material ranged in these samples from 20 to 194 mg, and when incubated with the enzyme, the range was 34 to 275 mg. This indicates that the percentage of total drug excreted apparently conjugated as the glucuronide remains essentially the same over a wide range of drug. When thioridazine is the administered drug, however, no glucuronide formation could be inferred because no additional chromogen was obtained after glucuronidase incubation. That thioridazine in the human is not

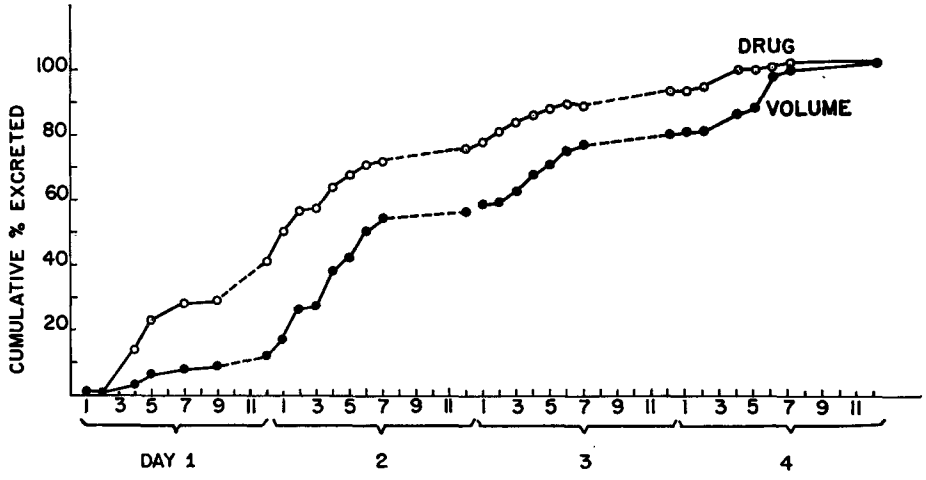

FIG. 3. Cumulative per cent plot—per cent total excretion and per cent total volume.

conjugated to any appreciable extent is supported by our observation that when ^{35}S-ring-labeled thioridazine is administered, less than 3 per cent of the metabolites in the urine are not extractable by chloroform.[6] This is remarkably different from the results obtained with chlorpromazine administration, where over 50 per cent of the urinary metabolites are not extractable with the nonpolar solvent.

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REFERENCES

1. E. E. HAYNES, *J. Lab. clin. Med.* **56**, 570 (1960).
2. C. L. HUANG and A. A. KURLAND, *Amer. J. Psychiat.* **118**, 428 (1961).
3. L. E. HOLLISTER, *Curr. ther. Res.* **4**, 471 (1962).
4. T. H. LIN, L. W. REYNOLDS, I. M. RONDISH and E. J. VAN LOON, *Proc. Soc. expt. Biol. (N.Y.)* **102**, 602 (1959).
5. S. EIDUSON and R. D. WALLACE, *Trans,, Second Research Conf. on Chemotherapy in Psychiatry, Veterans Administration*, **2**, 88 (1958).
6. S. EIDUSON and E. GELLER, *Biochem. Pharmacol.* **12**, 1429 (1963).

A sensitive and specific assay for the estimation of monoamine oxidase

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MONOAMINE OXIDASE (MAO) activity in tissue is usually measured by manometric[1] or fluorimetric[2] techniques. The former are laborious and require fairly large amounts of tissue; the latter, though more sensitive, still require milligram quantities of tissue and are subject to error because of potential

variation in endogenous levels of fluorescent material. We have developed a simple, sensitive, and specific assay for MAO, utilizing the measurement of deaminated ^{14}C-metabolites of ^{14}C-tryptamine. Sixty or more assays, each requiring as little as 5μg of tissue, can be performed in 3 hr.

Tissues are homogenized in chilled isotonic KCl, and 1–100 μl (10 μg– 1 mg) are used for assays. ^{14}C-Tryptamine (tryptamine-2-^{14}C-hydrochloride, New England Nuclear Co., 1·3 mc/mmole) is dissolved in water and stored at −4°. In a typical assay, 25 μl enzyme preparation, 25 μl (6·25 mμmoles, 10,000 cpm) of ^{14}C-tryptamine, and 250 μl 0·5 M phosphate buffer, pH 7·4, are mixed in a 15-ml glass-stoppered centrifuge tube and incubated at 37° for 20 min. The reaction is stopped by the addition of 0·2 ml 2 N HCl, and the deaminated radioactive material is extracted into 6 ml toluene by shaking. After centrifugation, a 4-ml aliquot of the organic layer is transferred to a vial containing 10 ml phosphor [0·4 per cent 2,5-diphenyloxazole and 0·005 per cent 1,4-di(2,5-phenyloxazole) benzene in toluene], and counted for 1–5 min in a liquid scintillation spectrophotometer. A small amount of ^{14}C-tryptamine (less than 0·3 per cent) is extracted by this procedure. A correction is made for this blank value (about 30–50 cpm) by incubating ^{14}C-tryptamine with boiled enzyme.

The reaction was linear with time for at least 20 min and with enzyme concentration over a range of 5–1000 μg liver (Table 1). Duplicate determinations of the MAO activity of 250 μg of many liver specimens differed by less than 2 per cent.

