

## A Study of Incorporation of $^{14}\text{C}$ -Dieldrin into Some Tissues of Mouse

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The mechanism of the development of poisoning due to dieldrin pesticides is poorly understood. Hence we have performed an experimental study of the incorporation of  $^{14}\text{C}$  labeled dieldrin into some tissues of mouse.

A dose of  $0.1 \mu\text{Ci/g}$  of  $^{14}\text{C}$  labeled dieldrin was administered to mice orally, and after a given interval mice were killed and the incorporated  $^{14}\text{C}$  into brain, heart, lung, liver, kidney, spleen and adipose tissue of omentum were determined.

The labeled dieldrin was already incorporated into each tissues after 1.5 hours and then gradually increased. Maximum incorporation of the  $^{14}\text{C}$  was measured after 48 hours. Liver and kidney incorporated more radioactivities per gram tissue than other tissue did. Unexpectedly we had not able to demonstrate that dieldrin pesticides had affinities to adipose tissue, kidney or central nervous system such as brain.

## XII. Metabolic Tracer III

### A new Method of Measuring Serum Cholesterol Using Tritiated Digitonin

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In metabolic disorders the level of serum cholesterol have much significance for the accurate diagnosis of the state and the prognosis of the disease. Up to the present various methods have been devised for measuring serum cholesterol. The data obtained with these methods, however, are not completely reliable because of the poor reproducibility and the instability of the methods.

We have devised and propose a new method for measuring serum cholesterol employing tritiated digitonin on the basis of the principle of Dr. Miller and Mr. Cardinal (Abbott Lab.) in U.S.A.

The details of our method are as follows: 0.5 ml. of serum or plasma was added to 5 ml. of acetone-ethanol (1:1) in a centrifuge tube and mixed thoroughly. The suspension was once brought to the boiling point in a hot water bath and cooled to room temperature. It was centrifuged and the extract was

decanted into a 10 ml. volumetric flask. The precipitate was washed with 5 ml. of acetone-ethanol. The wash solution was also added into the flask. The volume of the combined extract and wash solution was adjusted to 10 ml. with acetone-ethanol. This solution is used as is for free cholesterol assay and is hydrolyzed for total cholesterol assay.

The hydrolysis procedure is as follows: A 0.5 ml. aliquot of the above solution was placed in a graduated centrifuge tube and added 0.1 ml. of 9 N KOH solution. The solution was mixed and heated at  $45^\circ\text{C}$  for 45 minutes with shaking. After the solution was cooled, one drop of phenolphthalein was added and then 10% acetic acid solution was added dropwise to the solution until it became colorless with final addition of one more drop of the acetic acid.

Three ml. portions of tritiated digitonin solution, containing 45.2 mg. of tritiated digi-

tonin (7.1  $\mu$ Ci. per mg.), 772.0 mg. of non labeled digitonin and 500 ml. of 50% ethanol, was added to each tube, the volume was adjusted to 0.8 ml., the solution was mixed and the cholesterol digitonide was allowed to crystallize leaving over night at room temperature.

Two-tenth ml. aliquot of the supernatant was taken into a counting vial and 15 ml. of scintillator solution was added. The scintillator consists of 750 ml. of toluene, 250 ml. of methyl alcohol, 3 gr. of PPO and 100 mg. of POPOP. The treated samples were counted with a Tri-Carb liquid scintillation spectrometer with an efficiency of 15 to 20%.

A standard curve was drawn by plotting amount of cholesterol versus counts per minute in the supernatant aliquot by employing standard cholesterol samples. The values of

cholesterol of the samples were read on the standard curve. The standard curve show linear relationship with a straight line with a bend over the cholesterol level of 520 mg. per dl.

Sixteen serum samples were measured and compared with the Leffler method. The difference was almost under 10%. The reproducibility was examined for 5 serum samples and good results were obtained within a range of standard deviation of 1 to 4% except one with 8.0%. Ester ratio was also measured for 12 serum samples and reasonable results were obtained.

The present method is considered a good device for measuring serum cholesterol with high reproducibility and stability and can be employed in clinical studies which require precise data of serum cholesterol level.

### A Clinical Study with Tritiated Digoxin

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The extreme variation in both effective and toxic dosage of digitalis from one patient to another yields difficult problems in the daily practice of clinical medicine. The recent availability of radioactive digitalis has made it possible to investigate the metabolism of the compound more in details. We investigated the serum levels and excretion of digoxin after intravenous administration of tritium-labeled compound in the human subjects with renal insufficiency and diabetes mellitus.

Tritium-labeled digoxin was prepared by the Wilzbach hydrogen-exchange method and purified by column partition chromatography. Assays revealed specific radioactivities of 112  $\mu$ Ci. per mg.

Subjects studied are as follows: Three patients hospitalized with renal failure, five patients with diabetes mellitus and three control subjects, who had neither metabolic nor renal diseases.

The dose of tritiated digoxin injected was 0.23 mg., diluted in normal saline solution. The solution was administered intravenously. Specimens of venous blood were obtained from the opposite antecubital vein at 10 and

30 minutes; at 1, 2, 4, 6, 12 and 24 hours after injection. Urine was collected every 24 hours for eight to fourteen days. Stools were collected daily for seven to fourteen days.

Blood and urine specimens were treated with PE-611 scintillator, consisting dioxane, anisole, dimethoxyethane, PPO and POPOP. Stools were treated with Schoeniger combustion method. Counting was performed with a Packard Tri-Carb liquid scintillation spectrometer.

The time course of blood level of radioactivity was varying in each group. When compared control group with the other two groups significant decrease in clearance with higher blood level of radioactivity in 24 hours after injection was observed in the latter two groups. Among these the renal failure groups revealed highest level.

The excretion of radioactivity was further varying in each group. In ten days, urinary excretion of radioactivity was 65% in control group, 16% in the group of renal failure and 59% in the group of diabetes mellitus. Fecal excretion in ten days was 14% in control, 39% in renal failure and 15% in diabetes