dary bacterial invasion. Evidence both for and against this can be assembled, but perhaps the weight of argument is against this theory, because no one type of bacterial species occurred more frequently than any other. The third view is that the virus of 1918 influenza was of the same antigenic character as, for instance, virus A, but possessed unique biological properties (Burnet and Clark, 1942). Andrewes (1942) conceived of pandemic virus as being merely a more advanced grade from the virus of present experience with the added property of attacking the lung, but felt that antigenic mutation would alone explain the lethal effect of the viruses on the young adult. Burnet and Clark explain the latter phenomenon on the basis of excessive reaction of the inflammatory response to a strain of virus capable of spreading down into the alveoli.

Comparison of the properties of the various influenza A viruses encountered in the epidemics of different years certainly supports the view that virus strains of almost the same antigenic constitution can vary considerably in their virulence for experimental animals. It is a striking fact that whenever a considerable epidemic is under way no one seems to find it difficult to establish strains of virus A in the ferret, the clinical responses in the ferret are clear-cut, and passage is successful in a high proportion of instances. The viruses obtained from localized outbreaks of years such as 1939 and 1941 were by contrast difficult to transmit to ferrets, caused poor clinical reactions, and tended to die out on passage unless "nursed" along. We also know something of the variations which can be induced in the virus by simple transfer from one animal to another, so that the virus appears to be biologically plastic and capable of very considerable modification. The studies of Burnet and Bull (1943) on the changes which occur in the influenza virus immediately after isolation from human cases indicate the relative instability of the human virus and the considerable ease with which mutation can be induced. Should a strain of virus arise in nature, as a result, perhaps, of a particular combination of circumstances, which possessed both heightened power of spread and changed antigenicity, then a pandemic with particular mortality in the younger age groups might result. When all is said, however, our theories must remain unproved in the absence of opportunity of putting them to the test, and everyone must agree that ignorance is a more blessed state than knowledge born only of bitter experience of a new pandemic.

To summarize: I have tried to show how the knowledge of the virus agents active in the causation of influenza has begun to illuminate the problem of one of man's most implacable enemies. Whenever the influenza epidemics of recent years in Great Britain have been intense and widely spread they have been associated with that virus designated influenza A. Intervening epidemics have been associated both with virus A and with virus B, but a varying proportion of cases clinically similar to those associated with these viruses have remained of unknown aetiology. The clinical features of infection by the two known groups of influenza viruses have been described, and an attempt has been made to interpret the role of the virus in the pathogenesis of the human disease. The manner in which knowledge of the influenza viruses may help us to interpret pandemic influenza of the type experienced in 1918 has been briefly described, and emphasis has been placed on the variable biological properties of the different virus strains and on their power of rapid modification.

(A full list of references will be given at the end of Lecture II.)

We have received from the librarian of the U.S. Office of War Information, American Embassy (1, Grosvenor Square, W.) the printed report of a discussion on "Putting the Disabled Veteran back to Work." Those who took part included Dr. C. D. Selby, medical consultant, General Motors Corporation, and Dr. Harley L. Krieger, medical director, Ford Motor Company. The debate was held in the Mellon Institute on the occasion of the eighth annual meeting of the Industrial Hygiene Foundation, and the report is published by that body from 4400, Fifth Avenue, Pittsburgh, Pennsylvania. It forms Bulletin No. 2 of the Foundation's special series of publications.

DRYING PENICILLIN BY SUBLIMATION IN THE UNITED STATES AND CANADA

BY

EARL W. FLOSDORF, Ph.D.

*Assistant Professor of Bacteriology, University of Pennsylvania, and
Director of Research at the F. J. Stokes Machine Company*

Desiccation from the frozen state has now become well known as the result of its very wide and extensive application to the drying of blood plasma. The art itself is old, and dates back to Shackell in 1909. The subsequent history of the development of desiccation from the frozen state during the present century has been recorded in previous publications (Flosdorf and Mudd, 1935; Strumia *et al.*, 1941; Flosdorf *et al.*, 1945), and these include also reference to the work of Shackell (1909), Harris and Shackell (1911), Hammer (1911), Rogers (1914), Swift (1921), Sawyer *et al.* (1929), Elser *et al.* (1935), Reichel, Masucci, McAlpine, and Boyer (unpublished; see Flosdorf and Mudd, 1935), Reichel (1939), Greaves and Adair (1939), and Greaves (1942, 1944).

