Sterilization by dry heat¹

E. M. DARMADY, K. E. A. HUGHES, J. D. JONES, D. PRINCE, AND WINIFRED TUKE

From the Portsmouth and Isle of Wight Area Pathological Service

SYNOPSIS The advantages and disadvantages of three forms of dry heat sterilization are discussed. In addition a fourth method, consisting of heating by infrared rays *in vacuo*, is described. This method is particularly suitable for instruments used in the operating theatre, since it can replace an autoclave where a supply of steam is not available. Recommended times and temperatures for dry heat sterilization are detailed, and are related to the thermal death point of *Cl. tetani*. The dangers of recontamination during the cooling process are discussed.

Sterilization by dry heat has become increasingly popular in Great Britain. This is probably for two reasons: first, the need to sterilize small objects, particularly syringes, and secondly, the apparatus required is comparatively inexpensive when compared with the autoclave. It should also be added that shortly after the last war autoclaves were in short supply, and a number of people became increasingly interested in dry heat sterilization. Before considering the apparatus available it is as well to discuss the advantages and disadvantages of the method. It must be emphasized that sterilization by dry heat depends upon the penetration of adequate heat to the article as a whole; thus it is possible to sterilize such objects as syringes already assembled and presealed in their container, whereas steam and gases, such as ethylene oxide, can only be relied upon to kill organisms if the steam or gas comes into direct contact with the surface of the objects. Steam, therefore, is not suitable for such equipment as assembled syringes or for articles enclosed in a sealed tube. Another advantage of this method is that objects which are damaged by water or steam, e.g., powder or ointment, can be sterilized provided the heat penetrates to all parts of the substances. The disadvantages of the method are that, first, some of the apparatus, such as hot air ovens, takes a considerable time to reach sterilizing temperature, and the temperature and time necessary for sterilization must be considerably higher and longer than for steam. Secondly, much of the apparatus in use is unreliable and shows considerable variation in temperature during sterilization. Thirdly, the objects, particularly metal, may become oxidized at high temperatures or they may not withstand the temperature.

APPARATUS

The apparatus necessary to provide dry heat for sterilization can be considered under four headings. First, the hot air oven; second, the conveyor oven; third, conducted heat; and fourth, dry heat in the presence of a vacuum. It is proposed to examine each of these methods more critically.

1 HOT AIR OVEN Hitherto the most popular method for dry-heat sterilization has been the hot air oven. However the limitations of this apparatus have already been fully discussed (Ewald and Schmid, 1953; Darmady and Brock, 1954). These investigations showed quite clearly that a number of hot air ovens examined could not be relied upon to provide an even temperature throughout the oven, and indeed might not reach the temperature recorded by the thermometer. Thermocouples placed in syringes showed that there might well be a variation in temperature of 30 or 40°C. (Fig. 1). Investigations also showed that the time taken to reach the sterilizing temperature could be reduced if the oven was allowed to heat up first, and, if this was done, the temperature variation in the articles to be sterilized was not so great. Furthermore, it was shown that the provision of a fan shortened the time taken to reach sterilizing temperature and that the forced circulation of air ensured that the variation was reduced to a minimum (Fig. 2). Nevertheless, in spite of the improvement obtained by having a properly designed oven with a fan, the methods of loading were important, for it was found that unless objects were loosely packed, there would be a delay in heat penetrating to the centre of the load. It is essential, therefore, that every oven should be tested with thermocouples to determine the time taken for larger objects, such as petri dishes in copper canisters or containers holding vaseline, to reach the sterilizing temperature. Recently the British Standards Institution has laid down criteria for hot air sterilizing ovens. The specifications include the need to test the oven with a predetermined load, and that the temperature variation shall not exceed 5°C. at any time within 60 minutes after the heating-up period. Such ovens

¹From a lecture given by one of us (E.M.D.) to the School of Pharmacy, Chelsea College of Science and Technology, London, on 2 February 1960.

