walk for several weeks and then slowly improved. I am not convinced that this is a deficiency syndrome. A precedent febrile illness regarded as dengue occurred in Musselman's (1945) and Katz's (1946) patients, and Katz noted that the myasthenic symptoms were in inverse relationship to the severity of the fever. In my patient an infective toxin temporarily damaging the myoneural junction seems the probable cause.

Intoxication or Deficiency ?

Examples already given show that it is often difficult to distinguish between an intoxication and a deficiency. The verbal distinction is, of course, clear enough: a toxin is a harmful extra, a deficiency a harmful lack. But as soon as we get beyond the mouth difficulties arise. Food may be contaminated with a toxin, as, for example, Abyssinian wheat with flax-darnel (Brinton, 1945-6), or the food may be modified so as to become toxic, as the protein of agenzized flour causes canine hysteria (Mellanby, 1947; Moran, 1947). Or a normal constituent of food may be toxic only in the presence of vitamin or other deficiency—e.g., maize in pellagra.

Lathyrism gives rise to a spastic paraplegia which closely resembles the spastic syndrome occasionally seen in prisoners of war. Denny-Brown believes that lathyrism has not been proved experimentally to be due to *Lathyrus sativus per se*, and suggests that flour made from this pea may be deficient in tryptophan and that this may neutralize the action of a vitamin, the consumption of lathyrus thus bearing the same causal relation to lathyrism that the consumption of maize or cassava does to pellagra.

But maize is toxic only in the sense that it leads to a tissue deficiency of nicotinic acid, and it might be thought that the crucial question is the state of the nervous system. Even here, however, the distinction between intoxication and deficiency is not clear-cut. Peters *et al.* (1945) have shown that arsenite damages the pyruvate enzyme system in the nervous system, and from this sprang the discovery of dimercaptopropanol (British anti-lewisite, BAL), which was designed to resemble the tissue thiols, to combine in their stead with trivalent arsenic and so protect them. As we have seen, aneurin deficiency also damages the pyruvate enzyme systems, and both arsenical poisoning and beriberi are accompanied by a rise in the level of blood pyruvate. Thus it would seem that arsenical poisoning may act by causing a local enzyme deficiency, and aneurin deficiency by causing a local intoxication.

In the past we have tended to think that by describing a disorder as toxic we have explained it, but it was only our ignorance of the finer processes of the metabolism of the neurone that forced us to be content with such vague conceptions as "intoxication" and "toxic polyneuritis." All that we have learned about malnutrition should teach us that whatever happens to the nervous system—not only states of avitaminosis but head injuries, infections, vascular lesions, and demyelating disorders—will need increasingly to be considered not only from the standpoint of morbid anatomy but also in terms of the chemistry of the nerve cell.

(Figs. 1 and 4 are reproduced from Dr. R. C. Burgess's paper on "Deficiency Diseases in Prisoners of War at Changi" (*Lancet*, Sept. 21, 1946, p. 411), and Figs. 2 and 3 from the article on "Cerebral Beriberi" by Captain H. E. de Wardener and Dr. B. Lennox (*Lancet*, Jan. 4, 1947, p. 11).)

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BIOCHEMICAL APPLICATIONS OF STABLE AND RADIOACTIVE ISOTOPES*

BY

A. S. McFARLANE, M.A., M.B., Ch.B., B.Sc.

National Institute for Medical Research, Hampstead

With but one exception the body does not discriminate between natural elements and their stable or radioactive isotopes. In addition the stable isotopes, with the exception of deuterium, have no deleterious effects on living cells even in the highest concentrations. In the case of the radioactive ones it is possible to employ such low concentrations that most human experiments can be carried out with safety, and all animal experiments with impunity, since genetic effects of the radiations can be ignored. These simple considerations have made possible to the biochemist a new kind of experimental approach which he has not been slow to use.

