THE BEHAVIOR OF VIRULENT AND AVIRULENT STAPHYLOCOCCI IN THE TISSUES OF NORMAL MICE*

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Plate 4

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The experiments to be described in this and the following two papers are part of an attempt to investigate the effect of metabolic and physiologic disturbances on resistance to bacterial infections. One of the models used in our laboratory for this purpose being the experimental infection of mice with staphylococci, it proved necessary to acquire as a background more precise information concerning the natural history of this experimental disease in normal animals.

While not designed for the purpose of analyzing the mechanisms of pathogenicity of staphylococci, the experiments have brought out nevertheless a number of facts relevant to this problem. They have confirmed that the intravenous injection of virulent staphylococci into normal mice commonly results in a fatal outcome due to the rapid and progressive development of abscesses in the kidney (4). As expected, the strains of staphylococci found to be virulent for mice had the ability to coagulate human and rabbit plasma *in vitro* (7, 14, 16). However they all proved coagulase-negative in mouse plasma. One unexpected finding emerged from the comparative study of several strains endowed with various degrees of virulence—namely the fact that the non-virulent, coagulase-negative staphylococci tested were not killed *in vivo* as rapidly and as completely as could have been assumed from the results of phagocytosis experiments *in vitro* published by other investigators (13, 19). True enough these avirulent organisms were progressively cleared from the various organs, but hardly faster than were virulent coagulase-positive staphylococci. Like the latter, the avirulent organisms persisted for many days in the liver, lungs, and spleen—although in much reduced numbers. Even more surprisingly, they often multipled in the kidneys where they reached very large numbers in certain cases before disappearing in the natural course of events.

Taken together, the results suggest that, in the mouse at least, the differences in virulence between coagulase-positive and coagulase-negative strains of

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staphylococci are quantitative rather than qualitative. Indeed, it appears that the various strains can be arranged in a continuous spectrum with many intermediate degrees of virulence.

Bacterial Cultures:

All strains of staphylococci used in the present study produced a zone of hemolysis on human and rabbit blood agar plates, but they differed in many other characteristics. Some of these will be briefly mentioned for each individual strain:

Giorgio.—Aureus, coagulase-positive, phage type 7,47C[1] isolated at The New York Hospital from a case of osteomyelitis.[2]

Smith.—Aureus, coagulase-positive, phage type 44A·42E[1]; isolated by one of us in 1930 from a human case of osteomyelitis; has been maintained by repeated transfers in beef heart infusion–peptone broth in this laboratory since its isolation, with occasional passage through mice and recovery from the spleen. This same strain has been referred to by other groups of investigators under the designation S.A. 235.

O'Hara.—Aureus, coagulase-positive; described in reference 18. This strain has been shown to belong to phage type 52A.[1]

Stern and Air.—Albus, coagulase-negative.[2]

J.A.B.—Albus, coagulase-negative; isolated in 1951 by one of us from the normal skin of one of the laboratory personnel.

MAM.—Albus, isolated by Dr. D. Rogers from the normal skin of a laboratory worker at The New York Hospital. When first received in this laboratory from Dr. C. Le Maistre in 1953, strain MAM was coagulase-negative for rabbit and human plasma. It has been maintained since that time by repeated transfers in beef heart infusion–peptone broth. When tested again in June, 1955, it was still non-pigmented but proved moderately coagulase-positive in human plasma and slightly positive in rabbit plasma. At that time the subculture of this strain maintained at The New York Hospital was still coagulase-negative.

Infection Tests.—The cultures, grown in beef heart infusion–peptone broth for 18 hours at 37°C., were injected into one of the caudal veins. In general, they were diluted with physiological saline to a final volume of 0.2 ml.

The number of living cocci present in the culture at the time of injection was determined by plating appropriate dilutions on nutrient agar. In critical experiments duplicate series of dilutions and duplicate or triplicate series of platings were carried out for each dilution series. Calculations were made from plates giving 10 to 30 colonies. The results presented in the text are based on arithmetical averages of the values thus obtained.

The animals were albino mice of the so called Rockefeller Institute Swiss strain, bred at The Rockefeller Institute. They were weaned when approximately 25 days old. Unless otherwise noted, they were used for the infection tests within 1 week after weaning; their weight varied from 12 to 16 gm. at the time of use.

During the course of experimentation, the mice were generally kept in groups of 10 in metal cages, equipped with metal grids, without sawdust or other litter. Food (in the form of fox chow pellets) and water were given *ad lib.* unless otherwise noted.

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[1] For the phage typings of the various strains we are indebted to Dr. C. Bernsten of the Cornell Medical Service at the Bellevue Hospital. Dr. Bernsten used for these typings the collection of staphylococcus phages obtained from Dr. J. Blair (1).

[2] We wish to put on record our gratitude to Drs. D. E. Rogers, C. Le Maistre, and R. McCune of The New York Hospital, for their generosity in supplying us with the cultures of strains Giorgio, MAM, Stern, and Air, used in the present work, and for making available to us many of their unpublished findings concerning the behavior of these strains in experimental animals.

Observations on Virulence.—Three separate types of observations were made:

(*a*) Histopathological characteristics of the lesions; (*b*) Survival time following intravenous injection of known doses of cultures; (*c*) Quantitative determination of the numbers of staphylococci that could be recovered from the liver, lungs, spleen, kidneys, and heart blood at various intervals of time after infection with known doses of culture. The animals were killed with chloroform and autopsied 1 minute after death. The organs were then immediately emulsified with a teflon grinder (10) in 5 ml. of Earl's balanced ionic solution adjusted to pH 6.8, and the tissue suspension further diluted in 0.1 per cent bovine albumin in tap water, or in Earl's solution. Two culture techniques were used to determine the numbers of colonies that could be recovered from the organs and the blood: (*a*) a calibrated loopful of the appropriate dilution was spread over the surface of conventional plates of nutrient agar (1.5 per cent agar); (*b*) 0.2 ml. of appropriate dilution was added to 1.8 ml. of soft nutrient agar (0.3 per cent agar) in a small tube; the staphylococcus colonies remained isolated and suspended in the soft agar and could be readily counted (within the range of 1 to 30) after 18 hours' incubation. The results obtained by the two techniques were identical within the limits of error of the dilution techniques.

