COAGULASE AND HEMOLYSIN TESTS AS MEASURES OF THE PATHOGENICITY OF STAPHYLOCOCCI[1]

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Except in the case of abscesses and certain skin infections, early studies of staphylococci furnished no definite information concerning their relation to disease. The term "skin cocci," used to designate albus varieties found in the skin, was in common use and even to-day persists in such trinomials as "*Staphylococcus epidermidis albus.*" On the other hand, it was recognized early that cultures from pyogenic foci usually revealed pigmented types. This gave rise to a separation of the species into varieties based on their pigment production. It was generally considered that the white strains were not pathogenic. In this paper we will outline methods which we consider of value for the differentiation of pathogenic from non-pathogenic staphylococci.

Many authors have shown that *Staph. albus*, and many of the intermediate-colored varieties, could be derived from *Staph. aureus*. Pinner and Voldrich (1932) also demonstrated that, of the strains which they examined, the yellow were more pathogenic than the white. Duran-Reynals (1933) showed that the albus variants differed from aureus parent strains in hemolysin production and the liquefaction of gelatin. He also found that all the very invasive strains were of the aureus variety while about 82 per cent of his non-invasive strains were of the albus variety.

While a number of investigators have studied the pathogenicity of staphylococci, the results have been somewhat confusing as the following survey of the literature will demonstrate.

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It is well known that *Staph. aureus* produces a toxin which, under usual cultural conditions, is constant for the strain (Panton and Valentine, 1932). That this may not always be true was shown by Bigger (1933) who demonstrated that the lysin-producing power may vary from time to time. He suggested that this was due to the presence of both lysogenic and non-lysogenic strains in his cultures, the relative overgrowth of one or the other of which led to alterations in the lysogenic power of the whole culture. Most authorities consider that the outstanding feature of a staphylococcus toxin is its hemolysin (staphylolysin) and, on this basis, Burnet (1929) introduced a method of titrating the toxin which was based on its hemolytic activity. He showed that, using rabbit erythrocytes, the hemolytic titre of a toxin was proportional to its toxicity for rabbits. This was confirmed by Panton and Valentine (1932) and Dolman (1932), who stressed the fact that the hemolytic titre may not be reliable with the cells of species other than rabbits. Panton and Valentine concluded that the human and rabbit hemolysins were distinct.

Parker (1924) considered the hemotoxin to be distinct from the rabbit lethal factor and this view was confirmed by Burky (1933) who observed that one of his strains lost its hemolytic power coincident with an increase in its lethal factor. This suggested that while the hemolysin and the rabbit lethal factor are closely related, some factor other than hemolysis may be responsible for the pathogenic effects of certain strains. Burky described three strains. The first was strongly hemolytic and killed rabbits within 24 hours with a general toxemia but no abscess formation. The second strain was only moderately hemolytic and the rabbits either survived or died in a week with multiple abscesses and loss of weight. The third strain was not hemolytic and had no effect on rabbits.

Jones (1930) produced dissociants of *Staph. albus* which were either hemolytic and non-proteolytic (non-gelatinolytic?) or non-hemolytic and proteolytic (gelatinolytic?).

The parallel between the hemolysin content of staphylococcus toxin and the amount of skin-necrosing toxin present was dem-

onstrated by Panton (1932) and Panton and Valentine (1932). Dolman (1932) showed that the heat lability of the dermonecrotic property is marked and closely corresponds to that of the hemolytic property for rabbit erythrocytes. Nicolle and Cesari (quoted by Burnet, 1929) found that their killing toxins also produced marked skin lesions when injected subcutaneously. On the other hand, Weld and Gunther (1931) concluded that the necrotoxin was distinct from the hemotoxin or the leucocidin and Burnet stated that filtrates having no skin-necrosing power may be active hemolysins but that no skin-active filtrates lacking hemolysin had been reported.

Panton and Valentine (1932) showed that, on the basis of 22 strains, the leucocidin was distinct from the hemolysin and divided staphylococci into the four following types based on these two factors:

GROUP	PATHOLOGIC CHARACTERS	LEUCOCIDIN	HEMOLYSIN
1	Produces severe lesions	Strong	Weak
2	Boils and carbuncles	Strong	Strong
3	Sycosis	Weak	Strong
4	Mainly saprophytic	Weak	Weak

This classification was also used by Panton (1932). These authors found that the leucocidin was related to the acute killing toxin. But according to Weld and Gunther (1931), strains having negative hemolysin reactions but positive leucocidin had no lethal effect on rabbits. Julianelle (1922) showed that the hemolytic titre for horse red cells did not correspond to leucocidin production but his comparison may not be of value, because, as previously mentioned, only the red cells of rabbits have been shown to be reliable in studying the toxicity of staphylococci. Finally, Pike (1934) showed that leucocidin differed from the substance which had a depressing action on phagocytes and stated that leucocidin may be identical with hemolysin. On the basis of Van de Velde's experiments, he concluded that leucocidin was not a primary cause of virulence. In this connection, it is of interest that Gay and Oram (1933) found that the most

potent streptococcus leucocidin might be given in relatively large amounts to rabbits without obvious effect.