Radioactive Phosphorus

One of the earliest isotopes to become available was radioactive phosphorus (P^{32}), and of course it was administered as sodium phosphate by various routes to animals and its excretion observed and an analysis made of the skeleton and various organs to determine where and for how long it was retained. It was soon established that only a small fraction—less than 10%—is excreted in twenty-four hours, and of the total amount injected only a small proportion is present in the lymph and plasma a few

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hours after injection. The remainder has disappeared into the cells of the body, especially of the skeleton, the liver, and the intestine.

Precisely how this phosphorus is taken up by the cells is a matter of some general importance. By whatever route it is injected a high concentration of P^{32} occurs within a few minutes in the lymph and plasma. From the lymph the cells take up phosphate at a steady rate and discharge it back at the same rate into the same medium. There is no discrimination between ingoing and outgoing phosphate, and complete mixing takes place within the cell. The process consists, in fact, of a continuous diffusion in both directions across the cell wall, radioactive phosphate passing out of the cell long before all the inactive phosphate in it has been replaced. If the concentration of radioactive phosphate in the lymph is kept constant—and there are several experimental tricks for doing this—the conditions are simplified and it can be shown that the radioactivity per gramme of phosphate in the cell rises exponentially with time. Since the exponential curve is almost linear in the first part of its course, a simple measurement of the specific activity of cellular phosphate, after a time interval which is short in relation to the time required to replace all the phosphate, is sufficient to determine the steady rate of exchange of phosphate in the cell. For example, after four hours the activity of the phosphorus in the tibia of a rat is one six-hundredth that of the phosphate maintained constant in the lymph, and we may conclude that in four weeks an amount of phosphorus passes into and out of this bone equal to the total weight of phosphorus in it. This is called the replacement or turnover time. In a few cases where the turnover time is short it is possible to make allowances for the non-linearity of the growth curve and to obtain exact turnover values; but in general this is not possible, and the results obtained by assuming linearity are known to be of the right order only. However, to know the larger turnover times accurately and the shorter ones approximately has proved to be a matter of great importance to our understanding of the chemistry of the body.

Phosphorus Compounds

The phosphorus taken up by the skeleton is laid down for the most part in inorganic form, but elsewhere in the body it is incorporated into a large and still expanding group of complicated organic compounds. Outstanding among these are the phospholipids which are present in the blood plasma and in all cells. By transfusing radioactive plasma phospholipids to a dog and observing their rate of disappearance from the recipient's plasma, Chaikoff and his collaborators (Fishler *et al.*, 1943) have shown that the plasma phospholipids are replaced in six to ten hours. The liver is the main site of synthesis and degradation of these substances, since removal of the liver prolongs the turnover time to 33–160 hours. Removal of the intestine, on the other hand, has no effect. Although the liver produces enough phospholipids to renew the plasma reservoir inside ten hours it requires fifty hours to renew its own phospholipids. The intestine renews its phospholipids in forty hours, but the brain, which is rich in these substances, requires four weeks for their replacement.

Another important phosphorus compound of the body is nucleic acid, of which two main types are present—one characteristic of the nuclei, and the other found in high proportion in the cytoplasm of all cells. The P in the nuclear form is exchanged very slowly—about the same as in bone—but in the cytoplasmic form it is exchanged ten times as quickly. That is the position in normal adult cells, but when the cells are dividing—e.g., in embryonic tissues,

in regenerating tissues, or in malignant growths—the position is vastly changed, the nuclear P exchange increasing ten to fifteen times. This confirms and establishes on a quantitative basis general impressions derived from the histology of the tissues; but the tracer method can go further than this—it can distinguish the rate at which nucleic acid P^{32} builds up inside a cell from that at which it disappears. In this way—and in no other—it can be shown that growth from any of the causes mentioned results not from an increased rate of synthesis of cell compounds but from a diminished rate of disintegration, so that the organism reaches maturity when it has accelerated its intracellular breakdown processes until they are as rapid as the synthetic ones.

