GRADING OF COAGULASE-POSITIVE STAPHYLOCOCCI IN THE CONTROL OF HOSPITAL CROSS-INFECTION

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INTRODUCTION

Coagulase-positive staphylococci are probably responsible for the majority of severe septic lesions encountered in hospitals at the present time. One of the difficulties in controlling these infections is that staphylococci also cause a large number of trivial infections and, further, can be isolated from a high proportion of normal persons who carry the organisms in their anterior nares and on their skin. Unfortunately, there is no clear evidence that it is possible to recognize those staphylococci which are particularly liable to cause severe infections.

Knott & Blaikley (1944) introduced a system of grading coagulase-positive staphylococci based on a few readily identifiable features—namely, production of pigment, coagulase and haemolysin, gelatin liquefaction and the rate of fermentation of mannitol, lactose, sucrose, maltose and glucose. Strains most active in these respects were assigned to Grade A and progressively less active strains to Grades B, C, and D. In a study of staphylococci isolated from the patients and staff of the Department of Obstetrics at Guy's Hospital it was found that active lesions were mainly produced by staphylococci of Grades A and B, while Grades C and D appeared to be of far less significance. In attempting to control outbreaks of sepsis special precautions were therefore taken whenever staphylococci of Grades A and B were isolated from patients or from the nose, throat and hands of members of the nursing and medical staff. There is no doubt that the system of grading was of great practical value and that by its means it was possible to effect a great reduction in the incidence of serious infections in mothers and infants.

Since 1944 the practice of grading all staphylococci isolated from the patients and staff of the maternity department has been continued. The methods used have been similar to those described by Knott & Blaikley. The chief modification has been that fermentation reactions have been confined to the use of mannitol, because in the earlier work this substrate appeared to be of greatest differential value. It follows that the rate of mannitol fermentation has been a major consideration in assigning a staphylococcus to a particular grade.

Over the last 14 years many thousands of staphylococci isolated from the patients and staff have been examined and graded. Recently doubt has been

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expressed whether the grades to which staphylococci have been assigned have had any relationship to their pathogenicity and whether the system of grading is still of practical value in attempts to control infection.

In the present work an attempt has been made to reassess the usefulness of this system of grading. The staphylococci studied were all coagulase-positive (*Staphylococcus pyogenes*).

MATERIALS AND METHODS

Source of strains

The staphylococci were isolated in the course of routine work by the Department of Bacteriology and the Department of Clinical Pathology. The principal sources are referred to in the text.

Mannitol fermentation

The medium prepared by us consisted of peptone (Evans) 1·0 g., NaCl (A.R.) 0·5 g., mannitol (Kerfoot's Biochemical Reagents) 1·0 g., distilled water 100 ml.; the pH was adjusted to 7·5 and 1·0 ml. of freshly prepared Andrade's indicator was added; the medium was dispensed in 3 ml. amounts in sterile bijou bottles and sterilized by steaming for 30 min. on 3 successive days. The medium used in the routine work, however, was supplied by the Southern Group Laboratory, Park Hospital, London, S.E. 13. The inoculum varied in different experiments but 0·02 ml. of an 18 hr. digest broth culture was commonly used as a convenient standard inoculum. The bottles were incubated at 37° C. and inspected at 24, 48 and 72 hr. Normally the results were recorded as 'positive' when the indicator was a bright fuchsia red (pH 5·2 or less) and 'negative' when the colour was less intense. In certain comparative experiments the colour was matched to bottles of known pH.

Coagulase production

The tube coagulase test was performed using 0·9 ml. of fresh human plasma diluted 1:10 with normal saline and inoculated with 0·1 ml. of 18 hr. digest broth cultures. The tubes were incubated at 37° C. and the results recorded as 'negative' or 'positive' at 1, 2, 3 and 6 hr. All comparative tests were performed with a single batch of plasma prepared by pooling fresh oxalated plasma from several different patients.

Haemolysis

In routine work 5 % horse blood agar plates were used. In all comparative tests cultures were made on 5 % sheep blood agar plates incubated aerobically for 18 hr. at 37° C. and the size of the zone of haemolysis recorded as + + +, + +, + or −; the plates were then kept at room temperature for a further 24 hr. and the presence or absence of 'hot–cold' β-lysis was noted.

