Freezing and Transport of Sera in Liquid Nitrogen at −150°C to −196°C

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The development of a methodology for epidemiological-serological evaluation of mass yaws campaigns by the World Health Organization and the consequent need to transport large collections of sera for examination in overseas reference laboratories (in addition to field or laboratory testing in the tropical countries where the sera were obtained) have led to a reappraisal of the technical and practical aspects of currently used methods for the transport of sera. This was made the more necessary by the occurrence of such problems of immediate importance as specimen infection and serum denaturation during transport.

Experience has shown that the use of solid CO_2 (dry-ice) for serum preservation is expensive and not very practical and that the cost of freeze-drying under field conditions is prohibitive. The use of thermo-insulated jars and wet ice is not always sufficient to prevent important changes in the temperature of specimens and the denaturation of sera; and normal shipment of specimens by air or surface mail has been shown sometimes to entail repeated freezings and thawings—known to be deleterious to antibodies.

Deep-freezing by liquid gases at very low temperatures for the preservation of biological material has, over the past decades, been gradually extended from being mainly a research tool to becoming also the basis for many practical techniques. Outstanding examples with the application of liquid nitrogen, for instance, are its use for the preservation at −150°C to −196°C of bull sperm for artificial insemination in animal husbandry programmes, for the banking of whole blood for transfusion purposes, and for the establishment of type-culture collections of bacteria, parasites, viruses, tissue cell lines, etc., thus ensuring instant availability of uncontaminated, unaltered reference material.

It was thought that modern cryogenic techniques involving low temperatures of this order might also be usefully applied to the long-distance transport of human serum. This note briefly describes the successful adaptation for this purpose of liquid nitrogen transporter-refrigerator originally used for the preservation of bull sperm.

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FIG. 1
EXTERNAL ASPECT OF LIQUID NITROGEN REFRIGERATOR-TRANSPORTER

Description of the apparatus, its development and use

The transporter-refrigerator that has been developed (LR-10A-6)[a] is a superinsulated double-walled container made of aluminium, which gives it structural strength and light weight. Fig. 1 shows

[a] The Union Carbide Company Europa SA, Geneva, Switzerland, provided valuable assistance in all phases of the development of this equipment for serum transport. Acknowledgement for valuable technical advice is also made to Professor A. P. Rinfret of the Linde Research Laboratories, Tonawanda, N.Y., USA; to Dr. S. Christiansen, Scientific Adviser, WHO Serological Reference Centre, Copenhagen; and to Dr H. A. Nielsen, Director, Treponematoses Department, Statens Seruminstitut, Copenhagen, Denmark.

its external aspect. Its small size, carrying handle and carrying frame facilitate portability. A slotted ring cap surrounding the top of the neck tube provides a convenient method of suspending inside the container six canisters each holding a number of ampoule racks. A porous plug of low heat-conduction material reduces liquid nitrogen loss due to evaporation and protects the neck tube.

Fig. 2 shows a cross-section of the refrigerator-transporter with its different elements. Fig. 3 shows how the ampoules are clipped in the rack, and how the rack is inserted into the canister; and Fig. 4 shows insertion of a canister into the refrigerator-transporter.

FIG. 2
CROSS-SECTION OF REFRIGERATOR-TRANSPORTER

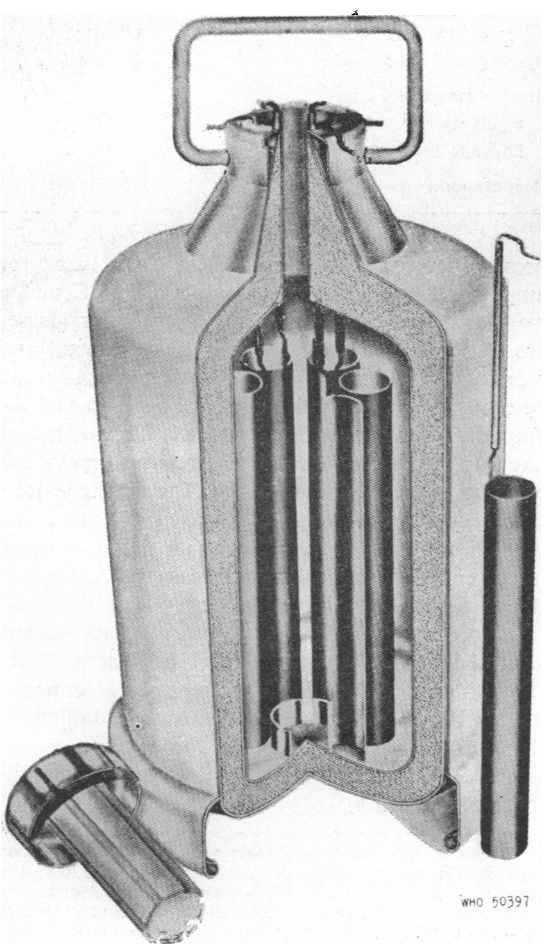
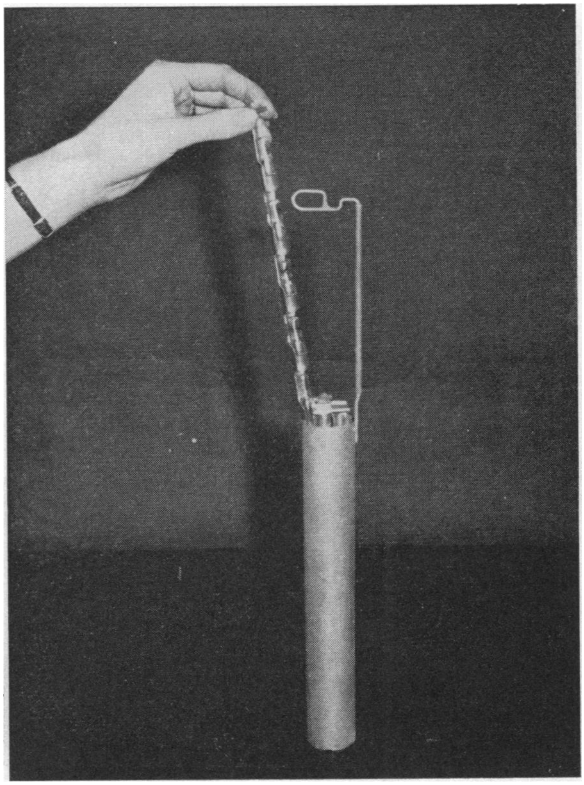