By 1935, as a result of the development made by these various workers and of our own contributions (Flosdorf and Mudd, 1935), desiccation from the frozen state was available for general production of various biological products. Hence when the war came the art had been developed and was ready for use with blood plasma; of course, under the stimulation of a project like this, the process was soon applied on a grander scale than ever before.

Earlier efforts to evaluate the possible clinical usefulness of penicillin were abandoned because its liability seemed to make it unsuited as a therapeutic agent (Clutterbuck *et al.*, 1932). However, when later research had resulted in demonstration of the effectiveness of penicillin (Chain *et al.*, 1940; Abraham *et al.*, 1941), it was natural that desiccation from the frozen state should be called upon to stabilize it; meanwhile, drying this way had undergone the necessary development. In many respects the problems with plasma and penicillin were similar and parts of equipment even identical. On the other hand, certain peculiarities in the nature of penicillin introduced problems quite different from any encountered earlier.

Actually, solutions of penicillin are not so labile that they cannot be evaporated at about room temperature without loss in potency, but they foam badly. Desiccation from the frozen state avoided this problem. This also allowed a more highly soluble product to be obtained.

On the other hand, if penicillin is to be dried while frozen, it is necessary to hold the temperature lower even than that which is required for many other biological products, including blood plasma. This is not because of the biological liability, but because of physical characteristics. Penicillin does not remain in a completely unsoftened condition unless the temperature is below about -20°C ., and preferably below -25°C .. The exact temperature varies somewhat with the degree of purification and with the concentration of the product; nevertheless, even the least exacting of preparations require a lower temperature than plasma. If penicillin is not kept at such low temperature, because of softening there is bubbling and frothing to a partial extent, and this is sufficient to introduce problems of practical control which are difficult to overcome. Consequently, it is simpler to maintain a lower temperature.

Drying in Bulk or in Ampoules and Bottles

Drying of a biological product intended for parenteral injection may be carried out either in bulk or in the final container in which the product is to be stored and distributed. However, drying in bulk has not met with general favour because of the obvious problems of maintenance of sterility in subdividing dry powder into ampoules for distribution. The problem is made more difficult because of the hygroscopic nature of dry penicillin, the size of doses requiring accurate weighing of small quantities. In spite of this, attempts were made to dry penicillin in bulk for transfer of dried powder to final ampoules, and these represent the first time this has ever been tried in routine large production of a biological product. To-day, however, the trend has returned to one of drying in

the final container as proposed in 1935 (Flosdorf and Mudd, 1935). In some cases preconcentration in bulk by partial drying from the frozen state is used with transfer of concentrated liquid to ampoules for final drying. This reduces the size of the final ampoule required, particularly where the original penicillin solution is very dilute. Concentrating in bulk in this way does not offer the same problems of sterility as are encountered by complete drying in bulk, inasmuch as a representative sample of the concentrated liquid is readily enough obtained for bacteriological culturing. If it is found sterile, then there is reasonable assurance that the entire batch is sterile, in contrast with the ineffectiveness of proper bacteriological sampling of dry powders. Improvements in the process of extraction now permit production of concentrated solutions without evaporative concentration.

If final containers such as ampoules or bottles are used for drying, close attention must be given to the necks selected, so that they are adequate in size for the escape of the vapours. These conditions have been fully described (Flosdorf and Mudd, 1935).

Freezing Conditions

There are no particular limitations with regard to the temperatures for freezing other than that they be low enough to ensure complete solidification and fast enough to avoid partial separation of concentrated penicillin. As indicated above, temperatures of -20° and -25° C. represent the maximum which can be used if an unsoftened product is to be dried.

