ON THE DIFFERENTIATION OF THE STAPHYLOCOCCI, WITH SPECIAL REFERENCE TO THE PRECIPITIN REACTIONS.

By LEONARD S. DUDGEON, C.M.G., C.B.E., F.R.C.P. (Lond.),
Professor of Pathology, University of London,
and J. W. HOPE SIMPSON, M.B. (Cantab.), M.R.C.P. (Lond.)

(*From the Department of Pathology, St Thomas's Hospital.*)

Introduction.

This work was undertaken for the purpose of investigating the staphylococci found to be pathogenic to man, but more especially those cocci isolated from cases of septicaemia and pyaemia. It was hoped that as a result of this study a step might be taken towards the preparation of a reliable anti-serum which would be a therapeutic asset of the greatest possible value.

There appears to be a very definite failure on the part of the medical profession to recognise how serious and how frequent are the acute infections caused by these cocci.

The classification of the staphylococci has always been a much-debated problem, beset with many difficulties, owing to the inconstancy of pigment formation, the indefinite cultural reactions, the failure to obtain true serological distinctions with many strains, and the extreme variations in pathogenicity.

This has led to many different attempts by various observers to differentiate the staphylococci.

Andrewes and Gordon (1906) concluded that there were four main types found in man, classing the pathogenic *S. albus* and *S. aureus* together in their large *Staphylococcus pyogenes* group, the remaining three groups being non-pathogenic or only feebly pathogenic and comprising white cocci found in the skin, scurf, saliva, and dust. Some of the strains which they examined, however, could not be fitted into any of these groups, and were regarded as "atypical."

Winslow (1908) classed the parasitic cocci into *Aurococcus* and *Albococcus*, basing the distinction on the difference in pigment, smaller growth on solid media, and more vigorous liquefaction of gelatin shown by the orange cocci. Buchanan (1915) and the Committee on Classification of American Bacteriologists considered the division into Orange and White, as two separate genera, justifiable.

Dudgeon (1908), on the other hand, regarded the orange and white forms of the pathogenic cocci as varieties of a single species, and in this connection it is interesting to note that Winslow, Rothberg and Parsons (1920), after a

detailed study of 180 strains (100 white and 80 orange), of which 104 were pathogenic, came to the conclusion that the typical pathogenic staphylococcus is *aureus*-coloured, vigorously ferments carbohydrates, and liquefies gelatin, and that from this type proceeds every degree of variation, with a progressive weakening of pathogenic and biochemical activity until reaching the saprophytic type of white coccus commonly found on skin and in dust. The latter observers attempted to draw up a rough scheme of classification, basing their types on differences of colour, of fermentation of lactose, and of liquefaction of gelatin.

Cummins and Cumming (1913), using Gordon's tests, found that in some cases, on repeating the reactions at a later date, consistent results were not obtained, and this led them to use serological means of differentiation, employing the opsonic index method and agglutination tests used by Dudgeon in 1908. They concluded that colour was a matter of specific importance, the "yellow" and greyish strains being serologically distinct from the average opaque white *S. albus*, while the majority of the latter were essentially similar.

Hine (1922), by the use of agglutination reactions with 90 strains, found that these could be separated into *S. pyogenes* and *S. epidermidis* groups, the latter being mostly white, and failing to acidify mannite. The *S. pyogenes* group fell into three serological types, the first comprising 90·2 per cent., the second 6·6 per cent. and the third 3·2 per cent. of the strains, while the *S. epidermidis* group contained two types only, 60 per cent. and 40 per cent. respectively. He noted that 30 per cent. of cocci tested were doubtful by agglutination tests alone, but fell into line after employment of absorption tests.

Mellon and Caldwell (1926), by means of agglutination and absorption tests, have described an *S. albus* strain which they regard as a primitive "common ancestor" type from which may have sprung distinct strains of *S. albus* and *S. aureus*, and found that while many of the *S. albus* strains studied were similar to the white component of the "parent" strain, relatively few of the *S. aureus* strains were closely related to the *S. aureus* groups present in the common ancestor. They hold that other "common ancestor" types with multiple agglutinogens may be found, and that the *S. aureus* characteristics are probably developed from such a type *pari passu* with increase of virulence, and that this would make classification based on a single set of characters very difficult.

In view of these conflicting opinions, it was decided to bring to bear a new line of attack upon the problem, and to continue the work of Dudgeon and Bamforth (1925) on the staphylococcal precipitin reactions, with the object of ascertaining whether the staphylococci could be separated by this means into distinct groups, or if any correlation could be found between precipitin formation and any other characteristics such as pigmentation, sugar fermentation, gelatin liquefaction, haemolysis, or pathogenicity to animals.

