Meeting of the
Committee on Research Projects
November 6, 1940 4:30 p.m.

Present: Dr. Stetson in the Chair
Dr. MacNeal, Dr. Ottenberg
Dr. Stetten, Dr. Corwin, Dr. Drew, Dr. Taylor
Miss Kivimaki, Recording Secretary

SURVEY OF PLASMA DRYING PROCESSES

Dr. Drew gave the following account of his visit to Philadelphia and of his study of the dried plasma situation:

The drying of human plasma or serum for storage or shipment has certain advantages which, from the point of view of this Association's present undertaking, are worth real consideration. These advantages are:

1. The more complete stability of the plasma or serum for long periods of time.

2. The ease of handling and the prevention of loss from breakage, especially under war-time movements.

The method of drying sera from the frozen state has been successfully used in bacteriology for preserving the complement for a period of about ten years. James A. Graigie (Brit. Jour. Exp. Path. 12:75: April 1931) reported on the method of drying complement from the frozen state which forms the basis of all of this work. At a decidedly later date Reichel, Mascucci and Boyer (Jour. of Immun. 1935) introduced the term lyophile, meaning solvent-loving, and broadened its connotation to mean specifically the rapid dehydration of biological products from the frozen state in a high vacuum. Since that time modifications of this fundamental method have been presented under the name of the Cryochem process (E. W. Flosdorf and S. Mudd, Jour. of Immun. 34:469:1938). The word itself is meant to denote dessication in vacuo from the frozen state by means of chemicals. In contradistinction to the earlier process, the dehydration is carried on through the medium of $CaSO_4$. Certain advantages were reported for this change in technique. The reasons for the change were: the elimination of the cost of dry ice used around the container of the original lyophile apparatus, and the operation of the machine requires less care. Later a third type of pump, the Desivac system, was developed with the idea of simplifying the operation, decreasing its cost and increasing the capacity. (Flosdorf, Stokes and Mudd, J.A.M.A. 115:1095: Sept. 28, 1940). One of the chief advantages of this pump, in the opinion of its creators, is that it will operate with much less vacuum pressure than the other two forms and that its pressure is created more rapidly.

Because of the confusion that has been added to this picture of drying plasma by the disagreement on patent rights, and by discussions of priority in the development of certain parts of the process, it was felt that a first hand observation of the various processes would clear the picture for us.

To that end, on November 2nd we spent the afternoon with Dr. Aims McGuiness of the Philadelphia Serum Exchange, which is situated at the Childrens' Hospital, 1740 Bainbridge Street, Philadelphia. Dr. McGuiness uses paid donors from whom he takes 250 cc of blood in a closed technique at each phlebotomy. The blood is allowed to clot, the 2-hole stopper is removed in a dust-proof ultra-violet

lighted room, the bottle is flamed, and a solid rubber stopper is inserted. This blood is then allowed to stand for 24 hours in an ice box at a temperature of 4 degrees. At the end of this time the clot is broken up, and after centrifugation for 20 minutes in a #2 International Centrifuge, the plasma is drawn off and re-centrifuged for another 20 minutes. The plasma is then placed in square storage bottles and samples from each bleeding are tested for sterility. Immediately following the transference of the serum from the centrifuge bottles to the small individual storage bottles, the serum is frozen in CO_2 and methylsol mixture and put into an ice box at 5 degrees until ready for pooling. When pooling is to be done, the frozen serum is allowed to thaw at room temperature. Following the pooling of 15-30 bloods, the serum is re-distributed into 50cc ampules which are then rapidly frozen on a "Shelley" machine, which consists of a manifold arrangement of revolving suction nipples on an endless chain, while the ampules are revolved in a dry ice and methylsol mixture. This pre-frozen serum is then placed in the vacuum chamber of the Cryochem Process, the vacuum is turned on, and drying proceeds at a rate commensurate with the amount of $CaSO_4$ in the machine. Each 100 lbs. of $CaSO_4$ are capable of absorbing the moisture from two litres of serum. At the end of this time hot air (180 degrees) is blown through the $CaSO_4$ to free it from its moisture and prepare it for subsequent use. This machine has a capacity of about 5 litres in 24 hours. When the material has been dried, the glass ampules are plugged with vaccine caps and as an added factor of safety, enclosed in hermetically sealed tin containers to prevent the absorption of air through the rubber caps. The costs in this process are of particular interest. Under the present set-up, if the whole laboratory staff were working continuously, one litre of normal serum costs approximately $100 to process and dispense in 10cc vials. The itemized cost is approximately as follows:

Labor	$20.00
Material	
Distilled water	8.80
Vials and labels	5.00
Files	.40
Instruction sheets	2.00
Boxes	.75
Desitubes	3.50
Dry ice	1.00
Donors' fees and serum tests	60.00
	$101.45

At the present rate of production on its small scale, the actual cost is now between $160 and $180 per litre of processed plasma.

