Certain compounds inhibited the iodide-diiodotyrosine exchange. The anti-thyroid drugs, 2-thiouracil and 2-mercaptothiazoline¹¹ as well as the compounds 4-thiouracil and sodium thiosulfate stopped the reaction for a time dependent on the concentration of the reactants and the temperature. Thus at 50° C. with 2-thiouracil in 0.05 per cent. the concentration of diiodotyrosine, only about 2 per cent. of the normal exchange for one hour was found. On the other hand, at 37° C., the addition of as little molecular iodine as 1 per cent. of the total radioiodide (diiodotyrosine to radioiodide equivalent ratio of 10 to 1) decreased the time from five hours to one hour for 65 per cent. exchange to completion. These inhibition and acceleration effects might both be expected since first, the thio compounds react readily with iodine¹² and second, the diiodotyrosine exchange with iodine occurs much faster than with iodide.

In view of the literature concerning the deuterium exchange reactions of tyrosine^{13,14} as well as the lack of radioiodide exchange of certain aromatic iodine compounds,^{15,16} the exchange of compounds related to diiodotyrosine was tried. In 50 per cent. aqueous methanol solution at 50° C., exchange was found between iodine-radioiodide mixtures and 3,5-diiodo-p-cresol, 2,6-diiodophenol, 4,6-diiodophenol and 2,4,6-triiodophenol.

The mechanisms of the two exchange reactions do not appear to be simple. The reactions may be related and it is possible that iodine is an active agent in the iodide-diiodotyrosine exchange. Further, the exchange reactions perhaps may not occur directly but may depend on some intermediate step. The fact that the iodide-diiodotyrosine exchange was affected by inhibitors and accelerators may be associated with the variable induction period noted at the lower temperatures. Some difficulties were encountered in obtaining exact duplication of the data; this is probably associated with the above effects and with the need for more closely controlling concentrations and pH. However, the data obtained were in fairly good qualitative agreement.

The results do not indicate that exchange reactions necessarily occur in vivo among various iodine compounds. On the contrary, certain of our experiments suggest the unlikelihood of such a possibility.

¹¹ E. B. Astwood, *Jour. Pharm. and Exp. Therap.*, 78: 79, 1943; also private communication.

12 E. B. Astwood, W. H. Miller and R. O. Roblin, Jr., unpublished.

¹³ D. Rittenberg, A. S. Keston, R. Shoenheimer and G. L. Foster, *Jour. Biol. Chem.*, 125: 1, 1938.

A. R. Moss and R. Shoenheimer, *ibid.*, 135: 414, 1940.
F. Juliusberger, B. Topley and J. Weiss, *Jour. Chem. Soc.*, 1295, 1935.

¹⁶ H. A. C. McKay, Nature, 139: 283, 1937.

However, we feel that this work does show the need to insure that results of metabolic studies are not distorted by exchange reactions occurring during subsequent *in vitro* procedures.

The l-diiodotyrosine used in these studies was either recrystallized Eastman Kodak Product or material synthesized here using either active or ordinary iodine. The radioiodine used was obtained in four lots from Columbia University and we wish to express our appreciation to the Cyclotron Staff for their cooperation.

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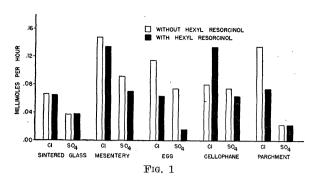
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EFFECT OF HEXYL RESORCINOL ON DIF-FUSION OF CHLORIDE AND SULFATE THROUGH SINTERED GLASS AND OTHER MEMBRANES

HEXYL resorcinol inhibits absorption of chloride in the ileum and promotes that of sulfate from a solution of sodium chloride-sodium sulfate, each component one-half isotonic with respect to blood. The purpose of the experiments reported here was to determine what effect hexyl resorcinol has on the diffusion of chloride and sulfate through membranes in vitro.