Methods.

Fifty separate strains of *S. aureus*, and 30 strains of *S. albus* were investigated.

These strains were derived from cultures made from various acute and chronic infections in man. They were not in any way "selected" except that a few strains were discarded on the grounds that their presence in the material examined had no causative connection with the pathological process. They were provisionally classed as *S. aureus* and *S. albus* on grounds of colour only. All were Gram-positive cocci and grew well on the ordinary media.

Cultural Reactions.

Colour of Growth. When, as sometimes happened, there was a difficulty in deciding whether a given strain was *S. albus*, or a poorly pigmented *S. aureus*, it was found that a clear differentiation could be made by planting the strain on to Dorset's egg medium containing 1 per cent. glucose. "Albus" growths were always white in colour on this medium, whereas strains whose colour was doubtful on agar or blood agar invariably showed some degree of orange pigmentation on this egg medium within two or three days.

Other methods of colour differentiation, such as the colour of the deposit after one month's growth in beef broth, or on 3 per cent. peptone agar were tested, but the egg medium was found to be the most satisfactory.

Five of our strains were examined by the method suggested by Bigger, Boland and O'Meara (1927) to see if variant colonies occurred from broth cultures of varying age.

Two well-pigmented strains, *S. aureus* Nos. 1 and 15, were plated on agar plates after 24 hours' growth in broth at 37° C., and again after 6 days' growth. Every colony was found to be "*S. aureus*" in colour, "smooth" and non-viscid.

Three poorly pigmented strains, *S. aureus* Nos. 20, 21, and 26, were examined in the same way, and with the same result. These three strains were again examined after one month's incubation in broth. The colonies of Nos. 21 and 26 were all orange coloured, but *S. aureus* No. 20 produced a fair proportion of colonies resembling *S. albus* in appearance. On examining these with a lens, a small area of yellowish pigment could be seen in the centre of the colony, and six of the whitest colonies were picked off on to egg slopes. On this medium they all produced the "*S. aureus*" colour, but to a somewhat varying degree. Except for colour, these pale colonies in no way differed from the pigmented ones.

Inspissated Blood Serum. All strains were inoculated on to blood serum slopes and examined after 10 days' incubation at 37° C. No digestion of the medium occurred.

Liquefaction of Gelatin. All strains were tested for liquefaction of gelatin in a medium containing gelatin 15 per cent., sodium chloride 0·25 per cent., and peptone 1 per cent. in 1 per cent. of lemco broth.

Tests were first made by incubating the inoculated tubes for 10 days at 37° C., then transferring to the ice safe, and noting if liquefaction was maintained. Any strains failing to liquefy by this method were planted on to gelatin slopes and incubated for 14 days at 22° C. Of the 50 *S. aureus* strains 32 liquefied gelatin at 37° C. and the remaining 18, on re-testing, liquefied at 22° C. Of the 30 *S. albus* strains, 13 liquefied at 37° C., 6 at 22° C. and the remaining 11 failed to liquefy by either method.

Sugar Fermentation. Every strain was tested in 1 per cent. of lactose and mannite, in 1 per cent. of lemco broth, containing 1 per cent. of sugar-free peptone. The reaction was adjusted to *p*H 7·5 before sterilisation, and 1·0 c.c. of a 0·06 per cent. solution of phenol red was added as the indicator. The inoculated tubes were incubated at 37° C., examined every other day, and the final readings taken after 10 days' incubation.

Lactose was acidified, without gas production, by every *S. aureus* strain after 24 hours' growth, except in the case of *S. aureus* No. 36 which took 48 hours. The acid reaction remained after 14 days' incubation. Of the 30 *S. albus* strains, all except three (Nos. 14, 27 and 29) acidified lactose, but No. 2 took nine days and No. 13 ten days to acidify. The acid reaction was permanent, as in the case of the *S. aureus* strains.

Mannite. Of the *S. aureus* strains 47, or 94 per cent., acidified this medium without gas production, and 3, or 6 per cent., remained neutral. Of the *S. albus* strains 12, or 40 per cent., acidified this medium without gas production, 6, or 20 per cent., remained neutral, and 12, or 40 per cent., rendered the medium alkaline.

