A summary of the reactions of 150 strains of staphylococci is given, illustrating the value of the coagulase test as a means of differentiating between pathogenic and saprophytic strains.

My thanks are due to Prof. J. H. Dible and Dr. R. Cruickshank for advice and criticism; to Dr. P. Sharpey-Schafer, under whose care the patient was admitted to hospital; and to the Chief Medical Officer, London County Council, for permission to publish the case.

REFERENCES

Chapman, G. H., Berens, C., Peters, A., and Curcio, L. (1934). *J. Bact.*, **28**, 343.
Cruickshank, R. (1937). *J. Path. Bact.*, **45**, 295.
Fisk, A. (1940). *Brit. J. exp. Path.*, **21**, 311.
Loeb, L. (1903–4). *J. med. Res.*, **10**, 407.
McCrea, H. M. (1940). *Proc. roy. Soc. Med.*, **33**, 679.
Mendell, T. H. (1939). *Arch. intern. Med.*, **63**, 1068.
Much, H. (1908). *Biochem. Z.*, **14**, 143.

THE PRESENCE OF DIFFUSING FACTOR AMONG THE METABOLITES OF AEROBIC MICRO-ORGANISMS

BY

J. UNGAR, M.D.

AND

A. L. BACHARACH, M.A., F.I.C.*

(From the Bacteriological and Biochemical Departments, Glaxo Laboratories Ltd.)

The presence of "diffusing factor" has been reported in: (i) mammalian testis extract (Hoffman and Duran-Reynals, 1931; McClean, 1930, 1931), (ii) snake and spider venom (Duran-Reynals, 1936), (iii) leech extract (Claude, 1937), (iv) malignant tumours (Boyland and McClean, 1935), as well as in cultures of the following micro-organisms: (v) staphylococcus (Duran-Reynals, 1933), (vi) streptococcus (Duran-Reynals, 1933), (vii) *Clostridium welchii* (McClean, 1936), (viii) *Cl. oedematis maligni* (*Vibrion septique*) (McClean, 1936), (ix) pneumococcus (McClean, 1936). Whether or not the diffusing activities evinced by these organisms and tissues are due to the same substance, or are even physiologically identical, cannot be stated with certainty, if only because the methods of test—to which we refer again below—have varied somewhat widely. We wish at the outset to make it clear that our references to "diffusing factor" and "diffusing activity" are to the substance that, in the simple but lucid definition of McClean and Morgan (1933), "dramatically increases the permeability of the skin to injected fluids and particles." All our tests, qualitative or quantitative, have been carried out by the procedure described elsewhere (Bacharach, Chance, and Middleton, 1940) for testicular diffusing factor (t.d.f.): the results of these tests are not necessarily the same as would have been obtained by different methods.

The presence of diffusing factor in bacterial cultures is of interest in itself to those investigating the metabolic processes of micro-organisms or applying the results of their work to medical practice, and has possible applications in therapy. Pharmacologically, diffusing factor appears to be inert: it has no effect on blood pressure (Christensen, 1938) and its action is not antagonized by histamine or adrenaline (Madinaveitia, 1939). Its injection into the cerebrospinal canal, however, markedly hastens the peri-axial spread of cerebrospinal fluid (Cohen and Davies, 1939). Apart from the possibility, to which considerable attention has been given by McClean (1941c), that diffusing factor produced by micro-organisms might affect their pathogenicity, there is also the question whether non-toxic preparations of the active substance might not be used as an adjunct in therapy—along with, for example, anaesthetics and narcotics, or in electrophoretic treatment—to reduce the quantities required for a given effect or the time required for an effect to be manifested.

