mainly due to the great disproportion of the optical density of the Hb A and Hb A$_2$ bands. In order to obtain measurable amounts of Hb A$_2$ for elution without staining at least 20 to 30 μl of a 10% Hb solution should be applied, preferably as one long streak or a series of shorter streaks (depending on the size and form of cellulose acetate available). If a micro-densitometer is available, obviously smaller samples can be applied. Satisfactory elution can be achieved if the sample volume to substrate surface (length of application site) ratio is not too high. Overloading leads to incomplete elution which in turn will result in relatively too low Hb A values and in consequence too high Hb A$_2$ values. About 2 μl per cm of application line creates conditions compatible with adequate elution. The Hb A$_2$ band is cut out close to the band margins. The haemoglobin trail between the HbA and Hb A$_2$ bands belongs to the Hb A band. The cut-up fragments of the Hb A$_2$ band (or bands) are eluted (preferably with agitation) in 3 to 4 ml of barbitone or Tris buffer (as for electrophoresis) or Drabkin's solution. Similarly the corresponding A band or bands is cut out from the same strip and placed in 30 to 40 ml of eluent. The eluate is centrifuged and the supernatant read in a suitable colorimeter at 413 mμ or 540 mμ, in cuvettes with at least 1 cm light path.

Alternatively, the bands can be stained, eg, with Ponceau S, eluted and the eluate is read at the appropriate wavelength, eg, 525 mμ for Ponceau S. The advantages of staining the bands is that much smaller volumes of the sample can be used; only about 3 to 4 μl of the haemolysate needs to be applied, bearing in mind that 1 μl/cm of the application line should not be exceeded. Care should be taken that the stromal protein bands are recognized and not mistaken for haemoglobin and subsequently measured together with Hb A$_2$.

The choice of method is largely a matter of personal preference. Using the techniques described, the normal range of Hb A$_2$ varied between 1·5 and 3·3% and did not exceed 3·4%.

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Apparatus for shell freezing liquids in the standard blood transfusion bottles

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Lyophilization (drying from the frozen state) is widely used for the preservation of biological materials. The physics of the drying process requires that for the best results there should be a large surface for evaporation, with a minimal depth of frozen material. This has led to the so-called 'shell freezing' in which the container is rotated during freezing in such a way as to coat the inside surfaces of the bottle with the material to be dried. There have been several attempts to deduce theoretically the physical structure of the frozen mass which provides the optimum conditions for the drying (Rowe, 1964) and the quality of the product (Greaves, 1946; Rosenburg, 1964). In the preparation of proteins with biological activity, the quality of the product, ie, speed of solution on adding water and the preservation of biological activity, are more important than achieving the maximum speed of drying.

The best material appears to result from prefreezing in such a way as to produce small crystals (Greaves, 1946). It has been found that if the material is to be dried in the cylindrical bottles of the type commonly used in the Blood Transfusion Service good results are obtained by rotating the bottle about its vertical axis during freezing, the speed of rotation being high enough to cause the liquid to rise up the walls of the bottles and distribute itself evenly over the inner periphery. During the freezing period the centrifugal force causes the ice crystals, as they are formed, to move towards the centre of the bottle, away from the cold outer surface, thus ensuring that small ice crystals form. Greaves (1946) describes a method of spin freezing using air as coolant but a liquid coolant such as alcohol offers many advantages.

We found that apparatus available commercially for vertical spin freezing was extremely expensive and cumbersome and lacked versatility. Some designs did not make provision for maintaining sterility within the bottle being frozen. We set out to design an apparatus which would incorporate the following points:— (1) It should be relatively inexpensive and compact. (2) The cold bath should be readily adaptable to other purposes. (3) The spinner assemblies should be independent units permitting easy replacement in the case of breakdown. (4) It should be possible to use bottles with a bacteriological filter cap in place ready for drying under sterile conditions. This implies that the bath liquid must not

splash the cap of the bottle. (5) Attachment and removal of bottles should be simple and quick, and avoid the possibility of frostbite.

