradle of radium is the Czecho-Slovakian town Joachimstal; it was from Joachimstal pitchblende ore that Professor and Madame Curie isolated that element. The Austrian Government, the owners of these mines, generously supplied Professor Rutherford not only with radium, but also with the by-products of radium production, equally important for the worker in the field of radioactivity. One of the most significant by-products is radium D, which has a half-life period of 20 years and is found associated with the very substantial amounts of lead present in pitchblende. The Austrian Government presented to Professor Rutherford several hundred kilograms of such "radio-lead". In view of its association with very large amounts of lead, which absorb the radiation emitted by radium D, this precious radioactive material nevertheless proved to be almost useless. When I met Professor Rutherford one day in 1911 in the basement of the laboratory where the radio-lead was stored, he addressed me in his friendly and informal way, saying: "My boy if you are worth your salt, you try to separate radium D from all that lead". In those days, I was an enthusiastic young man and, on immediately starting to attack the problem suggested to me, I felt quite convinced that I would succeed. However, although I made numerous attempts to separate radium D from lead and worked for almost two years at this task, I failed completely. In order to make the best of this depressing situation, I decided to use radium D as an indicator of lead, thus profiting from the inseparability of radium D from lead. Suppose that we dissolve 1 g of lead in the form of nitrate in water, add radium D of negligible weight showing a radioactivity of one million relative units (an electroscope being used to measure the activity), and proceed to carry out the most intricate operations with this "labelled" lead. If we then ascertain the presence of one radioactive unit in a fraction obtained in the course of these operations, we

must conclude that 1/1000 mg of the lead atoms present in the lead nitrate we started from, are now present in the fraction.

Radium D cannot be separated from lead, but it can easily be obtained in the pure form from lead-free radium salt samples or from radium emanation, since radium D is formed in the course of the disintegration of these radioactive bodies and can readily be separated from them. At that time, the Vienna Institute of Radium Research had more radium and radium emanation at its disposal than any other institution. This fact induced me, late in 1912, to start work at the Vienna Institute in collaboration with Dr. Paneth, assistant at that Institute, who himself had made very extensive and abortive trials to separate radium D from lead. The first application of labelled lead[1] was the determination of the solubility of some very slightly soluble lead compounds such as lead chromate. In these experiments, not radium D but another isotope of lead, thorium B, was applied as an indicator. Labelled lead chromate was obtained by adding a solution of 100,000 relative units of thorium B to lead nitrate containing 10 mg of lead and converting the nitrate thus labelled into chromate.

After the saturated solution of this compound had been held at the desired temperature in a thermostat for a sufficient time, its composition was ascertained by evaporating a few cubic centimetres to dryness and measuring the activity of the almost invisible residue in the electroscope. From the number of units of thorium B found, the amount of lead was calculated, one unit corresponding to 10^{-6} grams of lead; finally, the solubility of the lead chromate in moles per litre ($2 \cdot 10^{-7}$) was computed.

The radioactive method is extremely simple, having the advantage that the presence of foreign ions in no way interferes with the measurements. The method may be applied without difficulty, for example, in determining the solubility of lead sulphate in the presence of calcium sulphate.

Simultaneously with the said experiments, we used labelled lead and labelled bismuth (the radioactive bismuth isotope radium E can easily be obtained from radium, radium emanation, or radio-lead) in an investigation of the manner in which unweighable amounts of metals are precipitated during electrolysis[2]. The application of the well-known Nernst formula

$$e = \frac{RT}{n} \log_n \frac{c}{C}$$

was extended to concentrations of $10^{-8} N$ and even lower.

On the basis of Nernst's theory, we should expect an interchange to take

place between the metal of the electrodes and the ions in solution. The existence of such an interchange was demonstrated[3]. While, in the case of lead peroxide, the interchange was found to take place between the outermost layer of molecules of the geometrically calculated electrode surface, in the case of the lead electrodes numerous layers of molecules were found to participate in the interchange process. This finding is to be interpreted as a result of local currents due to variations in the structure of the metallic surface. An interchange between atoms of a lead foil and the lead ions present in a solution was found to occur very rapidly, while the lead ions adsorbed on colloidal lead particles were found to interchange at a slow rate only[4].

In contrast to metallic surfaces, Paneth[5] found that in the case of salt crystal surfaces the interchange was restricted to the uppermost molecular layer of the crystal. On this observation he based an important method for the determination of the surface areas of crystalline powders[6].

When lead sulphate is shaken with its saturated solutions, a constant kinetic exchange occurs between the molecules of lead sulphate in the solution and those on the surface of the solid. If the solution contains marked molecules, after equilibrium has been attained the numerical ratio of marked molecules on the surface to those in solution is identical to that of total molecules on the surface to total molecules in the solution. As the distribution of the labelled molecules is determined by means of radioactive measurements, and as the lead sulphate content of the saturated solution is evaluated by the usual methods of analytical chemistry, the amount of lead sulphate present in the uppermost molecular layer can be computed. When the weight is known of a unimolecular layer of lead sulphate of 1 cm^2 area, the surface of the crystal powder can be calculated from the above data.

Among the numerous applications of radioactive indicators by Paneth I wish to emphasize the importance of his discovery of the existence of bismuth hydride[7] and lead hydride[8]. After he gained experience regarding the best method of preparation and the stability of radioactive bismuth hydride and lead hydride, he succeeded in preparing these compounds from inactive bismuth and inactive lead, respectively.

Self-diffusion

The conception of the diffusion of a substance into itself, self-diffusion, was introduced by Maxwell. No further use was made of this concept until fifty

years later, when the method of radioactive (isotopic) indicators was introduced. The possibility of measuring self-diffusion by following the rate of penetration of the lead isotopes ThB or RaD into lead soon suggested the measurement of the self-diffusion in liquid and solid lead, using ThB or RaD as indicators. The measurements of the self-diffusion coefficient in liquid lead[9] gave the result anticipated from the known diffusion rates of lead in mercury and other related elements. The diffusion rate in liquids is primarily determined by the radius of the diffusing particle and the viscosity of the liquid: thus, the replacement of a diffusing metal present in small concentration by another related metal will not appreciably influence the rate of diffusion. A very different behaviour was revealed, however, when the self-diffusion in solid lead[10] was measured, using RaD as indicator. In the first experiments carried out in collaboration with Gróh, we soldered a piece of radio-lead to the bottom of a rod of ordinary lead, whereafter the system was kept at 280° for 140 days. After the lapse of that time, we cut the system into four equal parts, rolled the four lead pieces into thin plates, and placed them in an electroscope. No diffusion of the radio-lead into the ordinary lead could be ascertained, showing that the self-diffusion rate in lead must be at least several hundred times smaller than that of gold in lead, as determined by Roberts-Austin.