There is a further complication involved in large-scale production of penicillin in contrast with plasma. Because of the larger bulk of material in a single bottle in the case of plasma, the contents will not thaw quite so quickly when removed from the freezing-bath. With penicillin the amounts are so small in the individual containers that this is not the case. Unless special precautions are taken there is considerable hazard of partial thawing after removal from the freezing-bath or from the frozen storage vaults. This is during the loading of the drying chambers and during evacuation to pressures that enable the frozen state to be maintained by evaporative cooling. For that reason, drying chambers of the type used widely for blood plasma (Flosdorf *et al.*, 1945) have been modified to the extent that they can be chilled to sub-freezing temperatures. The product, after being loaded into the chambers, is held in a frozen condition in this way until the proper degree of vacuum is obtained. Then the chilling medium is removed from the jackets of the chambers and the heating medium circulated at proper temperature.

A still simpler procedure has now been introduced which greatly reduces the labour of handling the bottles. The bottles containing the penicillin as liquid are introduced into the chilled drying chambers. As soon as the penicillin has been frozen, which may be within as little as half an hour, the chamber is evacuated and then heated in the usual fashion.

Vacuum Conditions for Drying

It is necessary to set up conditions of vacuum whereby most of the air is removed from the system; otherwise the drying would take an impossibly long time. Under these conditions the ice in the product evaporates directly to the vapour phase.

The water vapour which evaporates from the frozen product may be removed by a number of means, but, in large production, only two basically different methods are being used. Either the water vapour is pumped directly by means of large steam ejector pumps (Flosdorf *et al.*, 1945; Flosdorf and Stokes, 1941) or, alternatively, large refrigerated condensers are used in combination with mechanical vacuum pumps. In the latter case the condenser removes the water vapour and the mechanical pumps serve principally to keep the air out of the system. Chemical desiccants or adsorbents may also be used in place of the refrigerated condensers, but these have not been employed on a production scale for penicillin except in certain combinations with other equipment which will be discussed below.

In any event it is necessary by means of the direct pumping system or the refrigerated condenser to set up a partial pressure of water vapour below that of the penicillin at the temperature at which it is being dried. For temperatures of -20° to -25° C. this means a vapour pressure of the order of

500 to 750 μ . Consequently, the steam ejector or condenser must produce pressures below this, and, on the surface of things, one might expect that the lower the temperature produced by the condenser or the higher the degree of vacuum produced by the steam ejector the more rapidly drying would proceed. However, this is not the case. As drying proceeds the receding surface of the layer of ice within the penicillin becomes covered with a porous outer layer of dried material, which means that the vapours must escape through orifices. According to the laws of adiabatic gaseous flow through orifices—the Napier equation—a pressure of water vapour produced by the condenser or by the steam ejector which is 55% of the vapour pressure of the penicillin will result in as rapid drying as can be obtained (Flosdorf *et al.*, 1945). This we have confirmed by experiment, and it means in effect that a degree of vacuum of the order of 250 to 400 μ is adequate. This applies only to the first stage of drying from the frozen state, which is in reality a process of sublimation of the ice contained in the product. Nevertheless, this does cover the removal of most of the moisture.

However, after all of the ice has been removed from the penicillin we have the second stage of drying, which is one of removing the final traces of moisture from the dry product. The hygroscopicity or the vapour pressure of the dry penicillin varies widely with the preparation in question and depends, among other things, on the degree of purification and concentration. This vapour pressure of the final dry product determines the final degree of vacuum which is required; generally it has been found that from 50 to 100 μ is sufficient.

One procedure which has met with some favour is to use steam ejectors or refrigerated condensers in the first stage of drying for removal of the bulk of the water vapour from the frozen penicillin solution. This affords the most economical way of carrying out the process, since a vacuum of no better than 250 μ is required. To complete the drying, a chemical desiccant is used. By choice of the proper one, partial pressures of water vapour as low as a fraction of a micron are readily obtainable, and make it possible to reduce the final content of moisture in penicillin to as low a level as needed (Flosdorf *et al.*, 1945). Inasmuch as the amount of actual water to be taken up by the desiccant in this second stage is small, weights of desiccant which are reasonable will last for a long time before replacement or regeneration is necessary.

