

THE PRESERVATION OF PROTEINS BY
DRYING.

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**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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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.

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R. I. N. GREAVES, M.D.

- (from the Drying Unit of the Medical Research Council)

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GENERAL INTRODUCTION

Protein molecules are the basis of all living material, and "life" itself would seem to involve the continual building up and breaking down of these molecules. No protein molecule has yet been synthesised; its large size, complex structure and instability have so far provided the biochemists with insurmountable problems.

Outside the living organism, protein molecules tend to aggregate and become insoluble—a sequence of events known as "denaturation." Denaturation is influenced by time and temperature, and the susceptibility of different protein solutions to denaturation is very variable. As extreme examples, milk may be boiled with the formation of but little insoluble residue, whereas the white of an egg is completely coagulated by heating to 70° C. Any factor which tends to increase the chances of aggregation of the molecules of a protein solution will also tend to hasten the process of denaturation; for example, a solution of pure crystalline egg-albumin will be denatured by gentle shaking. Conversely, the lower the temperature of storage, up to a point, the longer may a protein solution be preserved in its native state. As the temperature of storage is lowered, a marked improvement in preservation is found to occur once the freezing point is passed, and below the "eutectic" point, where the whole mass becomes solid, preservation is excellent. The effect of temperature on storage may be illustrated conveniently by the preservation of the complement activity of guinea-pig serum; the labile constituent, complement, is destroyed in 12 hours at room temperature, in 24 hours at 0° C., in 6 months at -10° C., and it shows no change after a year if the serum is stored at -25° C. Since the chief salt in serum is sodium chloride, with a eutectic of -21.4° C., there will be no liquid phase at -25° C. and the serum will be virtually dry. Thus, for perfect storage, protein solutions must be preserved either frozen below their eutectic point, or dry.

Proteolytic bacteria will also destroy protein solutions. This destruction may be avoided by sterile technique, or by the addition of an antiseptic. Since most antiseptics derive their power from the fact that they are protein coagulants, their usefulness for the preservation of protein solutions is limited. Bacteria will not multiply, however, when frozen or dried, so that this cause of protein disintegration will be avoided under either of these conditions.

Low temperature storage is widely used for the preservation of foods and biological materials, and its use is becoming even more general. It is, however, costly and not always practicable, its chief disadvantages being the liability to mechanical failure and the lack of mobility. For these reasons the alternative method of desiccation is being increasingly adopted.

The removal of water from protein solutions requires the use of energy, and we have already seen that heat is one of the main factors in speeding up the process of denaturation. The problem of drying is therefore one of applying the necessary heat with the minimal destruction of protein. How much destruction can be allowed, will depend on the nature of the material

being dried. With proteins that are cooked for food, some denaturation is obviously allowable; here the problem is one of getting the dried food to the consumer in such a form that its taste and solubility are not greatly changed when compared with those of the fresh material; but with many biological materials no denaturation can be tolerated during the drying process, and with modern procedures this drying without denaturation can be achieved.

In regard to foods, chief interest has centred round the drying of milk, eggs, meat, vegetables, fruit and pectin. The value of dried milk for infant feeding is well known, the advantage being chiefly the control of bacterial growth that results from desiccation, and fortunately taste is of little importance in this case. For general use, however, all methods which avoid change of taste are too expensive as yet for products where low price is essential. Fats present a further difficulty. In the first place, they tend to separate on drying unless previously homogenised, and secondly, they turn rancid very easily, so that dried full-cream milk must be dispensed in a costly oxygen-free gas-pack. It is interesting to speculate on a comparison of the cost of production and distribution between a properly dried full-cream summer milk and the present practice with liquid milk, which involves winter feeding of cattle, and costly and wasteful daily deliveries. The same considerations apply to eggs, but since here the basic product is more expensive per unit volume than milk, there is better reason to hope that more suitable (if costly) methods of preservation may soon be devised.

With meat, fruit and vegetables, the problem is further complicated by the fact that the water is contained in cells, the cell membranes inhibiting evaporation unless ruptured; enzyme activity must also be neutralised, and, in the case of fruit, it is often found that the factor associated with the taste is volatile and distils off with the water.

In the medical field we are fortunate in that the primary cost of the materials we wish to preserve is high, and consequently the actual cost of desiccation is of little importance. Freeze-drying is therefore the method of choice for preserving most therapeutic substances. The materials in this field which have been successfully preserved in the dry state are numerous, and may conveniently be classified under the following headings:—

- (a) Biological standards.
- (b) Therapeutic immune sera prepared in animals.
- (c) Immune and convalescent sera prepared in man.
- (d) Diagnostic antisera.
- (e) Human serum and plasma, for transfusion.
- (f) Unstable drugs—e.g., sodium and calcium salts of penicillin.
- (g) Hormone preparations.
- (h) Bacteria, viruses and yeasts—bacteria may be retained in their virulent form, free from variation.
- (i) Vaccines.
- (j) Human breast milk.

PART I.—METHODS OF DRYING

The problem of drying protein solutions resolves itself into how to supply the necessary energy for evaporation in the form of heat, without destroying the protein. It has already been stated that denaturation is a time-temperature phenomenon; thus, one method of approach is to expose the liquid in so thin a film that drying is very rapid. As the dry material is stable, the aim of the "high-temperature-short-time" methods is that drying should be

complete before the temperature of the solid has had time to reach a dangerously high level. Two methods are in common use, namely *roller* and *spray* drying; of the two, a good spray dryer is to be preferred as causing less damage to the proteins. These methods are comparatively cheap in operation, and are widely used for the drying of milk and eggs.

An alternative procedure would be to remove the water by chemical means. If, for example, serum were added to absolute alcohol in the cold, the water would leave the proteins, which would be precipitated; this precipitate could then be washed with ether to remove the alcohol, and the ether finally evaporated off, leaving a dried protein.

If warm dry air is blown across the surface of a liquid, evaporation is fairly rapid at normal room temperature. Attempts have been made to utilise this principle, the difficulty being to get a sufficiency of surface with reasonable sterility; the most successful results have followed the use of "Cellophane" tubes.

Under normal conditions water vapour molecules are continually leaving the surface of a liquid, but the majority collide with the molecules of gas above the surface of the liquid and so ricochet back. If the kinetic energy of the liquid molecules is increased by the application of heat, the tendency is for more molecules to escape, and evaporation becomes more rapid. Conversely, if the gas above the surface is rarefied, there are fewer collisions, so that more molecules escape, taking with them their latent heat of evaporation, with the result that, for the same heat input, evaporation will proceed at the same rate but at a lower temperature. By working, therefore, at a reduced pressure it is possible to dry protein solutions reasonably rapidly at a temperature well below their coagulation point.

As drying proceeds, however, the solution concentrates and eventually a glue-like scale is left, which it is very difficult to dry completely; further, the solubility is low and solution is never quite complete. Nevertheless, proteins which will stand this treatment may be well preserved if sufficient trouble is taken to get the scale preparation really dry—a process which may take anything up to six months.

As the pressure of air is further reduced, the temperature of evaporation for the same heat input becomes lower and lower, till the liquid freezes and evaporation proceeds as a sublimation of water vapour from the solid state. This procedure of "freeze-drying" gives a product quite different from that obtained by low-pressure evaporation from the liquid state. The dry solid shows little reduction in volume compared with the liquid; the protein, being present as a light spongy mass, is rapidly soluble; and denaturation even of the most delicate proteins cannot be detected.

Though the low temperature at which desiccation is carried out is itself beneficial as far as the protein is concerned, it seems that the real reason for the excellence of this method is the fact that, as the solid state is maintained throughout desiccation, the salts and protein molecules do not concentrate; this avoids the harmful effect of high salt concentrations on the proteins, and the increasing tendency of the protein molecules to aggregate as their concentration increases.

It is clear that freeze-drying is the method of choice for the desiccation of all valuable biological materials. The process is, however, the most complex and expensive of all methods of drying.

The different methods of drying protein materials will now be critically surveyed in greater detail.

High Temperature Methods

(a) *Roller or Film Drying*.—Roller dryers were introduced both in Britain and Germany during the war of 1914–18, for the drying of potato flour.

After that war the driers were sold cheaply to milk factories, and, within limits, were found to be capable of producing a marketable dried milk at a low cost.

Today there are many different models available, but the principle of operation is well shown by the "Just Hatmaker Double Drum Drier". This apparatus consists of two polished steel drums, set with their axes horizontal and parallel and separated by a space of approximately 0.02 inch. The drums are heated by steam and rotate in opposite directions. The milk is fed into the trough between the two cylinders, and is spread out as a surface film over the cylinders as they rotate. The speed of rotation is variable, averaging about 12 revs/min, and is set so that for any temperature of the drums the thin film of milk is dried in about 3/5th of a revolution. The water vapour evolved is removed by an extraction fan, and the dried film removed from the drums by knife blades accurately set along their length. The thin crumpled sheet of dried milk is collected in hoppers, and then reduced to a powder by bolting and sifting.

In the design of these driers it is important that the vapour evolved should not be allowed near the dried product, as it will wet it; the hot vapour should, however, be led over the incoming milk to preheat it, thus effecting an economy in running costs. It will be appreciated that the setting of the knives requires great care, for if a portion of the powder is not removed from the drums it will continue to cling to the hot surface and become charred.

The knives must be made of steel which is not too hard, or else the wear on the drums will be excessive. It is usual for a single knife to be used for the whole length of the drum, with adjustment screws every 1½ inches; such knives are liable to "chatter", with the result that the drums have to be rotated at a slower speed. In the "Kestner" pattern, multiple knives are used, each with its own adjustment.

Milk dried by this process has a solubility of about 80 per cent. if whole milk is dried, the majority of the cream separates as fat. The two main causes of deterioration of dried milk are the presence of excess moisture, and the development of rancidity due to the oxidation of the fat. Whole milk is less prone to rancidity when roller-dried than when spray-dried, and since the moisture content can be kept low, the keeping properties of roller-dried milk are good. Its nutritional properties are likewise good, and it is widely used for infant feeding. In general, it may be said that the higher the temperature at which cow's milk for infant feeding is dried, the better do the infants thrive; this is probably related to the fact that the curd formed in the infant's stomach from boiled cow's milk is much finer than that from fresh cow's milk and therefore offers a greater surface for the action of the digestive juices.

In roller drying, the milk is not able to cool by evaporation, so that desiccation takes place at the boiling point; in the majority of the plants, dry steam is used for heating the rollers and the dried product is subjected to very high temperatures. That the milk is not wholly destroyed is due to the fact that the time of contact with these high temperatures is so short.

Attempts to decrease the cost of drying have been made by pre-concentrating the milk, but on the whole they have failed, since the concentrated milk seems to be more sensitive to high temperature than milk of normal concentration.

Other attempts to improve the quality of the roller-dried product have been along the lines of shortening the time of heat contact by decreasing the depth of the film; this has usually involved attempts to spray the liquid milk on the drum, but films of uniform thickness are difficult to obtain by this method. By carrying out the roller-drying process in a partial vacuum, so that the temperature of drying is maintained at a lower level, an improved quality of dried milk has been obtained. In this process, the rollers are contained in a chamber in which a vacuum, of the order of 27 to 28 inches of mercury, can be

maintained. In this reduced pressure, the speed of evaporation is so increased that there is cooling of the liquid during drying, and only the relatively stable dry solid is momentarily exposed to the high temperature of the drum. Although the drum may be heated by dry steam at 14 to 15 lb. per sq. in., the temperature of the liquid milk never rises above 114° F.

Milk dried by this method is much more soluble than that dried by the open roller method, and it shows no insoluble residue. Though the capital expenditure is greater than for the open roller method, the running costs can be reduced, since pre-concentrated milk can be used for this process. The method has been further improved in the vacuum band drier, in which the milk is distributed in a thin film on a continuously circulating band, so that much larger heating surfaces can be employed than with the drum methods.

Roller drying has proved too drastic a method for the drying of therapeutic reagents, and even in the vacuum roller process temperatures are too high for sensitive proteins.

(b) *Spray Drying*.—Of the high temperature drying methods, spray drying is the least destructive to proteins, and the method has even been applied with some success for drying therapeutic antisera, human serum and plasma, and penicillin solutions. The principle of spray drying is that the liquid, finely divided into minute droplets so that the surface is maximal, is injected into a stream of hot dry air; evaporation from the surface of these droplets is so rapid that they do not attain the temperature of the air till the particles are dry, and therefore heat resistant. The time taken is so short that concentration is minimal; and provided that the air temperature is not too high, denaturation is slight and the powder has many of the characteristics of freeze-dried material. For a perfectly uniform product, however, the size of the droplets must be identical and they must escape from the air-stream at exactly the same moment; it is these requirements which have never been satisfied by spray-drying technique.

An immense amount of research has been undertaken in order to obtain uniform droplets of liquid. This has not yet been achieved by any form of air-atomised jet; the nearest approach to uniformity has resulted from the use of the "Kestner" disc. The disc is spun at a high speed (5,000-20,000 revs./min.), and the liquid dripped on its centre. The liquid is thrown out centrifugally and leaves the edge of the disc as a fine mist. In the "Krause" system the milk is fed from below. The disc atomiser, in addition to producing a more uniform size of droplet, has no narrow orifices which might clog, and it is easy to clean; moreover, since the size of particles can be altered by controlling the speed of the disc, the bulk density of the final product can be varied to suit circumstances. The particles, however, are not absolutely uniform, nor has a disc been developed which is suitable for small quantities.

Wilkinson, Bullock and Cowen (1942) described a small spray-drier as suitable for the drying of human serum and plasma for transfusion. It is therefore convenient for our purpose to consider this apparatus from the point of view of the difficulties encountered, how they were met, and to what extent successfully. This is important, for in certain respects the spray-drying methods are very convenient; for example, the apparatus is comparatively cheap in initial cost, when compared with freeze-drying plants; operation is a continuous process, and the final dried product, unlike the freeze-dried product, shows a marked reduction in volume over the liquid material.

In their article the authors state: "The principle of our plant is that the plasma or serum is passed through a suitable atomiser into a stream of sterile warm air, the water being evaporated from the surfaces of the droplets. The main advantages of this process are: (a) the rapid evaporation obtained on

account of the very large superficial area exposed at the surfaces of the droplets, and (b) the temperature of the material does not rise above the wet-bulb-temperatures of the drying air until drying is almost complete, so that drying may be done quickly at temperatures much lower than those required in other processes carried out at normal pressure, and the danger of impairing the product by over-heating is avoided.

"The apparatus, all of which can be sterilised before and kept sterile during use, consists essentially of a large drying chamber into which the plasma or serum is blown in a very fine umbrella spray from an atomising jet in the top of the chamber, into a current of warm sterile air circulating within the chamber. The dried plasma or serum collects at the bottom of the chamber, and also in a second smaller chamber or cyclone, the moisture being carried away in the outgoing air stream."

Before starting a drying cycle, sterile air heated to 230° F. is blown through the apparatus for one hour; since the apparatus is heat-insulated, this raises all parts of it to a temperature sufficiently high to give adequate sterilisation. The air inlet to the fan is through a thick felt syrup-strainer, covered with two layers of cotton wool and cotton cloth arranged alternately. This alone is probably an adequate filter for a fan sucking as much as 250 cu. ft. of air per minute; but even if it is not perfect, there is little likelihood of an occasional organism getting past the electric heaters without being destroyed. The fact that the dried products always proved sterile would be anticipated on theoretical grounds.

Wilkinson used a standard type of air-atomiser, giving very variable particle size; and this, because of its narrow orifice, was liable to clog in use, so that the procedure had to be under constant observation.

With unequal particles, the heavy ones, which take longest to dry, fall out of the air stream first. If the air temperature is raised so that these particles are dried properly, the smaller particles will be overheated; therefore, in order to avoid the denaturation of the small particles, Wilkinson was forced to reduce the hot air temperature to between 70° and 80° C., with the result that the final moisture content of the dried product was 10 per cent. A powder containing 10 per cent. residual moisture would have poor storage properties, so that it was necessary to dry this powder further, in a high vacuum over a chemical desiccant such as phosphorus pentoxide, and the removal of so large an amount of water in this way is by no means a cheap process.

Another problem with spray driers is that a portion of the powder tends to stick on the side of the chamber, where it gets completely denatured by its prolonged contact with the hot air. Some of this powder eventually drops off and so contaminates the final product.

Nevertheless, Wilkinson was able to dry scale-pepsin, muscle extracts, human plasma-saline, plasma and serum, without demonstrable denaturation; and guinea-pig serum and human blood-grouping serum, without loss of complement-activity or titre, respectively.

It is perhaps as well to emphasise that the usual tests for protein denaturation, though sensitive for testing minor changes in the bulk of the protein, are not nearly so reliable for detecting radical change in small amounts of the protein; and, in fact, Wilkinson's dried plasma when reconstituted contained numerous notes, presumably of denatured protein.

If spray-dried powder is examined microscopically it is found to consist of tiny hollow spheres of dried material. The majority of these spheres rupture; some do not, however, and the moist air they contain will rapidly spoil the protein. The removal of this moist air is difficult; if the powder is vacuum-packed, the vacuum is slowly lost as the air diffuses out, and the exhaustion

has to be repeated on several occasions; similarly, if it is nitrogen-packed, the oxygen content slowly rises and the pack has to be repeated.

On re-resolution the spray-dried powders are found to be very difficult to wet. This would cause great difficulties were it not that the trouble is largely avoided by vacuum packing.

It is thus apparent that spray-drying has several grave disadvantages when applied to delicate and expensive proteins in small amounts. Nevertheless, when used for human serum and plasma it has one great advantage over freeze-drying; in the absence of freezing, the lipo-protein complexes are not disturbed and the solution is clear on reconstitution. Freeze-dried material is always cloudy on re-resolution, unless a preliminary ether-extraction of the fat is carried out by the technique of MacFarlane (1943a).

For details and references on the roller- and spray-drying of milk, the reader should consult Scott (1932).

Chemical Drying

Chemical drying consists of mixing the protein solution with some chemical which has a greater affinity than the protein for water. The principle is largely used for the preparation of protein fractions from sera, particularly in the concentration of therapeutic antisera. In this process, the protein solutions are mixed with strong solutions of ammonium or sodium sulphate, and various protein fractions are precipitated by altering the concentration of the salt and the reaction of the solution. These precipitates can be filtered off and dried on blotting paper, but of course are highly contaminated by the salting out chemical. Unfortunately, the chemicals can be removed only by dialysis, which involves re-resolution of the protein, so that this method cannot be used for drying protein solutions.

Hardy and Gardner (1910) showed that if serum cooled to 0° C. were sprayed into a mixture of absolute alcohol and ether at -10° C., the proteins precipitated without denaturation and the lipoids were removed. The precipitate could be separated from the alcohol-ether mixture by filtration, and then washed with ether to remove all the alcohol; any remaining traces of ether could be removed from the precipitate by placing it in a desiccator in a partial vacuum over sulphuric acid, when the ether combines with the acid.

This procedure gives a beautifully dry powder, showing no denaturation: the powder is rapidly soluble and, having lost its lipoids, it gives a clear liquid on re-resolution. Bick (1943) has used this principle for drying human serum, and as the result of clinical trials she deduces that the product is suitable for transfusion purposes.

There are, however, difficulties in this technique. The amount of alcohol-ether mixture required is very great, so that for working on any large scale a solvent-recovery plant would have to be used. The temperatures must be very accurately controlled, and for large-scale work the operators would probably have to work for long hours at low temperatures.

If it were not considered necessary to remove all the lipoids, the process could presumably be altered by the omission of the ether, working with alcohol alone as in the Harvard technique of Cohn for the preparation of serum fractions by alcohol precipitation. The precipitated protein could be separated from the alcohol by filtration, or by centrifugation on a refrigerated "Sharples" centrifuge. The removal of the last traces of alcohol could be effected either by vacuum desiccation, using a cold condenser of "dry-ice" at -78° C., or better still, since alcohol is still liquid at this temperature, a condenser of liquid air at -184° C., or else by washing the precipitate with ether and removing the ether with sulphuric acid as in the Hardy and Gardner process.

Desiccation from the Liquid State

The principles involved in desiccation from the liquid state are naturally identical with those already discussed under the headings of roller- and spray-drying, though the approach to be discussed now is quite different. Two distinct methods can be recognised; (a) distillation at reduced pressure, (b) the use of large surfaces by means of semi-permeable membranes.

(a) *Distillation at reduced pressure.*—This was the method originally used by Ehrlich for the preservation of antitoxin standards. A measured quantity of the antitoxin was placed in a glass bulb, this bulb being connected by glass tubing to a second, similar, glass bulb containing a chemical desiccant. This double ampoule was then evacuated to a reduced pressure by a hand pump, and sealed off. In the course of time, the water vapour found its way to the desiccant, with which it went into chemical union and was retained; and the antiserum was reduced in the course of months to a dry glue-like scale. At this stage, if required, the union between the two ampoules could be sealed off in a flame, leaving the bulb containing antitoxin in a suitable condition for transportation. This "scale preparation" was but slowly and incompletely soluble, and there was some loss of antitoxin content during the drying; but the loss from all ampoules was the same, so that it was possible to use this method for the preparation of standards. Desiccation would have been accelerated if the process could have been carried out at lower pressures; but if lower pressures were applied, the serum frothed violently and an unmeasured quantity was invariably lost.

Martin (1896) approached the problem from a different angle; his apparatus consisted of a large wide-necked bottle connected *via* a similar vessel and an ice-condenser to a water pump. His bottles were immersed in a water-bath which was maintained at $+37^{\circ}$ C., while serum was sucked slowly through a bacterial filter into the first vessel by means of the vacuum. On its introduction to the vacuum, the serum frothed violently, but to a certain extent the frothing could be controlled by adjusting the rate of flow; any froth which escaped from the first bottle was collected in the second. By frothing, the serum distributed itself into an enormous surface, so that evaporation and drying were very rapid. Again a scale preparation was obtained which was slow in re-solution.

Hartley, Eagleton and Okell (1923) applied Martin's method in a slightly modified form, and reduced a large variety of substances of immunological interest to the dry stable condition. The method failed with complement, but satisfactory preparations were obtained by drying guinea-pig serum in desiccators, at low pressure, over sulphuric acid, phosphorus pentoxide being substituted later as the chemical desiccant.

Hartley (1936) then made the discovery that the frothing, which was the main trouble if low pressures were used in the Ehrlich process, was largely due to the liberation of dissolved gases; he found that if the pressure was reduced till bubbles just started to appear, and was then held stationary for a while, the bubbles would disappear and would not reappear till the pressure was reduced to a lower level. In this way, eventually, a quiescent solution was obtained even at fairly low pressures, and, since there was no danger of losing material by this system of "controlled de-gassing", Hartley adapted the process for the drying of standards of therapeutic sera. As a desiccant he used phosphorus pentoxide, and the standards were dispensed in special "Pyrex" ampoules with their necks plugged with cotton wool to maintain sterility, many being accommodated in a large, laboratory type, desiccator.

The method of Martin (1896) has been revived in recent years for the preparation of desiccated human plasma for transfusion, first by Edwards, Kay

and Davie (1940), and, more recently, samples of desiccated human plasma, which appear to have been dried in this way, have been sent from the U.S.S.R. Human plasma desiccated by this method appears as lemon-yellow crystals with glassy surfaces; this glassy appearance is due to the fact that the liquid has concentrated to a hard glue-like scale. The removal of the residual moisture through this scale can be accomplished only over a very long period, and as a result these preparations usually have high residual moisture contents, of the order of 5-10 per cent.; they therefore tend to deteriorate with age. This deterioration is shown by the fact that their solution time, which is never very rapid, gets progressively longer, and more and more protein becomes insoluble. This material, therefore, cannot be considered ideal for transfusion purposes, particularly since speed of re-solution may be an important factor in emergency transfusion work. But what of the proteins? Even immediately after drying, 100 per cent. re-solution is not obtained, showing that some denaturation has occurred, and this becomes progressively greater with time. Definite protein changes can be shown by electrophoretic analysis.

But in spite of these deficiencies, Edwards, Kay and Davie (1940) claim that they have used the material successfully for the treatment of human casualties, so that the chief criticism of the product would seem to be on the grounds of its poor solubility and keeping qualities. In favour of the product, it should be stated that there is no alteration of the protein-lipoid complex on drying, so that the material is clear on re-solution.

