(12), showed that at no time did Cs^{137} retention of animals maintained on 10- and 100-fold cesium intake levels differ significantly from the Cs^{137} retention of the control animals. However, significant differences ($P = <0.01$) between retention of animals on the 1000-fold cesium intake level and all other groups were found on day 23 and every day thereafter. Periodic weighings showed that 10-fold and 100-fold cesium intake levels had no deleterious effects on the growth pattern of the animals, but after 50 days there was no increase in body weights of animals on the 1000-fold cesium intake level (Fig. 2).

Computer analysis of the biological retention data for the control group gave the following retention equation:

$$R_t = 12.81\, e^{-0.90541\, t} + 39.21\, e^{-0.06734\, t} + 47.97\, e^{-0.048741\, t}$$

where R_t is the average biological retention for cesium on any day t; the coefficient and exponent of each term are the intercept and rate constant, respectively; and e is the base of natural logarithms. Integration of this function from 0 to ∞ times gives the total area which, in turn, is proportional to the equilibrium level attained during continuous ingestion to a constant dietary level of stable cesium. For example, the equilibrium level for stable cesium in the control animals would be about 14 times the daily cesium intake. Similar calculations show that the equilibrium concentration of cesium in the highest cesium intake group would be approximately half the LD_{50} toxicity value(13) for a single intraperitoneal injection of cesum chloride. Presumably, the increase in retention and perhaps the disruption of growth pattern are responses to cesium toxicity. These observations suggest the possible application of whole-body counting technics to studies of metabolic responses to materials in sublethal toxicity ranges.

Summary. Increased dietary cesium does not enhance excretion of parenterally administered Cs^{137} by rats and, because of apparent toxic action, may decrease normal Cs^{137} excretion at dietary concentrations exceeding $6.5 \times 10^{-3}\%$.

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Identification of a New Metabolite of Imipramine.* (27462)

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Recent studies indicate that imipramine (Tofranil), which is employed for the relief of anxiety neurosis, undergoes substantial metabolic changes in the body. According to Herrmann *et al.*(1,2), imipramine is subject to oxidative hydroxylation of the aromatic nucleus

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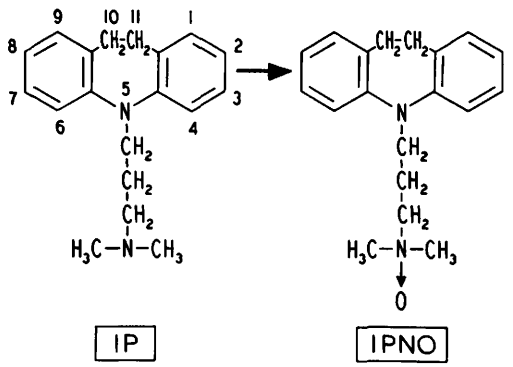

FIG. 1. Structures of imipramine (IP) and imipramine-N-oxide (IPNO).

as well as to side chain demethylation. Somewhat similar forms of transformation have also been demonstrated for chlorpromazine(3,4,5). By means of chromatographic technics the Swiss workers(1,2) were able to identify the following imipramine metabolites: (a) nor_1imipramine, (b) nor_2imipramine, (c) 2-hydroxyimipramine, (d) 2-hydroxy-nor_1imipramine and the glucuronic acid conjugates of (c) and (d).†

Preliminary studies on the metabolism of imipramine have led us to identification of a new metabolite, *viz.*, imipramine-N-oxide (IPNO, Fig. 1), whose structure reflects side chain oxidation similar to that observed for chlorpromazine(6). The evidence for this compound as well as its urinary excretion values are reported herein.

Experimental. Urine specimens. Urine was supplied by Hillside Hospital patients who were receiving 150-300 mg of Tofranil daily. Rigorous precautions were taken to exclude all other forms of medication. All urine specimens were routinely checked for proper drug intake(7), and creatinine determinations were carried out to verify the 24-hour collections. Control urines were provided by hospital personnel who were not receiving any medication.