TABLE I

Bacterial Populations Yielded by Three Strains of Staphylococci in Beef Heart Infusion-Peptone Broth

Strain	Coagulase	No.* of colonies recovered from cultures of indicated ages			
		18 hrs.	2 days	3 days	7 days
Smith	++++	48	63	51	54
"	++++	45	69	60	51
MAM	+	30	41	44	30
"	+	39	51	57	30
JAB	−	4	6	12	9
"	−	9	9	15	12

* Figures to be multiplied by 10^8 to give numbers of colonies obtained per milliliter of culture.

Population Size Reached by Various Strains of Staphylococci in Vitro.—The strains of staphylococci used in the present study were found to differ somewhat in the rate and extent of their multiplication in beef heart infusion–peptone broth, as well as in their survival in this medium. These differences are illustrated in Table I, which presents the results of quantitative bacteriological studies with three representative strains: Smith (strongly coagulase-positive); MAM, (weakly coagulase-positive); JAB (coagulase-negative).

Tubes containing 5 ml. of beef heart infusion–peptone broth were inoculated with one loopful of a 3 day old culture in the same medium; four tubes were used per strain. The cultures were incubated at 37°C. and the numbers of colonies determined after 18 hours, 2 days', 3 days', and 7 days' incubation. The enumeration was carried out by diluting the culture to 10^{-6} in three successive steps (0.5 ml. of culture or of dilution in 50 ml. of diluent); two further operations in tenfold steps gave final dilutions of 10^{-7} and 10^{-8}. The numbers of living organisms (or aggregates of organisms) were determined by spreading the contents of calibrated loopfuls or depositing calibrated drops, on nutrient agar surface. The colonies were counted after 24 hours' incubation at 37°C. Two tubes of culture were used for each

daily test; two independent series of dilutions were done for each tube, and three platings for each dilution. The arithmetical averages for each tube are presented in Table I.

As appears from the results presented in Table I, the Smith culture multiplied more rapidly and more extensively in beef heart infusion–peptone than did culture JAB; culture MAM was intermediate. Similar tests carried out with other strains of staphylococci have shown that the coagulase-positive strains multiply more rapidly and survive longer in beef heart infusion–peptone broth than do the coagulase-negative strains. This finding is consonant with results very recently published which suggest that, in general, the latter strains have more exacting growth requirements than the former (6).

TABLE II

Comparative Virulence for Mice of Seven Strains of Staphylococci

Strain	Coagulase	Dose injected*	Deaths‡
		ml.	*per cent*
Giorgio	++++	0.1	100
"	++++	0.03	25
Smith	++++	0.1	30
O'Hara	++++	0.1	20
MAM	+	0.2	0
"	+	0.1	0
JAB	−	0.6	0
"	−	0.2	0
"	−	0.1	0
Air	−	0.1	0
Stern	−	0.1	0

* Intravenously; overnight culture.

‡ Within 1 month after infection.

Lethal Dose of Various Cultures of Staphylococci for Normal Mice.—In many experiments carried out in this laboratory during the past 3 years, doses ranging from 0.03 to 0.6 ml. of 18 hour old cultures of various strains of staphylococci have been injected by the intravenous route into normal mice. Whatever the dose injected, no death caused by the staphylococci occurred within 1 month after infection when the organism was either coagulase-negative (strains JAB, Stern, Air), or only weakly coagulase-positive (strain MAM). In contrast, all coagulase-positive organisms caused a certain percentage of deaths when the injected dose was 0.1 ml. of culture. Table II presents in an abbreviated form some of the characteristics and virulence for mice of the various strains used in the present study. Table III presents the time and number of deaths occurring in several independent experiments among mice inoculated with 0.1 or 0.03 ml. of culture of the two coagulase-positive strains Smith and Giorgio.

For reasons of convenience, it has been the practice in most of the work in

this and the following two papers to terminate the experiments 2 weeks after infection. Within this lapse of time, and when the infective dose was 0.1 ml. of an 18 hour old culture of the Smith strain, the numbers of deaths per group of 10 animals in individual experiments have varied from 0 to 5, with an average of 2.5 for 15 experiments. As indicated in Table III, the results of three experiments with the coagulase-positive Giorgio strain indicate that the percentage of deaths with this strain was always larger under the same conditions.

The Fate in Mouse Tissues of Staphylococci of Various Degrees of Virulence.— It is well known that the tissues of normal animals possess a clearing mechanism

TABLE III

Virulence for Mice of Two Coagulase-Positive Strains of Staphylococci

Strain	Dose	Time of death*										Survivors‡
		days										
Giorgio	0.1 ml.	3	3	5	5	5	7	7	7	8	8	0
"	0.1 ml.	3	4	4	4	5	7	7	7	9	—	1
"	0.1 ml.	4	5	5	5	5	8	8	8	9	23	0
"	0.03 ml.	6	8	19	—	—	—	—	—	—	—	7
"	0.03 ml.	9	23	—	—	—	—	—	—	—	—	2
Smith	0.1 ml.	5	7	7	9	11	19	—	—	—	—	4
"	0.1 ml.	6	8	13	—	—	—	—	—	—	—	7
"	0.1 ml.	9	14	—	—	—	—	—	—	—	—	8
"	0.03 ml.	—	—	—	—	—	—	—	—	—	—	10
"	0.03 ml.	23	—	—	—	—	—	—	—	—	—	9

* Experiments discontinued one month after infection; — indicates survival.

‡ Out of 10.

highly active against staphylococci. It has been recently shown furthermore that coagulase-negative staphylococci are rapidly killed *in vitro* by human and rabbit polymorphonuclear leucocytes and macrophages, whereas the coagulase-positive strains can survive and indeed multiply within phagocytic cells (13, 19). These facts suggested that the differences in virulence between coagulase-positive and coagulase-negative strains might be merely a reflection of the ability of the cocci to survive within phagocytic cells *in vivo*. In order to test this hypothesis, experiments were instituted to compare the fate of various strains of staphylococci in the organs and in the blood of normal mice.

