It is reasonable to conclude that, although diffusing factor is produced by micro-organisms during their growth and liberation of diffusing factor is associated with growth, its production is independent of the rate of toxin production.

In the *Staph. aureus* groups we have so far found that only toxigenic strains produce diffusing factor, but that the production of toxin and of diffusing activity are independent seems clear from the Chart. The two properties of the staphylococcus culture are also differently affected by heat and formalin, as shown by the experiments summarized in Table III.

Heating staphylococcal diffusing factor for thirty minutes at 45° C. destroys the activity, while heating at 55° C. destroys it in only ten minutes. At those temperatures staphylococcus toxin is but slightly affected, as shown by the α -haemolysin titre. Formalin at 0.3% concentration and room temperature does not affect the diffusing factor of staphylococcus cultures, but at that concentration slightly accelerates the inactivation of the factor at 37° C. The effect of the two factors, formalin and heat, was examined by McClean (1936) on diffusing factor from *Cl. welchii*, with similar results.

TABLE III

Description	Mean Bleb Area (3) in sq. cm.	Titre of α -haemolysin
(1) <i>Staph. toxin</i> kept in refrigerator ..	6.60	1/512 C
(2) <i>Staph. toxin</i> + 0.3% formalin kept in refrigerator ..	6.23	1/128 TR; 1/16 AC
(3) <i>Staph. toxin</i> at room temperature ..	7.30	1/512 C
(4) <i>Staph. toxin</i> + 0.3% formalin at room temperature ..	6.47	1/64 TR; 1/2 AC
(5) <i>Staph. toxin</i> at 37° C. ..	4.33	1/2 TR
(6) <i>Staph. toxin</i> + 0.3% formalin at 37° C. ..	3.43	—

C=complete haemolysis. AC=almost complete haemolysis. TR=traces of haemolysis.

There was a rough correlation between the action of formalin on diffusing factor and on the α -haemolysin, as shown in Table III, but inactivation of the α -haemolysin is more pronounced.

Charcoal, which adsorbs staphylococcus toxin, also adsorbs the diffusing factor completely from filtrates of staphylococcus toxin.

Summary and Conclusions

Results of a modified semi-quantitative method for evaluating diffusing activity by the rabbit bleb procedure are described.

Diffusing factor was found among the pathogenic group of aerobic micro-organisms only in staphylococcus, pneumococcus, and streptococcus.

There is no correlation between the general invasiveness or toxigenicity of a wide series of pathogenic micro-organisms and their production of diffusing factor.

Production of diffusing factor is correlated with time of incubation, but is independent of the rate of toxin production.

Formalin at 0.3% concentration does not affect the diffusing activity of staphylococcus cultures. At 37° C. the diffusing activity diminishes; heating the diffusing factor for thirty minutes at 45° C. destroys it.

We wish here to express our gratitude to Prof. A. Miles and to Dr. D. McClean for supplying us with certain strains used in these experiments, and especially to the latter for much helpful comment, criticism, and discussion.

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THE STERILIZATION OF SURGICAL RUBBER GLOVES

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Much has been written from different points of view about the sterilization of surgical gloves. One of us (D. A. S.), finding himself responsible for the sterilization of the gloves at a busy hospital, searched for facts that would ensure sterility while causing the least possible damage to the rubber. The growing shortage of raw material added increased urgency to the problem. After scrutiny of the literature he found that he had gained some knowledge on the destruction of micro-organisms by heat and an acquaintance with many opinions, which were not clearly accompanied by evidence and frequently were conflicting.

Two questions required answering: the first was, "Using a standard autoclave, what are the minimum requirements of pressure and time to enable sterility to be guaranteed?"; the second was, "If more than one technique is satisfactory, which is least detrimental to the rubber?" The value of other methods of attaining sterility, such as boiling or the use of chemical action, also requires investigation, but these methods present problems that are best considered separately.

Bacteriological Technique

While the various chemical colour changes may be valuable in routine sterilization it is essential that for experimental work of this nature the indicator should consist of highly resistant sporing organisms. Cotton-wool swabs liberally soaked in a mixture of soil and water were dried and inserted into the inner recesses of a glove. After the test the swab was plated and incubated aerobically and anaerobically for periods varying from four to eight weeks. In no case did a culture which had remained sterile for seven days become positive at a later date. At first cultures of *Staph. aureus* were also included, but, as anticipated, this coccus proved far less resistant than the soil organisms.

Autoclaving Technique

Packing the Gloves.—Each test was carried out personally according to the following principles. The glove containing the test culture was placed in a cotton packet with another glove; this packet was packed in the centre of a batch of thirty similar packets in a standard drum. The drum was cylindrical, 14 inches in diameter and 5 inches deep, and was provided with apertures at the periphery which could be closed by a sliding band; the lid remained shut during the sterilization. The details were exactly those used in the normal sterilization in the Infirmary, and in fact the test drum was often included with a routine batch.

