HOST RESISTANCE AND STAPHYLOCOCCAL INFECTION

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DEDICATION

To the general practitioner in Troon, Scotland who made medicine sound exciting to a schoolboy and to the many doctors since who have shown how exciting medicine really is.

THESIS

That host factors play a large part in the eventual outcome of staphylococcal infections caused by "virulent" or "avirulent" cocci.

SUMMARY OF DISSERTATION

The host response to infection by staphylococci has been studied by death rates, histological studies and the fate of the cocci in the tissues. Different strains of staphylococci have shown a spectrum of virulence detected by quantitative differences in their multiplication in the kidney. When the host metabolism is disturbed by fasting, fasting with glucose solutions to drink or by increased metabolic activity caused by thyroid hormone or dinitrophenol then the animals are more susceptible to staphylococcal infection. Lactate solution given by mouth to fasted animals is beneficial in correcting the susceptibility to infection caused by fasting. Undernutrition, the result of protein or caloric restriction, does not affect the susceptibility of mice to staphylococcal infection.

Renal disease is related to death in human cases of staphylococcal infection. It is possible that a mouse virulence test for staphylococci can be developed.

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INTRODUCTION

> "----the presence or absence of virulence was considered to be a well defined or intrinsic quality of the microbial species under consideration. The problem in reality is much more complex."
>
> R. J. Dubos "The Bacterial Cell" 1946 (1)

Clearly the contact of a pathogen and its host does not of itself constitute sickness. In other words infection is not synonymous with disease. It is well known that many people harbor virulent staphylococci in their nose but it is unusual for them to fall ill with a staphylococcal disease. With many reports now made from all over the world of increasing resistance of staphylococci to antibiotics the time is ripe for a better understanding of the natural defences against this organism.

The Diseases due to Staphylococci

Staphylococcal infections (2-4) assume a variety of clinical pictures but by far the commonest occurrence, either alone or in combination with other disease, is the presence and multiplication of staphylococci in the nose. This organism causes a variety of skin diseases namely furunculosis, carbuncles, pemphigus neonatorum and impetigo. Rarely, it may be the cause of a variety of scarlet fever. (5-6) If the breast is considered to be a specialized skin organ then breast abscess belongs in this category.

Staphylococcal tonsillitis is a fairly common ailment and its diagnosis is becoming more frequent now that throat cultures are taken routinely in tonsillitis. Most common of the serious staphylococcal diseases is pneumonia. This is particularly prevalent in young children and in adults after influenza.

Septicemia is a rare disease comprising some 21% (7) of all septicemias. By far the commonest complication is kidney orperirenal abscesses. Other complications include involvement of the heart, liver, spleen, peritoneum, and central nervous system.

Osteomyelitis is undoubtedly a complication of septicemia or bacteremia but due to its distinctive characteristics it is usually considered separately.

Staphylococcal infections are pre-eminently infections of man. Infections in animals do occur but are rare and may indeed only represent a disease of man transmissable to animals. These diseases include bovine mastitis, botryomycosis in horses, purulent synovitis in turkeys and a disease of the udders of swine clinically resembling actinomycosis. (8) There is also a septicemia of newborn tick infested lambs. (9)

Tests for the Pathogenicity of Staphylococci

Criteria have been established to distinguish between "virulent" and "avirulent" staphylococci. Greater or lesser degrees of confidence are placed in the production of pigment, coagulase,

hemolysins and hyaluronidase, phosphatase and fibrinolysin. Other criteria used are mannitol fermentation, serological grouping, phage typing and precipitin reaction. These have been claimed to be, to a varying degree, successfully correlated with virulence. (10-13) Clearly, however, virulence must include a consideration of host defences and host susceptibility. On a theoretical basis, therefore, tests for leucocidin and dermonecrosis should be better. It is unfortunately true that these tests are not more successful than the in-vitro ones. The tests for enterotoxin do not come in to the present discussion.

Virulence as Conditioned by the Host

A study of the human disease shows that in certain cases host susceptibility exists. In some cases it may even be more important than the bacterial criteria of pathogenicity. In furunculosis, Montgomery (14) has discussed the importance of diet in the patient with boils. The name of pemphigus neonatorum indicates that host specificity is important. Smallpox owes some of its deadliness to the susceptibility of the pocks to staphylococcal infection.

A peculiarly fulminant form of pneumonia in adults is due to infection of the lung by staphylococci following influenza. Experimentally it can be shown that at least one virus--vaccinia--will add to the virulence of staphylococci in the lungs of rabbits' (15)

In septicemia the age of the host is important as most

deaths occur in the very young and in those over forty. (7) The source of the infection is also important as the fatality rate in septicemia following skin infections is 82% whereas following osteomyelitis it is 56%(16) Is it possible that the bacteremic origin of osteomyelitis has made the host muster its defences and become more resistant to a second invasion of the blood stream? The condition of the host may account for the fulminating septicemias following operations or criminal abortions or in the puerperium. Another fact demonstrating this susceptibility is that staphylococcal septicemias are more common in a hospital for cancer (Memorial Hospital, New York City) than in a neighboring general hospital (New York Hospital, New York City) (17)

Diabetes is the best known disease in which a change in host metabolism favors the occurrence of infections. In the treatment of 268 cases of acidosis occurring amongst 3009 cases of diabetes 117 cases died--a mortality of 43.7%. (18) Seventy-six per cent of the complications were due to infection. The data of these authors has been studied and from the diagnosis given it can be inferred that 5.9% of cases of acidosis die from staphylococcal infection. This figure may be an underestimate and 10.4% may be a more correct figure. This study was, of course, made before the era of antimicrobial agents.

TABLE I

MORTALITY IN THE TREATMENT OF DIABETIC ACIDOSIS

Group	No. of Cases	Mortality %	Probably due to staphylococcus Mortality %	Possibly due to staphylococcus Mortality %
Acidosis alone	129	24.8	--	--
Acidosis with complications	81	33.3	6.2	12.3
Complication sufficient to cause death	58	100.0	19.5	31.1
Totals	268	43.7	5.9	10.4

Approaching this problem from the opposite point of view it is interesting to note that in the treatment of 35 cases of staphylococcal septicemia 29 died (83%) six of which were diabetics (17%). (19)

Indeed all criteria of staphylococcal virulence may be absent yet the illness can be fatal. There are several records of fatal disease due to staphylococcus albus. (20-21) Mathew has described in detail two cases with subacute bacterial endocarditis due to coagulase negative staphylococcus albus. Both cases showed typical subacute bacterial endocarditis and both responded to penicillin but succumbed later to myocardial complications. In one case the organism was isolated on seven different occasions from blood culture; once from marrow culture and once from culture of the patients acne. In this case there was also serological evidence of this organisms etiological role. The second case showed the organism on five occasions from blood culture. In both cases the same organism was recovered from the heart at autopsy. It was believed that co-incident acute rheumatic fever may have been a predisposing factor in both these cases. (22)

Another disease reputed to be as common in children as diabetes (23) in which susceptibility to the staphylococcus is marked is cystic fibrosis of the pancreas. The cause of death in these children is staphylococcal pneumonia.

PART I.

Studies of Staphylococcal Infections in Mice

In the past studies of the pathogenicity of this organism have been made in the rabbit and in the mouse. For reasons of economy and convenience the mouse was used in this study. Ogston, of Aberdeen, in his original investigations when he characterized and named the staphylococcus used both mice and chicken eggs for the work. (24) Since that time the organisms have been given subcutaneously, intramuscularly, intraperitoneally, and intravenously. (25-29) The characteristic finding at autopsy is abscesses of the kidney. (25) Death only follows the use of coagulase positive organisms and it is believed to be related to the amount of α toxin produced.

Death and Autopsy Studies

A detailed study of pathogenicity in the mouse was carried out. It was considered important to exclude various variable peripheral barriers in the inoculation. An unknown amount of multiplication of the inoculated dose takes place in the abdomen after intraperitoneal infection before a generalized infection occurs. Similarly, the absorption of intramuscular and subcutaneous doses is uncertain. For these reasons the intravenous route was used.

Methods

The staphylococci used were of varying types as shown in Table 2. They were maintained in stock culture in 1% Pfanstiehl

(Pfanstiehl Chemical Company, Waukegan, Illinois) peptone meat infusion broth (Pf broth) and were transferred to new broth every one to two months. A loopful of the stock culture was inoculated into 7 cc. of Pf broth and incubated at 37° C overnight. The number of organisms present in 0.1 cc of overnight cultures of these organisms is shown in Table 3. In this initial study staphylococcus Smith was used.

The mice were approximately three weeks of age and were albino mice of the Rockefeller strain. They were housed in metal cages on pine shavings and were fed purina chow (Ralston Purina Co., St. Louis, Mo.) pellets with water ad lib.

Intravenous Inoculation

The broth culture to be inoculated was diluted with an equal quantity of saline to increase the accuracy of measurement. Tuberculin (1 ml.) syringes were used with No. 27 needles (British Standard No. 27)

The mouse was allowed to walk into a glass cylinder with a ventilated opening. His tail was held with the left hand and fixed in place by a rubber cork with a lengthwise V-notch cut in its side. Tapping the tail with the right forefinger and wiping with 70% alcohol made the veins of the tail prominent. The veins which are easiest to enter are the two lateral ones. The tail was supported over the index finger of the left hand and was fixed there by the thumb. The needle penetrates the skin and vein at the shallowest angle possible. A successful injection is shown by the ease of injection, a pink "neon" like lighting up of the tail, and a small bleeding point on withdrawal. A wheal will develop if the vein has not been entered and there will be considerable resistance to injection.

Autopsy Procedure

Mice were killed with chloroform. The dead animals were pinned to an autopsy board covered with an impervious disposable paper. The fur was thoroughly wetted with 70% alcohol and the skin was cut and separated from the carcass from neck to pubis. Sterile scissors and forceps were used to cut the skin of all animals in a given batch. Fresh scissors and forceps were used to remove the organs of each individual mouse. Heart blood was removed by using a Pasteur pipette and rubber teat after exposing the heart by dissection.

