which is at least partially preserved in root organ cultures and may be responsible for their high content of nicotine (29 μg/mg dry tissue)[14] compared with our cell cultures. Callus tissue culture offers an experimental technique for investigating this relationship and, by separating the processes of alkaloid formation and accumulation, a means of avoiding a source of difficulty always present in work with whole plants.

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Aminoacetonitrile Action on the Inhibition of the Protein Synthesis produced in the Rat Liver by Dimethylnitrosamine

AMINOACETONITRILE partially protects the liver of the rat from necrosis observed in this organ 24 h after administration of dimethylnitrosamine[1]. In the experiments recorded here the effect of aminoacetonitrile was examined on one of the early lesions produced by dimethylnitrosamine in the rat liver, which consists of the inhibition of the protein synthesis[2]. This lesion is already detectable 3 h after administration of dimethylnitrosamine when the morphological lesions, observable under the light microscope, are missing or are scarcely noticeable[2].

Male albino rats weighing 200–210 g were used. All the rats were injected peritoneally with 0·4 ml. of water in which 40 μc. glycine-1-^{14}C and 200 μg unlabelled glycine were dissolved. A group of rats (controls) received only glycine-1-^{14}C; a second and a third group of animals 3 h before the injection of labelled glycine were injected intraperitoneally with 20 mg dimethylnitrosamine dissolved in 0·5 ml. water; the third group of rats at the same time as the dimethylnitrosamine and in the previous two days received a subcutaneous injection of 20 mg aminoacetonitrile in 0·2 ml. water.

All the rats were kept fasting for 3 h before administering the glycine-1-^{14}C and were killed by decapitation 1 h after its administration. The livers were quickly removed and plunged into ice-cold water; a fragment of each of them of about 2 g was weighed and homogenized in an ice bath with 18 ml. of ice-cold water. The proteins were precipitated from 8 ml. of the homogenate by addition of trichloroacetic acid and then redissolved at 40° in 15 ml. 1 N sodium hydroxide together with 15 mg of unlabelled glycine. After having removed the matter which is insoluble in sodium hydroxide, the proteins were again precipitated with trichloroacetic acid, then isolated according to the method of Rabinovitz, Olson and Greenberg[3].

The proteins were counted with a Geiger–Müller counter under constant geometry conditions and at infinite thickness. The results expressed as counts per minute are given in Table 1.

Table 1. SPECIFIC ACTIVITY OF THE LIVER PROTEINS (COUNTS PER MINUTE)

Control rats	Rats treated with dimethylnitrosamine	Rats treated with dimethylnitrosamine and aminoacetonitrile
454	221	466
386	280	365
440	301	410
473	288	590

These figures clearly show that aminoacetonitrile prevents the inhibition of protein synthesis which occurs in the rat liver even a few hours after dimethylnitrosamine administration; therefore, it can be stated that the protective action of aminoacetonitrile is already operative in this early lesion.

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Possible Significance of the Isoenzymes of Lactic Dehydrogenase in the Retina of the Rat

VARIOUS physico-chemical techniques have been recently used to reveal the heterogeneity of the enzyme lactic acid dehydrogenase. On the basis of investigations of this enzyme in tissues of the chicken, Cahn *et al.*[1] have suggested that, although five fractions are identifiable, three of these represent hybrids of the two major and discrete fractions, the M and H isoenzymes. This designation is derived from the observation that the former predominates in skeletal muscle, and the latter in heart tissue. These authors consider, in fact, that the five fractions arise from a permutation of four sub-units, each type containing a different proportion of the M and H enzymes, and being designated as M, $M3H1$, $M2H2$, $M1H3$ and H.

Futterman and Kinoshita[2], using zone electrophoresis on starch paste, separated five fractions from retinal lactic dehydrogenase, but were uncertain as to the metabolic significance of this finding; they were unable to detect any difference in behaviour pattern between the individual isoenzymes. In this laboratory, the technique of cellulose acetate electrophoresis was applied to the retina of the rat, and the existence of five isoenzymes was confirmed. Although each fraction will react with NADP, as well as with NAD, the activity is less with the former, and the relative activity remains the same throughout all the fractions. There is no reason, therefore, to distinguish their functions in terms of preferential usage of either coenzyme[3].

A striking feature of the experiments in this laboratory, however, was the changing electrophoretic pattern during development; LDH_5, the most positively charged fraction and corresponding to the alleged M enzyme, showed significantly greater activity in the young animal than in the adult, and was substantially reduced in both the

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