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THE CONCENTRATION AND DRYING OF PLASMA

BY

F. X. AYLWARD, B.Sc., Ph.D.

Research Biochemist

B. R. S. MAINWARING, M.A., B.Sc.

Research Biochemist

AND

JOHN F. WILKINSON, M.Sc., Ph.D., M.D., F.R.C.P., F.I.C.

Honorary Physician in Charge and Director

Department of Clinical Investigations and Research, University and Royal Infirmary, Manchester; Manchester and Salford Emergency Blood Transfusion Service

During the past few months the efficacy of plasma transfusions in the treatment of certain conditions, and especially in cases of shock and burns, has resulted in the formation of plasma storage banks. Consequently much interest has now developed in the possible use of dried plasma, since it is known that if the desiccation is carried out under suitable conditions the resulting powder redissolves in distilled water, reconstituting the original plasma.

Advantages of Dried Plasma

Several advantages are claimed for such a dried product. They are:

1. *Stability.*—Many experiments on proteins, including anti-toxins, etc., have shown that dried protein material stored *in vacuo* can be kept for very long periods without any demonstrable changes in properties. Thus it is probable that the dried plasma proteins can be kept almost indefinitely even at room temperature. "Liquid" plasma is, of course, relatively stable at room temperature and keeps well, especially if it is placed in the dark; but for prolonged storage a refrigerator is technically desirable, although not essential.

2. *Economy of Storage Place.*—Five to ten times the amount of plasma can be kept in a given space if it is in the dried form, and this is a matter of great importance when considering the refrigerator or storage space available in a large blood bank. It must, however, be stored in appropriately small containers.

3. *Ease of Transport.*—The stability of the dried product at room temperature and its smaller bulk are of importance, since the transport of large volumes of water and the necessity for mobile refrigerating units are avoided.

4. *Preparation of Concentrated Plasma.*—Recent work has shown that there may be many advantages in the use of concentrated plasma, which can readily be obtained by dissolving the plasma powder in sterile distilled water to give the required concentration.

On the other hand, we must offset against the above advantages the difficulties and expense of the drying process, the relative stability of ordinary liquid plasma, and the fact that in many centres there will be a sufficiently rapid turnover of plasma in the bank to discount the necessity for storage for more than a few weeks. Nevertheless dried plasma is undoubtedly of use in wartime for building up local reserves and for transport by sea and air in response to emergency demands, provided adequate

facilities exist for reconstituting the plasma when required. Its use as a source of concentrated plasma may become much more widespread when further clinical reports are considered.

Methods Available for Drying Plasma

Any method for drying plasma must fulfil certain conditions:

1. *Temperature Control.*—The drying must be carried out at a low temperature, in no circumstances greater than 37° C. and if possible below 0° C., so that denaturation of the plasma proteins cannot take place. It may be observed that denaturation is one of the most rapid of chemical reactions: whereas the speed of most reactions is doubled with a 10° C. rise of temperature the rate of denaturation is increased at a much greater rate (Schmidt, 1938). Even a small amount of denaturation renders plasma unsuitable for clinical use, and therefore it is impossible to place too much stress on the necessity for adequate temperature control of the drying process.

2. *Solubility.*—The dried product must dissolve readily and completely in sterile distilled water. The speed of solution appears to be related to some extent to the temperature of the final drying process: plasma dried from the frozen state usually yields a bulky porous powder which is readily soluble in water.

3. *Sterility.*—The dried plasma powder must be sterile. Sterility is achieved usually by obtaining plasma from blood under sterile conditions or by subsequent sterilization through a Seitz filter; the plasma is then kept sterile during the drying process and the powder must be sealed off in the vessel in which it is dried or, alternatively, transferred with suitable aseptic precautions to storage ampoules. If the plasma is dried and stored in its original container no economy of storage or transport space is effected. The problem of sterility is undoubtedly a difficult one.

The above conditions are necessary whatever the scale of the process, but when large volumes have to be dried certain other factors also assume importance. The plasma should be dried as rapidly as possible, with the minimum of labour and attention and the minimum capital outlay and running expenses.