(b) *The use of semi-permeable membranes—"pervaporation."*—Extruded "Cellophane" tubes have recently been introduced, and their convenience as semi-permeable membranes for laboratory use has been widely recognised. Thalhimer (1938) made use of these tubes for concentrating human serum for transfusion purposes, with a view to decreasing the volume for transport and, also, to increasing the osmotic pressure. He hung the "Cellophane" tubes filled with serum in a current of warm dry air; the water slowly diffused through them and was removed by evaporation from the surface, till eventually the serum was reduced to a dry scale in the tube. No expensive apparatus is required for this method of drying, which can readily be carried out with complete sterility; moreover, it is not necessary to have sterile water for re-solution, for the whole tube can be immersed in any water, when bacteria-free water will slowly diffuse through and dissolve the serum. Serum and plasma dried in this way have all the advantages and disadvantages of material dried from the liquid state.

Drying by this method is slow, but it can be speeded up by rotating the tubes in the hot air stream, and by increasing the surface area by arranging two tubes of different diameter one within the other, the serum being placed in the space between the two tubes (Hartman and Hartman, 1940).

In this country, Aylward, Mainwaring, and Wilkinson (1940) have described a technique for drying human plasma in "Cellophane" tubes.

Thalhimer (1939) has also described two methods of drying in "Cellophane" tubes from the frozen state and, since he is frequently misquoted, it is as well to draw attention to his methods here. One procedure is to hang up the tubes in cold rooms maintained below the freezing point, when evaporation will occur from the surface of the tube, in the same way as it would from a block of ice. But evaporation under these conditions is very slow, and Thalhimer found that the only way in which he could dry serum within a reasonable time was to keep the tubes very flat so that the thickness of serum was very small.

Alternatively, the "Cellophane" tube may be fitted with a rubber bung perforated by a glass tube, and the serum shell-frozen round the inside periphery of the "Cellophane" tube and then dried by sublimation from the frozen state through the glass tube, in the same way that any other open ampoule of serum can be dried. It is an entirely erroneous conception that

the rate of diffusion through the "Cellophane" would be anything like adequate for evaporation in a vacuum to be rapid enough to keep the material in the tube frozen.

High-vacuum Sublimation from the Frozen State

A new chapter in the story of the desiccation of proteins was opened by a communication of Shackell (1909). He showed that if protein solutions were first frozen and then introduced into a high vacuum in the presence of a desiccant, evaporation was so rapid that the material remained frozen, drying by sublimation from the frozen state without passing through the liquid phase. His dried material differed from that dried from the liquid state in that it showed little or no reduction in volume and was very rapidly soluble. By this method of desiccation he preserved guinea-pig complement, the immune serum of hog cholera, and rabbit brain containing the fixed virus of rabies; and, furthermore, he applied the method to the drying of meat, fruit and vegetables. Recent work has entirely substantiated Shackell's claims for the great value of his products.

Little more was heard of freeze-drying till Craigie's paper in 1931. Probably one reason for this was the scarcity of low temperature refrigerating machinery, and the fact that it was found impossible to introduce liquids into a vacuum sufficient to cause their freezing, without encountering uncontrollable frothing. Craigie (1931) placed small quantities of guinea-pig complement in large basins in desiccators, the bottom halves of which were filled with anhydrous calcium chloride. On evacuating the desiccators, the complement frothed violently till a degree of vacuum was reached at which the rate of transfer of water-vapour to the calcium chloride was so rapid that the material froze suddenly and continued to dry from the frozen state. Complement so dried did not lose its activity, but the bulky masses of the dried froth in the large basins had to be stored in vacuum desiccators—a fact which rendered the method of little practical value.

About the same time, Lancefield and Quastel (1929) described a similar method for drying proteins from the frozen state, using phosphorus pentoxide as a desiccant at ice-box temperature. In 1931, Adair and Robinson reported the successful drying from the frozen state of haemoglobin and crystalline egg-albumin in platinum basins, using phosphorus pentoxide in a high vacuum. Thus, the principle was introduced of making use of the speed of evaporation that can be obtained in a high vacuum, in the presence of a suitable desiccant, for freezing protein solutions, which thereafter dry from the frozen state. On account of the uncontrollable frothing, the method was gravely limited if storage in reasonably small containers was required and if it was essential that none of the material should be lost.

The first real advance in our knowledge came from a paper by Elser, Thomas and Steffen (1935), which reported their researches, over a number of years, into this problem. They started by freezing their material as a thin layer over the inside of spherical ampoules in a freezing mixture of ice and salt; the ampoules were then connected to a manifold containing phosphorus pentoxide, which was subsequently evaporated. When a sufficient degree of vacuum was obtained, the serum evaporated so quickly that it started freezing the ice and salt mixture; at this juncture, therefore, the ampoules were removed from the freezing bath, and, even with gentle warming from the air, the serum remained frozen till dry. These authors overcame the frothing difficulty in the same way as Shackell, by introducing the material in a frozen state into the vacuum.

They soon found, however, that for anything but small-scale work, phosphorus pentoxide was too expensive a desiccant; they accordingly introduced the refrigerated condenser. Originally, they did this by submerging

their manifold in a bath of dry-ice at -78°C. , assuming that water vapour would pass from the serum at, say -20°C. and condense at -78°C. , if the level of non-condensable gases was sufficiently reduced, by evacuating the system, to allow the free passage of water-vapour molecules to the condenser.

These experiments having proved a success, Elser *et al.*, with the co-operation of the Mulford Laboratories, constructed a large manifold in a cold-store, and, instead of submerging this in dry ice, they ran the evaporator coil of a mechanical refrigerator down its centre; the reason for this was to keep the path of flow of the water-vapour molecules as short as possible. Within limits, this apparatus worked well, but they had considerable difficulty in getting the numerous vacuum joints tight, and they ultimately abandoned manifolds for desiccator construction.

For their final apparatus, they used a large insulated steel box as a desiccator, and employed the evaporator coils of a mechanical refrigerator placed at the top of the box as their condenser. The serum to be dried was introduced on to the floor of the box, which was then evacuated. The serum frothed, and then froze, drying from the frozen state. These authors, however, abandoned the method for several reasons; first, they found that the desiccator was apt to leak; secondly, they were unable to instal automatic control of either the refrigerator or of the temperature of the frozen proteins; thirdly, as the solutions were frozen in a layer on the bottom of the desiccator, it was difficult to maintain sterile conditions when handling the dried product.

Real interest in the commercial and laboratory possibilities of freeze-dried material dates from the publication by Flosdorf and Mudd (1935) of their "Lyophile" process. Flosdorf and Mudd (1935) reverted to the manifold method of Elser, Thomas and Steffen (1935), but instead of refrigerating the manifold, they relied on condensation in a trap, placed in a bath of dry-ice in "Methyl-cellosolve" at -78°C. , between the manifold and the vacuum pump. As dry-ice was to be used in the condenser, they also made use of it for pre-freezing the serum in the ampoules. They described several types of apparatus, ranging in size from a small model for laboratory work to a large model for the commercial drying of large numbers of ampoules. Their laboratory model has been widely used, and within limits it has given excellent results. Its main deficiencies have been due to its proneness to leak at its many joints, and to the fact that the obstruction to the flow of water-vapour introduced by the manifold is so great that it is impossible to plug the ampoules in the interests of sterility, and still maintain the serum frozen.

The principal operation of this type of apparatus is as follows: First, stress is laid on the preliminary freezing; Elser, Thomas and Steffen (1935) had emphasised this point, suggesting that slow freezing might lead to concentration of salts and consequent injury to the proteins. By using baths of dry-ice, freezing is rendered very rapid; and, moreover, if the ampoule is rotated in the bath, the serum may be shell-frozen round the inside periphery of the ampoule, giving a maximum surface for evaporation during the drying process, together with a minimum depth. The frozen ampoules are then connected to the manifold, and are kept cold in a tray of dry-ice while the vacuum is being obtained. When the critical pressure is passed, the dry-ice trays are removed and the ampoules are heated by the warm air of the room. In spite of this, because they are evaporating so quickly, they remain frozen until evaporation slows down when dryness is reached. The water-vapour evolved is collected, in the dry-ice trap, as ice at -78°C. , with a vapour pressure of 0.0056 mm. Hg. Thus, the final dried protein at room temperature is in contact with water-vapour at 0.0056 mm. Hg. which gives a residual moisture content of less than 0.1 per cent. This is considered a low enough residual moisture content, and the ampoules may be sealed off *in vacuo*, while still on the manifold.

Meanwhile, Greaves and Adair (1936) were attempting to overcome the problem of frothing, so that the evaporative freeze method could be used for drying serum in reasonably small containers. In 1936, they described a method of dealing with the frothing problem. Hartley (1936) had already shown that frothing was chiefly due to the escape of dissolved gases under reduced pressure, and that these gases could be removed by a very slow reduction of pressure. Greaves and Adair (1936) found that sera could be "degassed" in 15-20 minutes, by reducing the pressure at a suitably controlled rate. When the resulting quiescent fluid was placed in a desiccator over phosphorus pentoxide and rapidly evacuated, it would "snap-freeze" and dry from the frozen state. In this way it was possible to dry individual doses in their final containers from the frozen state, without the use of any refrigerant. This was a great advantage, for in those days dry-ice was not easy to obtain in this country. The method, however, was not free from disadvantages. It was applicable only to small total amounts (21 ml. in an 11-inch desiccator), and occasional bottles were apt to bump at the point of freezing, ejecting the bulk of their contents. A further difficulty was that very small individual doses tended to dry out from the liquid state before freezing had occurred. Nevertheless, the method has been widely used, and in careful hands it has yielded good results.

Flosdorf and Mudd (1938) then published their "Cryochem" process, in which they abandoned their dry-ice condensers in favour of a chemical desiccant. They gave a good review of the relative virtues and failings of common desiccants, and introduced a new desiccant under the trade name of "Drierite." "Drierite" is anhydrous calcium sulphate, which will take up water rapidly to form the hemi-hydrate. On heating the hemi-hydrate to 180° C., the water is driven off and the desiccant can be used again. Excluding the initial cost, this desiccant is cheap to use, its only disadvantage being the large amount which is needed; nevertheless, on a costing basis, it is much cheaper than dry-ice.

The full advantages of a chemical desiccant cannot be gained if a refrigerant must also be used for pre-freezing the serum. Flosdorf and Mudd (1938) therefore advocate the snap-freeze method of Greaves and Adair (1936) following controlled degassing. Flosdorf and Mudd describe several models, from small machines with a capacity of 25 ml. per run, for the drying of cultures, to larger models with a capacity of 1½ litres per run. For testing the final moisture content, which should be below 2 per cent. and can be below 0.1 per cent. by this method, these authors recommend the vacuum-oven method of Flosdorf and Webster (1937). They summarise the virtues of the "Cryochem" method thus: "The process and apparatus may be used for all purposes for which the 'Lyophile' process is of value. It is much more economical, simpler to carry out, faster in drying, requires no storage of 'dry-ice', and can accordingly be applied to a wider range of products."

Meanwhile, Greaves and Adair (1939), who had been trying to increase the output of their process, had come to the conclusion that phosphorus pentoxide was too expensive to be used for more than small quantities. They therefore changed over to liquid air condensers in desiccators for quantities up to 250 ml., and finally adopted mechanically refrigerated condensers for quantities up to 5 litres. In their paper, in 1939, they describe an apparatus using a liquid air condenser, and also an experimental mechanically-refrigerated plant.

Their paper contains a full account of the physical principles of the process, and the first records of drying temperatures. The authors also discuss the influence of various variable factors. As a result of their experiments, they reached certain very definite conclusions as to desirable and undesirable points in the construction and operation of freeze-drying plants. Ice, even

at low temperatures, has such a relatively high vapour pressure, when compared with substances like cod liver oil, that there is no need to keep the drying and condensing surfaces in close proximity, as is necessary for molecular distillation; in fact, provided a free enough path is provided, the water-vapour may easily be conducted over comparatively long distances to the condenser. What is of great importance, however, is that this path should be as free as possible. Otherwise, a large pressure difference will build up between the drying surface and the condenser, with the result that for a given heat input and condenser temperature the serum will dry at a higher temperature. In practice, therefore, one should use bottles or ampoules with as large openings as possible and avoid the use of manifolds; if for any reason manifolds must be used, they should be kept as short in length and as wide in bore as practicable. If these principles are acted upon, it is possible to use comparatively high condenser temperatures, keep the bottles plugged, and still dry at a relatively low temperature.

The form of the vapour pressure curve of ice does not seem to be widely appreciated, with the result that some highly erroneous statements as to desirable condenser temperatures have been made. The vapour pressure curve of ice is shown in Fig. 1. To quote from the paper of Greaves and

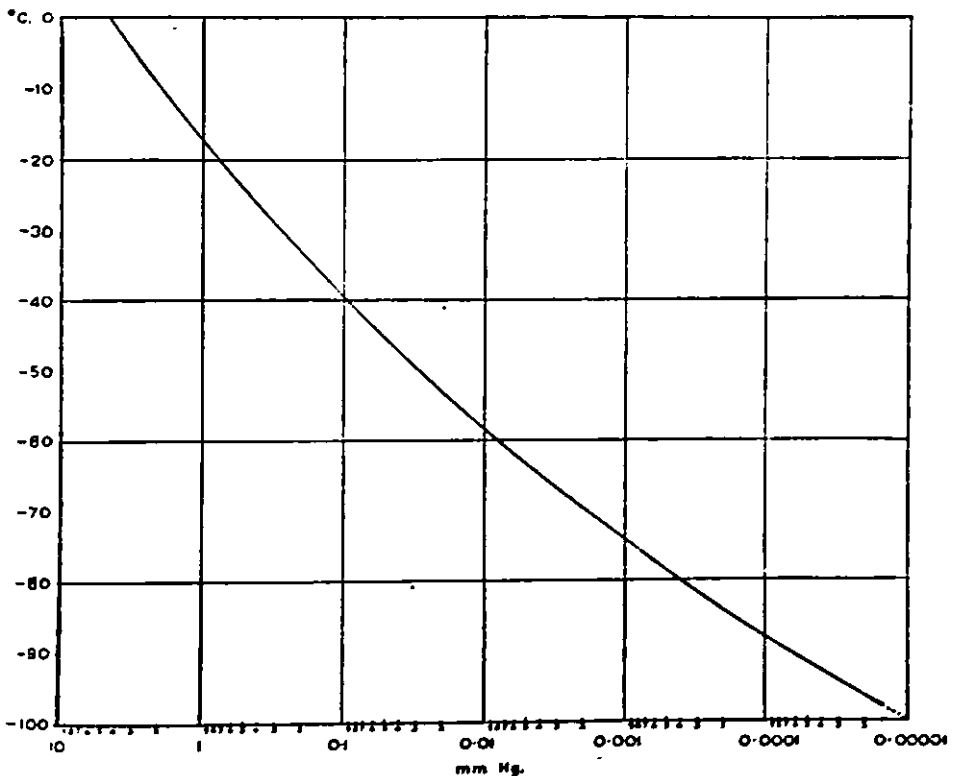


FIG. 1.—Vapour Pressure of Ice at Different Temperatures.

Adair (1939): "The serum temperature at -35°C . and the condenser at -45°C . gives a vapour-pressure difference of $0.173 - 0.061 = 0.112$ mm. Hg. under conditions in which obstruction is at a minimum. Assuming the vapour-pressure of ice at -184°C . to be *nil*, liquid air in the condenser would give the same vapour-pressure difference of 0.112 mm. Hg. when the serum is at a temperature of -39.5°C . Thus a drop of condenser temperature of 139°C . would cause a drop of 4.5 C. only in serum temperature." The temperature range, -40° to -45°C ., can be obtained with standard straight refrigeration,

whereas liquid air in bulk would be extremely costly. Perhaps this point may be stressed even better by quoting the drying conditions for human serum and plasma which were adopted at the Medical Research Council Drying Unit. Here the standard transfusion bottle, which has a relatively narrow neck, is used, and each bottle has to be fitted with an efficient bacterial cap in the interests of sterility. The condenser temperature is -40°C ., equivalent to V.P. of 0.1 mm. Hg. , and the drying temperature -21°C ., V.P. 0.7 mm. Hg. , giving a V.P. difference of 0.6 mm. Hg. If the condenser temperature were dropped to -184°C . for the same heat input and V.P. difference, the serum temperature would be -22.6°C .—i.e., there would be a lowering of 1.6°C . in drying temperature for a drop of 144°C . on the condenser. Fig. 2 illustrates this relation.

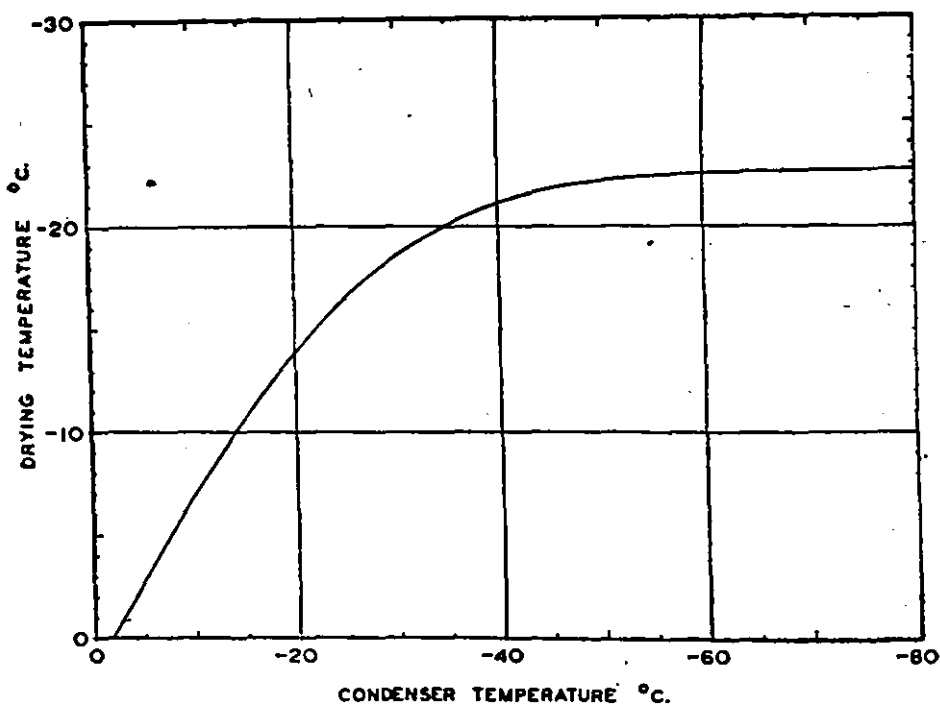


FIG. 2.—Relationship of Condenser and Drying Temperatures.

The relation between the condenser temperature and the drying temperature for the M.R.C. transfusion bottle, capped with the standard drying cap and heated at the rate of 7 watts per bottle.

Another point raised in the paper by Greaves and Adair (1939) was the speed of drying. For every gram of water removed, a definite amount of heat must be used; this heat must be neutralised by the refrigerator. Thus, if a definite weekly output is required, the refrigerator duty can be estimated. The maximum heat can then be applied to a small number of bottles over a short period, or to a large number over a long period; in each case the weekly output will be the same. In the first case desiccator space is saved but at the expense of drying at a higher temperature, and if obstruction must be introduced in the form of a bacterial filter, then surely it is the wiser plan to provide adequate desiccator space and dry the individual bottles slowly.

This paper also gives details of different methods of applying the required heat under controlled conditions, and suggests how the principle of the mechanically refrigerated condenser can be applied to almost all conditions of freeze-drying. These suggestions have now all been put into practice and will be discussed in a later section.

Knox (1939) applied the principles of Greaves and Adair (1939) to the dry-ice method of Flosdorf and Mudd (1936), keeping obstruction minimal by making his manifold a circular box with a central condenser. The result was an efficient apparatus for small scale work, which he applied successfully to the preservation of tumour viruses. A similar apparatus on a larger scale is described by Wyckoff and Lagsdin (1944).

With the outbreak of war, the problem arose of using human serum and plasma, as blood substitutes, for transfusion purposes. Previously, when only the problem of drying therapeutic antisera had been involved, an output of 10 litres per week had been considered large; now we were forced to think in outputs of 1,000 litres per week from a single plant. The result has been a rapid and very satisfactory development of large-scale plants for freeze-drying.

The first large-scale plant was described by Flosdorf, Stokes and Mudd (1940), and the method was called the "Desivac" process. Although 1 gram of ice will give off approximately 1,000 litres of vapour during evaporation at a pressure of 0.1 mm. Hg, there would theoretically be no need to remove the water vapour on a cold condenser if a pump, large enough to maintain this pressure against such an evolution of gas, were provided. On the other hand, the water vapour would be taken over into the pump and contaminate the oil. Flosdorf *et al.* overcame this difficulty by using a very large Stokes pump of special design, in which the oil was continuously passed through a high-speed centrifuge which separated the water from the oil, discharged the water and returned the oil to the pump.

The serum or plasma was distributed in 250 ml. amounts in bottles, shell-frozen round the periphery by rotating the bottles by hand in a bath of dry-ice, and then placed in liquid-jacketed compartments in a desiccator chamber. When the vacuum was adequate for cooling by evaporation to take place, liquid, warmed and thermostatically-controlled, was circulated through the jackets to supply the necessary heat of evaporation.

The obstruction to the flow of water vapour in this process was such that the additional obstruction caused by bacterial filters could not be tolerated. In consequence, the whole process had to be carried out in rooms sterilised by ultraviolet light, and all the attendants had to take the usual aseptic precautions. Best, Solandt and Ridout (1942) at the Connaught Laboratories in Toronto were operating such a plant successfully from the early days of the war. It was possible to maintain a fair output with the serum temperature at -10° C., but when the process was used for plasma, which required a drying temperature of -20° C., the heat input had to be so reduced that the drying cycle was doubled in length.

More recently it has been necessary to increase the drying capacity for serum and plasma in Canada, and this has been done by the insertion of mechanically-refrigerated condensers, the remainder of the equipment being unchanged (Flosdorf, Hull, and Mudd, 1945).

In the early stages of the war the "Desivac" principle was widely used for the large-scale production of dried plasma. As the scale of production increased, the pumps were superseded by 4- or 5-stage high-pressure steam ejector pumps, but the recent trend is to use these pumps for backing mechanically refrigerated condensers (Flosdorf, Hull and Mudd, 1945).

Along with the development of the steam ejector pump, Hill and Pfeiffer (1940) have introduced the "Adtevac" process. This relies on the water-vapour absorption powers of silica gel. In the past, silica gel has not been a popular desiccant, because it was incapable of taking up much moisture without showing a temperature rise which is coupled with loss of efficiency. Hill and Pfeiffer (1940), however, have overcome this difficulty by refrigerating their silica gel: even at quite moderate temperatures such as -20° C.,

the efficiency of the silica gel is enormously improved. In practice, by refrigerating their gel to -20°C. , they get the effect of a condenser mechanically refrigerated to -40°C. At this higher temperature the efficiency of mechanical refrigerators is greatly increased, so that it is possible to get the same duty out of a smaller compressor. But there still remain the problems of the bulk taken up by the silica gel, and the necessity of regenerating the gel; and in consequence the method is not readily applicable to really large-scale work.

Folsom (1942) has tried to adapt the original "Lyophile" process for the drying of transfusion fluid. He shell-freezes the plasma round the inside periphery of large flasks; each flask is then connected to its own condenser, which is another flask in a vacuum-flask containing dry-ice, and the far side of the condenser is connected to a manifold which, in turn, is connected to a small "Hyvac" pump. Each pump has up to 10 such condensers fitted to its manifold, the whole making up a unit. Each unit is comparatively cheap in initial cost, though the running costs on dry-ice would be heavy. It was Folsom's intention that each hospital should instal enough units for its own requirements, a procedure which has not found favour in this country. Again, this method would not be feasible for really large scale work.

Strumia and McGraw (1943) have worked on rather the same lines as Folsom, producing a small-sized unit for individual, or groups of, hospitals. They have developed a small and neat mechanically-refrigerated machine, which is entirely automatic, works off the electric mains, and provides for the pre-freezing and drying of 24 bottles of 250 ml. of plasma at one time. Their desiccator is water-jacketed, and the bottles of frozen plasma are in intimate thermal contact with it.

By heating the water in the jacket and controlling the heat of this jacket thermostatically, they have evolved a very simple method for dealing with the problem of heat control. In order to speed up the end process of the drying cycle, they allow the temperature of the water-bath to rise to $+80^{\circ}\text{C.}$ at the end of the run; they claim that this does not harm the proteins, and, at the same time, it results in a lower residual moisture content. It is interesting that they do not consider it necessary for the condenser temperature to even reach the -40° to -45°C. level advocated by Greaves and Adair (1939), claiming very satisfactory results with a temperature of -30° to -35°C. , with the result that they get an adequate duty from a smaller compressor. The use of these higher condenser temperatures is also supported by Flosdorf, Hull and Mudd (1945).