Extraction and chromatography. The unconjugated metabolite fraction containing IPNO was extracted with 3 volumes of ethylene dichloride (Eastman) from urine at pH 10.7. Moderate alkalinity was used in order to minimize decomposition of the imipramine derivatives. The ethylene dichloride extract was washed with 0.1 N NaOH, dried over anhydrous sodium sulfate and evaporated *in vacuo*. The residue was dissolved in methanol and suitable volumes were spotted on Whatman #1 or #3 chromatography paper for subsequent development. The following chromatographic systems were used: benzene-acetic acid-water (2:2:1), descending, 21-22°C(4); ethylene dichloride-benzene-formic acid (88%)-water (3:1:4:2), descending (8)‡; and ammonia (58%)-methanol (1:1 and 4:1), ascending. For the first 2 systems, untreated chromatography paper (#1) was used. For the AmMe systems, Whatman #3 paper was employed that had been treated with acetone containing 10% peanut oil. The chromatograms were routinely dipped in dilute Dragendorff reagent ("spraying reagent", ref. 9) or diazo stain. The diazo stain was prepared as follows: 0.4 g of Fast Red Salt GG (General Dyestuff Co.) was mixed with 4.0 ml concd. HCl and diluted to 100 ml with water. One volume of this solution was added to 2 volumes of acetone just before staining. The chromatograms were dipped and then dried with an air blower. The nitroprusside-acetaldehyde reagent for secondary amines (10) and ninhydrin-pyridine reagent(11) were employed as auxiliary stains. When individual spots were to be rechromatographed, they were either eluted with methanol or woven on to fresh chromatography sheets. For the quantitative studies, urine extracts were chromatographed in the BzA system along with a series of IPNO standards of graded concentration which had been extracted from control urine under similar conditions. The chromatograms were stained with Dragendorff reagent and the IPNO spots quantitated by visual comparison with the graded standards.

Electrophoresis. The samples were streaked at the anode end of Whatman #1 chromatography sheets and the electrophoresis con-

† The following abbreviations are used: IP = imipramine, IPNO = imipramine-N-oxide, nor_1 = desmonomethyl, and nor_2 = desdimethyl. Abbreviations for the chromatographic solvent systems are given in the footnote (*) to Table II.

‡ Separation of the organic and aqueous phases was facilitated by working in a warm room and directing a hot air blower at the separatory funnel containing the solvent mixture.

TABLE I. Identification of the Unconjugated Imipramine Metabolites.

Substance	R_f(BzA)	R_f(AmMe 1:1)	R_f(AmMe 4:1)	Diazo stain	Nitroprusside test	Standards with similar R_f's and color reactions
From urine						
1a	.17	.75	.67	rose	—	2-HO-IP
1b	.17	.80	.75	"	+	2-HO-Nor_1IP
2	.57	.81	.79	blue	—	IPNO
3a	.70	.16	.0	"	—	IP
3b	.70	.40	.05	"	+	Nor_1IP
Misc. st'ds						
Nor_2IP	.64	.55	.15	"	—	
β-HO-IP	.49	.57	.17	"	—	

ducted between glass plates for 3.5 hours at 20 milliamps using a 1% $KHCO_3$ buffer medium.

Synthesis of IPNO. Imipramine was liberated from the hydrochloride salt (2.83 g) with dilute sodium hydroxide and then extracted into ethylene dichloride. The organic extract was washed with dilute alkali, dried over anhydrous sodium sulfate and evaporated *in vacuo*. The syrupy residue was treated with a mixture of 1.35 ml of 30% hydrogen peroxide and 22 ml of ethanol. The clear solution was gently refluxed for 4 hours. At the end of that time it was evaporated *in vacuo* and the system was flushed out 4 times with ethanol. The residue was dried in a desiccator over P_2O_5 and then recrystallized from benzene. White needles were obtained which melted with decomposition at 120-123°C; yield, 54% of theoretical. Anal. Calcd. for $C_{19}H_{24}N_2O$ (1.5 H_2O): C, 70.56; H, 8.42; N, 8.66. Found: C, 70.64; H, 8.22; N, 8.33.

Results. Isolation and identification of IPNO. The unconjugated imipramine metabolite fraction obtained from alkalinized urine was chromatographed using BzA. Three spots were noted (Table I.) The first yielded a rose color with the diazo reagent, which is indicative of a phenol. It was resolved into 2 components (#1a and #1b) using AmMe 4:1, or by prolonged chromatography with EdBzF. These were identified as 2-hydroxy-imipramine and 2-hydroxy-nor_1imipramine by comparison with the reference standards. The second spot did not correlate with any established metabolite. The third spot was readily resolved by AmMe 1:1 into IP (#3a) and Nor_1IP (#3b). Aside from spot #2, the indicated assignments for the 4 identified compounds are in agreement with the earlier studies of Herrmann(1,2).