In principle the method of these experiments consisted in injecting by the intravenous route known quantities of culture, sacrificing the infected animals at stated intervals of time after infection, and determining by quantitative bacteriological techniques the numbers of living staphylococci present in the various organs. Preliminary tests revealed that even virulent coagulase-positive organisms disappeared rapidly from mouse tissues if the infective inoculum was

TABLE IV

Recovery of Living Staphylococci from Mice Infected with Various Doses of Coagulase-Positive Culture

Infective dose*	No. of mice (out of 4) from which staphylococci were recovered‡ at indicated times after infection					
	1 hr.	1 day	2 days	3 days	7 days	14 days
ml.						
0.002	4	4	2	1	0	0
0.02	4	4	4	4	1	1
0.05	4	4	4	4	4	2
0.10	4	4	4	4	4	4§

* Overnight culture of coagulase-positive strain "Smith" grown in beef heart infusion–peptone broth.

‡ The liver, spleen, lungs (both), and kidneys (both) of each animal were cultured for staphylococci, and the results pooled.

§ Including 2 deaths.

TABLE V

Log Numbers of Staphylococcus Colonies Recovered at Various Times after Infection from Mice Infected Intravenously with 0.1 Ml. Culture Smith*

Organ	Time after infection						
	2 min.	2 hrs.	1 day	5 days	15 days	21 days	36 days
Blood	5.85*	?	0‡	2.95	0	0	0
"	6.11	?	0	?	2.30	0	0
"	6.23	?	0	1.70	0	0	0
"	6.05	?	1.70	3.00	0	0	0
Liver	8.28	7.95	7.01	3.71	0	3.23	0
"	7.99	7.63	6.63	4.13	5.16	0	0
"	8.08	7.63	5.92	3.23	0	0	0
"	8.31	7.65	6.04	3.93	3.23	0	0
Spleen	6.96	6.80	6.03	0	0	0	0
"	6.72	6.23	6.13	3.23	3.71	0	0
"	6.13	6.51	5.83	0	0	0	0
"	5.83	6.01	5.88	4.13	0	0	0
Lungs	7.09	0	3.53	0	0	0	0
"	7.17	5.23	4.28	3.93	4.77	0	0
"	7.12	0	3.93	5.00	3.23	3.71	0
"	7.31	5.23	3.71	4.31	4.59	0	4.79
Kidneys	6.61	0	7.10	0	0	8.40	5.93
"	6.23	0	6.07	9.66	8.93	0	4.31
"	6.83	5.53	6.23	9.23	8.40	6.61	0
"	6.28	0	5.28	9.69	8.49	6.98	6.68

* The figures in the tables are the logarithms of 10 of the numbers of colonies recovered per milliliter of blood, or per whole liver, spleen, lungs (both), or kidneys (both)—for each animal tested.

Four animals were studied for each interval of time, except in cases where deaths had occurred (indicated by the letter D). The individual animals occupy the same relative position for each organ in the vertical columns; they are entered in the order in which they were tested.

‡ 0 indicates that no colony was recovered at any dilution tested. Because of the limitations of the enumeration technique, 0 really means that the logarithms of the numbers of living staphylococci per milliliter of blood or per organ was <3.23.

small. For example, it is seen in Table IV that staphylococci of the Smith strain could no longer be recovered from any of the organs tested 1 week after infection when the infective dose was 0.002 ml. of culture. For this reason, doses ranging from 0.03 to 0.2 ml. were used in all the experiments now to be reported.

TABLE VI

Log Numbers of Staphylococcus Colonies Recovered at Various Times after Infection from Mice Infected Intravenously with 0.03 Ml. Culture Smith*

Organ	Time after infection						
	2 min.	2 hrs.	1 day	5 days	15 days	21 days	36 days
Blood	5.30*	?	1.70	0	0	0	0
"	5.54	?	0‡	0	0	0	0
"	5.78	?	0	2.00	0	0	0
"	5.88	?	1.70	0	0	0	0
Liver	7.61	6.80	5.51	0	0	0	0
"	7.88	6.69	4.83	3.93	0	3.53	0
"	8.00	6.31	6.18	3.83	3.83	0	0
"	7.61	7.23	5.87	0	0	0	0
Spleen	6.08	6.13	5.83	0	0	0	0
"	6.71	5.93	7.23	0	0	0	0
"	6.13	6.46	5.82	0	0	0	0
"	6.18	6.72	5.46	0	0	0	0
Lungs	5.83	5.23	3.23	?	0	0	0
"	6.53	5.23	3.93	3.23	0	0	4.38
"	6.46	5.53	?	0	3.53	0	0
"	6.13	5.23	3.93	3.23	3.23	0	3.71
Kidneys	5.23	5.23	4.01	5.23	8.02	0	0
"	5.71	5.23	4.53	8.59	0	0	5.93
"	6.18	5.23	5.01	8.90	4.08	0	0
"	6.23	5.23	5.51	5.23	0	0	5.93

*, ‡ Legend as for Table V.

Tables V and VI present some of the results obtained in mice infected with either 0.1 or 0.03 ml. of culture of the coagulase-positive strain Smith. As can be seen, the pattern of findings was essentially the same irrespective of the dose injected. Moreover, the course of the infectious process was highly characteristic for each tissue tested despite large individual variations from animal to animal. It is obvious, for example, that the numbers of colonies recovered from the heart blood, the kidneys, and lungs were much smaller 2 hours than 2 minutes after infection. In the liver a profound fall in the bacterial population

also became apparent after 24 hours. Staphylococci disappeared somewhat more slowly from the spleen. Although living staphylococci were still present in the various organs for many days and even for weeks after infection, their numbers were very small in the blood, the liver, the spleen, and the lungs at any time after the 2nd day. There is no doubt, therefore, that the clearing

TABLE VII

Log Numbers of Staphylococcus Colonies Recovered at Various Times after Infection from Mice Infected Intravenously with 0.1 Ml. Culture Giorgio*

Organ	Time after infection			
	2 hrs.	6 hrs.	1 day	4 days
Blood	1.90*	2.00	2.30	0‡
"	1.54	1.63	2.30	1.85
"	1.58	2.30	2.08	D
"	2.60	1.40	2.30	D
Liver	7.61	7.46	5.73	5.43
"	7.99	6.75	6.40	6.38
"	7.18	6.68	6.63	D
"	6.53	7.08	5.92	D
Spleen	6.08	7.00	5.71	6.53
"	6.53	7.57	5.34	5.71
"	5.96	6.53	6.11	D
"	7.28	6.55	5.61	D
Lungs	6.21	5.80	5.53	6.28
"	5.95	6.05	5.89	6.43
"	5.51	6.38	5.72	D
	6.18	5.87	6.77	D
Kidneys	4.66	4.59	9.12	10.10
"	5.12	6.69	9.43	9.85
"	5.04	6.81	8.43	D
"	5.34	5.28	8.18	D

*, ‡ Legend as for Table V.

mechanism of these tissues in normal mice is highly effective against the coagulase-positive staphylococci of strain Smith.