Levinson and Oppenheimer (1944) also have developed the mechanically-refrigerated condenser, using "deep-freeze" cascade refrigerators, the second stage running on ethane. These compressors are stated to be capable of producing temperatures as low as -120°C. , though Levinson and Oppenheimer find it more economical, and quite satisfactory, to run them at -40°C. These authors have also introduced a system of heating, using radiated infra-red rays.

These deep-freeze cascade compressors have proved excellent for the pre-freezing of the plasma in a shell on the inside periphery of the bottle. The importance of rapid freezing in preventing salt concentration, and in keeping the crystal size small, has already been emphasised. This is particularly important with transfusion fluids, for the speed of re-solution is greatly affected by the crystal size on freezing. Originally, all the pre-freezing was done in dry-ice baths, by rotating the bottles by hand. The deep-freeze plant can, however, maintain an alcohol bath at -70°C. ; and by providing suitable means for rotating the bottles slowly on their sides, excellent pre-freezing can be done entirely mechanically.

Strumia and McGraw (1943) have produced a pre-freezer working at higher temperatures (-20° C.), which in other respects is the same as the deep-freeze method. At this temperature, however, the bottles are apt to super-cool, and then, instead of shell-freezing, freezing takes place into a solid lump. They overcome this difficulty by stopping the rotation at a suitable moment, and since the bottles are immersed in the cold alcohol for a depth of only about 1 cm., the serum freezes in a line along the length of the bottle; if rotation is now restarted, the plasma freezes in a satisfactory shell, though in the pictures they publish the crystal size is large.

The popularity of the mechanically-refrigerated condenser seems to be growing in the United States. In a recent personal communication, Flosdorf has stated that several large machines using compounded ammonia compressors to cool a brine tank, the brine from which is then circulated through the condensers and also through the heater heads in the early stages of the drying cycle, are being developed for the large-scale drying of penicillin (*see also* Flosdorf, Hull and Mudd, 1945).

In Britain, development has been entirely along the lines of mechanically-refrigerated condensers. Modelled on the experimental machine of Greaves and Adair (1939), "the final pilot plant" (p. 41), consisting of three units, was erected at the beginning of the war, and it ran continuously for three years. During this period, larger plants were developed, culminating in "the large drying plant" (p. 42) operated by the Medical Research Council Drying Unit, which was able to prepare 3,300 bottles of 400 ml. capacity of dried plasma each week.

Originally, all the drying was carried out in "medical flats" containing 200 ml. of serum or plasma, which were wedge-frozen in a cold room at -20° C. This, however, gave a large crystal, and a rather long period was required for re-resolution.

With the introduction by Greaves (1942) of the high speed vertical spin-freeze, which is carried out in a current of air at -18° C., and which gives a very small crystal size, it was possible to standardize the drying in the M.R.C. transfusion bottle, though in many ways this bottle is not ideal for the production of a dried product.

Byrom (personal communication), in Australia, has also worked on the lines of Greaves and Adair (1939), with very satisfactory results. He has modified the desiccator design so that it is in two halves, the top half lifting off to uncover a fixed heater head. He claims that this greatly simplifies the handling of the plant.

In the mechanically-refrigerated condenser method, energy must be applied to the serum to supply the heat for evaporation; energy must also be applied to the compressor. Thus, the work has to be done twice and the energy of the hot cycle of the compressor is wasted. This heat could presumably be utilised for heating the serum, but it would be rather too great in amount. MacFarlane (1943b) has patented a method whereby this heat is applied to the serum, the excess being neutralised by a small auxiliary refrigerator. Though this is sound engineering practice, it is scarcely applicable for our purposes. Over a long period at the M.R.C. plant, it required less than 2 units of electricity to freeze, store and dry a 400 ml. bottle of plasma. This cost is infinitesimal compared with that of collecting and preparing this amount of plasma, and even if the drying cost could be halved, the saving would still be insignificant.

All these war-time developments have been aimed at the drying of large numbers of large doses. Little attention has been paid to the drying of small doses. Here another problem is encountered, for during the time required to load and evacuate the chamber, these small quantities may start to thaw and may consequently froth—an accident unlikely to occur when large masses of ice are being handled. However, the need for drying small

quantities exists, particularly in the field of typing serum, therapeutic antisera, penicillin, yellow fever vaccine, convalescent sera, etc.

It was originally the custom in the M.R.C. Unit to dry these small quantities by placing them between the tins of the large heater heads, so that the large amount of cold in the transfusion bottles kept these small units frozen till the necessary degree of vacuum was attained. Research to find a satisfactory way of drying these small quantities independently from the large plant culminated in the vacuum spin-freeze method of Greaves (1944).

High-vacuum Spray-drying from the Frozen State

From what has been said as to the relative merits of the different processes already described, it is clear that if the spray-drying process could be carried out in a high vacuum so that drying took place from the frozen state, the method would be the ideal answer to many of the drying problems.

Some preliminary research was done by the author on this problem in 1939, and although no satisfactory solution was found for the various problems that were encountered, it may be of some value to record what these problems were.

If liquid were sprayed into a vacuum chamber which was evacuated to a pressure of the order of 0.1 mm. Hg and which contained a refrigerated condenser, the liquid particles should freeze very rapidly; and if these particles were subjected to radiant heat as they fell under the influence of gravity, they should dry extremely rapidly and fall as a dry powder into a suitable receptacle at the bottom of the chamber.

The first difficulty encountered related to the jet. An atomising jet was impracticable, since the air would destroy the vacuum, and a "Kestner" rotating disc atomiser could not be used, since the liquid would be frozen before it reached the disc. Consequently, fine needle jets were first tried, but these would not work, since the liquid invariably froze in the needle. Attempts to heat the jet were also unsuccessful; on one occasion when the orifice of the jet was being heated by a white hot platinum spiral, the spray was diverted so that it fell on the white hot wire and proceeded to freeze on it. Success was eventually achieved by making a jet from a minute hole in a piece of brass foil; thereafter, except for difficulty from the jet blocking with particulate matter (which was overcome by very thorough filtration of the liquid prior to spraying), no difficulty was experienced in making jets so fine that they discharged only 100 ml. per hour.

The spray made by these pin-point jets was allowed to fall through a radiant heat tube, and, using distilled water, the heat was increased in the hope that evaporation would be complete during transit through the heater. This was never achieved, and it was felt that a very long path would have to be provided. In the author's opinion, this was largely due to the fact that the particle size formed by this type of jet was very irregular.

The most successful experiment was as follows:—A 20-litre glass bottle was made to rotate slowly on its vertical axis in the vacuum chamber. The jet was placed at right angles on a length of pipe which went down vertically into the bottle through the neck. As the bottle rotated, so the jet was slowly raised and lowered. The bottle was warmed to 40° C. Thus, the liquid was sprayed against the side of the warm bottle, which it hit as frozen particles; these stuck. The combined slow rotation of the bottle, together with the vertical movement of the jet, caused a thin frozen film to be deposited uniformly over the wall of the bottle; this film had dried out before the next film was applied.

The introduction of four-stage steam-ejector vacuum pumps and radio-frequency heating might solve a number of these problems. The jet might be allowed to fire upwards at a slight angle, being heated by radio-frequency at

the top of its trajectory; the falling dry particles could be collected in a large dish heated to $+40^{\circ}\text{C.}$, so that if the particle size were not uniform the larger particles would have time to dry out. To provide an effective method of heating, very high radio frequencies, beyond the range which is at present commercially available, would have to be used.

If the jet were formed in a small chamber connected to the main chamber by a very small orifice only, it should be possible to introduce a controlled air leak through the jet; this would assist in atomising the liquid and would raise the pressure in the small chamber to 5 mm. Hg, so that there would be no chance of the spray freezing while in proximity to the jet; with a steam ejector pump, it should then still be feasible to hold a vacuum of the order of 0.1 mm. Hg in the main chamber, so that the particles would freeze and dry there from the frozen state.

PART II.—THE TECHNICAL ASPECTS OF FREEZE-DRYING BY CONDENSATION ON MECHANICALLY REFRIGERATED CONDENSERS

General Considerations

The first consideration in freeze-drying any solution is the temperature at which it must be held for sublimation of the material to occur from the solid state. The first procedure, therefore, is to measure the eutectic of the solution; this is found by placing some of the solution in a conductivity cell adapted for temperature measurement, and plotting conductivity and temperature on cooling and warming. The eutectic is shown by a plateau in the cooling curve coinciding with a sudden increase of resistance to an infinite level. This method works well with simple salt solutions, but with complex salt and protein solutions eutectics do not appear to be situated at temperatures that one might expect from consideration of the salt content alone. For example, with serum, whose main salt is sodium chloride, with a eutectic point at -21.4°C. , no plateau is found on a cooling curve below -1.5°C. though the resistance does not become infinite till very low temperatures are reached. A more practical approach is to dry at different temperatures and observe what happens, though a preliminary idea may be obtained by freezing, inverting the bottle, holding it at different temperatures, and noting whether any liquid separates from the ice at these temperatures.

When estimating eutectics by drying, the points to observe are: (a) any tendency for bubbles to form on the surface, (b) contraction in volume of the dried material when compared with the original liquid volume, and (c) "scalyness" of the dried material. From practical experience, it may be said that sera dry well if held below -10°C. in the early stages of drying, plasma at from -10° to -25°C. , depending on the citrate to protein ratio, and sodium penicillin at below -25°C. if impure (the purer solutions will dry satisfactorily at higher temperatures). Samples of broth may dry well at -30°C. , though in some cases it may be necessary to go as low as -60°C. to obtain perfect desiccation; this is important, since it is frequently convenient to dry virus and bacterial suspensions in broth.

A suitable temperature for desiccation having been chosen, the vapour pressure of ice at this temperature will be the vapour pressure of condensable gas as the drying surface; for drying to proceed, there must be a difference between the vapour pressure at the drying surface and that at the condensing surface. The speed of drying depends *only* on the rate at which heat is supplied to the material being dried. For a given heat input the V.P. difference between the drying surface and the condensing surface will depend on the amount of obstruction to the flow of water vapour. The vapour flow may be obstructed by: (a) high partial pressures of non-condensable gases—therefore the apparatus should be as leak-free as possible; (b) narrow necks of

bottles or ampoules; (c) long narrow paths for the flow of vapour, as in badly designed manifolds; and (d) the obstruction of a bacterial filter, which in many cases must be used in the interests of sterility. The last is a major factor, and in order that bacterial filters may be used, all other obstructions should be reduced as far as possible.

Some experimental figures will illustrate these points; in all cases the partial pressure of non-condensable gases was negligible, and the heat input per unit volume the same. The material used was 14 litres of serum, the total heat input 250 W., the condenser temperature -40°C ., and the vapour pressure of ice on the condenser was 0.1 mm. Hg.

	Drying temperature ($^{\circ}\text{C}$.)	V.P. of drying material (mm. Hg)	V.P. difference due to plant (mm. Hg)	V.P. difference due to bottle neck (mm. Hg)	V.P. difference due to cap (mm. Hg)	Total V.P. difference (mm. Hg)
(a) In open dishes	-38	0.12	0.02	—	—	0.02
(b) 35 M.R.C bottles, of 400 ml., uncapped. (Heat input 7.14 w/btl.)	-28	0.35	0.02	0.23	—	0.25
(c) Ditto, filter-capped	-21	0.70	0.02	0.23	0.35	0.60

If the temperature of the condenser were lowered to -184°C . by the use of liquid air, and if all the remaining factors were unaltered, the same vapour-pressure differences would give the following serum temperatures:—

Temperature.

- (a) Serum in open dishes -53°C .
 (b) Serum in uncapped bottles -31°C .
 (c) Serum in filter-capped bottles (see Fig. 5, p. 32) -22°C .

These figures illustrate a number of points. In the first place, as the majority of biological materials must be dried in a sterile manner, preferably in their final containers, it is clear that the obstruction due to the narrowness of the neck of the ampoule or bottle, and to the bacterial filter, is of such magnitude that the condenser temperature is of little importance if a reasonably high rate of drying is required. Lower drying temperatures could be obtained by reducing the heat input, but at the cost of very slow drying. It follows that for drying under sterile conditions at very low temperatures, it is not a practical proposition to dry in individual plugged containers, however low the condenser temperature may be.

The alternative is to dry in bulk, the condenser being close to the evaporating surface, and arranged so that it can be sterilised and isolated from the pumping system by the bacterial filter. In this way, high heat inputs, with rapid drying, can be achieved without raising the temperature of the material being dried appreciably above that of the condenser; but the price to be paid is that the dry material has to be distributed from the bulk, and the handling of bulk powders in a sterile manner is by no means as simple as the sterile handling of liquids.

The sequence—(1) material being dried, (2) bacterial filter, (3) condenser, (4) pumping system—is the more convenient method; and if suitable ampoules or bottles are selected, having wide necks relative to the surface area of frozen serum, temperatures as low as -30°C . can be obtained with fairly rapid drying, and with condensers no lower than -40°C ., provided the design of

the apparatus is good. For lower temperatures, the sequence must be altered to: (1) material being dried, (2) condenser, (3) bacterial filter, (4) pumping system; and, under these conditions, there is much to be gained by using condensers at very low temperatures.

If sterility is of no consequence the problem becomes very much simpler, and very high rates of drying can be achieved at temperatures differing but little from those of the condenser.

In the following sections, practical details, based on the experience gained by the M.R.C. Unit, while operating freeze-drying apparatus on both an experimental and a large scale over the past seven years, will be given under the appropriate headings.

Vacuum Requirements

(a) *Vacuum measurement.*—The importance of accurate vacuum measurement in the successful operation of freeze-drying procedures cannot be too strongly emphasised. The V.P. of ice at 0°C. is 4.2 mm. Hg, and at -80°C., 0.0004 mm. Hg; therefore one should have a method of measurement which is accurate over the range 10 to 0.001 mm. Hg. Simple mercury gauges are not suitable, though the McLeod mercury gauge is satisfactory over this range. The principle of the McLeod gauge is that a known volume of the rarified atmosphere is compressed into a capillary tube to atmospheric pressure, and the volume measured. During compression the condensable gases are liquified, so that the resulting measurement will be the partial pressure of the permanent gases. Thus, this type of gauge will neglect the water vapour pressure, the reading recorded being a measure of any leak in the apparatus plus the amount of dissolved permanent gas which is being evolved from the material being dried. The McLeod gauge has the disadvantage that it does not give a direct reading and cannot be made to record continuously.

The Pirani gauge is very suitable over the range 1 to 0.01 mm. Hg, but the readings obtained in the ranges 10 mm. to 1 mm., and 0.01 to 0.0001 mm. Hg, are less accurate unless special precautions are taken. The principle of operation of this type of gauge is that the electrical resistance of a wire is dependent on its temperature. If a current is passed through a wire, the latter will be heated, and the temperature that it attains will depend on its rate of cooling, which in turn, over a certain range, will be dependent on the pressure of gas surrounding the wire. If the gauge bulb is placed in one arm of a Wheatstone's bridge, the degree of "out-of-balance" can be recorded on a galvanometer, which, in turn, can be calibrated in mm. Hg by reference to a McLeod gauge in a vacuum system, dried by liquid air so that there is no partial pressure of condensable gases. Such a gauge gives a continuous record of the total pressure in a vacuum system.

In condenser drying systems, both gauges are useful—the Pirani to give the total pressure, the McLeod to give the partial pressure of non-condensable gases. The difference between the readings of the two gauges will be the water-vapour pressure. In practice we have found the use of the Pirani gauge alone to be adequate, since if the condenser temperature is known the water-vapour pressure is also known, and any variation of the Pirani reading from this water-vapour pressure will represent the residual pressure of permanent gases.

The response curve of the Pirani gauge is shown in Fig. 3, p. 22.

It will be seen that this gauge is best suited for the pressure range from 0.1 to 0.01 mm. Hg. Frequently the gauge filament is balanced by a fixed resistance in the opposite arm of the bridge, but if this is replaced by an identical filament at atmospheric pressure the gauge becomes independent of external temperature.

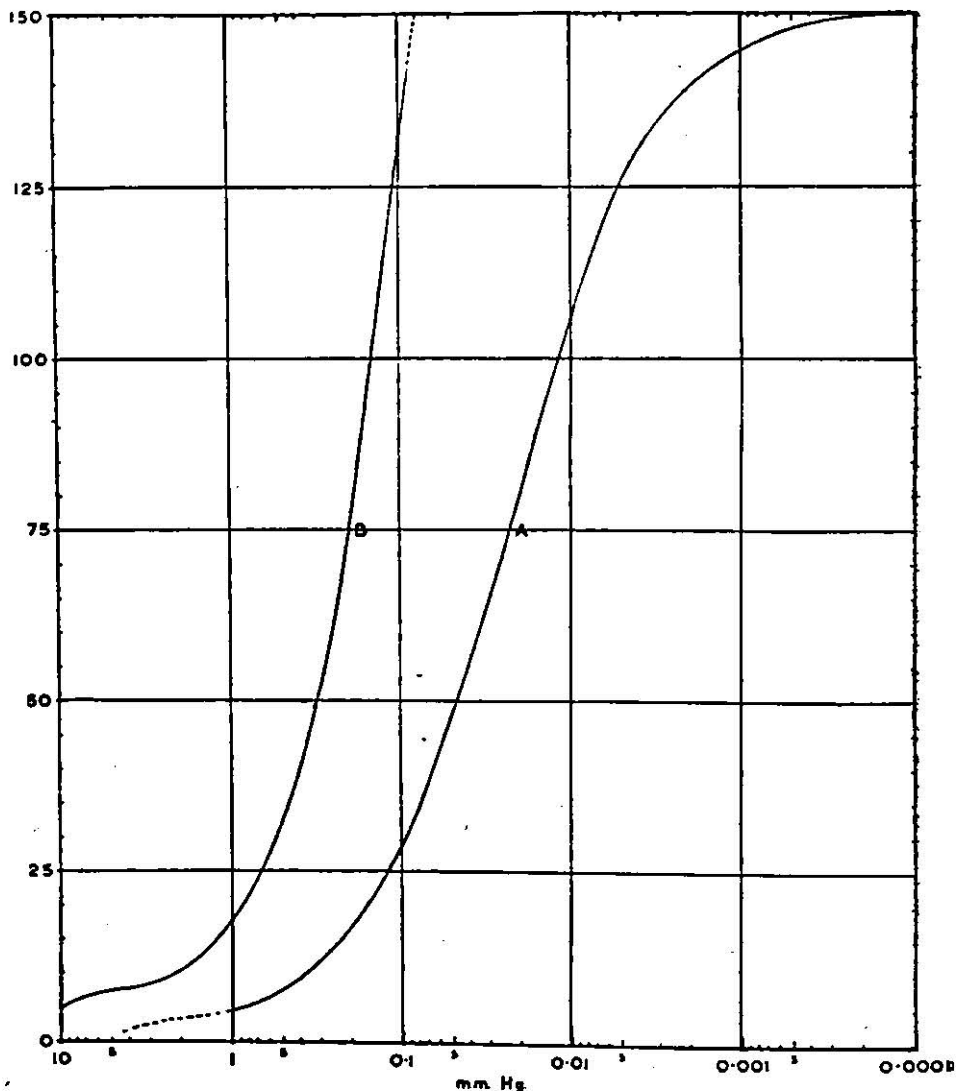


FIG. 3.—Pirani Gauge Calibration Curves.

A is the curve of a typical gauge on a constant current bridge operated at 26 mA.

B shows the effect, at the higher end of the scale, of raising the bridge current to 42.5 mA.

This gauge thus has two useful ranges : A from 0.5 mm. to 0.001 mm. Hg, and B from 5 mm. to 0.1 mm. Hg.

Within limits, the form of the scale can be varied by altering the working temperature of the filament. Thus, a fairly open scale between 10 and 0.1 mm. can be obtained by increasing the voltage and so running the filament hot, while the cooler the filament, the more open the scale is at the other end (if a sufficiently sensitive galvanometer is used). For accurate results it is essential that the working conditions of the bridge be checked whenever a reading is taken. This is most conveniently done with the same galvanometer, by fitting suitable switch gear. According to the precise arrangement used, the bridge can thus be operated either at constant voltage or at constant current. As the latter method involves raising the voltage slightly as the pressure falls, it gives a scale which is rather more open at the

lower end. If accurate readings below 0.001 mm. Hg are required, the balancing gauge should be evacuated to a known pressure, say 0.0001 mm. Hg, and the bridge balanced at this pressure. With some gauges there is a tendency for the characteristics to change with ageing of the filament, so that occasional rechecking against a McLeod gauge is indicated. Glass-coated filaments have been found to be more reliable in this respect.

A variation on the Pirani gauge is the thermocouple gauge, in which the temperature of the filament is measured by a thermocouple, pressures being read directly in terms of temperature.

The calibration of the Pirani gauge depends on the nature of the residual gases. Fortunately, the only gases normally met are air and water-vapour, which have practically identical thermal conductivities. If the material being dried contains organic solvents, then corrections may have to be introduced.

The Knudsen gauge, which operates mechanically, may be used as a substitute for the Pirani, since it also records the total pressure.

If it is desired to measure pressures below 0.0001 mm. Hg, then "ionisation" type gauges should be used.

(b) *Vacuum chambers.*—The necessity of providing as free a path as possible for the water-vapour to traverse, in its journey from the material being dried to the condenser, has already been emphasised. For this reason, manifolds, which usually introduce long narrow passages for the flow of water-vapour, should be avoided where possible. If manifolds must be used—and they have the advantages that heating is simplified and ampoules may be sealed off while still *in vacuo*—then the circular type of manifold, described by Knox (1939) or by Wyckoff and Lagsdin (1944), should be used. In both these types the manifold consists of a wide-bore tube, to branches on the outside of which the ampoules or bottles are attached; down the centre of this tube runs a second tube, which forms the condenser. Once it has left the ampoule or bottle neck, the vapour has only to traverse the annular space between the two tubes.

Our personal choice is in favour of suitably designed desiccator chambers as described by Greaves and Adair (1939). For small-scale work, chambers are constructed from glass filtration jars, with the bottoms cut off to give cylinders 7 in. in diameter by 11 in. long. The bottom of the chamber is a $\frac{1}{4}$ in. steel plate, through which the vacuum lead is taken; on top is a steel ring, and the lid is another steel plate, $\frac{1}{2}$ in. less in diameter than the outside of the ring, so that an angle is left between them in which the vacuum seal of "Apiezon Q" can be made. The glass cylinder is luted to the bottom plate and top ring by means of "Apiezon Q", and in operation the only seal to be made and broken is that between the ring and top plate.

Glass is convenient for experimental work, but for routine work steel cylinders are substituted for glass. The design is suitable for all sizes of chamber, from the small ones mentioned to large chambers 3 ft. in diameter by 6 ft. high. With large chambers, it is better to weld or braze the cylinders to the top ring and bottom plate.

This design of chamber has the great advantage that it enables pipes or electric leads to be inserted into the chamber wherever desired. Moreover, each chamber can be used for drying from almost any type of container, by providing suitable "heater-heads"; and if these are attached to separate top plates they will be readily interchangeable.

The fundamentals of the design of our chambers are shown in Plate I. The condenser coils occupy the bottom half of the chamber; they may be cooled either by direct expansion of a refrigerant gas or by cold brine being circulated through them. If the cylinder is welded to the bottom plate, the drip tray may be omitted, the thawed condensate being allowed to collect on the

floor of the chamber before removal by suction. The diagram also shows details of a heater-head to take the standard M.R.C. transfusion bottles.

The construction of the vacuum line is important. Inside the chamber it must be elevated from the floor, in order to prevent it from being filled with water during defrosting. The entrance to this lead must be screened to prevent water-vapour coming over to the pumps; the screening arrangements shown ensure that only gas which has been dried by passing through the coils can get into the vacuum line. A junction box of square section steel on the vacuum line after it has left the chamber is convenient for fitting vacuum gauges and taps. The tap on the main vacuum line should have as large a bore as possible.

(c) *Vacuum pumping system in condensation drying.*—Before evaporation from the frozen state can become reasonably rapid, it is necessary to remove the obstruction to the flow of water-vapour caused by permanent gases.

If the material to be dried has been pre-frozen, it is important to remove these permanent gases to a pressure below the critical with reasonable speed. It should be possible to obtain a pressure below 1 mm. Hg within 10 minutes if large individual doses are to be dried, though with small individual doses even this speed of evacuation may not be sufficiently rapid.