In the method of drying developed by Flosdorf and Mudd (1935) for the production of lyophil serum the serum is frozen in ampoules and the water is distilled off under high vacuum into a vessel cooled in solid

carbon dioxide and methyl cellosolve (temperature about $-70^{\circ}\text{C}.$). This type of apparatus is in general use in bacteriological laboratories, and in the simple form it is capable of drying about 100 c.cm. in ampoules in each twenty-four hours. Forms of this apparatus to deal with much larger quantities have been described. Later Flosdorf and Mudd (1938) developed their "cryochem" process, in which the frozen serum was subjected to a high vacuum, the water being distilled over and absorbed in specially prepared calcium sulphate. For the desiccation of 1 litre of serum 100 lb. of calcium sulphate is required, but this can be regenerated under suitable conditions. Knox (1939) has used a method similar to the lyophil method for the desiccation of various biological materials, and in his apparatus the rate of removal of the moisture appears to be about 100 c.cm. in twenty-four hours.

Greaves and Adair (1936) have described a simple method for the desiccation of small amounts of serum, using a high vacuum and phosphorus pentoxide as a desiccant; later (1939) they described a large-scale apparatus which is capable of drying 5 litres, either in bulk or in ampoules, the serum being frozen and the moisture distilled into a condensing system cooled with refrigerator coils at $-45^{\circ}\text{C}.$ Their paper contains a very full description of the physico-chemical aspects of the drying process. From the accounts given by the different workers it would appear that there is no difficulty in producing small amounts of sterile dried serum on a laboratory scale, but the drying of large volumes requires considerable capital expenditure in apparatus.

The above methods, which were all developed for the desiccation of serum, can also be employed with plasma.

In view of the possible importance of dried plasma or serum under the present emergency conditions it seemed desirable to investigate the subject further, the object being to devise a method for the concentration and drying of relatively large volumes with an apparatus that could be operated simply and cheaply in connexion with any blood bank.

Recently Edwards *et al.* (1940) have described a method of drying based upon the method of spray distillation *in vacuo*. Our own experiments have been founded on a similar principle and also on the principle of evaporation through a cellophane membrane.

Concentration and Drying of Plasma by Spray Distillation in Vacuo

We have been concentrating large volumes of liquids at temperatures below $37^{\circ}\text{C}.$, using the Reich apparatus, and this appeared to offer certain advantages over the apparatus of Edwards *et al.* for the concentration and desiccation of plasma. The apparatus shown in Fig. 1 is similar to that designed by Reich, except that the double glass water-pump has been replaced by a highly efficient water-pump made from a modified steam injector. Part of the apparatus (from D to K) was sterilized by autoclaving and was then attached to the condenser (B) and to a bottle of sterile plasma (L). The water bath surrounding the flask (J) was heated to $80^{\circ}\text{C}.$ and the plasma was drawn slowly in a fine spray into the distillation flask. In the initial stages the rate of evaporation was so great that despite the high external temperature the temperature inside the flask never rose above $20^{\circ}\text{C}.$ Careful control of the rate of inflow was necessary to check the violent frothing which took place owing to the liberation of dissolved gases. It is desirable to have the minimal amount of fluid in the flask and to modify the rate of inflow to equal the rate of distillation. In the final stages of drying the diminished rate of evaporation

resulted in a smaller temperature gradient between the two surfaces of the distillation flask, and the water bath was accordingly cooled at $35^{\circ}\text{C}.$

In this way 500 c.cm. of plasma was concentrated to about 50 c.cm. in thirty minutes (bath temperature $80^{\circ}\text{C}.$), and the concentrate was then reduced to a dry solid in a further sixty minutes (bath temperature $35^{\circ}\text{C}.$). The flask was then disconnected from the apparatus, plugged with sterile wool, and well shaken to break up the dried solid adhering to its walls (this is facilitated by the

FIG. 1

FIG. 1.—A, Rubber connexion to pump. B, Double-walled condenser. C, Rubber connexion. D, F, G, H, Ground-glass joints. E, Tap and sterile filter. J, 500-c.cm. distillation flask. K, Tap and screw clip. L, Plasma.