As an aid in reduction to proper final content of moisture the temperature of the penicillin may be raised towards the end of drying to increase the vapour pressure. This in turn raises the pressure which it is possible to use for obtaining final dryness. Although originally it had not been considered safe to take the final dry penicillin to above 40° C., there seems now to be evidence that the final product may be taken to considerably higher temperatures—even as high as 110° C. This, of course, simplifies the problem of obtaining low final moisture.

Final Degree of Moisture and Stability

Although exhaustive data are not yet available which relate the stability of penicillin at various temperatures to the actual content of moisture in the dry product (among other things, it varies with the degree of purification), it is generally agreed that the lower the content of moisture the greater the stability. There is considerable difference of opinion as to just what is the critical level, in some quarters this being placed as high as 7%. In the United States it is required by regulation that the product be reduced to below 2.5%, and there is some evidence that a final content of moisture of 0.5% is necessary for maximal stability.

Sealing of the Final Container

Obviously, if it is important to bring the content of moisture in the penicillin to a low point in the first place, it should be maintained low. A glass-sealed ampoule is always of first choice for a hermetically sealed container, but it does offer some difficulty in the clinical use of the material. For this reason bottles sealed with rubber stoppers have met with favour. When rubber-sealed bottles are used, however, it is necessary to relate the length of time the penicillin remains fully potent to the effectiveness of means of protection of the rubber stopper. Rubber and synthetic products are permeable

to moisture. In the case of products like plasma this is not significant, but with others which have a low content of solids it is highly important to give consideration to the amount of moisture going through the stopper.

For example, compare a 10-ml. amount of serum with a 300-ml. amount of plasma. If, under certain conditions of long storage, the 10 ml. of serum will be taken up to 8% moisture content, the 300-ml. amount of plasma will have its moisture content increased only by one-thirtieth of this—by about 0.27%. The reason is that there are 30 times as much solid matter by weight in the plasma container as in the small serum container, yet the amount of moisture permeating the stopper is the same. With penicillin, where the amount of solids may be only one-fifth as great as even in the 10-ml. quantity of serum, the moisture content could go as high as 40% (Flosdorf *et al.*, 1945).

Other Methods of Drying

As indicated above, the stability of penicillin is such that it may be evaporated as a liquid at room temperature without undue loss of potency. Recently, pre-concentrating dilute penicillin solution in bulk by means of dielectric heating in low-vacuum apparatus has been carried out successfully. This reduces appreciably the load on the equipment for final desiccation from the frozen state, particularly if the original penicillin solutions are quite dilute. After pre-concentrating in bulk in this fashion, the liquid product is distributed to phials in which the concentrated penicillin is frozen and then dried by sublimation.

It may be that before long it will be possible to dry the concentrated solutions of penicillin as a second step by means of dielectric heating on a practicable basis in the final ampoules. The reduction to a final moisture content of 0.5% must be accomplished as a third step in which a warm bath is used to heat, since the dielectric means become ineffective on the dry penicillin. The final product is less attractive in appearance. Solubility is slower, but fast enough for the purpose. The matter will then resolve itself simply into one of economy in handling a large number of ampoules in the second and third steps required with dielectric heating as compared with drying by sublimation.

Summary

Problems met in drying penicillin in contrast with those with blood plasma and other products are discussed. Procedures used in large production are described. Final moisture requirements and types of containers suitable for penicillin are discussed. Use of dielectric heating of penicillin is considered.

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The Research Board for the Correlation of Medical Science and Physical Education, which was formed in 1943 under the chairmanship of Brig. F. Howitt, has issued a first interim report. The material is arranged in four chapters, on maternity and child welfare, education and recreation, the Services (the normal recruit, the sub-standard recruit, and the sick, injured, and disabled), and a note on the work of the Industry Subcommittee by Brig. F. A. E. Crew. There is a foreword by the chairman, and the report concludes with some recommendations. Copies may be obtained from the Ling Physical Education Association, Hamilton House, Bidborough Street, W.C.1, price 2s. (postage 3d.).