Pigmentation

Cultures on nutrient agar plates were incubated aerobically for 18 hr. at 37° C. and then kept at room temperature for 3 days. The colour of the colonies was recorded as 'white', 'off-white', 'dull-gold', or 'bright-gold'.

Diffusible antigens

These were studied by the method of Elek & Levy (1950) using refined staphylococcal antitoxin globulin (Burroughs Wellcome and Co.).

RESULTS

Reproducibility of mannitol fermentation

In the course of this work many strains were tested for their ability to ferment mannitol on a number of separate occasions. It was found that the rate of mannitol fermentation often differed greatly from what had originally been found in routine work. Since this largely determined the grade to which the staphylococcus was assigned the reproducibility of results was investigated in some detail.

Observer error

The decision whether a strain has or has not fermented mannitol depends on the colour produced—the range of colours may vary from a bright fuchsia red to a faint pink. It was confirmed by means of a pH meter that this range of colour did in fact correspond to different pH values. In the present work, however, only bright red (pH 5·2 or less) was considered 'positive' and less intense colours were all considered 'negative'. With a little practice excellent agreement was achieved between the findings of two observers and between the findings of a single observer on two separate occasions. It was found that errors from this source amounted to less than 2%.

Inoculum size

When a sweep from a culture on nutrient agar was taken with a platinum loop and used to inoculate a set of six mannitol bottles the results were highly unpredictable and anomalous results were found in one or more of the six bottles with three-quarters of the strains tested. When, however, an extremely heavy sweep of culture was used then all strains were positive in 24 hr.

Experiments were therefore carried out with graded saline suspensions of washed cells harvested from 18 hr. broth cultures. Pipettes calibrated to deliver 0·02 ml. were used to inoculate mannitol bottles. Viable counts were made on blood agar plates.

By inoculating a known number of organisms in this way the reproducibility of the results was greatly increased. If the bottles were observed at 24 hr. it was usually possible to detect a fairly regular gradation from high acidity ('positive') with inocula of 50,000,000 organisms to low acidity ('negative') with inocula of 500 organisms or less, but even with a standard inoculum there was considerable variation in the results. Thus with an inoculum of 10,000,000 organisms in 0·02 ml. of saline one or more anomalous results were found with 20% of strains when the organisms were inoculated into sets of six bottles of mannitol. Nevertheless, it was decided to use 0·02 ml. of an 18 hr. broth culture as a convenient standard inoculum and assign a strain to a particular grade if five or six bottles gave identical results.

Effect of repeated subculturing

Strains were tested in sets of mannitol bottles immediately after primary isolation and examples of slow and fast mannitol fermenting strains were selected. These strains were repeatedly subcultured at 2–3 day intervals in peptone water and then retested. No change in the ability to ferment mannitol was noted. Similarly, strains which had been maintained for long periods of time by monthly subculture on nutrient agar slopes gave similar results when retested.

Influence of the medium

Various batches of medium were tested but provided it was freshly prepared and the initial pH was adjusted to 7·4–7·6 the variability of the results was the same. The use of specially cleaned glass-ware did not influence this variability. It was noted that medium prepared with an old batch of Andrade's indicator gave results less reproducible than usual.

Further investigations of the variability of results

At first it was thought that the variability might be an indication that cultures consisted of some fairly gross mixture of slow and fast fermenting strains. To test this eight sets of mannitol bottles (six bottles in each set) were inoculated with organisms derived from single isolated colonies of a given strain growing on a blood agar plate and compared with organisms derived from a sweep from the area of confluent growth on the same plate. A number of strains were tested in this way, but there was no evidence that selection of organisms from isolated colonies made any difference to the results. Similar experiments were made with staphylococci grown on mannitol neutral red agar plates. On this medium most strains showed a few colonies which differed from the majority in being much smaller and almost colourless. When, however, subcultures from representatives of the two types of colony were tested in sets of mannitol bottles the results were indistinguishable, and it is believed that the two types of colony represent a kind of variation which is frequently observed when staphylococci are grown on a medium which is not ideal.

Although there was no evidence that cultures consisted of any gross mixture of slow- and fast-fermenting strains, fast-fermenting strains were occasionally isolated from what had been considered to be slow-fermenting parents. These fast-fermenting strains appeared to be true variants as the property of fast fermentation could be passed in repeated subculture from single colonies on nutrient agar plates. The other properties of these variants were identical with those of the parent strain.