If the material being dried has originally been pre-frozen, freeze-drying will liberate its dissolved permanent gases as drying proceeds. If, however, the material is de-gassed before freezing, this evolution of gas during drying can be eliminated, so that if de-gassing has been carried out completely—and if the apparatus is free from all leaks—it is possible to run with the pump turned off, once the necessary degree of vacuum has been attained.

With condensation drying there is always a vapour pressure of ice at the temperature of the condenser; if the pumping system has a large clearance at this pressure, water vapour will be carried over into the pump and the oil will become contaminated. The ideal pumping system, therefore, will have a high speed of evacuation down to at least 1 mm. Hg, and a small clearance at the ice-vapour pressure.

Single-stage mechanical pumps have a good speed of clearance down to 1 mm. Hg, but from 1 mm. to 0.1 mm. the pumping speed falls rapidly, while from 0.1 to 0.01, which is about the maximum obtainable, the speed is infinitesimally small. Two-stage pumps have a good performance to 0.1 mm. Hg, moderate speed to 0.01 mm. Hg, and very small clearance from 0.01 to 0.0001, which is the maximum vacuum obtainable. For reasonably fast pumping speeds below 0.1 mm. Hg, mercury or oil diffusion pumps must be used, suitably backed by mechanical pumps. From atmospheric pressure to about 1 mm. Hg, a two-stage pump has practically no advantage over a single-stage pump, but as all the air taken through the first pump must also go through the second, they must both be of a similar capacity. For efficient backing below 1 mm. Hg, however, the second stage pump can be of much smaller capacity than the first; thus, if arrangements are made so that the second pump is operative only below 1 mm. Hg, great saving in cost can be effected.

Since a high rate of pumping is necessary in the early stages of evacuation, a good path must be provided from the chamber to the pump. Valves of adequate size, and as large piping as possible, should therefore be installed.

If a number of chambers is contemplated, should each chamber have its own pumping system, or can they all be connected to a common pumping system by manifolds? Ideally, each chamber should have its own pumping system in duplicate, but this is a counsel of perfection. On a costing basis, however, it is more efficient to have one adequate system for a number of

chambers than individual pumps all of which are too small for each chamber. In the large M.R.C. Drying Plant, one pumping system is connected to all the eight chambers. In this way, two large single-stage pumps give very rapid pumping down of any one chamber, while one pump backed by a small single-stage pump can deal with the evolution of gas during the drying cycles from all eight chambers. The eight chambers are coupled by a common 2-inch diameter manifold to the pumps — thus, when one chamber is being pumped down, the other chambers must be turned off. It would be more convenient to have two manifolds, one for pumping down and the other for holding.

The type of pump chosen should depend on the condenser temperature. With the condenser at -40°C ., the V.P. of ice being 0.1 mm. Hg, a large single-stage would be preferable to a smaller two-stage pump. Using a dry-ice condenser, a two-stage pump is indicated; whereas with liquid air a diffusion pump should be used. With the condenser at -40°C ., if a two-stage pump is used, there will be a tendency for water-vapour to come over into the pump, leading eventually to fouling of the oil. It is advisable, therefore, in these circumstances, to arrange for the water to be stripped from the oil either by heating or by centrifuging. The pumping arrangement used on the M.R.C. plant is shown diagrammatically in Plate II.

To summarise, the pumping system should be such that it has a rapid clearance from atmospheric pressure to 1 mm. Hg, and either a fair clearance at the vapour pressure of ice at the condenser temperature so that only a moderate amount of water vapour will come over into the oil of the pump (from which it must be removed), or, alternatively, small clearance at this temperature, so that oil stripping devices need not be used.

Heat Control

This is probably the most important single factor in the whole of the condensation freeze-drying technique. On a thorough knowledge of all the dependent variables involved must depend the whole design and running of the apparatus.

The latent heat of evaporation of ice at different temperatures can be calculated from Clapeyron's equation: $\frac{dP}{dT} = \frac{\lambda}{T} (v_2 - v_1)$, which gives a value of 672 cal./g. between -25° and -30°C . Within the accuracy of this analysis it is fair to assume an average value of 670 cal./g. of ice over the whole range. When Greaves and Adair (1939) tried to verify this figure in a condensation freeze-drying apparatus, they obtained the figure of 901.5 cal./g.; the higher figure was undoubtedly due to losses in the system, but it is wiser to adopt it when calculating the required duty of an apparatus.

From Clapeyron's equation it is clear that the speed of drying depends solely on the rate of application of heat. How quickly the heat can be applied will depend on: (a) the duty of the refrigerator at the condenser temperature required, (b) the highest "safe" temperature at which the material may be dried; for a given heat input, this temperature will depend on the degree of obstruction to the flow of vapour and on the condenser temperature, (c) the highest temperature at which it is considered safe to operate the heaters, and (d) the rate of transference of heat through the frozen material. If too high a heat input is used there will be a tendency to thaw at the point of contact of the frozen material with the heater surface.

During the early stages of drying a steady and balanced heat input can be arranged, but as drying proceeds the rate of heat absorption by the drying material will drop. We have found the following overall average figure useful for preliminary calculations: *one watt heat input will dry 1 ml. in 1 hour.*

For example, if it were required to dry 2,000 ml. in 10 hours the heat input required would be 200 watts, and the refrigerator duty must handle this load at the required temperature.

For drying to proceed, heat must be applied to the frozen material; this will produce a vapour pressure difference between the evaporating and condensing surfaces, which will be reflected in a temperature difference between these two surfaces. The system consists therefore of: (1) a pressure difference, (2) an obstruction, and (3) a rate of flow. The relationship of the three dependent variables in an electric circuit is shown by Ohm's Law:—

$\frac{P.D.}{R} = C$. In this system, the equation:

$$\frac{\text{Vapour pressure difference}}{\text{Obstruction to flow}} = \text{Rate of flow}$$

is at least approximately true. Since the rate of flow depends on the heat input in watts (Clapeyron's equation), the formula may be expressed as follows:

$$\frac{\text{Vapour pressure difference}}{\text{Obstruction to flow}} = K \times \text{watts.}$$

If we define the unit of obstructive resistance as that which, under a vapour pressure difference of 0.01 mm. Hg, passes vapour at the rate at which it is liberated by a heat input of 1 watt, then $\frac{P}{R} = \frac{W}{100}$

where: P = vapour pressure across the resistance in mm. Hg,
R = the obstruction expressed in the units proposed,
W = the heat input in watts.

It is not suggested that this analysis takes into account all the physical factors concerned, but over the pressure and temperature ranges normally encountered in this technique, calculations based on these assumptions are reasonably accurate in practice.

It will be seen that if R can be made infinitely small, W can become infinitely great, without producing any significant rise in P. Thus, in an ideal system, drying could theoretically be almost instantaneous. But in practice even if R were infinitely small, these ideal conditions could not be realised, for the infinitely great W would cause an extremely high heater temperature on account of the relatively low thermal conductivity of the frozen material, and melting would occur between the heat contact surface and the frozen material. Alternatively, if W becomes infinitely small, P will be small, irrespective of high values of R. In other words, it should be possible to dry with the material practically at the condenser temperature, even with relatively high R values, but drying under these conditions would take an exceedingly long time.

Referring to the temperatures already given on page 20 for drying under different conditions, it is interesting to apply this formula to the figures obtained experimentally, and to ascertain the various R values of the different parts of the system.

(1) *Serum in open dishes.*

$$P = 0.02 \text{ mm. Hg.}$$

$$W = 250 \text{ watts,}$$

$$\text{therefore, } R = \frac{0.02 \times 100}{250} = 0.008$$

This means that 0.008 units of obstruction exist in the system, the result of the presence of non-condensable gases and of the path the water-vapour has to take in order to reach the condenser.

(2) *Serum in open bottles.*

$$P = 0.25 \text{ mm. Hg.}$$

$$W = 7.14 \text{ watts per bottle, total 250 watts for 35 bottles.}$$

Here we have 35 resistances (due to the necks of the bottles) in parallel, and one resistance (that of the system) in series with this group. Considering one bottle only, the equation becomes: $P = \frac{RW}{100} + \frac{rw}{100}$

Where R = resistance of the bottle neck,
 W = heat input to each bottle,
 r = resistance of the system (determined above),
 w = total heat input to all the bottles.

$$R = \frac{100}{W} \left(P - \frac{rw}{100} \right)$$

$$= \frac{100}{7.14} (0.25 - 0.02) = 3.22$$

Therefore, 3.22 units of obstruction exist in the neck of the bottle, which has a diameter of $\frac{7}{8}$ in. and a length of $1\frac{1}{2}$ in.

(3) *Serum in filter-capped bottles.*—Here we have the same arrangement, but R may be subdivided into R_n (determined above), due to the neck of the bottle, and R_o , due to the filter-cap. P is now 0.6 mm. Hg, and the other data are as before. This gives $(R_n + R_o) = 8.11$, and $R = 4.89$ units.

Since the effective diameter of the cap is 2 inches, 3.14 sq. inches have a resistance of 4.89 units, and therefore the R value of these caps may be taken as 15.5 units per sq. inch.

How far may these measurements be used for calculating drying temperatures under other conditions? Another bottle which is frequently used for drying 10 ml. quantities has a neck of $\frac{1}{2}$ in. diameter.

Assuming that the R value varies inversely with the cross-sectional area of the neck of the bottle, since the length of the neck is so short, and the obstruction is probably chiefly due to turbulence at the internal orifice rather than obstruction to laminar flow, the R value for the neck of this bottle will be:

$$\frac{23}{7.14} \times \frac{49}{256} \times \frac{16}{\pi}$$

or 10 units within the limits of accuracy attainable.

The effective area of bacterial filter has a diameter of 1 inch or area of $\frac{7}{8}$ sq. inches. Since 1 sq. inch has an R value of 15.5 units, $\frac{7}{8}$ sq. inches of cap has an R value of about 20 units.

Therefore the R value of the neck and cap is 30, to which must be added P , due to the resistance of the system, times the flow from, say, 250 bottles at $\frac{1}{2}$ w. each = $\frac{0.008 \times 125}{100} = 0.01$, which is so small that it may be neglected.

Thus $P = \frac{30}{200} = 0.15$ mm. Hg, and if the condenser is at -40° C., the V.P. of the serum will be 0.25 mm. Hg, which is equivalent to drying at a temperature of -31° C.—a figure found to be correct experimentally to within 1° C.

The total R of this bottle when capped is 30, compared with an R value of 8 for the capped M.R.C. bottle. The M.R.C. bottle is thus nearly 4 times as efficient as the small bottle, but it is used for drying 40 times the amount. It will be realized accordingly that to dry 400 ml. in the M.R.C. bottle takes 10 times as long as to dry 10 ml. in the small bottle at the same drying temperature.

It is interesting to compare these two bottles when drying on different time schedules:—

Condenser Temperature — 40° C.

<i>M.R.C. bottle (capped) 400 ml. R = 8 (One bottle only in system. R of chamber neglected)</i>			<i>Small bottle (capped) 10 ml. R = 30 (R of chamber neglected)</i>	
<i>Time in Hours</i>	<i>Heat in Watts</i>	<i>Temperature of Serum</i>	<i>Heat in Watts</i>	<i>Temperature of Serum</i>
60	6.7	-21.8° C.	0.17	-36° C.
48	8.25	-20.0° C.	0.21	-35° C.
36	11.0	-17.2° C.	0.28	-34° C.
24	16.5	-13.2° C.	0.42	-32° C.
12	33.0	- 5.7° C.	0.84	-28° C.

These figures would hold good only if it were of no consequence what temperature the heater was allowed to attain.

Since drying takes place from the surface of the frozen material, and not from its point of contact with the glass or metal heating surface, heat must be transferred through the frozen material to the drying surface, but provided the thickness of the frozen mass is not too great, nor the heat input too large, there is very little temperature drop across it. As drying proceeds, there will come a time when the amount of evaporation taking place is not enough to keep the material cold and the temperature of the dried material will rise till it reaches the temperature of the heaters at the "dry point." Theoretically, therefore, if the heating is controlled from the serum temperature, it should be possible to neglect the heater temperature and yet not let the dried material rise above + 37° C. (or whatever is considered the safe upper limit to which to take the dried material). Under these conditions it should be possible to use as much heat as is consistent with a satisfactory drying temperature, and the duty of the refrigerator. Unfortunately, in practice, drying is not absolutely even, some areas drying out, leaving islands of frozen material; and if, by chance, the control point is in one of these frozen islands, the dry material may be exposed to very high temperatures.

The amount of heat which is absorbed at any given heater temperature is directly related to the surface area of evaporation.

The following graph (Fig. 4), taken from the paper by Greaves and Adair (1939), shows the heat absorbed by a surface area of 2,500 sq. cm. at different heater temperatures. It will be seen that 150 watts are absorbed when the heater is set thermostatically at 40° C.; if the heat input were raised to 300 watts, so as to double the rate of drying, the heater temperature would rise to 100° C. If this were done, there would be a risk that the dried material would also rise to 100° C. and be destroyed, unless some system of secondary relay, such as is described in their paper, were incorporated so that the heater temperature could be reduced to 40° C. when dryness approached.

Infra-red heating, as advocated by Levinson (1944), does not overcome this difficulty, but it is possible that the answer may lie in radio-frequency heating. This, however, would be an elaborate solution to a problem which is much more simply met either by providing more desiccator space so that the serum may be distributed with a larger surface for evaporation, or by using a longer drying cycle.

Further analysis of the graph shows that if the depth of frozen serum is kept to 1 cm., it can be dried conveniently in 24 hours by direct thermal conduction from heaters which do not rise above +40° C.

150 watts will dry 150 × 20 ml. in 20 hours = 3,000 ml.

150 watts can be applied to 2,500 sq. cm. without raising the temperature of the heater above 40° C.

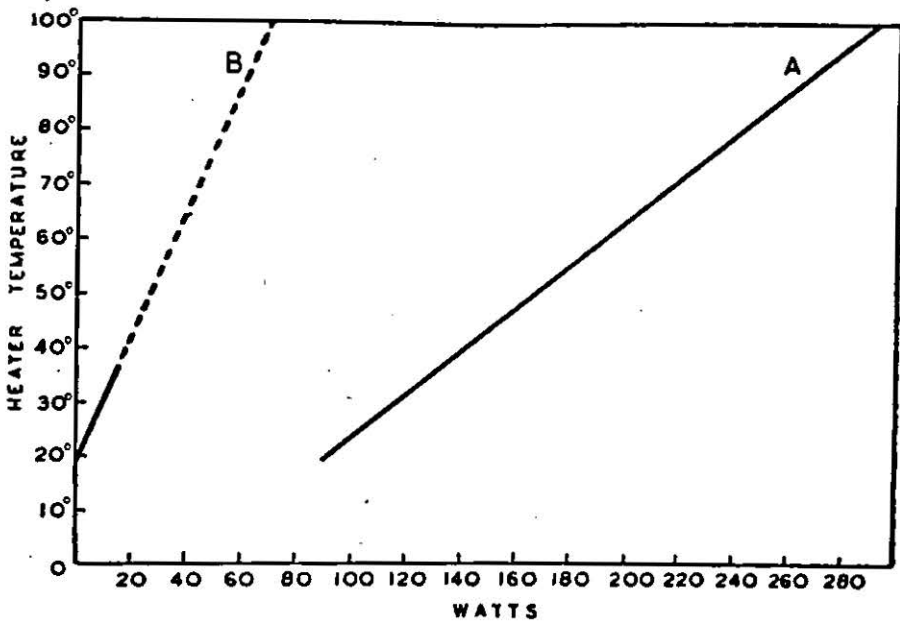


FIG. 4.—Rate of Heat Absorption at Different Heater Temperatures.

Therefore, the maximum depth that can be dried in 20 hours under these conditions is:

$$\frac{3000}{2500} \text{ cm.} = 1.2 \text{ cm.}$$

It is our custom to control the heat by a thermostat fixed to the heater head and never set above $+40^{\circ}\text{C.}$: under these conditions, the dried material cannot be submitted to a higher temperature.

This is probably a very conservative upper limit of drying temperature; Strumia and McGraw (1943) consider that a temperature of $+86^{\circ}\text{C.}$ will not harm the dried material, and Wyckoff and Lagsdin (1944) consider $+50^{\circ}\text{C.}$ quite safe.

In favour of these high end temperatures are the facts that the drying cycle is shortened and the residual moisture content of the final dried solid is lower.

Many different methods of applying heat have been advocated. When only small quantities are being dried, it is usual to provide no means of heating, as sufficient heat will be picked up by conduction and radiation. In the "Lyophile" and "Cryochem" methods of Flosdorf and Mudd (1936 and 1938), the ampoules or bottles are attached to a manifold and are thus exposed to room temperature (i.e., a heater at $+15^{\circ}\text{C.}$ to $+20^{\circ}\text{C.}$). Heating by this method is free from all criticism that the dried material may get overheated, but it is not readily controlled: for instance, if $+20^{\circ}\text{C.}$ proved too high a heat input for certain circumstances, the whole apparatus would have to be moved to a cold room; conversely, if $+20^{\circ}\text{C.}$ gave too long a drying cycle, the apparatus would have to be placed in a hot room.

In order to speed their drying cycle, Wyckoff and Lagsdin (1944) placed their apparatus in a hot room at $+45^{\circ}$ to $+50^{\circ}\text{C.}$ Flosdorf, Stokes and Mudd (1940), in their "Desivac" system (in which the bottles were contained in a desiccator), circulated liquid through jackets round each bottle and thermostatically controlled the temperature of this liquid. Strumia and McGraw (1943) placed their bottles in contact with the desiccator wall, which, in turn, was surrounded by a thermostatically controlled water jacket. Though

the mechanics of liquid heater-heads are difficult, they have one very great advantage: if a low-freezing liquid such as alcohol or "Methyl-cellosolve" is used, refrigeration is possible. With large individual doses there is little chance of the pre-frozen material thawing while the chamber is being evacuated and before the critical pressure is reached at which cooling by evaporation takes place, but with small individual doses this is not so, and it is helpful to have a refrigerated heater head into which to load the ampoules containing small amounts. When the critical vacuum has been passed, refrigeration of these heads is stopped, and heating begun. An alternative procedure is centrifugal freezing *in vacuo*, which will be described in the section on freezing.

We ourselves have always favoured the simplification of design that results from electric heating. This can be effected by winding resistance wire round tins in which the bottles are placed, using resistance mats if stages are to be heated, or heating by radiant heat in the case of vacuum spin-freezing apparatus.

Temperature Measurement

Temperature measurement of the serum during the drying cycle is essential for all experimental work and a valuable check during routine running. Thermocouples provide a convenient method for taking these temperatures and, if the thermocouples are connected to a recording galvanometer, a continuous record of the drying temperatures can be obtained.

The position of the thermocouple in the serum is a matter of some importance. With modest heat input and thickness of the serum, such as have been advocated, experiment shows that the temperature differences in the frozen serum are small. It is therefore our custom to fix the thermocouple against the glass of the bottle, at the thickest point of the serum layer. In this position, the thermocouple shows a maximum temperature during drying and gives an accurate indication of the dry point.

We have used copper-constantan couples, and have found it convenient to use long lengths of large diameter wire, so that all couples will reach back to the galvanometer, near which the constant temperature junction may be placed. It is common practice to use ice in water for the constant temperature junction, but such a junction requires constant attention. Since we are concerned only with the temperature range of $+50^{\circ}\text{C.}$ to -50°C. ; it has been considered preferable to hold the constant temperature junction at $+50^{\circ}\text{C.}$ This is achieved by heating oil in a thermos flask with a 1-watt heater, thermostatically controlled by a mercury contact thermometer operating through a "Sun-Vic" hot-wire vacuum switch. Such a constant temperature junction, once set, requires no further attention.

When drying in transfusion bottles, the temperature control bottle has to be spun-frozen with the thermocouple *in situ*. A 2-ft. length is taken from the standard thermocouple wire and passed through a hole drilled in the neck of the bottle, just below the thread for the screw-cap. The thermocouple is fixed to the glass inside the bottle at its bottom angle, with a little melted "Apiezon W" wax. The other end of the wire is fitted to a plug which is attached to the neck of the bottle with adhesive tape. The bottle is now filled with 400 ml. of serum, a drying cap screwed on to its neck, and the serum spun-frozen. On loading into the drying plant, the remainder of the standard length of the thermocouple is plugged into the control bottle plug, thus completing the thermocouple circuit.

With vacuum spin-freezing, it is necessary to take the serum temperature while the bottles are actually rotating. This involves using a slip-ring device, which we have not found satisfactory for use with thermocouples; in this case, therefore, resistance thermometers of very small size are used.

Sterility

For the drying of most biological materials absolute sterility is of paramount importance, particularly if the dried material is intended for injection into patients. Probably, of all important factors, this is the one which has received the least attention. The reason for this is not hard to seek, for any bacteriological filter is bound greatly to increase the obstruction of the drying system, and unless the latter is of the highest efficiency, it may be impossible to apply sufficient heat to ensure a reasonable rate of drying without causing thawing of the frozen material.

In the United States, since all material for human use must compulsorily be treated with an antiseptic, no attempt is made to dry in a bacteriologically closed container. The atmosphere of the drying room is kept as sterile as possible, by the use of ultra-violet light; and the assumption is made that if a few micro-organisms should get into the bottle, they will have no chance to multiply in the presence of the antiseptic, and will thus be of no significance.

This argument is probably perfectly valid, but it may not always be desirable to add an antiseptic, and the advantages of having the container bacteriologically closed throughout the whole process cannot be over-emphasised: sterility of the drying room and chambers can then be neglected, and the bottles can be handled with safety by comparatively unskilled personnel. Thus, in spite of the mechanical disadvantages, the whole of our procedure is based on drying from a bacteriologically closed container.

In the choice of a suitable filter, the aim is to secure maximum obstruction to the passage of particles with minimum obstruction to the flow of vapour. The most suitable type of filter has been found to be a pad composed of a layer of cotton wool between two layers of butter-muslin. Such pads, consisting of different weights of cotton wool, were placed over the necks of bottles of broth, which were then placed in an animal room with a fan blowing on to them; in this way, a minimal thickness of wool was found which could be relied upon to give complete sterility.

A sample pad was then sent to a commercial firm, who replied that since the weight of wool was nearly identical with that used in the civilian gas-mask filter, would these filters be suitable for our purpose? In the design of their filter, the anti-gas workers had had to solve the problem of removing minute particles of smoke, with a minimal obstruction to respiration, and it is of some interest that they should have decided on an almost identical filter weight to that which we had selected.

We now always use the standard gas-mask filters, if necessary trimming them to fit the various-sized bottle necks. The pad has an R value of 15.5 units per square inch. Fig. 5 shows the three ways in which this bacterial filter can be placed over the neck of the M.R.C. Transfusion bottles.

Method A shows the filter fitted over the neck, and attached with a rubber band, low down on the neck. Method B shows the filter attached to a perforated metal cap with a stainless steel ring. Method C shows the filter inserted in the metal cap.

Experiment has shown that the effective diameter of the filter is not the diameter of the bottle neck, but that the vapour diffuses out into the filter as far as its fixation point, giving approximately the effective diameters and R values shown. Assuming a heat input of 7 watts per bottle and a condenser temperature of -40° C., the drying temperatures (T) can be calculated.

It is clear that method C, though it provides very neat filter-caps, is not suitable. On small-necked bottles and ampoules we use method A, affixing the filter with a rubber band. When drying from the M.R.C. transfusion bottle, we use method B for convenience. In this method, the filters are made up

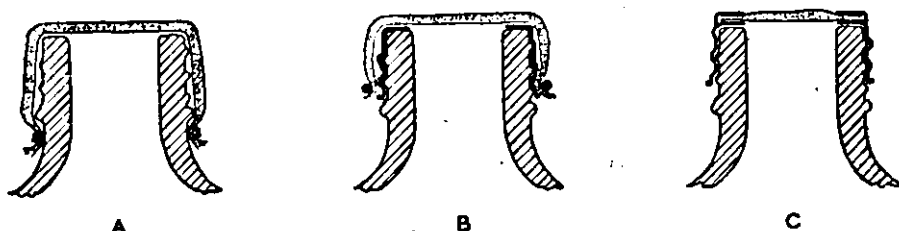


FIG. 5.—Drying Filter-caps.

A. Original arrangement still used for non-standard containers. The cotton-wool and muslin filter is held over the neck of the bottle by a rubber band.

B. The standard arrangement used for routine drying by the M.R.C. Unit. A perforated aluminium cap screws on to the neck of the bottle; a layer of adhesive tape round this prevents slipping, and the filter is held in position by a stainless-steel spring ring.

C. This arrangement with the filter inside the aluminium cap was tried but found to cause too much obstruction to the flow of water vapour.