This result necessitated the introduction of special methods of great sensitivity for measuring diffusion. Since the rate of diffusion is inversely proportional to the square of the thickness of the layer, we worked out methods for the measurement of the penetration of radioactive lead into ordinary lead layers to a depth of only a few microns. The α-particles emitted by ThB (more correctly, by its disintegration products, ThC and ThC′, which, however, attain radioactive equilibrium with the former within a few hours) produce scintillations on a zinc sulphide screen, the number of which is ascertained. The infinitesimal layer of ThB, which is in intimate contact with a lead foil placed below it, is then heated for a few hours to, say, 200°. If a diffusion of the ThB atoms into the lead foil takes place, the count of the scintillations will give a smaller value after the experiment than before. The range of the α-particles in lead being only about $30\,\mu$, a shift of a small percentage of the ThB atoms to depths less than $30\,\mu$ will suffice to reduce the counts of scintillations to a noticeable extent. This method, worked out in collaboration with Mrs. Obrusheva[11] was later replaced by a more sensitive and exact procedure applied in diffusion measurements in solid metals, and also salts, in collaboration with Seith[12]. ThB was condensed on a foil

or a single crystal of the metal, and the ionisation produced by radiation emitted by the radioactive body was measured. A slight diffusion of the ThB into the lead after heating sufficed to diminish the ionising effect registered by an electrometer. Instead of the ionisation produced by the α-rays, the ionisation produced by recoil particles accompanying the emission of α-rays was measured in some cases. The range of the recoil particles in lead being only about 10^{-5} cm, these measurements made possible the determination of diffusion coefficients as small as 10^{-12} cm^2 per day.

Our measurements led to the result that, while the diffusion coefficient of gold in lead is found to be $5 \cdot 10^{-3}$ $cm^2 day^{-1}$ at 165°, the coefficient of self-diffusion in lead at the same temperature is only 10^{-6} $cm^2 day)^{-1}$, the difference rapidly increasing with decreasing temperature. The change of the value of the coefficient of self-diffusion, D, in lead foils and single crystals is represented by the equation

$$D = 5.76 \cdot 10^5 \, e^{-27900/RT} \, cm^2 \, day^{-1}$$

Making use of this formula, we can show that, at room temperature, the atoms will change their places in a piece of lead on the average only once a day.

From the change of the coefficient of self-diffusion with temperature, the heat of activation of the diffusion process, the heat of loosening of the lead lattice, can be calculated. The value obtained and, for purposes of comparison, other thermal data are given in Table 1.

Table 1. Thermal data for solid lead

	kcal per g atom
Heat of melting	1.1
Energy content at the melting point	3.5
Heat of lattice-loosening	27.9
Heat of evaporation	36.2

Roberts-Austin measured the diffusion rate of gold in solid lead. His measurements gave the first quantitative determination of diffusion rates in solids. The high values he obtained, shown in Fig.1[13], led his contemporaries to consider diffusion in solid metals a comparatively rapid process. The introduction of the conception of self-diffusion and the subsequent development led to a very different view and also to the elucidation of the remark-

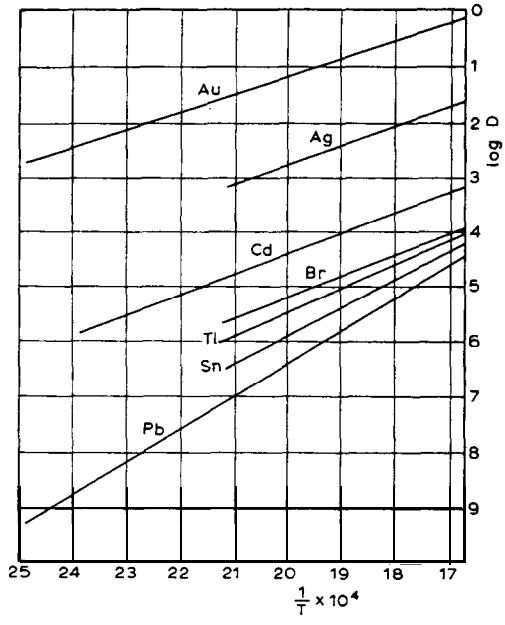

Fig. 1. Diffusion rates of metals in solid lead.

able nature of the gold-lead system investigated by that pioneer metallurgist.

The methods outlined above were also applied to determine the self-diffusion rate of Pb++ in solid lead chloride and lead iodide[12]. The variation of the self-diffusion rates with temperature can be expressed by the equations

$$D = 1.06 \cdot 10^{7}\, e^{-38120/RT} \text{ and } D = 3.43 \cdot 10^{4}\, e^{-30000/RT}$$

respectively. As first shown by Nernst, the ionic mobilities in an electrolyte solution, and hence the conductivity of the solution, can be calculated if the diffusion rates of the ions are known. We can apply the same ideas to solid electrolytes[14] and calculate, for example, the diffusion rate of Pb++ from the conductivity of lead chloride, on the assumption that the electrolytic conductivity of the salt is due solely to the transference of charges by the Pb++. The diffusion rate of Pb++ thus calculated is, however, many thousand times larger than the value found experimentally, showing that the chloride ions are almost exclusively responsible for the conduction of electricity in solid lead chloride. The above data permit the calculation of the transport number of Pb++ in solid lead chloride. Not far from the melting

point, at 484°, the transport number of Pb++ is found[15] to be 10^{-3}, at 273° 10^{-5}, and at 90° only 10^{-10}. By no other method can such small transport numbers be determined in any electrolyte.

In the case of lead iodide, the diffusion rate of Pb++ calculated from conductivity data, under the assumption that the whole conduction is due to Pb++, is in good agreement with the measurement of the self-diffusion rate of Pb++. This shows that, in contrast to the case of $PbCl_2$, the conductivity in PbI_2 at high temperatures is due almost exclusively to the transference of charges by Pb++. With decreasing temperature the role of Pb++ decreases and the transport number of I^- increases accordingly. At 260°, only 40% of the conductivity is due to Pb++ and 60 percent to the I^-; at 155° the share of the former is only 0.4 percent. In the case of lead iodide, Tubandt's beautiful method for the determination of transport numbers could also be applied. The values obtained agreed well with those found by the measurement of self-diffusion.

Formerly, the self-diffusion rates of only lead, bismuth and a few other elements could be determined. These elements have natural radioactive isotopes. The discovery of artificial radioactivity greatly enlarged the possibilities for the determination of self-diffusion rates. By making use of the radioactive bromine isotope, we can determine the self-diffusion rate of Br^- in AgBr just as we determined that of Pb++ in $PbCl_2$. Working at the Institute for Theoretical Physics at the University of Copenhagen, H. A. C. McKay determined the self-diffusion in gold. By the action of neutrons on gold a radioactive gold isotope, having the atomic number 198, can be produced. Neutrons having an energy of about 4 V are strongly absorbed in gold. A thin gold sheet exposed to the action of such neutrons will be more strongly activated on the side first struck by the neutron beam than on the opposite side. When the activated film is heated, the difference in the concentrations of the active gold atoms will decrease and, from the decrease of the activity difference shown by the two faces of the foil, the rate of self-diffusion in gold can be calculated[16].

Svante Arrhenius' theory of electrolytic dissociation

If we dissolve sodium chloride and the equivalent amount of sodium bromide in water and then separate the two salts by crystallisation, it would have been expected in the time prior to Arrhenius that the chloride ions

would retain their original partners, the same applying to the bromide ions. According to Arrhenius, however, each chloride ion has the same chance of associating with a sodium atom originally bound to chlorine as with one initially associated with bromine. The correctness of the much debated views of Arrhenius was shown in different ways; the most direct proof, however, was provided through the application of isotopic indicators[17]. When equivalent amounts of $PbCl_2$ and labelled $Pb(NO_3)_2$ (or *vice versa*) were dissolved and the two compounds were separated by crystallisation, the labelled lead ions were found to be equally distributed between chloride and nitrate ions.

