TABLE I. Effect of Wetting Agents on Pathogenicity of Enterobacteriaceae Inoculated Intraperitoneally into Mice.

Organism	Chemical and conc.	Vol inoculated, ml	LD_{50}* Exp.	LD_{50}* Control
Sh. dysenteriae	Deceresol-OT 1:500	.5	6.72	8.73
Sh. paradysenteriae	" "	"	6.30	8.63
Salm. typhosa	Duponol W.A. "	"	7.52	8.34
Sh. ambigua	" 1:600	1.0	6.81	8.55

* Logarithms to base 10 of No. of organisms killing 50% of inoculated mice in 72 hr, as estimated by the Reed-Muench method from 4 to 9 groups of 5 mice.

lated intraperitoneally into 5 mice: in no case did these mice show any sign of illness.

The median lethal doses were estimated by the Reed-Muench method, and the results, expressed as logarithms to base 10, are presented in Table I. It is clear that there was considerable reduction in the LD_{50} values for the experimental series of each test as compared with the corresponding control, indicating increases of approximately 100, 200, 7 and 60 times in pathogenicity of, respectively, *Sh. dysenteriae, Sh. paradysenteriae, Salm. typhosa,* and *Sh. ambigua.* The effect of Duponol W.A., though quite definite, seems to be less intense than that of Deceresol-OT. Further experiments are necessary to establish whether the enhancement of pathogenicity by wetting agents is peculiar to enteric bacilli inoculated intraperitoneally into mice as well as to elucidate the mechanism of the observed effect.

Summary. Two wetting agents, Deceresol-OT and Duponol W.A., when added to suspensions of *Sh. dysenteriae, Sh. paradysenteriae, Salm. typhosa,* or *Sh. ambigua,* consistently enhanced pathogenicity of the organisms for mice inoculated intraperitoneally.

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Biochemical and Pharmacological Studies with D- and L-5-Hydroxytryptophan. (23066)

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Following synthesis of 5-hydroxy-D,L-tryptophan (5HTP)(1) it was shown that this amino acid is the precursor of 5-hydroxytryptamine (serotonin)(2) and that this conversion is catalyzed in animal tissues by a specific decarboxylase(3). The amino acid itself has now been found in toad venom(4) in *Chromobacterium violaceum*(5) and in urine of a patient with malignant carcinoid.* It

* C. E. Dalgliesh, personal communication.

has also been reported that D,L-5HTP, when administered to animals, is taken up by tissues, including brain, and there decarboxylated to serotonin(6). Upon its administration 5HTP causes somatic, autonomic and behavioral changes which bear a striking resemblance to those produced by the hallucinogenic agent, lysergic acid diethylamide (LSD). It has been inferred that the pharmacological effects produced by 5HTP result from its conversion to serotonin. From previous studies on conversion of D,L-5HTP to serotonin it was apparent that only one of the isomers was decarboxylated to serotonin(3). It was therefore concluded that the observed pharmacologic effects following administration of D,L-5HTP were due to that antipode which could be decarboxylated to serotonin, presumably the L form. It has now been possible to obtain evidence that only the L form is converted to serotonin and that this conversion accounts for previously reported pharmacologic effects of D,L-5HTP(6).

Methods and materials. 5-Hydroxy-D,L-tryptophan and 5-benzyloxy-D,L-tryptophan were kindly supplied by Dr. Kenneth E. Hamlin of Abbott Laboratories and Dr. Gustav J. Martin of National Drug Co. The L-amino acid oxidase used was a crude dried rattlesnake venom extract obtained from Ross Allen Farms (*Crotalus adamantus*). The carboxypeptidase used was a purified preparation kindly supplied by Dr. John E. Folk. The hog kidney acylase was a commercial preparation kindly supplied by Dr. Leon Levintow. Both preparations were shown to be active when tested on carbobenzyloxyglycylphenylalanine. *Serotonin* in tissues was assayed by a method previously described(7). 5HTP decarboxylase activity was determined by the method of Clark *et al.*(3). L-amino acid oxidase activity was determined by measuring the generated 5-hydroxyindoleacetic acid† (5HIAA) as previously described(8).