GAS GANGRENE AFTER SECONDARY SUTURE OF SHELL WOUND OF THIGH

BY

J. HAMILTON BARCLAY, M.D., M.S., F.R.C.S.

Senior Honorary Surgeon, Royal Victoria Infirmary, Newcastle-upon-Tyne; Professor of Surgery, University of Durham

I am led to report this case for two reasons: (1) the fact that the gas gangrene followed secondary suture of the shell wound of the thigh; and (2) the fact that the patient recovered after apparently being moribund.

The former occurrence was most disconcerting, as the patient was very fit before the operation and, clinically, the wound looked healthy and suitable for suture and the swab was sterile. Many cases of gunshot wounds had been submitted to secondary suture, and this was the only one which caused any anxiety—all the others were successful. But it is important to record failures or accidents as well as successes. And, as Sir Ernest Rock Carling, with whom I have been in communication, remarks, "one accident does not vitiate the principle, and secondary suture is extremely important." It emphasizes the necessity for taking swabs not only from the surface but from the recesses of the wound. Apparently the organisms had been lurking just beneath the surface.

With regard to the latter occurrence, I have never in all my experience seen a patient so ill recover. I asked Prof. F. C. Pybus to see him with me, and we both agreed there was no chance of recovery. It shows that "where there is life there is hope."

Case History

On June 23, 1944, Capt. X., aged 30, and the crews of two tanks were resting beside their vehicles when a German shell burst near them, wounding seven men, including the captain. Assisted by another officer, he dragged the wounded men into slit-trenches, which were dug in the well-cultivated orchard of a French peasant. Capt. X. forgot to apply his field dressing to his wound, which became contaminated with soil. An hour after the bursting of the shell he was admitted to the regimental aid post with a lacerated wound of the skin and muscles of the right thigh, on its anterior aspect, the wound being about 4½ in. long by 2 in. wide. At No. 3 Casualty Clearing Station later that day the wound was found to be widely open but not suitable for surgical treatment, so it was dressed with sulphanilamide powder and vaselined gauze, and a back splint was applied. Two days later, in hospital, the splint was removed, as it was causing pain, and the wound was re-dressed. The patient's general condition was very good. Between the 23rd and 29th he received 3 c.cm. of A.T.S. and 20 g. of sulphanilamide.

On the 29th he was admitted to the Royal Victoria Infirmary, Newcastle-upon-Tyne, under my care. His general condition was still good. The wound of the thigh was rather dirty and putrid, and of a greenish colour. A swab was taken from the wound, and this produced cultures of mixed penicillin-sensitive organisms. Two days later another swab showed streptococci suitable for penicillin treatment. The patient complained of pain on micturition and frequency but no discharge.

On July 6 the wound was cleaner, and not everted so much. There was a very scanty colourless exudate. Dressing was done with penicillin powder and tulle gras, and the wound became cleaner and healthy-looking. A swab showed it to be sterile, so it was decided to carry out secondary suture. This was done on July 14, the edges being undermined and the skin approximated with silkworm-gut sutures. Proflavine and sulphanilamide powder were dusted into the wound. Next day there was a slight amount of serum oozing from the lower part of the wound.

On the 16th there was slight tenderness round the wound; some blood-clot was oozing, and this reappeared when cleared away. His pulse was 120, his temperature normal, and he was fairly comfortable. Some sutures were removed. By the next day a large amount of clot was escaping from the wound. The remaining sutures were removed and masses of foul-smelling clot evacuated. There was oedema over the knee and thigh, and the leg was very tender. His pulse was 108 and temperature 99° F. He was in pain and looked ill. A few bubbles of gas escaped from the wound. An intramuscular penicillin drip was set up in the left thigh. Sulphathiazole was given, together with 12,000 units of anti-*Cl.-welchii* serum, intravenously. That evening 22,500 units of polyvalent serum were administered. A blood drip was started at 09.30 hours, but by 10.30 hours a severe reaction to the A.G.G.S. took place, characterized by respiratory distress, vomiting, and urticaria. The blood drip was stopped as a precaution. The patient was gravely ill the whole day. A swab showed the presence of clostridia other than *welchii*, with sensitive *Staph. albus* and resistant faecal streptococci.