

THE PRESERVATION OF PROTEINS BY
DRYING.

MEDICAL RESEARCH COUNCIL

SPECIAL REPORT SERIES No. 258

**THE PRESERVATION OF PROTEINS
BY DRYING**

**WITH SPECIAL REFERENCE TO THE PRODUCTION
OF DRIED HUMAN SERUM AND PLASMA FOR
TRANSFUSION**

by

R. I. N. GREAVES

LONDON: HIS MAJESTY'S STATIONERY OFFICE

1946

(Reprinted 1949)

Universal Decimal Classification

66-047 : 612-11

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PREFACE

When war broke out in 1939, it seemed certain that blood transfusion would play an important part in the treatment of casualties, and elaborate arrangements had been made in advance for the collection and storage of human blood for Service and civilian needs. Citrated whole blood, as widely administered in medical and surgical practice, has the disadvantage that, unless it can be given fresh, it requires to be kept in a refrigerator and, even then, can safely be used for only a limited period after collection; moreover, whole blood transfusion, with the assurance of safety, necessitates careful blood-typing. The maintenance of adequate stocks of whole blood to meet a varying and incalculable demand involves the periodical rejection of time-expired material, with consequent wastage of a valuable product, the difficulties in the collection of which are well known. In addition, the transport of whole blood in liquid form in cold storage, for transfusion in the field, presents difficulties which must greatly limit its use. War conditions therefore created an urgent demand for a satisfactory blood derivative which would be stable, readily transportable and suitable for administration to any patients, irrespective of their blood groups.

Earlier studies—to which the author of the present Report and Dr. (now Sir Percival) Hartley, of the Council's staff, made important contributions—had shown that therapeutic sera could be preserved by drying without injury to their delicate protein structure and properties, and there was evidence from various sources that drying, by appropriate methods, could be applied also to the preservation of plasma or serum obtained from human blood; it seemed likely that such products would meet many war-time requirements, if they were found safe for use in transfusion after simple solution in water. A pilot plant, intended for use in drying tetanus antitoxin, had been erected in the Department of Pathology at Cambridge, in 1938, and experimental batches of human serum and plasma, dried in the plant and reconstituted with water, were given by transfusion to patients in 1939–40, and shown to be safe. The Council therefore decided to establish a Blood Drying Unit at Cambridge, on a production basis, by the enlargement of the pilot plant, and the Unit was placed under the direction of Dr. Greaves. The work of the Unit, which passed through several phases, is described in this Report.

The experience of working the original pilot plant provided data for the design of the "final" pilot plant, but many modifications were required for larger scale work, and war-time difficulties in obtaining certain articles of equipment necessitated some ingenious improvisations. The latter plant was, however, successfully operated from the Spring of 1940, and it provided large quantities of dried plasma and serum for Service and civilian requirements. To supplement its production, a plant of the same design was installed at the Wellcome Physiological Research Laboratories at Beckenham in 1941; in addition, the Army Blood Transfusion Service constructed a plant of their own, and the Scottish National Transfusion Association erected one at Edinburgh. However, the increasing demand for dried blood products for transfusion soon rendered the output even of these plants inadequate, and in 1942 it was decided to erect a new and bigger drying plant at Cambridge. A gift of £20,000 generously made to the Council by the Wellcome Trustees, was used for this purpose. The new production plant, which had a normal capacity of 2,500 bottles per week, was designed on the basis of the experience of the working of the final pilot plant, but many new features were incorporated in it. In order that this

plant might meet the Navy's requirements of dried material, the Admiralty obtained the necessary priorities for its construction and supplied Naval V.A.Ds. to assist in its operation. Since, however, a large part of the output was intended for use in civilian hospitals, it was considered appropriate that the Ministry of Health should share with the Council in the staffing and maintenance of the Unit.

From February, 1943, to September, 1945, close on half a million bottles of plasma and serum were dried, and the plant met the total requirements of the Royal Navy and the civilian population, together with a substantial part of the Army's needs and the home needs of the Royal Air Force.

The method adopted of drying by sublimation after "high speed vertical spin-freezing," followed by secondary desiccation, resulted in a very satisfactory product.

The dried products have proved extremely stable; material dried in 1940 and stored at room temperature is still in good condition. Supplies of dried serum or plasma were distributed to every theatre of war, and withstood the rigours of both arctic and tropical conditions. Either material could be reconstituted easily to a homogeneous solution, and allowed speedy transfusion under the most difficult conditions: the great value of these products for the resuscitation of war casualties has been amply proved, and there will be need for them in peace-time transfusion practice. Moreover, the experience gained by the Unit during the war has thrown light upon the fundamental problems involved in the drying, and preservation unaltered, of the unstable natural proteins, and it will be applicable in the future to the preservation of many biological products.

In addition to routine production, much experimental research was carried out by the Unit. This led to the discovery of the method of centrifugal vacuum spin-freezing, which has overcome many of the technical difficulties in drying and preserving protein substances in small quantities.

MEDICAL RESEARCH COUNCIL,
38 Old Queen Street,
Westminster, London, S.W.1.

7th August, 1946.

THE PRESERVATION OF PROTEINS BY DRYING WITH SPECIAL REFERENCE TO THE PRODUCTION OF DRIED HUMAN SERUM AND PLASMA FOR TRANSFUSION