In a very complete study of these phenomena, Burnet (1930) showed that the presence of small amounts of potassium and magnesium were necessary for, but that calcium was actively inhibitory to, hemolysin production. When the calcium was precipitated by the use of oxalated medium, the yield of hemolysin was identical with that produced by growth in an atmosphere of 20 per cent CO_2 (which is optimal for hemolysin production). Growth in oxalated medium gave rise to variants which produced hemolysin in the absence of CO_2. These variants retained their specific susceptibility to special phage. The hemolysin produced by the albus variants showed all the properties described for staphylococcal toxin. For these reasons, Burnet concluded that the various toxins were manifestations of one common toxin. A similar view was presented by Burnet and Freeman (1932), Gengou (1930) and Weld and Gunther (1931).

In dealing with food-poisoning strains, Jordan (1931), Dack, Jordan and Woolpert (1931) and Jordan and Hall (1931) found that both albus and aureus varieties were among those yielding toxic filtrates. Burnet (1930) reached a similar conclusion, and Panton, Valentine and Dix (1931) showed that the toxin production of *Staphylococcus aureus* appeared to be independent of the pigment which had neither toxic nor antigenic properties and which was of the nature of a carotin. Panton and Valentine (1932) demonstrated that there was no association between cultural characters and toxin production, one of their non-toxic strains giving "typical cultural reactions." From these observations, and from the experimental data illustrated in figure 1, it is clear that the color of any culture is not a reliable guide to its toxicity.

Granted that staphylococcal toxins are often hemolytic, there is conclusive evidence in the literature cited of the existence of toxic non-hemolytic strains. Pinner and Voldrich (1932) demonstrated that the golden yellow strains which they studied produced hemolysis, usually liquefied gelatin, coagulated citrated plasma and produced alkali. Since hemolysis and coagulase activity tests of staphylococci have been shown to roughly

parallel the pathogenic effects on rabbits (Pinner and Voldrich, 1932 and Daranyi, 1926), we included both tests in a study of the toxicity of this species. The test for alkali production was not as simple as that for coagulase activity and required several days for its determination. For this reason it was temporarily abandoned in favor of the test for coagulase activity.

We were particularly interested in the coagulase test because it had been overlooked by most writers on the subject, particularly English-speaking ones. Burnet (1930) referred to it as "Staphylokinase" and mentioned the fact that, after three hours incubation, only the aureus types showed coagulation but that the albus types showed clotting when left over night. It was interesting to note that rabbits killed by the acute killing toxin did not show intravascular thromboses and the heart blood clotted in the normal time (Burnet, 1929).

Gross (1933) called attention to the fact that, in operations during staphylococcic diseases, for example in surgical interventions on carbuncles or on osteomyelitis, blood coagulation was frequently accelerated. He pointed out that this observation was reported as early as 1908 by Much, who showed that the coagulating power was most pronounced in *Staph. aureus* infections. He mentioned other authors who found that only the pyogenic staphylococci possessed the coagulating power. He was able to confirm the fact that, to a certain extent, the test permits a classification of pathogenic and saprophytic staphylococci. An anti-coagulase was described.

Using hemolysis of rabbit blood agar, coagulation of citrated rabbit blood and coagulation of milk, with or without peptic digestion, and comparing these reactions with the effect of subcutaneous injection of the strains into rabbits, Daranyi (1926) divided his "more than 30 strains" into the following groups:

GROUP	MILK	PLASMA COAGULASE	HEMOLYSIS	RABBIT INOCULATION	CLASSIFICATION
1	Negative	Negative	Negative	No pus	Saprophytic
2	Positive	Negative	Positive	Pus in 6–10 days	Saprophytic
3	Positive*	Positive	Strong	Pus in 2–3 days, with focal necrosis	Parasitic

* Often peptonizing.

In order to test the relative importance of the hemolysis and coagulase-activity tests, we made comparative tests on over

