EXCHANGE REACTIONS OF DIIODOTYROSINE

RADIOACTIVE iodine has found increasing use in recent years in attempts to elucidate the mechanism by which iodine is metabolized by the thyroid gland. Generally, methods[1, 2, 3] previously worked out for the separation of the iodine-containing fractions, thyroxine, diiodotyrosine and inorganic iodide, have been utilized in these studies. Obviously, if exchange reactions can occur among these fractions during the isolation procedures or even under the conditions used for iodine metabolism, special caution is necessary in evaluating any results so obtained. The reported attempts to show that such exchanges can occur, although not very rigorous, were without success,[4, 5] nor was exchange observed by other workers in checks of their procedures.[6, 7, 8] Using radioiodine of 8-day half life, we have found exchanges between molecular iodine and diiodotyrosine and also between iodide ions and diiodotyrosine. These two reactions have been rather broadly investigated and some of the characteristics of each are briefly described in this preliminary note.

The exchange between iodine and diiodotyrosine was carried out in aqueous solution at 25° C. The amount of exchange occurring in a given time was determined by extracting the iodine with carbon tetrachloride and then measuring the radioactivity of the resulting two solutions. For this a small glass-jacketed Geiger counter tube[9] was used into which an aliquot of the solution was introduced. This procedure was proved satisfactory by an alternative method in which diiodotyrosine was isolated from the water layer and recrystallized to constant specific activity (counts/min./mg.) as determined by counting the solid with a mica window type counter tube. Although most of the work was concerned with the exchange from radio-diiodotyrosine to iodine, exchange in the opposite direction was also found.

In a given experiment the rate of reaction was not, as expected for simple exchange processes,[10] independent of time, but it usually decreased slightly. Both the relative and absolute concentrations of the reactants affected the reaction rate over a range of radio-diiodotyrosine concentrations from 1 to 10×10^{-8} mols/cc and iodine to radio-diiodotyrosine ratios from 2 to 130. Thus, for a radio-diiodotyrosine concentration of about 2×10^{-8} mols/cc a change of iodine from 5 to 60 fold excess increased from 18 to 62 per cent. the exchange to completion in 15 minutes at a pH of 2. It was of interest to note that when the above exchange at the higher ratio was carried out in the dark, the observed exchange was only 27 per cent. Normally, the exchange rate was independent of pH in the range 0.8 to 3.0, but increased at higher pH values. As an example, when the radio-diiodotyrosine concentration was about 1.6×10^{-8} mols/cc and the iodine to diiodotyrosine ratio 5 to 1, the exchange to completion in five minutes was 8 per cent. at pH 2, but 72 per cent. at pH 5.

The exchange between potassium iodide and diiodotyrosine in aqueous solution was observed to go in either direction, although radioiodide and inactive diiodotyrosine were usually used as the initial reactants. To determine the per cent. exchange to completion, diiodotyrosine was first isolated from the reaction mixture and recrystallized to constant specific activity as determined by counting the solid itself; comparison was then made between the specific activity found and that calculated for complete theoretical exchange.

The iodide-diiodotyrosine exchange was extremely slow at 25° C. However, runs at increasing temperatures gave increasing exchange rates until at 90° C. exchange was at least 97 per cent. complete in three minutes. At 50° C., where much of the work was done, an exchange was observed of 9, 29 and 92 per cent. to completion in 5, 15 and 90 minutes, respectively. For these experiments, a diiodotyrosine concentration of 2.2×10^{-6} mols/cc, a diiodotyrosine to radioiodide equivalent ratio of 10 to 1 and a pH of about 5 were used. No induction periods were encountered at the higher temperatures, but at the lower temperatures such as 37° C., they were of uncertain length; even at 50° C. they were sometimes found. The rate appeared dependent on the relative and absolute concentrations of reactants over the concentration limits 2 to 44×10^{-7} mols/cc. The reaction was sensitive to pH, with the maximum exchange rate at pH 4 to 5.5 at all temperatures. At pH 2, and 50° C., the rate was only about 5 per cent. of the maximum. At pH 7.5, the reaction mixture was held at 37° C. for over two days without significant exchange in contrast to an exchange of 62 per cent. in only six hours at pH 4.3.

[1] J. P. Leland and G. L. Foster, *Jour. Biol. Chem.*, 95: 165, 1932.
[2] A. B. Gutman, E. M. Benedict, B. Baxter and W. W. Palmer, *ibid.*, 97: 303, 1932.
[3] N. F. Blau, *ibid.*, 110: 351, 1935.
[4] P. Süe, *Compt. Rend.*, 212: 237, 1941.
[5] C. P. LeBlond and P. Süe, *Am. Jour. Physiol.*, 134: 549, 1941.
[6] I. Perlman, M. E. Morton and I. F. Chaikoff, *Jour. Biol. Chem.*, 139: 449, 1941.
[7] M. E. Morton and I. F. Chaikoff, *ibid.*, 147: 1, 1943.
[8] M. E. Morton, I. F. Chaikoff, W. O. Reinhardt and E. Anderson, *ibid.*, 147: 757, 1943.
[9] R. B. Barnes and D. J. Salley, *Ind. Eng. Chem. Analyt. Ed.*, 15: 4, 1943.
[10] H. A. C. McKay, *Jour. Am. Chem. Soc.*, 65: 702, 1943. Equation (2), p. 703.

Certain compounds inhibited the iodide-diiodotyrosine exchange. The anti-thyroid drugs, 2-thiouracil and 2-mercaptothiazoline[11] 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 iodine[12] 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. 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.

WILBUR H. MILLER
GEORGE W. ANDERSON
R. K. MADISON
D. J. SALLEY

STAMFORD RESEARCH LABORATORIES,
AMERICAN CYANAMID COMPANY,
STAMFORD, CONN.

[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.
[14] A. R. Moss and R. Shoenheimer, *ibid.*, 135: 414, 1940.
[15] F. Juliusberger, B. Topley and J. Weiss, *Jour. Chem. Soc.*, 1295, 1935.
[16] H. A. C. McKay, *Nature*, 139: 283, 1937.

EFFECT OF HEXYL RESORCINOL ON DIFFUSION 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.[1] 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 Anson[2] 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.