by

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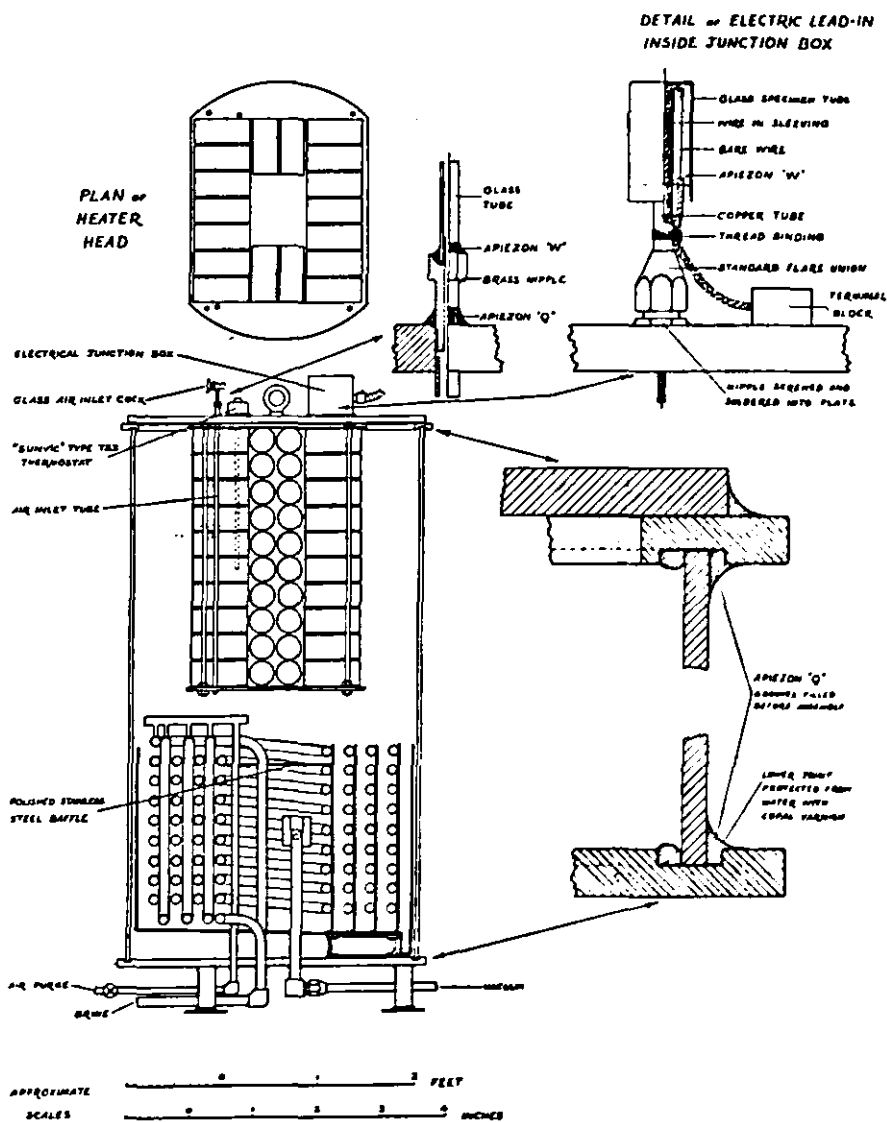
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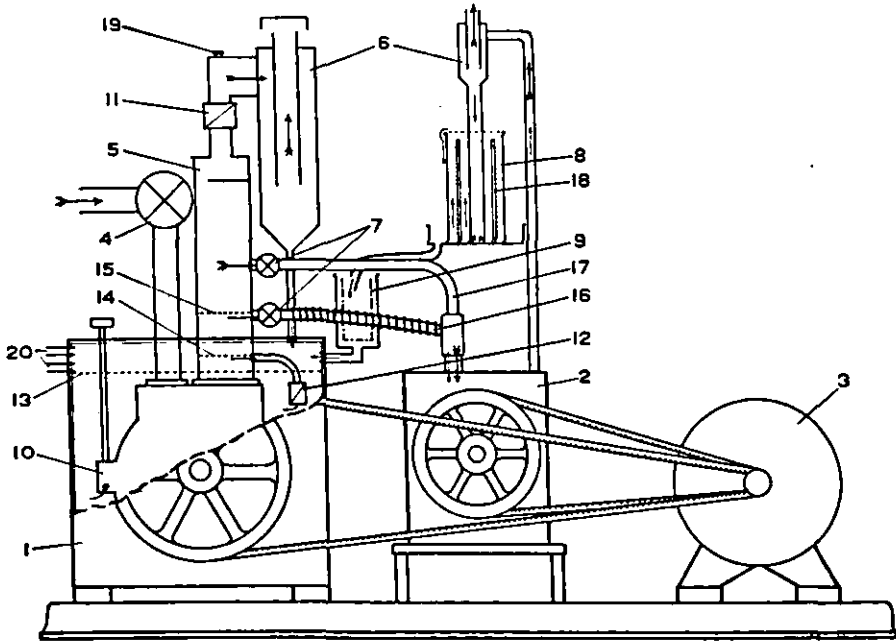
PLATE I



Design of Desiccator Chamber in the Large Drying Plant.

Part section of one of the desiccator chambers in the large drying plant, showing the electrically heated head to take 180 M.R.C. transfusion bottles and the brine-cooled condenser below.

PLATE II



Vacuum Pumping Arrangement, used in the Large Drying Plant.

1. Metrovac pump S.R. 4/40.
2. Speedivac pump No. 1.
3. 3-h.p. motor.
4. 2-inch Metrovic vacuum valve on inlet pipe.
5. Baffle box.
6. Cyclone oil separators.
7. Edwards vacuum cocks. Spindles sealed with Apiezon Q.
8. Oil stripper.
9. Oil strainer. Lint on gauze frame.
10. Oil inlet and flow regulator.
11. Automatic mushroom valve.
12. Ball non-return valve.
13. Oil level in main tank.
14. Oil level in baffle box. Single stage working.
15. Oil level in baffle box. Two stage working.
16. Oil way to backing pump. 21·4/12 mm. Tombac tube.
17. Air way to backing pump. $\frac{3}{8}$ - $\frac{5}{16}$ -inch copper tube.
18. Electric heater (400 watts) thermostatically controlled at 110°C.
19. Plug for replenishing oil seal on valve 11 when required.
20. Cooling water pipes to coil in main tank (not shown).

Air paths shown : >—> oil paths ———>

Pumping is started with the backing pump isolated by closing cocks 7. Air then escapes *via* valve 11 and the large oil separator; oil returns to the main tank *via* valve 12. When the pressure reaches about 10 mm. Hg the cocks 7 are opened. As soon as the pressure in the baffle box falls below atmospheric valves 11 and 12 close; both air and oil then pass on into the backing pump, air escaping from the small oil separator, oil returning *via* the stripper and the strainer to the main tank.

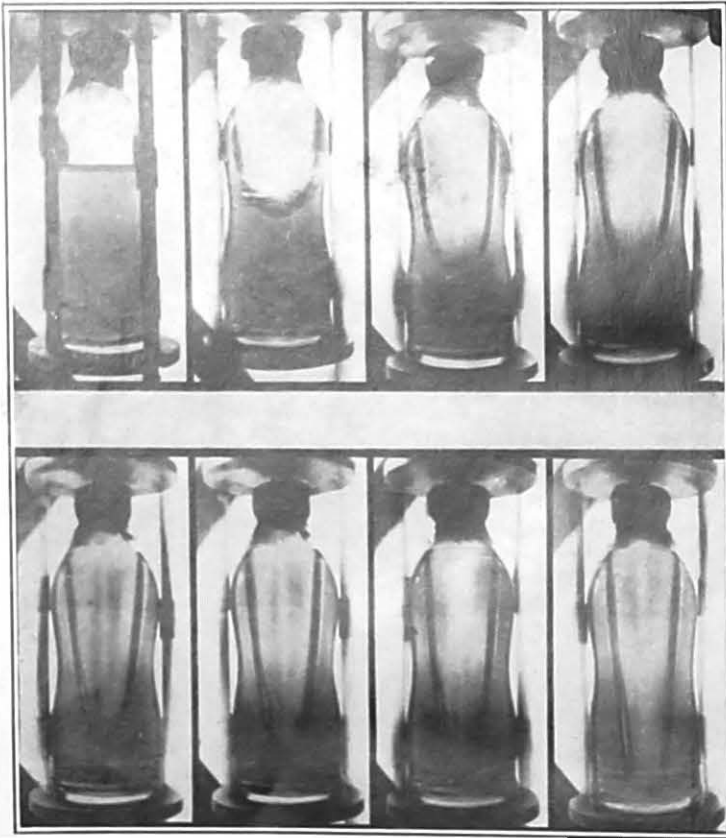
PLATE III



Making Drying Caps for M.R.C. Transfusion Bottles.

The drying caps are made from perforated aluminium caps with a layer of adhesive tape wound round them. The filter pads are fixed to the caps with stainless steel spring rings, and the surplus pad trimmed off with scissors.

PLATE IV



Stages in the Development of the Cones during Spin-freezing.

This series of photographs was taken with an interval of approximately 4 seconds between each exposure.