Death Rates

It was found that in practically all cases death occurred in

groups of ten mice observed for fourteen days after inoculation with

0.1 cc coagulase positive staphylococci (see Table 4). It is noteworthy, however, that the death rate varied from 0 to 4 per 10 mice in a number of experiments with the Smith strain. There was no evidence that this change was related to the season.

Using a strain of coagulase negative organisms (J. A. B.) we were not able to produce death with a similar dose. Indeed when the dose was increased up to 6 times that given to produce death with the coagulase positive organisms, no deaths occurred.

The organism which was a poor producer of coagulase (M. A. M.) likewise did not produce deaths.

Autopsies

Postmortem examinations were made in a great number of animals. In coagulase negative infections no lesions were found. With Smith and O'Hara strains the only abnormality found was abscesses of the kidney occurring two or more weeks after infection. Figure 1 shows the kidneys from a mouse killed 7 days after infection with 0.1 cc of staphylococcus Smith culture. Some, but not all, animals infected with the Giorgio strain also had abscesses present in the subcutaneous tissues and the heart. Animals infected with the MAM strain of intermediated virulence sometimes showed small localized abscesses in the kidneys.

Figure 1. Kidneys from a mouse killed seven days after intraveous inoculation with 0.1 cc. Staphylococcus "Smith". Abscesses are particularly prominent in the upper kidney.

TABLE 2

Characteristics of the Staphylococci Studied

Name	Source	Coagulase	Mannitol	Pigment	Hemolysis[1]	Phage
Giorgio	Case of Osteo-myelitis	+	+	aureus	+	7.47 C
Smith	Case of Osteo-myelitis	+	+	aureus	+	44A 42E
O'Hara	Case of Broncho-Pneumonia	+	+	aureus	+	
MAM[2]	Skin	±	±	albus	+	
JAB	Skin	Neg	–	albus	+	
AIR	Air	Neg	–	albus	+	
STERN	Skin	Neg	–	albus	+	

1. As demonstrated by growth on rabbit or human blood agar.

2. This was initially a coagulase negative strain which changed during in-vitro transfer.

TABLE 3

Log. Number of Organisms in 0.1 cc Overnight Culture.

Strain	Titer Log. 18 hour culture	Experiment No.
Smith	8.37	
Smith	7.96	
Smith	8.40	
Smith	8.30	
Giorgio	8.33	
Giorgio	8.35	
O'Hara	8.48	
M. A. M.	7.90	
M. A. M.	8.00	
J. A. B.	7.48	
Air	7.48	
Stern	7.33	

TABLE 4.

Effect of Virulence on the Number of Deaths in Groups of Ten Mice Infected Intravenously

Dose of Overnight Culture	Coagulase	Strain Name	Deaths*/10
0.1 ml.	+	Smith	3
0.1 ml.	+	Smith	2
0.1 ml.	+	Smith	1
0.1 ml.	+	Smith	4
0.1 ml.	+	Smith	0
0.1 ml.	+	Smith	2
0.1 ml.	+	Smith	2
0.1 ml.	+	Smith	4
0.1 ml.	+	Smith	4
0.1 ml.	+	Smith	5
0.1 ml.	+	Giorgio	9
0.1 ml.	+	Giorgio	10
0.1 ml.	±	MAM	0
0.1 ml.	±	MAM	0
0.1 ml.	±	MAM	0
0.1 ml.	-	JAB	0
0.1 ml.	-	JAB	0
0.2 ml.	-	JAB	0
0.6 ml.	-	JAB	0
0.1 ml.	-	Stern	0
0.1 ml.	-	Air	0

* at 14 days after infection

Methods

Animals were infected intravenously with 0.1 cc. of staphylococcus Smith. Two were killed one day and two were killed seven days after infection. A similar number of control animals were killed at the same time. Portions of the spleen, liver, lung and kidney were fixed in 10% neutralized formalin. Sections were made in the usual fashions and were stained with hemotoxylin and eosin.

Results

The sections of the spleen taken one day after infection already showed a well marked hyperplasia of the follicles. At this time in the liver very small foci of mononuclear cells were scattered throughout the parenchyma. There were no other significant changes in the organs. By seven days these foci had gone. In the lung similar small foci of round mononuclear cells were present and had gone by the end of a week.

The most striking histological changes were those present in the kidney. In the sections taken one day after infection, small cellular foci were present mainly in the cortex and usually closely juxtaposed to glomeruli. Small round cells and polymorphonuclears were present in about equal proportions. (Fig. 2) By the end of seven days the foci were frankly purulent. They were larger and were more numerous. Some of the neighboring ones had coalesced. By this time they were also present in the medulla, but the number of these foci was not quite as great as the number in the cortex. A further change was the presence of wedge shaped infarcts with their base at the kidney margin. Occasionally thrombosed vessels could be seen in the neighborhood of these infarcts. (Fig. 3)

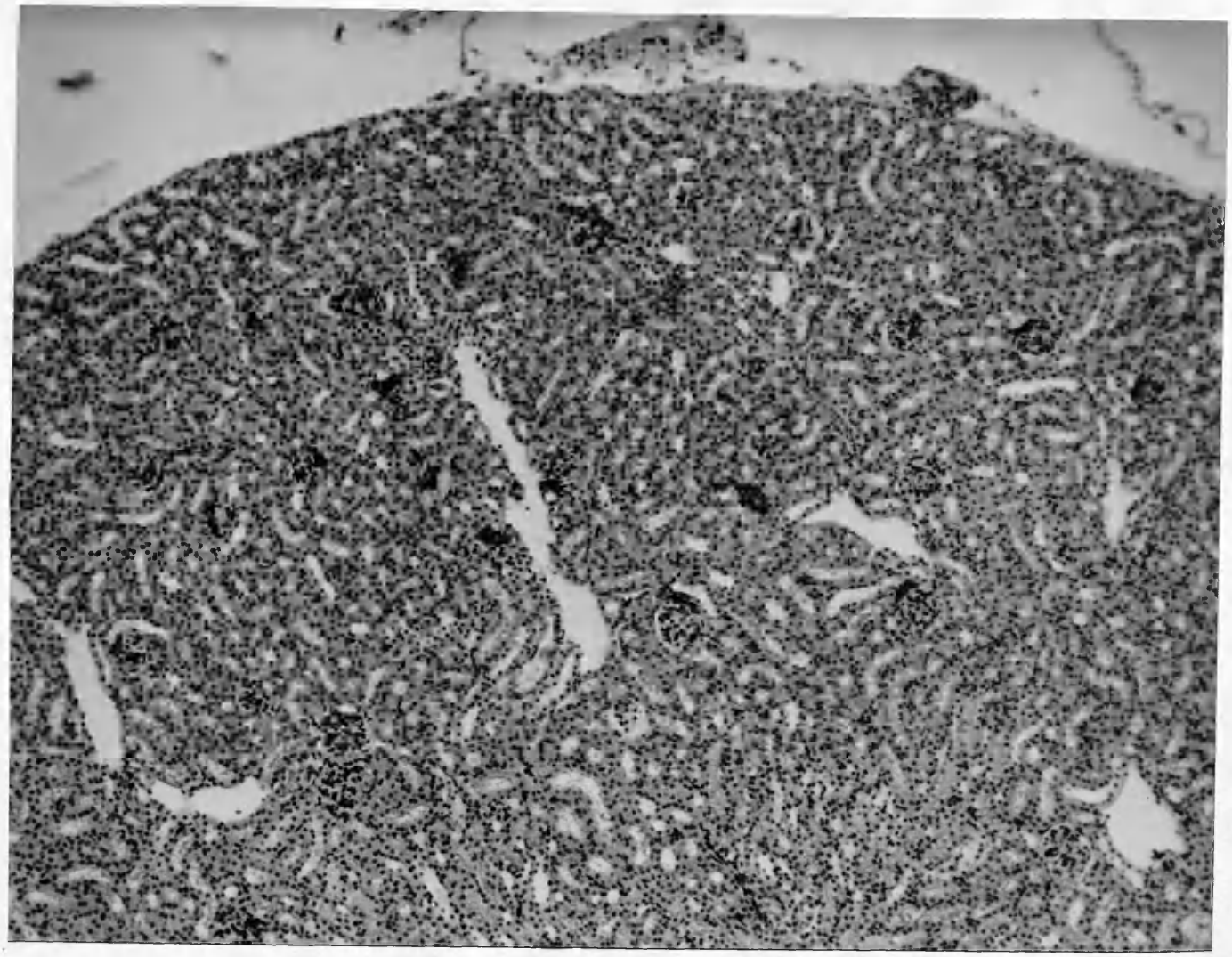

Figure 2. Histology of the kidney lesion one day after intravenous infection. Small cellular foci are present in the cortex.