	A.	B.	C.
Effective diameter of filter	3.5-in.	2.0-in.	0.88-in.
R value	1.6	5.0	25.6
Temperature when drying with a heat input of 7 watts per bottle of 400 ml. serum	-25°C.	-21°C.	-10°C.

on aluminium screw caps, having a $\frac{7}{8}$ in. hole punched out of their centre. A length of adhesive tape is tied round the screw, to give a better grip, and the filter is attached by means of a stainless steel spring ring, see Plate III.

Dry-weight Determinations

If a protein is dried from the frozen state in a high vacuum over phosphorus pentoxide it may be weighed daily and its progressive loss of weight observed. Such an experiment is recorded by Greaves and Adair (1936). It is found that the majority of the water is removed very rapidly, but that it may be several days with small amounts, or several months with large amounts, before a constant weight is obtained and the material may be considered completely dry. Only when it is absolutely dry can perfect storage be expected, and then only if steps are taken to prevent the dried material from being wetted. If the same material is dried from the liquid state this slow terminal loss of water is very greatly prolonged.

The determination of residual moisture content by vacuum desiccation and serial weighings is a laborious and lengthy process. Ovens at 110° C. are unsuitable for these materials, as fats and other substances will be lost. Flosdorf and Webster (1937) have found that for routine estimations of dry weight 22 hours at 50° C. and at a pressure of 0.1 mm. Hg gives approximately the same answer as the phosphorus pentoxide-vacuum method.

It is obviously impossible with a condensation drying method to obtain a completely dry preparation no matter for how long desiccation is prolonged, unless the condenser is at so low a temperature that its water vapour pressure is zero.

If desiccation is sufficiently prolonged, an equilibrium dry-point will be obtained which will depend on the temperatures of both condenser and dried material; lower residual moisture contents can be obtained by either lowering the condenser temperature or raising the temperature of the dried material.

The vapour pressure of water at +37° C. is 46.65 mm. Hg, while at -40° C. it is 0.1 mm. Hg. The "relative humidity" of the dried serum should be equal to the ratio $0.1/46.65=0.002$. There are no recorded data on the relationship between the water bound by dried serum and the relative humidity, but our measurements show that at this "relative humidity" the water bound by the dried serum is of the order of 5 mg./g. of dry solid.

With large amounts of material, unless adequate time is allowed for equilibrium to be established, this low level of residual moisture will not be reached; and that is one of the reasons why a secondary desiccation over phosphorus pentoxide for 3 to 4 days is considered desirable. One gram of phosphorus pentoxide has been found adequate for removing the residual moisture from the dry solids of 400 ml. of serum.

The Packing of the Dry Material

The majority of dried proteins are very hygroscopic. The percentage of residual moisture which can be permitted will depend on the particular protein it is desired to preserve, the likely temperature of storage, and whether or not prolonged storage is required. Dried plasma proteins with a moisture content below 1 per cent. of the dry-weight will show no obvious change after several years' storage at moderate temperatures, but this residual moisture content would be anything but satisfactory under certain conditions. There is fairly good evidence that, were it possible to dry a protein completely, it would remain unchanged almost indefinitely, even at moderately high temperatures; as the moisture content rises, so the preservation becomes less satisfactory. The first obvious evidence of unsatisfactory preservation of a plasma for transfusion is the formation of a gel on reconstitution, and imperfectly dried material may denature even more rapidly than the original solution. Theoretically, therefore, except for a short term policy, one should aim at getting very dry products, and, then, it is essential to ensure that the packing of the product shall not allow it to come into contact with water-vapour during storage.

In the clinical world the vaccine-type rubber stopper is much appreciated, and there is a demand for dried material in bottles capped in this way. Rubber is, however, an unsuitable sealing material for dried products, as it is permeable to some extent by water vapour, so that the dried material hydrates on storage. Very high moisture contents can rapidly result if such a package is stored in a humid atmosphere, and particularly if only small amounts are packed in each container. Flosdorf and Webster (1938) have measured the permeability of rubbers and "Neoprene" for air and water-vapour, and have shown that stoppers made from these materials cannot be relied upon for storing dry biological material for periods longer than six months, even if refrigerated to $+1^{\circ}$ C. Much more reliable storage can be obtained by packing the rubber-sealed glass container in a hermetically sealed can containing a desiccant such as "Drierite"—the "Desitube" (Flosdorf, Boerner, Lukens and Ambler, 1940).

It might be thought that a good capping solution or vacuum cement over the rubber might render the rubber seal more satisfactory, but Flosdorf, Hull and Mudd (1945) state that in their hands no capping solution or vacuum cement has proved satisfactory.

The ideal package is therefore an all-glass container, the product being perfectly dry and sealed in a high vacuum. Such high standards may not always be possible, but we have found that only under these conditions can blood grouping serum be relied upon to remain perfect under tropical conditions.

For transfusion materials, where the dosage is high, a glass sealed container is not very practical, particularly under war conditions. The American Army pack for dried human plasma may be taken as an example of a good method of packing without an all glass seal. The bottle of dried plasma is closed with a vaccine cap, and evacuated through a hypodermic needle. This bottle is then placed in a tin containing silica-gel, and the tin is evacuated and sealed. A similar tin contains a bottle of distilled water and the sterile "giving set."

Reconstitution of the dried material is effected by needling the two bottles, when the water is sucked into the bottle of dried material by the negative pressure.

In this country such a package has not been possible, and we have had to make a compromise with the rubber diaphragm and aluminium screw cap of the standard transfusion bottle. A vacuum pack is out of the question with these, for such a seal could not be relied upon and, if it failed, wet air would gain access to the bottle; the bottles are therefore packed with pure dry nitrogen to a slightly positive pressure through a plugged hypodermic needle. After nitrogen packing the needle is removed, the hole in the rubber being self-sealing. The hole in the metal cap, and the groove round the edge of the cap, are filled in with "Plasticine", and "Lassophane" tape is put over the "Plasticine" to keep it in place. For the Services, instead of the tape being used, the neck of the bottle is dipped in "N.F." capping solution. Such a seal is by no means perfect but has been reasonably successful. This is because the material is thoroughly dried by secondary desiccation over phosphorus pentoxide, and also because the bottles contain a relatively large amount of dry protein, so that any moisture gaining access to the bottle has a lot of dried material to wet. With small quantities, this method would not be nearly so successful.

The importance of the final packing cannot be over-estimated, and it is to be hoped that under peace-time conditions, compromise methods of sealing will be abandoned.

Refrigeration

In the design of the condenser in a freeze-drying plant the following points must be considered:—

1. The temperature at which the condenser should be operated;
2. The duty required at the determined temperature;
3. The design of the condenser coils;
4. The respective merits of direct expansion or brine circulation.

First let us consider the optimum temperature for operation of the condenser. A large number of factors has to be taken into consideration, but in the first case let us suppose that we desire to dry serum or plasma in the M.R.C. transfusion bottles on a 3-day cycle. It has already been pointed out that this requires a heat input of 7 watts per bottle, and that the R value is 8, giving a vapour pressure difference of about 0.6 mm. Hg.

Fig. 2 (p. 14) shows the drying temperatures with different condenser temperatures. This graph demonstrates that, so far as the drying temperature is concerned, there is little to gain by running the condenser below $-40^{\circ}\text{C}.$, and that not much is lost by having the condenser at $-30^{\circ}\text{C}.$ There are, however, other considerations which might favour the use of a lower condenser temperature; for instance, the higher the condenser temperature, the more water vapour will be taken over into the oil of the pump, and the higher will be the residual moisture content of the dried material.

But the duty of most commercial refrigerators falls rapidly below $-40^{\circ}\text{C}.$, and consequently it is much cheaper to provide means for removing excess moisture from the oil of the pumps by circulating it over heaters, than to attempt to obtain lower condenser temperatures. The residual moisture content can also be more easily lowered by raising the heater temperature at the end of the drying cycle, or by performing a secondary desiccation over a chemical desiccant such as phosphorus pentoxide.

Thus, for this particular purpose, the author favours a condenser temperature of between -30° and $-40^{\circ}\text{C}.$, the nearer to $-40^{\circ}\text{C}.$ the better. This does not mean that there are not circumstances in which higher or lower

condenser temperatures would be suitable. Where sterility is of small importance and cost is of great importance—as, for example, in the drying of milk—higher condenser temperatures could be used. To obtain the advantages of lower condenser temperatures together with sterility, however, the arrangement must be altered so that the bacterial filter is between the condenser and the pump, and the condenser must be capable of being sterilised.

The duty required of the refrigerator at the desired temperature is obtained from the calculated heat input, but allowance must be made for loss of efficiency through the insufficient lagging of pipes, etc.

The condenser coils should be so designed that a uniform distribution of refrigerant is obtained, and their surface should be such that, when fully loaded, the thickness of ice on them will not be greater than about $\frac{1}{4}$ inch. They should be so placed that they form an effective trap to prevent water vapour from entering the vacuum line.

The choice of type of refrigerator depends on the size of the plant and the desired temperature. For small scale and occasional work there is much to be said for the use of solid carbon dioxide or of liquid air, which gives very low condenser temperatures without involving capital expenditure; but for routine running on a large scale the expense involved would soon justify the capital cost of mechanical refrigeration.

The circulation of a secondary refrigerant has many points in its favour, but on small plants the loss of efficiency is great; for these, therefore, direct expansion is recommended.

It is claimed that the temperature of the coil is less variable if a secondary refrigerant is circulated through it than if direct expansion is used, and that therefore the distribution of ice is more uniform. Levinson (1944) goes so far as to say that the ice only condenses at the spot where the water vapour first meets the cold surface, and he therefore advocates a plate condenser. This has not been our experience: with direct expansion, and with well designed coils, the variation in temperature over the coil can be kept very small provided the "superheat" is kept low. The distribution of ice on the coil is remarkably uniform so long as the vacuum is good; any leakage leads at once to uneven distribution of the condensed ice.

Temperatures as low as -40°C . can be obtained moderately efficiently by the direct expansion of methyl chloride, or the "Freon" group of gases. The weakest link in direct expansion plants is undoubtedly the expansion valve. The automatic expansion valves of the constant temperature type at present available were not designed for working at these low temperatures, and the majority are found to be highly erratic in operation. Traces of moisture, or the use of oils with high wax contents, tend to make them stick, so that for reliable operation it is important, in our experience, to include a drier permanently in circuit, and to keep the system as free as possible from oil by the use of an oil return pot. Under these conditions the "Detroit" valve has been found to give reliable service. If the plant is not required to run unattended and automatically, a hand regulator will overcome these difficulties; an alternative solution is the use of a high-side float regulator, but this involves exact adjustment of the refrigerant charge at the working temperature. Down to -45°C ., compounded ammonia machines have been found satisfactory, particularly if fitted with liquid intercoolers.

If lower condenser temperatures are required, cascaded compressors must be used, so that a gas such as ethane or propane can be expanded in the evaporator coils. This adds greatly to the cost and complication of the refrigerator system, but by such means reasonable duty can be obtained at temperatures as low as -80°C .

In spite of the extra losses involved, brine circulation has much to be said in its favour for large-scale work. Two compressors can be used to cool the brine-tank, and if the equipment is planned so that one machine alone will carry the full duty of the plant, the other is always there as a standby, in the event of a mechanical break-down or sudden extra loads. Moreover, if the amount of brine is kept large, there is a large reserve of cold in the event of an electric mains failure, so that provided a small petrol motor is installed to work the brine pump to the desiccator chambers, quite long failures can be survived without in any way endangering the material being dried. Cold rooms and pre-freezing rooms can also be operated from the same brine tank, with the result that two large compressors, one being in reserve, can take the place of numerous small machines. Lastly, the efficiency of large machines is so much higher that the losses on the brine system are more than compensated.

Calcium chloride solution, of specific gravity 1.300, has been found satisfactory for circulating at temperatures down to -45°C .; below this temperature "Methanol" may be used.

The Freezing of Protein Solutions before Drying from the Frozen State

The method of pre-freezing the protein solutions has an effect on the solubility of the final dried material. Elser, Thomas and Steffen (1935) considered that slow freezing might lead to a partial denaturation of the proteins in serum on account of the concentration of the salts which occurs, even though this concentration is taking place at a low temperature. Flosdorf and Mudd (1935) stressed the importance of rapid freezing, and they used solid carbon dioxide in "Methyl-cellosolve" as the refrigerant.

The more rapid the freeze, the more amorphous in appearance is the final dried product, apparently because the ice crystals are then very small. Although denaturation has not been demonstrated as the result of slow freezing of serum, there is not the slightest doubt that the solubility of the dried material varies with the crystal size. Therefore, since speed of solution is usually important, care should be taken to obtain as small a crystal size as possible on pre-freezing.

On theoretical grounds, there are several reasons why serum which has been pre-frozen in small crystals should be more rapidly soluble after drying than serum which has been pre-frozen in large crystals. In the first place, the initial rate of solution should be directly proportional to the surface area of the interface between the dry protein and the water used for solution, so that, because of the greater surface of the material frozen in small crystals, solution of the dried product should be rapid. Secondly, if a film of water is placed between two crystals of a sparingly soluble salt, the time required for saturation by diffusion will be proportional to the square of the thickness of the film, so that if the thickness is halved, the time for saturation is decreased four times; the greater dispersion of small crystals should therefore greatly increase the speed of re-solution after drying. The greater surface energy of the finely divided particles must also aid re-solution.

As a general rule, it may be said that the more rapid the rate of freezing the smaller will the crystal size be, but the rate of freezing does not depend entirely on the temperature of the refrigerant. It is my experience that the most amorphous material results from the snap-freeze due to evaporation or the freeze occurring after supercooling.

Flosdorf and Mudd (1935) obtained satisfactory freezing by the use of dry-ice in "Cellosolve" as refrigerant baths. If the ampoules or bottles were slowly rotated in this mixture, with their axes nearly horizontal, the protein solution was shell-frozen round the inside periphery of the bottle; there was thus

a large surface for evaporation, with a minimal depth of frozen material. If dry-ice condensers are being used for drying, and dry-ice is thus available, this method of shell-freezing is extremely convenient for small-scale work. When large quantities are involved, the method is costly; and since 400 ml. of serum take at least 20 minutes to shell-freeze, manual rotation of large numbers of bottles becomes impracticable. However, the mechanics of an apparatus for slowly rotating the bottles on their horizontal axes in the refrigerant bath offer no great difficulties, and such methods have come into general use in America when large numbers of bottles have to be pre-frozen (Florsdorf, Hull and Mudd, 1945).

On a large scale, small cascaded ethane compressors capable of giving a reasonably good duty at -60° C. may be used to cool alcohol baths, thus replacing the use of dry-ice. Since these refrigerant baths consist of either "Cellosolve" or alcohol, the frozen bottle will have a film of the volatile organic compound on it after freezing. If such a bottle is to be placed in a vacuum chamber, this may spoil the vacuum. For this reason, if vacuum chambers are to be used, care must be taken to remove the film from the bottles; if manifolds are to be used, the difficulty does not arise.

It is clear that where vacuum chambers are to be used a method of freezing in air would have great advantages. In the early days of the M.R.C. Unit, when human serum was being dried in quantities of 200 ml. in 12-oz. medical flats, the bottles were placed in a cold room at -20° C. at a slight angle from the horizontal, so that the serum froze as a wedge. This method of freezing was slow and the crystal size was large and, although there was no evidence of denaturation, the time for re-resolution was long, being from 5-10 minutes.

Lanyon (1941) showed that the rate of freezing could be made rapid if a sufficient stream of cold air was passed over the bottles, and he introduced a method of shell-freezing serum or plasma in the M.R.C. transfusion bottles, by rotating them slowly at a small angle from the horizontal in a current of cold air, at -20° C., maintained by a fan. By this method a small crystal resulted, giving a rapidly soluble final product. At this temperature there is a great tendency for supercooling to occur, and, in practice, it was found that, particularly with filtered serum or plasma free from particles, a certain number of bottles did supercool and freeze suddenly in a solid lump.

Strumia and McGraw (1943) have largely overcome the difficulty of supercooling. They work with an alcohol bath cooled to -20° C. with a mechanical refrigerator. The bottles of plasma are placed on their sides, immersed to a depth of 1 cm. in the cold alcohol; they are then rotated slowly by mechanical means. After a short time, rotation is stopped and a strip of plasma freezes down one side of the bottle; rotation is then restarted, and uniform shell freezing follows. Supercooling is thus avoided but, judging by the published photograph, a rather large crystal size results.

Following the experience by the M.R.C. Unit of the Lanyon type spin-freezer, it occurred to us that the very rapid freeze which follows supercooling might be used to give a small-crystal structure. As a result, the high-speed vertical spin-freezing method was introduced by Greaves (1942).

In this method, the bottles are spun on their vertical axes at between 750 and 1,000 r.p.m. in a stream of cold air. The centrifugal force distributes the liquid uniformly round the inside periphery, leaving in the centre a conical air space which reaches to the bottom of the bottle (Plate IV). In this position the liquid, after supercooling, freezes suddenly, giving a very small crystal and a very rapidly soluble dried product. In order to get this small crystal size it is necessary to use clean filtered material and to pre-cool it to $+2^{\circ}$ C. in a cold room overnight. The temperature of the freezing room should be -17° C.; below this temperature cooling tends to be too rapid, so

that supercooling does not take place and the crystal size is larger; above this temperature freezing takes too long. This method has been used for some years by the Unit for pre-freezing transfusion fluids, and it has proved thoroughly reliable. Plate V shows a general view of the freezing room, which takes 108 bottles at a time on a 2-hour freezing cycle.

When large amounts are pre-frozen in each bottle, they do not rise much in temperature during their transfer from the storage chamber to the desiccator, or during the period of loading and attaining the necessary degree of vacuum in the chamber. For this reason, where the individual dose is large, pre-freezing either in dry-ice baths or by high speed vertical spin-freezing is at present the method of choice. Where the individual doses are small, there is a grave risk that some of the material may thaw during the transfer process and consequently froth on applying the vacuum; this is particularly liable to occur with materials such as broths or crude penicillin solutions which may contain components that will thaw even at -25°C .

This problem may be overcome by making provision for refrigeration of the heater heads, or by building the vacuum chamber in such a way that it is its own refrigerator. In the latter case, the ampoules do not have to be moved from the chamber which has been cooled to, say, -40°C ., and there is no chance of warming during the period of exhaustion. The provision of refrigeration to the heater heads greatly complicates the manufacture of the drying chamber, but it is a satisfactory method of overcoming the difficulty.

If liquid material is subjected to a vacuum in the presence of a desiccant, it first of all froths violently and then freezes with extreme suddenness owing to the loss of the latent heat of evaporation. Such a freeze is ideal, but enormous containers would be required to hold all the froth which forms. Hartley (1936) has shown that this frothing is largely due to the liberation of dissolved gases. The gases may be removed by reducing the pressure slowly, so that a quiescent solution is left, which, on a sudden further reduction in pressure, will snap-freeze, giving an excellent dried product. This method was used by Greaves and Adair (1936) and by Flosdorf and Mudd (1938). It has been widely adopted for small-scale work; for if a chemical desiccant such as phosphorous pentoxide or "Drierite" is used, it enables freeze-drying to be carried out successfully without the need for any refrigerants, and it is therefore particularly useful for occasional laboratory drying. The method, however, has its disadvantages; for occasionally individual samples bump badly at the point of freezing so that some material may be lost, and very small individual doses tend to dry out partially from the liquid state during the somewhat lengthy degassing procedure.

Greaves (1944), while trying to control frothing during degassing, found that quite a small amount of centrifugal force would completely inhibit frothing; 1,450 r.p.m. with a minimal radius of $\frac{1}{2}$ inch completely stopped bubble formation. Thus, if a suitable centrifuge is provided, the material to be dried can be loaded in liquid form into the chamber, the centrifuge started and the chamber evacuated. Freezing will then occur with extreme suddenness, as soon as the necessary degree of vacuum is attained. This method of freezing is extremely rapid, absolutely constant, and gives an excellent dried product. The centrifugal force is used for two different purposes—firstly, for preventing bubble formation, and, secondly, for distributing the liquid in such a way that it gives a large surface area for evaporation. On a small scale, the method permits freeze-drying with chemical desiccants without the necessity for any refrigerant. On a large scale, it avoids the necessity for pre-freezing and for low temperature storage rooms.

Where large numbers of small ampoules or bottles are to be dried, the mechanics of an arrangement for spinning each on its vertical axis would be

complex, but if the ampoules or bottles are placed off-centre and at a slight angle from the vertical, with the tops pointing slightly inwards, the liquid will freeze as a wedge on one side of the container; this wedge will provide an adequate surface for drying small amounts. Thus, in a chamber of the type described by Greaves and Adair (1939), a central rotating shaft can carry several aluminium discs drilled to take numerous bottles or ampoules (see Plate VI).

The method might even be used for bulk drying on a large scale. If a number of concentric cylinders were arranged on a common central shaft, and the liquid were placed in the spaces between the cylinders, on rotation it would be distributed as thin films on the inner surfaces of the cylinders, and these films would snap-freeze and dry from the frozen state.

PART III—THE DEVELOPMENT OF THE APPARATUS USED BY THE M.R.C. DRYING UNIT AND A REPORT ON THE WORK DONE BY THE UNIT

Introduction

My interest in freeze-drying originated in 1935, when I was confronted with the preparation of antisera in rabbits on a large scale. These sera lost their potency so rapidly under normal methods of storage that the development of methods for adequate preservation became a necessity. In the absence of refrigeration facilities, attempts were made to dry by the frothing self-freeze method by Craigie (1931) and Adair and Robinson (1931). This method worked well, but the dried material was so voluminous that difficulties of storage were soon encountered.

At this time, Hartley (personal communication) informed me that the frothing of liquids in a vacuum was largely due to the evolution of dissolved gases under the reduced pressure, and that by controlling the speed of evacuation quiescent fluids could be obtained. Applying this method, it was found that once a quiescent fluid had been obtained, a sudden further reduction of pressure caused automatic freezing without frothing. A method on these lines was therefore evolved for drying from the frozen state (Greaves and Adair, 1936; Flosdorf and Mudd, 1938).

Very soon the method, which could handle only 20 ml. of serum at a time, was found inadequate for our requirements. Large glass and metal desiccators were therefore made, on the lines of the vacuum chambers used in the Cavendish Laboratory (Cockcroft and Walton, 1932), and the chemical desiccant was replaced by a liquid air condenser (Greaves and Adair, 1939). This apparatus worked well with quantities up to 200 ml., but it was tiresome in operation, since the condenser had to be continuously recharged; it was therefore decided to explore the use of mechanically refrigerated condensers working at higher temperatures.

For this purpose, use was made of a discarded "Isodore" SO₂ compressor (Plate VII), and, although temperatures of only -20° C. were obtained with this apparatus, we were able to show that the principle was sound and were also able to work out methods for the automatic heating of the material being dried.

At this time, war in Europe seemed likely, and Dr. (now Sir Percival) Hartley was asked to arrange for the supply of large amounts of tetanus anti-toxin to meet a possible emergency. Since it was not known if, or when, this material would be required for use, steps had to be taken to see whether it could be preserved, if it were not needed for some time. On the recommendation of Dr. Hartley, who knew of our work, the Medical Research Council decided to finance the erection of a pilot plant based on the work we had already done. The apparatus described by Greaves and Adair (1939) was the result. (Plate VIII).

However, the conditions in Europe deteriorated and war became imminent; it therefore seemed clear that the tetanus antitoxin would not require to be preserved by drying, and consequently the plant designed on the experience gained with the original pilot plant was never constructed.

The Medical Research Council Drying Unit for Serum and Plasma

This Unit was established at the outbreak of war, to explore the use of dried serum and plasma as possible substitutes for whole blood for transfusion purposes.

Clotted blood, originally of group AB, which was not required for transfusion, was provided by the London Blood Supply Depots set up by the Medical Research Council on behalf of the Ministry of Health. The serum from this blood was separated, filtered and dried by the Unit at Cambridge, and returned to the London Depots for clinical trial. Reports of these tests will be found in the paper by Brown and Mollison (1940). The amount originally chosen as a suitable unit was 200 ml., since this was about the amount of serum which could be obtained from 1 pint of blood. Two hundred ml. could, moreover, be wedge-frozen conveniently in 12-oz. medical-flats in a cold room at -20° C., which was available in the Department of Pathology, Cambridge. Bottles of this type were chosen, therefore, as the standard pack for the dried product. At the same time, the distilled water for reconstitution was dispensed in M.R.C. transfusion bottles, so that serum, after reconstitution, could be administered with the standard "giving sets."