The course of events was more complex in the kidneys. As already mentioned, there was a profound fall immediately after infection in the numbers of colonies that could be recovered from this organ. Soon, however, the bacterial population began to increase and reached within a few days a level much higher than that found initially. It will be recalled that some 30 per cent of animals infected with 0.1 ml. of Smith culture died during the 4 weeks after infection (Tables

II and III). In the animals that survived, the numbers of colonies recovered from the kidneys progressively decreased; in fact, several of these animals apparently succeeded in eliminating the organisms altogether, at least as far as could be detected by conventional bacteriological techniques. Evidence of extensive bacterial multiplication in the kidneys was evident also in mice in-

TABLE VIII A

Log Numbers of Staphylococcus Colonies Recovered at Various Times after Infection from Mice Infected Intravenously with 0.03 Ml. Culture Giorgio*

Organ	Time after infection				
	2 hrs.	6 hrs.	1 day	4 days	21 days
Blood	2.78*	3.42	4.00	0‡	0
"	2.70	2.78	2.88	0	0
"	3.34	3.74	3.00	2.65	0
"	3.05	2.88	3.17	2.17	D
Liver	6.49	6.55	5.63	3.23	0
"	6.73	6.73	5.53	5.00	0
"	6.23	6.63	5.31	5.75	0
"	5.93	6.34	5.28	5.12	D
Spleen	5.34	6.08	6.28	0	0
"	6.34	6.09	5.00	3.71	0
"	6.53	6.40	4.71	4.31	0
"	5.93	6.28	5.28	4.28	D
Lungs	4.84	4.81	5.53	3.23	3.23
"	5.46	5.13	5.28	5.53	4.34
"	5.09	5.20	4.53	6.34	4.13
"	5.13	5.66	4.97	5.49	D
Kidneys	4.55	6.18	9.77	8.38	5.53
"	4.53	4.66	8.99	9.16	7.40
"	4.69	4.73	5.59	10.01	6.53
"	4.53	4.97	3.83	9.82	D

*, ‡ Legend as for Table V.

fected with 0.03 ml. of Smith culture but none of these animals died and most had become essentially free of staphylococci a few weeks after infection.

The behavior of the coagulase-positive Giorgio strain differed only in quantitative details from that of the Smith strain (Tables VII, VIII A, and VIII B). There was again striking evidence of a highly effective bactericidal mechanism against this strain in the various organs during the early phase of the infection. The heart blood, the liver, the spleen, and the lungs were almost freed of cocci after a few days. There was also initially a profound fall in the numbers of

colonies recovered from the kidneys. But the bacterial population in this organ soon began to increase in all animals tested, and the rate of increase was appreciably faster than had been observed with the Smith strain. As a result, all mice infected with 0.1 ml. of Giorgio culture died within a few days. Most mice having received only 0.03 ml. of the culture survived, and after an initial in-

TABLE VIII B

Log Numbers of Staphylococcus Colonies Recovered at Various Times after Infection from Mice Infected Intravenously with 0.03 Ml. Culture Giorgio*

Organ	Time after infection				
	1 hr.	1 day	2 days	5 days	7 days
Blood	3.60*	0‡	0	0	0
"	4.00	2.00	0	0	0
"	3.17	1.70	1.70	0	0
"	3.30	0	0	2.00	0
Liver	7.51	5.53	3.71	4.31	4.18
	7.49	5.59	6.71	3.23	4.55
"	7.68	5.18	6.34	4.28	4.18
"	7.28	5.43	4.51	4.49	3.53
Spleen	6.21	5.51	0	0	0
"	6.40	5.40	3.83	3.23	5.53
"	5.68	4.71	3.93	3.23	0
"	6.15	5.16	3.71	3.23	3.83
Lungs	5.01	4.85	0	4.94	3.23
"	5.46	4.46	4.65	4.08	5.71
"	5.01	4.43	3.23	5.40	5.71
"	5.53	3.83	4.53	5.82	4.71
Kidneys	5.28	6.66	7.57	9.31	7.71
"	4.98	7.14	8.46	8.82	9.13
"	5.13	7.46	8.69	9.34	8.28
"	5.13	3.83	7.21	9.28	8.53

*, ‡ Legend as for Table V.

crease, the numbers of staphylococcus colonies recovered from their kidneys progressively decreased (Tables VIII A and VIII B). In general, however, the kidneys still contained many living cocci 3 weeks after infection in this latter group of animals.

The results obtained with another strongly coagulase culture (O'Hara) are presented in Table IX. It is obvious that the trend of the infectious process was the same as with strains Smith and Giorgio.

Tables X and XI present the results of two experiments carried out ap-

proximately 1 year apart with the weakly coagulase-positive strain MAM. It is instructive to compare the results in Table X with those in Tables V and VI, as they correspond to experiments carried out simultaneously with several doses of cultures MAM (weakly coagulase-positive) and Smith (strongly coagulase-positive). It is obvious that, whatever the size of the infective dose, the be-

TABLE IX

Log Numbers of Staphylococcus Colonies Recovered at Various Times after Infection from Mice Infected Intravenously with 0.1 Ml. Culture O'Hara*

Organ	Time after infection						
	2 min.	1 hr.	1 day	2 days	5 days	7 days	14 days
Blood	5.48*	3.20	2.17	2.93	0	0	0
"	5.17	3.40	2.98	1.70	0	0	0
"	—	3.83	0‡	2.30	0	0	0
"	4.60	3.71	2.60	0	0	0	0
Liver	8.40	8.53	6.14	5.61	4.01	3.23	4.08
"	8.43	8.49	6.40	5.97	0	5.71	0
"	8.46	8.40	6.66	6.34	3.23	5.59	3.53
"	8.46	8.08	6.17	4.77	3.71	4.40	0
Spleen	6.40	6.95	5.53	3.71	0	0	0
"	7.31	6.63	5.57	4.13	0	0	0
"	7.40	7.08	5.63	4.49	3.23	3.23	0
"	7.49	6.46	6.07	0	0	0	0
Lungs	7.01	5.57	4.53	4.46	3.23	0	4.34
"	6.51	5.99	4.51	3.53	0	0	0
"	6.40	5.57	4.23	4.83	3.83	4.40	3.53
"	6.01	6.00	4.40	3.53	4.18	4.34	3.53
Kidneys	6.40	4.73	6.05	5.53	6.18	7.80	6.80
"	6.28	4.76	5.68	5.61	6.98	8.63	7.16
"	5.73	5.15	5.34	?	6.82	8.89	6.28
"	4.68	5.16	3.83	?	6.94	7.19	5.80