Five membranes were used: sintered glass, mesenteric, egg, Cellophane and parchment paper. In studying the sintered glass membrane, the method described by Northrop and Anson² was used. The mesenteric membranes were obtained from live dogs and used immediately, the egg membranes were prepared by dissolving the shell of eggs in 10 per cent. HCl, removing the contents of the egg and washing the membranes until free from chloride, and the Cellophane was du Pont's No. 600. This membrane, as well as the parchment paper, was soaked for 20 hours in distilled water. Twenty cc of the chloride-sulfate solution were placed in a test-tube, the open end of which was covered by a membrane. The tube was inverted in 10 cc of water and the solution allowed to diffuse 20 to 40 hours at 25° C. Chloride was determined by the modified Volhard-Harvey titration as described by Peters and Van Slyke (1932, p. 829), and sulfate by the method given by Kock (1937, p. 199). The results are depicted graphically in Fig. 1. Hexyl resorcinol was without effect on the sintered glass membrane, but in all other cases except two (no effect on sulfate diffusion through parchment paper

¹ R. L. Driver, Am. Jour. Phys., 135: 330, 1941. ² J. H. Northrop and M. L. Anson, Jour. Gen. Phys., 12: 543, 1929.



and an increase in chloride diffusion through Cellophane) chloride and sulfate diffusion was inhibited by the presence of this phenolic substance. It is possible that surface tension is involved in these studies. Hexyl resorcinol by virtue of its property to lower surface tension (decrease free energy) accumulates in the surface of water, and supposedly stops up the pores of the membrane keeping the ion from going through. If this interpretation of the data is accurate it is necessary that a revision be made in the general ideas of surface tension and passage through a dialytic membrane. A substance which lowers surface tension should not increase the passage of another substance through a membrane unless the two substances are miscible.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

ENDAMOEBA INVADENS AS AN AID IN THE STUDY OF ENDAMOEBA HISTOLYTICA

When it is desired to make more than a perfunctory examination of living trophozoites of Endamoeba histolytica, a method must be employed to maintain the microscope slide at a warm temperature. In large classes this is not practical, and the methods used detract from the study of the organism. For this reason, some laboratories have found it advantageous to introduce the exercise by studying a reptilian amoeba, Endamoeba invadens, since this parasite is adapted to survival at a temperature range of 10–33° C. Moreover, in most essential features of morphology, life cycle and pathogenicity, this organism very closely resembles the human pathogen.

With the exception of a few minor differences, the morphology of the trophozoite stage is strikingly similar to that of *E. histolytica*. The cytoplasm is dense, the karyosomal granules of the nucleus are situated centrally, and the nuclear membrane is lined with a thin, evenly distributed layer of discrete chromatin granules. The quadrinucleate cysts likewise are very comparable to those of *E. histolytica*, and their size, as well as the size of the trophozoites, is within the range of that of the human parasite.

The life cycle and pathogenicity of this reptilian amoeba also have been studied in detail.^{2, 3} The processes of encystation, excystation and metacystic development resemble very closely those published for *E. histolytica*. This is true particularly for excystation, where even minute details are in agreement entirely with those reported by Dobell for the human

¹ H. L. Ratcliffe and Q. M. Geiman, Science, 79: 324, 1934.

² Q. M. Geiman and H. L. Ratcliffe, *Parasitology*, 28: 208, 1936.

³ H. L. Ratcliffe and Q. M. Geiman, *Arch. of Path.*, 25: 160, 1938.

amoeba.⁴ The disease produced by *E. invadens* is comparable in its essential features to amoebiasis in man. However, in reptiles, irregularly outlined, undermining ulcers of the colon do not develop; liver involvement is more common in reptiles; and lesions of the stomach and upper part of the small intestine are peculiar to the reptilian disease.³

Endamoeba invadens can be cultivated easily at room temperature on a medium consisting of 0.3 per cent. gastric mucin in 0.5 per cent. aqueous "ground alum" salt, to which is added about 2 mg of sterile rice starch.³ For the convenience of laboratories not equipped to maintain stock cultures, this organism now can be obtained from the General Biological Supply House, and by placing orders in advance, it is possible to receive the material for class use without the necessity of subculturing.

It is recommended that laboratory exercises on *Endamoeba histolytica* be introduced by a preliminary study of *Endamoeba invadens*, since this reptilian amoeba so closely resembles the human pathogen, yet can be observed for long periods without the inconvenience of warming the microscope slide.

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School of Public Health, University of North Carolina 4 C. Dobell, *Parasitology*, 20: 357, 1928.

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