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Effects of chloral hydrate, paraldehyde, and ethanol on the metabolism of [^{14}C]-serotonin in the rat*

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THE MECHANISM of action for ethanol, chloral hydrate and paraldehyde remains unresolved. In man and experimental animals, the extended administration of these drugs produces a similar type of physical dependency. The abrupt discontinuation of any of these agents after prolonged use results in a devastating physical and psychological phenomenon termed "withdrawal". However, it has been a long-standing observation that dependency caused by any one of these drugs can be shifted to another to prevent the onset of withdrawal symptoms.[1]

Both ethanol and chloral hydrate produce hypnosis in animals, which can be prolonged by the administration of serotonin or other biogenic amines.[2,3] Rosenfeld[2] showed that the administration of serotonin (10 mg/kg) to mice pretreated with hypnotic doses of ethanol resulted in a striking increase in the sleeping time. Sublethal doses of serotonin caused subhypnotic amounts of ethanol to become hypnotic, without decreasing the over-all rate of ethanol metabolism. The induction of sleep in mice by injecting serotonin into animals pretreated with subhypnotic doses of ethanol or chloral hydrate is an apparent central nervous system effect. Paradoxically, the molecular characteristics of serotonin prevent this amine from passing the blood–brain barrier in significant amounts.[4,5]

There is mounting evidence that serotonin may be involved in the normal functioning of the central nervous system.[6–9] It has been suggested that alterations in amine metabolism may be involved in the synergistic action of biogenic amines and sedative hypnotics.[10] Previous investigations in this laboratory have shown that ethanol dramatically alters the metabolism of [^{14}C]serotonin by a partial diversion of the metabolism of 5-hydroxyindoleacetaldehyde from an oxidative route to a reductive path.[10] Recent experiments with rats have shown that chloral hydrate modifies the metabolism of intravenously administered [^{14}C]serotonin in a manner similar to that seen with ethanol.[11] Because of the relative inability of [^{14}C]serotonin to pass the blood–brain barrier, these data probably reflect peripheral changes in the metabolism of serotonin. Additional studies using rat brain tissues were conducted in order to circumvent the blood–brain barrier. The [C^{14}]-labeled amine was injected directly into the lateral ventricle of the brain.[11] After chloral hydrate administration, there was no significant change in brain tissue levels of [^{14}C]5-hydroxyindoleacetic acid, and only a modest rise in the "neutral" fraction. Considering the fact that the transfer of 5-hydroxyindoleacetic acid from brain to plasma in the rat is dependent upon a transport system,[12] brain levels of [^{14}C]5-hydroxyindoleacetic acid and [^{14}C]5-hydroxytryptophol may not be an accurate reflection of the effect of chloral hydrate on the metabolism of serotonin within the central nervous system.

In the present study, [^{14}C]5-hydroxytryptamine was injected into the lateral ventricle of the rat brain after treatment with chloral hydrate or ethanol. The urine was collected for 24 hr and analyzed for [^{14}C]5-hydroxytryptamine, [^{14}C]5-hydroxyindoleacetic acid and [^{14}C]5-hydroxytryptophol. These results are compared with those obtained after the intravenous administration of [^{14}C] serotonin to chloral hydrate- and ethanol-treated rats. The effects of paraldehyde on the metabolism of intraperitoneally injected [^{14}C]-serotonin are also reported.

Albino male rats were obtained from Houston Cheek Rat Company, Houston, Tex., and kept in stainless steel cages with two animals sharing a cage. When purchased, the animals weighed from 140 to 180 g, and were between 2 and 4 months of age. The rats were used only once and this was within 5 weeks of purchase. In all studies reported, the control animals were the same age as the test animals. For urine collections, individual animals were placed in stainless steel metabolic cages which allowed complete separation of urine and feces. At the end of a 24-hr collection period, the inside of the cage was washed with 20–40 ml of deionized water and the total urine volume brought to 100 ml. If not immediately processed, the urine was frozen and stored at −20°.