*, ‡ Legend as for Table V.

havior of the two strains was strikingly similar during the first phase of the infection. With both strains also, bacterial multiplication occurred in the kidneys after an initial profound drop in numbers of colonies recovered from this organ. The only detectable difference between the MAM and the Smith strains was that the former did not reach a population in the kidney as high as did the latter. Moreover, none of the animals infected with the MAM strain died, even when the infective dose was 0.2 ml. Thus, while the Giorgio strain seems to possess a greater ability than the Smith strain to multiply in the

kidneys and to cause the death of mice, the MAM strain is less virulent than either of these two cultures according to both criteria.

The results obtained with the coagulase-negative non-virulent strains Air and Stern, and JAB are presented in Tables XII, XIII, XIV, and XV. As was to be expected a profound and rapid fall in the numbers of colonies re-

TABLE X

Log Numbers of Staphylococcus Colonies Recovered at Various Times after Infection from Mice Infected Intravenously with 0.15 Ml. Culture MAM*

Organ	Time after infection						
	2 min.	2 hrs.	1 day	5 days	15 days	21 days	36 days
Blood	7.57*	?	0‡	0	0	0	0
"	7.05	?	0	0	0	0	0
"	6.85	?	0	0	0	0	0
"	7.36	?	0	0	0	0	0
Liver	8.01	7.94	7.06	3.23	0	0	0
"	8.63	7.46	6.20	3.53	0	0	0
"	8.83	8.21	6.87	3.23	3.23	0	0
"	8.76	7.95	7.11	0	0	0	0
Spleen	6.23	6.55	5.38	0	0	0	0
"	7.13	6.71	5.88	0	0	0	0
"	6.61	6.43	5.71	0	0	0	0
"	6.72	6.43	6.51	0	0	0	0
Lungs	7.31	6.81	?	3.83	3.93	0	0
"	7.18	6.72	3.83	0	3.71	3.23	0
"	7.53	6.23	4.23	?	0	4.53	0
"	7.57	6.81	3.71	0	4.77	0	0
Kidneys	7.22	5.23	4.38	7.11	6.95	4.89	5.00
"	6.65	5.23	3.53	5.23	7.43	7.38	6.11
"	6.81	5.23	5.53	9.03	0	7.11	0
"	6.90	5.53	5.77	7.02	6.53	5.71	5.73

*, ‡ Legend as for Table V.

covered from the various organs was observed soon after infection. But the coagulase-negative staphylococci persisted nevertheless for many days in the tissues even though in much reduced numbers. Indeed, the initial rate of killing was not obviously greater than in the case of the coagulase-positive virulent strains. It took approximately 3 weeks and in certain cases longer, for the various organs to free themselves of the coagulase-negative staphylococci. Equally surprising was the fact that bacterial multiplication occurred in the kidneys. However, it never proceeded as far as with strains Giorgio, Smith, or

TABLE XI

Log Numbers of Staphylococcus Colonies Recovered at Various Times after Infection from Mice Infected Intravenously with 0.1 Ml. Culture MAM*

Organ	Time after infection			
	1 min.	7 days	14 days	28 days
Liver	7.75*	0‡	1.45	0
"	7.62	0	4.53	0
"	7.53	0	4.60	0
Spleen	5.98	2.40	0	0
"	6.15	?	0	0
"	6.15	1.15	0	0
Lungs	6.53	2.93	1.75	3.97
"	6.75	0	3.30	4.81
"	6.35	0	3.05	1.45
Kidneys	6.23	4.93	0	0
"	6.20	4.93	4.68	4.85
"	6.15	5.85	4.15	0

*, ‡ Legend as for Table V.

TABLE XII

Log Numbers of Staphylococcus Colonies Recovered at Various Times after Infection from Mice Infected Intravenously with 0.2 Ml. Culture Air*

Organ	Time after infection					
	1 hr.	1 day	2 days	4 days	7 days	14 days
Blood	2.60*	0	0	0	0	0
"	2.00	0	0	0	0	0
"	1.70	0	0	0	2.70	0
"	0‡	0	0	0	0	0
Liver	6.57	0	0	3.23	0	0
"	6.11	0	0	0	0	0
"	6.38	3.23	0	0	0	0
"	5.71	0	0	0	0	0
Spleen	5.81	4.69	3.53	0	0	0
"	5.28	3.93	3.83	0	0	0
"	5.59	3.83	3.53	0	0	0
"	5.22	4.66	3.23	0	0	0
Lungs	3.83	0	3.83	2.23	4.31	0
"	4.01	0	0	3.83	0	0
"	3.83	0	0	0	3.71	0
"	4.01	0	5.68	0	4.01	0
Kidneys	4.40	4.89	6.01	5.49	3.93	0
"	4.18	0	3.53	0	0	4.72
"	3.53	0	0	0	6.31	4.53
"	0	3.13	5.12	6.01	5.49	0

*, ‡ Legend as for Table V.

TABLE XIII

Log Numbers of Staphylococcus Colonies Recovered at Various Times after Infection from Mice Infected Intravenously with 0.2 Ml. Culture Stern*

Organ	Time after infection					
	1 hr.	1 day	2 days	4 days	7 days	14 days
Blood	2.30*	0	0	0	0	0
"	2.54	0	0	0	0	0
"	0‡	0	0	0	0	0
"	2.17	0	0	0	0	0
Liver	6.69	0	0	0	0	0
"	6.31	0	3.23	0	0	0
"	6.46	0	0	0	0	0
"	6.66	0	0	0	0	0
Spleen	5.59	3.23	0	0	0	0
"	4.87	0	0	0	0	0
"	5.43	4.13	0	0	0	0
"	5.49	0	3.23	0	0	0
Lungs	4.53	3.23	3.23	3.83	4.01	0
"	3.23	3.53	0	0	3.71	0
"	3.23	0	3.53	0	0	0
"	3.23	0	0	0	0	0
Kidneys	4.18	0	0	0	0	0
"	4.01	0	0	0	3.23	0
"	4.53	0	0	0	0	0
"	4.38	0	0	0	0	0

*, ‡ Legend as for Table V.