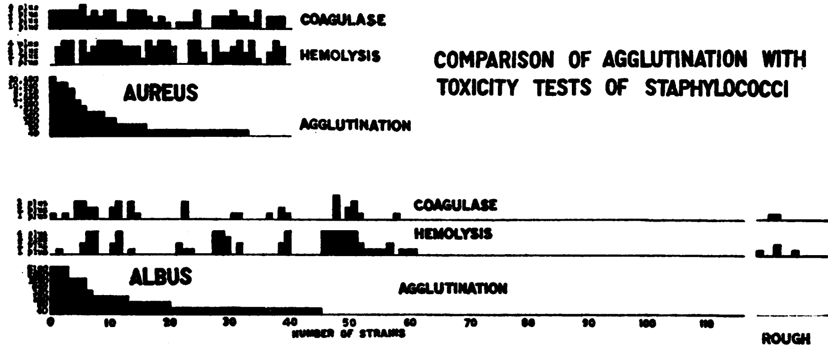

FIG. 1. COMPARISON OF TOXICITY WITH AGGLUTINABILITY

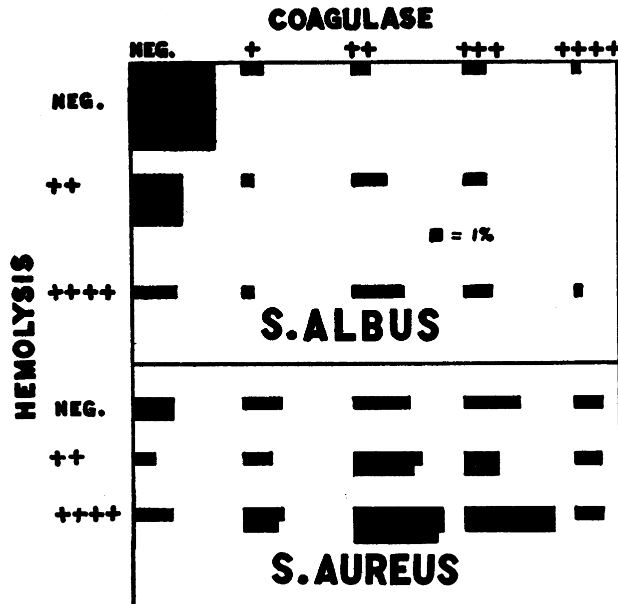

FIG. 2. COMPARISON OF HEMOLYSIS AND COAGULASE REACTIONS OF STAPHYLOCOCCUS AUREUS (368 STRAINS) AND STAPH. ALBUS (885 STRAINS)

5,000 strains using human plasma for the coagulase test and oxalated rabbit blood agar for the hemolysin test. This exten-

sive comparison was considered desirable since much of the previous contradictory evidence was thought to be due to the use by each author of a limited series of cultures. Much of the confusion appears to have arisen from the fact that although most strains contain these toxic fractions in parallel quantities, they

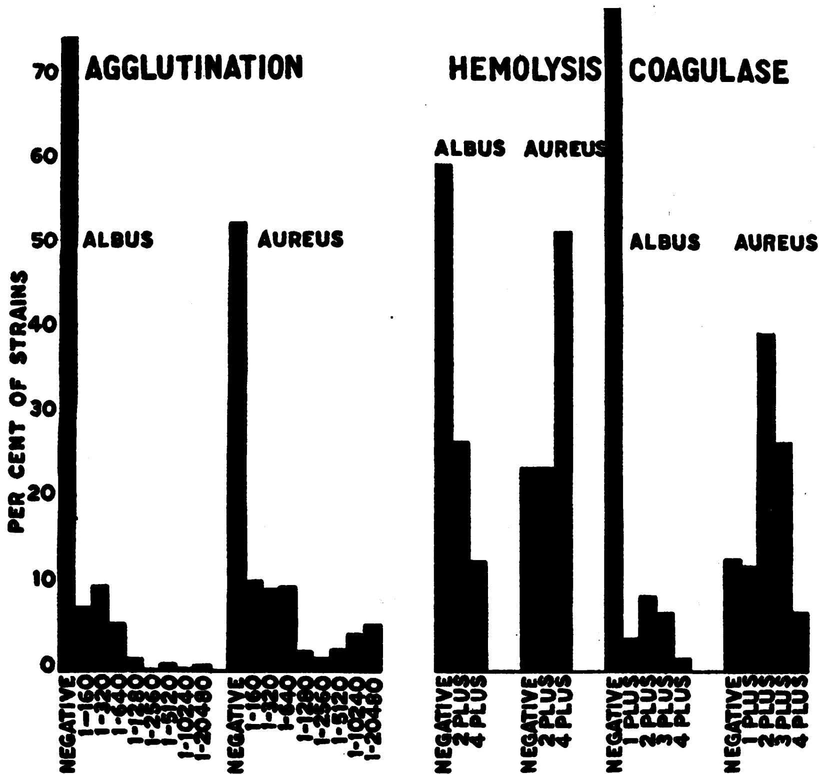

FIG. 3. COMPARISON OF SEROLOGICAL REACTIONS OF STAPHYLOCOCCI (368 AUREUS AND 885 ALBUS STRAINS)

may be present in unequal amounts in certain strains. By studying a large series of strains we found that, while the hemolysis and coagulase-activity tests usually gave parallel reactions, instances occurred in which one was strongly positive and the other entirely negative (fig. 1). In some cases where no hemolysin was detected, the strains possessed considerable coagulase

activity. Other strains were hemolytic but did not coagulate plasma. The strains which were lacking in one of these factors, were selected for animal inoculation.

For the coagulase test, one loopful of the twenty-four-hour culture on solid medium was mixed with 0.5 cc. of citrated human plasma and incubated for three hours at 37°C. The tubes were inspected at the end of one-half hour, one hour, two hours and three hours.

For the purpose of this study, porcelain white and creamy white strains were considered as *Staph. albus* while lemon yellow and golden yellow strains were considered as *Staph. aureus*.

The data recorded in figure 3 illustrate the larger number of positive hemolysis, coagulase and agglutinin reactions encountered in strains of *Staph. aureus* as compared with *Staph. albus*. The correlation of hemolysis with coagulase activity tests is shown in figure 2. The majority of albus strains gave weak or negative hemolysis and coagulase reactions while aureus strains are usually positive to both tests. The distribution of these positive reactions, particularly among the aureus strains, suggests that these two reactions may represent independent properties of various strains of staphylococci. Coagulase is more consistently a property of *Staph. aureus*. The difference between the agglutination reactions and hemolysis tests of the two types of staphylococci is less pronounced.