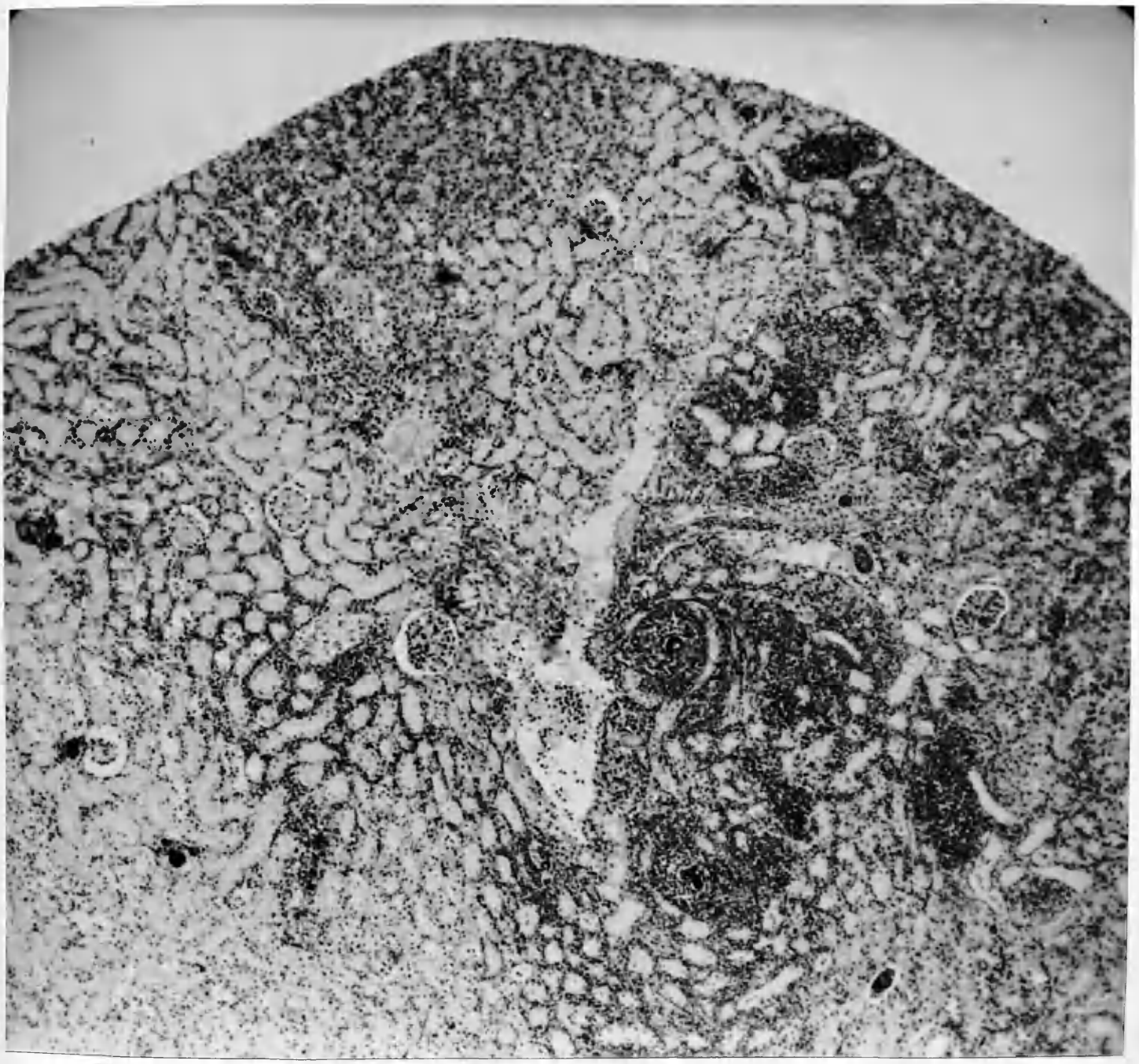

Figure 3. Histology of the kidney lesion one week after intravenous injection. A pale area of infarction is present in the upper left corner.

Studies of the Distribution of Staphylococci in the Organs of Mice

In order to obtain a detailed idea of the fate of staphylococci in mice an adaptation was made of a method originally used to study the fate of tubercle bacilli in the tissues.

Methods

Preparation of Tissue Emulsions

The method used was that of Fenner, Marten and Pierce (1949) and of Pierce, Dubos and Schaefer (1953), with certain modifications which are discussed below. (30, 31)

Organs removed at autopsy were ground in the presence of 5 cc. of Earle's solution[1], a modified Ringer's solution. Further tenfold dilutions were made in 0.1% bovine albumin which served to protect bacilli from toxic substances released by grinding or by autolysis of the tissues.

The grinding was done by Teflon[2] (a tetrafluoroethylene resin) grinders carefully machined to fit Pyrex glass tubes (6 1/4" x 3/4") which contained the 5 cc. of Earle's solution. The grinders[3] are fitted to stainless steel shafts which can be made to revolve at 1740 r.p.m. by a motor operated by a foot control[4] (Figure 4). Homogenization of tissue without an abrasive can be carried out in 30 seconds. Microscopic examination shows ruptured tissue cells, intact nuclei and bacteria which are well dispersed. Tenfold dilutions are made directly from these emulsions (0.2 ml. in 1.8 ml. dilution blanks)

[1]Earle's Solution:
NaCL 13.6 g.
KCL 0.8 g.
$MgSO_4 \cdot 7H_2O$ 0.2g.
$NaH_2PO_4 \cdot H_2O$ 0.29g.
10% $CaCl_2$ sdn 0.5 cc.
Dissolve in 100 cc's H_2O (distilled)
Dilute 1/20 before using and autoclave.

[2]Teflon DuPont trademark.

[3]Scientific Glass Apparatus Co., 100 Lake wood Terrace Bloomfield, N. J.

[4]Polo Laboratory Supplies, Inc., 81 Reade St., New York 7, N.Y.

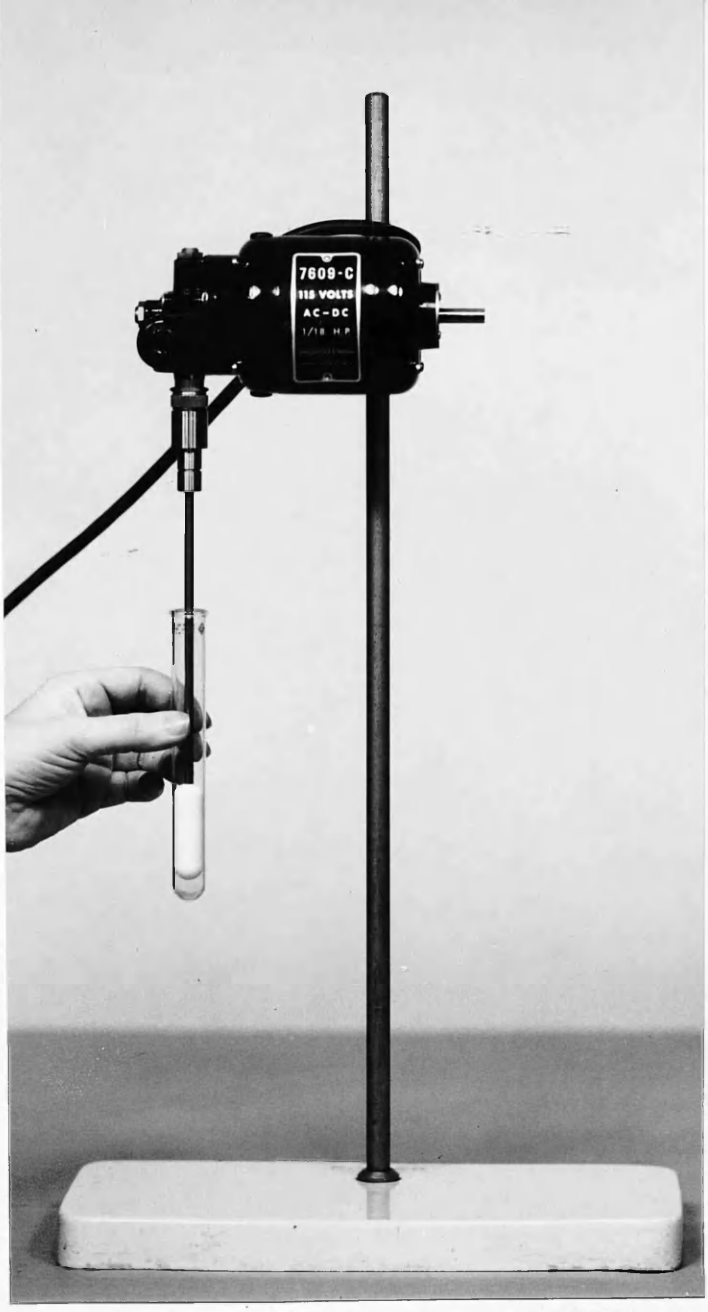

Figure 4. a) Teflon grinder and tube 6) Assembled for use.

Dilution Blanks and Counting

0.1% albumin was made from 5% albumin stock solution, which was rendered sterile by filtration. 1.8 cc. were dispensed into sterile Wassermann tubes using an automatic delivery 2 cc. syringe (Becton, Dickinson and Co., Rutterford, N. J.) in a sterile inoculating room.

The tissue emulsion titration material was placed on Pf agar plates using one standard platinum loopful for each dilution. Counts were made of the resultant colonies after 18 hours incubation at 37° C.

In some experiments counts were made of all the cocci in the tenfold dilutions of the organ extracts by a newly developed technique. (32) Agar was added to Pf broth (see below) to a concentration of 0.3%. After autoclaving and cooling to 50-56° C an equal quantity (1.8 ml.) of this agar solution was added to each of the tenfold dilutions. The agar was added with an automatic delivery 2 cc. syringe. Incubated in this soft agar each staphylococcus developed into a discrete colony. When the number of these was less than 20 or 30 counting was quite easy. Aeration was found to be adequate to give uniformity in the size of the colonies from top to bottom of the tube. Counts of the organisms present in organs of infected animals were made using both the plate method and the tube method. These simultaneous counts were found to give identical results within the limits of error of the methods.

Bacteriological Hood for Manipulation of Infected Materials

The grinding of mouse organs infected with staphylococci was carried out in hoods especially designed to protect workers against infection. The hoods are completely enclosed except for a space for the workers arms. They were kept under a slightly negative pressure by an exhaust motor pulling air from the chamber through a filter. The interior of the hood was sterilized by ultraviolet irradiation before and at the completion of each experiment. Irradiation was discontinued during the experiment.

Distribution of Coagulase Positive Staphylococci in the Different Organs of Mice Following Intravenous Injection.

In the preliminary phase of this study, experiments were carried out to determine what dose of staphylococci would result in the recovery of the cocci from some or all of the organs of mice over a two

TABLE 5

Number of Mice with Recoverable Staphylococci in Their Organs.

(Liver, Spleen, Lung or Kidney.)

Dose of Overnight Culture	Number of Mice with Staphylococci /4					
	1 hour	1day	2 days	3 days	7 days	14 days
.002 ml.	4	4	2	1	0	0
.02 ml.	4	4	4	4	1	1
.05 ml.	4	4	4	4	4	2
.1 ml.	4	4	4	4	4	4*

* Including 2 deaths.

week period. Coagulase positive organisms of the Smith strain were used. It was found as shown in Table 5, that 0.1 cc. of an overnight peptone broth culture was a suitable dose.

1. Staphylococcus "Smith"

Mice were infected intravenously with 0.1 ml. of overnight peptone broth culture (Staph. Smith) and were killed at intervals after infection. These intervals were one to two minutes, one hour, one, two three, seven and fourteen days. Enumeration (Table 6) of the staphylococci was carried out and these counts are listed in Table 7. The counts have been calculated as the total number of viable staphylococci per organ (in the case of the kidneys the count is for both organs) and these results are shown graphically in Figure 5. The lines in this figure are trend lines. The effect of a smaller dose is shown in Table 7E

2. Staphylococcus "O'Hara"

The same experiment was repeated with a different coagulase positive strain with largely identical results. These are detailed in Table 8.

