70. *A Synthesis of "Heavy"* dl-*Adrenaline.*

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All six hydrogen atoms of the catechol molecule exchange with deuterium oxide in alkaline solution, although replacement of the last is a very slow reaction. *dl*-Adrenaline containing 90% of its hydrogen atoms "heavy" has been prepared; its physiological action is almost indistinguishable from that of "light" *dl*-adrenaline.

Deuterium has been used as an indicator in many biological experiments and it has always been assumed that the substitution of deuterium for protium causes no change in the physiological action of the compound concerned. There is no reason to doubt the validity of this assumption, in general, although the majority of the substances examined (*e.g.*, fats in the metabolic studies of Schoenheimer and Rittenberg) are in the nature of foods, rather than compounds of specific physiological action. However, Erlenmeyr and Lobeck (*Helv. Chim. Acta*, 1937, **20**, 142) prepared acetylcholine bromide in which the hydrogen atoms of the acetyl group were replaced by deuterium atoms; and found the product to be slightly less active physiologically than the corresponding "light" compound. We therefore thought it would be interesting to attempt the preparation of a substance with marked physiological action in which all, or, at any rate, the greater part of the hydrogen was "heavy," and adrenaline was chosen for the experiment.

For this, "*heavy*" *catechol* was required. Although the exchange reactions of deuterium oxide with phenol (Ingold, Raisin, and Wilson, J., 1936, 1637) and with quinol, resorcinol, pyrogallol and phloroglucinol (Münzberg, *Z. physikal. Chem.*, 1936, *B*, 33, 23, 39; Geib, *ibid.*, 1937, *A*, **180**, 211) have already been studied, the case of catechol appears not to have been examined. The exchange reaction between deuterium oxide and catechol in alkaline solution was therefore investigated in a qualitative manner. All six hydrogen atoms of the catechol molecule are exchangeable, as was to be expected in view of the fact that each of the four nuclear hydrogen atoms stands in either the *o*- or the *p*-position to a hydroxyl group; but the attainment of equilibrium when all six have been replaced is very slow. Although a specimen of catechol containing 93 atoms % of its hydrogen in the form of deuterium has been obtained, the material which was used in the preparation of "heavy" adrenaline had a deuterium content of only 82 atoms % (*i.e.*, it approximated to the formula $C_6HD_5O_2$).

The "heavy" catechol was condensed with chlorodideuteroacetic deuteracid (prepared by the chlorination of trideuteroacetic deuteracid) to give "heavy" chloroacetocatechol containing 85·5 atoms % of deuterium (cf. Dzierzgovski, *J. Russ. Phys. Chem. Soc.*, 1893, **25**, 154). By treatment of this with a solution of trideuteromethyldideuteramine in deuterium oxide, "heavy" adrenalone was obtained (cf. Dakin, *Proc. Roy. Soc.*, 1905, *B*, **76**, 491). The sulphate of this base, in deuterium oxide solution, was reduced with deuterium in the presence of palladium–charcoal catalyst (Fig. 1); the resulting "heavy" *dl*-adrenaline contained 90 atoms % of deuterium (corresponding to the formula $C_9H_{1\cdot3}D_{11\cdot7}O_3N$).

Dr. J. Secker kindly carried out a physiological test on the product. Injection into a cat produced an elevation of blood pressure almost indistinguishable from that produced by a similar amount of "light" *dl*-adrenaline (Fig. 2). It is concluded that the replacement of protium by deuterium in adrenaline causes little (if any) change in the physiological action.

Experimental.

The isotopic analyses were carried out in the way described in this vol., p. 371.

Except where otherwise stated, the deuterium oxide used contained 99·6 atoms % of deuterium.

"*Heavy*" *Catechol.*—Catechol (0·5 g.) was placed in a bulb-tube, and deuterium oxide (1 g.) with sodium deuteroxide (5 mg. approx.) added; the cooled bulb was evacuated and sealed. After being heated for 4 days at 100°, the tube was cooled and opened, and the water (now only partially "heavy") distilled in a vacuum. A further quantity of deuterium oxide (1 g.) was added, and the above procedure repeated three times. The final residue was extracted with anhydrous ether, the extract dried with anhydrous sodium sulphate, the ether removed, and the residual "*heavy*" *catechol* recrystallised from anhydrous light petroleum (b. p. 80—100°); m. p. 104° (Found: C, 62·8; water, 50·2. $C_6HD_5O_2$ requires C, 62·6; water, 51·3%; isotopic analysis, 82 atoms % D). The water distilled in the exchange reactions contained a little catechol, and was used (instead of 99·6% D_2O) for the first two stages of the subsequent preparation. Thus, on an average, approximately 2 g. of 99·6% deuterium oxide were used for each 0·5 g. of catechol.