Haemolysis. Tests for haemolysis were made on blood-agar plates, prepared by adding 0·9 c.c. of oxalated human blood to a melted tube of 10 c.c. of agar at a temperature of 50° C., and immediately pouring out. Attempts were also made to produce haemolysis in a medium containing a suspension of human red cells in 1 per cent. peptone, 0·5 per cent. sodium chloride and 0·1 per cent. agar, both at 37° C. and at 22° C.

The blood media were inoculated from 24-hour agar cultures. On blood agar plates the results were read after 24 hours' incubation at 37° C. Thirty-four *S. aureus* strains, or 68 per cent., and 17 *S. albus* strains, or 56·6 per cent., were found to be haemolytic. In the liquid medium referred to no haemolysis could be demonstrated with any of our *S. aureus* or *S. albus* strains.

A haemolysin was prepared from 7 haemolytic *S. aureus* strains, Nos. 1, 2, 3, 20, 24, 27 and 28, by the method recently described by Bigger, Boland and O'Meara (1927).

S. aureus No. 1. No haemolysis was obtained with either sheep or human cells.

S. aureus No. 2. A trace of haemolysis occurred in a dilution of 1 in 4, and a faint trace at 1 in 8 with sheep cells, and a faint trace at 1 in 4 with human cells.

S. aureus No. 3. Marked haemolysis was obtained at 1 in 8, and a trace

at 1 in 16 with sheep cells, and a faint trace at 1 in 4 only with human cells.

In the case of *S. aureus* Nos. 20, 24, 27 and 28, haemolysis occurred with human cells in these dilutions:

S. aureus No. 20, *end point* 1 in 32, *S. aureus* No. 24, 1 in 16, *S. aureus* No. 27, 1 in 32, and *S. aureus* No. 28, 1 in 64.

Immunisation of Rabbits for the Preparation of Precipitin Anti-Sera.

All rabbits before use were tested against active *S. aureus* and *S. albus* antigens, and, whenever possible, against the antigen of the organism used for the injection. This was to guard against the possibility shown by Dudgeon and Bamforth that normal rabbit's blood may contain a factor which can react with staphylococcal precipitin antigens. Only rabbits which showed no reaction were used for immunisation.

S. aureus.

Two preliminary doses of vaccine were given at 5-day intervals, followed by a small dose of the living cultures. Serum was taken for testing 4 to 5 days later, *e.g.*:

11. v. 27. Inj. *S. aureus* vaccine No. 15 200 mill. intravenous.
16. v. 27. ,, ,, ,, 500 ,, ,,
21. v. 27. ,, living ,, 50 ,, ,,
25. v. 27. Serum taken and tested on 26. v. 27.

It was found that an efficient serum was not obtained until after the injection of *live* cultures. In most cases, following the injection of 50 million live *S. aureus*, the serum gave a good reaction against its own antigen, but in some instances larger doses of live *S. aureus* had to be given before any reaction appeared, while in two cases, Nos. 13 and 21, doses of 250 million live cocci failed to produce a serum which reacted against its corresponding antigen.

S. albus.

The procedure was at first similar to that of *S. aureus*, but it was soon realised that very few of the *S. albus* strains were pathogenic to rabbits, and hence in the later part of the work the preliminary vaccine injections were dispensed with. In two cases up to 5000 million living cocci were injected without effect. In general, however, it was found that if doses of 250 million living *S. albus* failed to produce an active anti-serum, further increase of dosage had no result.

Anti-Sera.

It was found that the period of maximum activity of the anti-sera varied with different strains, but seldom lasted longer than 3–10 days after the last injection of the live cocci, while the titre invariably fell shortly before the

death of the animal. The titre of stored serum also fell somewhat rapidly, even when stored in the ice safe, and was generally reduced by 50 per cent. after 2 or 3 weeks' storage. Hence it was necessary to test each fresh sample of serum against its own antigen before using it on other antigens, in order to safeguard against reduction of the titre.

PATHOGENICITY OF CULTURES TESTED.

Several strains were tested on rabbits with a single dose of living cocci, apart from the strains used for the production of anti-sera. A very marked difference was found between the pathogenicity of the average *S. aureus* strain to normal rabbits, and that of the average *S. albus* strain. Nineteen out of 25 *S. aureus* strains proved fatal to rabbits from a single injection of about 280 million cocci. Only 3 out of 18 *S. albus* strains caused the death of the rabbits from an injection of about 580 million cocci. Doses of 1200 million cocci of the non-pathogenic strains were injected without effect. Thus it will be seen that a much greater percentage of *S. aureus* strains are pathogenic than of *S. albus* strains, and further that the dose of *S. aureus* necessary to produce the death of rabbits is considerably less than the average *S. albus* dose.