Bound up with such considerations are the questions of the chemical nature and the specificity of diffusing factor. In some respects its properties are those of an enzyme system. Chain and Duthie (1939) found purified preparations of diffusing factor to possess marked mucolytic properties and suggested its identity with mucinase; but correlation between diffusing and mucolytic activity, whether this is determined by measuring the reduction in viscosity or in other ways, has not always been established. East, Madinaveitia, and Todd (1941) have recently shown that liberation of reducing substances does not go *pari passu* with reduction in viscosity, and suggest that two different enzymes may be involved: they have also demonstrated, by its action on β-phenyl-N-acetyl glucosaminide, the presence in testis extracts of an enzyme they call "β-glucosaminase," although it is admitted (Todd, personal communication) that "β-glucosaminidase" would be the more correct term. This enzyme is distinct from the substance (or complex) in the extract responsible for reducing the viscosity of hyaluronic acid. Robertson, Ropes, and Bauer (1940) examined preparations of *Cl. perfringens* and state that they hydrolysed mucoid (synovial fluid) in two stages: first there was a rapid decrease in viscosity, accompanied by a change in precipitability with aluminium hydroxide; then there was a gradual liberation of amino-sugars and reducing substances, reaching a maximum at twenty-four to twenty-eight hours. The work of Meyer and his colleagues (1941) suggests, on balance, a close correlation between diffusing activity and hyaluronidase content, as does the finding of McClean and Hale (1941) that the production of both hyaluronidase and diffusing activity by *Cl. welchii* or by streptococci is increased when these organisms are cultured in presence of hyaluronic acid. On the basis of existing knowledge it is not possible to say whether or not the increase in reducing power shown during the action of diffusing factor on mucoid is due wholly, or even in part, to the action of β-glucosaminidase. In any event, the relationship is not a simple one, and the matter must be considered still *sub judice*.

Unfortunately, lack of agreement as to the identity of diffusing factor even with a mucinase—or hyaluronidase, as most authors prefer to call it—and its differentiation from β-glucosaminidase have complicated the position, because different workers have been led to use different tests for the presence of diffusion-promoting substances. Apart from viscosimetric determinations and estimations of the amount of reducing substances liberated, a clot method has been described by McClean (1941b). In this the enzymic activity is measured by the extent to which, in the presence of acetic acid, the formation of a clot from mucoid is inhibited: there are also variants of the test on the skins of rabbits. Our method is based on the measurement of "blebs" produced in the skin of shaved rabbits after intradermal injection of the test solution. Full details have been published elsewhere (Bacharach, Chance, and Middleton, 1940), and it is here necessary only to indicate the quantitative limitations of the restricted tests reported below.

Between bleb areas of 4 and 7 sq. cm. the relationship between area and log dose is a linear one, these limiting values corresponding with about 0.4 and 2 of our units, respectively. Over this range the mean variance of an individual bleb was found to be 0.83. From this it follows that the means of three blebs differ from each other significantly at the 5% level when the difference exceeds 2 sq. cm.; with six blebs significance is established at the same level for differences of more than

* In this investigation Dr. Ungar had the assistance of N. McLeod and E. V. Welch; and Mr. Bacharach that of T. R. Middleton.

1.2 sq. cm. In these estimates the calculation of variance is based on the heterogeneous groups used in the experiments described by Bacharach *et al.* (1940)* ; this is certainly an overestimate when doses are distributed so as to counteract the effect of differences in sensitivity of different animals. A fairer estimate of variance can be calculated from the differences between replicate blebs produced by the same dose on one animal, and this estimate may be used in assessing the significance of the difference between mean values of bleb areas obtained under our conditions of test. Such a calculation shows that the means of two comparable groups of three blebs differ significantly ($P=0.05$), when the difference is greater than 0.57 sq. cm. for values below 4.5 sq. cm. ; the corresponding significant difference for values from 4.5 sq. cm. to 7 sq. cm. is 0.77, and for values above 7 sq. cm. 0.95 ; mean values above 8 are very seldom encountered. With six pairs of blebs the corresponding significant differences are 0.34, 0.44, and 0.55 sq. cm.

Consideration of these limits has led to the following scale of assessment, on the basis that the mean response at the mid-point of a grade should differ from that at the next by about twice the significant difference.