3. Staphylococcus "Giorgio"

This organism proved to be more virulent than the preceding ones and it was not possible to study it for the full fourteen day period due to the death of the animals. The recovery of the organisms from tissue homogenates is detailed in Table 9. The effect of a smaller dose is shown in Table 9B and 9C.

In the animals infected with "Smith" and "O'Hara" it is seen that the organisms are present in high titer in the spleen one hour after

TABLE 6.

Calculation of Titer — Plate Technique

Final titer equals — Observed number per loopful* of undiluted organ homogenate

x 1000 x 5/3

Logarithm of above to Base 10 — Ditto x $10^{3.23}$

Log Ditto + 3.23

*one loopful equals .003cc.

Tube Technique

Final titer equals — Observed number per tube of undiluted organ homogenate

x 5/1.8

Ditto x 2.8

Log Ditto + 0.45

Dilution of Blood

1 Drop Pasteur .02cc.

x 50 colonies/cc.

.02 cc in 1.8 cc 1/100.

TABLE 7A EXPT. 5.

Course of Infection in Mice Infected with 0.1 ml. I. V. Culture (Staph Smith)

1 hour	1 day	2 days	3 days	7 days	4 days
		Liver			
7.12	5.53	5.28	0	0	0
7.00	5.95	4.43	4.18	0	3.23
		Spleen			
6.18	5.51	4.49	4.18	0	0
	4.87	4.90	3.53	3.23	0
		Lung			
5.18	3.53	0	3.53	3.23	0
5.55	0	3.53	4.88	3.53	0
		Kidney			
4.08	7.03	5.34	4.55	9.08	
4.57	5.11	5.34	9.08	9.08	6.43

TABLE 7B EXPT. 7

Course of Infection in Mice Infected with 0.1 ml. I.V. Culture (Staph Smith)

1 hour	1 day	2 days	3 days	7 days	14 days
		Liver			
9.08	6.14	5.83	4.38	3.23	0
9.08	6.84		4.57	3.53	0
7.22	6.09	5.53	4.53	4.99	D
9.08	6.34	5.38	4.46	4.31	D
		Spleen			
6.66	6.02	4.01	3.23	0	0
6.97	6.04		4.18	0	0
7.19	6.01	3.71	4.13	4.84	D
7.23	6.23	3.23	3.53	3.71	D
		Lung			
5.43	4.46	3.23	4.08	5.34	0
4.98	4.68		0	4.28	0
5.68	4.01	4.87	4.87	6.38	D
5.28	6.80	3.93	3.93	4.13	D
		Kidney			
4.87	6.13	9.08	7.17	8.17	5.95
4.90	7.23		8.38	5.76	0
4.84	7.38	5.08	8.34	8.95	D
5.08	6.34	3.53	7.46		D

TABLE 7C EXPT. 27

Course of Infection in Mice Infected with 1 ml. I. V. Culture (Staph Smith)

1 day	2 days	3 days
	Liver	
5.51	4.77	5.53
5.86	4.38	3.93
5.57	4.95	4.79
	Spleen	
5.94	3.71	3.23
6.14	4.28	3.83
5.53	4.08	3.71
	Lung	
4.87	3.83	4.28
4.40	3.71	5.23
4.23	5.83	5.34
	Kidney	
5.51	3.93	9.99
7.14	8.09	8.55
6.34	8.18	8.81

TABLE 7D Expt. 115

Course of Infection in Mice Infected with 0.1 ml. I. V. Culture (Staph Smith)

2 min.	2 hours	1 day	5 days	15 days	21 days	36 days
			Heart Blood			
5.85	3.70	0	0	0	0	0
6.11	3.70	0	0	2.30	0	0
6.23	3.70	0	0	0	0	0
6.05	3.70	1.70	0	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
			Lung			
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
			Kidney			
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

TABLE 7E Expt. 115

Course of Infection in Mice Infected with 0.03 ml. I.V. Culture (Staph Smith)

2 mins	2 hours	1 day	5 days	15 days	21 days	36 days
			Heart Blood			
5.30	3.70	1.70	0	0	0	0
5.54	3.70	0	0	0	0	0
5.78	3.70	0	2.00	0	0	0
5.88	3.70	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	6.23	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
			Lung			
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	0	0	0
6.13	5.23	3.93	3.23	0	0	3.71
			Kidney			
5.23	5.23	4.01	5.23	0	0	0
5.71	5.23	4.53	8.59	0	0	5.93
6.18	5.23	5.01	8.90	0	0	0
6.23	5.23	5.51	5.23	0	0	5.93

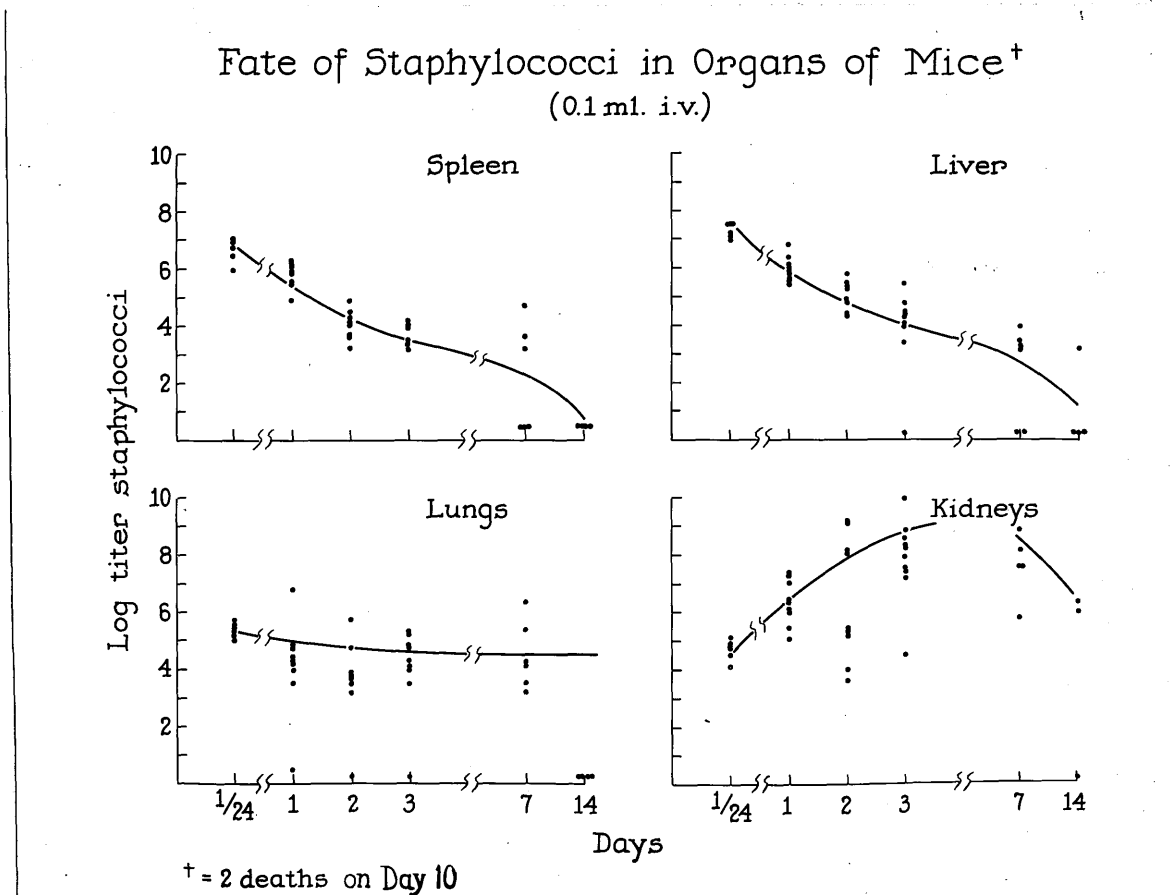

Figure 5. The distribution of Staphylococci in the organs of mice after intravenous infection of 0.1 cc. of Staphylococcus "Smith" culture.

TABLE 8A Expt. 5

Course of Infection in Mice Infected with 0.1 ml. I. V. Culture (Staph O'Hara)

1 hour	1 day	2 days	3 days	7 days	14 days
		Liver			
7.53	6.09	5.40	4.23	3.23	0
7.11	6.40	5.34	4.34	3.23	
		Spleen			
6.84	4.23	4.01	3.53	0	0
6.13	4.18	3.23	0	0	
		Lung			
6.04	4.28	4.13	4.61	4.53	4.38
5.46	5.38		4.28	3.53	
		Kidney			
5.05	5.40	6.23	6.63	7.46	8.53
4.89	5.49	7.00	5.72	3.53	

TABLE 8B Expt. 121

Course of Infection in Mice Infected with 0.1 ml. I. V. Culture (Staph O'Hara)

2 mins	1 hour	1 day	2 days	5 days	7 days	14 days
			Heart 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
			Lung			
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
			Kidney			
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

TABLE 9A Expt. 116

Course of Infection in Mice Infected with 0.1 ml. I. V. Culture (Staph Giorgio)

2 hours	6 hours	1 day	4 days
	Heart 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
	Lung		
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
	Kidney		
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

TABLE 9B Expt. 116

Course of Infection in Mice Infected with 0.03 ml. I. V. Culture (Staph Giorgio)

2 hours	6 hours	1 day	4 days	21 days
		Heart 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
		Lung		
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
		Kidney		
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

TABLE 9C Expt. 118

Course of Infection in Mice Infected with 0.03 ml. I.V. Culture (Staph Giorgio)

1 hour	1 day	2 days	5 days	7 days
		Heart 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
		Lung		
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
		Kidney		
5.28	6.66	7.57	9.31	7.71
4.98	7.14	8.46	8.81	9.13
5.13	7.46	8.69	9.34	8.28
5.13	3.83	7.21	9.28	8.53

infection. Thereafter they show a steady decline over a 7-14 day period until at 14 days we were unable to recover viable organisms by our technique. Starting at a slightly higher titer the fate of the organisms in the liver is the same. In both these cases the counts are reproducable in any one experiment and are moderately well reproduced between experiments.