Very different results were obtained in all cases in which the lead atom was joined to carbon. Between lead chloride and lead tetraphenyl in pyridine, between lead acetate and lead tetraphenyl in amyl alcohol, and between lead nitrate and diphenyl lead nitrate in aqueous ethyl alcohol, no change in the places of lead atoms could be detected, although in every combination investigated one of the molecular types was capable of electrolytic dissociation.

The lack of interchange of atoms present in organic binding (hydrogen atoms bound to oxygen or nitrogen being an exception, as shown by Bonhoeffer[81]), such as that of carbon atoms in glycogen or phosphorus atoms in lecithin with other carbon and phosphorus atoms respectively, was found to be of great significance for the application of isotopic indicators in biochemical research. Owing to the absence of such an interchange, the presence of labelled carbon atoms in glycogen molecules, or of labelled phosphorus atoms in lecithin molecules, extracted from the organs, proved that a synthesis of these molecules took place after the labelled atoms were administered. This principle enables us to distinguish between "old" and "new" molecules and to determine the rates at which molecules of the different compounds are built up and carried to the different organs.

A prompt interchange of the electrical charges between Pb++ and Pb++++ ions was found to take place in experiments where plumbous acetate and labelled plumbic acetate (or *vice versa)* were dissolved in glacial acetic acid and then separated by crystallisation[18]. The same holds for Tl+ and Tl+++ ion[19]. An interchange of lead atoms takes place between fused lead and fused lead chloride, lead oxide or lead sulphide[20].

After artificially radioactive isotopes became available as indicators, interchange processes were studied in numerous cases. A rapid interchange of charges was found to take place between Fe++ and Fe+++, Cu+ and Cu++, etc.[21].

Analytical chemistry

Analytical chemistry proved to be a fruitful field for the application of isotopic indicators. A knowledge of the total lead content of the earth's crust, for example, is of considerable chemical interest. In view of the small lead content of the average rock sample, the quantitative determination of its lead content involves some difficulties. These have been eliminated by making use of an isotopic indicator[22]. An amount of radium D, known in relative radioactive units, is added to the solution of the rock sample; the radium D is then recovered by electrolysis as peroxide. If 100 per cent of the added radium D is recovered, we may expect 100 percent of the lead present in the sample to have been recovered as well. If only 50 percent is recovered, for example, we have to multiply the amount of lead recovered by 2 in order to arrive at a correct analytical figure. The indicator method thus allows a correction for the shortcomings of the analysis. Such corrected analytical figures are seen in Table 2.

Instead of adding radium D to the solution to be analysed, we may add lead labelled by the presence of some radium D, for example 100 mg of lead having an activity of 1,000 units. If we subsequently isolate 10 mg of lead from the solution, this lead should show an activity of 100 units, under the assumption that the original sample does not contain lead. If the activity of the isolated 10 mg of lead is, for example, found to be 83 only, we have to conclude that the sample contains lead amounting to 20 mg.

In recent years, isotopic indicators have found an extended application in

Table 2. Lead content of igneous rocks

Rock types	*g lead per g rock precipitated by electrolysis*	*Percentage RaD recovered by electrolysis*	*Corrected value of g lead per g rock*
Gabbros and related types (composite of 67 samples)	$4 \cdot 10^{-6}$	80	$5 \cdot 10^{-6}$
Essexites and related types (composite of 40 samples)	$7 \cdot 10^{-6}$	80	$10 \cdot 10^{-6}$
Soda-granites and soda-syenites (composite of 26 samples)	$9 \cdot 10^{-6}$	73	$11 \cdot 10^{-6}$
Granite rocks (composite of 58 samples)	$18 \cdot 10^{-6}$	53	$30 \cdot 10^{-6}$
Basalt (Giant Causeway)	$4 \cdot 10^{-6}$	100	$4 \cdot 10^{-6}$

biochemical analyses. Schoenheimer and his colleagues[82] determined the leucine content in the protein of the rat by adding to the hydrolysate a known amount of leucine containing heavy nitrogen. This tracer was also used[83] in the investigation of the occurrence of the amino acids of the *d* series in cancer proteins; while Chargaff, Ziff and Rittenberg[25] used bases containing a known amount of ^{15}N in the analysis of the nitrogenous constituents of tissue phosphatides. Amino acids containing deuterium as indicator were used by Ussing[2,3] and the same tracer was applied by Rittenberg and Foster[24] in their determination of the palmitic acid content of rats' fat.

Early biological application

In contradistinction to the animal body, the uptake of mineral constituents by the plant is not followed by a loss of such constituents, and it was formerly considered that the ions taken up by the roots of the plant did not migrate in the opposite direction at all. The application of isotopic indicators, however, has shown that this is not the case. Ions taken up by the plant can be removed by an exchange process under the action of other ions present in the soil or in the nutrient solution. It was already found in 1923 that minute amounts of lead, labelled by the admixture of the lead isotope thorium B, when taken up by the roots of *Vicia faba,* could to a large extent be removed by an excess of non-labelled lead added to the nutrient solution[26]. Most other ions were found to be much less effective in removing the labelled lead ions from the plant.

In recent years, the behaviour of essential constituents of plants has been investigated, making use of artificial, radioactive ions as indicators; similar results were obtained. Mullins and Brooks[27] placed cells of *Nitella coronata* first in a solution containing radioactive potassium and later in solutions of different chlorides. Sodium and lithium were found to be much less effective in removing labelled potassium than potassium itself, whereas rubidium was more effective. Jenny and Overstreet[28] and Broyer and Overstreet[29] found that ionic exchange could take place during periods of, and under conditions favourable for, active solute uptake. It was also observed[30] that for each six phosphate ions taken up by the roots of growing wheat seedlings, one phosphate ion migrated from the roots into the nutritive solution.

Early in the twentieth century, the application of bismuth compounds in syphilis therapy came to the fore. This induced Christiansen, Lomholt and

de Hevesy[31] to investigate the absorption, circulation and excretion of labelled bismuth preparations. Lomholt[32] succeeded in showing that, of all the preparations investigated, bismuth hydroxide suspended in oil was most suitable for therapeutic application.

Successes achieved by Blair-Bell in cancer therapy, using lead compounds, induced the investigation of the partition of labelled lead compounds between normal and tumorous tissue[33]. Though this work gave a negative result, it nevertheless proved to be of great importance in the future development of isotopic indicators. It was in the course of these investigations that Schoenheimer became familiar with the method of isotopic indicators, which he applied several years later with such great success in the study of fat and protein metabolism and of numerous related problems. Never were more beautiful investigations carried out with isotopic indicators than those of the late Professor Schoenheimer, whose untimely and tragic death is much to be deplored. The discussion of the numerous important results obtained by Schoenheimer and Rittenberg and their collaborator[34] lies, however, beyond the scope of this lecture.

Heavy water

In 1931, Urey discovered deuterium, an isotope of hydrogen[35]. This important discovery made possible the labelling of hydrogen. Deuterium is not an ideal indicator, its properties differing appreciably from those of hydrogen. The latter has a unique position: it is the sole element met with, though only transitorily, as a naked nucleus in chemical reactions. Chemical forces do not suffice to remove all electrons from any other element. Differences in the structure of the nucleus will therefore make themselves more noticeable in the chemical behaviour of hydrogen isotopes than in the case of any other element. Furthermore, the difference between the mass of the hydrogen atom and that of the deuterium atom amounts to as much as 100 percent, while, for example, the corresponding difference between a ^{31}P and a ^{32}P atom is only 3 percent. The very small difference in the chemical properties of ^{31}P and ^{32}P remains at the present time within the errors of our experiments, whereas between hydrogen and deuterium the difference is quite appreciable. The same applies to H_2O and D_2O. Dilute "heavy" water, however, contains mostly DOH molecules which exhibit in their chemical behaviour a very great resemblance to HOH.

