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SHORT COMMUNICATIONS

Preliminary report on the metabolism of γ-butyrolactone and γ-hydroxybutyric acid

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RECENTLY we reported that when γ-butyrolacetone (GBL) is given by the i.v. route to the rat it is rapidly converted to γ-hydroxybutyrate (GHB), which accounts for the subsequent depression of the central nervous system.[1] It was therefore of interest to us to determine whether this conversion was simply the result of nonenzymatic, alkaline hydrolysis in the blood or whether it was catalyzed enzymatically in the body tissues. By means of an already described gas chromatographic method for identification and estimation of both GBL and GHB in rat tissues,[1] rat blood was found to convert GBL to GHB very rapidly, the half-time of conversion was less than 1 min. This activity in blood was localized in rat plasma, hemolyzed erythrocytes being inactive. The probability that we are dealing with a plasma enzyme came from three lines of evidence: (1) it could be completely inactivated by heating at 70° for 15 min. (2) it could also be inactivated by incubation with edathamil (EDTA, 10^{-3} M); and (3) there is no hydrolysis in isotonic saline–phosphate buffer at pH 7·4, even in 30 min (cf. Table 1).

TABLE 1. HYDROLYSIS OF γ-BUTYROLACTONE TO γ-HYDROXYBUTYRIC ACID IN VARIOUS MEDIA*

Medium	Per cent conversion of γ-butyrolactone ($1·3 \times 10^{-2}$ M) to γ-hydroxybutyric acid after an incubation time of 15 min	30 min	60 min
Rat plasma (1:10 dilution)	92	100	100
Hemolyzed erythrocytes	0	3	—†
Rat liver homogenate‡	87	94	—
Rat brain homogenate‡	—	1	—
Plasma + EDTA (10^{-3} M)	0	—	2
Plasma + EDTA (10^{-4} M)	62	—	80
Plasma heated to 70° (15 min)	—	0	—
Isotonic saline–phosphate buffer (pH 7·4)	0	0	0

* Incubated at 37° for varying periods of time. The diluting medium was isotonic saline–phosphate buffer (0·05 M at pH 7·4).

† Denotes no data available for this point.

‡ One gram tissue in 10 ml saline–phosphate buffer.

The fact that diisopropylfluorophosphate, prostigmine, and physostigmine did not block this hydrolysis in high concentrations (10^{-4} to 10^{-2} M) suggested that this enzyme is more specifically a lactonase than an esterase. This was further borne out by the ability of rat plasma to hydrolyze γ-valerolactone, but at a slower rate than GBL.

Other tissues of the rat such as brain, liver, kidney, heart, lung, skeletal muscle, intestine, and cerebrospinal fluid were examined for the presence of the lactonase. Of these, only liver (blood removed by perfusion) was found to have any substantial activity.

Preliminary radio-respirometric studies with ^{14}C-carboxyl-labeled GHB (sodium salt) have indicated that this compound is metabolized very rapidly in the rat. After the i.v. administration of radioisotopic GHB, respiratory $^{14}CO_2$ was detected within about 4 min, and a peak reached in 15 min. About 60% of the injected amount was recovered within 2·5 hr. In view of the report by Fishbein and Bessman[2] that GHB may enter the Krebs cycle, we sought to isolate ^{14}C-labeled succinic acid after

incubating rat brain and liver homogenates and blood with GHB-^{14}C (sodium salt) in the presence of malonic acid (2×10^{-2} M), added to block the Krebs cycle at the succinate level. Succinic acid was isolated from possibly interfering substances by passing an 80% ethanolic extract of the tissue over a Dowex-2-formate column, and eluting with 6 N formic acid. The eluate was then passed over a Dowex-50 column to remove interfering cations. Gas chromatography was used to separate and estimate succinic acid after methylation with diazomethane. By means of an effluent splitter, about 95% of the succinate peak was trapped in a vial containing an ethanolic PPO–POPOP* scintilllation-counting mixture and counted in a Packard Tri-Carb liquid scintillation spectrophotometer. One to two per cent of the ^{14}C-isotope of GHB was found in the succinic acid from brain and up to 6% in liver; no isotope could be detected in the blood succinate. In view of the negative findings of Walkenstein *et al.*[3] with regard to labelling of succinate by GHB-^{14}C *in vivo*, we feel that the small percentage of isotope found in brain succinate in our experiments was the result of random labeling of succinate by CO_2 fixation, which could have been overlooked by a less sensitive method.

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* Supplied by Pilot Chemicals Inc., Watertown, Mass., under the trade name "Liquifluor".

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A note on the relative toxic activities of tetrachloromethane and trichloro-fluoro-methane on the rat

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THE administration of carbon tetrachloride to rats (and to many other species) produces central necrosis and fatty degeneration in the liver (see Cameron and Karunaratne,[1] and Van Oettingen,[2] for early references). Carbon tetrachloride administration also results in many early disturbances in metabolic processes: for example, in protein synthesis (Smuckler, Iseri and Benditt,[3]); in nucleotide levels (Thielmann, Schulze, Kramer and Frunder,[4]; Slater, Sawyer and Sträuli,[5]); in serum enzyme levels (Rees and Sinha,[6]; Rees, Sinha and Spector, [7]); and in detoxication mechanisms (Neubert and Maibauer,[8]; Rees, private communication).

The metabolic disturbances which result in the accumulation of fat and in the histological appearanc of necrosis appear to proceed along largely independent pathways of development (See for example, Rees,[9]). Necrosis can be virtually prevented by various treatments (for example, adrenalectomy: Recknagel, Stadler, and Litteria,[10]; administration of certain drugs such as the phenothiazines: Rees, Sinha and Spector,[7]), whereas fat accumulation is little affected by such procedures. Since these 'protective treatments' also suppress several of the metabolic alterations usually observed after dosing with carbon tetrachloride (for example, nucleotide changes, Slater, Sawyer and Sträuli[5]; serum enzyme changes, Rees, Sinha and Spector[7]), it would seem not unreasonable to suppose that such changes have a role, however indirect, in the developing lesions which culminate in necrosis.