XXIV. THE DIALYSIS OF SMALL VOLUMES OF SERUM UNDER STERILE CONDITIONS.

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It has been found necessary during researches into the colloidal properties of blood-serum and of the component serum-protein fractions to have recourse to methods involving dialysis.

It was found [Vogé, 1928], when dealing with syphilitic serum, that the "syphilitic substance" could not satisfactorily be located in either the euglobulin or the pseudoglobulin fraction when Liefmann's method of "splitting" serum was adopted and that part appeared to exist in both fractions. Satisfactory results were obtained however when serum was placed in a collodion membrane and dialysed, the whole of the "syphilitic substance" being located in the fraction insoluble in water. In the method originally employed 10 cc. of serum were examined and dialysis had perforce to be continued for a considerable time to rid the serum of its salts, failing which complete separation was not obtained. This prolonged dialysis introduced other difficulties— increase in the fluid volume and bacterial contamination. These difficulties could be overcome by the use of a manometer and some antiseptic respectively, but it was considered feasible to attempt other methods involving smaller volumes of fluid and dialysis under sterile conditions, as the addition of antiseptics to serum may involve changes which will vitiate the experiment.

Methods involving electrodialysis have also been attempted, but great difficulty was found in regulating the temperature and so this method was abandoned.

A similar line of investigation has been adopted in order to determine in which serum fraction agglutinins can be isolated and it has been shown that they exert their influence in the euglobulin fraction [Vogé, 1929], a view which is in agreement with that advanced by Rosenholz [1928], and also by Kroeger and Hektoen [1927], the latter investigating the precipitin phenomenon. These authors state however that hydrolysis takes place and that the active fraction is liberated into the fraction soluble in water.

It is thus apparent that a simple and rapid method of dialysis under conditions as nearly as possible approaching sterility is required, especially in view of the use of such euglobulin-antibody fractions in prevention and treatment of disease.

The work of Rosenholz [1928], Cambessédès and Cochez [1928] and Vogé [1929] indicates that upon these lines there will be ground for a revaluation

of our present methods of preventive and curative medicine, since it has already been shown [Rosenholz, 1928] that such a fraction can be readily obtained and kept in a dry sterile condition.

EXPERIMENTAL.

The semi-permeable membranes are prepared from a solution which is made up as follows. 5 g. of pyroxylin are dissolved in 80 g. anhydrous ether and 20 g. absolute alcohol. This syrupy solution must be free from bubbles and must be kept in a wide-necked flask fitted with a rubber stopper. A Wassermann test-tube, $3" \times \frac{1}{2}"$, cleaned with aqua regia, washed thoroughly in water, alcohol and finally ether and dried, is used. It is necessary to observe these precautions or otherwise the membrane will adhere too firmly to the glass walls and cannot be separated without tearing. The tube is now filled with the pyroxylin solution, inverted, and the contents are allowed to drain. This process is repeated after the first coat has dried. Two coats of such a solution have been found to yield a membrane of sufficient strength and yet thin enough to allow rapid dialysis. The tube is now filled with sterile water and the sac gently separated from the glass walls with a blunt-pointed instrument.

A sterile 1 cc. pipette and rubber teat are now employed to withdraw 1 cc. of the serum to be dialysed. The point of the pipette is inserted within the mouth of the sac, the contents ejected and the tip of the pipette lowered until it is just below the level of the serum. In this position the sac is firmly bound to the pipette with linen thread and the pipette and sac are placed within a sterile bottle. The stopper fitted to the flask should be of rubber bored with three holes, one for the inflow of sterile water, the second for the pipette and the third for the outflow. It is an additional simplification if the stopper is already fitted to the pipette before the sac is fitted. The pipette and teat in the position indicated act as a manometer and so control the volume of liquid.

At the end of 18 hours a heavy flocculum, insoluble in water, is observed at the bottom of the sac; this is mixed with the supernatant fluid and the contents are withdrawn into the pipette by means of the teat. (Sterile pipettes are fitted with a plug of cotton wool at the upper end so that the teat can be

Fig. 1.

- A. Sterile distilled water.
- B. Rubber teat.
- C. Plug of cotton wool, sterilised with pipette.
- D. Sterile 1 cc. pipette.
- E. Outlet pipe dialysing apparatus.
- F. Sac, prepared as described in the text, bound to pipette so that the point is below the level of the fluid. At the end of 18 hours the solid water-insoluble euglobulin is observed at the foot of the sac.

removed to relieve the pressure and obtain suction.) The contents of the pipette are ejected into a sterile centrifuge tube and the solid removed from the liquid by centrifuging and decanting the supernatant fluid. The water-insoluble protein remaining is now emulsified in sterile saline and can be used for animal experiments.

Isolation of such material has successfully been carried out along these lines and subsequent injections into animals have been performed without complications due to bacterial contamination.

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