All drugs were freshly prepared immediately before using. Ethanol was administered as a 25% (w/v) solution in normal saline. Chloral hydrate was prepared from crystals and made up as a 2·5% solution in 0·45% sodium chloride. Paraldehyde was injected as a 5% solution in a 0·45% solution of sodium chloride. Because paraldehyde reacts with certain plastics, only glass syringes were used. Attention was given to mixing of the paraldehyde so that it would remain in solution upon

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standing. After the mixing of the drug, both the 5% solution of paraldehyde and the syringes for injection were cooled to below 18°.

The creatinine sulfate salt of [^{14}C]5-hydroxytryptamine was obtained in 0·05-mc amounts as a dry powder from Amersham/Searle, Des Plaines, Ill. For intraventricular injections, the isotope was prepared by diluting the 0·05 mc of the dry powder with 15 ml saline. The specific activity of the isotope was 39·6 mc/m-mole; therefore, each milliliter of solution contained 18·3 μg as serotonin "free base" and 3·33 μc ^{14}C activity. For intraperitoneal injection, the contents of the vial were diluted to a final volume of 50 ml with normal saline.

The isotope was administered intraventricularly into the lateral ventricle of the rat brain in control, chloral hydrate- and ethanol-treated animals. Each rat received an intraventricular injection of 0·03 ml of a sterile solution containing 0·55 μg [^{14}C]5-hydroxytryptamine (0·1 μc) according to a technique described by Noble *et al.*[13] The skull of the rat was exposed by a midsaggital incision and a small hole, just deep enough to pierce the skull, was made 1·5 mm lateral to the crossing of the coronal and saggital sutures by means of a pointed lancet. A 27 gauge needle having a rubber stop 3·5–4·0 mm from the tip was inserted through the skull hole into the lateral ventricle of the brain. After administration of the radioactive solution, the needle was allowed to remain in place about 5 sec, then withdrawn and the incision closed with stainless steel skin clips. For this procedure, the rats were anesthetized with CO_2. Intravenous injections were made via the tail vein.

In those rats receiving paraldehyde, 0·1 μc of the isotope was injected intraperitoneally in a 1 μc/ml sterile solution. This was done in order to deliver a precise volume large enough to be evenly distributed in the peritoneal cavity. In preliminary experiments, both the drug and isotope were injected intraperitoneally.

Ethanol was injected intraperitoneally at a dose of 4·0 g/kg. The ethanol-treated rats weighed 180–350 g. Chloral hydrate was injected intraperitoneally at a dose level of 250 mg/kg and the animals used weighed 320–380 g. The rats used in the paraldehyde study were injected with a dose of 750 mg/kg and weighed 124–355 g. In each experiment, control animals were injected with equal volumes of normal saline.

Hydrolysis of urine conjugates. After a 24-hr urine collection, a 50-ml aliquot was adjusted to pH 11 with 5 N sodium hydroxide. Solid barium chloride was added to remove inorganic phosphates and sulfates, which interfere with the enzymatic hydrolysis of sulfate conjugates. The barium precipitates were then removed by centrifugation. To each urine was added 50 mg EDTA and 100 mg ascorbic acid. The urine mixture was then adjusted with acetic acid to pH 5·5, and 0·1 ml Glusulase, a mixture containing both β-glucuronidase and sulfatase activity, was added. The urine–enzyme mixture was then incubated at 37° for 18–24 hr. After the incubation, the samples were adjusted to pH 7 and filtered.

Measurement of total ^{14}C activity. The total radioactivity in each urine collection volume was determined by counting 0·5 ml urine dissolved in 20 ml of a liquid scintillation solution previously described.[14] The radioactivity was assayed in a Packard model 3375 liquid scintillation spectrometer. Counting efficiency was determined by using the automatic external standard of the instrument and an established quench curve for the scintillator liquid. The per cent of the total radioactivity in the urine for a 24-hr period was calculated by knowing the precise activity of isotope injected.

