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Preparation of Stable Fibrinogen for Study of Streptococcal Fibrinolysin.

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In studies on streptococcal fibrinolysin it would be distinctly advantageous to have available a stable fibrinogen which could be maintained under storage conditions for a reasonable period.

Recently, Pomales-Lebron and Morales-Otero[1] have reported a technique for preserving whole plasma by a method involving freezing and desiccation in a high vacuum. Such preserved plasma, although shown to be suitable for fibrinolysis studies over a period of 8 months, must be secured from donors whose plasma is known to be susceptible to fibrinolysis. An obvious disadvantage of the method is that donors must be selected on the basis of a satisfactory preliminary test. Furthermore, we have observed that plasma clots much more slowly and undergoes lysis more slowly than does isolated fibrinogen.

Since the isolated fibrinogen fraction apparently does not carry the resistance factor which is present in many individuals, it is evident that pooled plasma from several individuals would constitute a satisfactory and convenient source of material if a method were available for preserving the isolated fibrinogen. Ferguson and Erickson,[2] in studies on blood coagulation, have preserved a highly purified fibrinogen, prepared from dog plasma, by a rather elaborate technic, involving prefreezing and subsequent desiccation in special apparatus. Fibrinolysis studies have not been reported with their product.

This report embraces the details of a simple method for isolating and preserving fibrinogen in such a state that it can be stored for considerable periods of time, maintaining all the while the ability to redissolve, and upon addition of thrombin, to yield a clot suitable for the determination of fibrinolysin in streptococcus cultures. The method is as follows: 1. Pooled plasma is diluted with an equal volume of distilled water, and filtered through a Seitz or Berkefeld

[1] Pomales-Lebron, A., and Morales-Otero, P., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 609.

[2] Ferguson, J. H., and Erickson, B. N., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 425.

filter. 2. The fibrinogen is precipitated by: a. Addition of one-fourth volume of saturated ammonium sulfate, or b. Addition of one and one-half volumes of saturated sodium chloride solution. 3. The resultant precipitate is separated by gentle centrifugation and placed in a Pyrex vacuum desiccator over phosphorus pentoxide. 4. The desiccator is then evacuated for one to 2 hours with a Cenco Hyvac pump. At the end of this time the stopcock is closed and the material allowed to remain in the desiccator overnight. 5. Upon removal, the dry material is easily broken up with a spatula or in a mortar. This dry material has been maintained at refrigerator temperature in a stoppered container for several months without evident deterioration.

In the above method, filtration serves to remove platelets and other solid material. In our experiments, satisfactory results have followed the use of either ammonium sulfate or sodium chloride as a precipitant. One precipitation with either apparently causes little or no denaturation of the fibrinogen. However, in the case of one lot of fibrinogen which was reprecipitated 6 times and then dried, the material, when redissolved, failed to give a satisfactory clot, indicating more extensive denaturation.

In this vacuum-desiccation, degassing and "snap-freezing" are accomplished in 5-10 minutes. The material has a porous and brittle consistency, (which probably facilitates resolution) and can be dissolved readily in M/100 phosphate buffer (pH 7.4) to yield a solution of the desired concentration.

Pooled, oxalated plasma, when stored for as long as 2 weeks before precipitation, often yields a product in which there is apparently some denaturation of the fibrinogen, since the dried material is somewhat less soluble and the resultant clot somewhat less firm. To compensate for this deficiency, it may be necessary to use more than the calculated amount of the dried material in order to make a satisfactory fibrinogen solution. In occasional samples we have noted the presence of some insoluble material which, while it does not interfere with fibrinolysin studies, may easily be removed by filtration if desired.

The dissolved fibrinogen clots readily (in a few seconds) upon addition of rabbit or human thrombin prepared according to the method of Tillett and Garner.[3] The clot is a stable one and does not undergo spontaneous lysis.

Tests were made with 40 strains of Group A streptococci, 2 of "Human" C, and 4 of Group G, using the technique of Tillett and Garner.[3] This technique involves the clotting of a mixture of di-

[3] Tillett, W., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

luted fibrinogen solution and streptococcus culture with thrombin, and the observation of subsequent lysis. Only one strain (Group A) failed to lyse fibrin clots from fibrinogen prepared in the above manner. This strain was also negative with a plasma clot. In tests made with known non-lysing strains of streptococci, the clots showed no sign of lysis after remaining overnight in the water bath. All other groups of streptococci tested, as well as several unidentified species of bacteria, failed to lyse the fibrin clot.

Preliminary experiments indicate that this fibrinogen preparation will also serve for the determination of staphylococcus coagulase.

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Effect of Adrenalectomy on the Growth of Mammary Glands in Underfed Albino Rats.*

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It has been found[1] that growth of hair is greatly accelerated by bilateral adrenalectomy in underfed rats. Since the mammary glands are similar in origin and location to the hair bulbs, the present experiments have been made to see if these glands, likewise, grow more rapidly after adrenalectomy in underfed animals.

After weaning at the age of 22 days, rats were isolated and thereafter fed 2 g of ground Purina dog chow each morning and 2 each evening. Rats gained very little weight on this quantity of food. Females were adrenalectomized via the dorsal approach usually when about 30 days old while unoperated litter mates served as controls. Three per cent NaCl solution was kept accessible for the adrenalectomized animals.

Usually 10 to 15 days after adrenalectomy, the adrenalectomized rats and their unoperated and underfed litter mate controls were killed and the skins of their ventral body walls were fixed in Bouin's fluid. The mammary glands were then dissected out, stained, cleared and mounted.

When the mammary glands (Figs. 1 and 2) of the adrenalectomized and control animals are compared, many more bud-like projec-

* I am indebted to Mr. Hobart Parkhurst for technical assistance in these experiments.

1 Butcher, E. O., *Endocrinology*, 1939, **25**, 787.