Centrifugation at Temperatures Near Freezing

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During the course of an investigation of isolated chloroplasts, it was necessary to centrifuge material at temperatures just above freezing (2°–5° C). A refrigerated centrifuge was not available but the procedure described here was found useful in maintaining the material at these temperatures during centrifugation.

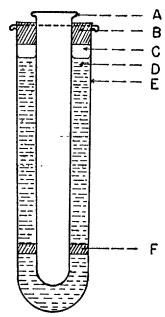

FIG. 1. *A*, 15-ml cellulose nitrate centrifuge tube. *B*, rubber washer. *C*, air space to allow for expansion of water on freezing. *D*, water in jacket. *E*, 50-ml cellulose nitrate centrifuge tube. *F*, rubber washer.

The double-walled plastic centrifuge tube shown in Fig. 1 was placed, together with a standard 50-ml metal tube holder, in the freezing compartment of a refrigerator and the water jacket was frozen solid before using.

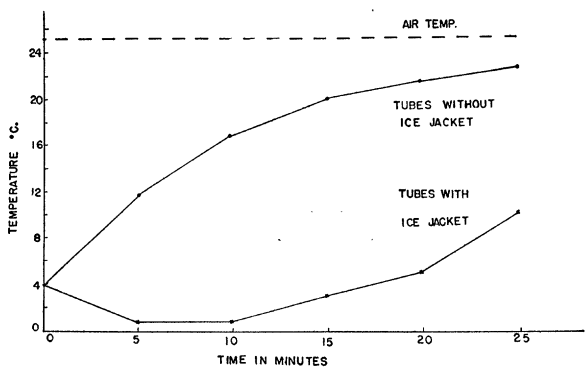

FIG. 2. Efficiency of the ice jacket in maintaining centrifuge tubes at low temperatures.

It was possible to maintain liquid samples in these tubes at 1°–5° C for 20 min of continuous centrifugation at a speed of 4200 rpm in a fixed angle centrifuge. The outside air temperature was 25° C. An initial chilling of the entire centrifuge head in the refrigerator did not prevent tubes without the ice jacket from reaching 12° C in 5 min (Fig. 2).

When the precipitate from the centrifugation was resuspended in the same tube and centrifuged again, it was found desirable to freeze the partly melted water-ice jacket by placing the tube in an ice-salt freezing mixture. By this technique a precipitate could be repeatedly washed and centrifuged at temperatures near freezing.

Thicker ice jackets can be made with larger-sized tubes. A permanent jacketed tube was made by replacing the rubber washers with plastic disks and sealing the top disk in place after adding water to the jacket.

Effects of Precipitates Formed by Insulin with Hyaluronic Acid and Mucoid from Vitreous Humor in Depressing Blood-Sugar Levels

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We have found that when insulin at pH 5–6 is added to dilutions of either hyaluronic acid or mucoid isolated from vitreous humor of ox eyes, precipitates are formed. We tested these at once for their effects on the blood-sugar level in the intact animal. Vitreous humor from fresh ox eyes was used, both to prepare hyaluronic acid (Meyer and Palmer methods [*3*]) and to obtain the mucoid (Suzuki method [*5*] modified by two of the writers [*1*]).

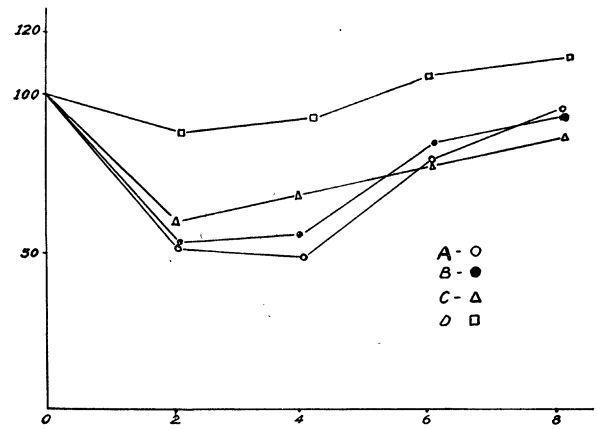

FIG. 1. Changes in the blood-sugar level following the injection of solution A, B, C, and D. Abscissas represent hours; ordinates, blood-sugar mg/100 ml.

Lilly insulin, 40 I.U./ml, with 2.0 mg phenol/ml, diluted with distilled water to 3.65 I.U./ml (*solution A*), was used as a standard of activity. Seven rabbits received intradermally 0.5 I.U./kg of this solution.

The precipitate formed by adding 1 ml of the original insulin to 10 ml of a solution of hyaluronic acid (100 μg/ml), kept for 12 hr in the refrigerator, centrifuged, rinsed with water, and resuspended in the original volume, was called *solution B*. Of this, 0.14 ml (theoretically equivalent to the values of insulin used) was given intradermally to two rabbits.

Solution C was prepared by adding 1 ml of the original insulin solution to 10 ml of a mucoid dilution (about 50 μg/ml), centrifuging the white flocculate formed, wash-