FIG. 1. *Temperature recordings in syringes sealed in aluminium containers in a gas-heated oven without a fan heated to 160°C. and loaded hot. Note that the variation of temperature was 40°C.*

FIG. 2. *Thermocouple recordings in syringes sealed as before in an electrically-heated oven fitted with a fan. Curves 13 and 18 denote tightly-packed syringes. With the exception of these syringes the overall variation was not more than 5°C. from 160°C.*

are particularly necessary for the sterilization of instruments or syringes where it is imperative that the overall variation of temperature should be reduced to a minimum. The principal disadvantage of the hot air oven is the time taken by the load to reach the sterilizing temperature and the danger that articles can be removed from the oven before the process is complete.

2 CONVEYOR OVEN The design and performance of the conveyor oven has been described earlier (Darmady, Hughes, and Tuke, 1957; Russell and Abdurahman, 1960). This apparatus consists of an insulated tunnel in which a series of 'infrared' heaters has been placed at predetermined positions. A metal moving belt carries articles to be sterilized. The advantages of the apparatus are, first, that it enables a continuous flow of work to be produced, and is therefore applicable to a syringe service which is processing at least 600 syringes a day. Secondly, articles of standard size receive the same heat treatment for the same length of time. Thirdly, because of the 'infrared' type of heaters used, heating up is reduced to the shortest possible time. Fourthly, the machine is simple in design and there is virtually nothing to go wrong, since the operator merely places the objects on the moving belt, and once it enters the tunnel there is no possibility of interference. It is, however, important to remember that the machine has been designed to sterilize a fixed standard load, and articles submitted to it will receive an equal amount of heat. Both the mass of the syringe and the surface of the outer container have a direct bearing on the amount of heat absorbed by the article to be sterilized. Thus when a 2 ml. syringe was placed in a glass test-tube the syringe reached a temperature of 180°C. in less than two minutes, whereas if the same size of syringe were placed in a polished aluminium container the time taken would be eight minutes. Again, if a large 20 ml. syringe were submitted to the same heat source and placed in a similar container the centre of the syringe would take a longer time to attain the required temperature than those of the smaller size. It is, therefore, possible that by treating the outer surface of the container in an appropriate way all the syringes will receive the same treatment for the same length of time. To ensure this in the Portsmouth Syringe Service, 2 and 5 ml. syringes are sterilized in a grey anodized aluminium container and the 10 and 20 ml. syringes are sterilized in a black anodized container. It is apparent that by altering the surface and colour of the container many other articles could be sterilized, provided the overall load is not exceeded. For example, four dressing forceps placed in a grey anodized 20 ml. container will reach sterilizing time at the same moment as a 20 ml. syringe in a black anodized container. Care should, however, be taken to ensure that the temperature achieved does not exceed 210°C. as the instruments may become oxidized during the cooling process. Examples of temperature recordings are shown in Fig. 3.

With these machines it is important that the voltage remain constant and they should all be fitted with a voltage stabilizer, since it has been shown that the temperature will vary by the square of the voltage variation. Similarly, the heat uptake in the syringes or instruments

FIG. 3. A three-dimensional graph showing the heat taken up of four syringes of different sizes and instruments in their containers during their passage through a conveyor oven. The hatched areas denote the time and level of temperature exceeding 180°C.; 2 and 5 ml. syringes were placed in dulled aluminium containers and the 10 and 20 ml. syringes in black anodized containers. The dotted line denotes instruments placed in polished aluminium containers, and should be compared with those in grey anodized containers.

will vary by the square of the distance from the heat source. The advantage of these machines is that they are mechanically sound and there are few parts to go wrong. In practice the elements, which are continuous strips, last for long periods of time, and indeed the machine used in the Portsmouth Syringe Service has only required new elements once during its life of six years. The only other mechanical failure that is likely to occur is in the electric motor used for the moving belt. The principal disadvantage of this machine is that the load cannot be varied and it is not suitable for the sterilization of instruments for the theatre, since the numbers and types will differ.

