Table I. The effect of dialysis on the inhibition of proteolytic activity by the 90,000 *g* supernatant fraction of *E. coli*

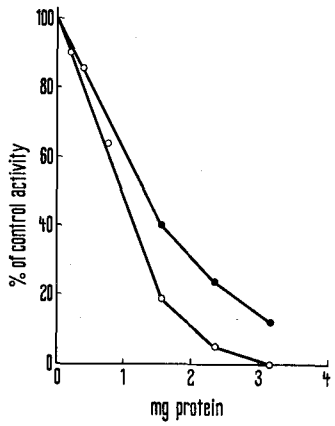
Incubation mixture	meq tyrosine released/ h/μg T or CT, $\times 10^3$	% of control activity
T, alone	0.975	100
T + 90,000 *g* supernatant fraction, undialyzed[a]	0.0	0
T + 90,000 *g* supernatant fraction, dialyzed[a]	0.108	9.6
CT, alone	0.825	100
CT + 90,000 *g* supernatant fraction, undialyzed[a]	0.292	35.5
CT + 90,000 *g* supernatant fraction, dialyzed[a]	0.296	35.1

[a] 2.33 mg protein.

Table II. The effect of heating on the inhibition of proteolytic activity by the 90,000 *g* supernatant fraction of *E. coli*

Incubation mixture	meq tyrosine released/ h/μg T or CT, $\times 10^3$	% of control activity
T, alone	1.07	100
T + 90,000 *g* supernatant fraction, unheated[a]	0.44	41.2
T + 90,000 *g* supernatant fraction, heated[a]	0.53	49.5
CT, alone	1.21	100
CT + 90,000 *g* supernatant fraction, unheated[a]	0.64	52.9
CT + 90,000 *g* supernatant fraction, heated[a]	0.71	58.7

[a] 1.56 mg protein. The supernatant fractions were heated for 10 min at 100 °C.



The inhibition of the tryptic and chymotryptic hydrolysis of casein by the 90,000 *g* supernatant fraction of sonicated *E. coli* cells. ○, T; ●, CT.

resemblance to the heat-stable inter-α-globulin[4] and α_2-globulin[5] observed in blood, which are also non-dialyzable.

The function of an inhibitor of proteolytic activity in an intestinal bacterium is subject to conjecture. It may function to protect the bacterium from degradation by the pancreatic proteases; it may regulate proteolytic activity in the *E. coli* cell; it may also serve as an evolutionary precursor to one or more of the mammalian or plant inhibitors of tryptic activity[14].

Résumé. Nous avons observé l'inhibition de l'activité tryptique et chymotryptique par extraits d'*Escherichia coli*. L'inhibition n'est pas détruit par chauffage et par dialyse.

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Inhibition of Deiodination of Diiodotyrosine in vivo; Relation to Catecholamine Biosynthesis

Various tyrosine analogues are specific inhibitors in vitro of the enzyme tyrosine hydroxylase[1], the enzymatic rate limiting step in catecholamine biosynthesis[2]. When given to animals[3] or man[4], most of these compounds inhibit noradrenaline (NA) production. The ring iodinated analogues of tyrosine, 3-iodo-L-tyrosine (MIT) and 3,5-diiodo-L-tyrosine (DIT) are among the most potent inhibitors of tyrosine hydroxylase in vitro[1]. However, when given to animals[5] or man[6], their inhibitory effect on catecholamine synthesis is weak. This lack of activity in vivo appears to be secondary to a rapid inactivation of these compounds by a specific deiodinating enzyme[5].

The present communication demonstrates that partial inhibition of the deiodination of the injected DIT by menadione (2-methyl-1,4-naphtoquinone) results in a marked fall in NA tissue levels.

Materials and methods. All compounds were administered by the i.p. route. DIT[7] was dissolved in 9‰ NaCl by the addition of 1 *N* HCl and the pH was adjusted to 2 with 0.5 *M* phosphate buffer pH 7. ^{131}I-labelled 3,5-diiodo-L-tyrosine was prepared according to Felber[8]. Fasted male Wistar rats, weighing 140–200 g, were used. Noradrenaline (NA) was essayed in tissues spectrofluorimetrically by the method of Crout et al.[9]