TABLE XIV

Log Numbers of Staphylococcus Colonies Recovered at Various Times after Infection from Mice Infected Intravenously with 0.1 Ml. Culture JAB*

Organ	Time after infection					
	1 hr.	1 day	2 days	3 days	7 days	14 days
Liver	7.83*	3.53	0‡	0	0	0
"	7.08	4.18	0	0	0	0
"	6.77	3.83	0	3.71	0	0
"	5.59	3.71	3.53	0	0	0
Spleen	5.81	4.31	3.83	0	0	0
"	6.18	4.77	4.34	0	0	0
"	5.71	4.80	3.53	0	0	0
"	5.59	4.57	0	0	0	0
Lungs	5.40	3.23	0	0	0	0
"	5.06	0	4.93	0	0	0
"	4.23	4.01	4.72	0	0	0
"	5.34	3.83	3.23	0	4.55	0
Kidneys	4.13	3.23	0	6.04	0	0
"	3.93	3.53	3.71	5.93	0	0
"	5.46	0	5.95	6.22	0	0
"	4.08	3.53	5.94	5.85	0	0

*, ‡ Legend as for Table V.

even MAM. Significant differences could be recognized amongst the various coagulase-negative strains; thus cultures of the JAB and Air strains yielded higher and more persistent bacterial populations in the kidneys than did the Stern cultures.

TABLE XV

Log Numbers of Staphylococcus Colonies Recovered at Various Times after Infection from Mice Infected Intravenously with 0.2 Ml. Culture JAB*

Organ	Time after infection				
	2 hrs.	6 hrs.	1 day	4 days	21 days
Blood	2.70*	2.17	0‡	0	0
"	2.40	3.05	0	0	0
"	3.00	2.17	0	0	0
"	0‡	1.70	0	0	0
Liver	6.92	3.00	3.23	0	0
"	7.63	6.73	4.43	0	0
"	7.99	6.57	4.46	0	0
"	7.69	6.38	4.63	0	0
Spleen	5.68	5.79	3.71	3.23	0
"	5.31	5.75	4.31	0	0
"	6.65	5.18	4.51	3.23	0
"	5.65	5.59	4.13	3.23	0
Lungs	4.72	4.46	4.18	0	0
"	5.00	4.43	0	?	0
"	5.18	4.85	0	3.53	0
"	4.94	4.01	4.88	3.23	0
Kidneys	4.13	4.01	4.43	6.04	3.23
"	4.38	4.61	0	3.71	0
"	4.46	3.93	3.53	0	4.13
"	4.28	3.23	5.53	4.61	0

*, ‡ Legend as for Table V.

Histopathology of Lesions Produced by Virulent Staphylococci.—Macroscopic observations made on the animals at the time of necropsy revealed no gross abnormalities except much enlarged spleens and the frequent presence of large abscesses in the kidneys. Microscopic studies were also carried out on mice sacrificed 1 or 7 days after intravenous injection of 0.1 ml. of culture Smith, the organs being fixed with 10 per cent neutralized formalin and the sections stained with hematoxylin and eosin.

The only pathological change detected in the lungs and liver were small foci of mononuclear cells which could be seen 1 day after infection, but were

no longer evident 7 days later. A well marked focal hyperplasia was already present in the spleen 1 day after infection and it became more intense as the infection proceeded.

In the kidneys, the first evidence of pathological reaction consisted of collections of mononuclear and polymorphonuclear cells largely confined to the cortex and usually bearing a close relationship to the glomeruli. True abscesses were present at later stages of the disease, mainly in the cortex, but also in the medulla. Infarcts formed in some areas, and occasionally thrombosed vessels could be identified (Fig. 1).

DISCUSSION

The power of staphylococci to cause disease in man and in experimental animals is well known to exhibit a high degree of correlation with several *in vitro* characteristics of these bacteria—for example the production of coagulase, of alpha toxin, and of pigment, and the ability to ferment mannitol and to hydrolyze phenolphthalein phosphate. It is still uncertain however whether these characteristics have any causal relation to, or are merely correlated with the property of virulence. One may assume that the alpha toxin contributes to the pathological manifestations of staphylococcal diseases even though playing little part in their initiation. On the other hand, it is widely held that coagulase facilitates in some way the establishment of staphylococci *in vivo*, either by rendering them more resistant to phagocytosis (7–9, 13, 14, 16, 19), or by protecting them against the bactericidal activity of serum (2, 17).

As mentioned in the introduction, the purpose of the experiments described in the present report was to provide a background for the study of the effect of physiologic and metabolic disturbances on the susceptibility of mice to staphylococcal infections. As a by-product, the findings have thrown some light on the natural history of these infections and thereby have contributed to the understanding of their pathogenesis.

Let it be stated at the outset that our findings are consonant with two well known aspects of staphylococcal infections in mice, namely the affinity of staphylococci for the kidneys, and the correlation between their virulence and their ability to produce coagulase. In brief, it can be stated that the virulent as well as the avirulent organisms injected by the intravenous route disappeared progressively and rather rapidly from most of the tissues. The virulent forms eventually multiplied in the kidneys, the death of the infected animals being in the great majority of cases due to the destruction of renal tissue as a result of abscess formation. Of the seven strains of hemolytic staphylococci used in our tests, only the three which were strongly coagulase-positive proved capable of causing death of mice within 1 month after intravenous injection of 0.1 ml. of culture. Of the other four strains, one was weakly coagulase-positive and three were coagulase-negative. Although the staphylococci of

these four strains persisted in the tissues of mice for prolonged periods of time after infection—in small numbers it is true—they failed to produce progressive disease even when the infective dose was very large.