The mortality from injections of living *S. aureus* and *S. albus* among vaccinated rabbits as compared with unvaccinated is as follows:

Vaccinated	Died	Survived	Total
S. aureus	8	4	12
S. albus	2	4	6
Unvaccinated	**Died**	**Survived**	**Total**
S. aureus	11	2	13
S. albus	1	11	12

These results appear to indicate that vaccination is of little value among rabbits injected with large doses of live staphylococci, but the vaccinated rabbits survive longer than the unvaccinated, and the pathological findings were, on the whole, different.

PATHOLOGICAL FINDINGS.

With few exceptions the majority of unvaccinated rabbits die within 2 or 3 weeks of injection, showing loss of weight, general weakness, abscesses in the kidneys, and, in two cases, abscesses in the heart muscle.

The vaccinated rabbits showed visceral lesions more rarely, and death was usually preceded by a definite flaccid paralysis, commencing in the hind limbs, and spreading to the fore limbs. It was found that animals did not survive this paralysis, so that in the later experiments they were chloroformed as soon as definite symptoms appeared.

Sections of the spinal cords of these paralytic rabbits showed varying degrees of leptomeningitis, with degeneration of anterior horn cells and a polymorph reaction spreading along the vessels of the cord. Staphylococci

were grown from the spinal meninges in several cases, and could be readily obtained from the visceral abscesses.

Blood cultures made in a few instances generally gave positive results. There was no essential difference in the lesions produced from injections of *S. albus* or *S. aureus*. On no occasion have we noticed any alteration in the colour or cultural characteristics of a strain after passage through rabbits. This point was specially investigated in connection with three pathogenic strains of *S. albus*, Nos. 8. 19 and 24, as it was thought that these might be strains of *S. aureus* with pigment formation temporarily in abeyance.

A few pathogenic strains of *S. aureus* and *S. albus* were inoculated into guinea-pigs and mice, but large doses failed to produce lesions in these animals.

Precipitin Reactions.

These were carried out by the technique described by Dudgeon and Bamforth (1925).

The rabbit sera were heated for 30 minutes at 55° C. before use. Each anti-serum was put up in dilutions of 1 in 5, 1 in 10, and 1 in 40, and quantities of 1·0 c.c. of diluted serum were added to 0·5 c.c. of the filtered antigens. The resulting serum dilutions were thus 1 in 7·5, 1 in 15, and 1 in 60.

Saline containing 0·25 per cent. of phenol was used for the dilutions and control tubes of serum and antigen were always employed. The reactions were carried out in the water-bath at 52° C. and the results were read after 24 hours.

Fifteen *S. aureus* and 15 *S. albus* anti-sera were prepared from rabbits by the methods described above, and, as far as possible, each serum was first tested against its corresponding antigen before testing it against other antigens.

Of the 15 *S. aureus* sera, 11 gave a positive result against the corresponding antigen; 2 were not tested owing to shortage of antigen, and 2 gave a negative result (Nos. 21 and 13). *S. aureus* antigen No. 21, however, failed to react with any anti-serum, and the antigen of *S. aureus* No. 13 reacted only with 1 out of 8 *S. aureus* anti-sera (No. 3), while anti-serum No. 13 reacted with *S. aureus* antigen (No. 3) only. Both these anti-sera (Nos. 21 and 13) were prepared from strains non-pathogenic to rabbits.

Of the 15 *S. albus* sera, 6 gave a positive result against the corresponding antigen, and 9 failed to react; of the antigens corresponding to these 9 anti-sera, 7 failed to react with any anti-serum. The remaining 2 antigens, *S. albus* No. 2 and *S. albus* No. 27, reacted with 1 out of 7 anti-sera, and 2 out of 8 anti-sera respectively.

Thus it will be seen that only 1 *S. aureus* serum, and 2 *S. albus* sera, failed to react with their corresponding antigens, if we exclude the 8 cases (1 *S. aureus* and 7 *S. albus*) in which the antigens remained persistently negative with all sera against which they were tested.

It was not found possible to test each serum against every antigen, as the amount of serum required for each reaction is at least 0·325 c.c., and in many cases the titre of the serum fell off rapidly *in vivo* before sufficient serum had

been obtained from the animal. This was especially noticeable in the case of those strains which were pathogenic to rabbits, and a rabbit which reacted well with its own antigen one day might fail to react when tested a day or two later. The difficulties were increased by the rapid falling off of the titre of stored serum.