Animal inoculation was made by injecting 5 cc. of an 18-hour culture of each monophasic toxic strain and non-toxic control strain in brain-heart infusion intravenously into healthy rabbits. Pinner and Voldrich (1932) had shown that 5 cc. of a broth culture of albus strains did not produce demonstrable lesions or apparent disease in rabbits. The results are recorded in tables 1, 2, 3, and 4 and figure 4. As previously suggested, the aureus strains were more frequently pathogenic than the albus strains. Consequently we have separated them in the tables in order to determine whether there is any significant difference between representative strains of these two varieties. It is important to re-emphasize that the strains selected for animal inoculation were selected from a large number of toxic strains only because

they lacked certain toxic phases. The results recorded in table 1 show that coagulating non-hemolytic albus strains are nearly

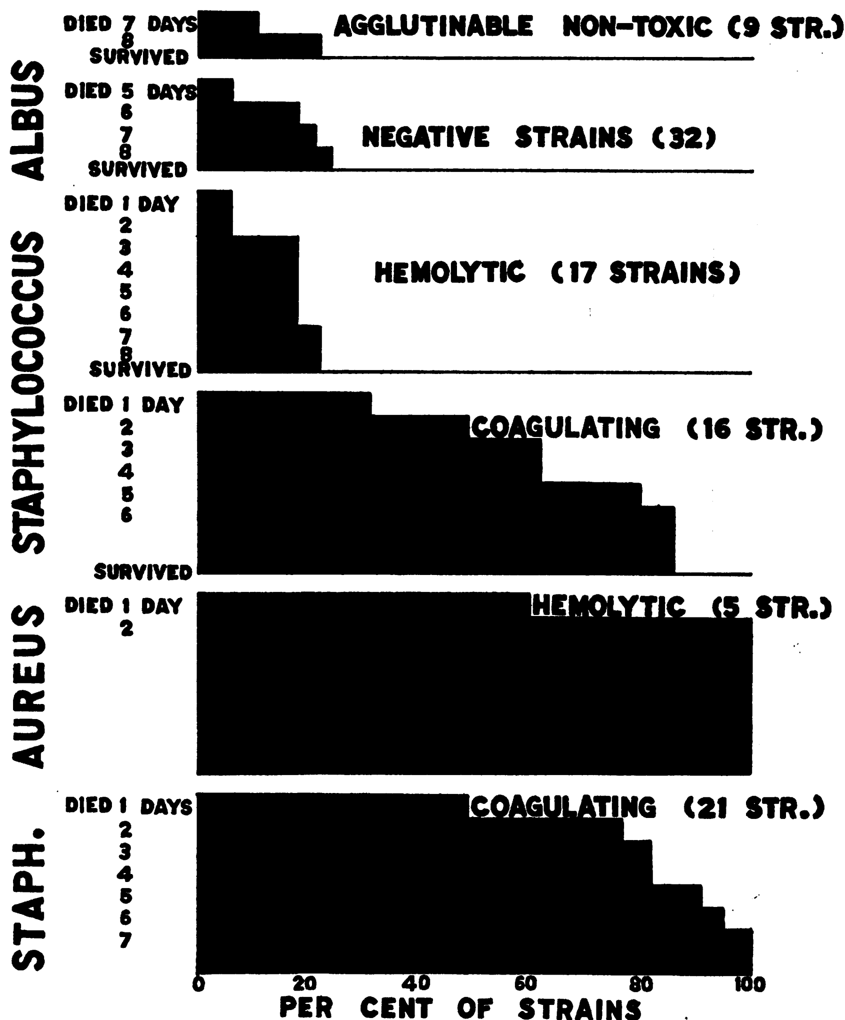

Fig. 4. Comparison of Hemolysis and Coagulase Activity Tests (and Some Agglutination Reactions) with Pathogenicity for Rabbits of 100 Strains of Staphylococci

always pathogenic for rabbits when injected intravenously, but that non-coagulating hemolytic albus strains are nearly always

TABLE 1

Injection of 5 billion live Staph. albus intravenously into rabbits

STRAIN NUMBER	HEMOLYSIS	COAGULASE*	AGGLUTINATION	EFFECT ON RABBIT	REMARKS
3212	0	+++	0	D 5	Coagulating non-hemolytic strains
3286	0	++	1:1280	D 2	
2949	0	++	1:640	D 1	
3189	0	++		D 1	
3190	0	++	1:640	D 1	
2832	0	+	1:10240	D 6	
3222	0	+	0	D 2	
2953	0	++++	0	Survived†	SR strain
3175	0	+++	0	Survived	
4526	0	+	0	D 1	
4529	0	+	0	D 2	
4577	0	++++	1:320	D 3	
4592	0	+++		D 5	
4643	0	++	0	D 5	
4806	0	++		D 3	
4816	0	++++	1:640	D 1	
4593	++++	0		Survived	Hemolytic non-coagulating strains
3207	++++	0	1:160	D 7	
2891	++++	0	0	D 3	
3252	++++	0	0	D 3	
3284	++++	0	0	D 1	
2975	++++	0	0	Survived	
3259	++++	0	0	Survived	
3270	++++	0	0	Survived	
3271	++++	0	0	Survived	
3326	++++	0	0	Survived	
3338	++++	0		Survived	
4562	++++	0	0	Survived	
4613	++++	0	0	Survived	
4681	++++	0	0	Survived	
4693	++++	0	1:320	Survived	
4735	++++	0	1:640	Survived	
4775	++++	0	0	Survived	

* Using human plasma.