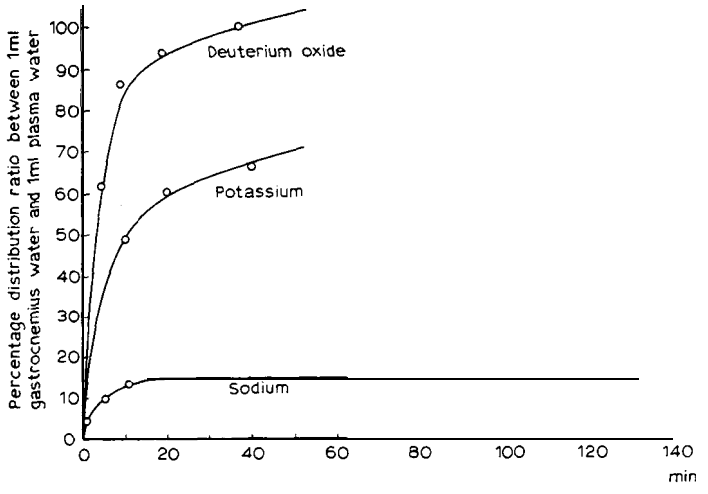

Fig. 2. Percentage distribution ratio of labelled sodium, potassium and deuterium oxide between plasma water and muscle water of equal weight.

In the study of the circulation of water in the organism, dilute heavy water can therefore safely be used as an indicator. In the determination of the life period of water molecules in the human organism, water containing ½ per cent heavy water was used[36]. While a small percentage (0.1) of the water was found to be excreted in so short a time as 26 minutes, the average life of the water molecules in the organism was found to be 13.5 days. In the excreted water, molecules were thus found which were taken in both a few minutes and several months before the investigation. By extrapolation, however, we arrive at the result that, though the number of water molecules present in an adult organism amounts to as much as ca. 10^{27}, the adult organism no longer contains a single water molecule taken up at birth.

The rate of admixture of administered water with the water present in the body was investigated in experiments on rabbits[37] and guinea pigs[38]. While the water reaching the circulation was found to enter into exchange equilibrium with the extracellular water (about ¼ of the weight of the rabbit) in the course of a few minutes, the penetration of the water molecules into the cells took some time. As is seen in Fig. 2, about 30 min passes before the exchange equilibrium is reached between the water administered and extracellular and intracellular water present in the muscles of the rabbit. In the guinea pig, 73 per cent of the water in the blood is exchanged for extracellular water every minute.

Time does not permit me to discuss the extended application of heavy hydrogen, heavy carbon and heavy nitrogen, and to treat the numerous important results obtained by the use of these isotopes as indicators.

Application of artificially radioactive isotopes

During the lengthy operations preceding the early experiments of self-diffusion in lead, we often discussed the great progress which might be expected if radioactive indicators of the common elements were made available to chemical and biological research. This wish, which seemed utopian in those remote days, was fulfilled by Frederic Joliot and Iréne Joliot-Curie's[39] fundamental discovery of artificial radioactivity, followed by Fermi's[39] work leading to the discovery of many more artificially radioactive isotopes. Soon after the announcement of these discoveries, we prepared the radioactive phosphorus isotope ^{32}P by neutron bombardment of carbon disulphide and used this isotope in collaboration with Chiewitz[40] in the study of phosphorus metabolism. In these experiments, 10 litres of carbon disulphide were used to absorb most of the neutrons emitted by a mixture of radium and beryllium kindly put at our disposal by Professor Niels Bohr. The ^{32}P formed was extracted by treatment with diluted nitric acid or with water, the carbon disulphide being immediately available after the extraction for further neutron-irradiation.

A few other radioactive isotopes, such as the radio-halogens can also be prepared by similar simple and convenient procedures. This is, however, not the case with the majority of radioactive isotopes. These were prepared in amounts sufficient to be utilized in indicator work only after the invention of the cyclotron[41]. Lawrence's highly significant invention also made available radio-phosphorus preparations of very much greater activity than could be obtained from neutron-sources containing as much as several grams of radium. The number of neutrons produced by the Berkeley cyclotron was stated by Birge[41] in 1939 to correspond to the ionisation produced by 100 kg of radium; since that date, a still more powerful cyclotron has been brought into use. In our later investigations, radio-phosphorus generously put at our disposal by Professors Niels Bohr, Lawrence and Siegbahn was used.

The preparations of radioactive isotopes of numerous elements prepared in the Radiation Laboratory at Berkeley and in other laboratories found an extended application as indicators. Radioactive iron prepared at Berkeley, for

example, was used by Hahn, Whipple and their colleagues[42] in extended studies of iron metabolism.

The application of cyclotron-prepared radio-carbon[43] revolutionized our views of the fundamental process of photosynthesis.

Radio-iodine[44] found an extended application in the study of the formation of thyroxine and diiodotyrosine; it led, *inter alia,* to the important finding that some thyroxine is formed in the organism even after total extirpation of the thyroidea[45].

Radio-phosphorus found, however[46], the most extensive application. This was due not only to the convenient mode of production and period of decay of this material, together with the low absorbability of the rays emitted by it, but mainly to the important part which phosphorus plays in a very great number of metabolic processes. These include skeleton formation, metabolism of carbohydrates and fats, cell division, and many other processes. The discussion of the role of phosphorus in metabolic processes is therefore well suited to demonstrate different applications of isotopic indicators in biological research. We shall therefore now describe some applications of radio-phosphorus. These examples represent only a small percentage of the investigations in which radio-phosphorus has been used as an indicator; many of the results to be discussed were obtained in Copenhagen.

Radio-phosphorus

Owing to the great sensitivity of the Geiger-Müller counter, which registers ^{32}P with an activity of only 10^{-6} microcurie, some of the radio-phosphorus administered can soon be located in all organs. Table 3 shows the distribution of ^{32}P in the organs of the rat 4 hours after subcutaneous injection of labelled sodium phosphate[47].

While 4 hours after the administration most ^{32}P is found in the skeleton, muscles, liver and the digestive tract, with increasing time more and more ^{32}p becomes incorporated with the skeleton; 98 days after the start of the experiment, 92 percent of all ^{32}P present in the rat, which corresponds to about one half of the total amount administered, is found in the skeleton. This result may be seen in Table 4. Most phosphorus taken up with the food, in so far as it is not excreted, ultimately finds its way into the skeleton, where it replaces "old" phosphorus which interchanges with the phosphorus present in other organs or is excreted.