Our attention was now focused on the second spot. This substance appeared to be homogeneous by available criteria, resisting all attempts at further resolution. It yielded negative primary and secondary amine tests, and was found to occupy the same position relative to imipramine on the BzA and AmMe chromatograms that chlorpromazine-N-oxide occupies relative to chlorpromazine(6). It was therefore carefully compared with synthetic IPNO in 8 solvent systems (Table II). The 2 substances could not be distinguished from each other by chromatography or co-chromatography. Their color reactions were identical. When subjected to paper electrophoresis, the two substances moved at similar

TABLE II. Chromatographic Identification of Imipramine-N-Oxide in Urine.

Solvent system*	R_f Urine metabolite (#2)	R_f IPNO standard
BuEt	.80	.80
BzA	.57	.57
AmEtA	.70	.70
AmMe 4:1	.79	.79
BzF	.11	.11
BuA	.81	.80
AmMe 1:1	.81	.81
EdBzF	.52	.52

* BuEt = n-butanol-ethanol-water (15:2:5), ascending. BzA = benzene-acetic acid-water (2:2:1), descending. AmEtA = ammonia (2.5%)-ethanol-acetic acid (200:15:3), ascending. AmMe 4:1 = ammonia (58%)-methanol (4:1), ascending. BzF = benzene-formic acid (88%)-water (1:1:1), descending. BuA = n-butanol-acetic acid-water (12:3:5), ascending. AmMe 1:1 = ammonia (58%)-methanol (1:1), ascending. EdBzF = ethylene dichloride-benzene-formic acid-water (3:1:4:2), descending.

TABLE III. Percent of Imipramine Excreted as Imipramine-N-Oxide.

Patient	Sex	Imipramine dosage* (mg/day)	IPNO excretion (%)†
WH	♀	150	1.8
FW	♀	150	1.2
WS	♂	250	1.9
JB	♂	300	1.7
CS	♀	300	2.1
		Mean ± stand. dev.	1.7 ± .3

* Administered and expressed as the hydrochloride.

† Each value represents the mean for two 24-hr urine specimens.

rates towards the cathode (6 cm. in 3.5 hours). All other standards tested, including IP, Nor_1IP, Nor_2IP, 2-HO-IP, 2-HO-Nor_1IP and β-HO-IP, remained at the origin. From these data we conclude that compound #2 is imipramine-N-oxide.

Stability of IPNO. Urine specimens from patients on imipramine therapy were analyzed immediately after collection and again after storage for 7 days in the refrigerator. There were no indications of any change in the IPNO content. The IP and 2-HO-IP concentrations also remained constant, which shows that IPNO does not arise spontaneously from these compounds. When IPNO was dissolved in normal urine and stored in the cold for 2 weeks, no qualitative or quantitative changes could be demonstrated. Refrigerated solutions of IPNO standards which had been extracted into ethylene dichloride (see *Experimental*) remained stable for 2 weeks or more.

Urinary IPNO excretion values. The daily IPNO excretion values for 5 patients on imipramine medication are given in Table III. The patients excreted an average of 1.7% of the administered drug as the N-oxide. The excretion was independent of the drug dosage in the limited range indicated.

Discussion. The imipramine metabolites isolated from human urine (Table I) exhibit ring hydroxylation, N-oxidation and monodemethylation of the side chain. There was no evidence in this pilot study for the presence of nor_2imipramine, which has been reported in rabbit urine(2). Our failure to detect Nor_2IP in human urine is probably due to the difference in species. However, it may be significant that the synthetic preparation was unstable in solution. As a consequence, there is some question whether Nor_2IP would be detected if present as a minor metabolite in urine. It may be noted for purposes of comparison that humans do not excrete chlorpromazine as nor_1- or nor_2-chlorpromazine, but rather as the desmethyl sulfoxides(5).

Summary. Imipramine-N-oxide (IPNO) has been identified as a metabolite of imipramine. The product was isolated from human urine by solvent extraction and chromatography. Its identity with authentic IPNO was established by their similarity in color reactions and electrophoretic behavior, and by paper chromatography and cochromatography in 8 solvent systems. Approximately 2% of administered imipramine is voided as the N-oxide.

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