† Survived 10 days.

D, died (number following symbol indicates number of days after inoculation).

non-pathogenic for rabbits. As far as these experiments were concerned, there was no significant difference between the agglutinable and the inagglutinable strains. Regardless of their

TABLE 2

Injection of 5 billion live Staph. albus intravenously into rabbits

STRAIN NUMBER	HEMOLYSIS	COAGULASE*	AGGLUTINATION	EFFECT ON RABBIT	REMARKS
3339	0	0		Survived†	Non-hemolytic non-coagulating strains (some weakly hemolytic strains have been included)
3342	0	0		Survived	
3341	0	0		D 6	
3319	0	0	1:160	D 8	
3330	++	0	0	D 8	
3320	++	0	0	Survived	
3316	++	0	0	Survived	
3231	++	0	0	Survived	
3230	++	0	0	Survived	
3227	++	0	0	Survived	
4807	++	0		D 7	
2834	0	0	1:640	Survived	
3163	0	0	1:640	Survived	
3167	0	0	1:640	Survived	
4585	0	0		D 6	
3328	0	0	0	Survived	
3322	0	0	0	Survived	
2854	0	0	0	Survived	
3151	0	0	0	Survived	
3159	0	0	0	Survived	
3154	0	0	0	Survived	
3139	0	0	0	Survived	
3142	0	0	0	Survived	
4820	++	0	1:320	Survived	
4821	0	0	1:320	Survived	
4657	0	0		D 5	
4660	0	0		D 8	
4719	0	0	0	Survived	
4744	0	0		D 5	
4791	0	0	0	Survived	
4813	0	0	1:640	D 7	
5038	0	0	1:160	Survived	
5025	0	0		Survived	
5027	0	0		Survived	
4996	0	0		Survived	
4994	0	0		Survived	
4992	0	0	0	Survived	
4990	0	0	1:320	Survived	
5012	0	0	0	Survived	
5039	0	0	0	Survived	
5022	0	0	0	D 6	

* Using human plasma.

† Survived 10 days.

D, died (number following symbol indicates number of days after inoculation).

agglutinability, the non-hemolytic, non-coagulating strains were usually non-pathogenic for rabbits (table 2). There was a uniform pathogenicity of the positively reacting aureus strains regardless of whether the toxin was a coagulase or a hemolysin (table 3).

TABLE 3

Results of injection of 5 billion live Staph. aureus intravenously into rabbits

STRAIN NUMBER	HEMOLYSIS	COAGULASE*	AGGLUTI-NATION	EFFECT ON RABBIT	REMARKS
3066	0	++++	1:1280	D 1	Coagulating non-hemolytic strains
2923	0	+++	1:160	D 5	
2815	0	+++	1:320	D 3	
3314	0	++++	0	D 2	
3275	0	++	1:640	D 1	
3329	0	++		D 1	
3333	0	++	0	D 6	
3248	0	++	1:640	D 2	
3327	0	++		D 7	
4810	0	++	1:640	D 1	
4795	0	++		D 1	
4767	0	++++		D 2	
4747	0	++	0	D 1	
4742	0	++	1:640	D 1	
4548	0	+	0	D 5	
4590	0	++	1:320	D 1	
4665	0	++		D 1	
4669	0	+++	1:160	D 1	
4672	0	+++	1:1280	D 2	
4695	0	++++	0	D 2	
4704	0	++++		D 2	
3070	++++	0	1:640	D 1	Hemolytic non-coagulating strains
3072	++++	0	1:640	D 2	
3246	++++	0		D 2	
3279	++++	0	1:640	D 1	
4777	++++	0	0	D 1	

* Human plasma.

D, died (number following symbol indicates number of days after inoculation).

From a summary of these reactions (table 4), it will be seen that none of the rabbits inoculated with hemolytic or coagulating aureus strains survived eight days and only 12 per cent of those inoculated with coagulating albus strains survived this period.