On arrival of the blood-clots from the Depots, the serum was poured off into centrifuge tubes and, after spinning, the supernatant fluid was syphoned into a pool. This pool was then Seitz-filtered through 8 cm. E.K. pads, placed on Buchner funnels and covered with Winchester quart bottles which had had their bases cut off and which acted as reservoirs for the unfiltered serum. Serum, rather than plasma, was used at this stage, since it was found impossible to filter plasma, owing to its clotting during the process.

At first, the original pilot plant was used for the drying and was arranged to accommodate twenty-four 12-oz. bottles at a time; two runs were done each week, so that the weekly output of dried material was 48 bottles of 200 ml., or 9.6 litres. Since these initial experiments proved encouraging, it was decided in December, 1939, to increase the scale of production of dried serum.

By enlarging the diameter of the drying chamber from 14 to 16 inches it was found possible to accommodate 60 instead of 24 bottles. With three machines the output was therefore 72 litres per week on two runs per week, or 84 litres per week on continuous running. Clearly, the filtration of this amount of serum could not be performed with the crude filtration arrangements then existing, and a Filtration Unit, to work with the Drying Unit, was therefore set up in the Department of Zoology. The separation of the serum from the clot was now done at the Blood Depots, and since the supply of AB donors could no longer meet the demand, recourse was had to pooling serum from Groups A and B donors—this procedure being found to remove effectively the iso-agglutinins from the serum.

A bacteriological check on the sterility of the serum throughout the whole period of preparation was made by Miss M. Mackay, who also investigated the possibilities of working with disinfectants. She concluded from her experiments that "it would appear that disinfectants can have only a limited value in controlling the bacterial growth in stored serum. Of those tried the most effective was proflavine." (Mackay, 1941.)

As a result, all blood substitutes have been treated in this country without antiseptic, but with very rigid sterility control throughout the whole processing.

The final pilot plant came into operation in March, 1940. It was based on the original pilot plant and consisted of three units. The chambers were made of steel steam-pipe 16 in. in diameter by 4 ft. 3 in. long, instead of the original 14-in glass cylinders. The refrigerators were 9 " W.H. Hallmark " methyl-chloride plants as in the original model. A " Cenco Hypervac " pump was fitted to one chamber, and an " Edward's B.1." rotary two-stage pump was shared by the other two chambers. The original " Cenco Megavac " pump was connected to a series of three secondary desiccators, made by mounting the original glass cylinders on a common steel base-plate. The heater heads were made to accommodate sixty 12-oz. medical flats in individual tins, and the heater mats were wound on a jig and placed between the layers of tins. The general construction and layout of this plant can be seen from Plates IX, X, XI, and XII.

This plant operated continuously without alteration until June, 1941. During this period the demand for dried material was rapidly growing, and the Wellcome Foundation undertook to increase the output by constructing a plant at the Wellcome Physiological Research Laboratories, Beckenham. This plant was similar to the final pilot plant and consisted of three units, but the capacity was increased by enlarging the diameter of the chambers to 24 in.

The main demand for the dried material at this period was for the Forces overseas, and in January 1941 the authorities of the Army Blood Transfusion Service decided to construct their own drying plant. They wished, however, to use the standard M.R.C. transfusion bottle for the drying process, and Captain E. C. G. Lanyon R.A.M.C., of the Army Blood Transfusion Service, devised a method of pre-freezing in these bottles (Lanyon, 1941). Originally the Army plant consisted of two units, but of a larger size than the Wellcome units. Four-cylinder, as opposed to the three-cylinder, methyl-chloride " Hallmarks " were used as refrigerators, and the chambers were 2 ft. in diameter by 6 ft. high. These chambers would accommodate 100 M.R.C. transfusion bottles, each containing 400 ml. of plasma, or 40 litres per load; and since Lanyon was able to reduce the drying cycle to 48 hours, this gave a weekly output of 280 litres. Since then, this output has been doubled by installing two further units.

Meanwhile, numerous requests had been received that the M.R.C. Unit should change to the standard M.R.C. transfusion bottle so that uniformity of apparatus could be achieved, and also because in the clinical use of the dried material the original 200 ml. dose had been found to be too small. After initial experiments with Lanyon, it was decided to make the change; but, as already described in the section on pre-freezing (p. 37) Lanyon's method proved unsuitable for routine work. The method of high-speed vertical spinning was therefore introduced by the M.R.C. Unit (Greaves, 1942).

The only alteration that had to be made to the final pilot plant was the construction of new heater heads to take the transfusion bottles (Plate XI). It was found possible to accommodate 35 bottles in each machine, and to run on a 3-day schedule, giving a weekly output of 98 litres. On this schedule the final pilot plant operated from June 1941 to December 1942, when the new " large drying plant " came into operation.

One unit similar to those of the Army plant was also erected at the Royal Infirmary, Edinburgh, to deal with the Scottish demand for dried material.

Extension of the Drying Unit

In the early part of 1942, difficulties were being encountered in the Ministry of Health Regional scheme for the issue of liquid serum and plasma as blood substitutes, because the instability of these liquid products was giving rise to

great wastage. The result was a greatly increased demand for the dried products.

The Wellcome Trustees, therefore, made a gift of £20,000 to the Medical Research Council for the establishment of a Drying Unit which should meet the demands of the whole country—estimated at the time to be of the order of 2,500 bottles of dried material per week.

The Medical Research Council entrusted the construction of a plant capable of this output to their Unit at Cambridge. The new plant was to absorb the routine work of the final pilot plant, thus liberating the latter for purely experimental studies. In order that the new plant might also 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.

The contract for the plant was placed in June 1942, and trial runs were made in December of that year. The plant went into routine operation on February 1st, 1943.

The Design of the Large Drying Plant

The problem put to us was to maintain an output of 2,500 bottles of dried material per week, and to provide for an increase, if necessary, up to double this amount. Since it would clearly be impossible to arrange a steady intake to the plant at a corresponding rate, it was realized that cold storage would be necessary for at least 5,000 bottles. For that purpose, a temperature of -25° C. was chosen, partly because this is below the eutectic of serum and plasma, and partly because it would allow a good margin of safety for the operation of loading the vacuum desiccators.

Five thousand bottles per week means that 714 bottles per day must be spun-frozen. This operation could be spread over the 24 hours—an arrangement which, with a spinning cycle of 2 hours, would require spinning accommodation for 60 bottles at a time. Lanyon's improved model of Greaves' spinner holds 36 bottles, so it appeared that two of these spinners should suffice. In order to have an adequate margin of safety, however, three were installed. Thus the normal daily load of 360 bottles could be handled with four spinnings per day, taking 8 hours; and even on the larger output only 16 of the 24 hours would be needed for spinning. This gives a reasonable margin of time for servicing the spinners and defrosting the room.

Lanyon (personal communication) has shown that if the heaters are set at $+40^{\circ}$ C., and 10 watts of heat are applied to each bottle, the plasma temperature reaches $+40^{\circ}$ C. in 32 hours. If, at this stage, the heater thermostat is reduced to $+30^{\circ}$ C., the whole drying cycle, including defrosting the condenser, can be completed and restarted in 48 hours. This is probably the fastest rate at which it is desirable to operate the drying process, so that to give an output of 5,000 bottles a week there must be primary desiccator space for 1,428 bottles. This would require 14 Army-type chambers, each of which can accommodate 100 bottles.

If the chambers were made 3 ft. in diameter, instead of 2 ft., 180 bottles could be accommodated, so that eight chambers (with a total capacity of 1,440 bottles) would be adequate. On the lower output for which the plant was chiefly to be designed, if two chambers were loaded each day, this would allow a 3-day drying cycle, plus one day for defrosting and any servicing of the chambers which might be required. Moreover, by loading 3, 3, and 2 chambers, in rotation, on successive days an intermediate output of 3,330 bottles per week could be obtained, still maintaining a 3-day drying cycle, but abolishing the servicing period. It appeared, therefore, that eight chambers,

together with a fair-sized storage room, should give all the flexibility that would be needed.

Refrigeration would be required for the spinner room, the storage room, and the eight primary chambers. These could all be supplied, as in the previous plants, by individual direct-expansion machines; but it seemed that the point had been reached at which it would be economical to abandon the unit system and embark on central refrigeration by cooling a brine tank to -40° C. and circulating the cold brine. The advantages of this scheme seemed easily to outweigh the slight loss of efficiency involved in using secondary, as opposed to primary, refrigeration. If two compressors were installed of such capacity that one alone could manage the load, the other would act as a standby for any mechanical failure of the leading compressor, and could be made to cut in automatically if the load should become too great for the one machine. Only when running on the 5,000 bottle-per-week schedule, would the period of operation of the second compressor become considerable.

Another advantage of the central refrigeration method would be obtained by having a large brine tank. Then, in the event of a prolonged electric supply failure, it would be necessary only to drive the desiccator brine-pump by a small petrol engine, in order to keep the condensers cold and the serum safe from thawing for a considerable period.

On the vacuum-pumping side, a rather similar problem arose; was it better to have individual pumps for each chamber, or a large pump for all eight chambers? It was essential that it should be possible to evacuate a chamber to below the critical vacuum in about ten minutes, and, with the enlarged chambers, this would require a fairly large pump. Once drying had started, provided the apparatus was leak-free, the amount of non-condensable gas to be removed would be small. It was therefore decided to connect all eight chambers through a common manifold to two large pumps, each big enough to remove the non-condensable gases evolved from all eight chambers (Plate II). In this way both pumps could be used for pumping down a chamber after loading, but, after that, it would be necessary to run only one, the other acting as a standby. An old motor-car engine, connected to one pump, completed the arrangements for guarding against loss in the event of electric-supply failure.

Secondary desiccators for the removal of the last traces of moisture, and for the final nitrogen-packing, had also to be provided. As a $+1^{\circ}$ C. room already existed, no provision had to be made for pre-cooling bottles before spin-freezing.

It was clear that personnel would be a major difficulty, and it was equally clear that with 1,500 bottles at risk at any moment, it would be inadvisable to leave the plant untended, lest a breakdown should cause a serious loss. An adequate alarm system would, however, remove the necessity of having more than one person on duty for the greater part of the 24 hours, and would allow the watcher to sleep, so that he could work the next day. A system (Plate XIII) was devised, therefore, that would give warning of: (1) an electric-supply failure, (2) a rise of brine temperature, or (3) any rise of pressure in the vacuum system. The whole apparatus is briefly described in a paper by Greaves (1944).

Technical Details of the Large Drying Plant

(a) *Refrigeration*.—The calculated mean load on a 2,500 bottle-per-week basis was 76,000 B.T.U's. per hour, for drying, freezing and storing. This heat neutralization was required at a brine temperature of -40° C. An atmospheric, water-cooling tower was necessary, since there was no water available except the mains supply, and this would have been very costly to use for cooling, as well as being liable to failure from enemy action. High condenser temperatures had therefore to be anticipated, and this fact, coupled

with the low evaporator temperature required, indicated the use of a compounded ammonia compressor with a liquid intercooler.

The "J. & E. Hall", 9 in. × 6 in. and 6 in. × 6 in., compressor, if run at 475 r.p.m., is capable of neutralizing 72,000 B.T.U's. per hour at -40° C. This was a little short of our requirements, but since a larger sized machine could not be fitted into the only space that was available for an engine room, and since two compressors were to be installed, this size was decided upon.

Plate XIV shows the arrangement of the two machines. It will be seen that control is by two high-side float regulators. This works well provided the gas charge is very accurately adjusted, and so long as the plant is kept thoroughly purged of air. In the presence of air, there is marked overheating of the high pressure cylinder; this cannot be adequately controlled by the liquid-injection system shown, since the condenser is normally almost empty of liquid, and the liquid line transmits a mixture of liquid and gas. In the absence of a liquid trap on the suction line, it is found that with the charge correct for one machine, "priming" occurs when two machines are run, so that the charge has to be adjusted as a compromise.

If, however, the hand regulator is used instead of the high pressure float, more ammonia can be put in the machine so that there is a liquid level in the condenser. This liquid is subcooled, so that lower intercooler temperatures are produced; and since the liquid line is now full of liquid, liquid injection to the high pressure cylinders can be used if required. This arrangement, though it gives an improved performance, requires more attention, and therefore the float control is more commonly used.

The oil return from the intercooler produced difficulties, which were eventually overcome by partially closing the H.P. suction stop-valve and venting the crank-case to the suction side of the valve. A difference in pressure of 2-3 lb. per sq. inch across the valve is adequate to maintain a continuous return of oil to the crank case.

The two machines are controlled automatically by the temperature of the brine. We have found it convenient to set the leading machine to cut in at -41° C. and out at -43° C.; the standby machine is set to cut in at -39° C. and out at -42° C. The temperature of the incoming and outgoing brine from the tank is continuously recorded by platinum, resistance thermometers on a Model "C" Cambridge recorder. Plate XV shows the two compressors in the engine room of the large plant, and Plate XVI the instrument panel.

(b) *Brine circulation.*—The brine is calcium chloride solution of S.G. 1.300. Five 1 h.p. pumps are used to circulate it. One pump circulates the brine to the storage room and another to the freezing room; these two pumps are fitted with cross-over valves, so that in the event of a breakdown on one pump, the other can be used for either room.

Two pumps are used on the brine circulation to the desiccator chambers, one being a standby for the other. One of these pumps is fitted with a petrol as well as an electric motor, so that the petrol motor may be used during any electric failure.

The fifth pump is on the defrosting circuit, which consists of a flash-heater containing three electric immersion-heaters, two of 6 Kw. rating and one of 3 Kw. rating, giving a total of 15 Kw. The splitting of the heater elements into three gives flexibility to the rate of defrosting; for instance, if it were required to defrost the freezing room slowly overnight the 3 Kw. heater only would be used, whereas, if rapid defrosting of three or four desiccator chambers were required, the full 15 Kw. would be used.

(c) *The freezing and storage rooms.*—Entrance to the two rooms is through an air lock kept below freezing point. Much of the moisture which enters

with the outside air is collected on the air-lock coil, with the result that defrosting of the chambers is not required so frequently as would otherwise be necessary. The air-lock also supplies limited storage space, below 0°C ., for material whose temperature of storage is not critical.

The freezing room (Plate V) is 11 ft. long by 4 ft. wide by 7 ft. high. At the far end is the cooling unit behind a baffle. The air in the room is circulated by two fans mounted on the baffle, the top fan sucking the warm air from ceiling level, and the bottom fan blowing cold air along the floor. The temperature of this room is controlled at -18°C ., and its temperature is continuously recorded on a recording galvanometer operated from a platinum resistance thermometer. The walls of both the freezing and storage rooms are lagged with 12 inches of cork.

The storage room is 11 ft. by 9 ft. 6 in. by 7 ft. high, and can accommodate 3,500 frozen bottles of serum or plasma stacked in crates. This is smaller than was originally planned, but is as large as the available space would permit. Cooling is by grids behind baffles, no fan being used. The temperature is controlled automatically at -25°C ., and is recorded continuously by a platinum resistance thermometer and recording galvanometer.

(d) *The primary desiccators.*—These are eight in number (Plate XVII). Each chamber is made from a piece of lap-welded steam pipe, 3 ft. in diameter by 6 ft. long. At the bottom, this tube fits into a groove in a piece of $\frac{1}{4}$ in. steel plate, and, at the top, into a groove in a $\frac{3}{4}$ in. steel ring.

On the steel plate is arranged the coil of the cold brine condenser, and this is surrounded by a drip tray, the bottom of which is sloped into a sump to facilitate the removal of the water which collects after defrosting. In the centre of the condenser coil, guarded by a baffle, is the vacuum exit pipe. This pipe, together with the two pipes to the condenser, is welded into the bottom plate.

On erection, the grooves in the bottom plate and top ring are filled with "Apiezon Q" and the cylinder put in position; on evacuation, the cylinder beds down in the groove, forming vacuum-tight joints by squeezing out most of the "Apiezon Q". The joints are luted off with this material, and then given a coat of copal varnish to keep out moisture.

The top of the desiccator is formed by a $\frac{3}{4}$ in. thick steel plate of radius $\frac{1}{2}$ in. less than that of the ring, thus providing a groove for making the seal, with "Apiezon Q", which has to be broken and remade each time the chamber is opened. On this top plate is mounted the heater-head. Plate I shows the detailed construction of these chambers.

The heater-heads are constructed from 180 tins which fit the transfusion bottles closely. Round each tin is wound a length of No. 24 S.W.G. constantan wire, and three banks are connected in series-parallel, in such a way that a dissipation of 10 watts per tin shall not raise the temperature of the constantan wire above $+60^{\circ}\text{C}$. *in vacuo*.

The tins could either be arranged as a honeycomb, or touching at four points; in either case, the same number of tins can be accommodated in the available space. The four-point method was chosen, since this left a space between each tin which previous experience had shown to be immensely valuable for the drying of material in small containers. Elsewhere it has been pointed out that one of the problems in drying small individual doses, which have been pre-frozen, is that they tend to thaw during the period of evacuation. If, however, they are loaded between the tins, they are kept frozen by the 80 litres of ice which surrounds them. In these spaces, we have dried large numbers of 0.25 ml., 0.5 ml., and 1 ml. doses of typing serum, and even 10 ml. bottles of convalescent serum.

The tins are enclosed in a galvanised-iron case; mounted on this case, and leading out through the top plate, is a metal pocket in which is fitted a

"Sunvic" TS. 3. thermostat; this is connected to control the heater circuit through a "Sunvic" F. 102 -4 'M.' hot-wire vacuum switch. In operation this thermostat is set at $+30^{\circ}$ C., and the heater current adjusted on a "Variac" transformer so that each tin receives 7 watts. These conditions give a convenient three-day drying cycle. A control bottle containing a thermocouple is included in each machine load, and these thermocouples are connected to a eight-point, model A, Cambridge recorder so that a continuous record of the drying temperature is obtained.

In the outlet brine-pipe is inserted a platinum resistance thermometer, the reference point of which is another resistance thermometer in the main brine tank. The difference between the temperature of the brine at its entrance and exit from the chamber is thus measured, and is also recorded on an eight-point, model A, Cambridge recorder, thus serving as a direct check that the brine flow through each condenser is satisfactory. At maximum heat load, the differential is 3.5° C.; as the heat is reduced, this differential falls till, at the dry-point, the differential is less than 0.5° C. The differential temperature might be used for estimating the dry-point, but it is not as accurate an index of this as the information which may be gained from the serum control thermocouple. The temperature recorded by the latter rises during the later stages of drying and finally steadies, when the dry-point is reached, at the level at which the thermostat is set.

The vacuum pipe is connected by a $\frac{3}{8}$ in. "Audco" vacuum tap to the 2 in. vacuum manifold. Between the tap and the chamber is inserted a steel block, on which is mounted a Pirani vacuum gauge.

(e) *Secondary desiccation.*—On removal from the primary chambers, the dried material contains 0.4 per cent. of its dry weight of residual moisture (Plates XVIII and XIX).

The first operation is to remove the drying cap of cotton wool and butter muslin, with which the bottle has so far been fitted for sterility purposes, and to replace this by the standard aluminium cap with a rubber diaphragm, as used in the M.R.C. transfusion sets. During this operation some moisture from the atmosphere will be absorbed by the dried material. The aluminium caps have a hole punched in the centre; through this hole and through the rubber diaphragm is introduced an hypodermic needle, to which is attached a bacterial filter. The needled bottles are now returned for secondary desiccation.

The secondary desiccators are similar to the primary desiccators, except that they have no condenser coils and the tins for the bottles have no electric winding. The chambers are therefore only 3 ft. 6 in. high.

Under each nest of tins is a sheet-iron shelf, and phosphorus pentoxide, distributed as a thin layer on plates, is placed on these shelves. Each bottle requires 1 gramme of phosphorus pentoxide.

The chamber is then evacuated to 0.02 mm. Hg, and is held at this vacuum for four days. The residual moisture escapes slowly through the hypodermic needle and is taken up by the desiccant. At the end of four days, the vacuum in the chambers is replaced by dry, oxygen-free, nitrogen from a cylinder, to a slightly positive pressure. The head of the desiccator is then raised and the needles removed from the bottle caps. The hole in the metal cap and the space round the skirt of the cap are filled with "Plasticine", and then the neck of the bottle is dipped in "N.F." capping solution, or alternatively sealed with "Lassophane" tape, to give an hermetical seal.

Work Done by the Final Pilot Plant and by the Large Drying Plant.

(a) *The final pilot plant.*—The final pilot plant, with its own Filtration Unit, started routine production in June, 1940, issuing dried serum in 12-oz. medical

flats each containing 200 ml. In the 12-month period from June, 1940, to June, 1941, when the change to drying in the transfusion bottle was made, the distribution of the medical flats of dried serum was as follows:—

Sutton Blood Supply Depot	912
Luton Blood Supply Depot	392
Slough Blood Supply Depot	1,064
Maidstone Blood Supply Depot	816
Sector I	864
Sector II	912
Reserve Store	384
<i>Total sent to London Area</i>	5,344
Royal Navy	2,248
Army	8,654
R.A.F.	2,484
<i>Total sent to Services</i>	13,386
Miscellaneous	127
<i>Total No. of medical flats supplied</i>				18,857

In June, 1941, a change was made to drying 400 ml. quantities of serum spun-frozen in M.R.C. transfusion bottles. From this period till routine production was taken over on the large drying plant, the distribution of bottles of the dried material was as follows:—

Royal Navy	13,015
Army	745
<i>Total No. of M.R.C. transfusion bottles supplied</i>					13,760

(b) *The large drying plant.*—A trial run of a fortnight's duration was carried out in December, 1942, but routine drying did not begin till February 1st, 1943. The plant was continuously in operation thereafter until September, 1945, when owing to the fact that large stocks had been built up, coupled with the cessation of hostilities in Europe, the plant was closed down. During this period, plasma and serum, supplied by the Army, Navy, Air Force and civilian centres, were dried, distribution being arranged to meet the most urgent needs of the moment. The distribution of M.R.C. transfusion bottles of dried blood products, during the whole of this period, was as follows:—

Royal Navy (serum supplied by Navy)	76,848
(serum contributed from civilian organisations)	5,768
Army (plasma supplied by Army)	23,820
(plasma contributed from civilian organisations)	40,123
R.A.F.*	—
London area	62,032
Newcastle area	5,483
Leeds area	14,461
Nottingham area	11,793
Cambridge	10,832
Cardiff	7,959
Birmingham	23,957
Liverpool	14,147
Manchester	7,850
Polish Red Cross	2,016
Miscellaneous	1,553
Reserve Store	10,058
<i>Total No. of M.R.C. transfusion bottles supplied</i>			318,700

* The home needs of the Royal Air Force for dried blood products were provided indirectly through various centres; figures are not available.

During the three years, 1943, 1944 and 1945, the numbers of bottles of blood products dried were 117,302, 139,430 and 61,968 respectively. The losses due to all causes, such as oversize bottles, damaged bottles, overfilled bottles, technical faults in the plant, etc., amounted to only 1,271 bottles (0.46 per cent.) over the whole period, and, in many of these instances, the blood product was recovered. In addition, the drying of blood-typing serum was carried out as a routine.

(c) *Miscellaneous*.—This includes bottles of serum dried experimentally. Some of these were dried with different antiseptics added, for the experiments of Mackay (1941). Amongst other materials dried experimentally were cat, dog and goat sera, and plasmas, for various workers experimenting on shock in animals.

Other materials dried in smaller quantities during this period included human blood-typing serum, measles convalescent serum, ether-extracted serum, haemoglobin solution, pregnancy mare serum, foot-and-mouth disease vaccine, and therapeutic antisera. Some bottles of dried serum were also dissolved in 200 ml. of liquid serum and redried; this gave the equivalent of 400 ml. of serum in each bottle.

The Conversion of the Final Pilot Plant for Centrifugal Vacuum Spin-freezing

When the large drying plant came into full routine operation, it became possible to use the final pilot plant for experimental work.

Although we were able to dry numerous small batches of material between the tins of the large plant, it was felt that a satisfactory method for dealing with these small doses, independently of the large plant, was an outstanding requirement of the freeze-drying technique. This was further emphasised when it was found that certain batches of sodium penicillin could not be dried in the routine way, nor were routine methods applicable to the drying of broth cultures of viruses or bacteria.

In an effort to solve this problem, we evolved the method of centrifugal vacuum spin-freezing (Greaves, 1944), which has been described on p. 38. By this method it was possible to load the drying apparatus with liquid material, and all pre-freezing and low temperature storage were rendered unnecessary.