Results. Enzymatic resolution of D,L-5HTP was attempted. However, solutions of N-acetyl-5-benzyloxy-D,L-tryptophan and of N - chloroacetyl-5-benzyloxy-D,L-tryptophan, when incubated with carboxypeptidase at 37° for 16 hours did not lead to ninhydrin positive material(9). The same negative result was observed when hog kidney acylase was employed (10). Attempts to prepare salts of optically active acids with esters of 5-benzyloxy-D,L-tryptophan were discontinued when it was found that debenzylation occurred during Fischer esterification. Resolution of N-acetyl-5-benzyloxy-D,L-tryptophan was successfully accomplished by recrystallization of the brucine salt from 95% ethanol, as indicated by attainment of constant optical rotation ($(\alpha)^{D}_{20}$-6.1, c, 1.0 ethanol). However, deacetylation without racemization or destruction was impossible.

It was found possible to demonstrate resolution of the quinine salt of N-carbobenzyloxy-5-benzyloxy-D,L-tryptophan (m.p.-132°).‡ A solution of this salt was prepared by dissolving 4.4 g of N-carbobenzyloxy-5-benzyloxy-D,L-tryptophan and 3.2 g of quinine in 100 ml of 95% ethanol. On cooling there was deposited 2.1 g of crystals which were recrystallized twice from the same solvent to yield 0.8 g of crystalline salt. The salt was decomposed by the addition of excess 2 N HCl and extraction of the indole component with 100 ml of ethyl acetate. The organic solvent when evaporated left 0.42 g of one antipode of N-carbobenzyloxy-5-benzyloxytryptophan, yielding after hydrogenolysis 120 mg of the crystalline amino acid (Fraction A containing antipode D). In order to obtain the other antipode the mother liquor (100 ml of 95% ethanol) from the first crystallization of the quinine salt, was concentrated to 70 ml, left standing overnight at 5°C and filtered from additional D-salt. The filtrate, after removal of the quinine and hydrogenolysis of the

† Since these preparations are devoid of catalase the keto acid is nonenzymatically decarboxylated by the peroxide. 5-Hydroxyindole-pyruvic acid does not differ from 5-HIAA with regard to color reactions and extraction pattern.

‡ While this investigation was in progress Dr. Marvin Armstrong kindly sent us a manuscript (*J. Org. Chem.* in press) in which he described the successful resolution and the isolation of the two antipodes of 5HTP in chemically and optically pure form, using the quinine salt of N-carbobenzyloxy-5-benzyloxy-D,L-tryptophan.

TABLE I. Enzymatic Reactivity of D,L and D and L-5-Hydroxytryptophans.

Enzyme	Sample	% conversion	
L-amino acid oxidase*	DL-5HTP	49	5HIAA
	Fraction B (L)	80	
	" A (D)	0	
5HTP decarboxylase†	DL-5HTP	50	Serotonin
	Fraction B (L)	82	
	" A (D)	3	

* 4 mg of snake venom were incubated with 100 μg of either D or L 5HTP or with 200 μg of D,L-5HTP, in 3 ml at pH 7.4 and 37°C for 1 hr. Deamination was complete within this period.

† 2 ml of a 100,000 × g guinea pig supernate were incubated with 300 μg of each antipode in 3 ml at pH 8.1 and 37°C for 1 hr. Decarboxylation was complete within this period.

benzyl groups, yielded 100 mg of crystalline amino acid (Fraction B containing mainly antipode L). Due to the paucity of the isolated material further purification by recrystallization was not attempted. By comparing molecular extinctions of the α-nitroso-β-naphthol chromophores(11) with that of a standard preparation of 5-hydroxy-D,L-tryptophan, Fractions A and B indicated a purity of 80-85% with regard to 5HTP; this was taken into account in the following experiments.

Fractions A and B when subjected to analysis by paper chromatography indicated only one indole component identical with authentic D,L-5HTP. Evidence concerning the optical identity of the two resolved antipodes and of the optical purity of each is presented in the section on enzymatic studies.

