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SHORT COMMUNICATIONS

Notes on the reduction of biological acetylations with A-methopterine

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In a recent publication[1], some interesting effects produced by A-methopterine on biological acetylation processes were reported. This folic acid analogue inhibited the acetylation of sulfanilamides and isoniazides in pigeon liver extracts, and lowered considerably the acetylated sulfanilamide blood levels of rabbits.

This report describes the effects produced by prolonged treatment with A-methopterine on the concentration in liver and the cellular distribution of the prosthetic group of the acetylating enzyme.

The experiments were performed on 20 young rats of our stock having an average weight of 120–150 g, and fed on a semisynthetic diet of a known composition, which contained 2 mg/kg folic acid (i.e. a quantity far in excess of normal requirements). A water suspension of A-methopterine (4-amino-N_{10}-methyl-pteroilglutamic-acid) was administered orally with the aid of a small esophageal probe in doses of 30 mg/kg.

Treatment was carried on for 7 days; 24 hr after the administration of the last dose, the animals were bled to death. Their livers were aseptically removed, cleansed in physiological solution, weighed and divided into two sections of known weight; the smaller section was employed to determine the concentration of co-enzyme A and total proteins; the larger one was centrifuged in accordance with standard procedure for the separation of nuclei (at 500 r.p.m.), mitochondria (at 3000 r.p.m.) and microsomes (at 20,000 rev/min).

The co-enzyme A contents was determined by Kaplan and Lipmann's method[2], while proteins were assessed by the method employed by Lowry *et al.*[3]. Results are shown in Fig. 1.

Table 1. Distribution of co-enzyme A in the different fractions of liver homogenate taken from animals treated with A-methopterine

Animal group	Homogenate fraction	Co-enzyme A	
		μ/g liver	μ/100 mg/proteins
Controls (8)*	Total homogenate	154·2 ± 6·1	85·6 ± 4·1
	Mitochondria	85·0 ± 4·8	157·6 ± 8·8
	Nuclei	29·7 ± 1·5	92·8 ± 3·2
	Microsomes	5·4 ± 0·2	26·7 ± 1·5
	Supernatant	33·1 ± 1·8	47·2 ± 2·2
Treated (10)*	Total homogenate	62·9 ± 5·5	41·8 ± 3·7
	Mitochondria	19·5 ± 1·2	38·2 ± 3·0
	Nuclei	20·8 ± 1·6	86·5 ± 7·4
	Microsomes	2·9 ± 0·2	24·1 ± 2·3
	Supernatant	19·4 ± 1·4	32·1 ± 2·9

* No. of animals in each group.

It may be noted that co-enzyme concentration fell by 59 per cent when based on weight and by 51 per cent when based on protein concentration; it would therefore seem clear that diminished co-enzyme A activity cannot be considered a result of a possible cellular fibrosis or necrosis. The mitochondrial fraction of co-enzyme A was most affected by A-methopterine treatment, its concentrations falling to values 77 per cent below normal. A lesser reduction was noted in co-enzyme A contents of the nuclei, microsomes and supernatant bodies; in all of these there was a considerable

fall in the protein content suggesting that widespread liver damage caused by A-methopterine. Although the methods employed in these investigations could not determine whether A-methopterine exerts its action by blocking the biosynthesis of co-enzyme A, or by inhibiting this latter's acetylating effect, it would appear likely that, given the doses used, both mechanisms were involved to a substantial degree. This hypothesis would seem to be supported by the fact that, in animals that were treated with either panthotheine (an intermediate product in the biosynthesis of co-enzyme A) or with adenosintriphosphate, the effect of A-methopterine on the acetylating processes was much smaller.[4].

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The effect of disulfiram on the metabolism of normetanephrine-1-^{14}C in the guinea pig

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THE oxidation of acetaldehyde is inhibited, both *in vivo* and *in vitro* by disulfiram*, a drug used in the treatment of alcoholism.[1] The oxidation of β-substituted acetaldehydes, which are intermediary metabolites of the catecholamines and related compounds,[2] may also be inhibited by this substance. This possibility was investigated in the guinea pig using normetanephrine, the O-methyl derivative of norepinephrine.

Normetanephrine-1-^{14}C, with a specific activity of 1·0 μc/μmole, was synthesized from vanillincyanohydrin-1-^{14}C by reduction with $LiAlH_4$.[3] Female guinea pigs weighing between 350 and 450 g were injected with 100 μg of this substrate and their urines collected during a 24 h period over $CHCl_3$. A portion of the combined urines was extracted with 4 vols. of alcohol–acetone (1 : 1) and the extract applied to a strip of Whatman no. 1 filter paper and developed overnight in *n*-butanol–acetic acid–water, (4 : 1 : 1). The dried chromatogram was scanned in a gas flow counter coupled with a recording device. The relations of the separated radioactive metabolites are shown in scan *A*, Fig. 1. The major

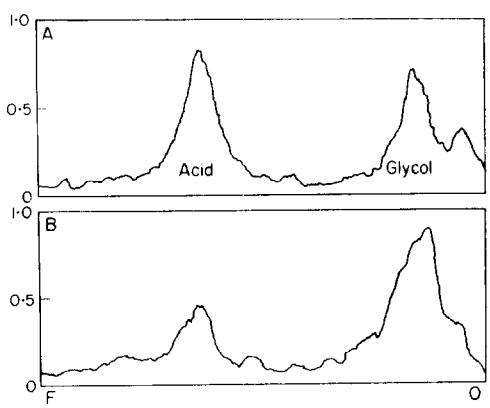

FIG. 1. Distribution and relative amounts of radioactive metabolites on the scanned chromatograms of urines from guinea pigs given normetanephrine-1-^{14}C. Scan *A*: untreated animals. Scan *B*: animals injected intraperitoneally with 200 mg of disulfiram per kg daily for 3 days prior to the administration of normetanephrine-1-^{14}C. Descending chromatography in *n*-butanol–acetic acid–water (4 : 1 : 1). *O* and *F*: origin and front.

* Disulfiram (tetraethylthiuram disulfide) was kindly supplied by Ayerst Laboratories.