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Effect of Allylisopropylacetamide on Liver Metabolism

THE administration of allylisopropylacetamide (AIA) causes porphobilinogenuria in rabbits¹, but it is not known whether this is caused by an increased synthesis of porphobilinogen (PBG) or a decreased utilization. PBG is synthesized in the liver via δ -aminolævulinic acid (ALA) from glycine and acetate or succinate and it seemed possible that a study of the effect of AIA on the metabolism of carbon-14-labelled glycine, acetate and succinate by rabbit liver might throw a light on the problem.

Four male rabbits (2.5–3.0 kgm.) each received 300 mgm. of AIA by stomach tube twice daily for 6 days. The urine from each animal then contained about 14 mgm./100 ml. of porphobilinogen (24 mgm./24 hr.) and 7.5 mgm./100 ml. of ALA (13 mgm./24 hr.). Four rabbits not treated with AIA were controls. The animals were killed, and chopped preparations² of the livers incubated with substrates labelled with carbon-14 as indicated in Table 1. The tissue was incubated for 3 hr. at 37° in air, killed and extracted with ethanol, and the ethanol-aqueous extracts chromatographed in two dimensions using phenol-water as the first solvent and *n*-butanol-propionic acid-water as the second. Radioactive substances on the chromatograms were located by radioautography and counted directly on the paper with an end-window counter³. The carbon-14 contained in each compound was expressed as a percentage of the total carbon-14 in the cell extract.

There was a decreased total incorporation of the isotope into the soluble intermediaries. Nevertheless, with labelled acetate and succinate as substrates considerably more carbon-14 was present in glutamic acid in the AIA-treated animals compared with the controls; this was also true for alanine when labelled succinate was used as substrate. When [2:3-¹⁴C] succinic acid was the substrate, the radioactivity in glutamic acid and alanine was about twice as great in both groups of animals as with [1:4-¹⁴C]

succinate as substrate (Table 1). This is to be expected if these two amino-acids were derived from succinate via the tricarboxylic acid cycle and transamination. No other obvious differences in distribution of carbon-14 were seen between the tissues from AIA-treated and the control animals in the incorporation of carbon-14 into aspartic acid, glucose, proline, γ -aminobutyric acid, valine, lactic, malic and fumaric or citric acids. The lack of increased incorporation from labelled acetate and succinate into aspartate in the AIA-treated animals compared with the controls suggests that there was no increased passage of carbon atoms from the keto-acids into the corresponding amino-acids derived from them by transamination. The preferential incorporation of carbon-14 into the alanine and glutamic acid may indicate that there is an abnormality of the further metabolism of these amino-acids in the AIA-treated animals, but it seems unlikely that this is directly related to the mechanism responsible for the porphobilinogenuria.

It was impossible to interpret the experiments using [2-¹⁴C] glycine because the material decomposed to a variable extent during the purification and chromatographic procedures.

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Inhibition of Emotional Reactions by Phenothiazine Tranquillizers

PHENOTHIAZINE tranquillizers inhibit conditioned responses of various laboratory animals. Since this action can be demonstrated by means of a great variety of methods, it is generally considered to be characteristic of these compounds. Some tranquillizers also diminish 'emotional reactions'¹⁻⁴; but these effects have not been studied so much, mainly due to a lack of simple and adequate methods. Thus, views are still divided whether depression of the emotional reaction is a primary effect or an effect secondary to the sedative action of such drugs^{3,4}. Any new technical procedure which permits the study of the influence of a drug on emotional behaviour independent of its general depressive effect, therefore, appears interesting.

Table 1. TOTAL INCORPORATION AND DISTRIBUTION OF CARBON-14 IN ALANINE AND GLUTAMIC ACID

Group of animals	Control			AIA-treated		
	[2- ¹⁴ C]acetate	[1:4- ¹⁴ C]succinate	[2:3- ¹⁴ C]succinate	[2- ¹⁴ C]acetate	[1:4- ¹⁴ C]succinate	[2:3- ¹⁴ C]succinate
Glutamic acid	11.1 $\pm$ 2.0 (6.7–16.3)	0.19 $\pm$ 0.05 (0.12–0.31)	0.39 $\pm$ 0.10 (0.30–0.67)	20 $\pm$ 3.7 (13–27)	0.73 $\pm$ 0.17 (0.50–1.2)	1.5 $\pm$ 0.30 (1.1–2.4)
Alanine	4.0 $\pm$ 0.75 (1.7–5.1)	2.6 $\pm$ 0.44 (1.4–3.4)	4.2 $\pm$ 0.6 (2.4–5.0)	5.2 $\pm$ 0.67 (0–1.7)	4.6 $\pm$ 0.67 (2.8–5.8)	8.5 $\pm$ 2.0 (4.2–13)
Total carbon-14	95 $\pm$ 48 (35–240)	1,700 $\pm$ 280 (1,100–2,300)	1,600 $\pm$ 250 (900–2,000)	65 $\pm$ 9.2 (40–79)	1,500 $\pm$ 320 (990–2,400)	1,400 $\pm$ 270 (900–2,200)

Samples of rabbit liver (containing about 10 mgm. dry weight of tissue in a vol. of 0.25 ml.), derived from four control animals and from four animals treated with AIA, were incubated in air for 3 hr. at 37° with 5 μ c. of either 1.01 μ moles [2-¹⁴C]acetate, 0.46 μ moles [1:4-¹⁴C]succinate or 0.46 μ moles [2:3-¹⁴C]succinate. The total carbon-14 incorporated into the sum of the soluble metabolic intermediates is expressed as 10⁻³ $\times$ counts/min./25 mgm. dry wt. of tissue. The radioactivity found in glutamic acid and alanine is expressed as a percentage of the total carbon-14 in the sum of the soluble metabolites. All results are given as the mean of 4 observations $\pm$ S.E. (range).