FIG. 3
INSERTION OF AMPOULE RACK INTO CANISTER



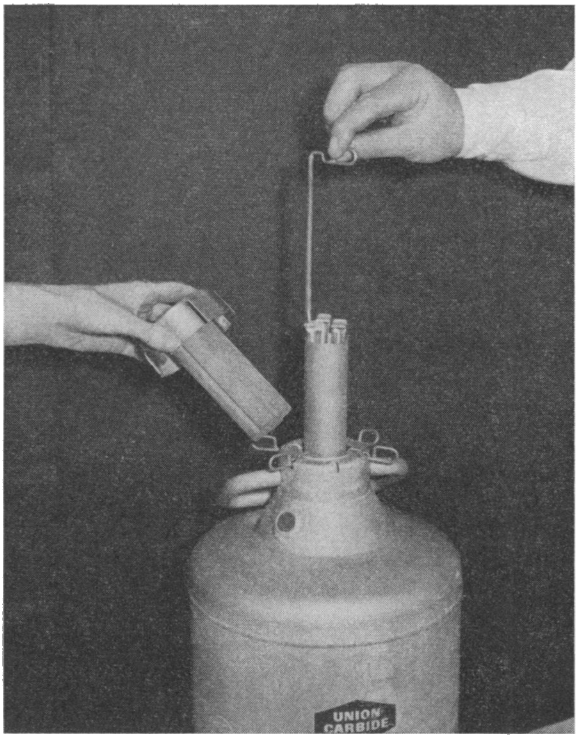
The measurements and technical specifications are given in the table.

The LR-10A-6 has a liquid nitrogen capacity of 9.4 litres with canisters, racks and ampoules inserted. The low evaporation rate of liquid nitrogen (0.35 litre per day) gave a holding time without nitrogen refill of more than three weeks on the average in 12 long-distance air transports of sera between Nigeria (Lagos) and Denmark (Copenhagen) or France (Paris). The refrigerator-transporter was sent by air from Copenhagen or Paris filled with liquid nitrogen, and on arrival in Lagos it was transported to remote rural areas, where field operations took place, so as to receive, freeze, and airship sera back to the overseas laboratories concerned. Its use in the field and the two-way trip were found to require an average of less than two weeks under the plan of operations prepared in advance.

While the preservation capacity of the LR-10A-6 is some 120 ampoules of 5 ml or some 200 ampoules

FIG. 4

INSERTION OF CANISTER INTO REFRIGERATOR-TRANSPORTER



SPECIFICATIONS OF THE LR-10A-6 REFRIGERATOR-TRANSPORTER FOR SERUM FREEZING AND SHIPMENT AT −150°C TO −196°C IN LIQUID NITROGEN

Height	53 cm
Outside diameter	27 cm
Diameter of neck tube	5.08 cm
Canister size	3.8 cm × 27.5 cm
Canister capacity	1.88 dm^3
Ampoule capacity:	
1.2-ml racked ampoules	200-250 ampoules
5-ml racked ampoules	120-130 ampoules
Liquid capacity:	
without canisters	10.4 litres
with canisters and ampoules	9.4 litres
Weight empty	6.4 kg
Weight with liquid nitrogen, canisters and ampoules	16.8 kg
Nitrogen evaporation rate per day	0.35 litres
Holding time without refill	21-25 days
Storage temperatures:	
below N_2 level	−195.8°C
above N_2 level	−147°C
Heat evaporation	48 Kcal/kg

of 1.2 ml, we have limited its use to 75-80 sera in 5-ml ampoules over long distances. This corresponds to the number of sera drawn at individual survey points in the field, as predetermined by the statistical sampling design used. Allowance must be made for initial liquid nitrogen use when the sera are directly frozen on insertion of the canisters containing the ampoule racks into the container, as well as for the amount needed to preserve these frozen specimens at −150°C to −196°C during the subsequent transport.