Even under ideal full-time working conditions and at no cost for donors and serum tests, serum treated in this manner would cost between $40 and $50 per litre. The Cryochem outfit for small-scale production can be created at a cost of about $1,000 per litre unit. To handle 100 litres a day would require at least 30 such units and perhaps 40. Certain technical details have been added to the original machine as put out by F.J. Stopes Machine Co. by C.H. Barr of the Sharp and Dohme Co. in order to facilitate the regeneration of the dessicant. After the inspection of this plant, which is very well constructed and well run by an enthusiastic and able person, one is left with the feeling that even on a small scale, to do the processing well is a relatively expensive procedure.

The morning of Monday November 4th was spent at the Bryn Mawr Hospital with Dr. Max Strumia who has been actively engaged in the study of plasma and serum for about fourteen years. Dr. Strumia uses for his drying process an adaptation of the original lyophile process which has been created for him by Dr. John Reichel, formerly of the Mulford Laboratories. Drying is carried out from the frozen state in a single drying chamber condenser unit operated under high vacuum and low temperature. With this apparatus working 8 hours a day, he feels that he can handle 150 phlebotomies of 500ccs each a week. That is a total of 35-40 litres of plasma a week, or close to one litre an hour. In his apparatus the heavy brass vacuum pump is surrounded by a water jacket in which the water is kept at a temperature of 37 degrees C. The plasma is put into this chamber in a pre-frozen state at a temperature of about -40 degrees. The condenser is submerged in a mixture of carbon dioxide ice and methyl cellosolve. A vacuum of approximately 110-150 micromillimeters of mercury is created by a one-half horse power pump. At the end of this drying process the plasma is guaranteed to have a composition which included protein of at least 7%, prothrombin of 75-80%, fibrinogen 60% and complement 90%. When, in order to reduce the bulk of the final product, the first powder is reconstituted and then lyophiled a second time, there is considerable loss in the complement, fibrinogen and prothrombin content as well as a lessening of solubility. For the treatment of shock these losses are offset by the compactness of the material and the greater ease with which it can be transported. For ordinary clinical work the secondary condensing process is inadvisable. Dr. Strumia felt rather strongly that plasma was definitely superior to serum because of the former's freedom from reactions, either the direct reaction in the form of pain in the chest and back accompanied by chills and fever, or the delayed reaction occurring in 7-10 days in the form of "serum sickness", which is characterized by urticaria, oedema and general malaise.

Several parts of the equipment at Bryn Mawr are worthy of special note. The large #3 International Centrifuge with specially built trunion cups capable of holding 800cc of plasma at a time, gives after centrifugation the largest yield of plasma-citrate mixture we have seen, amounting to better than 60% of the total blood volume. These caps will be available for use in other laboratories at an early date. The apparatus for pooling plasma consists of a long steel needle built on the principle of a catheter, with a somewhat sharpened but closed end, the inlet being situated about one quarter inch proximal to the tip, this allowing the needle to touch the cells yet not sucking them over. This needle is held in position by a piece of glass tubing which is constructed in two pieces to make a sort of runway for about two inches, the rest of the needle being enclosed in a sheathe of soft rubber, the lower end tied to the glass scabbard and the upper end to a spot about one inch below the upper end of the needle. This whole apparatus can be autoclaved and does for a closed method for obtaining plasma when the paragon rubber for one of the glass rods in the 2-holed stopper is used as a membrane and punctured. The small containers for shipping the concentrated form of dried material are those developed by the Sharp and Dohme Co. known as the Vacule ampule, which is capable of being stoppered and then sealed with a flame. Some of the single processed material was brought back to Dr. Scudder for clinical trial.

Following the visit to Dr. Strumia, we saw Mr. C.H. Barr of the Sharp and Dohme Co. Dr. Joseph Schneider, Director of Production of the Mulford Plant, and Mr. Barr were kind enough to show me through their entire plant, where the lyophile process is used on a massive scale in the production of biologicals of all types. The complete plant is refrigerated in that section where this work

is done. A specially built serum centrifuge, holding litre trunion cups, is used in the centrifugation process. A huge manifold, capable of taking about ten 5-litre cans of material at one time for drying, is attached to a condenser which is maintained at a temperature of -120 degrees C. by a refrigerating system which utilizes ethane as the refrigerant in association with their ammonia ice-making plant. The purpose of installing this system of refrigeration was: first, to save expense of the tremendous quantities of carbon-dioxide snow necessary for this volume of work, and secondly, because of the lowered temperatures obtainable by this method. All of the serum is filtered through Berkfeld filters by one man who has done the same job for 31 years, which probably accounts for the fact that their infections are very few. This material, which is frozen at the Glen Olden plant, is sent on to the main labs of the Sharp and Dohme Co. in Philadelphia, where Dr. William A. Feirer is the Medical Director. Here, in dust-proof, air conditioned ultra-violet lighted rooms, their technicians allow the frozen material to thaw out before running it through culturing processes or reconstituting the bulk lyophilized serum before lyophilization in the ampules for dispensing. This final process of drying is carried out in a rather large unit consisting of ten vacuum chambers, each capable of handling 14 litre bottles (7 litres of plasma) at a time, with a processing time of 60 hours. These vacuum condensers are all attached to a single huge condenser. Refrigeration is again supplied by a 1½ ton refrigerator which utilizes ethane as a refrigerant. (This refrigerator is made by the Winkler Ice Machine Co. of Philadelphia). In the early steps of the drying process in this plant, chloroform is used as an antiseptic because it is felt that by the time the process is complete it will have completely disappeared, but in the early stages offers very good protection. Merthiolate 1-10,000 is added to the serum before final lyophilization. Each container is tested for a vacuum before it is released by a Lepel high frequency coil. In a vacuum under such treatment a phosphorescent glow is seen which will not appear if the air is present. For large production this apparatus seems to be the best available at the present time.

