

Time (hr)	Case No.			
	1	2	3 <sup>a</sup>	4
0	0	0	0	0
2	Not detectable	0.34	0.45	0.12
6	1.10	0.78	2.40	0.60
12	1.40	0.33	1.20	0.06
24	1.40	—	—	—

**Table** Levels of oxytetracycline in serum samples of patients after oral administration of the amoebic antibiotic<sup>1,2</sup>

<sup>1</sup>Results are expressed in  $\mu\text{g/ml}$  serum.

<sup>2</sup>Three capsules of the amoebic antibiotic were administered to patients suffering from amoebic dysentery. Zero hour serum samples served as controls. Samples were stored in a refrigerator till they could be analysed later on the same day.

<sup>3</sup>Icteric samples.

John's Medical College, for his continuous support and encouragement, and Miss K. S. Uma for her skilled technical assistance. The research work was supported by a grant from Pfizer Ltd, to the Department of Pharmacology, St John's Medical College.

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## The development of aluminium containers for the storage of blood in liquid nitrogen for the immunization of Rh<sup>-</sup> volunteers

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The deliberate immunization of volunteers for the large-scale production of high-titre anti-D immunoglobulin is now a well recognized procedure in any blood transfusion programme, and efforts designed to reduce the risk of transmitting disease during this procedure have hitherto been restricted in this department to the careful selection of 'accredited donors', ie, those whose previous five donations have been transfused and the recipients monitored for the succeeding six months. This approach is limited as it does not exclude the possibility that the donor may be incubating a recently acquired transmissible disease at the time when blood is withdrawn. Glycerolized blood may be stored in a frozen state for prolonged periods during which time surveillance of the donor's health can be maintained. Despite the introduction of large volume frozen units for transfusion purposes (Pert, Schork, and Moore, 1965; Rowe, Eyster, and Kellner, 1968; Krijnen, Kuivenhoven, and De Wit, 1970; Jenkins and Blagdon, 1971; Mitchell and Muir, 1972) hitherto no small-volume system has been developed which is suited to the immunization of volunteers for the production of anti-D. The following communication describes the introduction of such a system, based on readily available components which can operate economically in any laboratory where liquid nitrogen is available.

#### Materials and Methods

Screw-cap aluminium containers (20 × 40 mm, New Style) were obtained from the Metal Box Co, Cowlares Industrial Estate, Glasgow, N1. Silicone rubber sheet (1/16 in. × 11 in. × 11 in. grade TC-156) was obtained from ESCO, 14-16 Great Portland Street, London, W1. A two-hole metal punch (5 mm dia. × 11 mm centres) was obtained from J. Hamilton, Star Works, Pollard Street, Manchester. Latex rubber fingerstalls (approximately 20 mm diameter) were obtained from a local surgical retailer. Storage trays with 36 spaces

Received for publication 22 May 1973.

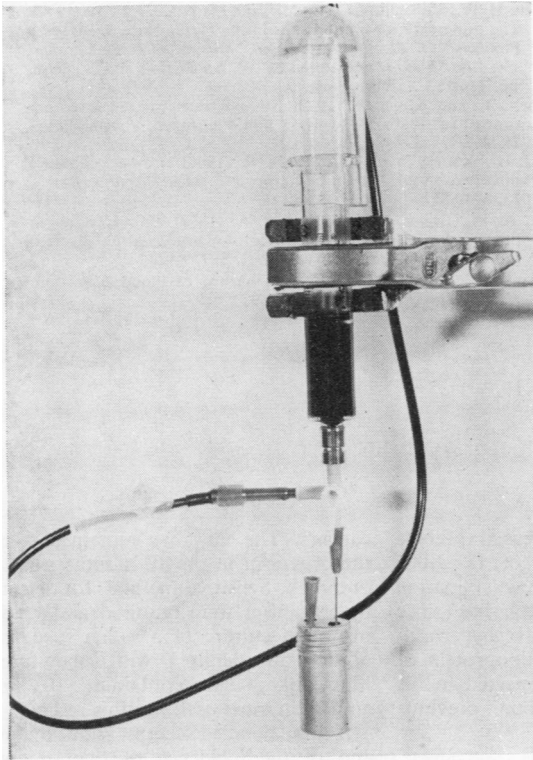


Fig. Syringe and three-way tap assembly for dispensing aliquots.

(VF/B) and vertical files for six trays (VF/3B) were obtained from Luckhams, Burgess Hill, Sussex.

After the PVC liners had been removed and a two-hole pattern punched in the cap, the cans were washed in an alkaline laboratory detergent (RBS 25), rinsed in 1% (v/v) acetic acid, and finally washed three times in pyrogen-free distilled water. Discs of 1/16 in. thick silicone rubber sheet were punched out with a cork borer (number 12, 22 mm dia) and after boiling inserted into the caps. The assembled cans, containing 0.2 ml of pyrogen-free distilled water, were autoclaved at 120°C and 16 psi for 30 minutes with autoclave tape over the puncture sites. Packed cells (200-250 ml), usually less than 24 hours old, housed in the original donation bag (Tuta Ltd), were glycerolized with 200 ml of freezing protective agent (45% w/v glycerol, 3.6% w/v sorbitol, and 0.9% w/v NaCl) via a Fenwall AE-2 plasma transfer line whilst mounted on a rotary shaker. The glycerolized blood was then removed through a three-way tap attached to the transfer line in 7 ml aliquots using a Luer-lok syringe and injected into the cans through a 1½ in. 21 SWG needle. A 3/8 in. 26 SWG needle was used as an