By raising the reaction temperature from 100° to 200°, allowing 7 days instead of 4 days at each stage, and by subliming (not extracting or recrystallising) the product, a specimen of "heavy" catechol was obtained which gave an isotopic analysis of 93 atoms % D.

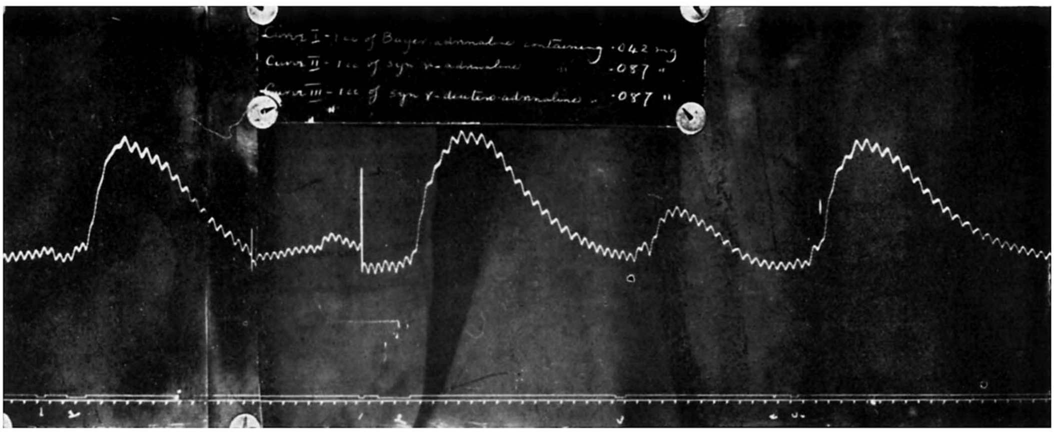
Chlorodideuteroacetic Deuteracid.—Trideuteroacetic deuteracid was chlorinated in the presence of an iodine–red phosphorus catalyst, while being heated on the water-bath. On fractionation, the distillate of b. p. 180—190° crystallised on cooling, giving chlorodideuteroacetic deuteracid, from which the remaining iodine was removed by standing over potash in a vacuum (isotopic analysis, 92 atoms % D).

"*Heavy*" *Chloroacetocatechol.*—Freshly distilled phosphoryl chloride was redistilled from a little quinoline (to remove hydrogen chloride), and the product fractionated out of contact with moisture. "Heavy" catechol of deuterium content 82 atoms % (0·12 g.), chlorodideuteroacetic deuteracid (0·11 g.), and purified phosphoryl chloride (0·08 g.) were heated together for 8 hours at 55—60°. Hot deuterium oxide (1·3 g.) was then added and, after the solution had been kept overnight in the refrigerator, the "heavy" chloroacetocatechol (0·06 g.) was collected, washed with deuterium oxide, and dried. When recrystallised from deuterium oxide, it had m. p. 172° (isotopic analysis, 85·5 atoms % D).

"*Heavy*" *Adrenalone.*—The above "heavy" chloroacetocatechol (0·12 g.) was mixed thoroughly with a solution of trideuteromethyldideuteramine (24%) in deuterium oxide (0·35 g.) and kept for 2 days at room temperature. The crude product (0·07 g.) was collected, and washed with deuterium oxide until the washings were no longer coloured. It was dissolved in a small volume of a hot dilute solution of "heavy" sulphuric acid in deuterium oxide, filtered while hot, and allowed to cool; "heavy" adrenalone sulphate then crystallised, and was collected, washed with a little ethyl deuteralcohol (prepared by the method of Erlenmeyr, Schenkel, and Epprecht, *Helv. Chim. Acta*, 1937, **20**, 368), and dried. The mother-liquors yielded a further quantity of this material on the addition of ethyl deuteralcohol, followed by anhydrous ether.

"*Heavy*" dl-*Adrenaline.*—The deuterium gas for the reduction was generated by the action of deuterium oxide on clean sodium in the apparatus illustrated in Fig. 1. The glass bulbs 10 and 11 of capacity 250 c.c.

FIG. 2.