By contrast the titer in the lung is substantially lower b ut it maintains this level without variation throughout the experimental period. We have been able to culture organisms from the lung weeks after infection. The tissues of the lung are more fibrous and are more difficult to grind than those of the spleen or liver. We believe this may be the cause of the greater spread in our results with this organ.

Quite the opposite developments take place in the kidney compared with the spleen and liver. Initially, the titer is very low but there is a progressive multiplication of the staphylococci. A peak titer is reached about the 6th day. Most of the deaths occur between days 7 and 10. Those animals that recover show a fall in titer after the 6-7th day which may be related to the development of antibody. We have not tested for the presence of antibody. It is noticeable that the results at any one period vary through a moderately wide range. This is greater during the later periods. It is felt that the development of abscesses must be associated with the destruction and death of an unpredictable number of cocci near the center of the abscesses. It appears reasonable to believe that the severe disorganization in the kidney is the immediate cause of death.

Distribution of Coagulase Negative Staphylococci in the Different Organs of Mice Following Intravenous Injection

These organisms grew rather less well in Pf broth than did the coagulase positive ones. To compensate for the slight difference 0.2 cc. of an 18 hour culture was used in most cases. As will be seen from the data presented this resulted in the recovery from the organs, just after inoculation, of titers very similar to those found when the coagulase positive organisms were used.

1. Staphylococcus "JAB"

This organism was injected in the usual fashion and titers at various times after inoculation are shown in Table 10

2. Staphylococcus "Stern"

The data obtained from this study are listed in Table 11.

3. Staphylococcus "Air"

Table 12 contains titers at various intervals after inoculation with this strain.

The clearance pattern for these strains is not unlike that of the coagulase positive ones. A difference however is evident in the behavior of the cocci in the kidney in the time subsequent to the clearance period.

Distribution of Staphylococci of Intermediate Coagulase Activity in the Different Organs of Mice Following Intravenous Injection

The organism used in this study has an unusual history. When initially received it was coagulase negative and Mannitol negative.

TABLE 10A Expt. 116

Course of Infection in Mice Infected with 0.2 ml. I.V. Culture (Staph JAB)

2 hours	6 hours	1 day	4 days	21 days
		Heart Blood		
2.70	2.17	0	0	0
2.40	3.05	0	0	0
3.00	2.17	0	0	0
D	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
		Lung		
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
		Kidney		
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	3.32	5.53	4.61	0

TABLE 10B Expt. 110

Course of Infection in Mice Infected with 0.1 ml. I.V. Culture (Staph JAB)

1 hour	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
		Lung			
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
		Kidney			
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

TABLE 11 Expt. 119

Course of Infection in Mice Infected with 0.2 ml. I. V. Culture (Staph Stern)

1 hour	1 day	2 days	4 days	7 days	14 days
		Heart 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
		Lung			
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
		Kidney			
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

TABLE 12 Expt. 119

Course of Infection in Mice Infected with 0.2 Ml. I. V. Culture (Staph AIR)

1 hour	1 day	2 days	4 days	7 days	14 days
		Heart 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
		Lung			
3.83	0	3.83	3.23	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
		Kidney			
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	3.23	5.12	6.01	5.49	0

After numerous subcultures in Pf broth it was found to be slightly coagulase positive and Mannitol positive. As it has retained its albus character and phage type contamination can probably be ruled out. The strain from which it was derived has maintained its coagulase negative character. The experiements to be described were carried out with the mildly coagulase positive sub-strain.

Staphylococcus MAM

This strain which does not kill mice gave titers not unlike those with the lethal Smith strain. The titer in the kidney however, failed to reach the heights of the "Smith" infected animals. The titers are detailed in Table 13.

The average titers in the kidney are listed in Table 14 for the seven strains tested and it is evident that while there is a difference in titer between coagulase positive and negative strains there is also a significant variation present within these groups.

TABLE 13A Expt. 46

Course of Infection in Mice Infected with 0.1 ml. I.V. Culture (Staph MAM)

1 hour	1 day	2 days	3 days
	Liver		
7.49	4.38	3.53	0
7.20	0		0
8.00	3.53	4.08	0
	Spleen		
6.03	3.83	3.23	0
6.66	3.53	0	3.23
6.59	3.23	3.23	3.23
	Lung		
5.28	0	0	0
4.66	0	5.81	0
5.00	0	5.61	0
	Kidney		
4.97	5.40	0	4.88
4.98	4.34	8.46	5.98
4.49	3.23	0	5.02

TABLE 13B Expt. 56

Course of Infection in Mice Infected with 0.1 ml. I.V. Culture (Staph MAM)

1 hour	1 day	2 days	4 days
	Liver		
6.85	4.68	3.30	
6.98	4.05	3.35	0
6.99	4.85	2.93	0
	Spleeen		
5.68	3.10	2.96	3.05
5.89	2.99	2.62	1.56
6.30	3.30	2.73	1.45
	Lung		
3.90	2.81	0.75	3.13
3.40	2.30	1.35	2.35
4.15	2.53	1.13	1.93
	Kidney		
3.60	2.83	5.07	0
3.35	2.99	6.53	4.60
3.45	3.62	2.35	0

TABLE 13C Expt. 60

Course of Infection in Mice Infected with 0.1 ml. I. V. Culture (Staph MAM)

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
	Lung		
6.53	2.93	1.75	3.97
6.73	0	3.30	4.81
6.35	0	3.05	1.45
	Kidney		
6.23	4.93	0	0
6.20	4.93	4.68	4.85
6.15	5.85	4.15	0

TABLE 13 C Expt. 108

Course of Infection in Mice Infected with 0.1 ml. I. V. Culture (Staph MAM)

1 day	2 days	3 days	7 days	14 days
		Liver		
4.82	3.93	3.53	0	0
5.80	5.38	4.80	0	0
5.84	4.08	3.71	0	3.71
3.71	4.34	3.23	0	0
		Spleen		
3.53	0	0	0	0
3.83	0		0	0
4.18	3.71	0	0	0
0	3.53	0	0	0
		Lung		
0	3.53	0	4.40	4.08
5.16	0	0	4.18	0
3.23	0	6.53	5.01	3.93
0	5.18	0	3.23	0-
		Kidney		
3.53	6.40	5.40	6.40	6.31
4.84	5.87	6.53	6.23	6.13
6.20	5.34	5.68	6.12	5.95
5.20	6.38	5.80	6.90	3.23

TABLE 13E Expt. 105

Course of Infection in Mice Infected with 0.15 ml. I.V. Culture (Staph MAM)

2 Min.	2 Hrs.	Day 1	Day 5	Day 15	Day 21	Day 36
			Heart Blood			
7.57	4.70	0	0	0	0	0
7.05	4.70	0	0	0	0	0
6.85	4.70	0	0	0	0	0
7.36	4.70	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
			Lung			
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	6.53	0	0
			Kidney			
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	5.23	5.77	9.02	6.53	5.71	5.73

TABLE 14

Staphylococcal Logarithmic Titers in Mouse Kidneys with Strains of Different Virulence

Strain	1-2 min.	1-2 hrs.	1 day	2	3	4	5	7	14
Giorgio		5.04*	8.79			?9.97			
Smith	6.49	4.72	6.37	6.10	8.02		9.53	8.21	7.64
O'Hara	5.77	4.79	5.30	6.09	6.23		6.75	7.25	6.91
M. A. M.	6.19	4.14	4.24	4.64	5.61			5.91	4.35
AIR		3.03	2.03	3.66		2.37		3.93	2.31
J. A. B.		4.40	2.56	4.15	6.01			0	0
Stern		4.30	0	0		0		0.81	0

*Each result is the average of readings made on 4 to 12 animals.

Discussion

From the preceding data it is apparent that the fate of the animal is dependent on a series of events almost entirely confined to the kidney. The main part of this conflict takes place at a time somewhat remote from the initial infection, in fact, after a very efficient clearance of the cocci from the tissues. It is striking that the virulent and avirulent cocci both multiply in this specific in-vivo environment. The difference is shown only by the titer reached at a particular time in this organ. It is possible that kidney growth inhibitors or stimulants for staphylococcal pathogenicity does parallel the coagulase activity of these organisms, it is difficult to be convinced that such correlation is more than fortuitous. The preceding data presents new advances of approach to the problem of virulence.

A knowledge of the events occurring in the experimental disease in the mouse will enable the physician to give a prognosis related to the stage of the human disease--staphylococcal septicemia. A knowledge of the underlying process may also improve treatment. In persistent septicemia the finding and drainage of a kidny abscess may well result in the cure of the patient's disease.

Differences in virulence for various diseases have been attributed to various qualitative differences in the injected bacteria. At times this has been considered to be the only factor. It has also been inferred that the initial fate of the organisms is the cardinal factor in their eventual virulence. Virulence was thought to be due to a combination of invasiveness and toxigenicity. Invasiveness was usually used

in a prompt and relentless sense and the toxic effect was also thought to be early and continuous.

Recently certain examples not unlike the present investigation have come to light and a certain unity can be detected in their results. Lurie[33] studied bovine and human strains of tubercle bacilli in rabbits. He was surprised to find that both strains grew in the lungs. The difference was that the bovine strain which kills the animals multiplies to a much higher titer in the lungs. Theiler[34] in a study of virulent and avirulent yellow fever virus in mice showed that the virulent strain differed in being able to grow to a much higher titer in the brain than did the avirulent strain. In the case of mouse pox or extromelia the place where the difference in titer is apparent is the internal organs. The virulent form multiplied here much better than did the avirulent form in experimental work carried out by Fenner[35]. Avirulent forms of pasturella pestis studied by Meyer[36] in mice and guinea pigs failed to multiply in the liver and spleen, as well as the virulent form. Karzon in an excellent study of virulent and avirulent strains of Newcastle virus showed that the virulent form differed from its attenuated relative in its ability to multiply in brain tissue.[37] To this group we can now add the staphylococcus in relation to its ability to reach a critical titer in the kidney.