Table 3. Distribution of ^{32}P between different organs in a rat, 4 hours after subcutaneous injection of labelled phosphate (Weight of the rat: 188 g)

Organ	*Percent ^{32}P present*	*Specific activity*
Bones	22.6	0.020
Muscles	18.7	0.191
Liver	17.6	0.475
Digestive tract	15.9	0.365
Skin	11.1	0.192
Lungs and heart	6.3	0.317
Blood	2.5	0.558
Kidneys	2.4	0.370
Spleen	1.3	0.256
Brain	0.02	0.032

Table 4. Percentage total ^{32}P found in some organs of rats

Organ	*Time after distribution of ^{32}P*						
	Hours		*Days*				
	1/2	*4*	*10*	*20*	*30*	*50*	*98*
Muscles	18.3	19.4	25.8	28.8	25.2	12.1	3.6
Total skeleton	19.1	23.4	43.1	43.1	51.8	76.5	92.0

From these results, however, no conclusions can be drawn concerning the extent of renewal of the skeleton, as the labelled phosphorus, i.e. phosphorus administered throughout the experiment, may be incorporated wholly or principally in the upper molecular layers of the apatite-like crystals which form the mineral constituents of the skeleton. We can determine the extent of renewal of the bone mineral phosphorus by comparing the ^{32}P content, i.e. the radioactivity of 1 mg bone mineral P, with the radioactivity of 1 mg free plasma P. Were the bone phosphorus entirely renewed in the course of the experiment, the ^{32}P would be distributed equally between the free P atoms of the apatite-like bone crystallites and the free P atoms of the plasma, the latter being the direct or indirect source of the bone phosphorus. If only 1 per cent of the bone apatite P were renewed in the course of the experiment, the specific activity of the bone apatite P would be only 1/100 of that of the free plasma P.

The determination of the degree of renewal based on the said administration is made difficult by the fact that the specific activity of the free plasma

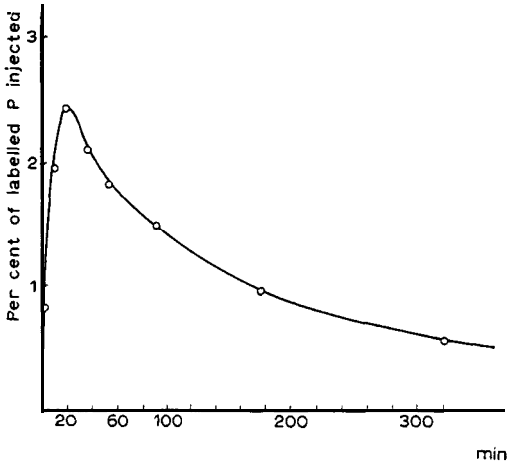

Fig. 3. Change in the specific activity of the plasma inorganic P after subcutaneous injection of labelled phosphate into a rabbit.

phosphorus varies throughout the experiment. After administration by the mouth or by subcutaneous injection, the specific activity first increases and subsequently decreases with time, while after administration by intravenous injection it first decreases very rapidly, and later at a moderate rate, as seen in Figs. 3 and 4[48]. The determination of the extent of renewal of the mineral P in the skeleton is much facilitated by keeping the free plasma P activity at a constant or almost constant level. This can be attained by repeated injections of varying amounts of labelled phosphate throughout the experiment. Making use of this technique, the data given in Table 5 were obtained for

Table 5. Extent of renewal of the mineral constituents of the bone in the course of 50 days

	Percentage renewal
Femur epiphysis mineral P	29.7
Femur diaphysis mineral P	6.7
Tibia epiphysis mineral P	28.6
Tibia diaphysis mineral P	7.6
Costa mineral P	27.5
Femur phosphatide P	100

the extent of renewal of the different parts of the skeleton of the adult rabbit in the course of 50 days[49]. It appeared that 72 per cent of the epiphysis and 93 percent of the diaphysis remained unchanged after this period, while 29

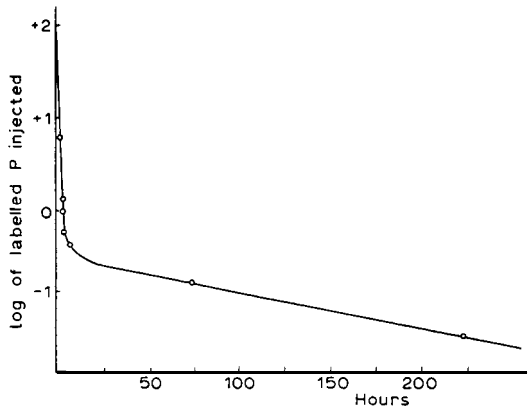

Fig. 4. Change with time in the logarithm of the labelled P content of the plasma after intravenous injection of labelled phosphate into a rabbit.

and 7 percent respectively were renewed not once, but, at least to some extent, repeatedly.

The restricted extent of renewal of the skeleton is due to the fact that while the P atoms of the uppermost molecular layer of the bone apatite crystals can promptly interchange with the free P atoms of the plasma (actually not the P atoms, but the phosphate ions interchange), a renewal of the main part of the apatite P can take place only when the crystal is dissolved and when crystals are formed from the plasma; from a labelled plasma, labelled crystals are formed. This "biological" recrystallization of the skeleton crystallites is a slow process. Moreover, we have to consider that if only the outer part of the crystal is renewed, this process can often be repeated without affecting deeper molecular layers of the crystal.

No data are available concerning the extent of renewal of the human skeleton; the relative rates of renewal of different parts of the skeleton were, however, determined by Erf[50].

The problem as to whether and to what extent the P atoms of the dental enamel are renewed has been a subject of extensive investigations[51] which led to the result that, though some ^{32}P is found to be present in the enamel after administration of labelled phosphate, the extent of renewal of the enamel phosphate is almost negligible. Regarding the extent of replacement of the phosphorus present in the constituents of the dentine, about one millionth part of the food phosphorus was found to be located in the mineral constituents of the dentine of each tooth.

The bone tissue growing in a labelled organism is bound to become labelled. Of the labelled phosphate administered by mouth, after the lapse of 3 days, 2 percent was found to be present in the rapidly growing incisors of the rat[52]. As seen in Fig. 5, these phosphate ions are mostly found in the

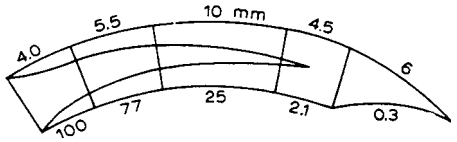

Fig. 5. Distribution of labelled phosphorus in the incisor of a rat killed 3 days after the administration of the phosphorus. The figures below give the relative amounts of labelled phosphorus present in 1 mg of fresh tissue in the section in question. The figures above give the length of the section in mm.

incisal part of the incisor, though a minor part are located at the apical end remote from the pulpa.

Permeability investigations

The above-mentioned rapid decrease in the plasma activity following intravenous administration of ^{32}P is to a large extent due to the interchange of plasma phosphate with the phosphate of the extracellular fluid. From this fact it follows that the capillary wall is readily permeable to phosphate; similar results were obtained for the other labelled ions investigated. Sodium ions, which are mainly confined to the extracellular space, enter into exchange equilibrium with the plasma sodium within 20 minutes[53,84,85]. This may clearly be seen from Fig.6[53]. For potassium and for phosphorus, elements mainly located in the tissue cells, a longer time is required for the attainment of such equilibrium[54]. The low rate at which exchange equilibrium between the cellular and extracellular phosphorus is reached in the animal organism is mainly due to a very low rate of renewal of large parts of the skeleton.