Hence only 2 sera, *S. aureus* No. 15 and *S. albus* No. 8, were tested against every antigen, and for this purpose one rabbit in the case of *S. aureus* No. 15, and three rabbits in the case of *S. albus* No. 8 were used. The number of antigens tested with the remaining 28 sera varied from 2 to 71 with an average of 18·0 per serum.

At the outset it was hoped that it might be possible to classify the strains tested into several large groups with common precipitation factors, or at any rate to distinguish between *S. albus* and *S. aureus* strains by this means, but it was soon realised that though for the most part *S. albus* antigens failed to react with *S. aureus* sera, there were several exceptions to this rule, and also that *S. albus* sera were almost as efficient as *S. aureus* sera when tested on *S. aureus* antigens.

It was also shown that in many cases a given anti-serum reacted more strongly with other antigens than with its own antigen, thus tending to throw doubt on the value of any attempt to differentiate between one strain and another on the grounds of the precipitin reaction alone.

On reviewing the reactions as a whole, however, without attaching undue importance to the variations of individual strains, certain general tendencies have been observed, and a close degree of correlation between the virulence of a strain and its tendency to form precipitins has been noticed.

In Tables I, II and III the results are so recorded as to include in column A those reactions with a lower limit of "+," and in column B those with a lower limit of "trace," all reactions weaker than these being taken as negative.

A good average reaction may show 1 in 5 +++, 1 in 10 ++, 1 in 40 +, 1 in 80 Trace, 1 in 160 Very faint trace, but in some cases a zone was observed, *e.g.* 1 in 5 Trace, 1 in 10 ++, 1 in 40 +, 1 in 80 Trace. Reactions marked +, ++, +++, or ++++ show a deposit of increasing amount at the bottom of the tube, but usually with some turbidity of the supernatant fluid. "Trace" reactions show a fine precipitate spread throughout the tube and appreciable by the naked eye. "Faint trace" and "Very faint trace" are smaller precipitates, only distinguishable with a hand lens.

Table I.

Antigens	Anti-sera	Total reactions	A	%	B	%
S. aureus	*S. aureus*	267	167	63·0	187	70·6
S. aureus	*S. albus*	178	88	49·2	108	59·5
S. albus	*S. aureus*	83	14	16·8	18	21·6
S. albus	*S. albus*	137	27	19·7	33	24·0

The above table shows that *S. aureus* antigens with *S. aureus* anti-sera give by far the largest percentage of positive results, whereas *S. albus* antigens,

with either *S. aureus* or *S. albus* anti-sera, show a low percentage of positive reactions. It would seem from these figures that the selection of the antigen is of far greater importance in the reaction than the anti-serum. This point will be further elucidated in considering the reactions of those antigens in which the virulence of the strain to rabbits has been determined, and which are taken separately in Table II.

Table II.

No.	Antigens	Anti-sera	Total reactions	A	%	B	%
19	Pathogenic *S. aureus*	*S. aureus*	112	83	74·1	93	83·0
	Ditto	*S. albus*	80	40	50	51	63·7
6	Non-pathogenic *S. aureus*	*S. aureus*	34	9	26·4	11	32·3
	Ditto	*S. albus*	18	4	22·2	5	27·7
3	Pathogenic *S. albus*	*S. aureus*	12	6	50	6	50
	Ditto	*S. albus*	19	5	26·3	8	42·1
15	Non-pathogenic *S. albus*	*S. aureus*	42	4	9·5	7	16·6
	Ditto	*S. albus*	71	12	16·9	13	18·3

In Table II is shown a very striking difference in the number of positive reactions obtained with antigens made from pathogenic strains, as compared with antigens from non-pathogenic strains of *S. albus* and *S. aureus*.

The difference is more marked in the reactions with *S. aureus* anti-sera than with *S. albus* sera, and this may possibly be due to the fact that 11 out of 15 *S. aureus* anti-sera were prepared from pathogenic strains, as compared with 3 out of 15 *S. albus* anti-sera.

When we analyse the results obtained with the anti-sera prepared from pathogenic cocci as compared with the non-pathogenic, it would appear that the anti-sera prepared from the pathogenic strains have no advantage over the others—in fact, the converse appears to be true, especially in reactions with *S. albus* antigens.

As might be expected, *S. aureus* anti-sera are more active than *S. albus* anti-sera with *S. aureus* antigens, but less so with *S. albus* antigens. The great difference between *S. aureus* and *S. albus* strains is apparent when the antigens are compared, as contrasted with the anti-sera, as is shown in Table III. The anti-sera derived from pathogenic strains are shown separately.

Table III.