A hypothesis can now be stated that pathogenic organisms have a target organ where they grow to a higher titer than elsewhere in the body. As variants of these organisms exist which grow in the same organ but not to such a high titer and are also avirulent then the in-vivo environment of such an organ holds the key to the pathogenicity of the virulent organisms.

PART 2.

Periods of strife associated with dietary limitations are also periods of prevalence of infectious disease often in epidemic proportions. In individuals severe wasting diseases may be complicated by infectious incidents. In both these cases periods of chronic and acute deprivation of food are inextricably intermingled. Experiments have been carried out where protein or caloric limitation has been brought about and has been followed by infection. This pure type of deficiency is not common in human affairs and it was therefore thought profitable to re-examine this area and also related areas of experimentation such as reduced carbohydrate intake to try to understand the nature of the host response to infection.

Methods

The cultures, media and techniques have already been described. One change was made in the caging of the mice. For these experiments all mice were kept in metal cages and were bedded on metal grids to avoid them eating their bedding to gain nourishment. This also effectively prevented coprophagy. When pellets were given they were kept in metal baskets which made the total removal of food easy.

Drinking Solutions: Control mice were given normal saline solution to drink (a 0.9% solution of reagent grade of sodium chloride in tap water) since other solutions were acid in nature and required the addition of sodium hydroxide to bring them to an acceptable pH for drinking. A 1% solution of lactic acid was adjusted to a pH of 6.8 by the addition of 0.1 or 1.0 Normal sodium hydroxide solution. Sodium citrate was similarly made and adjusted in pH.

Results

Effect of limiting the food intake of mice

Chronic undernutrition of mice was brought about in two ways. Mice were fed in groups of five either a diet containing

only 4% of skimmed milk (with 45% white flour, 30% cerelose and 20% peanut oil with 1% salt mixture) or a weighed amount of standard diet (see page 82) so that each mouse obtained 3 grams daily. The latter figure was decided upon when observation showed that healthy mice in this age group ate on the average 8 grams daily. Mice were weighed in groups at intervals as shown in Table 15. The several diets noted above were continued until sacrifice on the second or third or fourteenth day after infection.

In Table 16 are noted the days of death of the mice in the control group and mice in the group receiving the diet containing 4% milk. The mice were infected intravenously with 0.1 ml. of the Smith strain 32 days after the diet was started the dietary regimen being continued after infection. The fate of the staphylococci in the liver and spleen was determined 48 and 72 hours after infection. The results are presented in Table 17.

In a similar fashion the days of death of mice in the control group and in the group receiving 38% by weight of their normal diet are recorded in Table 18. The fate of the cocci in the tissues in the undernourished group and the control group are shown in Table 19.

In both these tests the animals receiving suboptimal feeding showed no increased susceptibility to infection. Also the fate of the cocci in their tissues was similar to that in the controls.

Effect of Acute Fasting

Early studies showed that mice could withstand starvation up to 56 hours without mortality.

TABLE 15 Expt. 1.

WEIGHT OF GROUPS OF MICE

Group	Day 1	Day 5	Day 14
Control	21*	21.8	26
	21.2	25	29
	19	21.2	26.2
	19	21.6	26.2
3 gms. daily	20	23	17.4
	20	22.6	17.4
	16.6	22.2	16.4
	16.6	22.6	18.8

*Each figure is average weight in grams of a group of 5 mice.
Diet started Day 1. Infection Day 32.

TABLE 16 Expt. 106

DAYS OF DEATH OF ANIMALS

Control	7	9	11	11	14	S	S	S	S	S
Diet with 4% milk.	S*	S	S	S	S	S	S	S	S	S

*S Sacrificed 14 days after infections.

TABLE 17

Susceptibility to Staphylococcus Infection of Mice Fed a Diet Low in Skim Milk

	Mice fed ad lib diets containing							
	33% skim milk				4% skim milk			
Wt. of individual mice*	25	25	24	23.5	22	19	17	16
Staphylococci in liver**	5.53	4.23	4.87	5.49	5.40	4.72	5.31	5.63
Staphylococci in spleen	4.51	3.83	3.23	4.79	3.83	3.93	4.01	4.75
Survivors at 14 days		5 out of 10				10 out of 10		

*Weight in grams at time of Sacrifice for Bacteriological Studies 48 hours after infection.

** Logarithm to base 10 of calculated number of colonies recovered per whole organ.

TABLE 18 Expt. 105

DAYS OF DEATH OF ANIMALS

Control	4	7	9	10	S*	S	S	S	S	S
3 grams of diet daily	7	7	8	11	14	S	S	S	S	S

*S Sacrificed 14 days after infection

TABLE 19

The Effect of Food Restriction on the Susceptibility of Mice to Staphylococci

	Mice fed at lib	Mice fed 3.5 gm. Daily
Weight Change*	plus 6.8 gm.	plus 0.8 gm.
Staphylococci in Liver**	5.34, 5.23, 5.69, 5.91, 3.93	5.73, 5.08, 5.61, 5.49, 5.72
Staphylococci in Spleen	4.08, 4.94, 4.96, 4.49, 0	4.34, 4.99, 4.53, 4.13, 5.04
Survivors in 14 days	6 out of 10	5 out of 10

*Average (in grams) per mouse over a period of 3 weeks

**Logarithm to base 10 of Calculated number of colonies recovered per whole organ two days after infection. The figure 0 corresponds in reality to L3.23.

If infection with staphylococci is preceded by varying lengths of fasting, mortality is increased when the period of fasting is 36 hours or longer. No deaths occur in fasted uninfected controls. The animals were infected, as in previous experiments, with 0.1 cc. of overnight culture of Staph. Smith. Deaths occurred in the fasted group both at an earlier time and in greater quantity than in the unfasted controls. This is well shown in the graph of cumulative mortality (Figure 6) Details of this and similar experiments are shown in Tables 20 and 21.

The Fate of Staphylococci in the Tissues of Fasted Animals

The fate of the cocci in the spleen and liver for six days after infection has been determined by the grinding technique. It will be seen in Table 22 that an increased number of organisms survive in the organs of fasted animals during the first three days and in some cases during the fourth day after infection.

By suitable timing, mice were fasted for varying intervals before concurrent intravenous infection with staphylococci. The animals were all killed three days after infection and counts were made by the usual procedures of the surviving cocci in each case. The individual counts and the mean counts for each group of six animals is shown in Table 23 It is seen that more than 18 hours and less than 48 hours fasting is required to uncover the increased susceptibility to infection after fasting.

Fasting and Cumulative Mortality from Staphylococcal Infection

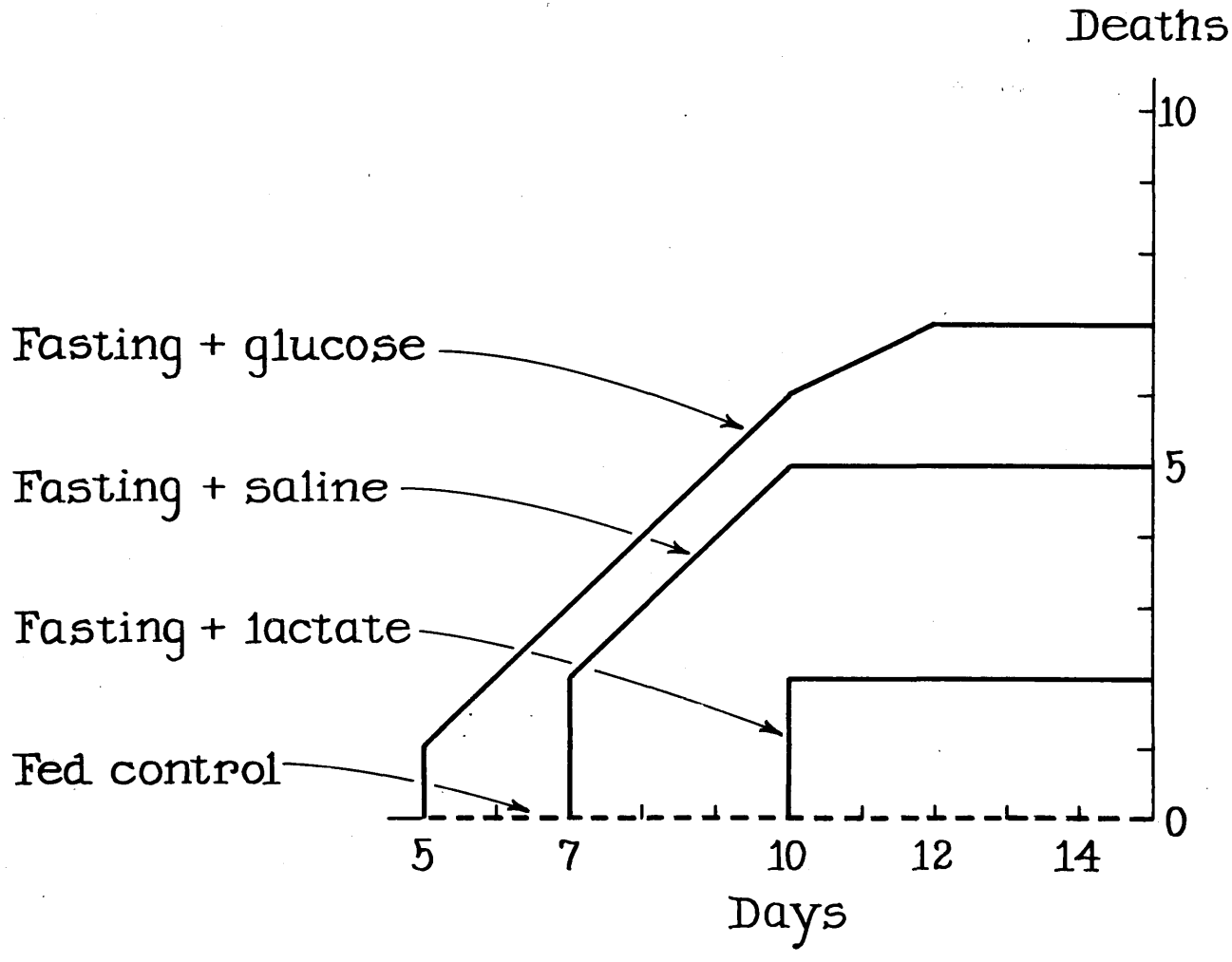

Figure 6.

