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The Flocculation of Lecithin by the Extracts of Adrenal Glands.

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In a previous publication[1] I have described the following reaction: The lipid of adrenal glands is flocculated in an aqueous emulsion in the presence of living tissue, oxydized adrenalin and H_2O_2. After sedimentation of the floccules it is possible to determine the presence of cholin and phosphoric acid in the supernatant liquid. It was observed that this phenomenon occurs very quickly, especially at body temperature, that oxydized adrenalin is not necessary, and that H_2O_2 only increases the speed of this reaction but does not cause it. Instead of living tissue a native preparation of blood albumen was used, which was found to be necessary and sufficient.

In this way the reaction became very simple: A lipid emulsion of adrenals is flocculated in the presence of native blood albumen. Another lecithin (lecithin *ex ovo* Merck) did not show this reaction. A very important condition is the integrity of the albumen; if this is denaturated the reaction is negative. The optimal temperature is body temperature; lower temperature inhibits the reaction; a higher one causes a denaturation of the proteins, so that the flocculation does not appear. The albumen does not enter into the reaction with the lipids; neither is it destroyed. After the reaction and after filtration it is possible to denature the albumen or to use it for a new reaction. It acts probably as a catalyzer (ferment?).

It was always possible to determine after flocculation and filtration, a certain amount of cholin and phosphoric acid in the clear solution, which obviously originates from the decomposition of lecithin. But this decomposition is perhaps only an accompanying phenomenon; the main manifestation is the flocculation.

Further I investigated why only the lipids of the adrenals flocculate upon coming in contact with proteins, and other lecithins do not. There are two possibilities: Either the lecithin from the adrenals is different or it is accompanied by a compound, which is the real condition for the reaction as described above. I therefore prepared pure lecithin from the adrenal glands according to Erland-

[1] *Ctp. rend. des séances de la Soc. Biol.*, **103**, 647.

son, i. e., an alcohol extract is prepared, evaporated, the residue dissolved in ether and the concentrated ether solution precipitated with acetone. The solution in ether and the precipitation with acetone are repeated several times and both fractions, i. e., the precipitate and the solution were used. The precipitate which represents a lecithin of high purity after the evaporation of acetone was emulsified in water. The emulsion did not show the above described reaction. The acetone-ether solution was evaporated in a vacuum and the residue dissolved in water. This added to the lecithin emulsion of the adrenals and to the albumen immediately caused a flocculation of the lecithin. This flocculation in the presence of the acetone-ether solution appeared also if we used a lecithin of different origin (e. a. lecithin *ex ovo* Merck). It is apparent that the flocculating reaction is not caused by the lecithin but it depends on a compound in the adrenal glands which accompanies the lipids in their solvents.

We prepared an extract which prevents the going over of lipids of lecithin nature into the solvents and which at the same time denatures the proteins. From dissected adrenals an extract with saturated cadmium chlorate solution in 96% alcohol was prepared, after 24 hours the extract was evaporated in a vacuum, from the residue after removing the fats and cholesterin through simple filtration an ether extract was prepared. This ether extract was evaporated in a vacuum and the residue dissolved in water. The solution prepared in this way showed the above mentioned qualities. The substance itself is of medium hard consistency, yellowish color, does not crystallize, smells like acetic acid, has a slight acid reaction and gives a cacodylic reaction. On account of technical difficulties it was not possible to test it on epinephrectomized cats. The cortin prepared by Hartman also gives the flocculating reaction. Hartman and Brownell[2] prepared a substance from the adrenals by ether extraction which they call cortin and which keeps epinephrectomized cats alive.

A short time before Swingle and Pfiffner[3] prepared an extract by a more complicated process, which contained the cortical hormone and was shown to keep epinephrectomized cats alive in good condition. Concluding from the publications of the authors, their

[2] Hartman and Brownell, *Am. J. Physiol.*, 1930, **93**, 655; PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 938.

[3] Swingle and Pfiffner, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 510; *Science*, 1930, **1**, 321.

preparation has some of the same qualities as above described substance, in that it is soluble in solvents of lipids, unstable in alkaline solutions and gives as it was found only for cortin the flocculating reaction. Swingle and Pfiffner's cortical hormone was not examined because its preparation is very difficult and I could not obtain a market preparation.

If we assume that the cortical hormone regulates the relation between the lipids of the tissue and the medium, it is possible to suppose that the substance which flocculates the lecithin in the presence of albumen is, if not the same, closely related to the cortical hormone.

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Bubble Recorder for Mariotte Bottles.

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The recording of bubbles passing through a Mariotte bottle by means of a tambour offers considerable technical difficulties, as for instance, in the lung perfusion, described by Sollmann and von Oettingen;[1] nor does the device, worked out by Atzler and Frank[2] give satisfactory records.

Very good results have been obtained with the following device which utilizes the oscillation of the fluid in the inlet tube of the Mariotte stopper to close a circuit between a pair of wire electrodes adjusted in the lower end of the tube. The electrodes consist of copper wires fused into narrow glass-tubes for insulation, with projecting platinum tips. One wire is adjusted to about one millimeter above the lower rim of the tube, the other wire dips about 0.5 cm. into the fluid. The 2 electrodes are held together by a rubber-ring through which a pin passes, which rests upon the upper rim of the Mariotte tube (Fig. A). By shifting this ring up and down, the electrodes can be adjusted to different levels; they are connected with a current of 110 volts and with a relay arrangement,

[1] Sollmann, T., and von Oettingen, W. F., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 692.

[2] Atzler, E., and Frank, L., *Pflüger's Arch. ges. Physiol.*, 1920, **181**, 141.