No.	Anti-sera	Antigens	Total reactions	A	%	B	%
11	Pathogenic *S. aureus*	*S. aureus*	210	125	60·0	141	65·8
	Ditto	*S. albus*	63	5	7·9	8	12·7
4	Non-pathogenic *S. aureus*	*S. aureus*	57	42	73·7	46	80·7
	Ditto	*S. albus*	20	9	45	10	50
3	Pathogenic *S. albus*	*S. aureus*	92	47	51·1	54	58·7
	Ditto	*S. albus*	55	7	12·7	9	16·4
12	Non-pathogenic *S. albus*	*S. aureus*	86	41	47·7	54	63·0
	Ditto	*S. albus*	82	20	24·4	24	29·3

From the results of the precipitin reactions as a whole, we are led to the conclusion that, whereas an efficient anti-serum can be obtained by the

immunisation of rabbits with strains of *S. aureus* or of *S. albus*, a far higher proportion of *S. aureus* strains are efficient in this respect than of *S. albus*; on the other hand, when an active *S. albus* anti-serum is obtained, it reacts as efficiently as an *S. aureus* anti-serum on *S. aureus* and *S. albus* antigens. In this connection it may be of interest to refer to the results obtained by Dudgeon and Bamforth with the serum of patients suffering from serum sickness which showed a higher percentage of positive reactions than the serum of patients suffering from a known *S. aureus* infection with *S. aureus* antigens.

The striking differences between *S. aureus* and *S. albus* antigens and between antigens prepared from pathogenic and non-pathogenic strains cannot be explained by a selective action of the anti-sera, but rather to the greater power of certain strains to elaborate an efficient antigen from peptone broth. This property appears to be developed in proportion to the increase in virulence, biochemical activity and pathogenicity to rabbits.

On the Formation of Precipitin Antigens by the Staphylococci.

It would appear from the results obtained by us that those strains of staphylococci which produce the most efficient antigens are those which were pathogenic for rabbits. The order of efficiency was as follows:

(1) Pathogenic strains of *S. aureus* 19 in number.
(2) ,, ,, *S. albus* 3 ,,
(3) Non-pathogenic ,, *S. aureus* 6 ,,
(4) ,, ,, *S. albus* 15 ,,

It must be fully understood that the staphylococci which we have investigated were obtained from infective processes in man. Our distinction of pathogenic and non-pathogenic is based on the experimental observations on rabbits with the dosage already referred to. We found also that the most deeply pigmented strains of *S. aureus* produced more efficient antigens than the feebly pigmented cocci.

On the Nature of the Precipitin Deposits.

The deposit in the various tubes, which was examined microscopically in 47 cases, taken at random, was either "granular," like fine sand, or "flocculent," resembling cotton-wool. A few showed an uniform turbidity throughout the tube, the deposit on centrifugalisation resembling the granular type.

Microscopically, the "granular" deposits were seen to consist of fine clusters of crystalline needles, together with a variable number of thin rhomboidal plates, which could only be properly identified under a 1/12″ objective.

The "flocculent" deposits consisted microscopically of clumps of amorphous debris.

In a minority of the tubes a mixture of both types of deposit was present.

Eleven deposits obtained from precipitin reactions with other organisms were also examined as controls. The results are recorded in Table IV.

Table IV.

Anti-sera	Antigens	Result: Amorphous	Result: Crystalline	Result: Amorphous and crystalline
S. albus	*S. aureus*	5	5	3
S. albus	*S. albus*	11	0	1*
S. aureus	*S. aureus*	8	11	3
B. coli	*B. coli*	2	0	0
B. Shiga	*B. Shiga*	6	0	0
B. Friedlander	*B. Friedlander*	3	0	0

It will be noticed that the crystalline deposit occurred only with an *S. aureus* antigen and an *S. albus* or *S. aureus* anti-serum with one exception (*) where a few crystals were present in a large amorphous deposit formed by *S. albus* anti-serum and *S. albus* antigen.

Summary and Conclusions.

(1) Fifty *S. aureus* and 30 *S. albus* strains obtained from infective processes in man have been investigated. Such cultural reactions as were completed did not differentiate between strains of *S. albus* and *S. aureus*, or serve to group these cocci; we found, however, that 94 per cent. of the *S. aureus* strains fermented mannite, as opposed to 40 per cent. of the *S. albus* strains. These results are in accordance with the findings of Cummins and Cumming (1913) and Hine (1922).

(2) A close degree of correlation between pathogenicity for rabbits, pigment formation, and biochemical activity was noticed among the *S. aureus* strains. Out of 19 pathogenic strains of *S. aureus*, 9 showed medium pigmentation and 10 were strongly pigmented.