TABLE 20

Fasting Hours	Cumulative Number of Deaths on Day			Survivors on Day 14
0	I	I	I	9 out of 10
36	3	3	5	5 out of 10
0	I	3	5	5 out of 10
36	8	9	10	0 out of 10
0	0	0	0	10 out of 10
48	I	4	4	6 out of 10
0	0	0	0	8 out of 8
48	2	5	5	3 out of 8
0	I	I	I	5 out of 6
36	2	2	3	3 out of 6
0	I	4	4	6 out of 10
36+12*	3	5	5	5 out of 11
0	2	4	4	5 out of 9
36+12*	6	7	8	2 out of 10

*Animals deprived of food 36 hours before infection and 12 hours after.

TABLE 21

Effect of Fasting on Survival Time of Mice Infected with Staphylococci

Fasting (hours)	Days of Death
0	6*, 23, -*, -, -, -, -, -, -, -.
once 36**	6, 7, 7, 12, 14, 18, 19, -, -, -.
0	5, 9, 9, 12, 14, 27, 28, -, -, -.
twice 36**	5, 5, 5, 5, 5, 5, 7, 7, 9, 11.

*In the first experiment the mice were deprived of food for 36 hours immediately prior to infection, in the other experiment the animals were deprived of food on two occasions, the second time being for 36 hours 1 week after infection.

All fasted but non-infected animals were alive and well at the end of the experiment.

**The numbers indicate days after infection at which death occurred. The sign - indicates survival; experiment discontinued 30 days after infection.

TABLE 22

Effect of Fasting on the Fate of Staphylococci in the Liver and Spleen of Mice.

Time After Infection	Organ	Number of Staphylococcus Colonies Recovered from Individual Mice	
		Fed Continuously ad lib	Fasted for 36 +12 hours
2 days	Liver	5.28, 4.46, 4.61, 4.93, 4.79, 4.43.	6.06, 5.86, 6.15, 5.38, 5.13, 6.18.
4 days	Liver	3.71, ?, 4.34, 0, 4.51, 3.23.	5.53, 3.23, 5.16, 4.73, 5.17, 4.13.
2 days	Spleen	4.01, 4.53, 4.08, 4.13, 4.13, 5.13.	5.18, 4.83, 5.02, 5.34, 3.93, 5.23
4 days	Spleen	0, ?, 3.23, 3.23, 4.08, 0.	3.53, 0, 3.53, 3.23, 0, 0.
1 day	Liver	4.88, 5.61, 5.28, 4.92, 5.57, 6.03.	6.12, 5.51, 6.02, 5.28, 6.31, 5.40.
3 days	Liver	?, 3.23, 4.34, 3.93, 4.28, 4.91	5.92, 4.59, 5.51, 5.38, 5.34, 4.63.
1 day	Spleen	5.77, 5.43, 5.21, 6.18, 5.31, 6.04.	5.41, 5.97, 5.49, 5,49, 4.63, 4.23.
3 days	Spleen	4.28, 4.08, 3.93, 0, 4.75, 3.93.	5.68, 3.53, 4.95, 3.83, 5.01, 4.53.
1 day	Liver	6.28, 5.99, 6.40, 6.72, 7.15, 5.93.	7.17, 6.65, 6.51, 6.97, 7.57, 6.57.
3 days	Liver	5.43, 5.17, 5.09, 5.85, 4.91, 5,46.	6.40, 7.46, 6.14, 5.55, 4.63, 5.95.
1 day	Spleen	6.34, 6.02, 6.15, 6.28, 6.83, 5.93.	6.28, 5.77, 6.23, 6.12, 6.33, 5.80.
3 days	Spleen	5.15, 4.93, 4.75, 5.51, 4.08, 4.57.	5.09, 6.09, 5.12, 5.00, 4.38, 5.50.

The figure 0 corresponds in reality to less than Log. 3.23.? indicates that the culture was lost by accident or contamination. The table gives the results of three consecutive experiments in which the fasted mice were deprived of food for 36 hours before and 12 hours after infection.

TABLE 23.

Effect of Length of Fasting Period on Fate of Staphylococci in the Liver of Mice.

Fasting Before Infection	Log. Number of Staphylococcus Colonies from Individual Livers 3 days after Infection						Mean
0 hours	3.23	3.83	4.28	4.31	4.38	4.73	4.13
3 hours	3.83	4.08	4.18	4.34	4.51	4.89	4.31
6 hours	3.83	4.08	4.46	4.55	4.80	4.81	4.19
18 hours	3.53	3.93	4.01	4.01	4.43	4.82	4.12
48 hours	4.01	4.43	4.57	5.14	5.23	5.89	4.88

In order to determine the amount of refeeding that is required to abolish the deleterious effect of fasting, groups of mice were refed for varying periods after a 48 hour fast. The timing was such that all mice were infected at the same time. Following 24 hours of feeding mice were almost returned to their normal degree of resistance to infection. The details of this experment are shown in Table 24.

The Effect of Glucose to Drink During Periods of Fasting on Susceptibility to Infection.

In an attempt to restore the several parts of the diet one by one to fasted animals glucose was first used. It was given in the drinking water as a five percent solution in saline. This partial replacement of the diet surprisingly did not help restore the animals resistance but caused a further deterioration in their susceptibility. In Figure 7 the cumulative mortality of mice receiving saline to drink or glucose to drink is shown. Again it is obvious that deaths occur sooner and in greater quantity in the starved as compared with the fed group but this is also true when the mice receiving glucose to drink are compared with those receiving saline when both groups are fasted. The details of this and similar experiments are given in Table 25. This is not true when the animals are fed and given glucose to drink. The fate of the cocci in the tissues is shown in Table 26.

TABLE 24.

Effect of Refeeding on Deleterious Result of Fasting

Refeeding in hours	Log titer Staphylococci in liver 2 days after infection I. V. with 0.11 cc. Staph Smith					
	Average	Individual Results				
0	5.46	5.98	5.40	5.28	5.23	5.14	4.86
6	5.87	6.08	6.05	5.88	5.82	5.77	5.21
12	5.76	6.17	5.68	5.69	5.61	5.53	5.53
18	5.82	6.15	5.83	5.61	5.59	5.59	---
24	5.65	6.18	5.53	5.51	5.34	5.28	4.84

TABLE 25

Effect of Glucose and Lactate on the Survival Time of Fasted Mice Infected with Staphylococci.

Fasting (hours)	Drinking Fluid	Time of Death after Infection Days	Survivors at 14 Days
0	Saline	6, -, -, -, -, -.	5 out of 6
36	Saline	6, 7, 12, 14, -, -.	2 out of 6
36	5% Glucose	4, 6, 9, 13, -, -.	2 out of 6
36	1% Lactate	6, 10, 13, -, -, -.	3 out of 6
0	Saline	-, -, -, -, -, -, -, -.	8 out of 8
36	Saline	6, 7, 8, 9, 10, -, -, -.	3 out of 8
36	5% Glucose	2, 7, 7, 8, 9, 10, 11, -.	1 out of 8
36	1% Lactate	8, 9, -, -, -, -, -, -.	6 out of 8
0	Saline	-, -, -, -, -, -, -, -.	8 out of 8
48	Saline	7, 7, 10, 10, 10, -, -, -.	3 out of 8
48	5% Glucose	5, 7, 7, 10, 10, 10, 12, -.	1 out of 8

TABLE 26

Effect of Glucose on the Fate of Staphylococci in the Organs of Fasted Mice

Days After Inf.	Organ	Log Numbers* of Staphylococcus colonies recovered from mice: Fed continuously ad lib	Fasted**	Fasted but receiving glucose
1, L*		4.84, 5.61, 5.28, 4.92, 5.57, 6.03	6.12, 5.51, 6.02, 5.28, 5.13, 5.40	6.40, 6.00, 6.97, 6.23, 6.09, 5.91
1, S		5.77, 5.43, 5.21, 6.18, 5.31, 6.04	5.40, 5.97, 5.49, 5.49, 4.63, 4.23	5.34, 6.43, 5.86, 5.34, 6.31, 4.93
3, L	?	3.23, 4.34, 3.93, 4.28, 4.91	5.92, 4.59, 5.51, 5.38, 5.34, 4.63	4.23, 0, 4.80, 3.83, 3.23, 3.23
3, S		4.28, 4.08, 3.93, 0, 4.75, 3.93	5.68, 3.53, 4.95, 3.83, 5.01, 4.53	3.23, 3.23, 4.59, 3.93, 4.83, 3.53
1, L		6.28, 5.99, 6.40, 6.34, 7.15, 5.93	7.17, 6.65, 6.51, 6.97, 6.57, 6.57	6.34, 7.28, 6.76, 6.71, 6.11, 6.49
1, S		6.34, 6.02, 6.15, 6.28, 6.83, 6.73	6.28, 5.77, 6.23, 6.12, 6.53, 5.80	6.86, 6.83, 7.17, 6.51, 6.92, 6.57
3, L		5.43, 5.17, 5.09, 5.85, 4.91, 5.46	6.40, 7.46, 6.14, 5.55, 4.63, 5.95	6.53, 5.51, 5.73, 5.55, 5.06, 4.46
3, S		5.15, 4.93, 4.75, 5.51, 4.08, 4.57	5.09, 6.09, 5.12, 5.00, 4.38, 5.38	5.66, 4.28, 4.85, 4.96, 4.28, 3.71

L*: liver
S: spleen

*Figures represent logarithms to base 10 of calculated number of colonies per whole organ. 0 corresponds in reality to L3.23. The results are those of two consecutive experiments.

**Animals fasted for 36 hours before and 12 hours after infection. The received either physiological saline or 5 percent glucose as drinking fluid during the fasting period.