Class	3 Pairs of Blebs	6 Pairs of Blebs
0	Up to 4·5 sq. cm.	Up to 4 sq. cm.
+	4·5 to 6 sq. cm.	4 to 5·5 sq. cm.
++	6 to 7·7 sq. cm.	5·5 to 7·4 sq. cm.
+++	Over 7·7 sq. cm.	Over 7·4 sq. cm.

We have examined a considerable number of aerobic micro-organisms on this semi-quantitative basis. They include saprophytes and pathogens of toxigenic, non-toxigenic, invasive, and non-invasive types, different species of a common genus, different types or varieties of a common species, a typical yeast, several moulds, a certain number of toxin and toxoid preparations, and two samples of bacteriophage. The yeast (*Saccharomyces cerevisiae*) and *Pseudomonas pyocyanea* were examined as specially active elaborators of cellular enzymes, which are found in their broth cultures, and bacteriophage because of its intense biological activity. At least two independent tests were almost always carried out on preparations from the same organism (strain, variety, etc.), so that the number of samples examined exceeds, sometimes considerably, the number of strains indicated in column 3 of Table I ; when there was any ambiguity, results were checked by further tests. In Tables II and III the figures in parentheses indicate the number of blebs from which the means have been calculated.

Our experiments have been designed to examine certain selected aspects of diffusing activity as shown by these bacterial cultures. Broadly, three matters have been investigated: (1) In the cultures of which micro-organisms can diffusing factor be detected? (2) At what time does diffusing factor appear in the cultures, and what is the relation between the amount of diffusing factor present in the medium and the amount of toxin and number of micro-organisms? (3) How does the influence of formalin and heat on diffusing factor compare with their effects on toxin production by the same micro-organisms?

Pathogenic micro-organisms develop their specific action in the infected host by local invasion with consecutive extensive tissue damage (e.g., anaerobic wound infection), by multiplying in the body of the host and causing fatal bacteriaemia (sepsis), or by producing at the place of the infection a toxin which affects a specific sensitive tissue of the body (diphtheria, tetanus). The virulence of the micro-organisms can be shown (to characterize it in simple terms) by their invasiveness or by their toxigenicity, which may be quite distinct properties. In some micro-organisms both those properties can coexist (as in septicaemia, pyaemia). The pathogenic action, whether toxigenic or invasive, is enhanced by substances produced in the active phase, such as fibrinolysins, coagulase, leucocidin, and other enzymes.

The question arises whether diffusing factor produced by pathogenic micro-organisms also acts as a factor in their pathogenic effect. We have therefore examined a range of aerobic micro-organisms for the presence of this factor in their cultures. We are aware of the limitation applying to conclusions drawn from effects observed *in vitro* ; all we claim is that these experiments give certain comparative information as to bacterial properties.

In Table I is arranged a list of the micro-organisms used, with an indication of the type of their pathogenic activities. Column 2 gives the description of the bacterial product tested ; column 3 indicates the number of strains of each species used ; and column 4 shows whether diffusing factor is present, with an indication as to its concentration. In column 5 the type of virulence of the micro-organisms is roughly indicated—i.e., their production of an exotoxin or their invasiveness.