FIG. 2

FIG. 2.—A, Hot-plate. B, Air inlet. C, Cellophane tubes. D, Fan. T, Thermometers.

addition of sterile beads to the flask). In this and similar experiments yields of 8 to 10 grammes of solid were obtained from 100 c.cm. of plasma. The light yellow solid so obtained is readily soluble in sterile distilled water, giving a clear solution similar to the original plasma.

Although the results obtained were satisfactory the method was not without its difficulties. Constant attention was required to control the rate of spraying and the external temperature. The frothing in the initial stages was troublesome, but it can be controlled in large part by preliminary degassing, as suggested by Hartley (1936). It was found necessary to limit each batch to 500 c.cm. of plasma with the flask employed (500 c.cm.), as with larger amounts the layer of material in the flask did not dry easily. Undoubtedly one of the main drawbacks to the method is the necessity for resterilizing the apparatus after each batch of 500 c.cm. Thus the apparatus, in its simple form at any rate, is not suitable for operation with very large volumes of plasma.

The difficulties of temperature control can be overcome to some extent by a modification of the condenser, enabling it to be cooled with solid carbon dioxide in a solvent or by refrigerator coils. Alternatively the flask containing the concentrated material can be removed under sterile conditions and attached to a manifold of the Flosdorf-Mudd type of apparatus. With either of these modifications the dried plasma has to be removed from the 500-c.cm. distillation flask, which is unsuitable as a storage vessel.

In order to overcome some of the difficulties in the above process we have carried out the drying in two stages. In the first of these the plasma was concentrated to one-quarter or one-fifth of its volume in the Reich apparatus as above, but without special sterile precautions. In the second stage the concentrate was sterilized by passage through a Seitz filter, collected in batches in sterile ampoules, and dried, as described later, using the Flosdorf-Mudd lyophil technique. We have prepared dried plasma by this technique, but the rate of filtration

of concentrated plasma varies greatly from one batch to another (probably on account of the variations in fat content), making such a process unsatisfactory as a routine procedure. While investigating this matter further we tried out a different and simpler technique, described below, by which the plasma can be concentrated under sterile conditions.

Concentration and Drying of Plasma by the Cellophane Tube Method

The process of dialysis depending on the passage of water and other substances of low molecular weight through a cellophane membrane is a familiar laboratory process; it does not appear to be so well known that when a cellophane bag containing a solution is suspended in air a rapid concentration of the solution takes place, due to the passage of fluid through the membrane followed by evaporation at the surface. This phenomenon was first described by Kober (1917, 1918), and it has been used for concentrating various biological solutions, including serum (Thalhimer, 1938; Terry, 1939).

We have applied this method to the concentration of plasma. Cellophane tubing 2.6 cm. in diameter was cut into 100-cm. lengths and one end of each was tied tightly with fine string or thread. Into the other end a 5-cm. length of glass tubing of slightly smaller diameter was inserted and similarly tied. The glass was covered with grease-proof paper and the whole apparatus was sterilized by autoclaving for thirty minutes at 15 lb. pressure. After being allowed to cool the cellophane tube was suspended, sterile plasma was poured in through the glass tube, and the cellophane was then tied off a few centimetres below the glass, which was now cut off. A number of such tubes were suspended in a current of warm air as shown in Fig. 2. The temperature at the bottom of the tube was not allowed to exceed 35° C.; at the top it was considerably less, while inside the cellophane tube it was 5° to 10° C. less than the corresponding external temperature, due to the cooling effect of evaporation.

In this way 500 c.cm. of plasma in each tube was concentrated to about 100 c.cm. in forty-eight hours. During the process the more concentrated material settled to the bottom, and it was therefore of advantage to invert the tubes occasionally. The sterile plasma, thus concentrated to 20% of its original volume and containing 50% of solid, was either stored for clinical use or dried by the Flosdorf-Mudd lyophil method.

Some experiments were carried out in which the process of concentration was continued further to obtain a dry product. A resin-like solid was obtained, as compared with the porous powder which resulted from the other methods. This solid did not appear to be perfectly dry, and some difficulty was experienced in removing it from the tube. Various modifications of the apparatus, including one wherein the cellophane tube can be rotated horizontally, have been tried to improve the final product.

