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Effect of allylisothiocyanate on amino acid incorporation in rat liver microsomes*

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THE SEEDS of various plants of the Brassica family have been shown to be goitrogenic when fed to rats.[1, 2] The active component is considered to be a mixture of esters of isothiocyanic acid in the form of glucosides. These esters, commonly called mustard oils, have a high content of allylisothiocyanate (AITC) which is also goitrogenic.[3]

Leblova-Svobodova and Kostir[4] found that AITC treatment of germinating seedlings increased the content of free amino acids and decreased protein nitrogen, suggesting an inhibition of protein synthesis. Studies by Ahmad *et al.*[5] showed that rats fed AITC had reduced activities of some liver and kidney enzymes. In view of these reports, it appeared to be of interest to determine the effect of AITC on amino acid incorporation into rat liver microsomes.

Methods and materials

Adenosine triphosphate (ATP), guanosine triphosphate (GTP), creatine phosphate and creatine phosphokinase were from Sigma Chemical Company. ^{14}C-1-DL-leucine was obtained from Radiochemical Centre, Amersham, London. Sephadex G-25 Fine was a product of Pharmacia, Uppsala, Sweden.

Two groups of young male albino rats, 40 days old (obtained through the courtesy of the Pakistan SEATO Cholera Research Laboratory) were kept on a standard natural stock diet[6] for 7 days. At this time the rats weighed 70–80 g. The rats of one group were then injected intraperitoneally with 2 mg of AITC in 2 ml of distilled water per 100 g of body weight. The rats of the other group were treated as controls and administered only distilled water. Food consumption of the control rats was restricted to that of the AITC-treated rats. This procedure was continued for 7 days. On day 8, experimental and control rats were sacrificed and the incorporation of C-1-DL-Leucine by liver microsomes determined.

The livers were homogenised individually in 10 ml of ice-cold buffer[7] and cell debris, nuclei and mitochondria were precipitated by centrifugation at 19,000 *g* for 10 min. The supernatant solution was centrifuged at 19,000 *g* for 10 min to remove the last trace of mitochondria.

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Microsomes were prepared from the supernatant by centrifuging at 135,000 *g* for 70 min.[8] The supernatant was later used as cell sap after passing through a sephadex G-25 column.[10] The microsome pellets were suspended in 0·25 M sucrose buffer and removed by centrifugation at 135,000 *g* for 70 min. The latter pellets were suspended in a small volume of 0·25 M sucrose buffer and heavy aggregated particles were removed by centrifugation at 850 *g* for 10 min.

Samples of microsomes containing 2 mg protein (estimated by Biuret method) were incubated in test tubes for 30 min at 37°, with cell sap containing 7·5 mg protein. The incubation medium consisted of 2·8 ml of 0·25 M sucrose tris buffer pH 7·6 containing 2 μmoles ATP, 0·5 μmole GTP, 14·8 μmoles creatine phosphate, 20 μg creatine phosphokinase and 1 μc ^{14}C-1-DL-Leucine. The total volume was 3 ml. At the end of incubation the tubes were chilled and treated with an equal volume of 0·6 N $HClO_4$ containing 1 mg of unlabelled DL-Leucine.

After two washings with 0·3 N $HClO_4$ the RNA was hydrolysed by incubation in 0·3 N KOH for 1 hr at 37°. The protein was then recovered quantitatively by acidification of the digest, separated by centrifugation and redissolved in alkali using the same volume of 0·3 N KOH in all tubes.

Results and discussion

From Table 1 it can be seen that AITC treatment reduced the incorporation of leucine to about one-ninth that of the control microsomes. These results are in agreement with the observations of

TABLE 1. INCORPORATION OF ^{14}C-1-DL-LEUCINE INTO RAT-LIVER MICROSOMES

	No. of rats	Counts per 100 sec per mg microsomal protein*	Incorporation (%)
AITC-treated	6	91 ± 6	4
Control	6	869 ± 76	36

* Mean ± S.E.

Leblova-Svobodova and Kostir.[4] Although the mechanism of inhibition is not known, we have shown[5] that AITC reduces liver xanthine oxidase and kidney D-amino acid oxidase. The reduction of amino acid incorporation into microsomal protein may be due to suppression of activity of enzymes associated with microsomal protein synthesis.

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