TABLE I

Description of Micro-organism	Description of Substance	No. of Strains	Diffusing Activity	Properties of Micro-organism
1. Staph. pyogen., albus, aureus	Toxin grown on synth. agar	5	++ to +++	Virulent (toxigenic, invasive)
2. Str. pneumoniae Types I, II, III, IV	Filtrate of 48-hr. broth cult.	6	0 to ++	Virulent (invasive)
3. Str. scarlat. Types I, II, IV Griffith	Filtrate of 24-hr. broth cult.	4	+ to +++	Virulent (toxigenic, invasive)
4. Str. Group C haem.	,, ,,	2	+ to ++	Virulent (invasive)
5. Sac. cerevisiae	Filtrate of 48-hr. broth cult.	1	+++	—
6. Penicillium species	Filtrate	3	—	—
	Czapek's medium	1	++	
7 Br. abortus Bang	Filtrate of serum broth	2	0	Virulent (invasive)
8. Br. melitensis	,, ,,	2	0	,, ,,
9. Str. scarlat. Type III Griffith	Filtrate of 24-hr. cult.	1	0	Virulent (toxigenic, invasive)
10. Mycobact. tuberculosis, human type	Filtrate of glycer. liver broth cult.	1	0	Virulent
11. Cor. diph. mitis, intermed., gravis	Toxin grown on broth	7	0	Virulent (toxigenic)
12. Str. haem. Group A untyped	Filtrate of 24-hr. broth cult.	1	0	Virulent (invasive)
13. Bact. typhosum Vi strain	,, ,,	5	0	,, ,,
14. Bact. paratyphosum B	,, ,,	4	0	,, ,,
15. Bact. paratyphosum A	,, ,,	1	0	,, ,,
16. Bact. aertrycke	,, ,,	1	0	Virulent
17. Bact. shigae	,, ,,	5	0	Virulent (toxigenic)
18. Bact. flexneri	,, ,,	3	0	Virulent
19. Vibrio cholerae	,, ,,	2	0	,,
20. B. anthracis	,, ,,	2	0	Virulent (invasive)
21. Haem. pertussis	Filtrate of 48-hr. broth cult.	4	0	Virulent
22. Haem. influenzae	,, ,,	4	0	Virulent (invasive)
23. Neiss. gonorrhoeae	,, ,,	1	0	Virulent
24. Ps. pyocyanea	Filtrate of 24-hr. broth cult.	4	0	—
25. B. subtilis	,, ,,	1	0	—
26. Bact. coli	,, ,,	1	0	—
27. Influenza virus	Filtrate of mouse lungs	1	0	Virulent
28. Staph. antivirus	Broth filtrate	1	0	
29. Bacteriophage	Filtrate	4	0	Active against Bact. typhosum; Bact. paratyphosum A, B; B. coli
30. Sat. solution of sulphanilamide	—		0	

For explanation of the significance of 0, +, ++, +++, in recording diffusing activity, and for the meaning attached by us to such terms as virulent, invasive, etc., see text.

* An opportunity may be taken here of correcting three previously undetected errors in that paper. (i) In describing the preparation of the haemoglobin indicator solution the authors intended that the solution of haemoglobin (p. 1467, line 1) should be treated with sufficient sodium chloride to make it isotonic; (ii) the abscissae in Figs. 1 and 2 refer to microgrammes (μg.) of preparation A, not to milligrammes; (iii) on page 1470, below line 12, the " probable limits of error " at the two levels should be reversed (for $P=0.95$, 83.5 to 120%; for $P=0.99$, 79 to 127%) and be described as " fiducial limits."

In this paper we apply the term "invasive" in the sense widely used by bacteriologists to characterize the ability of certain micro-organisms to enter the body through a "porta invasionis," to multiply in the body, and eventually to be deposited in different organs by means of the circulation. McClean and his associates give to the term "invasive" a different and more specific meaning, applying it to the ability of micro-organisms to invade the tissue locally, to multiply there and cause destruction of tissue, as shown in cases of gas gangrene or staphylococcal infection. Under this altered definition, invasiveness and spreading activity are closely related, and production of the spreading factor is one of the properties characterizing the "invasive microbe."

From Table I it appears that there is no relation between diffusing activity and the toxigenicity or invasiveness (as defined by us) of the micro-organisms tested. Some potent toxin producers, such as *Corynebacterium diphtheriae*, showed no diffusing activity, thus confirming a fact to which McClean (1941a) has drawn attention; on the other hand, certain micro-organisms of high invasiveness, such as *B. anthracis, Bact. typhosum, Brucella melitensis,* also showed no diffusing activity. The only species yielding diffusing factor were staphylococcus strains (both albus and aureus), pneumococcus, and streptococcus. The amounts of diffusing factor produced differed in different types and strains, but its presence was correlated with virulence to mice or rabbits.

