the recruiting response may be greater during the fast-wave sleep stage than during wakefulness or the stage of sleep associated with high-voltage, slow-wave electrocortical activity.

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HÆMATOLOGY

Antihæmophilic Globulin in Frozen Plasma

IN a recent communication[1], I described the effect of certain conditions on the stability of freeze-dried factor VIII (antihæmophilic globulin). Owing to the antigenicity of animal material and the restricted supply of human factor[2], other sources of this labile factor have been sought. Plasma, kept frozen after being separated from fresh blood, is now being widely utilized in the management of hæmophilia. This raises for consideration the effect of different methods of freezing on factor VIII and other blood-clotting factors.

Plasmas collected by the same person on the same day showed unequal loss of factor VIII activity after being stored in a deep freeze at −30° C for 48 h. In some cases this amounted to 50 per cent. The following two conditions were thought to contribute to this unexpected finding.

(1) *Variations in the composition of plasma.* These arise mainly from differences in: (*a*) the concentrations of protein, lipoids, minerals, etc., in the donor's plasma; (*b*) the packed cell volumes; (*c*) the ratio of acid-citrate-dextrose solution to the amount of blood collected in each bottle.

(2) *Rate and uniformity of cooling and thawing.* The rate of freezing varied according to whether the M.R.C. bottle was surrounded by air or liquid (Table 1). Frozen plasma (400 ml.) thawed in 4–4½ h at room temperature (18°–20° C). Under running tap-water (18° C), thawing occurred in 1 h. This was shortened to 30 min in a thermostatically controlled water-bath at 37° C. On lowering the temperature, the composition of a solid–liquid system changes by the deposition of solid(s). The separation of solid is accompanied by the evolution of heat which diminishes the rate of cooling. The temperature remains constant until the system is completely solid whereupon cooling proceeds further. The reverse occurs during thawing. These changes may lead to the development of *p*H, salt concentrations or other conditions which, depending on the temperature and the period (reduced by freezing and thawing in liquid, as mentioned here) during which they operate, adversely affect the blood-clotting factors. Some of these changes are given in Table 2.

Table 1. TIME REQUIRED FOR FREEZING 400 ML. PLASMA IN M.R.C. BOTTLE

Temperature (° C)	Surrounding medium: Air	Liquid
	Freezing time (min)*	
−20	180–240	60–70
−30	150–200	45–55
−40	150–170	30–40
−65	—	8–10†

* Complete solidification of contents. Longer periods are required for the contents to reach a homogeneous temperature equal to that of the surrounding medium.
† In order to avoid breakage of bottle, contents were mixed by rotating the bottle in the freezing liquid. In all other instances the bottle was not handled (usual procedure for freezing plasma in deep freeze in blood banks).

Table 2. LOCAL VARIATIONS IN PLASMA COMPOSITION DURING THAWING

Test	Sample* 1	2	3	4 (control)
*p*H	6·75	6·72	6·69	6·70
Total protein (g/100 ml.)	9·8	4·6	2·5	7·2
Blood-clotting factors (% of normal) †:				
Factor II, V	150	70	45	100
VII	150	80	45	110
VIII	120	45	8	80
IX	160	65	35	95
X	150	75	40	100
XII	170	80	40	100

* Freshly collected plasma frozen in a deep freeze at −30° C for 24 h. Liquid samples were obtained at intervals without mixing contents. The control (sample 4), however, was obtained after complete thawing and mixing of contents.
† For 100 per cent standard, fresh plasmas (collected in A.C.D. mixture) from 5 normal subjects were pooled.

That the foregoing two conditions affect the stability of factor VIII in frozen plasma was deduced from the consistency achieved and the negligible loss occurring on rapid pre-freezing of plasma in liquid and thawing in a bath at 37° C.

The effect of storage for longer periods was also investigated. At −30° C factor VIII activity showed 20 per cent and 50 per cent loss after 3 and 6 months respectively. Preservation at −40° C reduced this by approximately 20 per cent. Plasma factor IX activity, as measured by the thromboplastin generation test, was similarly affected. On the other hand factor IX, evolved after coagulation[3], was comparatively stable, less than 10 per cent being lost after 6 months at −30° C. Repeated thawing and re-freezing caused marked reduction in the activity of plasma factors V, VIII and IX, but had no significant effect on other blood-clotting factors. The latter were also highly stable at −30° C.

It would now appear that for maximal activity of factor VIII, fresh plasma should be pooled, rapidly frozen in liquid, and stored at −30° C or at −40° C for a period not exceeding 3 and 4 months respectively. Thawing with continuous mixing of contents at 37° C is desirable. Re-freezing of thawed material should be avoided.

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Oxidation of Reduced Glutathione by Acetylphenylhydrazine, Hæmoglobin and Erythrocytes

INCUBATION of erythrocytes, from individuals having the sex-linked, inherited deficiency of glucose-6-phosphate dehydrogenase, with oxidant drugs such as acetylphenylhydrazine (APH) produces a drop in the level of intracellular reduced glutathione (GSH)[1]. Beutler *et al.*[2] consider that APH itself has no effect, but modifies cell hæmoglobin to a complex, not methæmoglobin, which oxidizes GSH. Allen and Jandl[3], on the other hand, have indicated that these two compounds oxidize one another, and that the APH oxidation products aid GSH oxidation.

This communication describes the effects of APH, hæmoglobin and red cells on GSH in air and nitrogen. Heparinized human blood was centrifuged, the red cells washed in 0·9 per cent sodium chloride and used to prepare saline suspensions, 0·1 per cent saponin hæmolysates and