Measurement of [^{14}C]5-hydroxytryptamine. Duplicate 4-ml aliquots of the hydrolyzed urine were mixed with 4 ml of 0·02 M NH_4 acetate, pH 7·5. The urine samples were then transferred quantitatively to 6 × 80 mm, CG-50 NH^+_4 ion-exchange resin colums and allowed to pass through the columns under hydrostatic pressure. The columns were then washed with two 5-ml washes of 0·02 M NH_4 acetate, pH 7·5. The [^{14}C]5-hydroxytryptamine was then eluted from the columns using 20 ml of 3 N NH_4OH, and the eluate was collected in counting vials. The eluate was evaporated in an exhaust Precision Thelco oven at 110° to a volume less than 0·5 ml and then taken into solution with 20 ml of the liquid scintillator. The samples were counted and the data expressed as per cent of the total ^{14}C activity in the 24-hr urine sample. There was no loss of radioactivity during the evaporation. This was established by adding a known amount of [^{14}C]5-hydroxytryptamine standard to a sample prior to evaporation, and measuring the total activity after evaporation.

Measurement of [^{14}C]5-hydroxyindoleacetic acid. Duplicate 5-ml aliquots of the hydrolyzed urine were transferred to extraction tubes which contained 4 ml of deionized water, 4 g NaCl, and 3 ml of 3 N HCl. The sample was extracted with 25 ml of fresh ether by shaking on an automatic shaker for 20 min, centrifuged at 2000 rpm for 10 min, and then 20 ml of the ether was removed and transferred to a final extraction tube containing 1·5 ml of 0·5 M phosphate buffer, pH 7·0. The [^{14}C]5-hydroxyindoleacetic acid was extracted from the ether into the buffer by shaking the mixture for 20 min. After a 10-min centrifugation, a 0·5-ml aliquot of the buffer was removed and counted in 20 ml of the liquid scintillator.

Measurement of [^{14}C]5-hydroxytryptophol. After adjustment of the hydrolyzed urine to pH 7·0, duplicate 5-ml aliquots of the urine were transferred to extraction tubes containing 4 g NaCl and 30 ml of fresh ether. The 50-ml screw-cap centrifuge tubes were shaken for 20 min and then centrifuged

for 10 min. A 25-ml fraction of the ether was removed and washed twice with 3 ml of 0·5 M sodium phosphate buffer, pH 7·0, in order to remove any traces of [^{14}C]5-hydroxyindoleacetic acid. Twenty-ml aliquots of the washed ether were transferred to counting vials, evaporated to dryness, and counted by liquid scintillation techniques previously described.

Efficiency of assays. The efficiency of the 5-hydroxytryptamine assay was established by adding a known amount of [^{14}C]5-hydroxytryptamine to the urine and calculating recovery on the basis of radioactivity in the column eluate. The recovery varied only slightly for the resin used, and normally ranged from 75 to 90 per cent. For the extraction procedures, authentic standards of 5-hydroxytryptophol and 5-hydroxyindoleacetic acid were carried through the procedure and determined chemically. There was no contamination of one fraction by the other, and an extraction efficiency of 85 per cent was established for both compounds.[10] Counting efficiency was applied in the final calculation for each metabolite and data were expressed as a per cent of the total ^{14}C activity in the urine.

The effects of chloral hydrate on the metabolism of intraperitoneally administered [^{14}C]-serotonin are summarized in Table 1. Chloral hydrate markedly reduced the amount of urinary [^{14}C]5-hydroxyindoleacetic acid from a control value of 48·02 per cent to a test level of 35·30 per cent. The increase in urinary [^{14}C]5-hydroxytryptophol reflects a shunting of the intermediate indole aldehyde to an available alternate reductive pathway. This alteration in the metabolism of serotonin by chloral hydrate could be explained by the inhibitory action of chloral hydrate on aldehyde dehydrogenase.[15]