In other experiments it was shown that washed suspensions of slow-fermenting strains harvested from 4 hr. cultures in mannitol-containing medium were similar in their mannitol-fermenting abilities to control suspensions grown in peptone water. This seemed to confirm that rapid fermentation of mannitol was unlikely to be an adaptive mechanism.

Although rapid fermentation by a normally slow-fermenting strain could sometimes be explained by growth of fast-fermenting variants, it is extremely difficult

to explain slow fermentation by a normally fast-fermenting strain. Subcultures from cultures showing unexpectedly slow fermentation have so far had the same fast-fermenting properties as the parent strain.

Relation of grades to pathogenicity

It was clear from the investigations of mannitol fermentation already carried out that it was often impossible to obtain reproducible results. It now seemed important to see whether mannitol fermentation could be related to pathogenicity, as judged by other means.

Survey of staphylococci isolated from a maternity department

For some years a separate record has been kept in the Department of Clinical Pathology of the source and grade of all coagulase-positive staphylococci isolated from the Department of Obstetrics at Guy's Hospital. It was decided to examine this record and see what information it gave on the distribution and possible significance of the different grades.

Table 1. *Staphylococci isolated from carriers*

	Grades to which staphylococci were assigned				
Source	A	B	C	D	Totals
Nurses and midwives (nose)	335	110	26	13	484
Dressers (nose)	21	3	2	12	38
Nurses and midwives (throat)	5	0	0	1	6
Dressers (throat)	1	0	0	1	2
Dressers (hand)	4	1	2	10	17
Mothers (nose)	297	56	7	36	396
Mothers (throat)	26	1	1	4	32
Totals	689	171	38	77	975

Table 2. *Staphylococci isolated from cases of infection*

	Grades to which staphylococci were assigned				
Source	A	B	C	D	Totals
Babies (eye infections)	82	1	4	12	99
Babies (skin infections)	21	2	0	0	23
Babies (other infections)	21	1	0	1	23
Mothers (various infections)	17	0	4	0	21
Totals	141	4	8	13	166

A period of 2 years (27 August 1956 to 26 August 1958) was chosen at random and the staphylococci isolated during this period were classified under the various headings shown in Tables 1 and 2. The time spent by a member of the nursing staff in the obstetrics department varied from a few days to many months, and as the staphylococci isolated from a particular person were not always of the same grade

throughout this period, it was thought that a more accurate picture of nasal carriage would be obtained if every positive culture were treated as a separate observation. When staphylococci were isolated on more than one occasion from other sites in an individual, only one strain has been included in the totals; this strain has been classified under the grade assigned to it on the first occasion it was isolated.

The strains shown in Table 1 ('Strains isolated from carriers') were derived from routine swabs taken in the maternity department. Nose and throat swabs were taken from mothers on admission, from nurses and midwives at intervals of 2–3 weeks and from students at the beginning of their period as dressers; students had an additional swab taken from their hands. It will be seen that 689 out of 975 (70·7 %) of the strains from carriers belonged to Grade A.

The strains shown in Table 2 ('Strains isolated from cases of infection') were derived from infections in which *Staph. pyogenes* was the probable causative organism. The majority of these cases (145 out of 166) were infections of infants and included 99 cases of eye infections ('sticky eye', conjunctivitis and styes), 23 cases of infection of the skin (septic spots, infected blisters, small boils) and 23 miscellaneous infections (abscesses, infections of the umbilicus, urinary infections, etc.). Only 21 strains were derived from infections in the mother; these included infection of surgical wounds, breast abscesses, urinary infections and boils. It is probable that staphylococci were also largely responsible for a number of other infections of the mothers, notably local infections of the perineum and lower vagina. Staphylococci were often recovered in mixed culture from these sites, but it was thought unwise in a retrospective survey of this kind to include these infections amongst those which were certainly due to *Staph. pyogenes*. It will be seen that of the 166 strains considered in Table 2 141 (84·9 %) belonged to Grade A.

Staphylococci from the noses of nurses and midwives

The results of nose swabs from nurses and midwives showed whether the grade of staphylococci varied when repeated observations were made in the same individual. By examining the records of senior members of the staff (sisters, charge midwives and staff midwives) eleven individuals were selected who had been swabbed at fairly regular intervals over periods of from 4 to 23 months and for whom positive results had been found on five or more occasions. The findings are summarized in Table 3.

