SPECIAL ARTICLES

THE ABSORPTION AND DISTRIBUTION OF INSULIN LABELLED WITH RADIOACTIVE IODINE[1]

THE measurement of the absorption rate of insulin from the site of depository injections is of practical interest. While it is usually estimated by its indirect effect on the blood sugar level, Beecher and Krogh[2] have studied the absorption of insulin after an injection of protamine insulin into a rabbit's ear, using methylene blue as a tracer. The distribution of insulin in the body has been studied only by determining the insulin content of blood and pancreas by the insufficiently accurate method of extraction followed by biological assay. Since biologically active and crystalline insulin derivatives have been prepared containing heavy atoms, as iodine and arsenic,[3] it became possible to prepare a radioactive insulin derivative containing radioactive iodine. We have attempted to follow the absorption and distribution of insulin after injection, by using insulin-p-azoiodobenzene containing about 2 azo groups per insulin molecule.

EXPERIMENTAL

We were faced with the problem of introducing a sufficient amount of radioactivity with the smallest number of iodobenzene azo groups into insulin so that the resulting derivative be as nearly similar to insulin as possible and yet have sufficient radioactivity for measurement in the small amount of insulin which is tolerated by the experimental animals.

Preparation of Iodoaniline. Morton's[4] micro technique was used in the synthesis of iodoaniline according to the following reactions:

(1) $KIO_3 + 5\,KI + 6\,HCl \rightarrow 3\,I_2 + 3\,H_2O + 6\,KCl$

(2) $I_2 + C_6H_4(NH_2) + NaHCO_3 \rightarrow I\,C_6H_4(NH_2) + NaI + H_2O + CO_2$

Radioactive iodine containing isotopes of 8-day and 12.6-hour periods was prepared by the deuteron bombardment of tellurium.[5] The iodine was extracted from the tellurium target by adding potassium iodide as carrier, dissolving it in nitric acid and then distilling into carbon tetrachloride from which it was extracted with sodium thiosulfate. A solution of the radioactive sodium iodide containing less than 30 micrograms of iodine was evaporated to a volume of about 0.05 cc. The iodine was liberated by acidification with hydrochloric acid and addition of an excess of potassium iodate, both the acid and the iodate being contained in about 0.01 cc. A small excess of aniline was added after first making alkaline with sodium bicarbonate and the mixture was stirred intermittently for a half hour. Since the sodium iodide which is formed in this reaction must contain at least half of the radioactivity, iodine was again liberated by the addition of potassium iodate and acid, using about half the amounts of reagents used before. In one experiment this was repeated a third time, this procedure allowing for the introduction of more than half of the available radioactivity.

Preparation of the Insulin Azo Derivative. The mixture was acidified with hydrochloric acid, cooled to 0° and the iodoaniline diazotized by the addition of sodium nitrite. The reaction was practically complete in half an hour. Five mg of insulin dissolved in the minimum amount of N/10 hydrochloric acid were then added, and the solution brought to a pH of from 8–9 for coupling. The solution was allowed to stand for at least an hour and sometimes over night. The insulin azo dye was then precipitated three times at its isoelectric point in the presence of non-radioactive potassium iodide, iodophenol and p-iodoaniline. From this material a solution was prepared containing 80 units of insulin per cc.

Absorption Rate Determination. Ten rabbits were injected subcutaneously with 2/3 units/kg. One or two of the animals were killed at given intervals and the skin at the site of the injection and corresponding parts of the abdominal wall was excised. Blood samples for sugar determination were taken just previous to killing the animals, and at the same time from all the other animals which had not as yet been operated on. Ten mg of potassium iodide were added to the skin samples as carrier and the skin and added iodine oxidized with a chromic and sulfuric acid mixture.[6] After reduction with oxalic acid the iodine was distilled into carbon tetrachloride. The completeness of the recovery of iodine was checked by titration with sodium thiosulfate. Activity measurements on the titrated aqueous solution were made with a Geiger counter.

The technique of following the rate of absorption could possibly be simplified and made more accurate by carrying out the experiment in intact animals and applying the Geiger counter with appropriate filters directly to the site of injection. This technique proved to require a higher specific radioactivity than was present in the preparations originally made.

[1] This investigation was aided by a grant from the Josiah Macy Jr. Foundation.

[2] H. K. Beecher and A. Krogh, *Nature*, 137: 458, 1936.

[3] E. H. Lang and L. Reiner, SCIENCE, 93: 401, 1941.

[4] A. A. Morton, "Laboratory Technique in Organic Chemistry," McGraw-Hill, New York, 1938.

[5] The authors are indebted to Professor Robley D. Evans, of Massachusetts Institute of Technology, and Professor John R. Dunning, of Columbia University, for their kindness in preparing the radioactive iodine.

[6] Joseph G. Hamilton and Mayo H. Soley, *Am. Jour. Phys.*, 127: 557, 1939.

