

## On $^{15}\text{N}$ -Glycine incorporation In-vivo to the Various Kinds of Organs of Mice

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To get any information about the turn-over of protein or amino-acid metabolism under the stressful condition, the  $^{15}\text{N}$  incorporation in-vivo to the various kinds of organs was estimated in mice with burns.

As the control experiment, 1 ml of 10% solution of  $^{15}\text{N}$ -glycine (26 atom % excess) was injected intraperitoneally in DDY-strain male mice (20 g of body weight).  $^{15}\text{N}$  concentration in the protein fraction, extracted from the tissues, was measured with Mass-spectrometer RM1-2 type. The  $^{15}\text{N}$  incorporation in-vivo was estimated in various kinds of tissues such as liver, pancreas, kidney, intestinal mucosa and skeletal muscle during the same course from 8 to 72 hours after the injection. The data, obtained at 8 hours after the injection, showed that  $^{15}\text{N}$  incorporation was the highest in the intestinal mucosa, followed by the orders of pancreas, liver and kidney, and that it was definitely low in skeletal muscles. In the protein fraction of the intestinal mucosa and pancreas, revealing the initially active incorporation of  $^{15}\text{N}$ , the concentration was much more rapidly decreased associated with the time course after the injection compared with the decrease in livers. On the other hand,  $^{15}\text{N}$  incorporation to the protein fraction of skeletal muscles was gradually increased associated with the time course after the injection of  $^{15}\text{N}$ .

Then, the  $^{15}\text{N}$  incorporation in-vivo was estimated in mice with the burn.  $^{15}\text{N}$ -glycine

was given intraperitoneally with the similar fashion as the control group, individually at 1 hour, 6 and 14 days after the burn, and the incorporation in-vivo was measured, also, individually at 8, 24 and 72 hours after the injection in various kinds of organs. In livers,  $^{15}\text{N}$  incorporation to the protein fraction was increased immediately after the burn, and the increase was more markedly found in the group with the injection of  $^{15}\text{N}$ -glycine on the post-burn 6th day. It was returned to the control value 14 days after the burn.  $^{15}\text{N}$  incorporation to the protein fraction of intestinal mucosa or kidney was not increased up to 72 hours after the burn. In the pancreas, the definite increase of  $^{15}\text{N}$  incorporation was revealed both in two groups that  $^{15}\text{N}$ -glycine was given on the post-burn 6th and 14th day. On the other hand,  $^{15}\text{N}$  incorporation to skeletal muscles was decreased after the burn, and returned to the control value in the group with the injection of  $^{15}\text{N}$  on the postburn 14th day. The pattern concerning the changes of  $^{15}\text{N}$  concentration in each organ associated with the time course, was essentially similar as the control even after the burn.

These data could support the results of the authors' previous experiment, revealing that the accelerated turn-over of nucleic acid metabolism, estimated from the  $^{32}\text{P}$  incorporation, was shown not in skeletal muscles, but in livers of mice with the burn.

## Clinical Application of in Vitro Labeling Technique with Tritiated Thymidine and Cytidine

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We have already reported in vitro labeling technique with microautoradiography using tritiated thymidine and cytidine in order to reveal the nucleic acid synthesis of human

malignant tumors.

We have also studied the effect of radiation on those tumors.

Several kinds of tissue culture media have

been compared and now are sure that Eagles' amino acid solution is much better than Y.L.E. solution. The grain counts by Eagles' solution is several times as many as that by

Y.L.E. solution, as for as tritiated thymidine is concerned. So, chemical application is now possible with short exposure time and less amount of isotope.

## Cholesterol Metabolism in the Alloxan Diabetic Rabbits

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Hypercholesterolemia is often associated with diabetes, especially with acute alloxan diabetic animals.

In an attempt to explain these findings, an investigation of the rate of cholesterol turnover in alloxan diabetic rabbits were made.

Control and acute alloxan diabetic rabbits were treated by injection with cholesterol-4- $^{14}\text{C}$  and acetate-1- $^{14}\text{C}$  intravenously and the serum obtained at various time intervals was extracted with chloroform-methanol mixture.

The total lipid so obtained was separated into cholesterol ester and free cholesterol by silica gel column chromatography.

About 50% of injected labeled cholesterol disappeared during 48 hours in the controls whereas only 20%, in the diabetic rabbits. The maximum cholesterol ester specific radioactivity occurred after 16 hours in the controls and after 35 hours in the alloxan diabetic animals.

The peak specific activity for serum free cholesterol after injection of acetate-1- $^{14}\text{C}$  occurred after 6 hours in the controls and after 9 hours in the diabetic rabbit.

Cholesterol ester specific radioactivity increased gradually in the both groups during 24 hours, but no gross differences were observed in serum cholesterol specific radioactivity between the control and the alloxan

diabetic rabbits.

Radioactivity of liver and gut cholesterol of control rabbits 24 hours after injection of acetate-1- $^{14}\text{C}$  was significantly greater than that of the alloxan diabetic ones, whereas kidney and aorta of the both groups had similar cholesterol radioactivity. From these findings, defective cholesterol synthesis by the alloxan diabetic rabbits was found to be present in liver and gut and delayed appearance of radioactive cholesterol ester in the serum of the diabetic animals is attributed to the disturbance of the incorporation of cholesterol into liver.

A few enzyme activities associated with cholesterol metabolism were studied with tritiated 3 $\alpha$ -hydroxy- $\Delta^7$ -cholenate and 3 $\beta$ -hydroxy- $\Delta^5$ -cholenate as substrates.

The enzyme activity of  $\Delta^7$ -steroid reductase which converts 7-dehydrocholesterol into cholesterol is lesser in the alloxan diabetic rabbits than in the controls and the activity was not enhanced with the addition of nicotinamide adenine dinucleotide phosphate plus glucose-6-phosphate. While, the activity of 7 $\alpha$ -hydroxylase, which plays a role in conversion of cholesterol into 7 $\alpha$ -hydroxycholesterol had not much difference in both groups, but in the diabetic animals, its activity was not restored sufficiently with the addition of nicotinamide adenine dinucleotide phosphate plus glucose-6-phosphate.