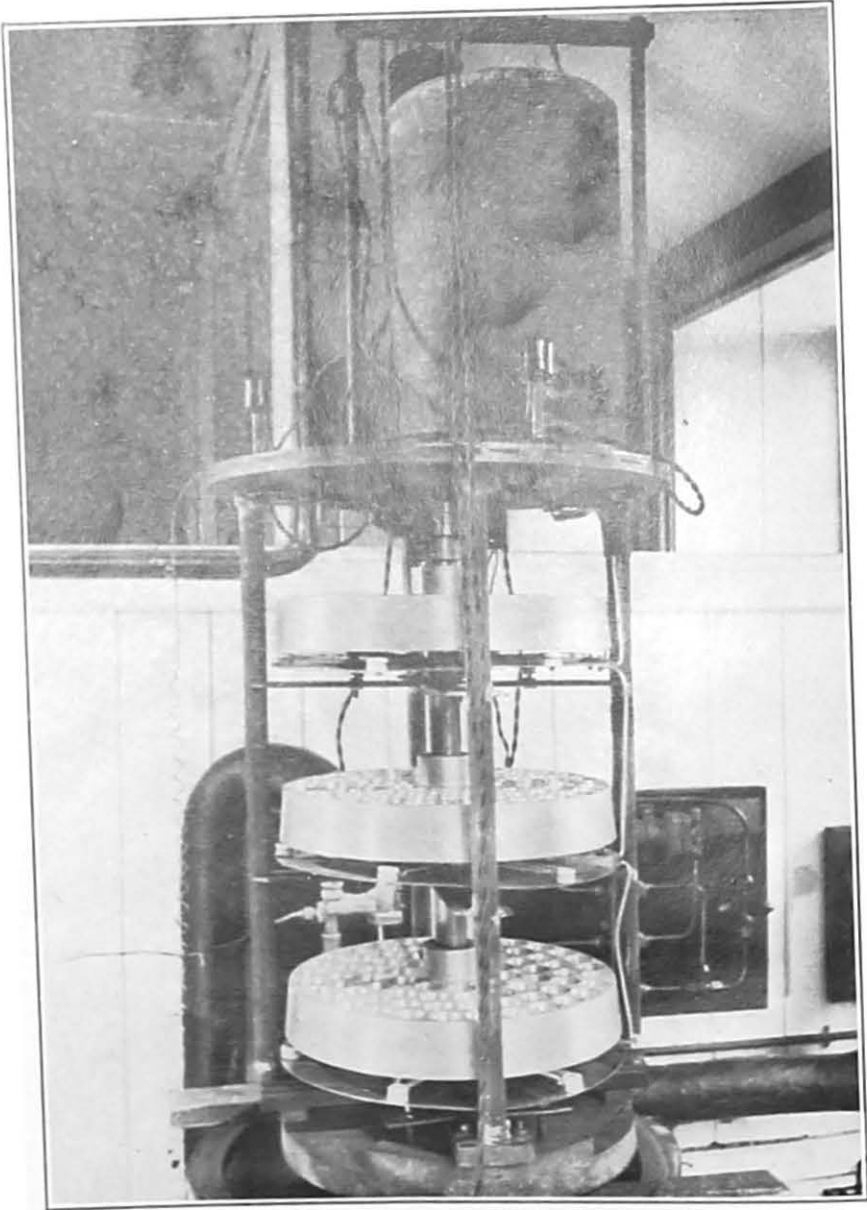
PLATE V



Vertical Spin-freezers in the Freezing Room of the Large Drying Plant.

This cold room is maintained at -18°C . by a current of air which is kept circulating over the brine coils; these are behind the diaphragm seen at the far end of the room. There are three spinners, each of which can take 36 M.R.C. transfusion bottles, a total load of 108 bottles per run of two hours.

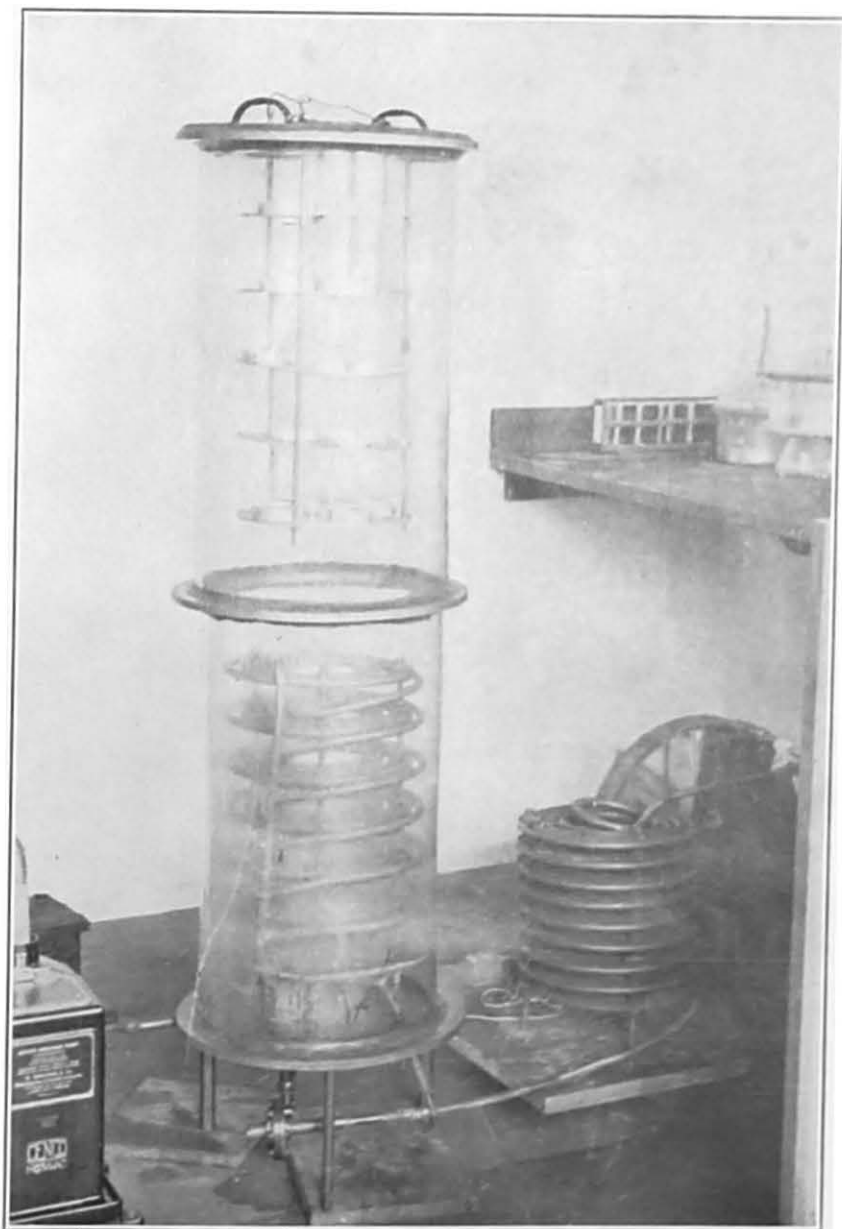
PLATE VI



One of the Heater Heads for the Final Pilot Plant, after its Conversion for Centrifugal Vacuum Spin-freezing.

This head is for centrifugal vacuum spin-freezing 225 bottles, each containing 10 ml. of fluid. It consists of three aluminium discs on a central shaft which is rotated at 1,450 revs./min. by the electric motor under the top cover. Each disc has 75 holes drilled in it at an angle of 7° from the vertical. The cast-iron slip rings and Morganite brushes for the resistance thermometer can be seen at the top of the shaft. Beneath each disc are the heater spirals and radiant heat screens.