TABLE 1. EFFECTS OF CHLORAL HYDRATE ON THE URINARY METABOLITES OF INTRAPERITONEALLY ADMINISTERED [^{14}C]SEROTONIN

	Per cent of total ^{14}C in urine*	
^{14}C Metabolites	Control	Chloral hydrate
5-Hydroxytryptamine	4·35 ± 0·50	3·82 ± 1·49
5-Hydroxyindoleacetic acid	48·02 ± 3·55	35·30† ± 3·67
5-Hydroxytryptophol	10·77 ± 1·41	18·63† ± 0·87
Total	61·95 ± 2·39	56·71 ± 2·66

* The per cent of the i.p. administered isotope recovered in 24 hr for the control rats was 75·88 and 76·90 for the chloral hydrate-treated rats. Values are expressed as mean ± S.D. Each group consisted of 11 animals; significance of data was determined using the Student *t*-test.

† $P < 0·001$ (highly significant).

Previous experiments in our laboratory have established that ethanol ingestion alters the metabolism of orally or intravenously administered [^{14}C]5-hydroxytryptamine.[11] This alteration occurs at the point of metabolism where 5-hydroxyindoleacetaldehyde is acted upon by aldehyde dehydrogenase and alcohol dehydrogenase (Fig. 1). Apparently, chloral hydrate acts in a manner similar to ethanol in altering the metabolism of [^{14}C]-serotonin in the rat, i.e. blocking the further oxidation of 5-hydroxyindoleacetaldehyde.

Table 2 shows the effect of chloral and hydrate ethanol on the metabolism of intraventricularly and intravenously administered [^{14}C]-serotonin. The most significant single observation was the increase in urinary [^{14}C]5-hydroxytryptophol by the animals receiving chloral hydrate and ethanol. There was a slight but significant decrease in the excretion of [^{14}C]5-hydroxytryptamine by the chloral hydrate-treated animals.

In control animals injected intraventricularly with [^{14}C]-serotonin, [^{14}C]5-hydroxytryptophol represented only 5·09 per cent of the total urinary ^{14}C radioactivity. This was less than half the amount of [^{14}C]5-hydroxytryptophol found in urine of control rats that were injected intravenously with the [^{14}C]-labeled serotonin. In addition, the amount of ^{14}C activity excreted as 5-hydroxyindoleacetic acid was 1·67 times higher in animals receiving [^{14}C]5-hydroxytryptamine intraventricularly than in the group receiving the isotope intravenously. These data support recent observations that serotonin is oxidized primarily to 5-hydroxyindoleacetic acid in the rat brain, and very little is reduced to 5-hydroxytryptophol.[11]