The technical problems which were met and overcome included: (1) The avoidance of out-of-balance diffi- culties caused by the bottles being irregular in diameter, effected by using a self centring system with angular constraint. (2) Undue turbulence of the bath liquid, which was avoided by immersing the bottles up to the shoulder. At $-40°C$ industrial alcohol is quite viscous and little splashing occurs. (3) For rapid freezing an adequate reserve of refrigeration was required. The 'stand by' temperature should not be so low that the bottles crack on initial immersion. Instead of using a large condensing unit with a thermostat, two smaller compressors were used. One only is switched in during standby periods; both operate during spin freezing. In practice the bath temperature is maintained between -40 and $-45°C$, and does not rise above $-38°C$ during prolonged use. (4) The bath, when used as a simple spin-freezer, has one end of the alcohol tank bridged with a Grant type unit containing two stirrers, which run con- tinuously (Fig. 1). This unit can be lifted off the bath and replaced with a Grant type thermostatic control unit enabling the bath to be thermostatically controlled at
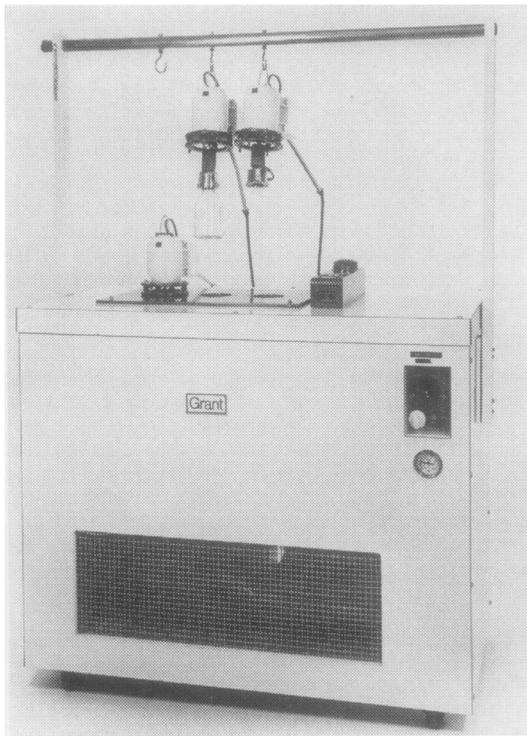
any temperature between $-40°C$ and $+60°C$ at a tem- perature differential of $\pm$ $0.2°C$. (The industrial alcohol must be replaced with water if to be used at temperatures above $+10°C$, owing to the fire risk.) A dial thermometer is fitted to the bath to indicate the liquid temperature. A safety cut-out is fitted that will switch off the bath if the temperature rises above a preset level, and this unit is fitted with a relay that will sound an alarm if de- sired. A nylon-coated duralumin lid locates positively
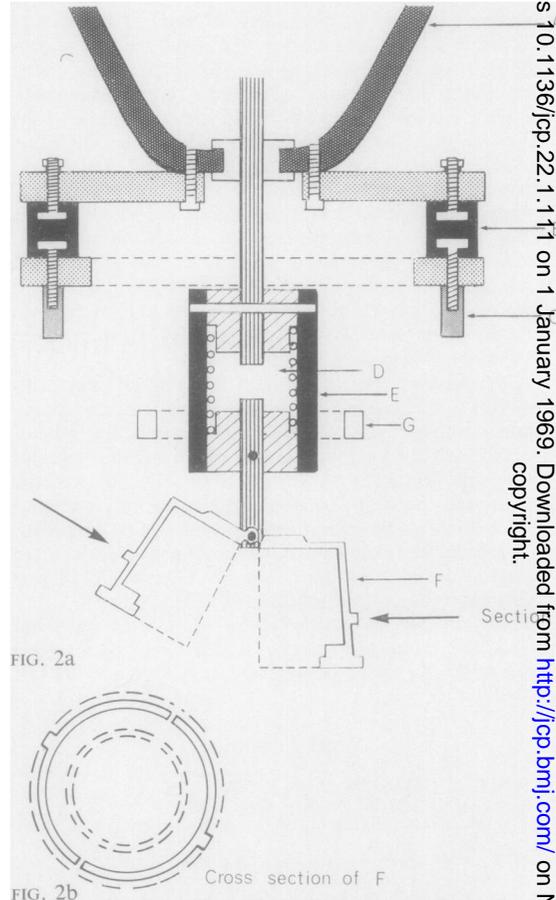

FIG. 1. *Photograph of complete shell freezing unit, showing one spinner unit in position for freezing, one with a standard blood transfusion 250 ml blood bottle attached, and one with the chuck open ready to receive a bottle.*