On the other hand, over 75 per cent of the rabbits inoculated with non-coagulating albus strains survived. This was true both of the hemolytic and the non-hemolytic albus strains. Carrying this analysis still further, the "survival time" of the rabbits is plotted in figure 4. The hemolytic aureus group was most pathogenic. There was only a slight difference in the pathogenicity of the coagulating aureus and the coagulating albus strains. The hemolytic albus group was only slightly more pathogenic than the non-hemolytic, non-coagulating albus group. In view of the observation of Burnet previously referred to, viz., that oxalated medium alters the hemolytic activity of staphylococci,

TABLE 4

Summary of animal inoculation experiments

ORGANISM	NUMBER OF STRAINS TESTED	COAGULASE	HEMOLYSIS	DEATHS IN LESS THAN 10 DAYS	
				Number	Per cent
Staph. albus (74 strains)	16	+	0	14	88
	17	0	+	4	23
	32	0	0	8	25
	9	0	0	2	22*
Staph. aureus (26 strains)	21	+	0	21	100
	5	0	+	5	100

* Agglutinating strains.

we decided to investigate staphylococcal hemolysins with a view to attempting a differentiation of the non-toxic albus hemolysin from the toxic aureus hemolysin. It was considered possible that the use of oxalated blood for hemolysis tests had permitted hemolysis by strains whose mechanism of hemolysis differed from the factors responsible for the hemolytic activity of aureus strains. Two procedures suggested themselves: (1) The use of defibrinated blood, and (2) the use of heparin as an anticoagulant. Parallel observations of the three types of blood revealed no significant difference (table 6).

The presence of a coagulase, even in small amounts, was associated with toxicity for rabbits. There was no significant differ-

ence in the pathogenicity of strains giving a ++++ reaction (clotting plasma in one-half hour), those giving a +++ reaction (clotting plasma in one hour), those giving a ++ reaction (clotting plasma in two hours) and those giving a + reaction (clotting plasma in three hours). The time factor does not seem to be strictly constant and should be interpreted with caution (table 8).

TABLE 5

Comparison of hemolysis of staphylococci growing on blood agar prepared from different types of blood

LABORATORY NUMBER	TYPE	OXALATED BLOOD	DEFIBRINATED BLOOD	HEPARINIZED BLOOD	INCUBATION PERIOD
					hours
5216	Aureus	++++	++++	++++	48
5221	Aureus	+++	+++	+++	48
5224	Aureus	++	++	++	48
5225	Aureus	++	++	++	48
5228	Albus	0	0	0	48
5231	Albus	+	+	+	48
5204	Albus	+++	+++	+++	48
5197	Albus	++++	++++	++++	48
5198	Albus	++++	++++	++++	48
5233	Aureus	++++	++++	++++	48
5234	Albus	0	0	0	48
5235	Aureus	++++	++++	++++	48
5236	Albus	0	0	0	48
5237	Aureus	++++	++++	++++	48
5238	Albus	+	0	+	24
5239	Albus	++	0	+	24
5240	Aureus	+++	++++	+++	24

These findings stress the importance of separating staphylococcus strains on the basis of color, hemolysin and coagulase production. The agglutinin reaction will receive treatment in a later paper. The color determination is important because, if the strain is yellow, it is probably pathogenic, while, if it is white or creamy white, it is probably non-pathogenic. In certain cases it was noted that while the twenty-four-hour growths on proteose lactose agar appeared to be creamy white, the mass culture ob-

tained by gathering the growth of an entire plate into a small area had a distinct yellow color. Many of these strains were quite toxic. This suggested that some of the anomolous reactions might have been due to errors in the determination of the color. Since the pigment had been shown to be a carotin, we attempted to modify the colors of surface growths by incorporating substances which might possibly contain mother substances for this class of pigments. Among the substances tried were corn meal, carrot infusion, carotene crystals and cod-liver oil. None of these were capable of enhancing the production of the yellow pigment in pale yellow strains. The color was slightly intensified on Loeffler's medium but the maximum color development was observed by incubating the strains for forty-eight hours instead of twenty-four hours, as suggested by many authors. By this technic, many of the supposed albus strains were found to be of the aureus type.

The determination of the coagulase is important because, regardless of its color, a coagulating strain is probably pathogenic. The determination of the hemolysin is important because, if the strain is yellow, it is probably pathogenic, while, if it is white and does not coagulate plasma, it is likely to be non-pathogenic. Consequently, we believe that these tests should be included in a study of the pathogenicity of staphylococci.

Both albus and aureus strains may be pathogenic for rabbits, but many of these pathogenic strains may not be agglutinable by the homologous serum. On the other hand, a few of the albus strains, which would have been considered of doubtful significance on the basis of the hemolysis and coagulase tests, were agglutinable. However, we were unable to demonstrate pathogenicity for these non-toxic agglutinable strains by the method used.

Since the Shwartzman phenomenon is considered a function of bacterial toxins, strains whose effect on rabbits was known were tested for their ability to elicit the phenomenon by the following technic:—A twenty-four-hour growth of the monophasic strain on proteose lactose agar (lactose agar in which Difco proteose peptone had been substituted for the usual peptone) was washed off with

one per cent phenol in saline and the suspension was diluted to a concentration of 50 billions per cc. After testing for sterility, 0.1 cc. was injected subcutaneously into the depilated flank of a rabbit. The following day, 3 cc. was injected intravenously into the same animal and the site of the subcutaneous inoculation was watched for several hours.