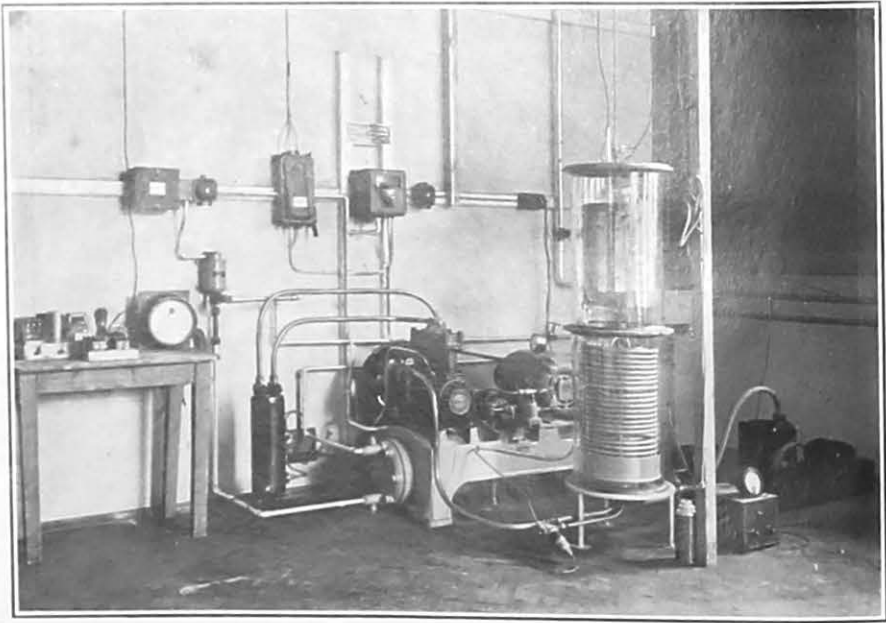
PLATE VII



The Experimental Drying Plant.

The desiccator is made from glass cylinders, and the refrigeration unit is a small SO_2 unit. The lowest temperatures obtained with this plant were around -20°C ., but the results obtained showed that the principle was sound and enabled the preliminary experiments on automatic heat control to be carried out successfully.

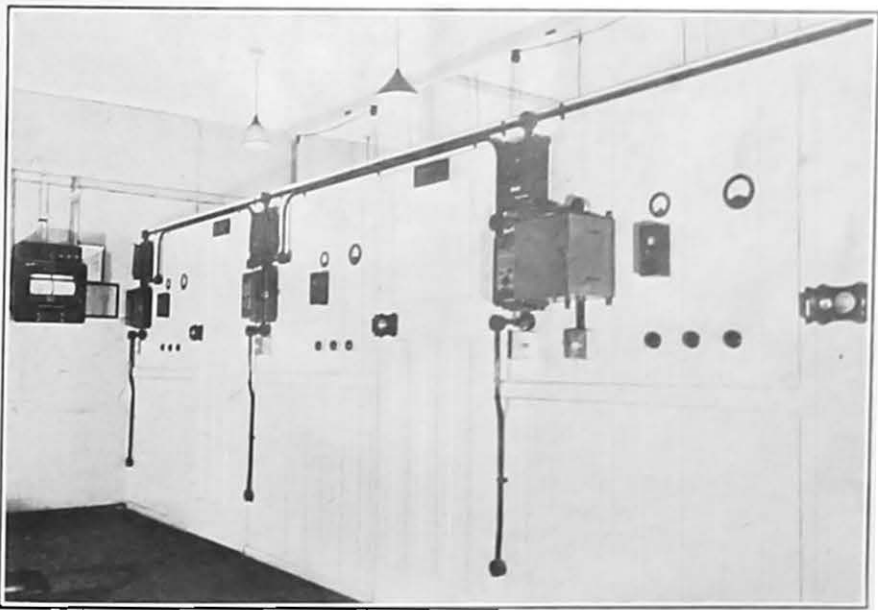
PLATE VIII



The Original Pilot Plant.

The SO_2 compressor has been replaced by a 3-cylinder "Hallmark" methylchloride machine giving a temperature below -40°C . with a heat exchange of 250 watts. This machine could dry 5 litres at a time from a 25-litre bottle.

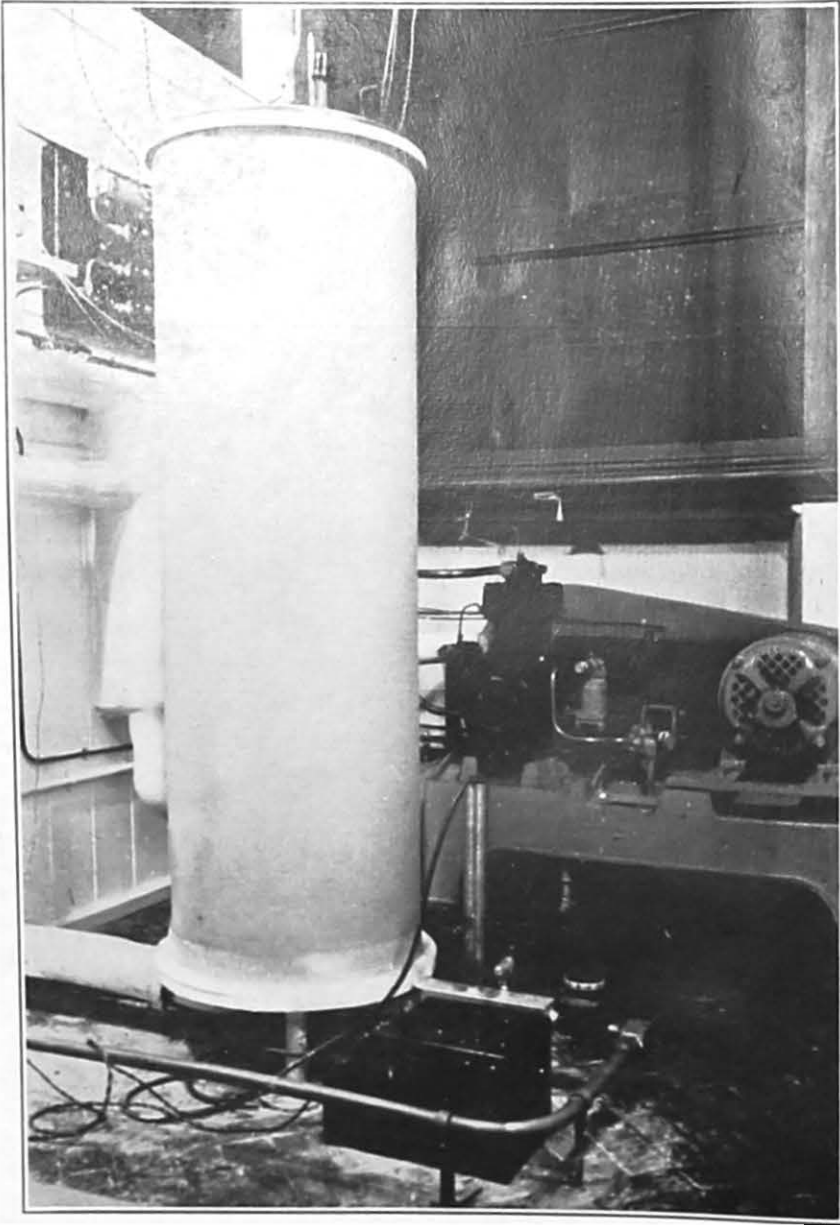
PLATE IX



The Final Pilot Plant. General Lay-out.