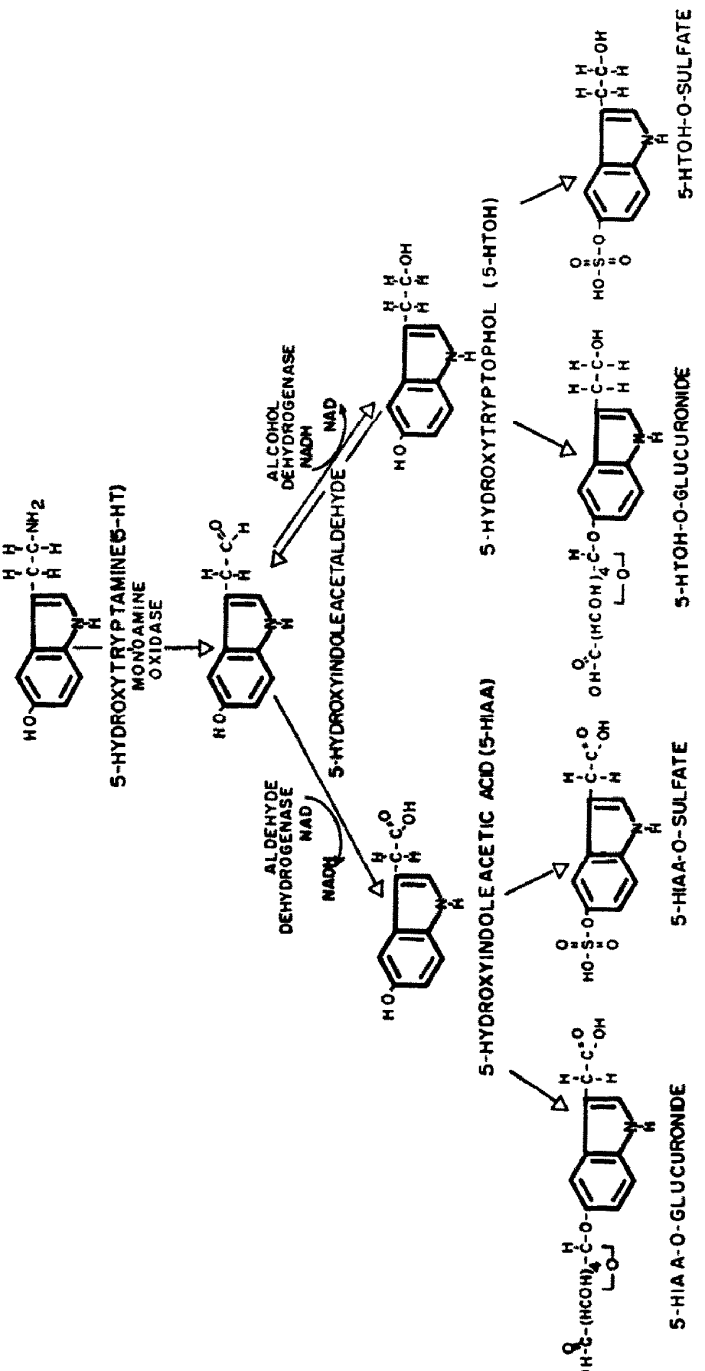

FIG. 1. Metabolic pathway for 5-hydroxytryptamine in the rat.

TABLE 2. EFFECT OF CHLORAL HYDRATE AND ETHANOL ON THE METABOLISM OF INTRAVENOUSLY AND INTRAVENTRICULARLY ADMINISTERED [^{14}C]-SEROTONIN

^{14}C Metabolites	Per cent of total ^{14}C in urine*					
	Control		Ethanol		Chloral hydrate	
	i.v.	i.c.†	i.v.	i.c.	i.v.	i.c.
5-Hydroxytryptamine	19·74 ± 2·90	12·01 ± 1·11	17·22 ± 1·31	12·47 ± 1·31	20·84 ± 1·65	9·13 ± 1·65
5-Hydroxyindoleacetic acid	41·49 ± 1·83	69·35 ± 2·06	36·86 ± 2·85	67·96 ± 4·14	28·19 ± 2·12	70·58 ± 2·64
5-Hydroxytryptophol	13·01 ± 1·08	5·09 ± 0·46	19·08 ± 1·83	8·35 ± 0·98	20·60 ± 3·49	10·50 ± 1·08
Total	74·24 ± 1·94	86·46 ± 2·21	73·16 ± 2·32	88·79 ± 2·64	69·63 ± 2·42	90·21 ± 1·98

* The per cent of the i.c. dose recovered in 24 hr was 79·12 ± 3·90 for control animals, 74·35 ± 4·44 for ethanol-treated rats, and 75·86 ± 8·73 for chloral hydrated-treated animals. The per cent of the i.v. dose recovered for the same time period was 50·47 ± 8·32, 50·00 ± 5·94, and 48·84 ± 3·63. Values are expressed as mean ± S.D. Each group consisted of 8 animals.

† The abbreviation i.c. is used to denote intraventricular (intracranial) injection.