(3) CONDUCTED HEAT. In a previous communication, Darmady, Hughes, Jones, and Tuke (1958) described experiments with an apparatus suitable for conducted heat sterilization. This consisted of a thermostatically controlled hot plate on to which an aluminium block had been fixed. Holes were bored to take six 2 ml., two 5 ml., and one 10 ml. Nuffield containers. Obviously these holes can be varied to suit individual needs. Cutting instruments were placed on a flat tray fitted with a lid on the top of the block. It was covered by a hinged, resin-bonded, fibre-glass insulating cover. Investigations showed that the temperature variation when tested by thermocouples was

FIG. 4. A thermostatically controlled hot plate with an aluminium block used for the sterilization of small numbers of instruments and syringes in their containers.

$\pm 1^\circ\text{C}$. and so provided the most accurate form of controlled heat that has yet been found (Fig. 4). In the apparatus described, the trays were placed on top of the hot plate, and it was shown that instruments laid flat in them could easily be sterilized. This form of apparatus is particularly suitable for those who wish to provide heat at a precise temperature or to sterilize a small number of instruments or syringes. To save time it was found that it was better to set the aluminium hot plate at a standard temperature (190°C .) and maintain it continuously. In the machine described it was found that a syringe in its container could be sterilized in 22 minutes. Since then, further experiments have shown that the container need not fit the bore holes accurately, and will be adequately sterilized in the same time.

HIGH-VACUUM, INFRARED STERILIZER

The three types of apparatus previously described have not proved suitable for sterilizing operating instruments, first because the hot air oven takes time to reach the required temperature and the working space is usually restricted; secondly, because the conveyor oven is not suitable for varying loads and may cause oxidation of the instruments, and thirdly because the capacity of conducted heat equipment is strictly limited. Although steam at high pressure is suitable for the sterilization of instruments, a survey of the hospitals in the Wessex Regional Hospital Board area showed that about one-third were not equipped with a steam supply, and that designers of modern hospitals, particularly in tropical countries, were anxious, since central heating was not required,

not to include one. One architect stated that to equip a hospital with a special steam supply to the theatres might cost as much as £75,000.

It was therefore decided to try and devise equipment which would be capable of sterilizing instruments by dry heat using electrical power. Such a piece of apparatus has now been developed.

It consists of a double-ended rectangular chamber measuring 30 in. $\times$ 14 in. $\times$ 5½ in. (clear) fitted with a tray capable of carrying on average 70 instruments and two stainless steel bowls 14 in. in diameter. The roof and floor of the sterilizer are fitted with four infrared heaters of 1 kW. and four of 0.5 kW. respectively (eight heaters in all). The cycle, which is completely automatic, is as follows:—

1. After loading the chamber the doors are closed, and the starter switch engaged. To prevent accidental contact the switch has to be held for at least 10 seconds.

2. A vacuum pump now exhausts the chamber to a pressure of 1 or 2 mm. Hg absolute pressure. As soon as this is reached, and if it is obtained (usually in three and a half minutes), the heating elements are switched on, the vacuum maintained, and the heat applied. The temperature of the chamber was found to reach a maximum of 280°C .

3. At the end of the predetermined time of heat treatment, the heaters are turned off and the vacuum broken by refilling with nitrogen gas through a filter from a cylinder. This fulfils a twofold function: first it ensures that the instruments are not oxidized during the cooling cycle and secondly it helps to cool the instruments so that they can be handled with safety. In one model, a further vacuum was drawn and again replaced by nitrogen. This, however, did not greatly improve the cooling.

In order to determine the efficiency of the machine and to provide a satisfactory time cycle, a number of investigations were carried out. The method of testing the apparatus was as follows:—

EXPERIMENT 1 The tray was fully loaded with at least 70 stainless steel instruments or syringes. A number of these were smeared with either dried soil suspended in serum and allowed to dry at room temperature or with *Cl. tetani* spores suspended in broth dried in a similar manner. Many of the instruments were so arranged that infected instruments were shielded from direct radiation from the heaters. In the first machine heaters were provided only on the roof of the chamber, and although the cycle was exactly as described, the heat treatment was applied only for two to three minutes.

In six consecutive experiments carried out over a number of days on 61 instruments and nine syringes infected with soil, only 10 and eight respectively proved to be sterile; of 12 syringes and slides infected with *Cl. tetani*, N.C.T.C. 5411, all proved to be sterile.