As seen from the above examples, the method of isotopic indicators can be utilized with advantage in permeability investigations. It is with the aid of isotopic indicators that we best can measure the permeability of phase boundaries, since other methods do not indicate solely the resistance of the phase boundary to the penetration of ions, but a more complex phenomenon. Prior to the application of isotopic indicators, the high potassium content of the

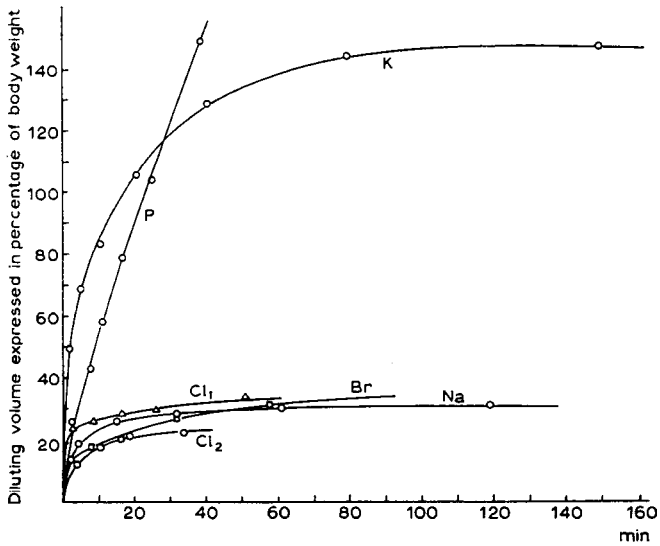

Fig.6. Rate of disappearance of various labelled ions from the plasma.

erythrocytes of most species and their low sodium content were interpreted as being due to the impermeability of the erythrocyte membrane to potassium and sodium ions. The application of isotopic indicators, however, has disclosed the fact that potassium ions in the erythrocyte interchange quite easily[55] with those present in the plasma, and the same applies to the sodium ions. The high concentration of potassium and low concentration of sodium found in the erythrocytes of most animals can thus not be explained as being due to an impermeability of the corpuscle membrane to these ions.

Not only the resistance of phase boundaries to labelled ions, but also that to molecules of different kinds, can be measured with the aid of isotopic indicators. The rates of interchange of phosphatides present in the plasma and in different organs were determined in the following way[56]. Labelled phosphate was administered to a rabbit. After the lapse of 2 days, when the plasma contained an appreciable amount of labelled phosphatides, part of the plasma of another rabbit (rabbit II) was replaced by the labelled plasma. On following the decrease with time in the activity of the phosphatides extracted from the plasma of rabbit II, it was found that half of the plasma phosphatide molecules had interchanged in the course of few hours with phosphatide molecules present in the organs of the rabbit. An investigation of the activity of the phosphatides isolated from the organs led to the result that a very

substantial part of the labelled phosphatide molecules injected was found in the liver.

Rate of formation

The rate of formation of labelled organic phosphorus compounds differs much for various compounds and varies greatly with the organ in which they are located. The labile P of adenosine triphosphate, for example, is renewed at a very remarkable rate[57,86], the second P atom being renewed somewhat more slowly than the terminal atom[58]. Hexose monophosphate, present in the red corpuscles, was found to be largely renewed within a few minutes[57]. The formation of labelled phosphatides takes place in the liver and in the intestinal mucosa of the rabbit at a much more rapid rate than in the brain and more quickly than in any other organ[59]. Desoxyribose nucleic acid, on the other hand, shows a behaviour opposite to that of the phosphatides. Its extent of renewal in the liver of adult rats is very low[60,61,87], amounting to only about 0.1 percent in the course of 2 hours. In the spleen and in the intestinal mucosa, the renewal of the desoxyribose nucleic acid is 20 and 30 times, respectively, more rapid than in the liver. High figures for the rate of formation were found in rapidly growing tissue.

X-rays were found to obstruct the formation of labelled desoxyribose nucleic acid molecules[61]. Following irradiation with 300 r or more both in the sarcoma and in the organs of the rat formation figures were obtained amounting only to about half of the value observed in the controls. In the study of the reduction in labelled nucleic acid formation under the action of X-rays, a new line of attack was opened in the study of the action of such radiation on cell division.

We calculate the extent of renewal of the compound in question, for example creatine phosphoric acid, by comparing the specific activity of the creatine phosphorus at the end of the experiment with the average specific activity of the free phosphorus present in the tissue cells during the experiment. This calculation is based on the assumption that the labelled free phosphate, or the phosphate of a donor whose P enters rapidly into exchange equilibrium with the free P present in the cells, is incorporated in the creatine phosphate molecule present in the cells of the organ investigated. In the case of phosphatides, the possibility cannot be entirely excluded[45,46] that a precursor of the phosphatides containing labelled phosphate is transferred from another organ into the organ investigated. In such a case, the calcula-

tion of the extent of the renewal of the phosphatide molecules would necessitate knowledge of the specific activity of the precursor P. The renewal figures obtained must therefore be interpreted with caution. Another more pertinent reason for the cautious interpretation of the results obtained is that the molecules of some organic compounds may possibly be built up within the phase boundary, where the specific activity of the free P may appreciably differ from that of the intracellular free P.

While we measure the rate of renewal of phosphatide molecules with respect to their phosphate content by employing ^{32}P as an indicator, the rate of renewal of the fatty acid constituents is determined by the use of deuterium[62] as indicator, and the new-formation of the choline content by applying ^{15}N as a tracer[63]. A molecule can clearly be renewed in various ways.

Site of formation of phosphorus compounds in the organism

Origin of yolk phosphatides

We shall first consider the site of formation of some constituents of the hen's egg. Where in the organism are the phosphatides found in the yolk synthesized? This question can be answered by comparing the specific activity of phosphatide P extracted from the yolk and from the different organs a few hours after administration of labelled sodium phosphate[64].

The results of an experiment in which the hen was killed 5 hours after subcutaneous injection of labelled P are seen in Table 6. The specific activities of the yolk phosphatide and the ovary phosphatide P were very low, showing that only a small part of the phosphatide molecules present in the said phosphatides had been formed within the last 5 hours. The plasma phosphatide P had a much higher specific activity than those extracted from the ovary and the yolk, while the liver phosphatide P had a higher specific activity than the plasma phosphatide P. The gradient indicating the presence of phosphatide molecules formed within the last 5 hours, thus falls off in the direction from the liver, through the plasma, into the ovary.

The conclusion that the formation of the phosphatide molecules of the plasma mainly occurs in the liver[59,64,65] is strongly supported by the results obtained in the study of fat phosphorylation in the hepatectomized dog by Chaikoff and his colleagues[66]. These authors injected labelled sodium phosphate intravenously immediately after removal of the liver. Practically no

Table 6. Specific activity of phosphatides extracted from the organs of a hen

Organ	*Relative specific activity (Activity of inorganic plasma P = 1)*
Liver	0.54
Plasma	0.43
Ovary	0.039
Yolk	0.0035
Intestine	0.11
Spleen	0.1

phosphatide ^{32}P was recovered in the plasma as late as 3-6 hours after extirpation of the liver; at these times 0.4 percent of the injected ^{32}P had been incorporated into phosphatides of both kidneys and about an equal amount into the whole small intestine.

Considerations similar to those applied to the origin of the phosphorus compounds of the yolk were used in an investigation of the phosphorus compounds of milk[67]. As seen in Table 7, the milk phosphatides were found to have a much higher specific activity than the plasma phosphatides, indicating that the phosphatides must enter the milk from a source other than the plasma and must thus have been synthesized to a large extent in the mammary gland. The determination of the specific activity of the mammary gland phosphatides revealed a very high value, even higher than those found for the kidney and liver phosphatide P.