(3) Anti-sera were prepared by inoculating rabbits with living cultures, 24 hours old, of the various strains. The *S. aureus* rabbits and a few of the *S. albus* received two preliminary doses of the specific vaccine, but little or no protective action was afforded from the doses of living cocci injected.

(4) Nineteen out of 25 cultures of *S. aureus* killed rabbits, but only 3 out of 18 cultures of *S. albus*.

(5) Fifteen strains of *S. aureus* and 15 of *S. albus* were used for the preparation of anti-sera, and tested against as many antigens as possible, by the methods described by Dudgeon and Bamforth.

(6) Cultures of *S. aureus* produced efficient anti-sera more readily than *S. albus* cultures, but when an active *S. albus* anti-serum was produced, it appeared to be as effective as the *S. aureus* anti-sera.

(7) A marked difference in efficiency between *S. aureus* and *S. albus* antigens was noted. *S. aureus* antigens were almost three times more active than *S. albus* antigens.

(8) Pathogenic strains of *S. aureus* and *S. albus* produced more efficient precipitin antigens than non-pathogenic.

(9) From the microscopical examination of the deposit formed in the precipitin reactions, a difference was noted between *S. aureus* and *S. albus* antigens.

(10) The study of the precipitin reactions has failed to show any hard and fast dividing line between staphylococci of different colours, or of different cultural reactions. We are inclined to agree with Winslow, Rothberg and Parsons (1920) and with Dudgeon's original view in 1908, that the pyogenic, Gram-positive staphylococci must be regarded as members of one common family.

This is represented at one end of the scale by the strongly pigmented *S. aureus*, pathogenic to man and rabbits, haemolytic, liquefying gelatin, fermenting lactose and mannite, and forming active precipitin antigens. At the other end of the scale we find a non-pigmented *S. albus*, feebly pathogenic to man, non-pathogenic to rabbits, often non-haemolytic, failing to liquefy gelatin, failing to ferment mannite, and without the power of forming precipitin antigens from peptone broth, or of producing active anti-sera in rabbits.

Between these extremes there appear to be a large number of strains which possess many factors in common, but without sufficiently persistent characteristics to enable them to be classified by any exact methods.

It would appear possible that the characteristics shown by the typical active strains of *S. aureus* may be developed by passage from patient to patient from the less active *S. albus* forms *pari passu* with increase in virulence, and that the formation of precipitins goes hand in hand with a more specialised parasitic activity developed by such strains.

APPENDIX.

The Cultural and Precipitin Reactions of the Strains Examined.

The *S. aureus* strains were isolated from cases of abscess, bronchitis, furunculosis, septicaemia, arthritis, osteomyelitis, enteritis, nephritis, tonsilitis and rhinitis.

The *S. albus* strains were obtained from similar sources with the exception of furunculosis, and the addition of acne, sycosis, mastitis, cervicitis and conjunctivitis.