EXPERIMENT 2 The machine was modified so that four heaters were placed on the roof and four on the floor of the chamber. Heat treatment was applied for two to three

FIG. 5. The temperature recordings of a typical cycle of the high-vacuum, infrared sterilizer.

minutes as in the previous experiments, and the instruments were loaded as before. Out of the 18 infected instruments with spore-bearing soil only 11 proved to be sterile.

EXPERIMENT 3 For this investigation the same machine as described in experiment 2 was used, but the heat treatment was increased to seven minutes. All the 100 instruments infected with *Cl. tetani* proved to be sterile, although one out of 12 infected with soil gave a positive culture.

EXPERIMENTS 4 AND 5 The same machine was used, but the heat treatment was reduced to four and six minutes respectively. With the cycle set at four minutes, 144 out of 153 tests using *Cl. tetani* and at six minutes, 35 out of 37 using dried soil, proved to be sterile. For this reason it was decided that it was necessary to provide the extra safety margin and therefore the time cycle should include heat treatment for at least seven minutes, making a total cycle of 15 minutes. Temperature readings are shown of a typical run in Fig. 5. Unfortunately it was not possible to obtain temperature recordings of the instruments *in situ*. However, it was clear from our investigations that

using the time cycle recommended the surface heat was more than adequate to ensure complete sterility.

The repeated heat treatment has so far not caused properly made stainless steel instruments conforming to B.S.I. standards to deteriorate, although some of the instruments have been exposed on 100 or more occasions.

The makers claim that the advantages of this machine are that the provision of a vacuum of 2 mm. Hg absolute during the heating cycle prevents first, the convection of currents normally found in an hot air oven and responsible for heat variation in the chamber; second, a build-up of pressure within the chamber so that the chamber and doors need not be built to withstand it; third, oxidation of the instruments. Furthermore, the heating process is accelerated, since heat is not taken up by the air. Throughout the experiments care was taken to provide varying loads of instruments, and the infected instruments were placed in such positions that they were shielded from direct radiation. It is apparent that many of the smaller articles will reach the required temperature more quickly than larger articles, but as the temperature of the heaters is constant, the danger of overheating is minimal. From the experience gained it seems that this apparatus would be suitable as a sterilizer when high pressure steam is not available, and has the advantage that the time of the sterilizing cycle is not greatly increased over that of the high-steam sterilizers on the market at present.

BACTERIOLOGICAL CRITERIA

At the time of the introduction of the conveyor oven, the normally accepted standard for sterilization by dry heat was a holding time of one hour at 160°C. However, a survey of the literature (Darmady and Brock, 1954) showed that the recommendations varied considerably (Table I), and it was decided to investigate the problem, since it was clearly not practicable to provide a conveyor belt to maintain a sterilizing holding time for one hour at 160°C. It was therefore considered vital to sterilize the instruments at a higher temperature for shorter periods of time. For the purpose of this investigation, the thermostatically controlled hot plate fitted with an aluminium block was used, as has been described earlier and in detail elsewhere by Darmady, Hughes, Jones, and Tuke (1958).

Before each test, the thermostat was set at the desired temperature, and checked with thermocouples. The test organisms were dried on glass slides and placed in small Pyrex test-tubes plugged with cottonwool. The tubes were placed in the holes of the