Table 3 shows the number of months during which an individual was studied, the number of nose swabs examined and the results of these in the order in which they were examined. It will be noted that only one individual (S.W.) carried staphylococci of the same grade throughout the period of observation. Of the remaining ten individuals, five carried staphylococci of two grades, four carried three grades and one individual (S.S.) carried all four grades. When the grade has changed there is no evidence that this has been a persistent change. Although it is possible that the variation in these results sometimes represents a change in the strain of staphylococcus present in an individual's nose, it is believed that most of it can be explained by the inherent variability of the method of grading.

Table 3. *Staphylococci isolated from the noses of senior nurses and midwives*

	Period of observation in months	No. of nose swabs examined	Results of nose swabs in the order in which they were examined	Grades A	B	C	D
S.D.	23	27	AOOOAOOAAAAAAOAOAOOODOOOOOB	10	1	—	1
S.M.W.	16	19	AABAOOAAAAAAAAOOOOO	11	1	—	—
S.W.	14	15	AAAAAAOAOOAAAAA	12	—	—	—
C.M.F.	13	15	BOBOODOOOOOOAAA	3	2	—	1
S.M.R.	11	19	OOOBOBOABBDABAOOBAB	4	7	—	1
S.M.B.	10	17	OOOOBAAOOOOAAOBAO	5	2	—	—
S.O.	9	13	AABAOBOAAAABO	7	3	—	—
S.B.	8	11	OOOAOOOBAAB	3	2	—	—
S.S.	7	15	COAAOBODAOAAAAA	8	1	1	1
S.M.O.	6	14	BOBAOOOBAOOBOO	2	4	—	—
C.M.B.	4	9	AAAAOABCA	6	1	1	—
			Totals	71	24	2	4

A, B, C and D refer to the grade of staphylococcus isolated. O indicates absence of coagulase-positive staphylococci.

Staphylococci recovered from a Burns Unit

A series of 102 strains of staphylococci had been collected in the course of a study of infection occurring in a Burns Unit (Penikett, Skinner & Knox, 1958). Forty-four strains had been isolated from infection and 58 strains from the air. The grades of these strains were as follows: Grade A, 79; Grade B, 10; Grade C, 4; Grade D, 9.

Distribution of strains

Table 4 summarizes the distribution of all the strains so far considered. They have been classified into two groups, namely, those belonging to Grade A and those belonging to other grades.

When the figures in Table 4 are examined the feature that stands out is that in all groups more than 70 % of the strains belong to Grade A. It is also noted that the proportion of strains belonging to Grade A is greatest in the group of strains

Table 4. *Proportion of Grade A strain in different populations*

	Source of strain: I. Carriers in maternity dept. (see Table 1)	II. Sepsis in maternity dept. (see Table 2)	III. Noses of senior nurses and midwives (see Table 3)	IV. Air and burns	Totals
Grade A	689 (70·7 %)	141 (84·9 %)	71 (70·3 %)	79 (77·4 %)	980 (72·9 %)
Grades B, C and D	286 (29·3 %)	25 (15·1 %)	30 (29·7 %)	23 (22·6 %)	364 (27·1 %)

χ^2 = sum of $\frac{(\text{observed} - \text{expected})^2}{\text{expected}}$ = 16·89, n = 3, P = < 0·01. The contribution to χ^2 by Group II = 12·30.

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derived from cases of infection. When the figures are analysed statistically it is found that this difference is significant.

By examining a large number of strains in this way it is probably justifiable to conclude that staphylococci assigned to Grade A are more dangerous than staphylococci of other grades. The question then arises whether this information is of practical value in attempts to control outbreaks of infection.

Many of the strains isolated in the last 2 years from other departments in the hospital were regarded as pathogenic in that they were recovered in pure culture from serious septic conditions. The staphylococci responsible included examples of all four grades with a predominance of Grade A. It is doubtful if it is wise to analyse the findings since, on the whole, these were sporadic infections and no adequate control cultures from carriers are available.

In one particular outbreak of sepsis studied by Penikett, Knox & Liddell (1958) there was strong evidence from phage-typing and other epidemiological data of the probable chain of events which led up to the outbreak, and adequate controls were available in the form of staphylococci isolated from the skin, nose and throat of healthy carriers. The source of the infection appeared to be a carrier among the nursing staff who was somehow responsible for infecting materials and instruments in the operating theatre and infection from this source was then transmitted to surgical patients.