The rats were divided into 4 groups. Animals of group I received no drug. Rats of group II were given 2 injections of menadione (50 mg/kg) at intervals of 16 h. Rats of group III were given 2 injections of menadione (50 mg/kg)

at intervals of 16 h and 200 mg/kg of DIT 1 h after each administration of menadione. Rats of group IV received 2 injections of DIT (200 mg/kg) at intervals of 16 h. A few animals of groups III and IV received ^{131}I-labelled-DIT with the second injection of DIT. Aliquots (0.2 g) of liver from animals which were given ^{131}I-labelled-DIT were dissolved in 0.2 ml 30 *N* KOH and the radioactivity was determined. The liver was chosen for radioactivity determination because this organ is very rich in deiodinating enzyme. In addition, identification of the radioactive compounds from the liver extracts was performed by paper chromatography with the solvent system *n*-butanol – acetic acid – water (12:3:5), and autoradiography of the chromatograms.

Results. (1) Effect of menadione on DIT deiodination. Identification by paper chromatography of the radioactive compounds of the liver from ^{131}I-labelled-DIT treated animals (groups III and IV) gave a single band of radioactivity with the same Rf as DIT. Therefore the concentration of this amino acid could be measured in the liver extracts (Figure 1). 1 h after the second injection of this amino acid, the concentration of DIT in the liver was significantly higher in the menadione-pretreated than in control rats; however, 4 and 8 h after this injection, the difference in DIT levels between the 2 groups of animals was no longer significant.

(2) Effect of DIT on NA concentration in the heart and brain stem. The effect of DIT on NA levels in the heart and brain stem is shown in Figure 2. Our results indicate that the tissue concentration of NA is slightly reduced after 2 injections of DIT (rats of group IV). However, the menadione-treated animals (rats of group III) 4 h after the second injection of DIT had significantly lower NA tissue levels than the rats treated with DIT only (group IV).

Preliminary studies showed that animals of group II, which were given 2 injections of menadione (50 mg/kg) at 16 h intervals without DIT, had normal NA tissue levels.

Discussion. BASTOMSKY and ROSENBERG[10] have shown that a variety of electron acceptors inhibit the deiodination of DIT in vitro, presumably by oxydation of NADPH which is rate-limiting for this reaction. Menadione has been found by these authors to be a very active inhibitor of DIT deiodination in vitro. In the present work, the

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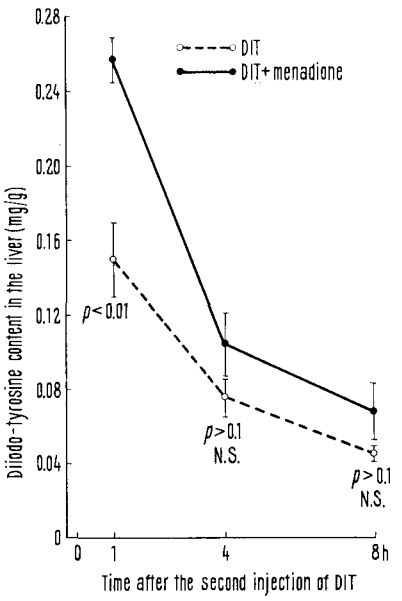

Fig. 1. Effect of menadione on diiodotyrosine (DIT) content in the liver of rats treated with labelled DIT (specific activity: 50,000 to 400,000 cpm/mg). DIT (200 mg/kg) was given twice (at 1 and 17 h). Menadione (50 mg/kg) was administrated twice (at 0 and 16 h). Each value represents the average (± S.E.M.) of 5–7 experiments. N.S., non-significant.

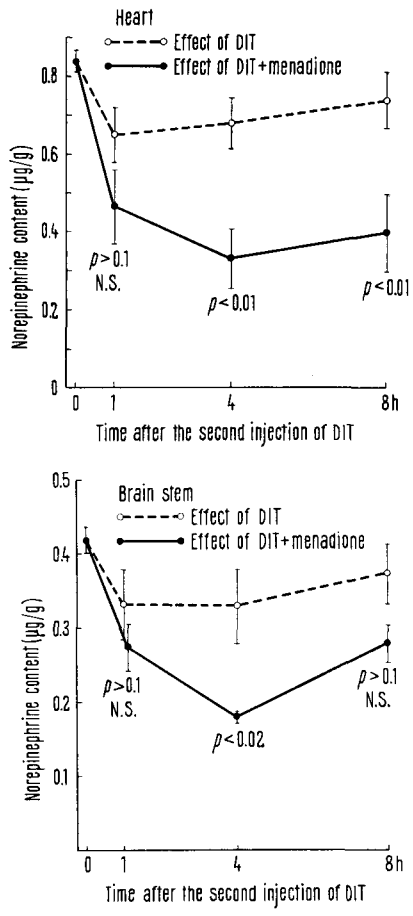

Fig. 2. Effect of diiodotyrosine on tissue levels of noradrenaline in rats pretreated with menadione. Each value represents the average (± S.E.M.) of 5–7 experiments. The protocol of the experiments is described under Figure 1.

menadione-treated rats show a slower initial rate of disappearance of DIT from the liver; this effect of menadione very likely results from inhibition of the deiodinating enzyme; however, direct proof for this mechanism is still lacking and a toxic effect of menadione on the liver cannot be excluded.

