Incorporation of Labeled Pyrimidine into Cells

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In the process of the biosynthesis of DNA-thymine, there are two different pathways leading to the synthesis of thymidilic acid (TMP),: formation via phosphorylation of exogenous thymidine (TdR), which is salvaged, and formation from deoxytidilic acid (dCMP) via deamination reaction to dUMP and subsequent methylation to TMP. Of these two pathways, it is highly probable that the major route of supply of endogenous TMP is predominatly through the reactions mediated by the dCMP deaminase and synthetase.

In most of the works concerning the effect of the synthesis of DNA, the degree of incorporation of a particular radioactive precursor (mainly labeled thymidine) into the DNA molecule has been used as a measure of the rate of DNA synthesis.

However the rate of the incorporation of this exogenous thymidine into DNA must be affected by changes of the other route of TMP synthesis.

Indeed, it is possible that the decreased supplies of TMP from dUMP may be resulted in the increasing incoporation of the exogenous thymidine into DNA.

For this reason, observations on the incorporation of exogenously administered thymidine or its analogues into DNA were carried out in different conditions.

Radioactive precurosors (TdR-^{14}C, BUdR-^{14}C, ^{131}IUdR) were dissolved in suspensions of yeast cells. Following the administration, a certain aliquot was picked up from time to time and radioactivities of extracted DNA were measured. And DNA of aminopterin (or 5-fluorouracil)-treated cells or of x-irradiated cells were extracted and their activeities were measured. Aminoputerin (or 5-FU) was believed to inhibit methylation of dUMP and to decrease TMP supply.

Increase in incorporation of thymidine-^{14}C (and other radioactive precursors) into DNA was observed both in aminopterin treated-cells and in x-irradiated cells.

These results csuggest that the exogenous TdR incorporation into DNA-thymine must be increased, owing to the decrease in *de novo* synthesis of TMP following aminopterin or x-radiation treatment.

Studies on the Metabolism of Tritiated Folic Acid

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Following an injection of tritiated folic acid (^{3}H-FA), its conversion to reduced active derivatives in the liver of rat and its urinary excretion in man were investigated.

Folic acid derivative were separated, using DEAE-Celeite column and determined by microbiological assay with L. cassi and P. cerevisial and their radioactivities were measured with Packard Tri-Carb Liquid Scintillation Counter.

Distribution of folic acid derivatives in the liver of rat fed a stock diet consists mainly of N^{5}-CH_3 tetrahydrofolic acid (N^{5}-CH_3 THF) and partly of N^{10}-CHO THF, N^{5}-CHO THF and THF. When 1 mg of ^{3}H-FA per kg of body weight was intraperitoneally injected in rat on a folic acid-deficient diet, distribution of microbiologically measured folic acid derivatives was the same as that in normal rat. On the other hand, the radioactivity was highest in $N^{5}CH_3$ THF, moderately high in N^{10}-CHO THF, slightly in N^{5}CHO THF,

THF, and $N^{5,10}$-CH_2 THF. Folic acid did not detected by both determinations. Two peaks which were detected with radiometry were likely to the degradation products of ^{3}H-FA.

When rats were treated with 10 mg of amethopterin, incorposation of radioactivity into these derivatives were extremely small and peaks due to degradation were not detected. But it was observed that small amount of folic acid was remained unchanged in the liver.

An aliquot of human urine collected in a brown bottle containing potassium ascorbate for 3 hours following an intravenous injection of 20 μg of ^{3}H-FA was applied on a column. Most of folic acid derivatives assessed by microbiological assay was folic acid and relatively large amount N^5-CH_3 THF, and small amount N^{10}-CHO THF, whereas the radioactivity was mostly in folic acid and relatively small in N^{10}-CHO THF and extremely small in N^5-CH_3 THF. Peaks of radioactivity due to, probably, degradation products were also observed. Thus, N^5-CH_3 THF apprearing in the urine following intravenously injected folic acid was mainly not derived from the injected folic acid.

Intravenous Pentose Solution Injection in the Surgical Patients

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In the 4th and 6th Anual Meeting of the Japanese Society of Nuclear Medicine, we had reported that, both P-incorporation into the hepatic RNA and S-incorporation into the hepatic RNA and C-incorporation into the hepatic ribosomal protein and serum albumin of mice were markedly increased in response to surgical stress. To evaluate the effects of xylitol injection in the surgical patients, we have studied the changes of these hepatic RNA metabolism following intraperitoneal injection of xylitol, both in the laparotomized mice and 70% hepatectomized rats.

H-incorporation in vivo of hepatic RNA during the periods from 3 to 12 hours after the 31 ml 10% xylitol and 10 μCi H-uracil administration per 20 g body weight, was as similar as the 10% glucose or saline solution injected control mice. However, in the laparotomized mice, the H-specific activity of hepatic RNA after xylitol injection was somewhat more remarkable than the glucose or saline solution injected groups. In the same time, TPN and DPN activity in the xylitol administrated mice liver was more rapidly decreased following surgical stress. These findings about RNA and pyridine nucleotides metabolism suggest that the metabolic flow via uronic acid pathway is stimulated first of all in order to metabolize xylitol, especially under the surgical stress.

In the 70% hepatectomized rats, H-incorporation of DNA in the regenerating liver following 30 μCi H-thymidine injection was also stimulated by xylitol administration in the early peak of cell devision. However, it should be solved by the further investigation why fatty acid deposition early after hepatectomy was most prominent in the xylitol injected rats.