doses of the mono-azo dye, Congo Red, produces effects quite similar to those of liver extract in cases of primary anemia. Our observations were made on two cases of untreated but mild Addisonian anemia, using intravenous injections of 1.5 per cent. Congo Red 4B in 6 per cent. dextrose. One patient received 60 ccm in five days, the other 90 ccm in ten days; both had a rise in reticulocytes and a fall in serum bilirubin comparable to that produced in similar cases by intramuscular injections of liver extract. Massa and Zolezzi continued treatment until blood regeneration was complete in nine of the fourteen cases which responded to Congo Red, but we felt that liver therapy was less troublesome to administer for continuous management.

Many normal guinea pigs exhibit a slightly delayed but sharp and sustained reticulocyte shower, following injection of liver extracts known to be potent in pernicious anemia. This response seems specific for the liver fractions valuable in therapy.[3,4] Congo Red injected daily for five days into the peritoneal space of guinea pigs produced a reticulocyte shower, maximal from 5 to 7 days after beginning treatment with 30 mgm of dye per pig daily. The reticulocytosis declined gradually, reaching the control level from 10 to 14 days after the peak. At that time large doses of potent liver extract were injected, but caused no further reticulocyte response. Congo Red not only produces the same effect on normal guinea pigs as potent liver extract, but the treatment, like that with liver, renders the animals refractory to liver therapy for a considerable period of time.

These results of injecting a mono-azo dye with colloidal properties can scarcely be accounted for by the widely current theory that pernicious anemia is cured, and the reticulocyte response of guinea pigs evoked, by providing a substance needed for the maturation of red corpuscles. Massa and Zolezzi[5] suggest that the dye prevents hemolysis by blocking reticulo-endothelial cells. While the theory that pernicious anemia results from over-active blood destruction and can be corrected by blocking the reticulo-endothelial system might be satisfactory to account for the blood disturbances of Addisonian anemia, it obviously fails to account for the glossitis and spinal cord lesions which often accompany the disease and are arrested by liver therapy. Congo Red is notably effective in neutralizing toxic substances (curare, strychnine, diphtheria and tetanus toxins) and it is more probable that in pernicious anemia and in normal guinea pigs it assists in detoxification of substances, probably enterogenous in origin, which are hemolytic. These observations make imperative a further exploration of the old theory that pernicious anemia is due to excessive absorption or deficient detoxification of noxious substances derived from the gastro-intestinal tract. While it is not improbable that the effective factors in liver are utilized in the detoxification of a toxin, it seems highly unlikely that Congo Red can supply material needed for production or maturation of red cells or for maintenance of neurones and lingual papillae.

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[3] B. M. Jacobson, SCIENCE, 80: 211, 1934.
[4] Y. Subbarow, B. M. Jacobson and C. H. Fiske, *New Eng. Med. and Surg. Jour.*, 212: 663, 1935.
[5] M. Massa and G. Zolezzi, *Gior. Clin. Med.*, 14: 1207, 1933.

DEUTERIUM AS AN INDICATOR IN THE STUDY OF INTERMEDIARY METABOLISM

MANY attempts have been made to label physiological substances by the introduction of easily detectable groups such as halogens and benzene nuclei. However, the physical and chemical properties of the resulting compounds differ so markedly from those of their natural analogues that they are treated differently by the organism. The interpretation of metabolic experiments involving such substances is therefore strictly limited.

We have found the hydrogen isotope deuterium to be a valuable indicator for this purpose. The fact that it occurs in the same proportion (1 atom of deuterium to 5,000 atoms of protium) in the hydrogen of ordinary water and of organic matter is in itself evidence that the living body is unable to distinguish the few organic molecules which contain deuterium from those which do not. Were the reverse the case, organic matter of biological origin would display differences in isotopic ratio.

We have prepared several physiological compounds (fatty acids and sterol derivatives) containing one or more deuterium atoms linked to carbon, as in methyl or methylene groups. Their physical properties are indistinguishable from those of their naturally occurring analogues by the methods commonly employed. As, however, the deuterium content of these substances or of their physiological derivatives can readily be determined from the properties of the water formed on combustion, their fate in the body can be followed even after considerable dilution.