Any role, therefore, of diffusing factor in promoting virulence of micro-organisms must be limited to a few species only. Among anaerobic toxin producers, diffusing factor has been detected in *Cl. welchii* and *Vibrion septique,* and to some extent in *Cl. oedematiens* (McClean, 1936), but not in others that are highly toxigenic, such as *Cl. tetani.* Diffusing factor is absent from most of the micro-organisms characterized by general invasion of the host. It is possible that there is some connexion between diffusing and necrogenic activities of the micro-organisms tested (to which McClean refers as local invasiveness); the latter is marked in staphylococcus, pneumococcus, streptococcus, and *Cl. welchii.* (The chemotactic effect of some metabolic products of these micro-organisms is known.)

We tested different phages of the *Bact. typhosum–paratyphosum* group for the presence of diffusing factor, with negative results; *Ps. pyocyanea,* showing high fermentative activity during its growth, also gave negative results. Of filtrates from our penicillium strains, one isolated as an occasional contamination on agar plates showed pronounced diffusing activity. Saturated aqueous solutions of sulphanilamide showed no diffusing activity, a fact that may be of importance in the local application of sulphonamides to wounds, as indicating that presence of the drug does not enhance the spread of the micro-organisms or their products. A strain of *Sac. cerevisiae* grown on synthetic medium showed only insignificant growth (after forty-eight hours) and no diffusing activity in the filtrate. The same strain grown on 1% glucose broth showed marked growth and yielded a filtrate that gave maximal diffusion. Here the presence of peptone and glucose enabled the latent property of the yeast to develop, showing that the appearance of diffusing factor in the medium depends on a sufficiently vigorous growth of the micro-organisms.

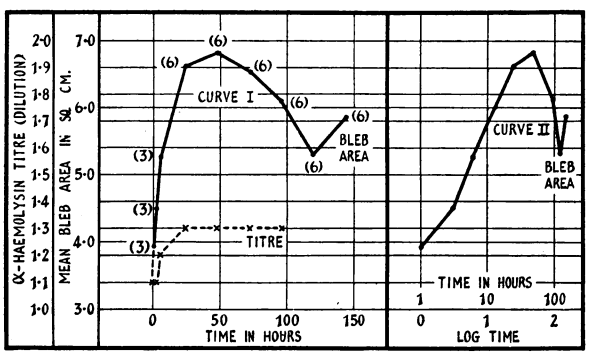
The influence of the medium on formation of diffusing factor by *Staph. aureus* was tested. There was a slight difference, due to the type of medium used, in so far as staphylococcus grown on synthetic medium in CO_2 gave somewhat enhanced figures. Passage through a Seitz filter has decreased the titre of diffusing factor in the filtrates, but passage through a Berkefeld filter caused only insignificant reduction, as McClean (1936) also found both for diffusing factor and for hyaluronidase.

Toxin	Mean Bleb Area (3) in sq. cm.	
A. Unseitzed	6·43 (++)	No effect
A. Seitzed	6·60 (++)	
B. Unseitzed	8·27 (+++)	Significant reduction
B. Seitzed	6·40 (++)	

A was produced from organisms grown on agar, B from organisms grown on agar-synthetic medium for staphylococcus toxin.

The addition of glucose to the medium had no apparent influence on the amount of diffusing factor in staphylococcus and pneumococcus filtrates. Micro-organisms, such as *Ps. pyocyanea, Bact. shigae,* and streptococcus, which did not produce diffusing factor on the usual medium, failed also to do so when glucose or serum was added.

The production of diffusing factor by *Staph. pyogenes* is shown in the Chart in relation to time and growth of the culture. Curve I shows bleb area plotted against time (in hours), with the number of blebs in parentheses; curve II



Graph showing the production of diffusing factor and α-haemolysin by *Staph. aureus* (227) in plain broth.

shows bleb area plotted against log time (in hours). It will be noticed that there is a close linear relation between the second, third, and fourth points on the second curve and that the departure from linearity of the first and fifth points is what would be expected if the usual sigmoidal relation were expressed by the curve. As it has been shown (Bacharach, Chance, and Middleton, 1940) that within the limits involved bleb area is lineally related to the logarithm of the amount of diffusing factor used, it follows that the amount produced in this culture was lineally related to time of incubation.

