AN APPARATUS FOR SPIN-FREEZING SERUM OR PLASMA IN CIRCULAR BOTTLES

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(With 5 Figures in the Text)

A TECHNIQUE for drying serum proteins from the frozen state has been described by Greaves & Adair (1939). For war requirements the procedure has been to dry in 12 oz. medical flats into each of which is put 200 c.c. of serum. The bottles are then placed in a -20° C. cold room on shelves slightly inclined to the horizontal. The inclination is to allow the maximum quantity of serum to be dried consistent with the exposure of a reasonable surface area from which to dry. This method of freezing produces a wedge-shaped block of serum in the bottle (Fig. 1) and is very efficient. Large amounts of serum for the use of the Army have been dried in this manner by Greaves and his colleagues.

Fig. 1. Sketch showing the wedge-shaped appearance of the frozen serum in the 12 oz. medical flat as at present in use.

From the point of view of the fighting services there are, however, a number of practical objections to the drying of serum in this form, and it would be a considerable advantage if serum could be dried in larger quantity in the round, waisted pint bottle which is standard for the issue of all transfusion fluids. In the first place, the 12 oz. medical flat contains only 200 c.c. of the dried product and a single case requiring transfusion may need as many as ten bottles which are only one-third full of the product to be transfused. In the second place, the Army has to operate in situations where the preparation of distilled water for the reconstitution of the dried product is impossible. In these circumstances, it is necessary to send with each bottle of the dried product a bottle containing enough suitable distilled water for reconstitution. Hence

Fig. 2. Sketch showing the appearance of approximately 400 c.c. of serum in a standard pint transfusion bottle, inclined at an angle so as to give the maximum surface. The thickness of the wedge at the bottom will be noticed.

Fig. 3. Sketch showing the appearance of 400 c.c. of serum spun-frozen in a standard 1 pt. blood transfusion bottle.

the bulk to be transported is very large. In the third place, the standard pint bottle in which all other transfusion fluids are transported necessitates the use of a rubber bung and apparatus for administration which can be used for any transfusion fluid, and with which the whole Army is familiar. A 12 oz. medical flat, on the other hand, requires a special bung and the apparatus for administration has to be adapted to this. It is, therefore, clear that if serum could be efficiently dried in an amount larger than 200 c.c. in the standard 1 pt. transfusion bottle considerable advantage would be gained, both in simplification of equipment and in the bulk of apparatus that has to be transported.

From the technical aspect it is difficult to dry a wedge of serum in a round waisted pint transfusion bottle because the wedge is thick and the surface area exposed is not large, neither is the amount that can be put into a bottle for drying under these conditions very much larger than can be contained in a 12 oz. medical flat. But if the serum be placed in the bottle and the latter inclined at such an angle that the fluid is just clear of the neck, and the bottle afterwards rotated at the same inclination during the period of freezing, a very much larger amount can be introduced and the rotation will presumably make the fluid leave a hollow core in the shape of a cone with its apex towards the bottom of the bottle (Fig. 2). In actual fact, however, the core is cylindrical (Fig. 3). This presents a relatively large surface area for drying without undue thickening at any point. By this method, satisfactory results were obtained with a standard transfusion bottle containing as much as 400 c.c. of serum set at an inclination of approximately 15° to the horizontal.

A diagram of the apparatus for spinning the bottles during the freezing process is shown in Figs. 4, 4a. It consists essentially of a series of rotating pads to which are attached spring clips to grip the bottoms of the bottles, the upper end of each bottle being supported on two free rollers. Each pad is mounted on a short length of steel shafting which passes through a phosphor-bronze bush, and to the other end of which is fixed a sprocket. The whole series of pads is rotated by an endless chain running over the sprockets. One of the sprockets is used as a driver and has a universal joint connecting it to a further length of shaft through which the power drive is transmitted. The drive may consist of an electric motor through reduction gear or by electric motor and suitable pulley ratios. The whole apparatus is mounted on a metal framework and is provided with a hinged base so that the inclination of the bottles can be altered at will. The upper end of the framework has attached to it a screw and handwheel for making adjustment to the inclination. The object in making provision for alterations in inclination is to enable bottles with varying amounts of fluid in them to be rotated and also because it was found that if, after freezing has commenced, the bottles are brought more nearly to the horizontal position, the core then becomes a truncated cone with the wider end

Fig. 4. Diagram showing the way the driving chain passes over all the sprockets.

Fig. 4a. Explanatory diagram showing details of an apparatus for rotating 1 pt. blood-transfusion bottles during freezing. *a*, handwheel and screw for adjusting inclination of the bottles; *b*, cold room wall; *c*, sprocket to carry driving chain; *d*, 1 pt. blood-transfusion bottle in position; *e*, spring clip to grip bottom of the bottle; *f*, rotating pad; *g*, phosphor-bronze bush; *h*, driving shaft; *i*, universal joint; *j*, driving sprocket; *k*, free roller for supporting front of bottle; *l*, post carrying roller; *m*, hinge to allow of alteration in inclination. Above is shown an end view of the bottle supported on its two rollers.

towards the bottom of the bottle, thus increasing still further the surface area. It is intended that the apparatus should stand in a freezing-room. The driving shaft and adjusting screw would be carried through the wall of the cold chamber. Any number of pads up to 25 can be incorporated but with more than this number it is necessary to supplement the main drive with a secondary chain from the driving sprocket. The general arrangement may be made to fit available space in the cold room.

Experiments have shown that blood bottles filled with 400 c.c. of serum, placed in a cold room held within the limits of -18 to -21°C . with an estimated air speed, derived from a fan, of a 100 ft. a min., and with a rotation speed of approximately 50 r.p.m., are completely frozen at the end of 2 hr. It was established that air circulation over the bottles is absolutely essential. Experiments carried out in stagnant air were a complete failure. As previously mentioned the frozen serum adhered to the periphery and the bottom of the bottle, leaving in the centre a cylindrical core of approximately $1\frac{5}{8}$ in. in diameter and 6 in. in length, thus presenting a surface area of approximately 31 sq. in. The texture of the frozen mass was found to be superior to that of serum frozen in a stationary condition. The crystals were smaller and the structure more uniform with the result that reconstitution is completed very rapidly.

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REFERENCE

- GREAVES, R. I. N. & ADAIR, MURIEL E. (1939). High-vacuum condensation drying of proteins from the frozen state. *J. Hyg., Camb.*, **39**, 413-45.

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