A number of problems arose during the development of this equipment for serum freezing and transport. A long-necked, 5-ml glass ampoule was designed to fit the racks of the canisters in the refrigerator-transporter, and the resistance and other factors of the ampoule glass were defined so as to avoid the glass becoming excessively brittle, cracking and breaking during the freezing process, during preservation or on removal. The glass [b] used for ampoules has a softening point of +783°C and a working point of +1165°C. Other similar glasses are somewhat harder at similar temperatures, and more highly thermo-resistant borosilicate glass could be used, but this would make it difficult to seal the ampoules with a blow-lamp, which is the only means likely to be available under the relatively primitive conditions obtaining in the field. The sealing process is important: care must be exercised to ensure that there is no leakage through which liquid nitrogen might enter, since this might result in breakage of the ampoule when it is removed from the refrigerator-transporter at the receiving laboratory. The canisters with the ampoule racks should be inserted very slowly into the container for freezing (at approximately 10 cm per minute) so as to avoid boiling of the nitrogen and exposure of the glass to undue strain. The correct insertion and removal technique must be learned by experience.

[b] Jena Fiolax hydrolytic glass with a heat conduction capacity (in cal/cm/sec/°C) of 2.83 × 10^{-3} at + 320°C and 1.1-1.3 × 10^{-3} at −196°C, and a linear thermal extension coefficient of 48 × 10^{-7} at + 20°C to + 30°C and 39 × 10^{-7} at −130°C to 0°C.

Improvements to the above techniques are possible, and experiments are being conducted on the use of serologically inert, freeze-resistant and sterilizable low-pressure polyethylene or polypropylene tubes with an airtight stopper or seal. We have found these to be a practical replacement for glass ampoules in our further studies.

There is no risk of explosion with the LR-10A-6, and it is accepted by public carriers for air shipment under IATA regulations.[c] The air freight cost is reasonable, e.g., US$ 40 between Lagos (Nigeria) and Copenhagen (Denmark). The total cost of liquid nitrogen for six shipments over the distances mentioned did not exceed US$ 35. The purchase cost of this refrigerator-transporter is also reasonable.

Discussion

The equipment described above has been found to provide a practical, efficient, safe and economical method of " bringing the field into the laboratory " for immunological investigations. It allows serological specimens to be frozen *in situ*, minimizing the risk of specimen infection and of protein denaturation. The ready availability of such " base-line " biological material also permits accurate evaluation by an overseas reference laboratory of serological test performance in a local field laboratory working under tropical conditions. In addition, this equipment makes it possible for scientific measurement procedures to be established for comparative studies of the influence of time and temperature exposures on serum components and antibodies under given conditions. Such evidence as is available suggests that the eutectic temperature of human serum is in the neighbourhood of −60°C and that preserved serum is not inert above that temperature; there is also evidence that some serum components become denatured at −40°C. Since laboratories throughout the world continue to store serum collections at temperatures of −20°C or higher in conveniently available commercial food refrigerators, further fundamental research in this area appears to be needed.

[c] International Air Transport Association (1965) *IATA regulations relating to the carriage of restricted articles by air*, Montreal, Section IV N, Article 1275. This article allows the carriage of up to 50 litres of liquid non-pressurized nitrogen on passenger and cargo aircraft.

Difficulties in the Management of Venereal Disease in Mariners

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The management of venereal disease in mariners is an international problem. Aside from the treatment of patients attending clinics, such management is concerned with three main functions—namely, (*a*) the prevention of export of venereal disease, (*b*) the prevention of import of venereal disease, and (*c*) the supervision of persons with venereal disease while aboard ship between ports.

The successful performance of these three functions depends on collaboration among all agencies that have responsibilities in this area and on knowledge of the varying factors concerned, information about some of which is considered below.

Prevention of export of venereal disease

Venereal disease acquired by mariners in one port is often treated in another, and distinct patterns of behaviour are associated with different venereal infections.[b] These patterns are governed, in part, by the incubation periods of the diseases. This factor determines, to a certain extent, where the patient will seek treatment, but an appreciable number of mariners will wait to seek aid until they reach a clinic that has been recommended to them.

Table 1 sets out the percentage distribution of places of risk of exposure, by diagnosis, of visiting and local mariners as seen at the venereal diseases clinics at North and South Shields, at the mouth of

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[b] Schofield, C. B. S. (1965) *Brit. J. vener. Dis.* **41**, 51.