TABLE 6

*The relationship of the Shwartzman phenomenon to other reactions of staphylococci**

ORGANISM	NUMBER	HEMOLYSIS	COAGULASE	AGGLUTINATION	RABBIT INOCULATION	SHWARTZMAN REACTION
Staph. albus	5025	0	0		D 9	0
Staph. albus	5027	0	0		0	0
Staph. albus	4996	0	0		0	0
Staph. albus	4994	0	0		0	0
Staph. albus	4992	0	0	0	0	0
Staph. albus	4990	0	0	1:320	0	0
Staph. albus	5012	0	0	0	0	0
Staph. albus	5039	0	0	0	0	0
Staph. albus	5038	0	0	1:160	D 9	2.0 x 2.0 cm.
Staph. albus	5022	0	0	0	D 6	0.7 x 0.6
Staph. albus	5061	0	+	0	0	2.0 x 1.5 (pustule)
Staph. albus	4980	0	++		0	0.5 x 0.5
Staph. albus	5138	+++	0	1:160	0	0.5 x 0.5 (papule)
Staph. albus	5135	+++	0	1:640	D 7	1.0 x 1.0 (papule)
Staph. albus	4964	+++	0	1:640	0	1.0 x 1.0 (macule)
Staph. aureus	5181	0	+	1:10240	D 4	0
Staph. aureus	5182	0	+	1:320	D 1	3.0 x 3.0 (papule)
Staph. aureus	5183	0	+	1:640	D	1.5 x 2.0 (nodule)
Staph. aureus	4844	0	+++	1:640	D 1	1.0 x 1.0 (pustule)

* These strains were not included in tables 1, 2 and 3 or fig. 4.

Preliminary tests had indicated that strains giving negative toxicity tests showed only a slight pinkish discoloration, while toxic strains produced markedly congested indurated areas which, in one instance, measured 3.0 x 3.0 cm. in diameter. Burnet (1931) obtained a negative reaction with a white staphylo-

coccus. A more extensive series of tests was undertaken. Ten strains of *Staph. albus* giving negative toxicity tests were injected intravenously into rabbits to confirm the results of these tests. The same strains were then tested for their ability to elicit the Shwartzman phenomenon. A fresh rabbit was used for each test. Monophasic toxic strains were then subjected to similar experimentation. The following results were obtained (table 6).

Two of the non-toxic control strains produced reactions. They were weakly pathogenic when introduced intravenously. Another non-toxic control strain killed the rabbit in nine days but gave a negative Shwartzman reaction. The rest of the non-toxic strains reacted negatively to both tests. All toxic strains except one gave positive Shwartzman reactions although two of

TABLE 7

Comparison of hemolysis and coagulase reactions of staphylococci

ORGANISM	NUMBER OF STRAINS EXAMINED	PER CENT OF STRAINS REACTING POSITIVELY	
		Hemolysis (++++ and +++)	Coagulase (++++, +++, ++ and +)
Staph. albus	1852	13.4	11.9
Staph. aureus	690	51.7	88.0

them failed to kill rabbits in ten days. Of interest are the reactions obtained with the hemolytic albus strains. The Shwartzman reaction was positive with all three although only one was pathogenic intravenously. These strains were also agglutinable but it is not likely that the agglutinogen was responsible for the Shwartzman reaction because an agglutinable non-toxic strain reacted negatively.

These observations give further support to the prevailing opinion that the Shwartzman phenomenon is activated by bacterial toxins and confirm Burnet's observation (1931) that the activity of a preparation as a Shwartzman reagent is apparently parallel to its killing power.

These results are of equal significance in strengthening the idea that albus strains are degenerate aureus strains. Most of the

TABLE 8

Comparison of coagulase tests on different occasions

DATE	PLASMA USED	STRAIN	RESULT
October 2	Rabbit no. 1	*Staph. aureus* no. 1	+++
7			++
12			++
21			++++
31			+++
November 1	Rabbit no. 2	*Staph. aureus* no. 1	+++
November 3	Rabbit no. 3	*Staph. aureus* no. 3	+++
8			++
11			+++
October 3	Human no. 1	*Staph. aureus* no. 1	+++
5	2		+++
7	2		+
	3		++
	4		++
12	5		+
	6		+
	7		+
15	8		+
	9		+
October 15	Human no. 10	*Staph. aureus* no. 2	+++
	11		+++
	12		++
	13		++
17	14		+++
	15		+++
18	16		++
19	17		+++
October 21	Human no. 18	*Staph. aureus* no. 3	++
	19		++
22	19		+
	20		++
	21		++
	22		++
	23		+
	24		+
	25		+
	26		+
	27		+

albus strains tested were lacking in pathogenic properties. The majority of the potentially toxic albus strains were less toxic than the aureus strains. The albus hemolysin is considerably less toxic for rabbits than the aureus hemolysin.

SUMMARY

Although most pathogenic types of staphylococci are hemolytic, and the hemolysis test is considered a reliable guide to the toxicity of the staphylococcus group, it is evident from the literature that certain pathogenic strains are non-hemolytic. In our series, only 51.7 per cent of 690 strains of *Staphylococcus aureus* were hemolytic.