S. aureus *Strains.*

No. of strain	Cultural reactions: Haemolysis on blood-agar	Lactose fermentation	Mannite fermentation	Gelatin liquefaction	Pigment on agar	Pathogenicity to rabbits	Precipitin reactions: *S. aureus* antigens and *S. aureus* sera: Positive	*S. aureus* antigens and *S. aureus* sera: Negative	*S. aureus* antigens and *S. albus* sera: Positive	*S. aureus* antigens and *S. albus* sera: Negative
1	H	A	A	L	Deep	+	13	2	7	7
2	H	A	A	L	„	+	7	2	3	3
3	H	A	A	L	Medium	+	8	2	4	2
4	H	A	A	L	Deep	+	3	0	3	3
5	H	A	A	L	Weak	0	4	2	3	1
6	NH	A	A	L	Medium	+	7	0	4	0
7	H	A	A	L	Deep	0	2	0	0	1
8	H	A	A	L	Medium	0	4	1	4	0
9	NH	A	A	L	Deep	+	3	3	2	2
10	H	A	A	L	„	0	5	2	2	1
11	H	A	A	L	„	0	5	1	2	1
12	H	A	A	L	„	+	5	1	2	1
13	NH	A	A	L	Weak	−	1	7	1	3
14	NH	A	A	L	Medium	+	3	3	3	1
15	NH	A	A	L	Deep	+	8	1	0	3
16	H	A	A	L	„	0	6	0	3	0
17	H	A	A	L	Medium	−	6	1	3	0
18	H	A	A	L	„	0	5	0	3	0
19	H	A	A	L	„	0	4	3	1	3
20	H	A	A	L	„	0	0	8	0	4
21	NH	A	N	L	Weak	−	0	8	0	4
22	NH	A	A	L	Medium	0	5	0	2	0
23	NH	A	A	L	„	0	4	1	4	0
24	H	A	A	L	Weak	0	4	0	2	1
25	H	A	A	L	Medium	+	4	0	2	0
26	NH	A	A	L	„	+	5	0	1	1
27	H	A	A	L	„	0	3	1	2	0
28	H	A	A	L	Weak	0	2	3	3	0
29	NH	A	A	L	Medium	0	0	5	1	2
30	H	A	A	L	Weak	−	2	2	1	2
31	NH	A	A	L	Medium	0	1	3	1	4
32	H	A	A	L	Deep	+	5	1	3	0
33*	H	A	A	L	Medium	0	2	3	1	2
34*	NH	A	A	L	„	0	2	3	1	2
35	H	A	A	L	„	+	2	4	1	3
36	NH	A	N	L	Weak	−	2	5	0	4
37	NH	A	A	L	Deep	0	2	2	2	2
38*	H	A	N	L	Medium	+	4	0	3	1
39*	NH	A	A	L	„	+	4	0	3	1
40	NH	A	A	L	„	0	4	0	3	0
41	H	A	A	L	„	0	4	0	3	0
42	H	A	A	L	Deep	0	4	0	2	0
43	H	A	A	L	„	+	4	0	2	0
44	H	A	A	L	Weak	0	4	0	2	0
45	H	A	A	L	Medium	0	3	0	2	0
46	H	A	A	L	Deep	+	3	0	3	0
47	H	A	A	L	„	+	3	0	3	0
48	H	A	A	L	„	0	2	0	1	2
49	H	A	A	L	„	0	2	0	2	1
50	H	A	A	L	Medium	+	2	0	2	1

H = haemolytic; NH = non-haemolytic; A = acid; N = neutral; L = liquefaction; + = pathogenic; − = non-pathogenic; 0 = not tested for pathogenicity.

* Nos. 33 and 34 and Nos. 38 and 39 are haemolytic and non-haemolytic cocci from the same cases.

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S. albus *Strains.*

No. of strain	Cultural reactions: Haemolysis on blood-agar	Lactose fermentation	Mannite fermentation	Gelatin liquefaction	Pathogenicity to rabbits	Precipitin reactions: *S. albus* antigens and *S. aureus* sera: Positive	*S. albus* antigens and *S. aureus* sera: Negative	*S. albus* antigens and *S. albus* sera: Positive	*S. albus* antigens and *S. albus* sera: Negative
1	NH	A	A	—	–	0	3	2	4
2	H	A	N	L	–	1	3	1	4
3	NH	A	A	L	–	2	1	0	3
4	NH	A	A	L	–	1	2	0	5
5	NH	A	N	L	0	0	4	0	5
6	NH	A	N	—	–	0	4	0	6
7	NH	A	A	—	–	0	3	0	5
8	NH	A	A	L	+	4	2	3	7
9	H	A	N	L	–	0	3	0	4
10	NH	A	Alk.	L	0	2	2	1	5
11	NH	A	N	—	–	0	3	0	6
12	H	A	Alk.	L	–	0	2	1	3
13	H	A	A	—	0	0	2	0	3
14	H	Alk.	A	—	0	0	3	0	4
15	H	A	Alk.	L	0	2	3	2	3
16	H	A	Alk.	L	0	1	4	2	3
17	—	—	N	—	0	0	1	0	4
18	H	A	A	L	–	3	2	3	2
19	H	A	A	L	+	2	3	2	3
20	H	A	Alk.	L	–	0	4	0	4
21	NH	A	Alk.	L	–	0	2	2	2
22	H	A	A	—	–	0	1	2	2
23	NH	A	A	L	0	0	1	2	1
24	H	A	A	—	+	0	1	3	1
25	NH	A	Alk.	L	0	0	1	2	1
26	H	A	Alk.	L	0	0	1	1	2
27	H	Alk.	Alk.	—	–	0	1	1	2
28	H	A	Alk.	L	0	0	1	1	2
29	H	Alk.	Alk.	—	0	0	1	1	2
30	H	A	Alk.	L	–	0	1	0	3

H = haemolytic; NH = non-haemolytic; A = acid; N = neutral; Alk. = alkaline; L = liquefaction; + = pathogenic; – = non-pathogenic; 0 = not tested for pathogenicity.

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