The cellophane tube method appears to be more suitable for the preparation of concentrated plasma and serum rather than of a dry product, and for this purpose it possesses many advantages over other methods. The apparatus is simple and cheap and can be installed in any laboratory on almost any scale. Unlike some of the other methods proposed, it involves little capital outlay, little personal attention is required during the process, and the running costs are low. As cellophane will not allow large molecules to pass through there is no danger of bacterial contamination during the process of concentration, providing sterile precautions are taken at the beginning and the ends of the tubes are tightly sealed.

The rate of evaporation depends upon a number of factors, the most important being the surface area of the tube, the external temperature, the humidity of the air, the efficiency of the fan, and the thickness and porosity of the cellophane. The rate given above can be increased by using tubing of smaller diameter, but this may not be so convenient. A simple method of setting up a large battery of tubes is to use a well-ventilated fume cupboard, with a suitable arrangement for the air intake to pass over a hot-plate or other simple heating device. (If the atmosphere is too humid calcium chloride trays may be required to dry the air before drawing over the tubes.)

We have dried the concentrated plasma obtained both by the spray distillation process and by the cellophane tube process described above, using the lyophil technique developed by Flosdorf and Mudd (1935). The details of the method need not be described here, as they have been treated fully by Flosdorf and Mudd (1935), Knox (1939), and Greaves and Adair (1939). The size and design of the apparatus depend on the quantities to be dried. In one experiment 80 c.cm. of plasma concentrated from an original volume of 500 c.cm. by the cellophane tube method was divided between two flasks and frozen in a mixture of solid carbon dioxide and alcohol, the flasks being rotated to get an even film of solid round them. The two flasks were then attached to a condenser cooled in carbon dioxide and alcohol and evacuated, using a Cenco Hyvac pump. In thirty hours a completely dry light solid was obtained that was readily soluble in water.

The apparatus we used was one usually employed for the drying of small amounts of serum or bacterial suspensions, and the rate of drying of the concentrated plasma was undoubtedly lower than that of normal serum. The rate of drying of concentrated plasma would be expected to be less than that for solutions with a lower solid content; the presence of sodium citrate and sodium chloride is also an important factor. We have no doubt, however, that with suitable modifications of the simple apparatus we used the speed of drying can be accelerated.

Discussion

The plasma employed in the above experiments was obtained from blood collected in a citrate-saline mixture (430 c.cm. of blood to 70 c.cm. of 2.5% sodium citrate and 0.85% sodium chloride), the plasma being separated off from the blood under sterile conditions after two days' storage at 4° C. Normally, in order to delay the onset of haemolysis, glucose is added to the blood which is to be stored for whole-blood transfusions (Aylward *et al.*, 1940), but in these present investigations it was omitted since preliminary experiments had indicated that the presence of glucose retarded the drying process. Furthermore, if plasma is separated after two days the amount of haemolysis, even in the absence of glucose, is negligible.

The desirability of separating plasma as soon as possible, so that it will have the minimal potassium and phosphate contents, has already been stressed (Mainwaring *et al.*, 1940). This precaution is considered to be particularly necessary in view of the large amounts of plasma now recommended for use in each case (see Medical Research Council War Memorandum No. 1, 1940).

While the clinical effects of serum and plasma transfusions are probably similar (Magladery *et al.*, 1940) there are certain advantages in collecting serum rather than plasma. Serum will dry somewhat more easily than plasma owing to the absence of fibrin, sodium citrate, sodium chloride (and possibly glucose). Further, the absence of fibrin removes the danger of clot formation both before drying

and after reconstitution. On account of their relative simplicity and cheapness we hope that the methods described here will be of use in laboratories where expensive large-scale installations are not possible; so far as we can judge, the final dried products are similar to those obtained by the lyophil process.

Recent clinical reports on the use of concentrated plasma and serum are very promising (Medical Research Council War Memorandum No. 1, 1940), and the cellophane tube method should be of great help in the preparation of such concentrated plasma and serum, which can be prepared without risk of contamination and stored in any plasma bank. Many of the advantages claimed for dried plasma also apply to concentrated plasma. Moreover, concentrated plasma is prepared with considerably less risk of bacterial contamination and much more easily than is the dried form, and can be diluted to the required strength without the short delay necessary for the solution of plasma powder.

