NOTES

METABOLISM OF ^{35}S -CYSTINE IN THE LIVER OF MICE FED ON LOW PROTEIN DIET CONTAINING LUTEOSKYRIN

Our preceding report (Morooka, Nakano and Uchida, 1966) described an increased acid soluble sulfur content of the liver in association with degeneration of the liver in the mice fed on a low protein diet, composed mainly of ground rice, added with luteoskyrin.

To analyse the possible cause of this abnormal increase in sulfur content, experiments were conducted with the incorporation of ^{35}S -cystine in to the mouse liver under different pathological conditions. An enhanced uptake of ^{35}S into the liver was observed in the mice fed on the low protein diet. In the mice fed on the low protein diet added with luteoskyrin, the incorporated radioactivity in the whole liver or in its acid soluble fraction remained constant for a long time and the incorporation into the acid insoluble fraction was delayed.

Materials and Methods:

Male DDD mice, four weeks' old, weighing 15-18 g were used. Five mice were allotted to each diet group. The animals were fed on each test diet for 30 days. Each mouse was housed in one cage. The animals were fed with diet and water *ad libitum*. As for the pathological conditions of the liver, refer to our preceding report (Morooka, Nakano and Uchida, 1966).

Three kinds of diet, diet I (the standard diet), diet II (the low protein diet) and diet II-L (the low protein diet added with luteoskyrin at 2 mg %), were used.

After feeding, the mice injected intraperitoneally with $2\mu\text{C}$ of L- ^{35}S -cystine (the Radiochemical Center Amersham, England: initial specific radioactivity, 36.1 mCi/mM) in a 0.2-ml dose. They were decapitated at 10, 30, 60, 120, 180 or 360 minutes after injection. After decapitation, the liver was removed, washed with cold saline and weighed. A portion from a fixed position of the liver was taken for histopathological examination. The remaining part was cut with scissors and frozen in acetone-dry ice. The frozen liver added with 5 % cold trichloroacetic acid (TCA) solution was ground at 0°C with a Potter-type homogenizer. The homogenized liver was subjected to centrifugation at 3,000 rpm for 10 minutes at 0-5°C. The precipitate was washed with cold 5 % TCA repeatedly. The radioactivities were determined with the whole liver homogenate, the supernatant (the acid soluble fraction) and the precipitate (the acid insoluble fraction) by means of a liquid scintillation spectrometer model SC-201 according to the method of Jeffy, Olubajo and Jewell (1960).

Results:

The time course curves of radioactivity in the total sulfur (TS) and the acid insoluble

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Fig. 1. Specific radioactivity of mouse liver.

(a) Whole liver and (b) acid insoluble fraction. ●—● Diet I;
■—■ Diet II; □—□ Diet II plus luteoskyrin.

Fig. 2. Specific radioactivity of acid soluble fraction of mouse liver.

●—● Diet I; ■—■ Diet II; □—□ Diet II plus luteoskyrin

sulfur (ISS) fractions are shown in Fig. 1; those in the acid soluble sulfur (ASS) fraction are shown in Fig. 2. The variations between the diet group II-L and I or II at each determination point were statistically analysed by the factorial analysis. The differences among the dietary groups were highly significant ($P < 0.01$).

The time course curve of ^{35}S uptake into the mouse liver under normal conditions seems to be similar to that reported by others (Tarver, 1951, Garrow, 1957, Skarzynski, 1959, Takase and Bito, 1963). The uptake of ^{35}S into both the acid soluble and insoluble fractions of the liver was on a higher extent in the mice fed on the low protein diet than those fed on the standard diet.

A piece of information is available on the metabolism of sulfur-containing amino acids in the liver of the animals administrated together with a toxic substance (Achord and Galambos, 1963). As shown in the present paper, the uptake of ^{35}S was greater and the incorporated ^{35}S remained longer in the necrotic liver of the mice fed on the low protein diet containing luteoskyrin than in the liver of the animals fed on diet I or II. The longer retention of ^{35}S in the liver may indicate a decreased rate of metabolism due to the decreased activity of cysteine desulfhydrase (Morooka et al. 1964). It is also likely that luteoskyrin is combined selectively with a sulfhydryl group in the active center of dehydrogenase (see Massy, Hofmann and Polmer 1962) resulting in a decreased rate of metabolic activity.

The different patterns of time course curve of ^{35}S uptake into the liver seem to reflect different intermediate metabolisms caused by the different dietary conditions.

Summary :

We compared incorporation of ^{35}S -cystine into the mouse liver in three different pathological stages, i. e., the normal liver, the liver with carbohydrate-food type degeneration caused by a low protein diet and the liver with initial necrosis caused by the low protein diet containing luteoskyrin. The incorporation of ^{35}S -cystine was on a much higher level and lasted much longer in the liver with initial necrosis than in the other two groups. The ^{35}S incorporated into these livers was found mostly in the acid soluble fraction. This may be an indication of impaired transference of sulfur containing amino acids into the protein.

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