Only about 50 per cent of an intravenous dose of [^{14}C]5-hydroxytryptamine was recovered in rat urine during a 24-hr period. This was most probably due to platelet binding of the amine.[16,17] After the intraventricular injection of the isotope, 76·44 per cent of the injected activity was excreted in 24 hr. The major portion of the excreted activity, approximately 70 per cent, was found to be [^{14}C]5-hydroxyindoleacetic acid. In addition, only 12·02 per cent of the urinary activity appeared as [^{14}C]5-hydroxytryptamine in the intraventricularly injected animals, while 20 per cent was found in the urine of the intravenously injected animals. These data would strongly suggest that more than half of the intraventricularly injected [^{14}C]-serotonin was metabolized before egress from the brain.

The level of urinary [^{14}C]5-hydroxytryptamine was approximately the same for control, ethanol-treated and chloral hydrate-treated animals that were injected intravenously. The anticipated alteration in the intermediate metabolism of [^{14}C]-serotonin was seen in the ethanol-treated rats. However, a greater decrease in urinary [^{14}C]5-hydroxyindoleacetic acid was seen in the chloral hydrate group than in the ethanol-treated rats. On a molar basis, 40 times more ethanol than chloral hydrate has been given the test animals to produce hypnosis.

Although a compensatory increase in the amount of [^{14}C]5-hydroxytryptophol was found in the urine of the ethanol-treated rats, this increase was not observed in the chloral hydrate-treated group (Table 2). This unusual observation might be explained by the following results. Chloral hydrate inhibits aldehyde dehydrogenase. Erwin and Deitrich[15] found that on a molar basis chloral hydrate was as effective as tetraethylthiuram disulfide (disulfiram) in inhibiting a partially purified monkey brain aldehyde dehydrogenase preparation. Their data suggested that the sulfhydryl groups are important for the activity of aldehyde dehydrogenase and that chloral hydrate and disulfiram *in vitro* produced a 50 per cent inhibition at a concentration of 5×10^{-5} M. This would explain the decrease in the formation of [^{14}C]5-hydroxyindoleacetic acid. The lack of a compensatory increase in urinary [^{14}C]5-hydroxytryptophol could be explained by using the results of Friedman and Cooper,[18] who demonstrated that chloral hydrate is rapidly metabolized to trichloroethanol and that alcohol dehydrogenase is the major catalyst in this metabolic process. They also showed that NADH was a required cofactor in the enzymatic metabolism of chloral hydrate. Thus, chloral hydrate would be competing with 5-hydroxyindoleacetaldehyde for both the available alcohol dehydrogenase and NADH. These observations suggest that the metabolism of the intravenously administered [^{14}C]5-hydroxytryptamine is blocked by chloral hydrate at both the aldehyde dehydrogenase level and the alcohol dehydrogenase level. Considering these findings, one might expect a decrease in [^{14}C]5-hydroxyindoleacetic acid without a compensatory increase in the urinary [^{14}C]5-hydroxytryptophol.

When [^{14}C]5-hydroxytryptamine was administered intraventricularly, more of the amine was metabolized than when the isotope was administered intravenously (Table 2). This is reflected by an increase in total ^{14}C metabolites in the urine and a decrease in the amount of the unmetabolized amine. There was no decrease in the amount of [^{14}C]5-hydroxyindoleacetic acid excreted for either the ethanol or chloral hydrate group. However, an increase was observed in the amount of [^{14}C]5-hydroxytryptophol excreted by both the ethanol and chloral hydrate group. Therefore, an inhibition of aldehyde dehydrogenase within the brain can only be inferred from the increase in urinary [^{14}C]-5 hydroxytryptophol.