The final pilot plant consists of three units each with its own 2 h.p. methyl-chloride compressor. The desiccators are 4 ft. 3 in. by 16 in. diameter. Each unit is housed in its own cubicle with the control gear on the front panel. The recording galvanometer can be seen at the far end of the room.

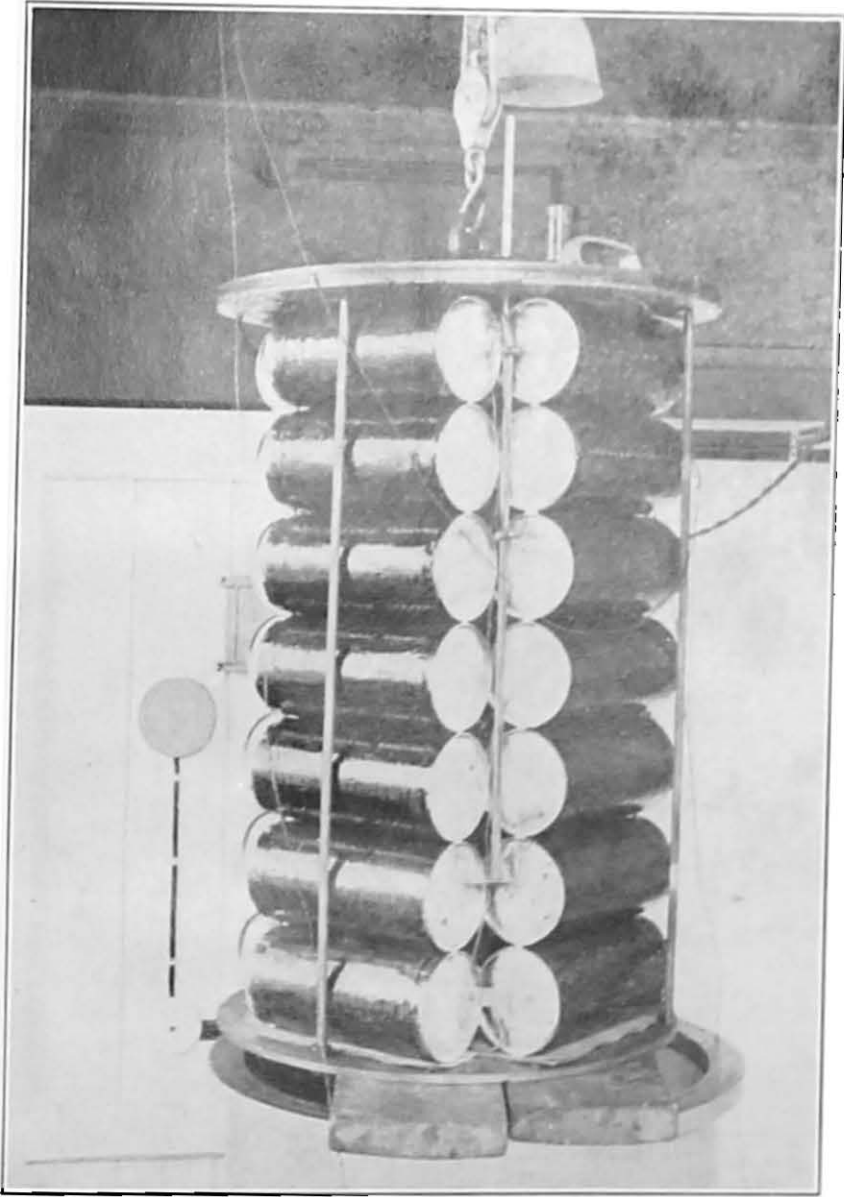
PLATE X



One of the Desiccator Chambers of the Final Pilot Plant.

These chambers are made from lap-welded steel steam-pipe. In the bottom half of each chamber is the condenser coil which is cooled by direct expansion of methyl-chloride. Interchangeable heater-heads enable these chambers to be used for drying from varying sized ampoules and bottles.

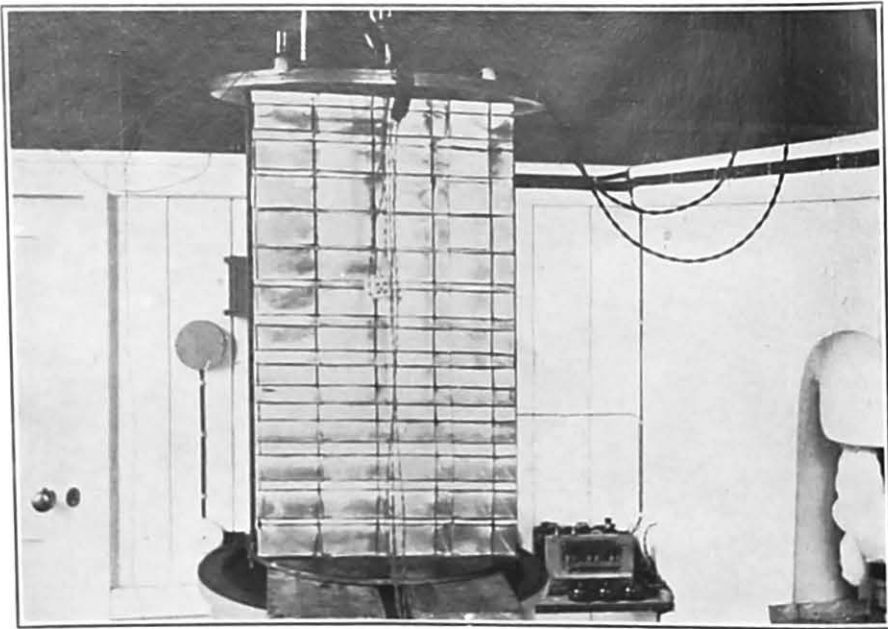
PLATE XI



One of the Heater Heads for the Final Pilot Plant.

This head is made to accommodate 35 M.R.C. transfusion bottles, each of which will hold 400 ml. of serum or plasma vertically spun-frozen round its inside periphery.

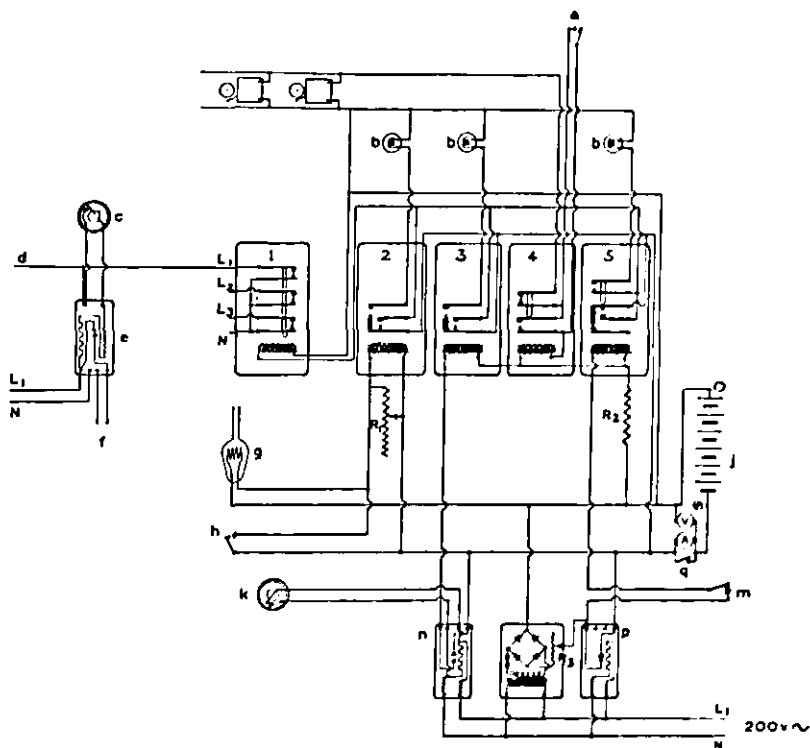
PLATE XII



One of the Heater Heads for the Final Pilot Plant.