FIG. 2a. *Cross section of one spinning unit.* A, *motor*; B, *antivibration mountings*; C, *locating pins to fit bath lid*; D, *flexible coupling*; E, *rubber constraint tube*; F, *chuck*; G, *chuck locking ring.*
FIG. 2b. *Cross section of chuck.*

on the top of the bath to accommodate the spinning units, and replaces the normal heavily insulated lid. (5) Each spinner unit is fitted with a capacitor start induction motor for rapid acceleration to the final speed of 950 rpm. The motor is fitted with a nylon coated alloy base plate and locating ring, which locates on the cabinet lid. A set of antivibration mountings are fitted between the baseplate and the locating ring to eliminate residual vibration. The bottle chuck is a quick

release chuck designed to accept both metal and lint-capped bottles. To overcome the out-of-balance load of the bottle, the chuck is fitted with a flexible coupling allowing the bottle to precess around its dynamic centre while spinning. For loading and unloading the spinners a horizontal bar is fitted above the cabinet on which the spinners can hang.

Details of the design are shown in the sectional diagram (Fig. 2). With this equipment the times for freezing solutions starting at room temperature were as follows: 100 ml of solution in a standard 540 ml transfusion bottle three minutes or, in a 250 ml bottle five minutes; 400 ml of plasma in a 540 ml bottle 15 to 20 minutes.

We wish to thank Mr I. C. Costar for the diagrams.

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CORRECTION

In Figure 1 of the paper by Helen McCullough, 'Semi-Automated method for the differential determination of plasma catecholamines' (*J. clin. Path.*, 21, 759), showing the flow system the waste tube (no. 10) should pass through the pump once only. Reagents for the various tubes and tube diameters in inches should be shown as follows: (2) Sample (0·045); (4) Acetate-Ferricyanide (0·065); (6) Air (0·056); (8) 2·5N NaoH (0·045); (9) Stabilizing reagent (0·030); (10) Waste (0·065).

Letters to the Editor

THYROID FUNCTION TESTS

I was most interested in the paper by Thomson, Boyle, McGirr, Macdonald, Nicol, and Brown (1968)[1] in which they describe difficulties they have experienced in the interpretation of some thyroid function tests. I feel, however, that their conclusions are too dogmatic, and shall appreciate space to reply to some of their statements.

Thyroid function tests fall into two groups, first those which aim to determine 'thyroid status', and secondly those directed at specific thyroid disorders independent of thyroid status. The commonest example of the second group is the diagnosis of Hashimoto's disease by demonstration of high titres of specific antibodies to thyroid components in the peripheral blood. A patient with Hashimoto's disease may be euthyroid, hypothyroid or, rarely, thyrotoxic, but the antibody findings are quite independent of this aspect. It is useful, when considering and comparing tests of thyroid function, to separate the two groups of investigations to avoid confusion.

The authors state in their synopsis that 'an uptake test and estimation of the serum protein-bound radio-active iodine (PB^{131}I), supplemented as required by the protein-bound iodine (PBI), remain the best routine tests of thyroid function'. This conclusion is not based on a comparison with the variety of newer tests now available, of which they used only the triiodothyronine resin uptake in a few cases. Although their statement might have been valid between 1963 and 1965, when the work was done, it does less than justice to the many authors who have published studies since then.

Recent work on the determination of thyroid status has largely been concerned with the direct measurement of levels of thyroid hormone in blood. Ekins (1960) and Murphy (1965) developed specific thyroxine assay techniques based on the saturation analysis principle and Nauman, Nauman, and Werner (1967) described a method for the determination of serum triiodothyronine also based on this technique. These methods eliminate inaccuracies inherent in PBI measurements by being specific for the hormones themselves. Following the extensive development by Robbins and Rall (1957) of the concept of protein-binding of thyroid hormones in blood, it has been recognized that thyroid function is most closely related to the concentration of unbound hormone. This level is dependent on the concentrations of thyroxine-binding globulin (TBG) and thyroxine-binding prealbumin (TBPA), as well as on the level of total thyroid hormone. Free thyroxine can be measured directly by equilibrium dialysis or ultrafiltration, and indirectly by methods derived from that of Hamolsky, Stein, and Freedberg (1957) which reflect the concentration of thyroxine-binding proteins. These have been well