It is clear also in all these studies that some injected phosphorus is excreted into the lower bowel, and it can be demonstrated that part of the phosphorus of our diet which is absorbed in the upper alimentary canal is re-excreted and mixed with unabsorbed phosphorus in the lower. An entirely different picture comes to light if this experiment is repeated using radioactive iron in the diet. Hahn *et al.* (1939) have shown that in a normal adult practically all the iron passes through the gut unabsorbed, but if the subject has been made anaemic new radio-iron is absorbed into the circulation. The new red cells which are produced differ from the old ones in having some of this radio-iron incorporated in the haemoglobin molecule. These radioactive cells persist in the circulation for some months, as do all red cells, and are finally destroyed in the spleen and liver, where their iron is stored. In time this iron finds its way back into the circulation for incorporation into new red cells. Here once again the tracer element is able to reveal something which could not have been discovered in any other way—namely, that the body is extremely conservative in relation to this particular constituent of the diet, endeavouring so far as possible to carry with it enough iron circulating internally to meet all its requirements. It is as conservative in relation to iron as it is liberal in relation to phosphorus. The mechanism by which the intestinal mucosa “senses” internal iron deficiency and responds by allowing some of the element to pass from the gut into the blood is not yet understood, but its beauty is impressive.

Glycine

A few days after administering the amino-acid glycine containing C^{13} in the methyl group and N^{15} in the amino-group to rats the isotopes are found in adjacent positions in the protoporphyrin of haemoglobin. This tells us much about the manner in which the body synthesizes the five-membered pyrrole ring and at the same time provides a convenient method of labelling the red cells of the blood. If N-labelled glycine is given to a human being the isotope is detectable in the red cells at the fourth day and the concentration rises steadily over a period of twenty-five days, remaining constant for a further fifty days, and dropping slowly to zero in the next 150 days.

Shemin and Rittenberg (1946), who first observed this behaviour, were quick to note its unusual character. Normally when a tracer is injected the concentration of labelled compound in a tissue or organ rises to a maximum as synthesis proceeds and immediately thereafter declines again as the labelled molecules are metabolized. The fifty-day period of constant isotopic porphyrin concentration can be explained only in one way. The porphyrin of the newly formed red cell is excluded from the metabolic reactions of the body and enters into these again only at the end of the life-span of the red cell. Statistical analysis of the results shows that the average life of a human red cell is 127 days. This method is not the only one leading to this result, but

it is particularly elegant in involving no more disturbance to the subject than is associated with feeding labelled glycine. The method further reveals that, when the red cell finally disintegrates, the porphyrin group, unlike iron, is not further utilized in the formation of new red cells but is excreted in the bile.

The body contains three important substances—choline, creatine, and methionine—with no obvious chemical relationship to each other except that each has a methyl group linked to the rest of the molecule through sulphur or nitrogen. Deficiency symptoms due to the absence of choline from the diet can be cured by giving methionine, and the absence of both choline and methionine from the diet affects the excretion of creatine as creatinine. There is clearly, therefore, a metabolic interdependence between these three substances, and a clue to its character is found by administering one of them with deuterium in the methyl group. The isotope is subsequently found in the methyl groups of the other two and nowhere else. It seems, therefore, that a methyl group linked through sulphur or nitrogen is handled by the body quite differently from a methyl group linked through carbon. We are introduced here to the idea of a vitamin-like importance in the diet of a particular radical. In not dissimilar fashion the amino-groups of all the amino-acids of the diet are merged in a metabolic pool from which the amino-acids of the tissues are derived. This is shown by administering N-labelled glycine or ammonia and finding the isotope in all the amino-acids except lysine of the tissue proteins.

Carbon

If carbon dioxide containing isotopic carbon is breathed in it is found that some of the carbon finds its way into glycogen in the liver and from there to other compounds of the body, thus disposing in one simple experiment of the belief that only plants and bacteria can utilize carbon in the form of carbon dioxide. Needless to say, biochemists in the last few years have investigated this phenomenon intensively, and no fewer than six distinctive chemical reactions are now known by which the body can fix carbon dioxide, the most important one being the condensation of carbon dioxide with pyruvic acid to give oxalo-acetic acid.