These facts appear at first sight to support the widely held view that coagulase plays an essential part in the virulence of staphylococci; they are also compatible with other findings reported by several independent groups of investigators. It has been established beyond doubt, for example, that coagulase-negative strains are very susceptible to the bacterial activity of phagocytic cells *in vitro* (13, 19). Under the proper experimental conditions a very large percentage of these avirulent organisms lose their ability to grow on nutrient agar within a few minutes after phagocytosis, and the survivors are killed shortly thereafter. In contrast, the coagulase-positive forms survive phagocytosis under the same conditions; indeed, they multiply within the phagocytic cells and eventually kill them. It has been reported furthermore that the coagulase-positive strains are better able than the coagulase-negative strains to multiply in normal plasma, the difference appearing to be due to the neutralization of some staphylococcidal property of serum by coagulase (2, 17). It could have been expected from these two independent types of observations that the tissues would rapidly destroy the coagulase-negative organisms and that contrariwise, the coagulase-positive forms would survive and begin to multiply *in vivo* shortly after infection. In reality, however, there was no indication in our experiments that the ability to produce coagulase affected the fate of the injected staphylococci in mouse organs during the initial phase of the infection.

Quantitative bacteriological techniques revealed that the pattern of distribution of the staphylococci in the various organs of animals sacrificed 2 minutes after intravenous infection was independent of the type of strain injected. A very large and rapid decrease in the numbers of living staphylococci that could be recovered from the tissues took place during the 2 hours that followed infection. During this initial stage the percentage sterilization was greatest in the lungs and the kidneys and somewhat smaller in the heart blood, liver, and spleen. Contrary to expectations in other words, the coagulase-positive staphylococci were killed rapidly at first just like the coagulase-negative forms in all organs including the kidneys. Even more surprising was the fact that some coagulase-negative staphylococci persisted for long periods of time—even though in much reduced numbers.

Differences correlated with the production of coagulase became apparent in the kidneys a few days after infection. Whereas most of the staphylococci had been cleared from this organ during the first phase of infection, their numbers began to increase after a few days—particularly in the animals infected with coagulase-positive strains. The bacterial proliferation in the kidneys was associated with the production of abscesses which destroyed the renal tissue and eventually killed the animals. Interestingly enough, bacterial multiplication in

the kidneys took place also in animals having received coagulase-negative staphylococci, but it was much more limited than in the case of coagulase-positive forms, and apparently never reached a level sufficient to bring about an extensive destruction of renal tissue.

It may be worth repeating here some peculiar facts concerning the strain MAM. When first isolated in The New York Hospital by Dr. D. E. Rogers, strain MAM was coagulase-negative and non-pigmented. When tested again by Dr. Rogers after having been maintained for 1 year in our laboratory, it was still non-pigmented but had become weakly coagulase-positive in both human and rabbit plasma. The MAM culture maintained during that year in the laboratories of The New York Hospital had remained coagulase-negative. The persistence of the non-pigmented character and the failure to kill mice make it unlikely that the acquisition of the coagulase property was a result of contamination with the three virulent strains in use in our laboratory, which are all pigmented and lethal for mice. In fact, there are a few reports in the literature of changes in cultural characteristics and in phage specificity of staphylococci occurring during cultivation *in vitro* under various conditions (5, 11).

Whatever the cause of its change in coagulase activity, the MAM substrain used in the present study has been of interest from the point of view of the study of virulence. None of the mice infected with this culture have died within the period of 1 month after infection during which they were observed; according to this criterion, therefore, the culture is not virulent. On the other hand, most mice infected with culture MAM exhibited marked bacterial multiplication in the kidneys and macroscopic abscesses in the renal cortex. The renal infection was not as overwhelming as observed with the strongly coagulase-positive strains (Smith, Giorgio, and O'Hara), but was more extensive than with the coagulase-negative forms JAB, Air, and Stern. Clearly then, MAM is capable of producing definite renal disease, even though the infection is apparently self-limited or at least evolves very slowly. It would be of interest to test the behavior of strain MAM in mice of different genetic make-up and in different metabolic states. The fact that staphylococci of the MAM strain can cause death in a certain percentage of mice treated with thyroid extract (15) strongly suggests that they could behave as fully virulent organisms under the proper conditions of test.

Differences in the degree of virulence could be recognized among the strongly coagulase-positive strains of staphylococci as well. Thus, with a given infective dose, the Giorgio strain proved more effective than the Smith strain in causing fatal disease in mice and in producing rapidly a large microbial population in their kidneys. Among coagulase-negative strains, on the other hand, the Stern strain has always yielded smaller bacterial populations in the kidneys than did the Air and JAB strains. If these observations can be substantiated by quantitative comparative studies on many strains, they will point to the possibility

of arranging staphylococci in a continuous spectrum of virulence. In mice, the degree of virulence seems to be controlled in part, at least, by the ability of the organisms to multiply within the specialized biochemical environment of the kidney, rather than by their ability to resist the bactericidal power of the organs during the first phase of the infectious process.

It must be emphasized in conclusion that none of the staphylococci used in the present study were capable of coagulating mouse plasma; their coagulase-positive character could be brought out only in human or rabbit plasma. It is surprising therefore that virulence for the mouse appeared nevertheless to be correlated with the power of the organisms to produce coagulase—a fact which casts doubt on the validity of the theories commonly invoked to explain the causal role for this substance in infection. It is also disturbing that the great resistance exhibited by the coagulase-positive strains *in vitro* against the bactericidal activity of phagocytic cells and of serum did not express itself in resistance to bactericidal power of the tissues *in vivo*. This discrepancy does not apply only to the mouse for, as shown by Dr. D. E. Rogers, in this laboratory, enormous numbers of virulent staphylococci are rapidly killed in the tissues of the rabbit immediately after infection, although these microorganisms can coagulate rabbit plasma and multiply within rabbit leucocytes when the tests are carried out *in vitro* (12). It is worth keeping in mind in this regard that coagulase has not yet been shown to be active *in vivo*, even in those animal species whose blood it can coagulate *in vitro*. For example, injection of large amounts of coagulase-positive staphylococcal cultures or filtrates into rabbits does not cause detectable intravascular clotting (3). There has not yet come to light therefore any convincing evidence for a mechanism whereby coagulase could affect causally the fate of staphylococci in the tissues, not only of mice, but also of other species susceptible to infection with coagulase-positive staphylococci. On the basis of present knowledge, coagulase production must be regarded as an index, rather than as a determinant of virulence.

SUMMARY

The fate of hemolytic staphylococci injected intravenously into albino mice was followed by determining quantitatively the numbers of living organisms present in the various tissues at different intervals of time after infection.