At the University of Pennsylvania Medical School in the Department of Bacteriology, Dr. E.W. Flosdorf has in operation at this time three types of drying machines. The first is the lyophile machine which utilizes a manifold with many outlets for handling small containers, carbon dioxide ice and methyl cellosolve being used as a refrigerant around the condenser. The vacuum is created by a Cenco Hyvac pump equipped with a pressure gauge of the McLoed type. The critical pressure in this apparatus is reached within 2-7 minutes after starting the pump. The optimum working range of 0.015 to 0.050 mm of mercury should always be reached in at least 20 minutes. About 90-96% of the water can be removed by this system. Such an apparatus with 50 outlets for containers can process an average of one litre per days for 4 days in a five day run. The new apparatus by Sharp and Dohme is definitely superior to this type and Dr. Flosdorf does not use it at the present time, but has gone over to the more constant use of the Cryochem process which is similar to that described in the visit to Dr. McGuiness.

The Desivac apparatus is the latest refinement in the freezing of blood. Here pumps of large volumetric capacity which circulate the oil used in the vacuum seal, rather than ordinary pumps which operate in a static bath of oil, are used. The oil from the exhaust or the atmospheric side of the pump is passed continuously through a device for separation of water from oil in the manner of a reversed cream separator. In this apparatus de-gassing or self-freezing may be simplified as in the Cryochem apparatus, or the materials may be pre-frozen before evaporation is started. The pump may be used with either

a manifold, a vacuum chamber or an autoclave-like oven such as that which you recently supplied to Dr. Best. This latter apparatus utilizes flat pans for the drying process, each containing a thin layer of the serum in containers covered like a petrie dish, with a small outlet for the loss of water vapor. Each flat pan sits on a water bath which is kept at a temperature of 37 degrees. The great advantage with this pump is that it reaches an optimum vacuum almost immediately and will work effectively at a level of 1.5 to 2mm of vacuum, which is a much higher level than is possible for the other two types of pumps to function at. Mr. Barr is of the opinion that eventually some of the water will not be removed by the separator and will return to the motor of the pump and lessen its efficiency. If this does prove to be so, it is the purpose of Dr. Best and Dr. Solandt to include a condenser between the drying oven and the pump, and thereby eliminate this defect.

In summary, it would seem that at the present time the Cryochem process is the least adaptable for large scale use. The Desivac apparatus has not had sufficient trial and the very large lyophilizing plant at Sharp and Dohme is the most suitable for immediate use. In the light of these observations it would seem that if the simple type of apparatus now being operated by Dr. Rhoads and Mr. Folsom at the Memorial Hospital could be perfected, it would prove as effective or more so, than any of the apparatus now in use and considerably cheaper. The large machine now being perfected by Mr. Folsom, with a capacity of approximately one litre per hour, is the fastest we have seen. It has yet to be demonstrated that it can work on long continued runs with efficiency.

REQUESTS FOR GRANTS

From Dr. Rhoads

After general discussion, it was voted that the request of Dr. Rhoads of November 1, 1940 for a grant of $1200 for the development of drying apparatus for plasma or serum be approved and recommended to the Board of Medical Control.

From Dr. Rosenthal

Re: Dr. Rosenthal's request for a grant of $1000. for the purpose of "continuing investigation with respect to a comparison of the effects of plasma and serum in the fluid and dried states as against blood",

It was the consensus of opinion that the proposed experimentation is of undoubted value, but that as it is being carried on in several institutions, Dr. Rosenthal be requested to furnish more specific information regarding the work he wishes to do and the reasons for it than is contained in his letter of October 24th before a grant can be considered.

From Dr. Vogelaar

Re: Dr. Vogelaar's request for a grant of $2500 to $3000 for experimental work in using various media, mixed with whole blood, for transfusion purposes;

It was voted not to approve this request as the proposed research is outside the present scope of the activities of this Association.

It was voted that the action of the Committee on the request of Dr. Rhoads be submitted to the members of the Board of Medical Control in writing, with the request that they express their opinions by November 8th, the date of the meeting of the Board of Trustees.

DISPOSAL OF OLD 250cc BAXTER BOTTLES

Dr. Drew reported that some hospitals have asked for the used Baxter bottles. Dr. Stetten stated that he would undertake the responsibility of authorizing that 250cc Baxter bottles, after use, should be given to those hospitals which have requested them.

The meeting adjourned at 6:00 p.m.

Recording Secretary