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.

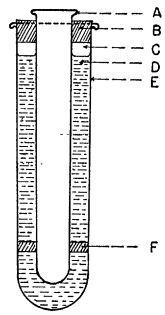

FIG. 1. *A*, 15-ml cellulose nitrate centrifuge tube. *B*, rubber washer. *C*, air space to allow for expansion of water on freezing. *D*, water in jacket. *E*, 50-ml cellulose nitrate centrifuge tube. *F*, rubber washer.

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.

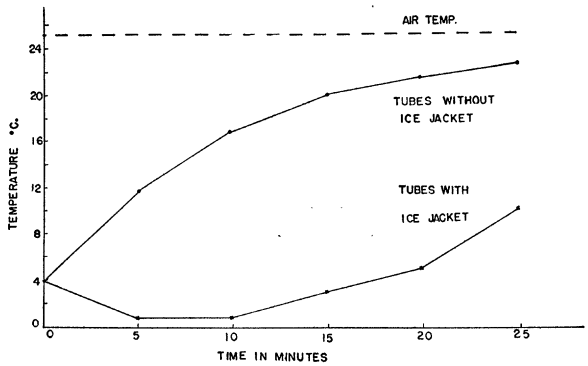

FIG. 2. Efficiency of the ice jacket in maintaining centrifuge tubes at low temperatures.

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*]).

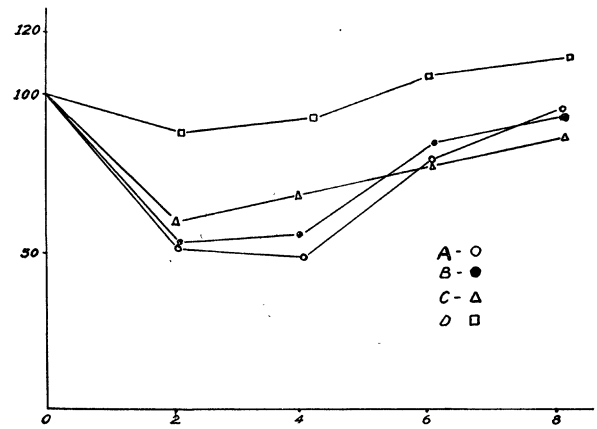

FIG. 1. Changes in the blood-sugar level following the injection of solution A, B, C, and D. Abscissas represent hours; ordinates, blood-sugar mg/100 ml.

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-

TABLE 1

TOTAL NITROGEN, PHENYLALANINE, AND HISTIDINE IN MUCOID, HYALURONIC ACID, INSULIN, INSULIN-MUCOID, AND INSULIN-HYALURONIC ACID

Materials analyzed	Nitrogen, %	Phenyl-alanine, %	Histi-dine, %
Vitreous humor mucoid ...	12.35	3.19	2.45
Hyaluronic acid	3.73	...	...
Insulin-mucoid precipitate .	13.97	6.45	3.87
Insulin-hyaluronic acid precipitate	12.86	...	...
Insulin	14.62	7.25	4.40

ing and resuspending it in the original volume of water. Of this suspension 0.14 ml/kg was injected intradermally in seven rabbits.

The supernatant fluid in solution C, after centrifuging, was separated and called *solution D*. From it 0.14 ml/kg was injected in a control rabbit.

Blood-sugar determinations were made (by the method of Hoffmann [*2*]) before each injection and every 2 hr afterwards, and the results were plotted together with relation to time, as shown in Fig. 1. From this, it will be seen: (1) that there was no noticeable difference between the effects of solutions A and B; (2) that solution C, although slightly less effective during the first 2 hr than A or B, exerted a more prolonged effect, and for this reason the original sugar values return more slowly to their former level; (3) that solution D was still active, but in a much lower degree.

We have planned an investigation of the nature of the precipitates formed. A priori, one is tempted to identify those formed by hyaluronic acid, mucoids or mucins, and described by Meyer and Palmer, as mere artifacts. For the time being we have conducted analysis of only the total nitrogen content (by micro Kjedahl) in the two above-mentioned precipitates, and also in mucoid, hyaluronic acid, and insulin (the last precipitated from the original solution by alcohol ether). Phenylalanine and histidine in the precipitates, insulin, and mucoid were determined by Stokes *et al.* (*4*) by microbiological method. Table 1 reports the results from duplicate determinations.

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Spawning of Oysters at Low Temperatures

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The assumption by Churchill (*1*), Galtsoff (*2*), Nelson (*5*), and others that 20.0° C is the minimum temperature for spawning of the oyster (*Ostrea virginica*) in nature was first questioned when it was established that oysters in Long Island Sound were spawning at temperatures ranging from 16.4° to 18.5° C (*4*). Systematic observations conducted since then by one of us have shown that in some years not only spawning but also larval metamorphosis, or, as it is commonly called, setting, took place in Long Island Sound before the temperature reached 20° C. Reports of these observations are in the official files of the U. S. Department of the Interior, Fish and Wildlife Service. However, this evidence was not corroborated by laboratory observations.

The first direct observation on mass spawning of laboratory oysters at a comparatively low temperature was made in May 1949. These oysters were kept for a period of 90 days in trays with running sea water, the temperature of which was steadily maintained at 15° ± 1° C. Because samples of gonads taken toward the end of the 90-day period began to show that the oysters were discharging some spawn, and because examinations of the bottom deposit disclosed fertilized eggs, it was decided to add sperm and egg suspension to one tray to see if the oysters would respond to the stimulation and begin spawning. Within a minute after addition of the suspension, the first male began to spawn. He was soon followed by two other males, and after 5 min three females were also spawning. Later on more oysters of both sexes began to spawn, and finally, after about 25 min almost all 20 oysters were spawning, and continued to do so for about 2 hr.

Addition of sperm and eggs to three other trays also induced mass spawning, with oysters of both sexes discharging large quantities of eggs or sperm. Throughout spawning, the water temperature in all trays remained steadily at 15.8° C. Fertilized eggs were collected and cultured; they showed normal development.

Obviously, spawning of a certain portion of the oyster population in northern waters may begin at temperatures much lower than 20° C. It is possible that the temperatures prevailing during the period of gonad maturation may determine the temperature at which the first spawning will take place.

As Thorson (*6*) points out, many marine invertebrates may have ripe sexual products outside the breeding period, sometimes months ahead of spawning. In our waters, such is the case in *Venus mercenaria,* which possesses ripe sperm and morphologically mature ova in November, although it does not spawn until the following July or August (*3*). Therefore, a distinction should be made between the temperature high enough to permit maturation of sexual products and the temperature at which spawning is possible. Sometimes such temperatures are several degrees apart. However, as our laboratory observations indicate, by conditioning oysters for a long period at a temperature just high enough for maturation of the gonads it is possible to induce spawning without an increase in temperature, thus bringing together, or to virtually the same level, the temperatures needed for ripening of gonads and for spawning.