This head is made to accommodate sixty 12-oz. medical flats, each of which will hold 200 ml. of serum frozen as a wedge on one side of the bottle.

PLATE XIII



Alarm System for the Large Drying Plant.

The G.P.O. relays and bells are operated by the 12-volt accumulator which is "trickle" charged from the electric mains.

G.P.O. Relay 1 cuts off the heater current of all eight chambers if any of the alarm relays should operate.

G.P.O. Relay 2 is the vacuum alarm relay operated by the vacuum alarm Pirani bulb. By adjusting the 200 ohm variable rheostat this relay may be set to operate at the desired pressure.

G.P.O. Relay 3 is the brine temperature alarm relay and is operated *via* a Sunvic relay from a thermostat in the brine-tank.

G.P.O. Relay 4 stops this bell ringing when operated by a push-button on the control panel.

G.P.O. Relay 5 operated *via* a Sunvic relay gives warning of an electric mains failure. Since this relay is only on one phase, the automatic vacuum valve which will operate on the failure of any phase is also connected in this circuit.

1, 2, 3, 4, 5. Telephone relays—all contacts shown in normal running position, Nos. 1-4 "out," No. 5 "in."

V 0-15 volts.

A 0-50 mA.

R₁ 200 ohms—adjusts pressure at which vacuum alarm operates.

R₂ 70 ohms.

R₃ 30 ohms—adjusts charging rate.

L₂, L₃ Connected similarly to the other chambers on the other two phases.

a Push-button to stop bells.

bb Indicator lamps—12 v. 3 w. car sidelamp bulbs.

c Thermostat in chamber (SUNVIC type TS3).

d Connected similarly to the other chambers on the same phase.

e SUNVIC relay type F 102-4M modified.

f To VARIAC type 100L and heaters in chamber.

g Vacuum alarm Pirani bulb on the vacuum manifold.

h Push-button to re-set vacuum alarm relay.

j 12 volt accumulator.

k Thermostat in brine tank—(SUNVIC type TS3).

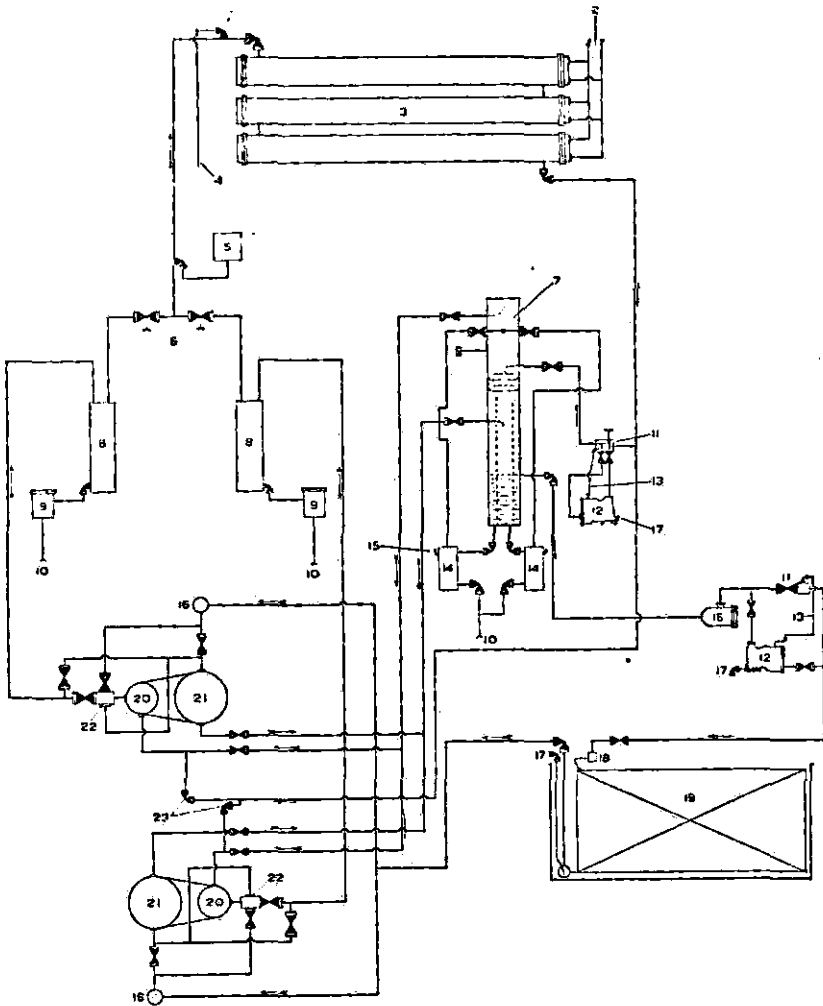
m Contacts on automatic vacuum valve.

n SUNVIC relay type F 102-5 modified.

p SUNVIC relay type F 102-5

q Push-button to read charging rate.