Paraldehyde induced an alteration in the metabolism of intraperitoneally administered [^{14}C]5-hydroxytryptamine. Because of variation in the results obtained from the paraldehyde-treated rats, this study was repeated four times and the data were compiled from the separate experiments. Control rats were used in each experiment and all control data were similar and consistent. Experimental data derived from the analysis of the urine of the paraldehyde-treated rats were divided into three groups as given in Table 3. This grouping was based in part on the effect of paraldehyde in altering the metabolism of [^{14}C]5-hydroxytryptamine to [^{14}C]5-hydroxyindoleacetaldehyde, and on the amount of the injected isotope recovered in the 24-hr urine collection. In group I, there appeared to be a marked inhibition of monoamine oxidase activity. This apparent decrease in the oxidative metabolism of [^{14}C]5-hydroxytryptamine was metabolically expressed as an increase in urinary [C^{14}]-5-hydroxytryptamine levels. There was a marked decrease in the amount of the [^{14}C]5-hydroxyindoleacetic acid excreted by the rats of group I. Conversely, there was a 2-fold increase in the amount of [^{14}C]5-hydroxytryptamine metabolized to [^{14}C]5-hydroxytryptophol. Only 37 per cent of the urinary activity was accounted for in group I as [^{14}C]5-hydroxyindoleacetic acid and [^{14}C]5-hydroxytryptophol. For the control animals, this value was 61 per cent.

Group II was comparable to the control group in that all three metabolites did not change in their relative concentrations. For group III, there was a decrease in the level of urinary [^{14}C]5-hydroxytryptamine, a slight decrease in the amount of [^{14}C]5-hydroxyindoleacetic acid, and an increase in the amount of [^{14}C]5-hydroxytryptophol. Additional studies are warranted to study the mechanism by which paraldehyde appears to alter the initial oxidation of [^{14}C]5-hydroxytryptamine by monoamine oxidase.

TABLE 3. ALTERATION OF [^{14}C]-SEROTONIN METABOLISM BY PARALDEHYDE IN THE RAT

^{14}C Metabolites	Per cent of total urinary ^{14}C activity*			
	Control	Paraldehyde		
		Group I	Group II	Group III
5-Hydroxytryptamine	5·12 ± 1·52	30·35† ± 7·60	4·84 ± 1·45	2·69† ± 0·22
5-Hydroxyindoleacetic acid	50·59 ± 3·37	15·14† ± 1·74	56·60 ± 6·54	41·16‡ ± 5·72
5-Hydroxytryptophol	10·89 ± 1·60	22·79† ± 8·66	11·33 ± 0·80	16·43‡ ± 2·15
Total	66·60 ± 2·16	68·28 ± 6·00	72·77 ± 2·93	60·28 ± 2·70

* The control values represent data from 10 animals. In the paraldehyde-treated animals, group I consisted of 8 animals, group II contained 10 animals, and group III had only 5 animals. The values are expressed as mean ± S.D. The per cent of the i.p. administered isotope recovered in a 24-hr urine collection was 81·68 ± 5·67 for the control rats, 45·84 ± 9·99 for Group I, 78·06 ± 3·84 for group II, and 73·46 ± 9·81 for group III.

† $P < 0.001$ (highly significant).

‡ $P < 0.01$ (significant).

Based on the findings of this study, we concluded that, in the rat, the intermediate metabolism of [^{14}C]5-hydroxytryptamine is altered by the administration of chloral hydrate. This alteration appears to occur at the 5-hydroxyindoleacetaldehyde level and is similar to the changes seen after ethanol injection. In addition to the "ethanol-like" effect of blocking 5-hydroxyindoleacetaldehyde conversion to 5-hydroxyindoleacetic acid, chloral hydrate apparently acts on alcohol dehydrogenase to limit the formation of 5-hydroxytryptophol.

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The tricyclic antidepressants—Inhibition of norepinephrine uptake as related to potentiation of norepinephrine and clinical efficacy

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THE CLINICAL activity of the tricyclic antidepressants has been attributed to a sensitization of central adrenergic receptor sites,[1] since the antidepressant activity appeared to be correlated with the ability of these compounds to potentiate both the peripheral response to exogenously administered norepinephrine[2] (NE) and the response to sympathetic nerve stimulation.[3] Imipramine was also reported to block the uptake of infused ^{3}H-NE in the heart by causing a decreased permeability of the cell or