Our results show a relationship between the rate of DIT deiodination and the effect of this amino acid on the catecholamine tissue levels. When the rate of disappearance of the injected DIT is decreased by menadione, a marked fall in tissue NA levels occurs; this result suggests that the concentration of DIT is high enough to compete with the normal substrate of tyrosine hydroxylase and to cause enzyme inhibition.

The question arises whether MIT and DIT, the physiological precursors of thyroxine, could play a physiological role in the regulation of catecholamine biosynthesis. This appears to be unlikely because these iodinated amino acids, synthesized in the thyroid gland, are not usually released into the circulation. However, in a few pathological conditions, MIT and DIT can appear in the peripheral blood. A defect in deiodination of MIT and DIT has been reported in a few cases of familial cretinism[11], in which a continued leakage of these amino acids from the thyroid gland occurs. Identification of MIT and DIT in the serum from patients with thyroiditis or thyroid carcinoma has also been described[12]. Studies are now in progress in our laboratory to see whether such patients could show a diminution of their catecholamine production rate[13].

Résumé. La diiodotyrosine est un puissant inhibiteur in vitro de l'enzyme limitant la synthèse des catécholamines, la tyrosine hydroxylase. Or, administrée in vivo à des rats, la diiodotyrosine n'inhibe que très peu la production endogène de catécholamines. Cependant, en ralentissant la rapide désioduration de la diiodotyrosine injectée par le ménadione, on observe une inhibition marquée de la synthèse des catécholamines in vivo.

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The Effect of X-Irradiation on the Citrate Content in Mouse Liver

Changes in the citrate content in liver during different physiological states are of interest for several reasons. It is known that citrate inhibits hepatic phosphofructokinase[1-4], and, as this enzyme may be regulatory for glycolysis, an elevated or decreased citrate content might play an important role in the regulation of this enzyme. On the other hand, citrate is an activator for acetyl-CoA carboxylase, a regulatory enzyme in fatty acid synthesis[5], and, moreover, citrate is considered to be the source of extramitochondrial acetyl-CoA for fatty acid synthesis via the citrate cleavage enzyme reaction[6].

Radiation effects of citrate metabolism in mammals are reported only by DUBOIS et al.[7], who found a decreased citrate content in fluoracetate treated rats after 800 R. Data on the effects of irradiation on citrate metabolism in mice are lacking.

It was therefore considered interesting to measure the content of citrate in mouse liver after whole-body X-irradiation with 690 R (LD 80/30 days).

Materials and methods. Male white mice of the institute were used. Water and standard food (Altromin) were provided ad libitum, 'starved mice' were fasted 24 h before killing, water was given ad libitum.

Mice were irradiated with 690 R (148 R/min), at 150 KV, 20 mA, filtered with 0.43 mm copper, at a target distance of 30 cm. The dose corresponds to a LD 80/30 days. The livers of 8 individual mice were removed by the freeze-stop technique under light ether anaesthesia. Each liver was placed in a mortar and ground after the addition of a fourfold volume of 5% trichloroacetic acid (TCA). After homogenization in a Potter-homogenizer for 2 min, the homogenate was centrifuged at 10,000 *g* and the supernatant kept.

From an aliquot of the supernatant corresponding to 0.8 g liver, the TCA was extracted 3 times with 7 ml ether each time, and the aqueous layer was brought to dryness in vacuo. In the residue the citrate content was estimated by the method of SPENCER and LOWENSTEIN[8].

Each point in the Figure represents the mean value of 8 livers, with standard deviation of the mean.

Results. The citrate content in the liver of normally fed mice was 0.333 ± 0.011 μmoles/g wet liver tissue (48 mice), and in 24 h starved mice was 0.259 μmoles/g wet wt. (48 mice). These amounts of citrate are of the same order as in rats, although the data reported on citrate contents in the livers of fed and starved rats are conflicting[8,9].

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