Increase of diffusing factor in the medium is rapid after two hours to about twenty-four hours, continues for the next twenty-four hours, and begins to decrease after forty-eight hours; this lowering of diffusing factor titre may be due to the influence of temperature at 37° C. There was no correlation between the increase of diffusing activity and the titre of α-haemolysin. Broth filtrates tested at a time when diffusing factor is markedly increasing did not show the presence of staphylococcus toxin as demonstrated by α-haemolysin or toxin effect on the rabbit; this confirms the independence of the two principles in broth cultures of staphylococcus. The correlation between the actual number of micro-organisms in the medium and the titre of diffusing factor is shown in Table II.

TABLE II

	Number of Micro-organisms	Mean Bleb Area (6) in sq. cm.
Staphylococcus aureus:		
5-hour culture	179×10^6 in 1 ml.	4·05 (0 to +)
24-hour growth	569×10^6 in 1 ml.	5·98 (++)
48-hour growth	569×10^6 in 1 ml.	7·57 (+++)

The number of micro-organisms was estimated by the opacity-tube method. Although allowance has to be made for some inaccuracy in counting by this method, it appears that the amount of diffusing factor increases in the first twenty-four hours concurrently with growth of the micro-organisms and still increases during the stationary period in the next twenty-four hours.

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) Staph. toxin kept in refrigerator	6·60	1/512 C
(2) Staph. toxin + 0·3% formalin kept in refrigerator	6·23	1/128 TR; 1/16 AC
(3) Staph. toxin at room temperature	7·30	1/512 C
(4) Staph. toxin + 0·3% formalin at room temperature	6·47	1/64 TR; 1/2 AC
(5) Staph. toxin at 37° C.	4·33	1/2 TR
(6) Staph. toxin + 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.

REFERENCES

Bacharach, A. L., Chance, M. R. A., and Middleton, T. R. (1940). *Biochem. J.*, **34**, 1464.
Boyland, E., and McClean, D. (1935). *J. Path. Bact.*, **41**, 553.
Chain, E., and Duthie, E. S. (1939). *Nature*, **144**, 977.
Christensen, J. F. (1938). *J. Path. Bact.*, **48**, 287.
Claude, A. (1937). *J. exp. Med.*, **66**, 353.
Cohen, H., and Davies, S. (1939). *Nature*, **143**, 285.
Duran-Reynals, F. (1933). *J. exp. Med.*, **58**, 161.
—— (1936). *Science*, **83**, 286.
East, M. E., Madinaveitia, J., and Todd, A. R. (1941). *Biochem. J.*, **35**, 872.
Hoffman, D. C., and Duran-Reynals, F. (1931). *J. exp. Med.*, **53**, 387.
McClean, D. (1930). *J. Path. Bact.*, **33**, 1045.
—— (1931). Ibid., **34**, 459.
—— (1936). Ibid., **42**, 477.
—— (1941a). *Lancet*, **1**, 595.
—— (1941b). *J. Path. Bact.*, **53**, 13, 156.
—— (1941c). *Lancet*, **1**, 797.
—— and Hale, C. W. (1941). *Biochem. J.*, **35**, 159.
—— and Morgan, W. T. J. (1933). *J. Path. Bact.*, **36**, 193.
Madinaveitia, J. (1939). *Biochem. J.*, **33**, 347.
Meyer, K., Chaffee, E., Hobby, G. L., and Dawson, M. H. (1941). *J. exp. Med.*, **73**, 309.
Robertson, W. van B., Ropes, M. W., and Bauer, W. (1940). *J. biol. Chem.*, **199**, 261.

THE STERILIZATION OF SURGICAL RUBBER GLOVES

BY

D. ASTLEY SANFORD, M.B., B.S., M.R.C.P., F.R.C.S.
Resident Surgical Officer

AND

H. A. COOKSON, M.B., F.R.C.P.Ed., F.R.C.S.Ed.
Pathologist and Bacteriologist
Royal Infirmary, Sunderland

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