airway (see fig). The can puncture sites and lid were then smeared with 1% (w/v) phenol in glycerol (Sherwin, 1956) and capped over with two latex fingerstalls. Freezing was by directly immersing a storage tray which had a capacity for 36 cans in liquid nitrogen for five minutes. Two trays were used per donation (maximum capacity 72 cans) and each vertical file thus held three different donations. Twelve such files will fit into the vapour phase of a Union Carbide 250L refrigerator, giving a maximum storage capacity of 2600 individual aliquots. After freezing of the donation, the health of each donor was followed by personal interview and serum bilirubin, SGPT, and serum HAA assays at monthly intervals for six months.

Cans were thawed by agitation in a 37°C water bath for two minutes, the fingerstalls discarded, and the puncture sites wiped clean with an isopropanol swab. The contents were transferred to an empty, sterile McCartney bottle via a 3½ in. 19 SWG spinal needle; after sterile handling of the stilette, it was reinserted in the spinal needle which remained *in situ* in the McCartney bottle throughout the washing process. The glycerolized blood was centrifuged in a swing-out rotor at 2500 rpm (700 × *g*) for five minutes and the supernatant glycerol removed. A hypertonic wash (sorbitol, 19% w/v in saline) of 20 ml was added and the centrifugation repeated. After removal of the sorbitol, a further two washes of 20 ml of normal saline were performed in a similar manner.

#### Comment

The optimum system, using 20% w/v glycerol at pH 6.7-7.0 and a sorbitol wash of 19% w/v, gave recoveries of 85 to 95% intact cells. Sterility tests were all negative and the system has been used in this department for 18 months without any complications. A range of antibody titres have been produced from negative to over 8000.

The introduction of the approach described above for the immunization of volunteers in the anti-D programme has several advantages. During prolonged storage in liquid nitrogen a more sophisticated approach to donor accreditation can be made. This has already proved useful within this study. Three weeks after donation one of the cell donors developed a 'flu-like' illness and, although HAA negative by radioimmunoassay, had an elevated serum bilirubin and SGPT; these cells were discarded. The thawing/washing procedure requires one hour and it is therefore possible to arrange immunization at any time convenient to the cell recipient. By increasing the range of specificities of the cell panel a closer red cell antigenic match between donor and recipient can

be achieved, so reducing the chance of other red cell antibodies forming in the recipient. Finally, the system devised has significant economical advantages: none of the original cell donation needs to be wasted, and the equipment and running costs have been calculated to be no more than 60p per aliquot (excluding labour).

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## Letters to the Editor

### A Simple Aid to the Administration of Blood Product Concentrates

We have found that nylon catheter mounts are useful for the efficient withdrawal from plastic transfusion bags of blood products such as cryoprecipitate and platelet concentrate.

The mounts (ref 700/180/Luer) are made by Portex Ltd of Hythe, Kent, and can be attached directly to a syringe. Their conical shape makes them a snug fit in the port of a blood bag. They are firm enough to pierce the membrane in the port but the end is not sharp and will not damage the wall of the bag. Their length (5 cm) is sufficient for the tip to lie just within the margin of the bag.

With one of these mounts, the contents of several bags can quickly be aspirated into a single large syringe. The mounts can be autoclaved for re-utilization.

I am grateful to Mr H. Byram, SRN, Superintendent of CSSD, Royal Lancaster Infirmary, who recommended these mounts.

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### Type III Hyperlipoproteinaemia and Sinking Prebeta Lipoprotein

In our study of the kindred of a patient with type III hyperlipoproteinaemia (*J. clin. Path.*, 1973, **26**, 163), we postulated that a son of the proband exhibited a stage in the development of the type III disorder. His electrophoretic strip revealed

chylomicrons and an increased prebeta band without lipoprotein of D < 1.006 with beta mobility. On re-examination one year later, this patient has since developed a definite type III hyperlipoproteinaemia in that beta lipoprotein of D < 1.006 has now been detected in his plasma; this confirms our previous hypothesis.

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### Neonatal Meningitis Caused by *Citrobacter koseri*

The outbreak described by Gross, Rowe, and Easton (1973) is of interest because we have recently encountered a similar outbreak with four cases in our own premature baby unit (Gwynn and George, 1973) and know of one further case in another baby unit in this city (Bridgwater, 1972, personal communication). *Citrobacter koseri* may therefore be a more frequent cause of neonatal meningitis than has been recognized in the past and its potential pathogenicity in baby units seems clear.

Studies in our own unit suggested that intestinal carriage was important. The organism was recovered from the bowel of several unaffected infants and also from a member of staff at one stage. The outbreak was controlled, without need for closing the unit, by regular screening of all babies and by isolating

carriers. The unit has remained free of the organism in the 10 months since the outbreak. Sensitive methods for detecting the organism were required and we find overnight culture of a saline suspension of stool in selenite F with subculture to MacConkey agar containing 10 µg ampicillin most useful in this respect.

A more detailed account of our methods and of the incidence of this organism in different situations is being prepared for publication.

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#### Correction

In the paper by Freedman *et al* (*J. clin. Path.*, 1973, **26**, 261-267) the illustrations shown for figs 1 and 2 have been transposed.