Arising out of this and other experiments much attention has been paid in recent years to acetic acid. In the past this has been little investigated because it is known to be present in insignificant quantities in the body and these are extremely difficult to measure precisely. With the advent of isotopes, however, an inefficient method of isolation may be made the basis of a precise method of estimation, as the following example will show. Supposing a compound containing C^{13} is given in the diet and we wish to know whether any part of this C^{13} is incorporated into acetate in the liver. A known weight of liver is mixed thoroughly with a known but excessive weight of normal acetate and the mixture is fractionated to recover a proportion of the acetate. At the same time a like proportion of labelled acetate is recovered—minute in amount but sufficient for mass spectrometric estimation. Multiplication of the result by the recovery factor tells us the weight of labelled acetate in the piece of liver. This has become an important general method of analysis known as the isotope dilution method.

When a fatty acid labelled in the carboxyl carbon is oxidized by the liver some acetate—but considerably more aceto-acetate—is found. This aceto-acetate is labelled in both carbonyl and carboxyl groups, indicating that it is formed by condensation of acetyl groups, methyl to

carboxyl. Aceto-acetate is not further utilized by the liver but is oxidized elsewhere. The kidney, among other organs, can oxidize it and is known also to take up acetate, but normally no aceto-acetate can be found there. By incubating kidney slices with labelled acetate in the presence of an excess of normal aceto-acetate and demonstrating the presence of isotopic aceto-acetate in the excess recovered, Medes *et al.* (1945) have shown that the oxidation of acetate is in fact through aceto-acetate, but the latter is normally further oxidized as soon as it is formed.

An alternative tracer procedure to the above has been to feed labelled acetate and to investigate the more complicated molecules of the body to find what has happened to that part of it not immediately excreted. By this procedure the carbon skeleton of acetate has been identified in the dicarboxylic amino-acids of the tissue proteins, in the fatty acids of the liver and depot fats, in cholesterol, and in glycogen. It has been shown (Bloch and Rittenberg, 1945) that although the carcass of a 100-gramme rat contains but a few milligrams of acetate, more than a gramme of acetate is produced in its liver every day.

Conclusion

These developments, which are largely if not exclusively derived from isotopic studies, represent substantial contributions to biochemistry. They reveal numerous unsuspected metabolic pathways, and if some of these are but little used normally—as, for example, the fixation of CO_2 —they may be of the greatest importance in disturbed metabolic states: e.g., in diabetes, where aceto-acetate and related ketone bodies are produced in abnormal amounts. They demonstrate that the quantity of substance in a tissue bears little if any relation to its metabolic importance, and that important intermediary metabolites may not even be present in detectable concentrations, thereby heralding a new dynamic point of view in which the body is conceived as in a state of incessant change, atoms and molecules displacing one another with surprising rapidity in every tissue. All that remains constant is the pattern into and out of which these atoms pass—a stereo-chemical relationship of atoms within molecules, and a spatial juxtaposition of molecules within tissues—which is specific for each individual and characteristic of living matter as a whole.

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The Annual Report for the year ending July 31, 1947, has been issued by the Royal College of Surgeons of England. It draws attention to the Supplemental Charter which was granted on May 20, and states that new by-laws have been submitted to the Home Office for approval. Fellows and members of the College have been co-opted to the Council in order to include various branches of practice not represented by the ordinary process of election. Arrangements have been made to hold special final Fellowship examinations in ophthalmology and otolaryngology, and the court of examiners is being enlarged to include the necessary specialists. Regulations for the Fellowship in dental surgery have been approved and the first 18 Fellows elected. The building of a residential hostel for post-graduate students is planned. During the year 158 Fellows have been admitted. After Jan. 1, 1949, candidates will not be admitted to the final Fellowship examination until three years after qualification and until they have served a period of twelve months in posts approved by the Council, six months of which must have been occupied in residence in a hospital recognized for the purpose.