One often encounters the view that the milk fat originates from the phosphatides of the blood, which are decomposed into fatty acid and inorganic P in the mammary gland. The inorganic P present in the milk should, according

Table 7. Specific activity of the phosphatide P extracted from the organs of a goat 4½ hours after administration of labelled sodium phosphate

Fraction	*Specific activity*
Milk	0.09
Plasma	0.02
Corpuscles	0.01
Mammary gland	0.13
Liver	0.09
Kidney	0.11
Plasma inorganic P	1.48

to this view, originate from phosphatide P. The fat content of goat's milk amounts to about 3 percent. Taking the ratio of fatty acid to phosphorus to be 20 : 1 in plasma phosphatides, the production of 3 percent fatty acid from phosphatides would set free 0.15 percent of inorganic phosphorus. This being about the inorganic P content of the milk, almost all inorganic P of the milk should originate from plasma phosphatide. A few hours after the administration of labelled phosphate, the milk phosphatides are only slightly active, while the milk inorganic P shows a strong activity. This is a decisive argument against the above view. The high activity already found for the milk inorganic P in the early stages of the experiment, is only compatible with the assumption that the milk inorganic P is derived from the plasma inorganic P. The latter acquires a high activity soon after subcutaneous injection of labelled sodium phosphate.

As a further example we may mention the origin of the phosphorus compounds in the chick embryo[68]. Physiological sodium chloride solution (0.1 ml) containing traces of labelled sodium phosphate was injected into fertilized eggs. Several days after incubation, the phosphatides and other phosphorus compounds were isolated in turn from the embryo and the yolk, their activity and their phosphorus content being determined. As seen in Table 8,

Table 8. Specific activity of P extracted from the hen's egg incubated for 18 days

		Specific activity
Yolk residue	Acid-soluble P	1.56
	Phosphatide P	0.016
	Protein P	0.058
Embryo	Skeleton inorganic P	1.66
	Phosphatide P	1.59
	Protein P	1.44

the specific activity of the embryo phosphatide P is very different from that of the yolk phosphatide P.

While the phosphatides of the yolk are scarcely active, the phosphatides extracted from the embryo are found to have a very strong activity. The phosphatide molecules present in the embryo must obviously have been newly synthesized. Similar considerations apply to the protein phosphorus present in the embryo.

Reaction path

The path taken by organically bound phosphate radicals in glycolytic processes was investigated by using labelled compounds prepared under the action of enzymes present in muscle juice or yeast. When labelled adenyl phosphate was added to fresh muscle pulp in which glycolysis occurred, no formation of active inorganic phosphate was found to take place, but active phosphate was detected in the Harden-Young ester formed during alcoholic fermentation.

In a study of the interaction of labelled adenosine triphosphoric acid with non-labelled hexose monophosphoric acid ester, which leads to the formation of fructose 1,6-diphosphoric acid ester, the labelled phosphate given off by the adenosine triphosphoric acid was found to be exclusively present in the fructose 1,6-diphosphoric ester. The fact that the free phosphate formed according to the equation:

adenosine triphosphoric acid + hexose 6-monophosphoric ester + H_2O → fructose 1,6-diphosphoric ester + adenylic acid + phosphate

was found to be inactive, indicates that the free phosphate originated exclusively from the hexose 6-monophosphoric ester[69].

When both hydrogen and labelled phosphate were transferred, neither of the two stable radicals of the cozymase molecule was found to be replaced by active phosphate. A similar negative result was obtained for the reaction

3-phosphoglyceric acid → 2-phosphoglyceric acid

in the presence of active phosphate and also for the conversion of glucose monophosphoric acid into glucose hexaphosphoric acid in the presence of active phosphate. The ester fractions were found[70] not to have taken up ^{32}P.

Does a less pronounced formation of new molecules take place simultaneously with the autolysis observed in tissue slices? By the employment of isotopic indicators this question can be answered. On shaking liver, kidney or brain slices for a few hours with a Ringer solution containing ^{32}P at 37°C, Chaikoff and his colleagues[71] found that the phosphatides isolated from the tissue slices contained ^{32}P; hence, side by side with an autolysis of the phosphatides in the tissue slices, an appreciable formation of labelled phosphatides also takes place.

The formation of labelled nucleic acid in slices of Jensen's rat sarcoma was likewise obtained[72] in the investigation of the formation of desoxyribose nu-

cleic acid *in vitro*, when these slices were shaken with labelled blood or labelled Ringer solution. About 0.1 percent of the desoxyribose nucleic acid molecules present in the tissue slices were found to be labelled after the lapse of 4 hours; these molecules had accordingly been formed during the experiment. The presence of hydrogen sulphide, azide or carbon monoxide inhibits the formation of labelled phosphatides. Addition of cyanide, fluoride or monoiodoacetate to labelled blood or labelled Ringer solution is also found to inhibit the formation of labelled nucleic acid.

Dynamic state of body constituents

The most remarkable result obtained in the study of the application of isotopic indicators is perhaps the discovery of the dynamic state of the body constituents. The molecules building up the plant or animal organism are incessantly renewed. In the course of this renewal, not only the atoms and molecules taken up with the food participate, but atoms and molecules located in one organ or in one type of molecule will soon be found in another organ or in another type of molecule present in the same or in another organ. A phosphate radical taken up with the food may first participate in the phosphorylation of glucose in the intestinal mucosa, soon afterwards pass into the circulation as free phosphate, enter a red corpuscle, become incorporated with an adenosine triphosphoric acid molecule, participate in a glycolytic process going on in the corpuscle, return to the circulation, penetrate into the liver cells, participate in the formation of a phosphatide molecule, after a short interval enter the circulation in this form, penetrate into the spleen, and leave this organ after some time as a constituent of a lymphocyte. We may meet the phosphate radical again as a constituent of the plasma, from which it may find its way into the skeleton. Being incorporated in the uppermost molecular layer of the skeleton, it will have a good chance of being replaced by other phosphate radicals of the plasma or the lymph, but it may also have the good fortune to find a more or less lasting abode in the skeleton. This will be the case when it becomes embedded in a newly formed apatite-like bone crystallite.

There are indications that, in the growing organism, the rate of new formation of the molecules is still greater than in a fully grown organism. It was found, for example, by making use of heavy nitrogen, kindly put at our disposal by Professor Urey, as an indicator, that in "old" leaves of the sun-

flower, which did not develop further during the experiment, 12 percent of the protein molecules present were renewed within 12 days. In growing leaves, the replacement of old protein molecules was found to take place at a higher rate[73].

Schoenheimer and Rittenberg[34] have shown, by applying labelled nitrogen, that the peptide linkages in the proteins of the animal tissue are opened and reclosed with great ease. They found that the protein molecules in the living body continually change and renew their structures. This discovery is one of the most surprising and outstanding results arrived at with isotopic indicators.

Excretion studies

Chemical analyses of the food and of the excreta permit the determination of the extent to which the organism is in balance. Chemical methods, however, cannot determine to what degree the substances found in the faeces originate from undigested food and to what extent they have been 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 with the aid of isotopic indicators.

The simplest procedure is the following[74]. At a suitable time after administration of labelled sodium phosphate, we determine the specific activity of the urine P and that of the faeces P. Both originate from the blood plasma and, provided that 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 faeces P were entirely of endogenous origin, it should show a specific activity equal to that of the urine P. If we find the faeces P to be less active than the urine P, the active faeces 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 faeces P and urine P tells us to what extent the endogenous faeces P has been diluted by food P.

The ratio 100 x specific activity of faeces P/specific activity of urine P gives the percentage of P in the faeces which originates from the body proper. In the case of human subjects, $\frac{3}{4}$ to $\frac{4}{5}$ of the P present in the faeces was found to originate from non-absorbed P.