The technique was originally worked out in a small glass desiccator, using phosphorus pentoxide as a desiccant, and it was felt that the final pilot plant should be modified for this method of drying. Since the most urgent problem was that of drying penicillin, the final pilot plant was modified for the centrifugal spin-freezing of 10 ml. amounts in 25 ml. bottles.

Accordingly, cast aluminium discs were made, 12 inches in diameter. Each of these was drilled so as to take 75 bottles inclined inwards at an angle of 7° from the vertical, and three of these discs were mounted on a $1\frac{1}{2}$ -inch diameter shaft. This shaft was mounted in self-aligning ball races; at the top end, the race was fixed to the top plate of the chamber; at the bottom, to a plate fixed to the top plate by four supporting legs of $\frac{3}{4}$ -inch pipe. The drive for the shaft was taken through 3 small V-belts to a $\frac{1}{4}$ -h.p. split-phase motor, mounted eccentrically above the top plate, and covered with another cylinder so that it could be evacuated with the rest of the chamber. The top half of the shaft was hollow, and in it was placed a thermostat for temperature control. Below each disc, were a heater winding and a radiant heat screen (Plate VI).

At first, some difficulty was experienced due to vibration, but this was overcome by mounting a heavy weight on the lower plate. Nevertheless, it was felt that a better form of construction would be that illustrated in diagram on Plate XX. This drawing shows an apparatus capable of drying 6 litres per day in 10 ml. amounts. The chamber design is altered so that the top half

lifts as a bell-jar. The bottom portion contains the condenser coils and the spinner head: the latter is supported on rubber feet so as to damp its own vibration.

Accurate temperature measurements of the material being dried are essential in experimental work, and are helpful in routine running; but these at first presented some problems. The first method tried was the use of thermocouples connected through slip-rings, one of copper and the other of constantan; this failed both mechanically and electrically. Experiments with slip-rings showed that the best results followed the use of "Morganite" brushes on cast-iron rings, but this combination excluded the use of thermocouples. The alternative was to use a resistance thermometer with these slip-rings, but the thermometer would need to have a fairly high resistance, and to be of small size so that it could be inserted into small bottles or ampoules. A resistance thermometer made from the coiled filament of a 15-watt electric light bulb was consequently tried, and has proved extremely satisfactory for recording the temperature of the material while freezing centrifugally or drying in a stationary position.

In operation, the centrifuge is kept running, if serum is being dried, till the temperature of the serum has fallen to -20° C.—a matter, on our apparatus, of half an hour. If penicillin is being dried, the centrifuge is kept running till the temperature has fallen to -30° C., which takes about 1 hour. When the centrifuge is stopped, the heating is started, the heater current being adjusted with due regard to the maximum, safe, drying temperature and to the speed of drying that is required.

SUMMARY

The technique of freeze-drying has been found to be admirably suited to the preservation of many biological fluids. Though the technique has been known for many years, it has only recently been developed to such a stage of efficiency that freeze-drying has become the procedure of choice for the preservation of a variety of biological materials both in the laboratory and on a commercial scale.

The preservation of most materials by this method is so good that it is too early, as yet, to say for how long perfect preservation may reasonably be expected in the case of any particular preparation, under varying storage conditions; nevertheless, the degree of stability of the products is such that some delicate preparations will withstand several hours' boiling in their dried form.

The basic principles underlying freeze-drying are essentially very simple. If a protein solution is dried by sublimation from the frozen state, the dried solid shows but little reduction in volume from the original material, is very light and friable and dissolves readily; on the other hand, if these solutions are dried from the liquid state, they reduce in volume, ending in glue-like scales which are relatively insoluble. The virtue of drying from the frozen state would seem to derive from two factors: firstly, the low temperature delays denaturation and, secondly, the solid state prevents concentration of salts; the latter might prove injurious to the proteins and also cause that aggregation of protein molecules, which is the first stage in denaturation.

The procedure of freeze-drying may be divided into two stages:—the primary desiccation, in which the bulk of the water is removed from the frozen material, leaving a relatively dry powder, and the secondary stage, which consists in the drying of the powder. On this secondary stage depends the ultimate preservation of the material; and good preservation can be expected only if this stage is really efficiently carried out. Efficient secondary desiccation is a lengthy procedure, and must be concluded by perfect hermetic sealing to prevent re-wetting of the powder. Since these two desiderata were difficult

to attain under wartime conditions, they have been largely neglected in favour of simple compromises, which, on the whole, have proved adequate for a short-term wartime policy. However, in the case of blood-typing serum, packed in small amounts and intended for storage under tropical conditions, we have found that no compromise is permissible; and this material in consequence, is now issued in $\frac{1}{4}$ ml. amounts, vacuum-packed in glass-sealed tubes. Even so, it is not possible to turn out a perfectly dry product, so that our criterion of durability has been that the serum shall not lose more than half its potency during five hours' boiling.

Where standard reagents are required, it would seem that even more attention should be paid to the secondary desiccation.

In the field of primary desiccation, vast progress has been made during the war years, stimulated mainly by the need for large amounts of dried human serum and plasma for transfusion purposes, and by plans for the drying of penicillin. The problems of primary desiccation may be discussed under three headings: (a) the preliminary freezing of the material, (b) the maintenance of a high vacuum against the evolution of water vapour during the drying process, and (c) the supply of latent heat of evaporation to the subliming solid.

Preliminary freezing of the material is necessary because, if the material were introduced in liquid form to a vacuum, violent frothing would occur, owing to the liberation of dissolved gases. Were this not so, the rapid evaporation that occurs during the freeze-drying procedure would abstract the latent heat sufficiently rapidly to cause freezing of the liquid. The appearance and solubility of the dried product largely depend on the methods used for pre-freezing the liquid. These should be such that freezing is rapid and small crystals are formed; such conditions were obtained by Flosdorf and Mudd (1935) by freezing in baths of dry-ice. On a large scale of production, dry-ice baths are expensive to maintain, and they have been largely replaced by alcohol baths, refrigerated to -60° to -70° C. by mechanical means. If, during freezing, the bottle is rotated on a nearly horizontal axis, the liquid can be frozen round the inside periphery of the bottle, thus giving a much more favourable surface-to-depth ratio and hence a shorter drying time. This procedure has been called shell-freezing by Flosdorf and Mudd (1935).

An alternative procedure is to freeze in an air-draught at -18° C., the bottles being rotated at a comparatively high speed on their vertical axes. In this way a cone is forced down through the liquid, and the liquid freezes around the inside periphery of the bottle as in shell-freezing. This procedure has the advantage that it can be carried out at a much higher temperature than shell-freezing, it has been called "high-speed, vertical spin-freezing" by Greaves (1942). Recently, Greaves (1944) has shown that if high-speed, vertical spinning of the liquid is carried out in the vacuum chamber, frothing is avoided and evaporation-freezing occurs. The advantage of this procedure is that all pre-freezing is abolished, the freezing becoming the first stage of the drying process itself.

The next problem is how to maintain an adequate vacuum, during primary drying, against the vast evolution of water vapour. On a small scale, chemical desiccants such as phosphorus pentoxide, calcium chloride, calcium sulphate and silica gel have given good results, but each has its disadvantage either of cost or bulk, so that none of them can be satisfactorily used for large-scale work. Direct pumping can be used, though the pump size has to be greatly increased for the purpose, and since the water vapour will be carried over to the pump if this is of the mechanical oil variety, means must be provided for removing this water from the oil. Such a procedure has been described in the "Desivac Process" by Flosdorf, Stokes and Mudd (1940), but for large-scale work 4- or 5-stage steam-ejector pumps are generally used.

Of all methods of removing water vapour, the refrigerated condenser is probably the most efficient. On a small scale, liquid air or dry-ice condensers are widely used with excellent results. On a large scale, liquid air becomes out of the question and dry-ice is very expensive, with the result that mechanically refrigerated condensers are now usually employed.

The latent heat of evaporation must be supplied to the frozen material for drying to proceed, and the speed of drying will depend on the rate at which this energy is provided. Here, a number of inter-related factors are involved, which are fully discussed in the section on Heat Control (p. 25). In the majority of cases, the factor of first magnitude is the design of the container in which the material is to be dried; in general, this should be wide, squat and with a large orifice. Compare, for instance, the standard M.R.C. transfusion bottle, which is not ideal as a drying container, with the pre-war Baxter "Vacoliter", which is: 400 ml. in the M.R.C. bottle, capped for sterility, can be dried in 72 hours, the drying temperature being -21° C. and the finishing temperature $+37^{\circ}$ C.; 500 ml. can, however, be dried in the Baxter bottle, also capped for sterility, in 36 hours, the drying temperature being -28° C. and the finishing temperature only $+25^{\circ}$ C. The Baxter bottle thus gives much more rapid drying at much safer temperatures.

Much controversy has centred round how best to apply the required heat. Drying takes place from the surface of the frozen mass, so that after a while there is left a layer of dried material on the surface, as the ice retreats. If heat is to be applied by conduction, it must be transmitted to the drying surface through either the frozen or the dried mass. Heat transference through both is poor, but it is better through the former than through the latter; the heat should therefore be applied through the frozen material. This will cause a temperature gradient across the frozen material, but, in drying in a bacteriologically-closed container, other factors so limit the amount of heat which can be applied, that there is no danger of the frozen mass being thawed where it is in contact with the glass. Drying in open trays with thick layers of frozen material, it may well happen that the temperature gradient will be so great that thawing at the point of contact of the heater with the frozen material will occur, and this will be the limiting factor in the speed of drying. There is no evidence that radiant energy assists materially; and since direct conduction of the energy is so easy to arrange, this would appear to be the method of choice. Radio-frequency di-electric heating has been suggested as an alternative but, to quote Flosdorf, Hull and Mudd (1945), "Such heat must not be generated within the frozen mass at a rate in excess of the rate at which the heat can be conducted through the ice to the evaporating surface where it is utilised; otherwise a pool of molten product will be formed within the mass of ice". Di-electric heating, therefore, would appear to offer no advantage.

Accordingly, if it is desired to increase the rate of drying, the only logical way is to increase the evaporating surface and, consequently, to decrease the depth of the frozen material. As a rough and conservative guide, it is suggested that, if a 24-hour drying cycle is desired, the depth of frozen material should be restricted to 1 cm.

Experimental work on freeze-drying was being carried out at the Department of Pathology, Cambridge, before the recent war, but most of the methods and apparatus described in this report were devised for routine production to meet war demands for dried materials. In consequence, much of the work has been of an *ad hoc* nature, and frequent compromises have had to be made to meet wartime shortages or lack of supplies.

This wartime production has, however, been a very valuable check on laboratory procedure, since so frequently it has proved quite impracticable to

apply a small-scale laboratory method on a production scale. Throughout, it has been our policy to produce the best possible product on a laboratory scale, and then to try to develop large-scale methods, as far as possible, on the same lines.

Looking back on the war experience, it would seem that primary freeze-drying on any scale now presents no serious problems. Centrifugal vacuum spin-freezing offers such enormous advantages on a small and medium scale, that further work in adapting this method to large-scale production would seem to be indicated. The difficulties and defects of the drying technique relate to the secondary drying and packaging of the dried material. Insufficient is known about the keeping qualities of different materials, with differing residual moisture contents and at different temperatures; nor has a container yet been perfected, which is efficient in preventing wetting of the dried product and is at the same time convenient for the clinician to use.

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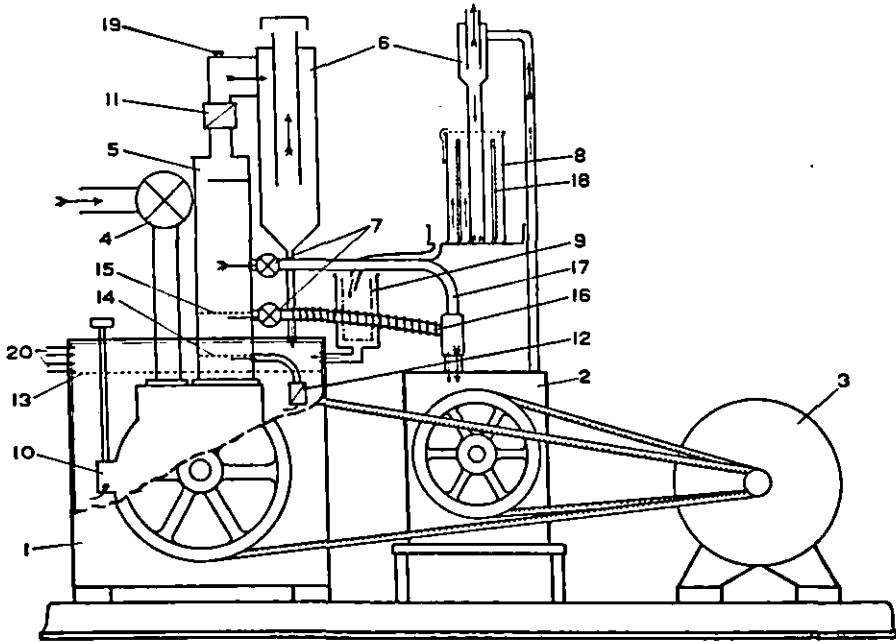
This work could never have been undertaken without the expert mechanical assistance of Mr. F. Mitchell, of the Department of Pathology, Cambridge.

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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 : $\triangleright \longrightarrow$ oil paths \longrightarrow

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.

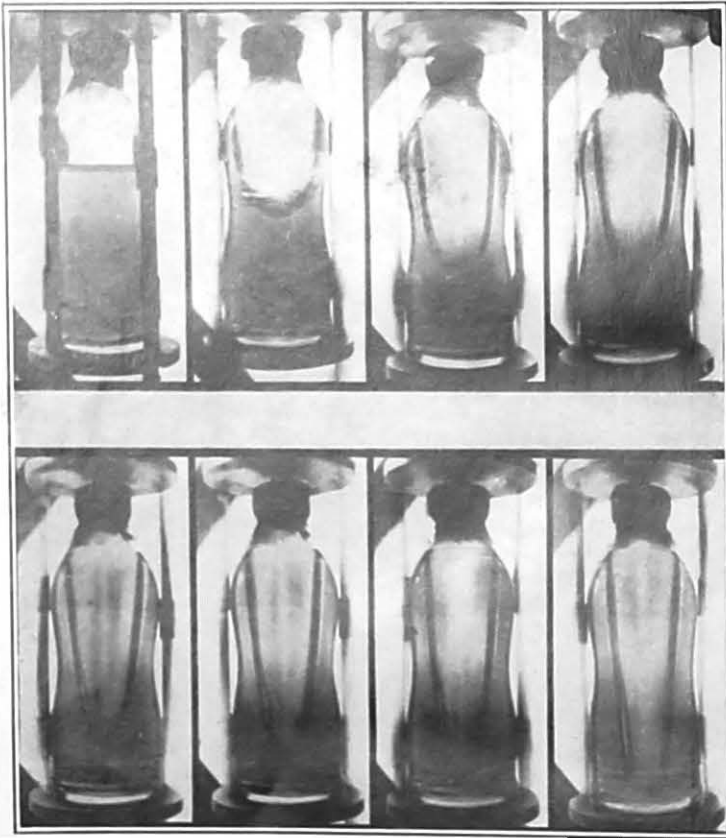
PLATE III



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.

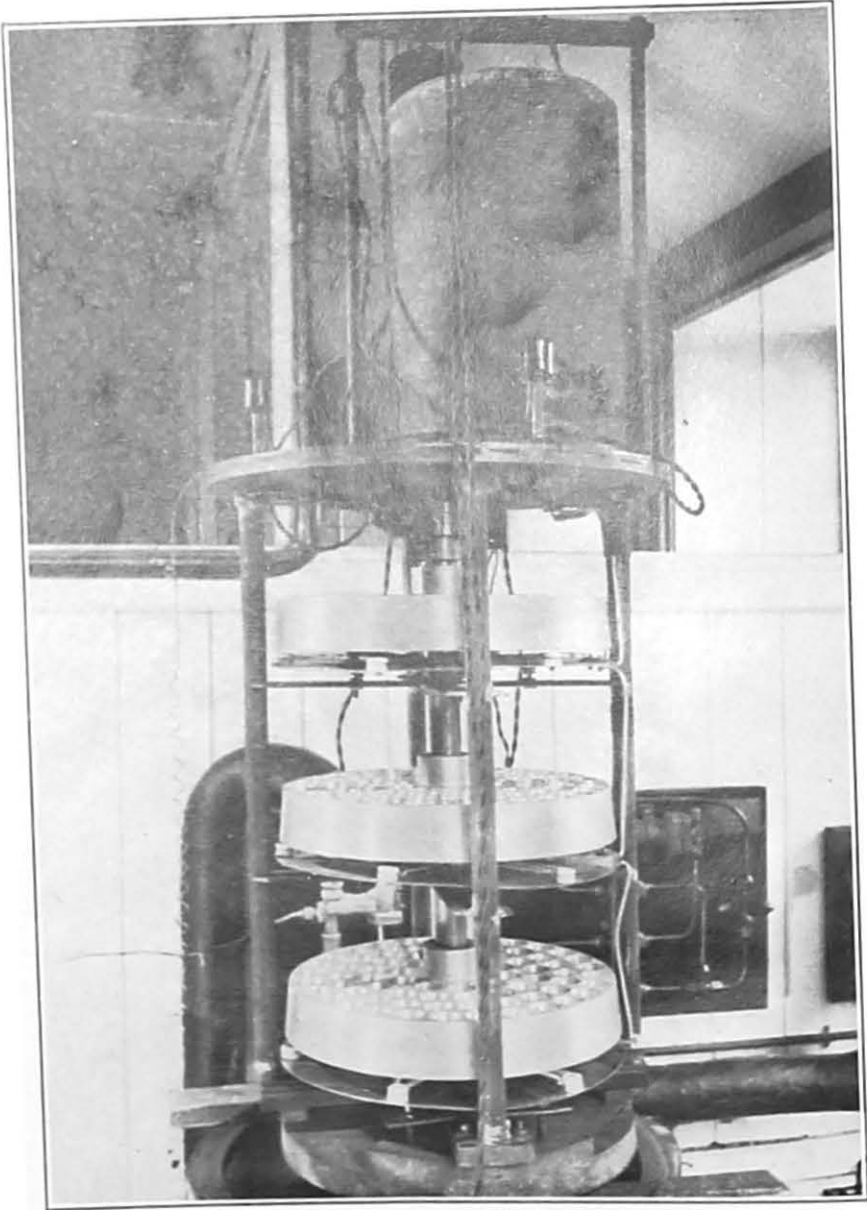
PLATE V



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.

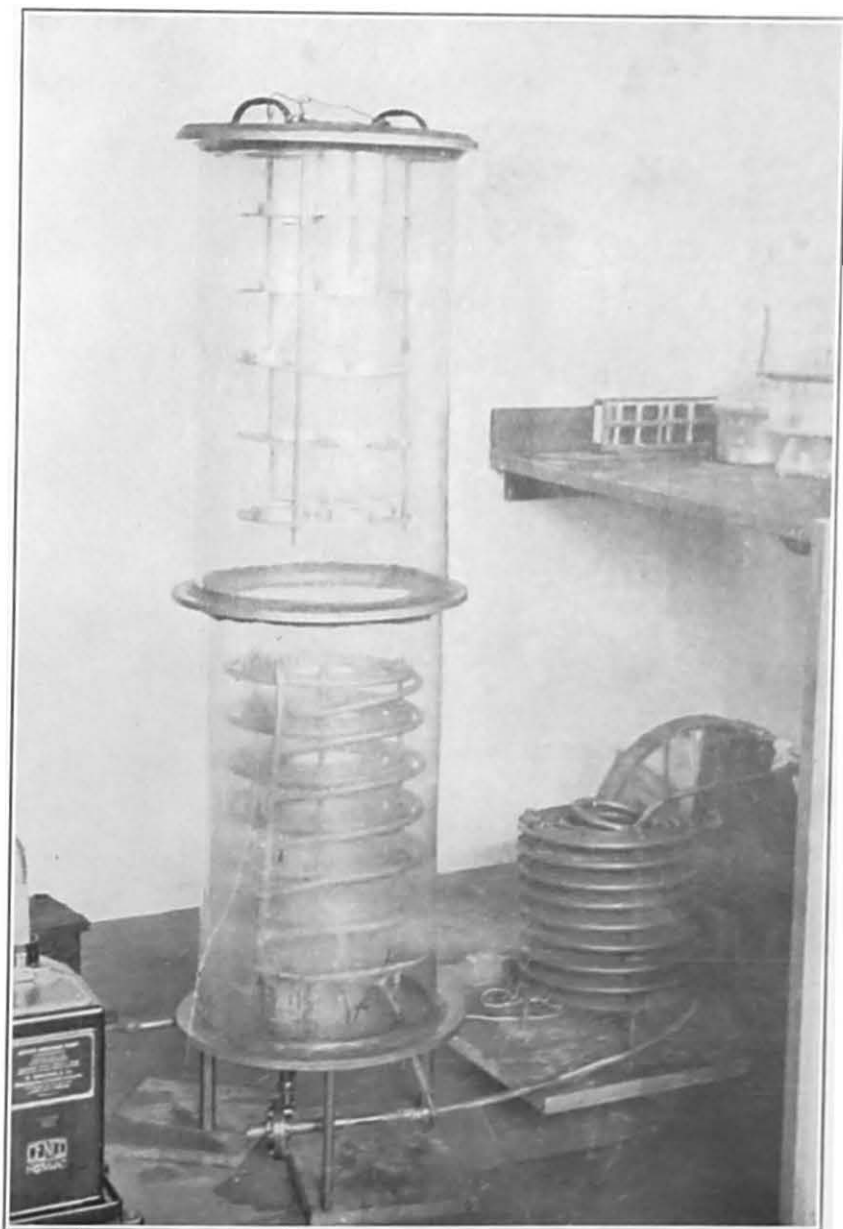
PLATE VI



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.

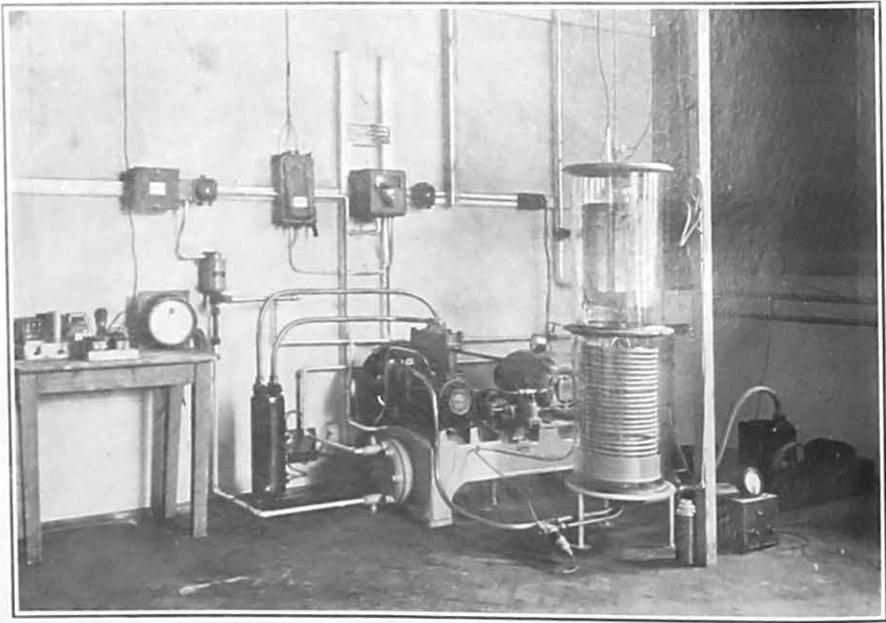
PLATE VII



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.

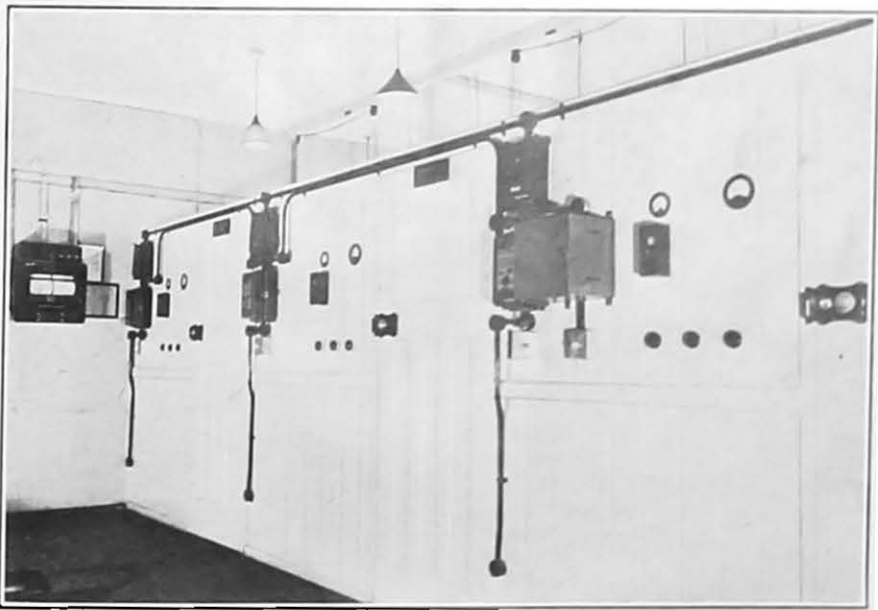
PLATE VIII



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.