In preliminary feeding experiments with different amounts of fat (linseed oil, partially hydrogenated with deuterium; the product had similar properties to olive oil) to mice, it was found that most of the fat, before being utilized, is stored in the fat depots; the fat burned in the body was not taken directly from that absorbed but from the fatty tissue.

The breakdown of the fat in the organism could be followed by deuterium analysis of the body fluids, since organic compounds containing deuterium, when burned, form an equivalent amount of heavy water. This is equally distributed in all body fluids. For example, after feeding mice for four days on a diet rich in carbohydrate with only 1 per cent. of fat, 60 per cent. of the absorbed fat could be recovered from the fat depots, and in the body fluids an amount of heavy water equivalent to 20 per cent. of the fat was found. In order to prevent an excessive storage of food material the total food intake was so limited that the animals lost weight during the feeding period.

The use of deuterium has made possible, for the first time, the recognition of cholestenone and coprostanone as intermediates in cholesterol metabolism. Earlier experiments with dogs have shown that administration of cholestenone gives rise to an excess excretion of either cholesterol or coprosterol, according to the nature of the basal diet. We have now found that ingested coprostanone is converted into coprosterol; after feeding coprostanone 4, 5–d_2 to a dog and to a human the coprosterol isolated from the stools contained large amounts of deuterium.

The number of possible applications of this method appears to be almost unlimited.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A BIOLOGICAL EFFECT OF IONIZED AIR

IN view of the contradictory results of certain experiments[1] designed to test the therapeutic and other biological value of ionized air, it has been thought desirable to try some simple experiment under well-controlled conditions which would give a definite answer to the question whether or not ionized air has any effect on living material. Accordingly, inbred cultures of wild-type *Drosophila melanogaster* have been grown in ionized air under various conditions.

In the first set of experiments ionized air was drawn through the culture bottle, which was closed by a rubber stopper. The air was ionized by a polonium source suspended in a glass tube, one end of which was closed with a wad of cotton, the other end leading without obstruction to the culture bottle. Since the source was never closer than 30 cm to the bottle, the weak gamma-radiation characteristic of polonium need not be considered. These experiments were performed in a damp, cool basement.

In a second series of experiments, performed in various rooms in another building, air was ionized in the culture bottles by means of a brush discharge between point and plane connected to a 5,000 volt transformer, each bottle being closed with a wad of cotton. In this case, as indeed in the previous one, the degree of ionization would vary at different points within the bottle, because of recombination of ions.

Each culture was started with flies from the inbred stock; neither these nor any of their ancestors had in any case been exposed in the experimental bottles, but exposure to highly ionized air was started within ten minutes after each culture was prepared. In the polonium experiments exposure was continuous, but in the second series exposure was stopped by turning off the current during about half of each day.

In both series of experiments a definite effect has been observed, consisting in the coloration and subsequent death of larvae. The coloration, reddish brown or rusty, appeared first at the posterior spiracles, and spread from this point through the larval body. The amount of coloration when death occurred was not uniform; the majority of affected larvae died when about half of the body was colored, although a few lived until coloration was practically complete, and many died with only a spot of color at the spiracles. Coloration did not advance after death.

Coloration never appeared in all larvae in a culture; it was not observed in larvae that remained in or upon the food medium at the bottom of the bottle, but only in those that crawled up the walls. Some larvae were colored in every bottle treated, and in several cases the majority of those on the walls. Once coloration set in, death of the affected larva followed within twelve to twenty-four hours. Within another day or two all larvae that could be seen in the bottle died. The adult flies remained alive, although in the second series, with the discharge, the adults showed an extreme debility, being scarcely able to crawl around after an exposure of two days or more.

That death of the larvae was not caused by a poisoning or other change in the food was proved in the following manner: A culture in which after exposure to ionized air all larvae that could be seen had died was allowed to stand filled with normal atmospheric air. Within two days larvae appeared, and seemed active and normal. None of these larvae were colored.

[1] Asperen, *Ann. Botany*, 44: 176, 989; Chouchak, *Rev. Gen. Bot.*, 41: 488, 465, 1929; Belak, Holik and St. Kelemen, *Zeitschr. Hyg. u. Infektionskrankh.*, 111: 5, 703, 1930. See also: Koller, *Jour. Franklin Inst.*, 214: 5, 543, 1932; Romanoff, SCIENCE, 81: 536, 1935.