TABLE I

TEMPERATURE LEVELS FOR HOT-AIR OVENS

Name	Hot-air Sterilizer
Bigger, J. W. (1949)	160°C. for 1 hour 140°C. for 3 hours 150°C. for 1 hour
British Pharmaceutical Codex (1949)	
British Veterinary Codex (1953)	150°C. for 1 hour
Burrows, W. (1949)	170°C. for 2 hours
American Public Health Association (1950)	160°C.–180°C. for 2 hours
Dubos, R. J. (1948)	160°C. for 90 minutes
Fairbrother, R. W. (1953)	160°C. for $\frac{1}{2}$ –1 hour
Gerhards, G. A. (1952)	140°C. for 1 hour (electric fan oven)
Gradwohl, R. B. H. (1948)	170°C. for 2 hours (gas oven)
M.R.C. Memorandum No. 15 (1945)	160°C. for 1 hour from cold
Mackie, T. J., and McCartney, J. E. (1953)	160°C. for 1 hour 180°C. for $\frac{1}{2}$ hour
McCulloch, E. C. (1945)	160°C. for 1 hour
Smith, D. T., and Martin, D. S. (1948)	150°C.–160°C. for 1 hour
Stitt, E. R., Clough, P. W., and Branham, S. E. (1948)	160°C. for 90 minutes (syringes must be separated)
Walter, C. W. (1948)	160°C. for 1 hour if instruments are clean 160°C. for 4 hours if oily or greasy
Whitby, L. E., and Hynes, M. (1951)	160°C. for 1 hour
Wilson, G. S., and Miles, A. A. (1946)	140°C.–160°C. for 1–1 $\frac{1}{2}$ hours 400°C. for 20–30 seconds
Weedon, B. (1941)	320°F. for 1 hour (electric oven without fan) (160°C.)

aluminium block. The time taken to reach the desired sterilizing temperature was found on each occasion to be nine minutes, the test tube then received heat treatment for varying lengths of time.

In all 1,472 tests were made and three groups of organisms used, the results being compared with those obtained from the literature (Darmady, Hughes and Jones, 1958). Although dried soil was found to be most resistant, it was thought more reasonable to relate any recommendation for time and temperature to the behaviour of *Cl. tetani*, which, in our hands, proved to be the most resistant of all pathogens to dry heat. The results showed that although two strains of non-toxigenic *Cl. tetani* (N.C.T.C. 5411 and 5413) were used, there was no difference between them. Four hundred and seventy-four tests showed that sterility was attained by exposure to 180°C. for one minute, to 170°C. for five minutes, to 160°C. for 12 minutes, and to 150°C. for 30 minutes. For this reason it was decided to base a safety margin on the results with *Cl. tetani* and that this margin should be one and a half times that which is needed to kill the organisms.

However, as the heat of objects placed in a hot air oven may vary as much as 5°C. it was thought that an added safety margin was imperative. Thus it was recommended that the holding time for the selected sterilizing temperature should be one and a half times

that required to kill all organisms at a temperature 10°C. lower than the temperature selected. For example, if the instruments were to be sterilized at 170°C. the holding time, exclusive of heating-up time, should be 18 minutes, or one and a half times the minimum lethal time (12 minutes) for *Cl. tetani* at 160°C.

Although the ability to kill a selected organism may be regarded as a satisfactory method of testing the efficiency of dry-heat sterilizing, there is a need to provide a visual and easy check for those employed in a central sterilizing service. For this purpose the Browne tubes III and IV have proved to be satisfactory, and a comparison is shown in Table II which sets out the recommended sterilizing times and the time taken for the Browne tubes III and IV to change colour. For the conveyor oven Browne's tubes No. IV are recommended as they more nearly conform to the recommended times and temperatures as set out in Table II.

TABLE II

Temperature	Time Recommended (min.)	Colour Change in Minutes	
		Browne Tube III	Browne Tube IV
160°C.	45	51–61	42–51
170°C.	18	26–31	21–33
180°C.	7 $\frac{1}{2}$	17–22	13–19
190°C.	1 $\frac{1}{2}$	7–11	4–10

¹ The first figure in each column denotes the time taken for the first tube listed and the second figure the last tube of 10 to change colour

RECONTAMINATION

Recently, Russell and Abdurahman (1960) have drawn attention to the possible risk of recontamination of syringes during the cooling process immediately after sterilization in the conveyor oven.

They have shown, for example, that a 20 ml. syringe container can aspirate 70 ml. of unsterile air while cooling, thus drawing attention to the absolute necessity for adequate sealing of the containers. It is equally clear that this kind of recontamination might occur with other kinds of dry-heat sterilized equipment. In order to test the possibility of this recontamination, the following experiment was undertaken:—