In studying complementary tests for their correlation with pathogenicity of these non-hemolytic strains, the importance of the coagulase-activity test was confirmed. Of 690 strains of *Staphylococcus aureus* tested, 88 per cent coagulated oxalated plasma. When 1852 strains of *Staphylococcus albus* were similarly tested, only 11.9 per cent gave the coagulase reaction. Pathogenic non-hemolytic strains coagulated oxalated plasma.

Although aureus strains are usually more pathogenic than albus strains, the color, which should be determined after forty-eight hours' incubation, does not always furnish a reliable guide to the toxicity of a strain. Certain aureus strains are non-pathogenic, while a few albus strains are pathogenic for rabbits.

Strains reacting positively to either the hemolysis or coagulase test were injected into rabbits. Negatively-reacting strains were similarly injected to serve as controls. A few negatively-reacting but agglutinable strains were also tested. The following results were obtained: (a) 75 per cent of 32 strains reacting negatively to the hemolysis, coagulase and agglutination tests did not kill rabbits in ten days; (b) 78 per cent of nine agglutinable strains which reacted negatively to the hemolysis and coagulase tests did not kill rabbits in ten days; (c) 77 per cent of 17 hemolytic strains of *Staphylococcus albus* did not kill rabbits in ten days; (d) 88 per cent of 16 coagulating *Staphylococcus albus* strains killed rabbits in less than ten days; (e) all the hemolytic and the coagulating strains of *Staphylococcus aureus* killed rabbits in

less than ten days; (f) of the rabbits which died, death occurred in a shorter time with the aureus than with the albus strains.

There was a close parallel between the results of toxicity tests, as determined by hemolysis and coagulase activity tests, the pathogenicity of these strains when injected intravenously and their ability to produce the Shwartzman phenomenon.

There was no difference in the behaviour of strains when tested for hemolysis on rabbit blood agar which had been prepared from either oxalated, heparinized or defibrinated blood.

The simultaneous use of hemolysis and coagulase-activity tests and a careful determination of the color after forty-eight hours' incubation seems to afford a reliable combination of tests for the study of the pathogenicity of staphylococci. Omission of any of these tests may result in failure to recognize certain toxic strains.

CONCLUSIONS

1. A knowledge of the hemolytic activity of staphylococci is insufficient as a single serologic test of pathogenicity of this species.

2. A determination of coagulase activity is an important adjunct to the hemolysis test. Coagulating strains are usually pathogenic regardless of their hemolytic activity.

3. Coagulase-positive strains are usually pathogenic, regardless of the color produced on solid media.

4. On the other hand, the importance of the hemolysis test depends on an accurate determination of the color produced by the strain. Hemolytic non-coagulating albus strains are probably non-pathogenic but hemolytic aureus strains are usually pathogenic, regardless of their coagulase.

5. For the serologic recognition of toxic types of staphylococci, it is imperative to use at least the hemolysin and coagulase activity tests and a careful determination of the color. With a combination of these three reactions it is possible to estimate the toxicity of a strain with a high degree of precision.

REFERENCES

Bigger, J. W. 1933 Jour. Path. and Bact., **36**, 87.
Burky, E. L. 1933 Jour. Immunol., **24**, 93.
Burnet, F. M. 1929 Jour. Path. and Bact., **32**, 717.
Burnet, F. M. 1930 Jour. Path. and Bact., **33**, 1.
Burnet, F. M. 1931 Jour. Path. and Bact., **34**, 45.
Burnet, F. M. and Freeman, M. 1932 Jour. Path. and Bact., **35**, 477.
Dack, G. M., Jordan, E. O. and Woolpert, O. 1931 Jour. Prevent. Med., **5**, 151.
Daranyi, J. V. 1926 Centralbl. f. Bakt. 1, Abt. Orig., **99**, 74.
Dolman, C. E. 1932 Canad. Public Health Jour., **23**, 125.
Duran-Reynals, F. 1933 Jour. Exper. Med., **58**, 161.
Gay, F. P. and Oram, F. 1933 Jour. Immunol., **25**, 501.
Gengou, O. 1930 Arch. Internat. de Med. Exper., **5**, 633.
Gross, H. 1933 Klin. Woch., **12**, 304.
Jones, L. R. 1930 Proc. Soc. Exper. Med. and Biol., **2**, 732.
Jordan, E. O. 1931 Jour. Amer. Med. Assn., **97**, 1704.
Jordan, E. O. and Hall, J. R. 1931 Jour. Prevent. Med., **5**, 387.
Julianelle, L. A. 1922 Jour. Infect. Dis., **31**, 256.
Panton, P. N. 1932 Lancet, **2**, 1019.
Panton, P. N. and Valentine, F. C. O. 1932 Lancet, **1**, 506.
Panton, P. N., Valentine, F. C. O. and Dix, V. W. 1931 Lancet, **2**, 1180.
Parker, J. T. 1924 Jour. Exper. Med., **40**, 761.
Pike, R. M. 1934 Jour. Immunol., **26**, 69.
Pinner, M. and Voldrich, M. 1932 Jour. Infect. Dis., **50**, 185.
Weld, J. T. P. and Gunther, A. 1931 Jour. Exper. Med., **54**, 315.