

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

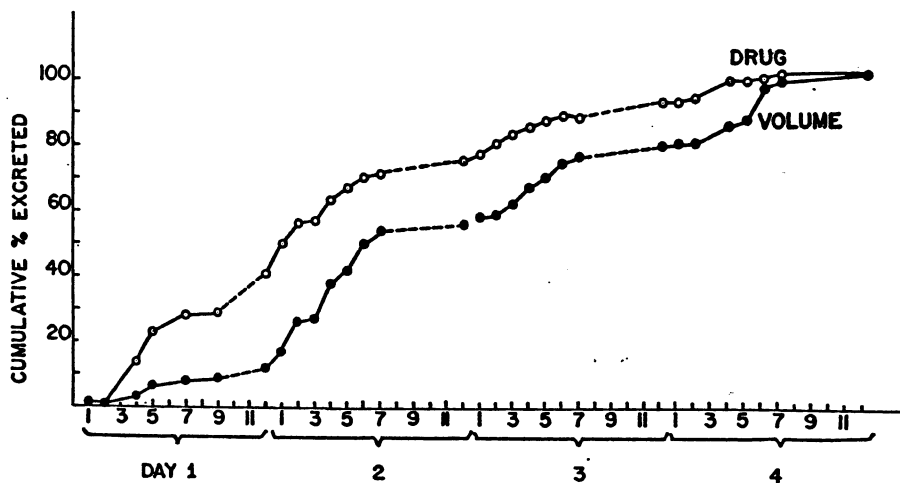


FIG. 3. Cumulative per cent plot—per cent total excretion and per cent total volume.

conjugated to any appreciable extent is supported by our observation that when  $^{35}\text{S}$ -ring-labeled thioridazine is administered, less than 3 per cent of the metabolites in the urine are not extractable by chloroform.<sup>6</sup> 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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#15

#### 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<sup>1</sup> or fluorimetric<sup>2</sup> 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  $^{14}\text{C}$ -metabolites of  $^{14}\text{C}$ -tryptamine. Sixty or more assays, each requiring as little as  $5\mu\text{g}$  of tissue, can be performed in 3 hr.

Tissues are homogenized in chilled isotonic KCl, and 1–100  $\mu\text{l}$  (10  $\mu\text{g}$ –1 mg) are used for assays.  $^{14}\text{C}$ -Tryptamine (tryptamine-2- $^{14}\text{C}$ -hydrochloride, New England Nuclear Co., 1.3 mc/mmmole) is dissolved in water and stored at  $-4^\circ$ . In a typical assay, 25  $\mu\text{l}$  enzyme preparation, 25  $\mu\text{l}$  (6.25  $\mu\text{moles}$ , 10,000 cpm) of  $^{14}\text{C}$ -tryptamine, and 250  $\mu\text{l}$  0.5 M phosphate buffer, pH 7.4, are mixed in a 15-ml glass-stoppered centrifuge tube and incubated at  $37^\circ$  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  $^{14}\text{C}$ -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  $^{14}\text{C}$ -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  $\mu\text{g}$  liver (Table 1). Duplicate determinations of the MAO activity of 250  $\mu\text{g}$  of many liver specimens differed by less than 2 per cent.

TABLE 1. ENZYMIC FORMATION OF  $^{14}\text{C}$ -INDOLEACETIC ACID FROM  $^{14}\text{C}$ -TRYPTAMINE\*

Liver aliquot, ( $\mu\text{g}$ )	cpm	$\mu\text{moles } ^{14}\text{-CIAA/mg liver}$
5	15 $\pm$ 4	3.80
10	29 $\pm$ 4	3.60
30	80 $\pm$ 5	3.33
100	266 $\pm$ 9	3.38
250	742 $\pm$ 15	3.76
1,000	2,805 $\pm$ 195	3.57
3,000	4,170 $\pm$ 110	1.81
10,000	4,215 $\pm$ 40	0.54

\* Enzyme preparation (25 $\mu\text{l}$ ) was incubated with 6.25  $\mu\text{moles } ^{14}\text{C}$ -tryptamine for 20 min at  $37^\circ$ . Data were corrected for blank values (32 cpm), obtained by incubating  $^{14}\text{C}$ -tryptamine with boiled enzyme. cpm are expressed as mean  $\pm$  average deviation of duplicate samples.

The radioactive metabolite of  $^{14}\text{C}$ -tryptamine produced by incubation with rat heart or liver was identified as  $^{14}\text{C}$ -indoleacetic acid (IAA) by ascending paper chromatography, in confirmation of the findings of Lovenberg, *et al.*,<sup>2</sup> who used nonradioactive material. Indoleacetic acid was separated from tryptamine in a butanol:acetic acid:water (4:1:1) system, and from indoleacetaldehyde 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<sup>3</sup> and appropriate cofactors, the chromatographic yield of indoleacetic acid was increased but the number of counts extracted into toluene was unchanged, indicating that indoleacetaldehyde 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 tranlycypromine (SKF 385). This compound is a potent inhibitor of MAO at doses that have no effect on diamine oxidase.<sup>3</sup> Tranlycypromine both *in vivo* and *in vitro* produced a 97 to 99 per cent decrease in enzyme activity.

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