The methods we have described for the preparation of concentrated and dried plasma are capable of many modifications, according to the scale on which the processes are to be carried out, and in view of the importance of the matter at the present time we have thought it wise to put forward our preliminary results at this stage. A detailed clinical report on the use of dried and concentrated plasma will be published later.

Summary

Two methods for the concentration and drying of plasma have been described: (1) using spray distillation *in vacuo*; (2) using the principle of evaporation through cellophane tubes under sterile conditions, followed by low-temperature drying of the concentrate.

These methods enable large amounts of plasma to be concentrated and dried without undue cost.

Serum may also be concentrated or dried by these methods, and may prove more satisfactory than plasma.

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F. G. Chandler, S. R. Gloyne, and D. V. Hubble (*Tubercle*, 1940, 21, 292) record the case of a female infant, aged 12 weeks, who had difficulty in breathing since birth, and was admitted to hospital for increasingly frequent attacks of cyanosis. Death took place two days later. The necropsy showed that the right lung had several shallow grooves suggestive of imperfect accessory lobulation, and that the greater part of the lung was involved in a cystic condition with a disorderly arrangement of the bronchi. Histologically the cystic condition was due to an abnormal arrangement of air spaces and bronchi.

DYSMENORRHOEA AND STERILITY PERSONALITY STUDIES

BY

E. WITTKOWER, M.D., L.R.C.P. & S.Ed.

Physician and Halley Stewart Research Fellow, Tavistock Clinic, London

AND

A. T. M. WILSON, B.Sc., M.D.

Physician and Rockefeller Research Fellow, Tavistock Clinic, London

Informed gynaecological opinions abound to the effect that personality factors are related to dysmenorrhoea and sterility in some way as yet undetermined, while some gynaecologists have gone further and suggest that psychological factors may actually be of primary importance in the production of both complaints. Conversely, many medical psychologists have noted that dysmenorrhoea and sterility are common constituents of various syndromes which are predominantly psychological, and that where psychological treatment is successful these gynaecological symptoms often disappear simultaneously with the others.

The patients to be described were submitted to a biographical study, occupying on an average two hours, covering the family background and, in rather more detail, the childhood characteristics, the reaction to various phases of adolescence, and the adult personality. Biographical studies of this type were made of 57 unselected patients with primary dysmenorrhoea, aged 16 to 40, 31 of them single; and of 30 patients with sterility of some years' duration, aged 21 to 45, similarly unselected. A suitable control group was found in 30 primigravidae, aged 19 to 40, who had never suffered from dysmenorrhoea; they were studied in an identical manner. Sedentary workers formed approximately half of each of the three groups. Complete verbatim reports were kept of each interview, and the data thus obtained from each group were later submitted to more detailed analysis and comparison (Table I).

TABLE I.—*Childhood Adjustment in Cases of Primary Dysmenorrhoea and of Sterility*

	Control (30 Cases)	Sterility (30 Cases)	Dysmenorrhoea (57 Cases)	
			Proportion (in 30 Cases)	Actual
Apparently well adjusted	19	9	5	9
Fairly well adjusted (e.g., concealed shyness)	8	7	7	13
Definitely ill adjusted:				
(a) Boisterous tomboy	2	3	11	21
(b) Resentful, aggressive				
(c) Miserable, non-aggressive				
(d) Miserable, ailing	1	11	7	14
(e) Exceptionally timid and shy				

Childhood Adjustment

It will be seen from the table that the control group of 30 normal primigravidae is mainly composed (27 out of 30) of women who give a history of having been as children well-adjusted or fairly well-adjusted types, familiar in the general population. The term "ill adjusted" was confined to children whose abnormality of emotional status was beyond discussion.

In the two groups who later complained of sterility and dysmenorrhoea there is a high proportion of ill-adjusted children as compared with the control group. Equally striking was the difference between the control group and the others in the degree of maladjustment of any particular type—for example, in the control group the