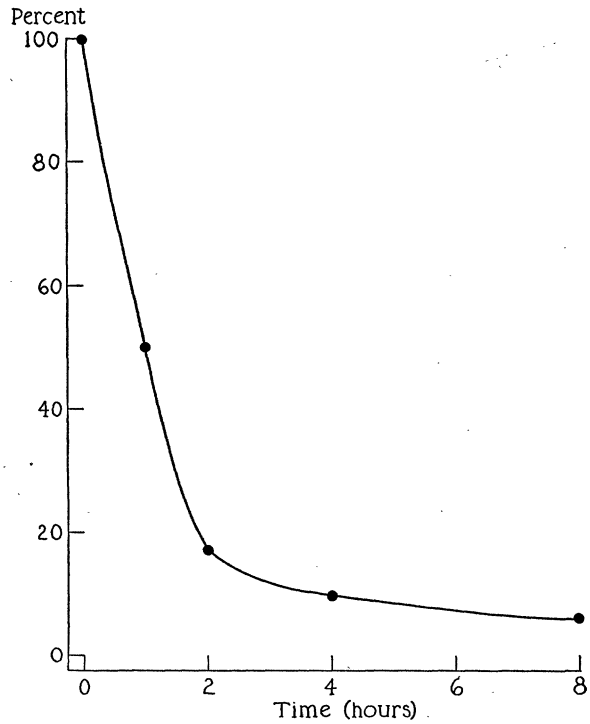

FIG. 1. Per cent. radioactivity remaining at site of injection.

In Fig. 1 the percentage of radioactivity remaining at the site of injection is plotted against time.

In Fig. 2 is shown the relation between the rate of absorption of radioactive insulin (*i.e.*, the percentage of radioactive material absorbed per hour since the last measurement) and the blood sugar at various times following the injection. From this graph it appears that the time of maximum rate of absorption is soon followed by the maximum drop in blood sugar. Also, as the absorption rate drops to very low values, the blood sugar rises to its original level. There is still a small but measurable activity when the blood sugar has risen to its original level. This may be due to the presence of some denatured insulin.

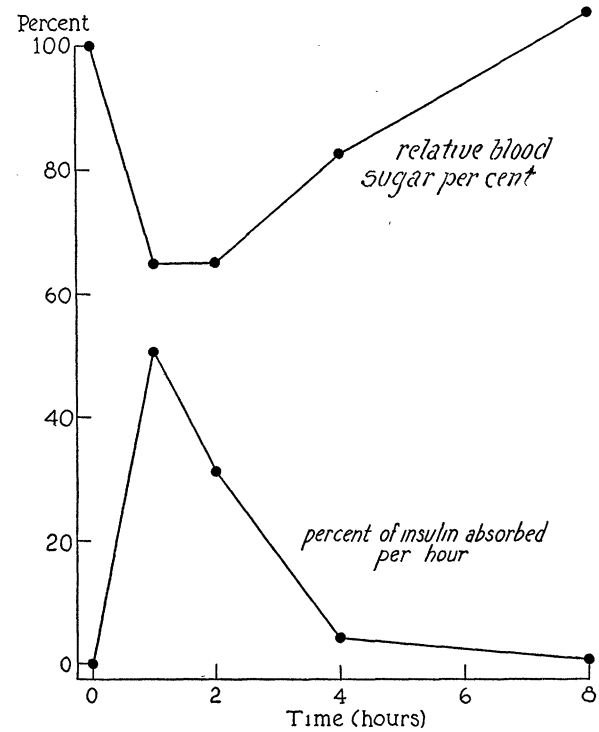

FIG. 2. Comparison of the rate of absorption of radioactive insulin with blood sugar levels.

Preliminary experiments were conducted on the distribution of the radioactive insulin, in rats injected intravenously and intracardially. An hour after injection the circulating blood contained a considerable fraction of the radioactive material. Relatively large quantities of radioactive material were found in the liver and kidneys, suggesting concentration of insulin by these organs.

Since part of the azo groups may be split off from the insulin by reduction in the body, distribution experiments of this type can be of value with regard to the physiology of insulin only if the rate of decomposition of the insulin derivative is also determined. However, as the reduction of azo compounds is relatively slow, it seems probable that in short experiments the distribution of the label would reflect the distribution of insulin in the body.

The technique described above represents a simple means for testing the rate of absorption of insulin depot preparations such as globin and protamine insulin.

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COLCHICINE INDUCED UNIVALENTS IN DIPLOID ANTIRRHINUM MAJUS L.

THE spindle inhibiting effect of colchicine in mitosis and meiosis in both animals and plants is well known. However, the extent to which colchicine may affect the chromosomes themselves is less definitely established. Spiralization seems to be influenced by colchicine and a fusion or stickiness of the chromosomes frequently follows colchicine treatment. A low frequency of induced chromosomal aberrations and an altered mutation rate may also result. A disturbing effect on chromosome pairing and crossing over has also been reported. Walker,[1] Levan[2] and Dermen[3] found uni-

[1] R. I. Walker, *Amer. Jour. Bot.*, 25: 280–285, 1938.
[2] A. Levan, *Hereditas*, 25: 9–26, 1939.
[3] H. Dermen, *Jour. Hered.*, 29: 211–229, 1938.