

The total thiocyanate and chloride available volumes of the normal cat are of similar magnitude. This distribution is not exclusively extracellular, inasmuch as CNS and chloride penetrate the red cell,¹¹ the digestive glands and are found in excessive quantities in the subcutaneous tissues of the skin (to be published elsewhere). In the muscles, chloride and thiocyanate are distributed through approximately the same volume of fluid. This volume differs from muscle to muscle and to some extent from animal to animal for any given muscle. It is not improbable that this distribution, at least in the thick muscles, is limited to the extracellular compartment.

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A Method for Preservation of Oxalated Plasma Clot for Fibrinolytic Tests.

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Oxalated plasma usually loses its ability to form a clot upon addition of calcium chloride when kept in the icebox for 72 hours.

We have been testing the fibrinolytic power of hemolytic streptococci immediately after isolation and at regular intervals thereafter to observe any changes in this property and its relation to other biological characteristics of the organism. A susceptible plasma clot from an individual was used repeatedly, necessitating a considerable number of bleedings, resulting usually in loss of time and material. For these reasons we decided to search for a method to preserve plasma while retaining its ability to clot readily and maintaining its susceptibility to lysis unaffected. This has been accomplished by the following method:

Human blood from a susceptible person was obtained and distributed in 5 cc amounts into small glass bottles, each containing 10 mg of potassium oxalate in the form of the dry powder. After mixing well by gentle shaking, the plasma was separated by centrifugation. The susceptibility of this plasma to the lytic action of hemolytic streptococci was tested, employing the original technic of

¹¹ Gregersen, M. I., and Stewart, J. D., *Am. J. Physiol.*, 1939, **125**, 142.

Tillett and Garner.¹ * A 16- to 20-hour-old culture in neopeptone broth containing 0.02% glucose to which a drop of defibrinated rabbit blood was added at the time of inoculation, was used in all the tests. Transplants were always made to this medium from the stock cultures on blood agar. From the growth thus obtained a second transplant to a tube of glucose neopeptone blood broth was made. The growth from this second transplant was actually employed in the test. Clotting took place within 10 minutes. Dissolution was complete in from 10 to 15 minutes.

The fresh plasma was distributed in 0.3 cc amounts into special all glass containers of 5 cc capacity.† The material was then dehydrated by the Cryochem process using the degassing-self-freezing procedure described by Flosdorf and Mudd.² The material was processed for 22 hours, at the end of which the tubes were sealed under the original vacuum. The dehydrated material was kept in the icebox.

We have had some difficulty in obtaining the material in perfectly frozen condition with the degassing-self-freezing procedure. The plasma had the porous appearance described by other authors in some cases, but in other instances it looked slightly gelatinous. We feel that in localities where dry ice can be obtained, initial freezing with it will greatly simplify the procedure and improve the solubility of the final product.

To perform the fibrinolytic tests enough distilled water was added to the tubes of dehydrated material to restore the original volume. Solubility was accomplished readily by gentle shaking. Samples of the plasma in the dehydrated form were tested at monthly intervals for its ability to form a firm clot and for its susceptibility to lysis. Similar technic and reagents as employed for testing the fresh plasma were used in all tests. The last sample tested (kept for 8 months in the icebox) clotted in 10 minutes, and dissolution was complete in 18 minutes.

The ability of plasma to clot after addition of calcium chloride and its susceptibility to lysis remained constant after 8 months when the material was dehydrated by the above procedure.

* Strain Co. of hemolytic streptococcus kindly sent to us by Dr. Tillett was used.

¹ Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

† Catalog No. 112—Cryochem Apparatus (Flosdorf-Mudd). F. J. Stokes Machine Co. of Philadelphia. A model No. 101 Cryochem apparatus was used.

² Flosdorf, E. W., and Mudd, S., *J. Immunol.*, 1938, **34**, 469.