ON THE PREPARATION OF CHOLESTERIN FROM BRAIN. By OTTO ROSENHEIM, Ph.D.

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THE method usually employed for the isolation of cholesterin from brain consists briefly in extraction with ether and subsequent precipitation of the ethereal extract with alcohol. The principal disadvantages of this method are the following:—

1. The ether extraction of freshly pounded brain is a very troublesome operation. The brain swells up and filtration and complete removal of the cholesterin is a most difficult and tedious process[1]. F. Baumstark[2] avoids some of these drawbacks by subjecting the whole brain to ether-dialysis, by means of which the water of the brain is gradually replaced by ether; an aqueous layer forms beneath the ethereal; but the time required for the removal of the water is according to him 1 to 3 months, and the extraction of cholesterin is incomplete.

2. Ether dissolves not only cholesterin, but large quantities of lecithin, kephalin, myelin, protagon, fats, etc., and the subsequent separation of cholesterin from these substances involves several operations. Further this separation is very incomplete, and Baumstark only obtained 35 per cent. of the total cholesterin contained in brain by this method. The rest is carried down with the precipitates of lecithin, etc. and can only be freed from them by the process of saponification, a fact which led Baumstark to assume the presence of cholesterin-ethers in brain (see following paper by Miss Tebb). G. Zuelzer[3] precipitated the phosphatides from their ethereal solution by means of acetone and effected in this way a more complete separation.

[1] Compare Gamgee's *Physiol. Chem.* I. p. 427. 1880.
[2] *Zeitsch. f. physiol. Chem.* IX. p. 145. 1885.
[3] *Ibid.* XXVII. p. 255. 1899.

It is probably owing to these disadvantages that gall-stones are usually recommended for the preparation of cholesterin instead of the more easily obtainable brain.

In the course of some work on the chemical composition of brain, it occurred to me that the previous removal of the water without the application of heat, and the subsequent extraction of the brain with a solvent which would only extract cholesterin would greatly facilitate the study and separation of the other constituents. The method finally adopted was incidentally found to be applicable to the preparation of cholesterin in large quantities. It briefly consists in the removal of water by means of plaster of Paris, and the subsequent extraction of cholesterin by means of acetone. Sheep's brain is passed through a mincing machine, and then mixed intimately with some sand and with about three times its weight of plaster of Paris. In the course of some hours the mass has set hard, and this is easily broken up into a coarse powder which lends itself readily to extraction. It is then treated repeatedly at room temperature with acetone. The first extract usually contains some water, and therefore less cholesterin. But from the second and third extracts the bulk of the cholesterin crystallises out in snowy-white crystals on spontaneous evaporation of the solvent, or on concentrating the extract by distillation. The cholesterin thus obtained is practically pure, and after recrystallisation from a mixture of alcohol and acetone (with the addition of a little charcoal) shows a melting point of 145—147° C. and an optical activity in chloroformic solution ($\alpha_{[D]} = -36{\cdot}6°$) corresponding to that of pure cholesterin.