Labelled red corpuscles

As seen in Fig. 7[75], labelled phosphate penetrates at a fairly slow rate into the red corpuscles. On entering the corpuscles, however, the newly arrived phosphate ions participate rapidly in the formation of acid-soluble organic phosphorus compounds which occur in a comparatively high concentration in the corpuscles. The formation of new acid-soluble phosphorus compounds in the corpuscles is largely associated with glycolytic processes occurring there and is attended by the destruction of an equal or almost equal number of "old" molecules. As a result of these processes, the specific activity of the labile P atoms of adenosine triphosphoric acid and that of the P of some other compounds will soon acquire a specific activity almost as high as that shown by the free P of the corpuscles, but much lower than that of the free P of the plasma. This fact and the fairly slow rate of penetration of phosphate through the corpuscle wall explain the low rate of loss of ^{32}P by labelled corpuscles when brought into contact with unlabelled plasma, and make possible the application of such labelled corpuscles in the determination of the total circulating red corpuscle content of the organism[76,77]. A detailed investigation of the erythrocyte content of human subjects, making use of labelled corpuscles, was carried out by Nylin[78,88]. He estimated, furthermore, the rate at which injected blood and the circulating blood are homogeneously mixed. Fig. 8 shows the result obtained by Nylin in an experiment

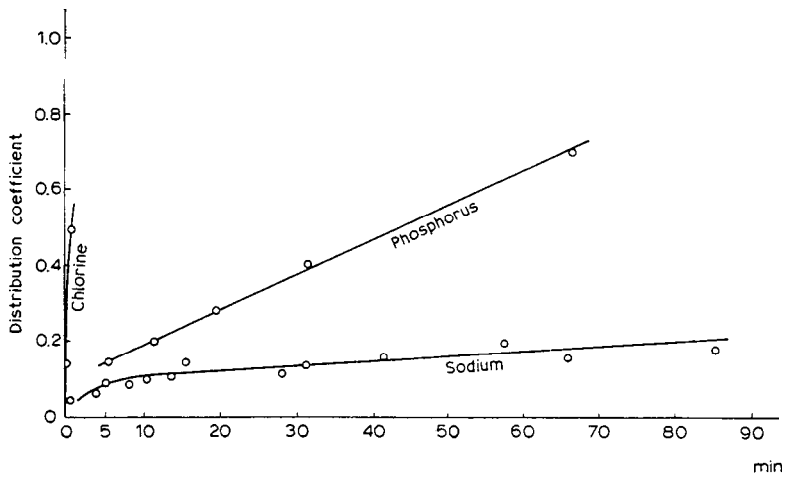

Fig.7. Distribution of labelled ions between corpuscles and plasma of equal weight at 37°.

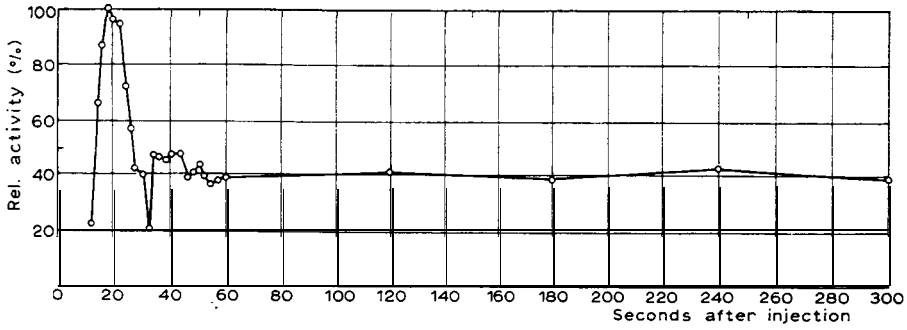

Fig. 8. Change of activity of the arterial corpuscles with time following intravenous injection of labelled corpuscles (G. Nylin).

where homogeneous distribution of the injected blood took only 60 seconds.

Corpuscles can also be labelled by introduction of radio-iron into the corpuscle haemoglobin. Such corpuscles were used by Hahn and his colleagues[79] in the determination of the red-corpuscle content of the dog. Radio-iron has a much longer half-life period than has radio-phosphorus; such "iron-labelled" corpuscles remain labelled for a much longer time than "phosphorus-labelled" corpuscles. While, however, the latter can easily be obtained by shaking blood with labelled phosphate, the former can be made only in the living organism, a fact which, together with the relatively great difficulty of obtaining radio-iron, restricts the applicability of iron-labelled corpuscles in the determination of the erythrocyte volume.

The determination of the total corpuscle volume of the organism demands only that the labelled corpuscles retain their labelling for some minutes; the determination of the life cycle of the corpuscles requires, however, the use of marked corpuscles which conserve their labelling for weeks. No worker has yet succeeded in achieving a labelling of mammalian red corpuscles that fulfils this condition. Iron-labelled corpuscles, although remaining labelled for a sufficient time, were found by Hahn and his colleagues[89] not to be suited to the purpose in hand. The life of the red corpuscles of the hen, however, was determined[80], making use of phosphorus-labelled corpuscles. In contradistinction to mammalian corpuscles, avian corpuscles contain large amounts of desoxyribose nucleic acid, and the nucleic acid molecules were found to remain unchanged throughout the life of the corpuscles. The newly formed corpuscles of a hen to which labelled phosphate is administered contain labelled desoxyribose nucleic acid.

By daily injection of labelled phosphate, the activity of the plasma phos-

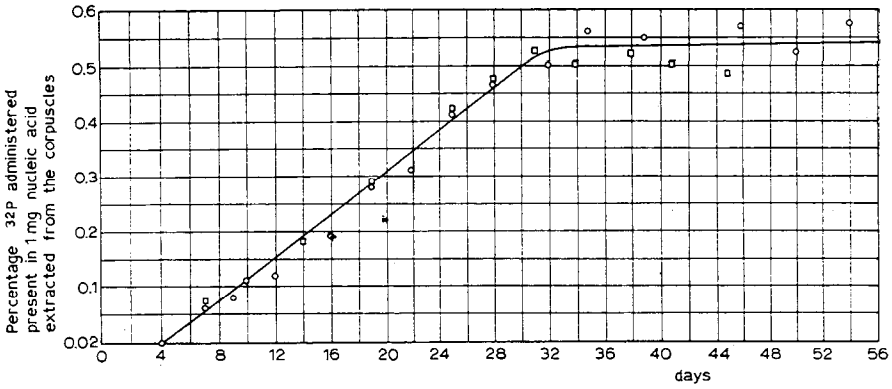

Fig. 9. Life cycle of the red corpuscles of the hen.

phate is kept at a constant level, and at suitable intervals the specific activity of the nucleic acid P extracted from the corpuscles is determined. Fig.9 illustrates the results obtained, including the fact that, after the lapse of about 33 days, the specific activity of the nucleic acid P became constant. This indicates that all corpuscles present in the circulation of the hen were formed during the experiment. In the corpuscle samples taken in the course of the first four days, only minute amounts of labelled nucleic acid were found to be present. This may be interpreted by supposing that the formation of the corpuscles in the marrow, up to the point of their release into the circulation, requires four days; 3.5 percent of the corpuscle content of the hen is thus built up daily.

I have attempted to give a short review of the earliest applications of isotopic indicators and to discuss a few examples of their earlier and more recent employment. Their use may be much extended in the time to come.

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