PLATE XIV

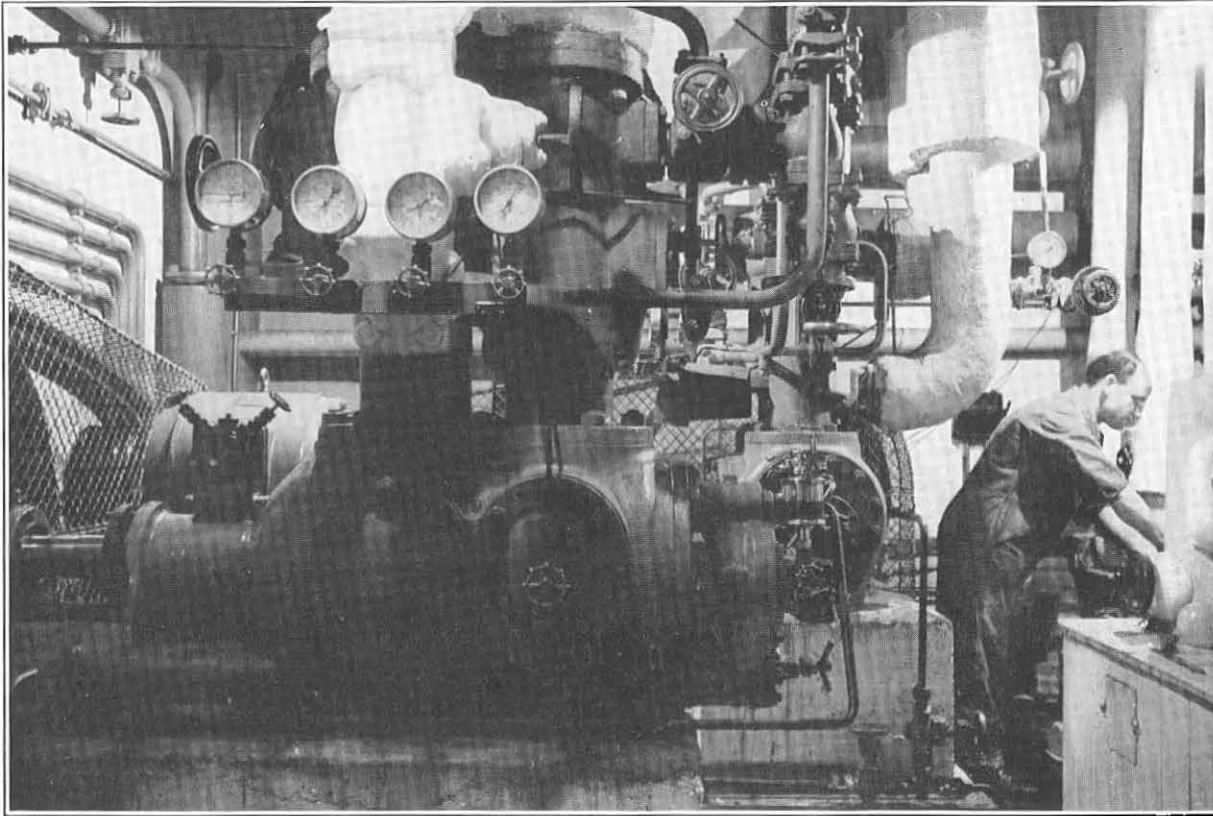


Ammonia Refrigeration Circuit in the Large Drying Plant.

1. Air purge valve.
2. Cooling water pipes.
3. Condenser.
4. Air purge pipe to exterior.
5. High pressure cut-out.
6. Non-return valves with lifting spindles.
7. Liquid intercooler.
8. Oil separators.
9. Float valves.
10. Oil return pipes to crankcases.
11. Hand regulators.
12. Float regulators.
13. Float regulator vent pipes.
14. Oil pots.
15. Charging valve.
16. Strainers.
17. Oil drains.
18. Liquid distributor.
19. Evaporator coils in brine tank. Three circuits.
20. High pressure cylinders.
21. Low pressure cylinders.
22. Safety discs.

Direction of flow { $\begin{array}{l} \longrightarrow \text{ gas.} \\ \longrightarrow \text{ liquid.} \end{array}$

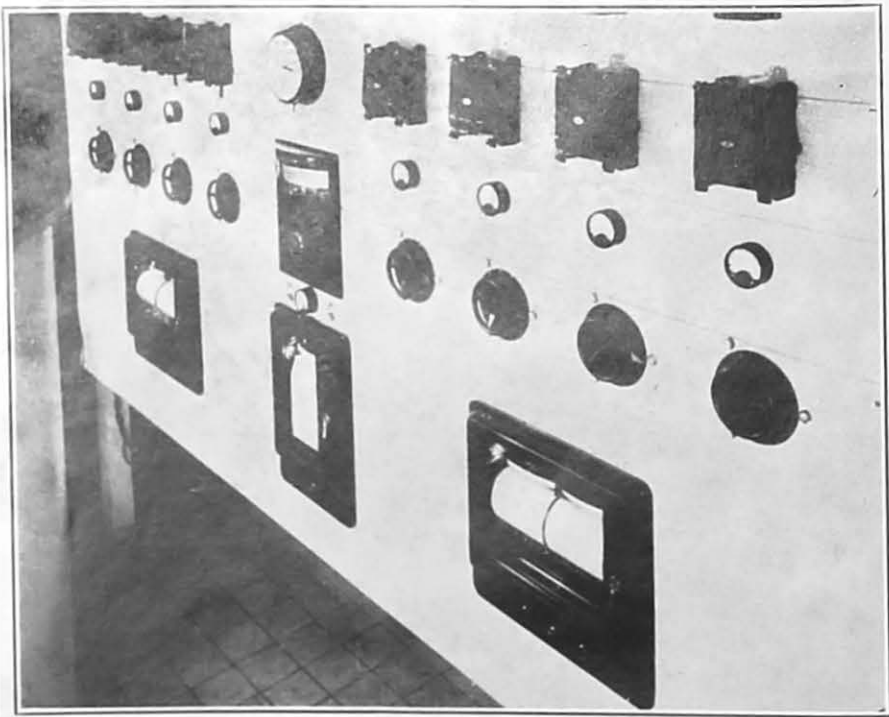
PLATE XV



The Large Drying Plant.

View of the engine room showing the two compounded ammonia compressors.

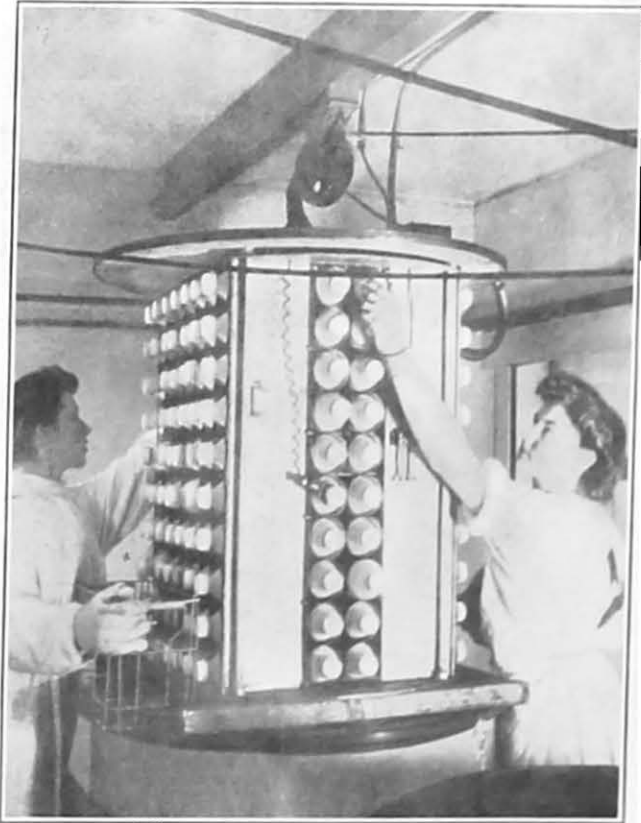
PLATE XVI



The Large Drying Plant.

The instrument panel showing the recording galvanometers and heat control circuits.

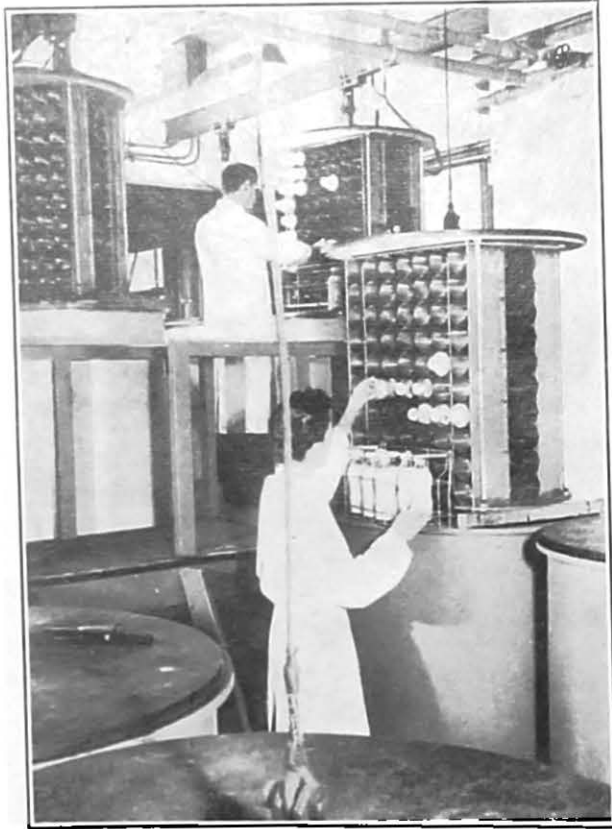
PLATE XVII



The Large Drying Plant.

