the mucosa; (2) ragged, round and slit-like ulcers; and (3) typical pin-point lesions.

From the above results it is observed that all of the 16 different "carrier" strains of *E. histolytica* were pathogenic to dogs. Every animal became infected and showed amebic lesions at autopsy. The severity of the infections was different for the various strains and for the individual experimental animal but all strains were shown to be pathogenic and in many cases very active tissue invaders. The gross lesions were very typical of amebic infections but in histological sections amebae were never observed beneath the muscularis mucosae. The average incubation period of 6 days is considerably shorter than the 11 days of Walker and Sellards[8] for their human experiments but this is probably due to the fact that the infections could be detected by cecal aspiration at a much earlier date. If the conditions of an amebic infection in the dog are at all comparable to those in man, it is probable that there is no such thing as a healthy carrier condition in man and that all individuals harboring the organism have amebic lesions in the large bowel.

Conclusions. From the evidence cited above it is highly probable that all "carrier" strains of *E. histolytica* are pathogenic and that "healthy human carriers" as well as those individuals exhibiting clinical manifestations of the disease should be regarded as clinical cases.

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Conversion of Dibenzyl Disulfide to Hippuric Acid in the Rat.

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It has been demonstrated that benzyl chloride, S-benzyl-*l*-cysteine and S-benzylglutathione, when administered to animals, yield in the urine N-acetyl-S-benzyl-*l*-cysteine.[1-3] S-benzyl-*d*-cysteine undergoes optical inversion in the rat, via the oxidative deamination, to yield N-acetyl-S-benzyl-*l*-cysteine.[4] The optical inversion of S-

1 Stekol, J. A., *J. Biol. Chem.*, 1938, **124**, 129.

2 Stekol, J. A., *J. Biol. Chem.*, 1939, **128**, 199.

3 Stekol, J. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 108.

4 du Vigneaud, V., Wood, J. L., and Irish, O. J., *J. Biol. Chem.*, 1939, **129**, 171.

benzyl-*d*-cysteine to the corresponding acetylated *l* enantiomorph has been shown to occur in man as well as the rat.[5] It was observed, however, that the yields of the benzylmercapturic acid in the urine of rats and man after the ingestion of S-benzyl-*l*-cysteine were much greater than those after the ingestion of S-benzyl-*d*-cysteine.[4, 5] This was attributed to partial destruction of the *d* isomer of S-benzylcysteine at some stage of its metabolism to which, apparently, the corresponding *l* isomer is either immune or exposed to a smaller degree.[5] This intermediary stage of the metabolism of S-benzyl-*d*-cysteine is most probably the formation of S-benzyl-thiopyruvic acid, which was undoubtedly formed *in vivo* before the asymmetric synthesis of N-acetyl-S-benzyl-*l*-cysteine occurred. S-benzyl-thiopyruvic acid is expected to be unstable (no successful synthesis of it has been accomplished as yet) and one of the probable breakdown products of S-benzyl-thiopyruvic acid could be benzyl-mercaptan or dibenzyl disulfide. It has been shown that S-methyl-γ-thio-α-ketobutyric acid easily decomposes in alkaline medium to yield methyl mercaptan.[6] Upon feeding S-benzyl-*d*-cysteine to man, hippuric acid was isolated from the urine in addition to the benzyl-mercapturic acid.[5] If our deductions concerning the instability of S-benzyl-*d*-cysteine *in vivo* are correct, *i.e.* if dibenzyl disulfide is one of the breakdown products of it *in vivo,* then the administration of dibenzyl disulfide to animals should result in the excretion of hippuric acid. The present work demonstrates this fact.

Six adult albino rats were fed 300 mg of dibenzyl disulfide mixed with a complete synthetic casein diet and the urine was collected under toluene over a period of 48 hours. The urine was acidified with HCl to Congo Red paper and extracted with several portions of ether. The ether extract was evaporated to dryness, the residue was dissolved in hot water, decolorized with Norit, filtered and cooled. The crystals were recrystallized three times from hot water. M.P. 187-188° (corrected); N, 7.67; hippuric acid: M.P. 187-188°; N, 7.82. To insure the completeness of the extraction, the ether extracted urine was evaporated *in vacuo* at 38° to a syrup, acidified with HCl and extracted with several portions of ethyl acetate. The extract was evaporated to dryness and the residue was purified as was described above. The substance isolated proved to be hippuric acid, N, 7.77, M.P. 188-189° (corrected). Total amount of hippuric acid isolated from the urine of rats which ingested 300 mg of dibenzyl disulfide was 250 mg, or about 71% of the theoretical

5 Stekol, J. A., *Proc. Am. Chem. Soc.*, 100th meeting, Detroit, Mich., 1940.

6 Waelsch, H., and Borek, E., *J. Am. Chem. Soc.*, 1939, **61**, 2252.

amount. No N-acetyl-S-benzyl-*l*-cysteine was present in the urine of rats after the ingestion of dibenzyl disulfide.

The results strongly support the suggestion that benzyl mercaptan or dibenzyl disulfide may be formed from S-benzyl-*d*-cysteine *via* S-benzyl-thiopyruvic acid *in vivo*. The results further indicate the possible source of hippuric acid when S-benzyl-*d*-cysteine is ingested by animals. In this connection it is of interest to mention the recent work of Wood and Fieser.[7] These workers synthesized the cysteine derivatives of 1,2-benzanthracene, 10-methyl, 1,2-benzanthracene, 3,4-benzpyrene and 10-methyl, 3,4-benzpyrene. The preparation of the substances was prompted by the suggestion that these carcinogenic agents may form the corresponding mercapturic acids *in vivo*.[8] Wood and Fieser[7] found that these cysteine derivatives easily break down in neutral aqueous medium to yield the corresponding disulfides, but the authors suggested the possibility "that the mercaptan and the cysteine derivatives of the present series may undergo eventual conversion to the mercapturic acids, possibly even through the intermediary of the disulfides." In the light of the present work it would appear, however, that the mercaptan and the cysteine derivatives of 1,2-benzanthracene and 3,4-benzpyrene, especially those of the 10-methyl derivatives, would yield *in vivo*, not the corresponding mercapturic acids, but the corresponding hydroxyl or carboxyl derivatives, either free or conjugated with sulfuric acid or glycine respectively. The above discussion is in accord with the possibility that, in the course of metabolism of certain carcinogenic agents, conjugation with cysteine may occur at some intermediate stage, even though the corresponding mercapturic acids may not be the end products of such metabolism.

Summary. Dibenzyl disulfide was fed to rats and hippuric acid was isolated from the urine and identified by analysis. The results support the suggestion that benzyl mercaptan or dibenzyl disulfide may be formed *in vivo* from S-benzyl-*d*-cysteine *via* S-benzyl-thiopyruvic acid. Certain phases of the metabolic transformation of sulfhydryl and cysteine derivatives of certain carcinogenic compounds are discussed.

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7 Wood, J. L., and Fieser, L. F., *J. Am. Chem. Soc.*, 1940, **62**, 2674.

8 White, J., and White, A., *J. Biol. Chem.*, 1939, **131**, 149.