Four sizes of containers were loaded with syringes and a piece of filter paper placed on top of the plungers. They were sealed with aluminium foil capsules and sterilized in the conveyor oven in the usual way. Other containers were processed in a similar way, but before being put into the oven the capsules were perforated by a needle prick. As soon as they emerged from the oven they were all placed in a container containing an aerosol of *Serratia marcescens* and allowed to cool. The seals were then removed and cultures made of the filter papers. Positive

cultures were obtained only from those syringes in which the cap had been deliberately punctured. In a further experiment, an inexperienced operator was asked to seal the containers, and they were sterilized and allowed to cool in a similar manner and once again positive cultures were obtained. However, those sealed in an automatic machine giving a fixed pressure or by an experienced operator ensured that recontamination did not occur. Russell and Abdurahman (1960) have suggested that although the possibility of recontamination is slight this can be overcome by placing a piece of cottonwool over the top of the syringe and sealing into position. This, however, is not desirable as small fibres may become jammed between the plunger and barrel causing the well-known horse shoe fracture of the barrel. This is particularly liable to occur in syringes that have been lubricated. Alternatively, these authors have suggested that cooling under high-intensity ultraviolet light would prevent recontamination.

Our thanks are due to Mr. M. Welling, of Messrs. Vacwell Ltd., for his help and advice during the development of a high-vacuum infrared sterilizer, and to Mr. P. J. Robinson, of Messrs. Prior and Howard, for the loan of apparatus. We are also grateful to the Wessex Regional Hospital Research Committee for a research grant, and we are indebted to Mr. G. Sykes, for suggesting the use of a three-dimensional graph.

REFERENCES

- American Public Health Association (1950). *Diagnostic Procedures and Reagents*, 3rd ed. New York.
- Bigger, J. W. (1949). *Handbook of Bacteriology*, 6th ed. Baillière, Tindall & Cox, London.
- British Pharmaceutical Codex (1949). Pharmaceutical Press, London.
- British Veterinary Codex (1953). Pharmaceutical Press, London.
- Burrows, W. (1949). *Jordan-Burrows Textbook of Bacteriology*, 15th ed. Saunders, Philadelphia.
- Darmady, E. M., and Brock, R. B. (1954). *J. clin. Path.*, 7, 290.
- , Hughes, K. E. A., and Jones, J. D. (1958). *Lancet*, 2, 766.
- , —, —, and Tuke, W. (1958). *Ibid.*, 2, 769.
- , —, and Tuke, W. (1957). *J. clin. Path.*, 10, 291.
- Dubos, R. J. (1948). *Bacterial and Mycotic Infections of Man*. Lippincott, Philadelphia.
- Ewald, H., and Schmid, W. (1953). *Öff. Gesundh.-Dienst.*, 15, 159.
- Fairbrother, R. W. (1953). *A Textbook of Bacteriology*, 7th ed. Heinemann, London.
- Gerhards, G. A. (1952). *Arch. Hyg. (Berl.)*, 136, 547.
- Gradwohl, R. B. H. (1948). *Clinical Laboratory Methods and Diagnosis*, 4th ed. Mosby, St. Louis.
- Mackie, T. J., and McCartney, J. E. (1953). *Handbook of Practical Bacteriology*, 9th ed. Livingstone, Edinburgh.
- McCulloch, E. C. (1945). *Disinfection and Sterilization*, 2nd ed. Lea and Febiger, Philadelphia.
- Medical Research Council (1945). War Memorandum No. 15. H.M. Stationery Office, London.
- Russell, F., and Abdurahman, R. (1960). *Scot. med. J.*, 5, 213.
- Smith, D. T., and Martin, D. S. (1948). *Zinsser's Text Book of Bacteriology*, 9th ed. Appleton Century Crofts, New York.
- Stitt, E. R., Clough, P. W., and Branham, S. E. (1948). *Practical Bacteriology, Hematology and Parasitology*, 10th ed. Blakiston, Philadelphia.
- Walter, C. W. (1948). *The Aseptic Treatment of Wounds*. Macmillan, London.
- Weedon, B. (1941). *Text Book of Sterilization*, 2nd ed. American Sterilizer Co., Erie, Pa.
- Whitby, L. and Hynes, M. (1951). *Medical Bacteriology*, 5th ed. Churchill, London.
- Wilson, G. S., and Miles, A. A. (1946). *Topley and Wilson's Principles of Bacteriology and Immunity*, 3rd ed. Arnold, London.