TABLE 1. ENZYMATIC FORMATION OF ^{14}C-INDOLEACETIC ACID FROM ^{14}C-TRYPTAMINE*

Liver aliquot, (μg)	cpm	mμmoles ^{14}-CIAA/mg liver
5	15 ± 4	3·80
10	29 ± 4	3·60
30	80 ± 5	3·33
100	266 ± 9	3·38
250	742 ± 15	3·76
1,000	2,805 ± 195	3·57
3,000	4,170 ± 110	1·81
10,000	4,215 ± 40	0·54

* Enzyme preparation (25μl) was incubated with 6·25 mμmoles ^{14}C-tryptamine for 20 min at 37°. Data were corrected for blank values (32 cpm), obtained by incubating ^{14}C-tryptamine with boiled enzyme. cpm are expressed as mean ± average deviation of duplicate samples.

The radioactive metabolite of ^{14}C-tryptamine produced by incubation with rat heart or liver was identified as ^{14}C-indoleacetic acid (IAA) by ascending paper chromatography, in confirmation of the findings of Lovenberg, *et al.*,[2] who used nonradioactive material. Indoleacetic acid was separated from tryptamine in a butanol:acetic acid:water (4:1:1) system, and from indoleacetalydehyde in an isopropyl alcohol:ammonia:water (8:1:1) system.

The assay was sufficiently sensitive to measure MAO activity in scrapings of human buccal mucosa. In this tissue, paper chromatography demonstrated that both acid and aldehyde were generated, indicating that in some subjects this tissue lacked an excess of aldehyde dehydrogenase. When such assay mixtures were incubated with a preparation of aldehyde dehydrogenase from guinea pig kidney[2] and appropriate cofactors, the chromatographic yield of indoleacetic acid was increased but the number of counts extracted into toluene was unchanged, indicating that indoleacetalydehyde has solubility characteristics similar to indoleacetic acid in an acid:toluene system. It is concluded that this assay can be used whether or not aldehyde dehydrogenase is present.

The enzyme specificity of this assay was investigated by determining the effect on hepatic MAO activity of pretreatment *in vivo* or preincubation *in vitro* with tranylcypromine (SKF 385). This compound is a potent inhibitor of MAO at doses that have no effect on diamine oxidase.[3] Tranylcypromine both *in vivo* and *in vitro* produced a 97 to 99 per cent decrease in enzyme activity.

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REFERENCES

1. N. H. CREASY, *Biochem. J.* **64**, 178 (1956).
2. W. LOVENBERG, R. J. LEVINE and A. SJOERDSMA, *J. Pharmacol. exp. Ther.* **135**, 7 (1962).
3. P. A. SHORE and V. H. COHN, JR., *Biochem. Pharmacol.* **5**, 91 (1960).

The reaction of β-propiolactone with guanosine, deoxyguanylic acid and RNA

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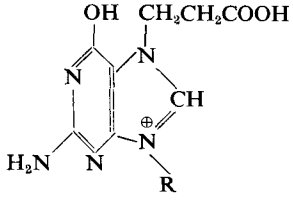
β-PROPIOLACTONE is a compound of considerable interest since it was shown to be mutagenic by Smith and Srb[1] in *Neurospora*, and was later shown to be a complete carcinogen for mouse skin by Roe and Glendenning[2] and Searle,[3] and was also found by Dickens and Jones[4] to produce sarcomas at the site of injection in rats. The latter authors[4] showed also that the compound reacts readily with cysteine at physiological pH yielding S-(2-carboxyethyl) cysteine.

Roberts and Warwick[5] reported briefly on the interaction of β-propiolactone with DNA, deoxyguanylic acid and guanosine *in vitro* and indicated that the products were derived from reaction at the N-7 nitrogen of the guanine moiety. The lactone is a highly strained molecule which, under the appropriate conditions, reacts readily by ring opening. This can occur in two ways yielding, in the case of amines, both amides and amino acids as shown by Gresham *et al.*[6] The relative yields of the products from the two competing reactions

have been found to vary with the amine, the solvent and the order of addition. In the case of the tertiary amines studied by Gresham *et al.* however, formation of the amino acid occurred exclusively. In view of the findings of Lawley[7] and of Brookes and Lawley[8] that the 7-nitrogen atom of the guanine moiety in nucleic acids, nucleosides or nucleotides is highly reactive towards alkylating agents of the haloalkyl type, it seemed likely that this position would also be prone to attack by β-propiolactone. Further, since this atom is a tertiary nitrogen atom one would predict on the basis of Gresham's work that the most likely product formed from its interaction with β-propiolactone would be the acid I and not the amide II.



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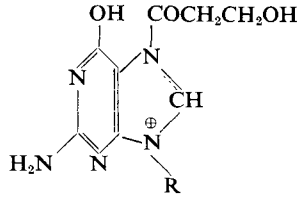

II

Analytical and spectral data has indicated that reaction at the N-7 position does occur in the case of guanosine and deoxyguanylic acid. The guanine in RNA reacts in the same way and the hydrolysis product is similar in all respects to 7-(2-carboxyethyl)guanine prepared unambiguously by the reaction of β-iodopropionic acid with deoxyguanylic acid.

MATERIALS AND METHODS

β-Propiolactone was obtained from L. Light & Co., and purified by redistillation. β-Iodopropionic acid was obtained from the British Drug Houses Ltd. Absorption spectra were measured with a Unicam S.P. 500 spectrophotometer. Paper chromatography was carried out on Whatman No. 1 filter paper using the following solvents by upward flow chromatography.

(1) Methanol–concentrated hydrochloric acid–water (7:2:1);
(2) Butan-1-ol–aqueous ammonia (d. 0·88)–water (86:2:12);
(3) Ethanol–water–aqueous ammonia (d. 0·88) (80:18:1).