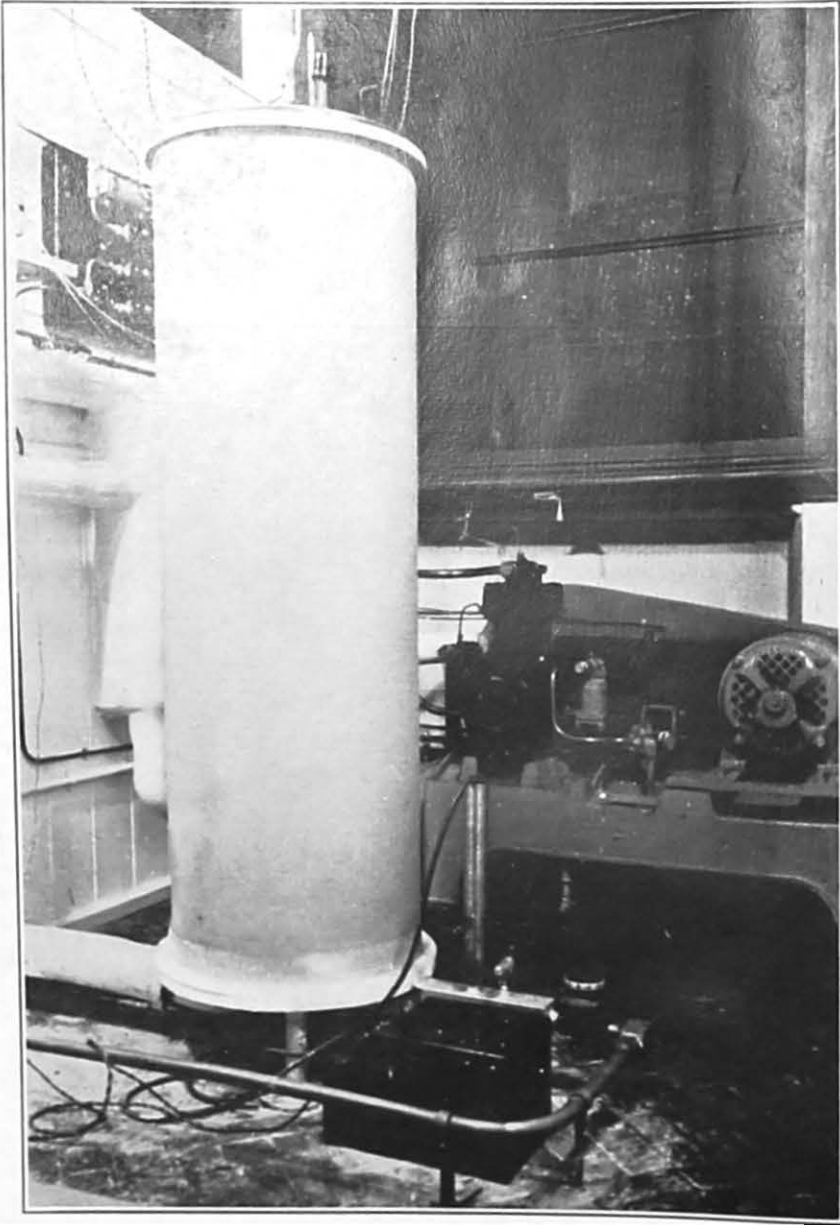
PLATE IX



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.

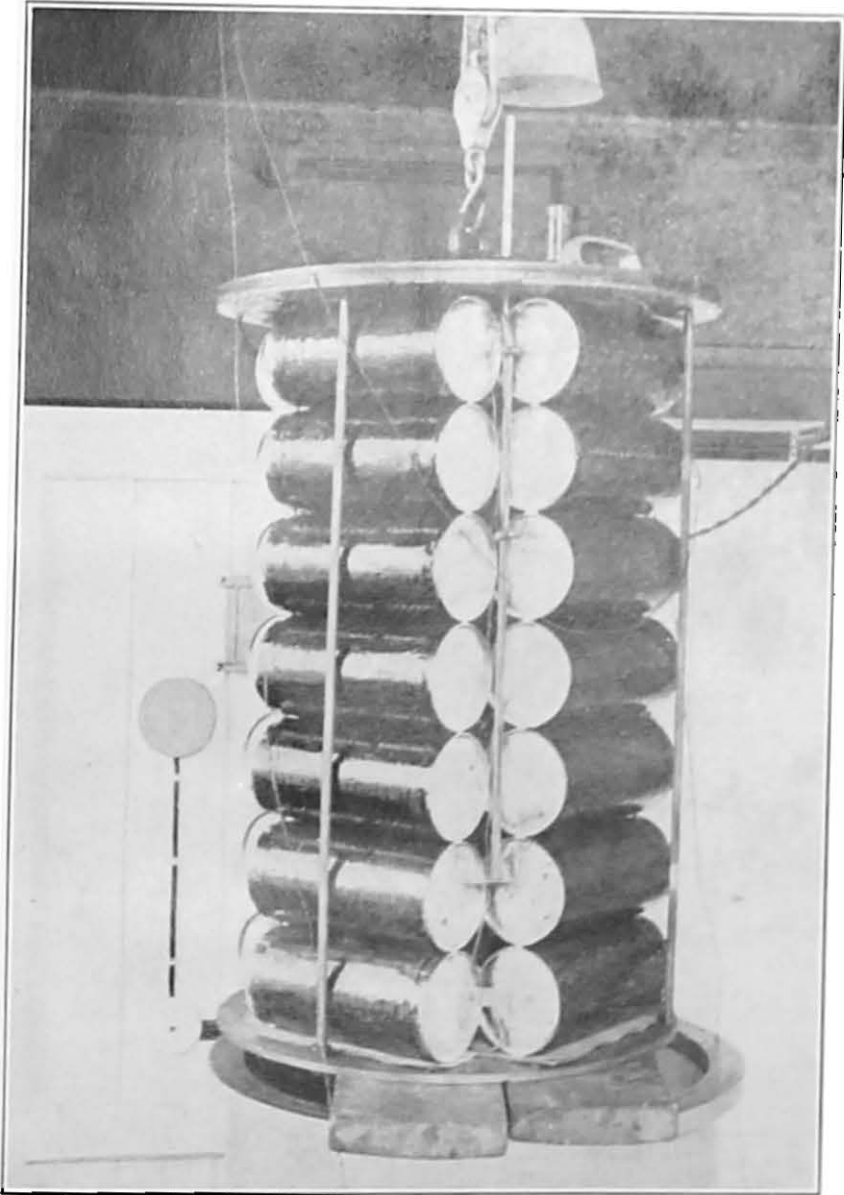
PLATE X



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.

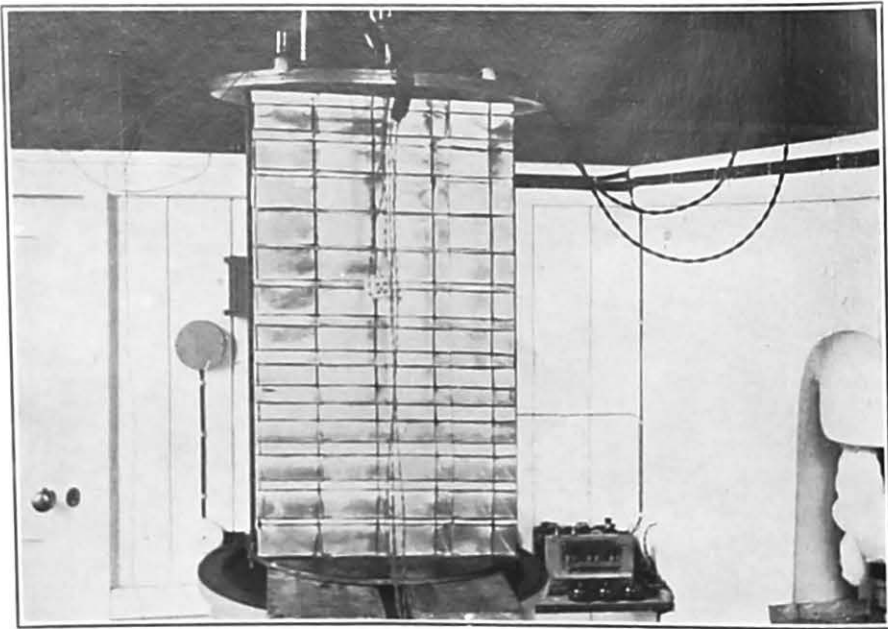
PLATE XI



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.

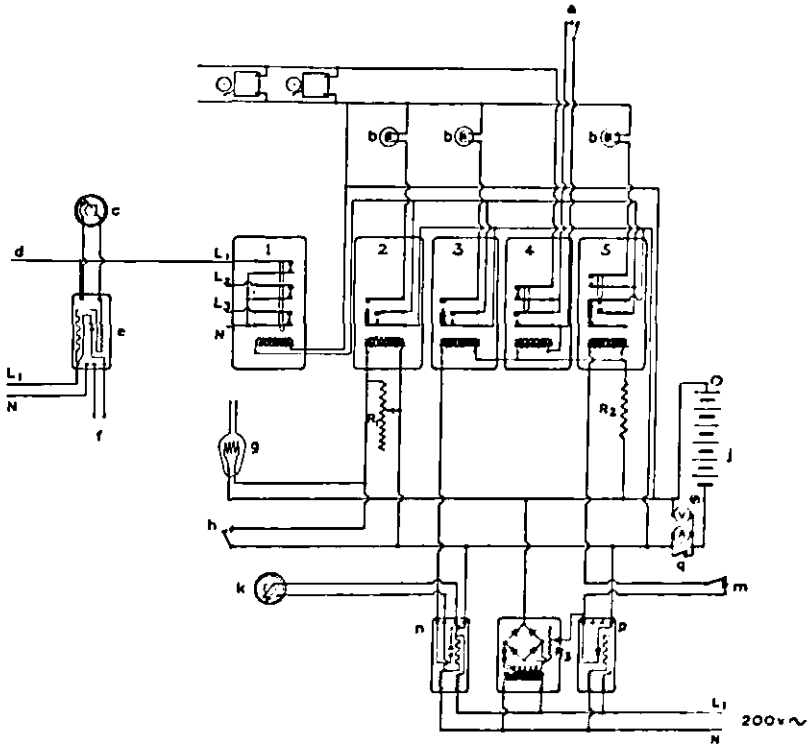
PLATE XII



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).

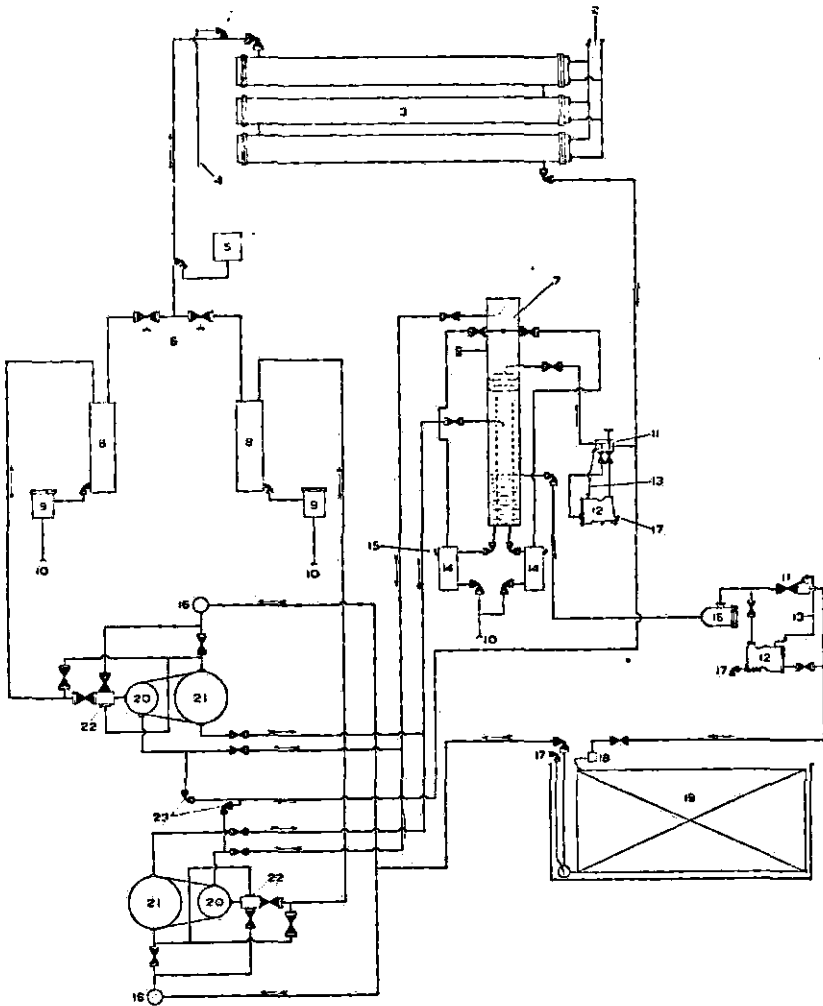
m Contacts on automatic vacuum valve.

n SUNVIC relay type F 102-5 modified.

p SUNVIC relay type F 102-5

q Push-button to read charging rate.

PLATE XIV

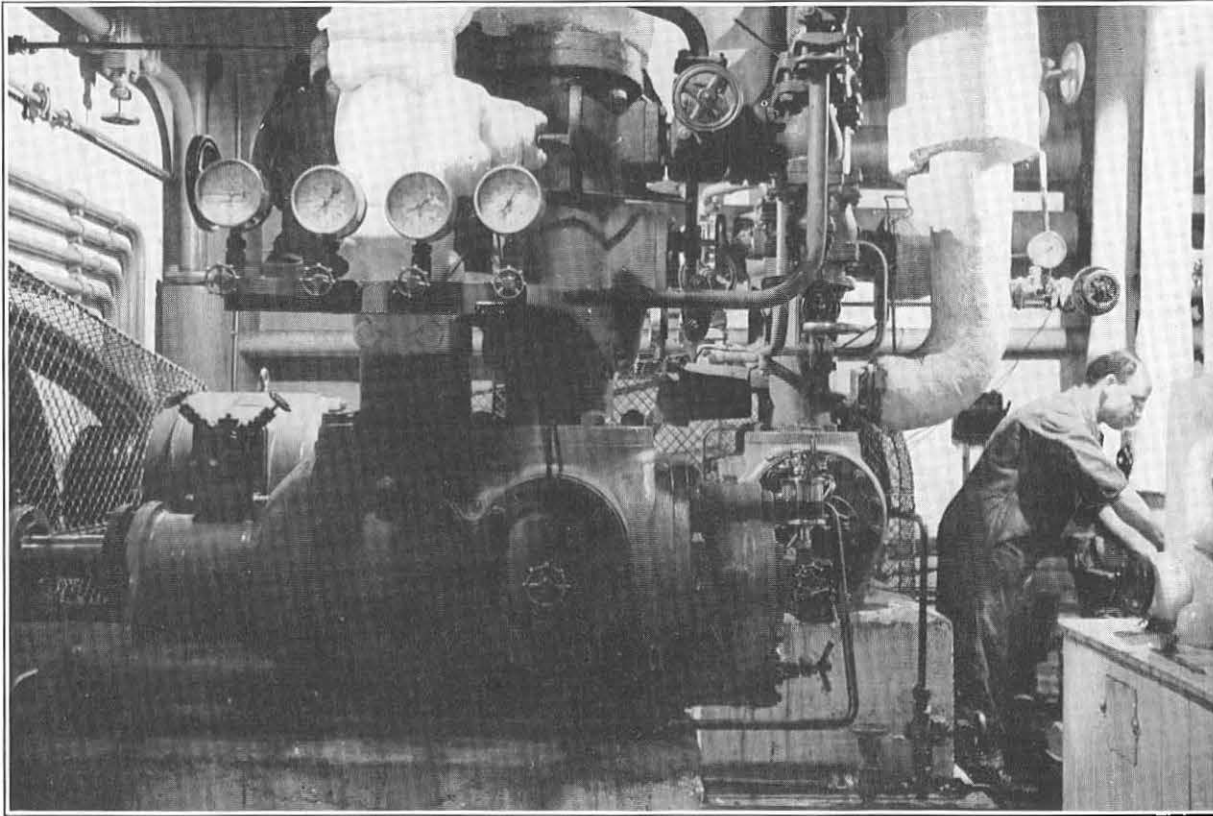


Ammonia Refrigeration Circuit in the Large Drying Plant.

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.

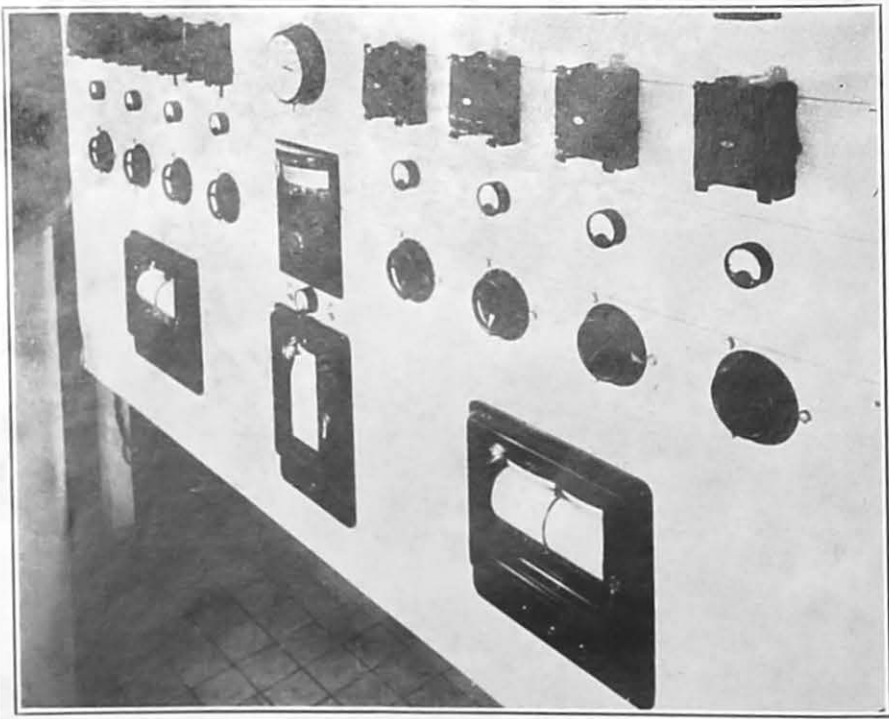
Direction of flow { $\begin{array}{l} \longrightarrow \text{ gas.} \\ \longrightarrow \text{ liquid.} \end{array}$

PLATE XV



The Large Drying Plant.
View of the engine room showing the two compounded ammonia compressors.

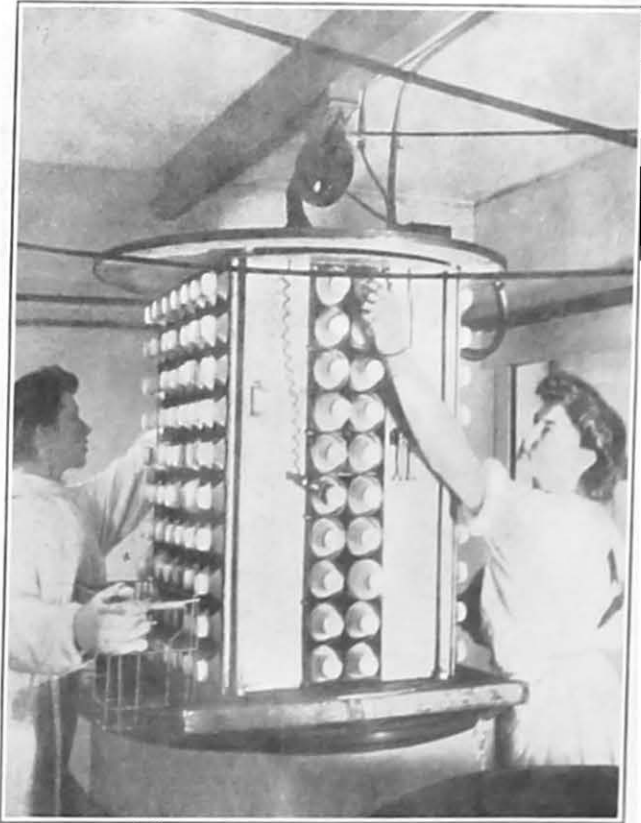
PLATE XVI



The Large Drying Plant.

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

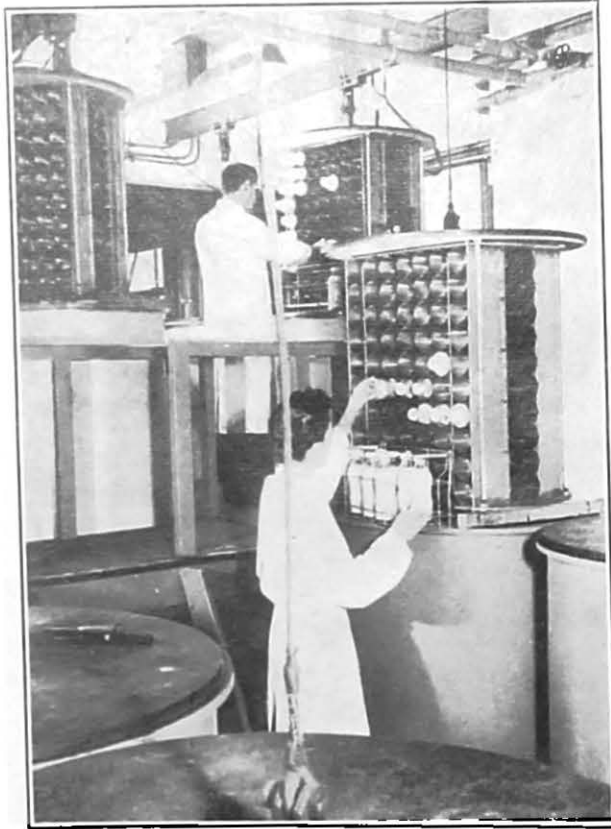
PLATE XVII



The Large Drying Plant.

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

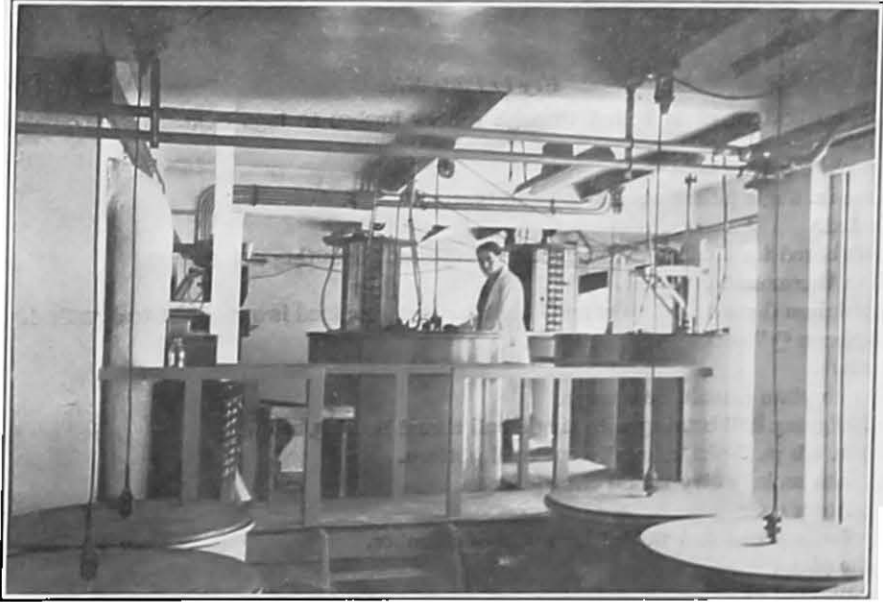
PLATE XVIII



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.