All the strains from healthy individuals, patients with sepsis and the nurse herself, who was probably a carrier of a highly infectious organism, were submitted to the mannitol fermentation test. Over 90 % of the staphylococci were assigned to Grade A and it was impossible on the grounds of grading to distinguish between strains from patients with sepsis, strains from the nurse and strains from healthy carriers.

Grading compared with diffusible antigens

The number of diffusible antigens produced by strains of staphylococci has been considered to be one of the best measures of their pathogenicity or virulence (Elek & Levy, 1950; Howard, 1954; Anderson, 1956). It was, therefore, decided to examine 100 strains selected at random and see if the number of diffusible antigens had any relation to the grades to which the organisms were assigned.

Table 5 gives the distribution of the strains according to the number of flocculation lines they produced. The mean number of lines was 6·50. This compares with means of 6·37 for strains from lesions and 5·19 for strains from carriers found by Elek & Levy (1950). In Table 6 strains belonging to the four grades have been classified into two groups: those producing six or more flocculation lines and those producing less than six.

It will be noted that there is no obvious correlation between the grades assigned to the strains and the number of flocculation lines. For example, with staphylococci of Grade A, previously considered to consist of particularly dangerous organisms, 11 out of 79 strains produced less than 6 flocculation lines. Conversely, of 21 strains belonging to the supposedly less dangerous Grades B, C and D, 16 strains produced 6 or more lines.

Table 5. *Distribution of strains according to number of flocculation lines produced*

Number of flocculation lines	Number of strains
2	1
3	2
4	3
5	10
6	25
7	44
8	13
9	2

Table 6. *Relation of grade to flocculation line production*

Grade	No. of strains	Number of lines produced	
		6 or more	Less than 6
A	79	68 (86%)	11
B	10	8 (80%)	2
C	6	4 (67%)	2
D	5	4 (80%)	1
Total	100	84	16

DISCUSSION

Over the years there have been many attempts to classify staphylococci, especially to see whether those strains which cause severe infections are characterized by any special properties that can be recognized in the laboratory.

From the earliest times it has been considered that those staphylococci which produce a golden pigment are in general more dangerous than those producing a white pigment. When strains have been studied further by fermentation reactions and other simple biochemical tests, various other properties have been noted which appear to parallel either the colour of the colonies or the ability of the organisms to cause disease.

The ability to ferment mannitol is one such test. This property has been frequently studied and there is general agreement that nearly all staphylococci isolated from suppurating lesions have the ability to ferment mannitol; other staphylococci, including most of those with white colonies, are less likely to have marked mannitol fermenting ability (Andrewes & Gordon, 1907; Dudgeon, 1908; Hine, 1922; Dudgeon & Simpson, 1928; Cowan, 1938; Christie & Keogh, 1940; and many other observers). Several of the early workers laid great stress on the differential value of this test, but it was soon realized that mannitol fermentation was also a property of strains which we would now describe as *albus* variants of *Staph. pyogenes*.

Many other properties of staphylococci have been studied and attempts made to correlate these with what has variously been called the 'virulence' or 'patho-

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genicity' of the organisms. Since, in spite of much experimental work, it has not proved possible to devise any convenient laboratory measure of virulence or pathogenicity the definition of these terms is essentially clinical. With this proviso certain conclusions have been drawn. Thus the production of coagulase has usually been considered to be the most useful single test of pathogenicity (Cruickshank, 1937; Christie & Keogh, 1940), but it is clear that quantitative estimation of this property is beset with difficulties (Lack & Wailling, 1954). The production of α-haemolysin is closely correlated with the production of coagulase and less clearly with virulence (Gillespie, Devenish & Cowan, 1939; Schwabacher, Cunliffe, Williams & Harper, 1945; Selbie, 1953; Lack & Wailling, 1954). The production of certain diffusible antigens has an absolute correlation with the production of coagulase and hence with virulence (Elek & Levy, 1950). The production of hyaluronidase (Schwabacher *et al.* 1945) and fibrinolysin (Lack & Wailling, 1954) are other properties which have been shown to be correlated with virulence. The classification of staphylococci by serological methods (Cowan, 1938, 1939; Christie & Keogh, 1940) or by phage-typing (Williams, Rippon & Dowsett, 1953) shows broadly that some groups of staphylococci are more dangerous than others.