Loading a primary chamber with frozen material. The control bottle with its thermocouple can be seen in the foreground.

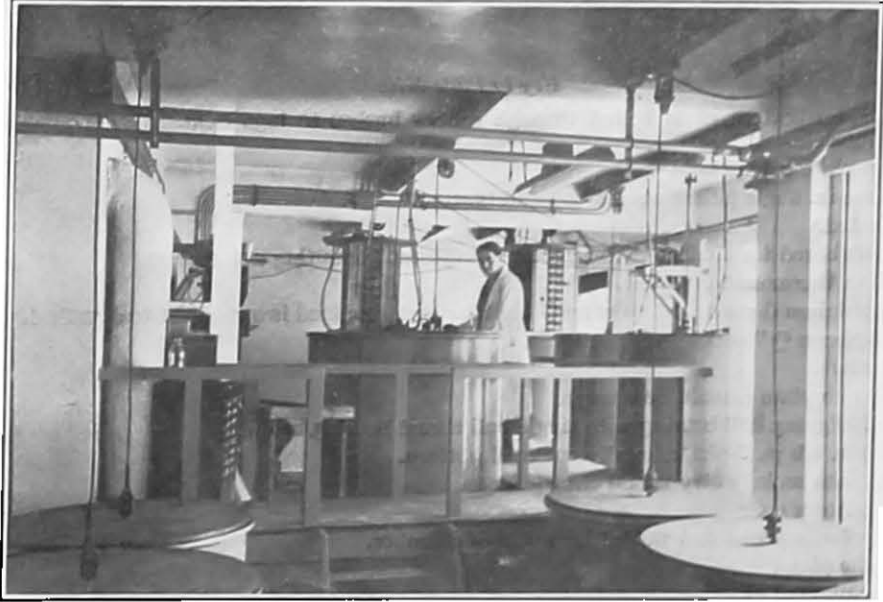
PLATE XVIII



The Large Drying Plant.

Transferring dried material from the primary to the secondary chambers.

PLATE XIX



The Large Drying Plant.

General view of the desiccator room, showing the secondary desiccators in the foreground and the primary desiccators with the loading staging in the background. The heater heads from the two chambers which are defrosting are raised.

Key to Plate XX.

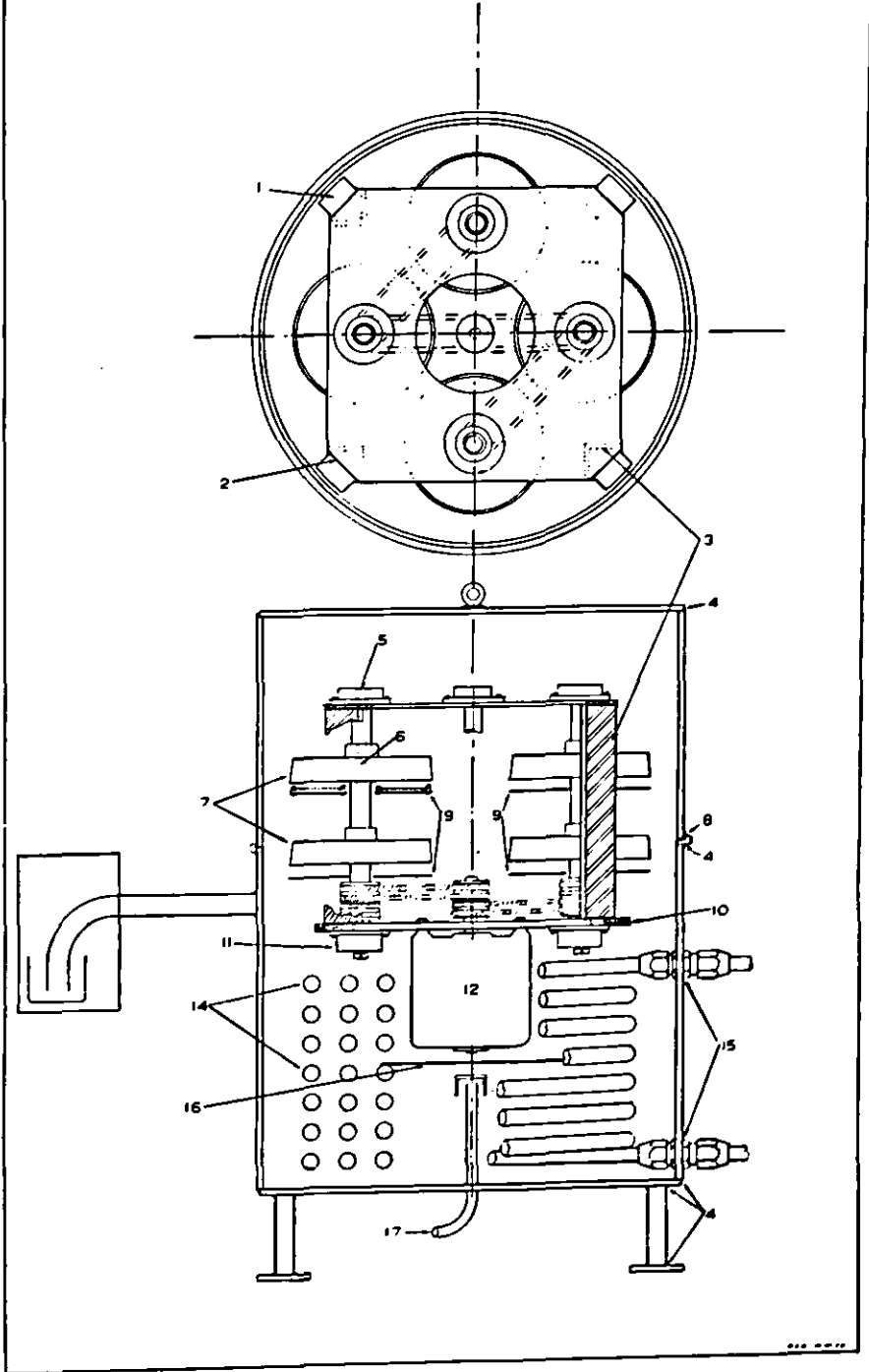
1. Lugs on bottom plate bolted through rubber bushes to lugs welded inside chamber (not shown).
2. Top plate cut off here.
3. Three in. angle pillars welded top and bottom.
4. Welded.
5. Shaft bored for contact thermometer thermostat (mounting not shown).
6. Oil in thermometer pocket.
7. Aluminium discs drilled to carry 75 bottles each, inclined inwards 7° from vertical.
8. "Apiezon Q" seal.
9. Heaters.
10. Bottom plate (should be heavy).
11. Self-aligning ball bearing with single ball thrust bearing below.
12. Motor, 1 h.p., 3-phase, totally enclosed pattern.
13. Vacuum sealed entry for all electric leads.
14. Condenser coils.
15. Nipples, screwed and welded into wall of chamber.
16. Baffle plate.
17. Vacuum outlet.

PLATE XX

GREAVES CENTRIFUGAL VACUUM DESICCATOR

ROUGH LAY-OUT OF SUGGESTED DESIGN

(OUTPUT 600 x 10 ml per day)



Centrifugal Vacuum Spin-freezing Apparatus in the Converted Final Pilot Plant.

Design of plant for daily drying of 600 ampoules, each containing 10 ml. by the method of centrifugal vacuum spin-freezing.