Irrespective of the strain of staphylococcus used, most of the organisms disappeared rapidly from the blood, liver, spleen, and kidneys. This was true even when the infective dose consisted of large numbers of virulent, coagulase-positive staphylococci, capable of producing a fatal disease in a high percentage of the infected mice. The initial rate of removal or destruction of staphylococci was particularly high in the lungs and kidneys. In all cases on the other hand, a few living staphylococci persisted in the various organs for several weeks after infection, even when the organisms were non-virulent and coagulase-negative.

Although virulent as well as avirulent staphylococci were eliminated ex-

tremely rapidly and efficiently from the kidneys during the initial stage of infection, the microorganisms soon began to multiply in this organ, causing abscesses first detected in the cortex. Death of the animals infected with virulent cultures appeared to be due to the destruction of renal tissue by these abscesses. The abscesses caused by the avirulent strains eventually became sterile, and healed.

No convincing difference could be recognized amongst seven strains in their resistance to the bactericidal power of the mouse tissues during the initial phase of the infection. In contrast, marked quantitative differences came to light in their subsequent behavior in the kidneys. The multiplication of the coagulase-negative staphylococci in this organ soon came to an end in all animals and never proceeded far enough to result in fatal disease. The staphylococci of a weakly coagulase-positive strain multiplied somewhat more extensively in the kidneys than did the coagulase-negative, but never sufficiently to cause the death of any animal within the period of observation of 1 month. The three coagulase-positive strains tested yielded the largest bacterial population in the kidneys and caused the death of many of the infected animals. These three virulent strains differed quantitatively amongst themselves with regard to both the rapidity and extent of their multiplication in the kidneys and the lethal power of a given infective dose.

Taken together, the findings indicate that the hemolytic strains of staphylococci can be arranged in a continuous spectrum according to their ability to cause disease in albino mice. Although virulence for these animals appeared to be correlated with the production of coagulase, it did not seem to depend upon the ability of this substance to interfere with the bactericidal mechanisms of the mouse organs during the early phase of the infection. Virulence manifested itself chiefly by the production in the kidneys of progressive abscesses originating from the few staphylococci which were not destroyed during the initial bactericidal reaction.

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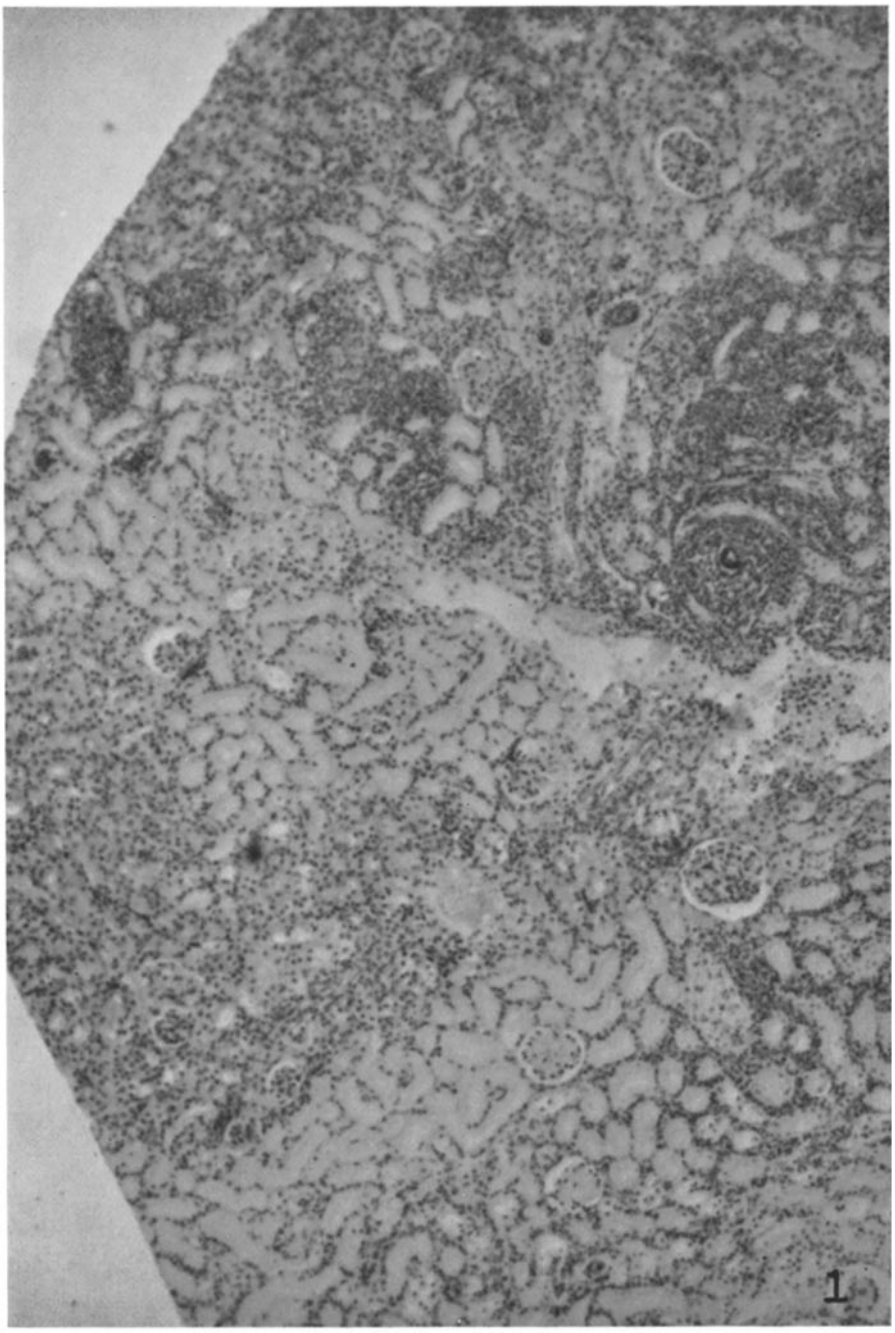
EXPLANATION OF PLATE 4

Renal lesions produced in mice by intravenous injection of 0.1 ml of coagulase positive staphylococci. Animal sacrificed 7 days after infection. Organ fixed with 10 per cent neutralized formalin. Sections stained with hematoxylin and eosin. Same size. See text.



(Smith and Dubos: Staphylococcal infections in mice)