Enzymatic studies. L-amino acid oxidase: Fractions A and B and D,L-5HTP were incubated with L-amino acid oxidase permitting the reaction to go to completion. As shown in Table I the racemic mixture yielded 49% of the theoretical amount of 5HIAA. Fraction B yielded 80% of the theoretical amount of 5HIAA whereas no 5HIAA was produced from Fraction A. Thus Fraction B was taken to be the L form. Apparently it still contained some of the D-antipode. However, Fraction A was apparently the pure D-antipode. 5HTP was found to be a much poorer substrate of hog kidney D-amino acid oxidase than alanine. *5HTP decarboxylase*: When the various forms of 5HTP were treated with crude preparations of guinea pig kidney decarboxylase the racemic mixture yielded 50% of the theoretical amount of serotonin (Table I). Fraction B was also decarboxylated and reacted to a similar extent as it had with L-amino acid oxidase. Little serotonin was formed from Fraction A.

These enzymatic findings identify the configurations of the two antipodes and substantiate the optical specificity of 5HTP decarboxylase. *Pharmacological studies.* The pharmacological effects produced by 5HTP, in previous studies(6) were explained by the production of serotonin *in vivo*. However, one could not rule out the possibility that some of the observed actions might be due to 5-hydroxytryptophan itself.

With the resolution of 5HTP it became possible to compare the pharmacological activities of D and L forms only one of which can be enzymatically converted to serotonin. Each of the isomeric forms of 5HTP and the racemic mixture were administered to mice. After 15-20 minutes those animals given the L form (Fraction B), or twice the amount of D,L-5HTP, exhibited marked tremors, piloerection, apnea, and hind limb abduction. After 35 minutes most of these animals were moribund. In contrast, those animals which had been given the D form (Fraction A) did not differ from the control animals. At the end of 45 minutes all the animals were sacrificed. The brains from all 4 animals in each group were pooled, homogenized and assayed for serotonin. The results are shown in Table II. Consistent with their observed central effects L and D,L-5HTP produced a large increase in brain serotonin. No increase in serotonin appeared in those animals

TABLE II. Serotonin Levels in Brain following Administration of the Various Forms of 5HTP.

Exp.	Brain serotonin, mg/g
Control	.8
D,L-5HTP	5.0
L- "	3.8
D- "	.8

Mice were pretreated with 10 mg of iproniazid phosphate (i.p.) 40 min. before administration of 2.5 mg of 5HTP (i.p.). They were sacrificed 45 min. after receiving 5HTP.

treated with the D form.

Discussion. The resolution of 5HTP into the D and L forms has permitted establishment of the optical specificity of one of the enzymes involved in the biogenesis of serotonin, 5HTP decarboxylase. It has also made it possible to further substantiate the claim that the central effects of 5HTP are due to its *in vivo* conversion to serotonin. It may also be of some interest that the anti-insulinase activity previously reported for D,L-5HTP has likewise been shown to be restricted to the L antipode.§

Summary. The D and L forms of 5-hydroxytryptophan were prepared. Only the L form was found to be acted on by 5HTP decarboxylase, to increase brain serotonin levels *in vivo*, and to be pharmacologically active.

§ Dr. Martha Vaughan, personal communication.

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Relation of Adrenal Weight to Social Rank of Mice. (23067)

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Recent work(2) has shown that mice in groups have significantly heavier adrenal glands than mice kept in isolation. Other work has shown that mice in a group will arrange themselves in an ordered social rank such that each mouse is dominant over those below it in rank(4,5). It seemed logical to investigate whether or not there is a relationship between social rank and adrenal weight. This report describes experiments designed to determine if there is such a relationship, after first performing pilot experiments to determine if the procedures used would in themselves produce changes in adrenal weight.

Methods. The mice used were "wild-strain" house mice (*Mus musculus*). These were descendants of wild mice captured in buildings and had been maintained in the laboratory for more than 10 generations. This strain is superior to albino mice for the present purpose, because past work(2) has shown that their adrenals and reproductive organs are more responsive to stressing stimuli. Male mice were weaned at 20 days and placed individually in jars, where they were kept until becoming sexually mature, usually at an age of 2 months and weight of 20-25 g. The jars were kept on shelves with partitions between the jars. Thus the mice were isolated visually, but not auditorily or olfactorily. The experimental design called for placing mice in groups for 4 hours each day, observing the social rank of each individual and then returning each mouse to its own jar for the remaining 20 hours of the day, and repeating this procedure daily. It was therefore important to determine by a pilot experiment whether daily handling would itself produce increase in adrenal weight and