Working the Autoclave.—The first vacuum is produced (the apparatus used in this work was capable of reducing

the pressure to -18 in. Hg). Steam is then admitted to a pressure of 5 lb. and maintained for one minute. A second vacuum is now produced. Steam is again applied at the pressure in use for sterilization. Drying is satisfactory after fifteen minutes of vacuum and fifteen of airing (an air-heating coil is used). The use of a double vacuum is essential if it is required to reduce deterioration of the gloves to a minimum. This is demonstrated by the following results: (a) Sterilization was adequate with a double vacuum if a pressure of 5 lb. was maintained for twenty-five minutes. (b) With a single vacuum it was found that at the same pressure forty minutes were required. (c) Since deterioration of rubber is proportional to the temperature, it occurs almost entirely during the process of steaming.

Some older types of apparatus are unable to produce a pressure as low as -18 in. Hg, so tests were carried out using a vacuum of ten inches: any instrument in good order should be able to reach this moderate figure. It was found that there was no loss of efficiency provided the double-vacuum method was followed. This is probably to be explained as follows: The first steaming results in condensation of water on the cold material that is to be sterilized; the second vacuum causes the water so formed to evaporate; the steam produced washes the last traces of air out of the drum. Such a process is most effective in eliminating air from the chamber and does not require a high degree of vacuum. That this process is occurring is suggested by the very much longer time taken to achieve the full vacuum after a short steaming than when the chamber contains air only. It can even be observed that this increase of time is roughly proportional to the quantity of material loaded into the sterilizer.

Bacteriological Results Obtained

The double-vacuum technique was employed on all occasions: (1) at a pressure of 20 lb. ten minutes were required for sterilization; (2) at a pressure of 10 lb. twenty minutes; and (3) at a pressure of 5 lb. twenty-five minutes. All results were repeated, and were accepted only if confirmed on three occasions. The combination finally accepted—No. 3 above—was tested and confirmed on ten separate occasions. It is significant that of five tests at 5 lb. for twenty minutes four remained sterile, and one culture became positive only after a period of incubation of five days. This suggests that a pressure of steam of 5 lb. maintained for twenty-five minutes gives a fair margin of safety.

Loss of Elasticity

Having established certain definite combinations of time and pressure that would guarantee sterility, it was now necessary to turn to our second question, "Which of these methods is least detrimental to the rubber?"

Sample strips of the cuff of a new surgical glove were cut to three inches in length and half an inch in width. The elasticity of these strips was measured and recorded; the strips were heated in the sterilizer and their elasticity again estimated. This measurement is made very simply by clamping each end of the strip, fixing one end, and adding a series of weights to a pan suspended from the other end. The length of the strip at different tensions is then recorded. Each test was carried out three times, but variations of over half an ounce on either side of the mean were not observed. The results have therefore been recorded for simplicity in the form of the mean of each group of test strips.

Group 1: Heating for 72 minutes at 20 lb.
 " 2: " " 144 " " 10 lb.
 " 3: " " 180 " " 5 lb.

The figure of 72 minutes was chosen arbitrarily to represent the period of heating that would occur with six steriliza-

tions, the generally accepted life of a glove. Any figure of the same order would have been suitable. Having chosen a time for one of the pressures, the remaining times were found from the ratios of the times of effective sterilization at the different pressures under examination. The respective losses of elasticity will therefore represent the efficiency of the different methods. For ease of comparison the results are here presented in graphic form.

These curves represent the results of stretching the test strips under increasing tensions.

A = Before heating.
 B = After heating for 180 minutes at 5 lb.
 C = " " " 144 " " 10 lb.
 D = " " " 72 " " 20 lb.

These curves demonstrate very clearly that the lower the pressure used the less the destruction of material, in spite of the longer time required.

The cost of fitting a regulator to the ordinary autoclave so that a pressure of 5 lb. may be automatically maintained should be roughly £30. The value of an average load of gloves is about £25.

Conclusion

These results suggest that, with adequate elimination of air from the chamber, steam at atmospheric pressure (0 lb.) might sterilize effectively with even less deterioration in the rubber. The investigations cannot be hurried, as long periods have to be spent waiting for the results of culture; therefore, because of the sense of urgency, already mentioned, it has been decided to publish the work in its present incomplete form. This speculation on the possibilities of cutting the pressure down to atmospheric level leads to an interesting conclusion: the gloves would be subjected to physical conditions similar to those of sterilization by boiling and yet would be dry at the end of the process. It is therefore not too much to hope that the long-standing controversy between the protagonists of "wetness against wastage" and those of "dryness desirable" might finally be settled in the realization of the dreams of both.

In order to determine the glycogen content of the liver in different conditions, D. S. MacIntyre, S. Pedersen, and W. G. Maddock (*Surgery*, 1941, 10, 716) removed small pieces of liver during abdominal operations on 33 patients. These were divided into four groups. In 10 patients with "normal livers," under spinal anaesthesia, the average value of hepatic glycogen was 3.15%, the lowest figure being 1.1 and the highest 6.3. In 7 similar cases, also operated on under spinal anaesthesia but given glucose in the evening and morning before operation, the average percentage of liver glycogen was 5.03, the values ranging between 3.19 and 7.56. Five patients with normal livers and operated on under nitrous oxide and ether anaesthesia, and given pre-operative glucose, were found to have an average liver content of glycogen of 4.72%. Four patients with what is described as "advanced hepatic pathology" were given 200 grammes of glucose in the twelve hours immediately before operation; the average percentage of hepatic glycogen in them was 2.87.