When one considers the results of all these different methods it is clear that pathogenicity and virulence depend on many different factors. Statistically it may be true that staphylococci which produce a large number of different toxins, enzymes and antigens are more dangerous than those which produce few. Clearly, it would be desirable to study some of these properties and attempt to grade the degree of danger of staphylococci as a routine clinical procedure. In effect this is what has been done by Knott & Blaikley (1944).

An essential feature of any practical system of grading is that it should present easily recognizable grades. Now any system of grading, which depends on sorting organisms into grades on the results of a number of different tests, must sometimes fail if the number of grades is less than the total number of possible combinations of results, the only exception to this being if some of the tests have a 100 % correlation between one another. For instance, if one uses three tests, each of which can be positive or negative, there should be $2^3 = 8$ grades. If the tests produce answers of greater complexity, such as recognizing four categories of a particular result, then a larger number of grades are possible.

It would be surprising if it were always possible to classify staphylococci into four grades on the basis of differing degrees of pigmentation, coagulase production, haemolysis and mannitol fermentation. In practice the grade assigned must often depend on a further decision as to which property or properties must be given most weight. Unless the rules for making this decision are clearly formulated, there can be no certainty that anyone else using the system of classification will agree on the grades assigned.

Even if the definition of the grades is precise, there is still the question of the reproducibility of the individual tests. In the present work only the fermentation of mannitol has been considered in any detail, and it was found that the reproducibility of this test left much to be desired. Similar variability may be inherent in the other tests.

The second feature of a system of grading of the kind considered here is that it should be of some practical value. There is no doubt that by taking special precautions when staphylococci of Grades A and B were isolated Knott & Blaikley (1944) succeeded in diminishing the rate of sepsis in a maternity department. The question arises whether their success was really due to the grading of the staphylococci or whether it was simply due to the fact that a high proportion of all coagulase-positive staphylococci belong to Grades A and B.

In the studies that we have made over 70 % of the staphylococci were of Grade A. This is a much higher proportion than that found by Knott & Blaikley (1944). For example, from Table 2 of their paper, in 142 strains from cases of infection the proportions were Grade A, 28 %; Grade B, 53 %; Grade C, 15·5 % and Grade D, 3·5 %; in 50 strains from the skin of healthy patients the proportions were Grade A, 2 %; Grade B, 8 %; Grade C, 34 % and Grade D, 56 %. The explanation for the higher incidence of Grade A strains in recent times is not clear. As far as is known there has been no significant change in the way the tests have been carried out. The grading of the results cannot always be a precise procedure and it is possible that the interpretation has changed over the years. However, it is very difficult to see that this can be the sole explanation and it seems highly probable that there has been a real increase in the incidence of staphylococci of Grade A.

We have confirmed that staphylococci from septic lesions are more likely to belong to Grade A than staphylococci from carrier sites, but this conclusion has only been possible after examining a large number of strains. The difference is certainly not an obvious one and there is no valid reason for assuming that the other grades are of low pathogenicity. Several workers (Elek & Levy, 1950; Howard, 1954; Anderson, 1956) have considered that the production of diffusible antigens is one of the best measures of pathogenicity and virulence. If grading were important as a measure of pathogenicity or virulence one would expect an obvious correlation between the grade and the number of flocculation lines. In our experiments this was not so.

From our results we feel that, although the system of grading was previously of value, today it offers no practical help to the clinician: indeed the system may in fact be misleading; for instance, to report a staphylococcus as Grade D may tempt the clinician to dismiss the organism as of little significance when it may be in reality highly dangerous. The information of use to the clinician seems to be whether *Staph. pyogenes* is present or absent, and if present some indication of the numbers of the organisms and their antibiotic sensitivities. In addition it is desirable that the laboratory should preserve all strains of *Staph. pyogenes* for several months, so that if an outbreak of infection occurs it is immediately possible to apply the more precise methods of phage-typing.

SUMMARY

The fermentation of mannitol by coagulase-positive staphylococci has been studied. It was found difficult to obtain reproducible results by methods which could be conveniently used in a routine department.

A method of grading based on the fermentation of mannitol has been examined.

Organisms of the supposedly most dangerous grade (Grade A) were isolated slightly more frequently from lesions than from carriers, but from all sources a high proportion of strains belonged to this grade.

It was not possible to correlate the grades to which staphylococci were assigned with their production of diffusible antigens.

In this hospital it seems that the proportion of strains assigned to Grade A has become very much higher than it was 15 years ago.

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