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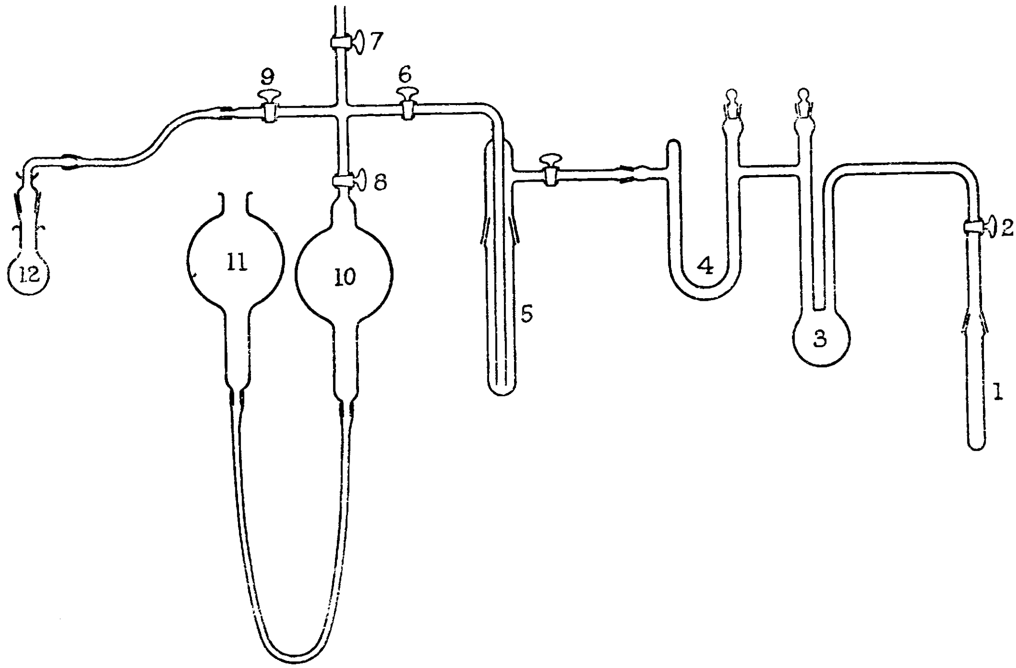
contained mercury, and clean sodium was introduced into the vessels 3 and 4. Deuterium oxide (approximately 0·6 g.) was placed in tube 1, and cooled strongly while the apparatus was exhausted by means of a high-vacuum pump connected at 7. Then vessel 3 was cooled in solid carbon dioxide–alcohol, and tube 1 allowed to warm so that the deuterium oxide distilled over into vessel 3. When the latter was allowed to warm, reaction occurred; the evolved deuterium was collected in the bulb 10 (trap 5 being cooled in liquid air). The reduction vessel

FIG. 1.



12, which could be shaken mechanically, was of capacity 5 c.c. and was connected to the deuterium storage vessel by a length of "aged" rubber pressure tubing. The palladium–charcoal (10%) was heated for 1 hour at 80°/15 mm. to remove as much as possible of the adsorbed moisture.

"Heavy" adrenalone sulphate (16 mg.) was dissolved in deuterium oxide (1·1 g.), palladium–charcoal (3 mg.) added, and the mixture shaken in an atmosphere of deuterium for 15 hours. The catalyst was then removed, and washed with a little deuterium oxide. To the filtrate was added a slight excess of anhydrous sodium carbonate; the separation of the product soon commenced. After standing for 2 hours out of contact with air, the "heavy" *dl*-adrenaline (10 mg.) was collected, washed with deuterium oxide, and dried in a vacuum desiccator. It formed a white powder, m. p. 208° (isotopic analysis, 90 atoms % D).

Physiological Test.—"Light" *dl*-adrenaline was prepared in a similar way to the "heavy" compound; and an aqueous solution of the sulphate prepared containing 1·74 mg. of the base per 10 c.c. 1·74 Mg. of the "heavy" base were weighed out into a 10 c.c. graduated flask, and dissolved in approximately 0·2 c.c. of deuterium oxide containing sufficient "heavy" sulphuric acid to neutralise the base. Meanwhile a cat was anæsthetised with "Dial" liquid compound; and prepared so that injections could be made into the femoral vein, while the arterial blood pressure could be continuously recorded. Fig. 2 shows the tracings obtained. The first peak (on the left) was obtained by injection of Bayer (optically active) adrenaline; after the canula had been washed out with saline, 0·5 c.c. of the "light" adrenaline solution was injected, followed by 2 c.c. of 0·9% sodium chloride solution (second peak). A further 2 c.c. of saline were then run in, to wash out the canula, leading to a slight rise in pressure, represented by the small peak. Then the "heavy" adrenaline (until now in deuterium oxide solution) was made up to 10 c.c. with "light" water, and 0·5 c.c. of the resulting solution immediately injected, followed by 2 c.c. of 0·9% sodium chloride solution. The resulting increase in blood pressure (last peak) was almost identical with that produced by the "light" *dl*-adrenaline. It was thus concluded that the physiological actions of *dl*-adrenaline, $C_9H_{13}O_3N$, and "heavy" *dl*-adrenaline, $C_9H_{1\cdot3}D_{11\cdot7}O_3N$, are approximately equal.

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