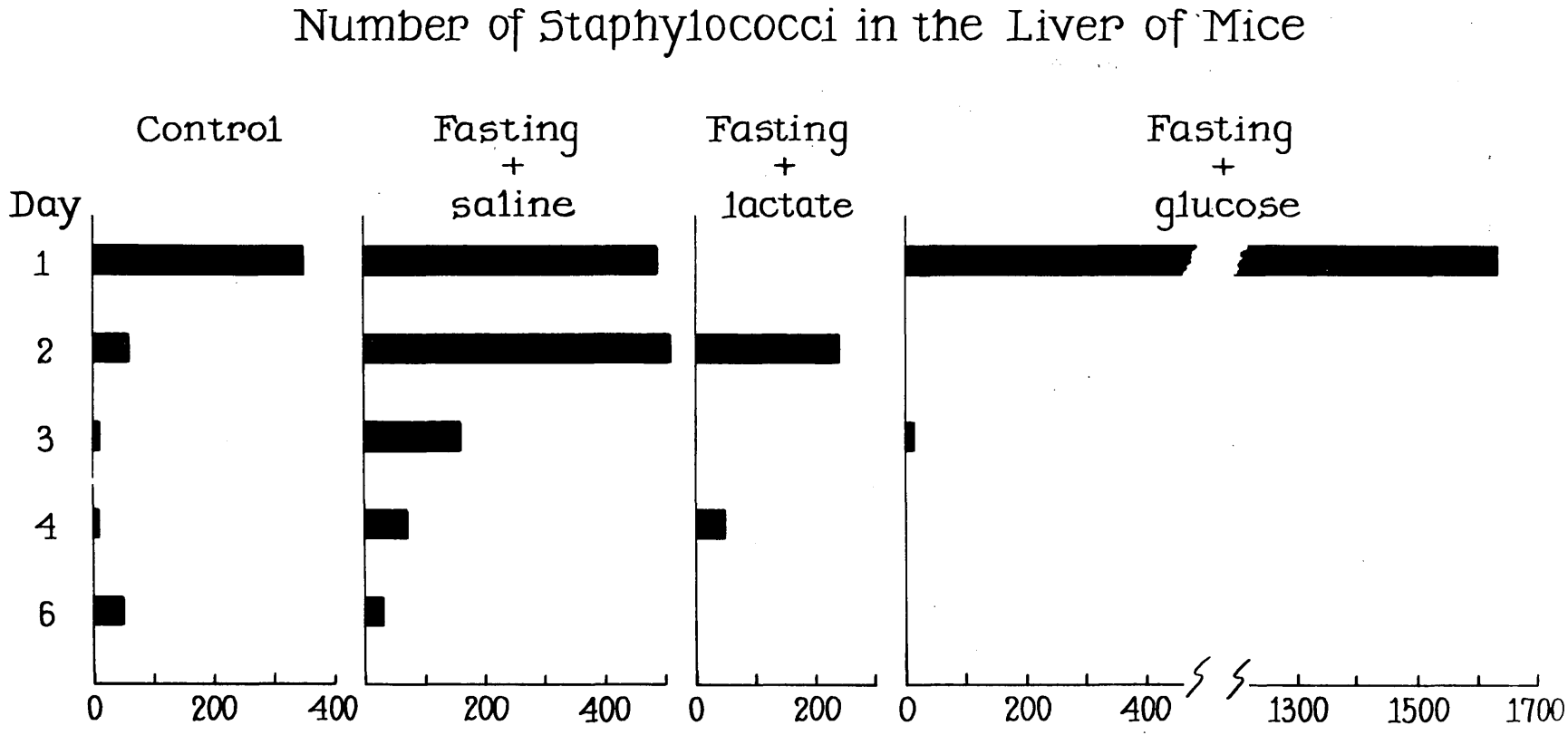
Number of Staphylococci in the Liver of Mice
Control
Fasting + saline
Fasting + lactate
Fasting + glucose
Day
1
2
3
4
6
0
200
400
200
400
0
200
0
200
400
1300
1500
1700

Figure 7.

The Effect of Lactate and other Tissue Metabolites in the Drinking Fluid of Fasted Animals on their Susceptibility to Infection.

When lactate was present in the drinking fluid there was evidence of protection against the deleterious effect of fasting in regard to resistance to infection. Table 25. The fate of the cocci is shown in table 26.

Other fluids were given in a similar fashion without detectable effect. These were sodium succinate, sodium citrate and sodium ketoglutarate.

DISCUSSION

It is evident that chronic undernutrition produced in two different ways does not affect the susceptibility of mice to staphylococcal infection. In contrast acute fasting makes mice more susceptible. Glucose by mouth enhances this susceptibility and lactate decreases it. In all these cases increased numbers of cocci can be demonstrated in the tissues.

In human starvation (38) scarlet fever, diphtheria, dysentery, typhoid, typhus, cholera and tuberculosis are the diseases most often increased in prevalence. The experimental evidence regarding susceptibility to infection has been reviewed (38). Its obvious here that we have deaths in an early period so that antibody formation is unable to be connected with the susceptibility we have found. The few studies done on animals show results which are not consistent and are difficult to explain. In summary, it may be said that despite a strong clinical impression that starved patients are more susceptible to infection and do poorly when they get infected, there is little documental evidence to back up the statement.

While this work was in progress, the author was approached by Major

Paul Teschan regarding the death of severely wounded soldiers in Korea who had been so severely wounded that shock had led to acute renal shut down. The only nourishment they received was intravenous glucose. Despite high and continued doses of antibiotics from time of the initial trauma these men died of severe and uncontrolled sepsis. He has since published these observations (39) and has stated, "Evidence of massive infection was the prominent finding at autopsy and thought to be the main cause of death in the majority of fatal cases after the artificial kidney became available."

It is possible that this work provides a clue to the susceptibility of diabetics to infection. In uncontrolled diabetics the deposition of glycogen in the liver and other tissues is blocked and glucose accumulates in the circulating blood. By the devices described in this section it is possible that a very similar biochemical "milieu interneux" is produced. The ratio of glucose to glycogen may well be the decisive factor but much more investigative work in this field is required.

The experiments regarding carbohydrate metabolism were continued in a slightly different field. It was decided to feed thyroid hormone to increase the turnover of carbohydrate. The results of these experiments have been of great interest.

Methods

The mice were infected intravenously with the Smith and MAM strains as described earlier.

Diets: The basic diet used consisted of 66% of white flour, 33% of powdered skim milk and 1% of salts. The salt mixture has the following composition: KCL 42.5 g; Na_2CO_3 42.5; $MgCO_3$ 14.3; $CaCO_3$ 25.0 g; ferric ammonium citrate 5.5g. To 100 grams of this diet were added 100 cc. of 7.5 gelatin. (dissolved in cold and then warm tap water). The thyroid diet was prepared by adding 600 mgm. of thyroid U. S. P. (Lilly, Indionapolis, Indiana) to 1000 g of dry portion of the diet. The dry portions of the diet were thoroughly mixed and sieved before adding the gelatin. This gave a final concentration of 300 mgm. per kilo of diet. This was the standard thyroid diet. Other thyroid diets contained less hormone as indicated in the text. The thyroxine diet was made by adding a 0.1% solution of thyroxin (E. R. Squibb and Sons, New York) in 0.1% sodium carbonate solution so that there was 1 mgm. of thyroxine per kilo of diet.*

The di-iodotyrosine diet was made by adding 3:5;di-iodotyrosine (National Biochemical Corporation, Cleveland 1, Ohio) to the diet in the solid phase. The amounts used are indicated in the text.

The iodine diet Potassium iodide (Reagent grade, Merck and Co., Inc., Rahway, N. J.) was added so that the diet contained 1-3000 mgms/ Kilo of diet.

The methyl thiouracil diet. This chemical (from National Biochemical Corporation, Cleveland, Ohio) was added to the diet so that each kilo contained 0.8 gm.

The effect of thyroid on uninfected mice

Mice were fed for three weeks on diets containing from 10 to 2,400 mgm/Kilo of thyroid in the diet. The deaths in each group are recorded in Table 27. A dose of 300 mgm. per kilo,

(* Sodium carbonate up to and including 5 gms/kilo has no effect on normal or infected mice.

TABLE 27 Expt.

Effect of Thyroid by Mouth on Normal Mice

Thyroid in mgm/kilo* of diet	Deaths after 21 days of diet.
2400	10/10
1200	6/10
600	4/10
300	3/10 0/12 0/6 0/10
100	0/12 0/6
30	0/12 0/6
10	0/12 0/6

*refers to total weight of solid and liquid portions of diet.

although slightly toxic in some groups, was finally chosen as this has been shown by Burn (40) to produce maximum increase in oxygen uptake in mice. When the mice were fed and watered regularly and not crowded in the cages deaths did not occur. This dose was sufficient to prevent the normal weight gain of uninfected animals.

The effect of thyroid on infected mice

1. Coagulase Positive Staphylococci

Animals which had been fed thyroid or control diet for a period of a week were infected intravenously with 0.1 cc. of overnight culture of Staph. Smith. Thyroid feeding was continued throughout the experiment. It was evident that the thyroid treated mice died of their infection both earlier and to a greater extent than did the control mice. This is well shown in Figure 8 and details of several experiments are tabulated in Table 28

That this enhancing effect of thyroid can be produced by lower doses can also be shown. (Table 29)

Studies of the Distribution of Staphylococci in Organs of Thyroid Treated Mice

A more thorough study of the effect of coagulase positive staphylococci on thyroid treated mice was made by the grinding method previously described. Mice were fed a diet containing 300 mgm/kilo of dessicated thyroid for one week and then were infected intravenously with 0.1 cc. of overnight culture of Staph. Smith. Thyroid feeding was continued until the end of the experiment. These were compared with

TABLE 28 Expts. 21 & 35

Effect of Thyroid by Mouth on Mice Infected with Staphylococci

Thyroid in mgms.	Days of Death*	Total Deaths.
0	S S S S S S S S 5 11	2
300	SSS 1 5 5 5 11 11 11	7
0	S S S S S S S S S S	0
300	S S S 1 5 5 5 7 9 9	7
0	S S S S S S S S S S	0
300	S S S S 1 5 5 7 8 9	6

*S Survived until 14 days after infection.

TABLE 29 Expt. 21

Dose Response to Oral Thyroid of Infected Mice

Throid mgm/kilo	Days of Death	Total Deaths
0	S S S S S S S S 5 11	2
300	S S S 1 5 5 5 11 11 11	7
150	S S S S 5 5 5 6 7 11	6
75	S S S S 5 5 6 7 11 11	6
37	S S S S 5 5 5 5 6 11	6

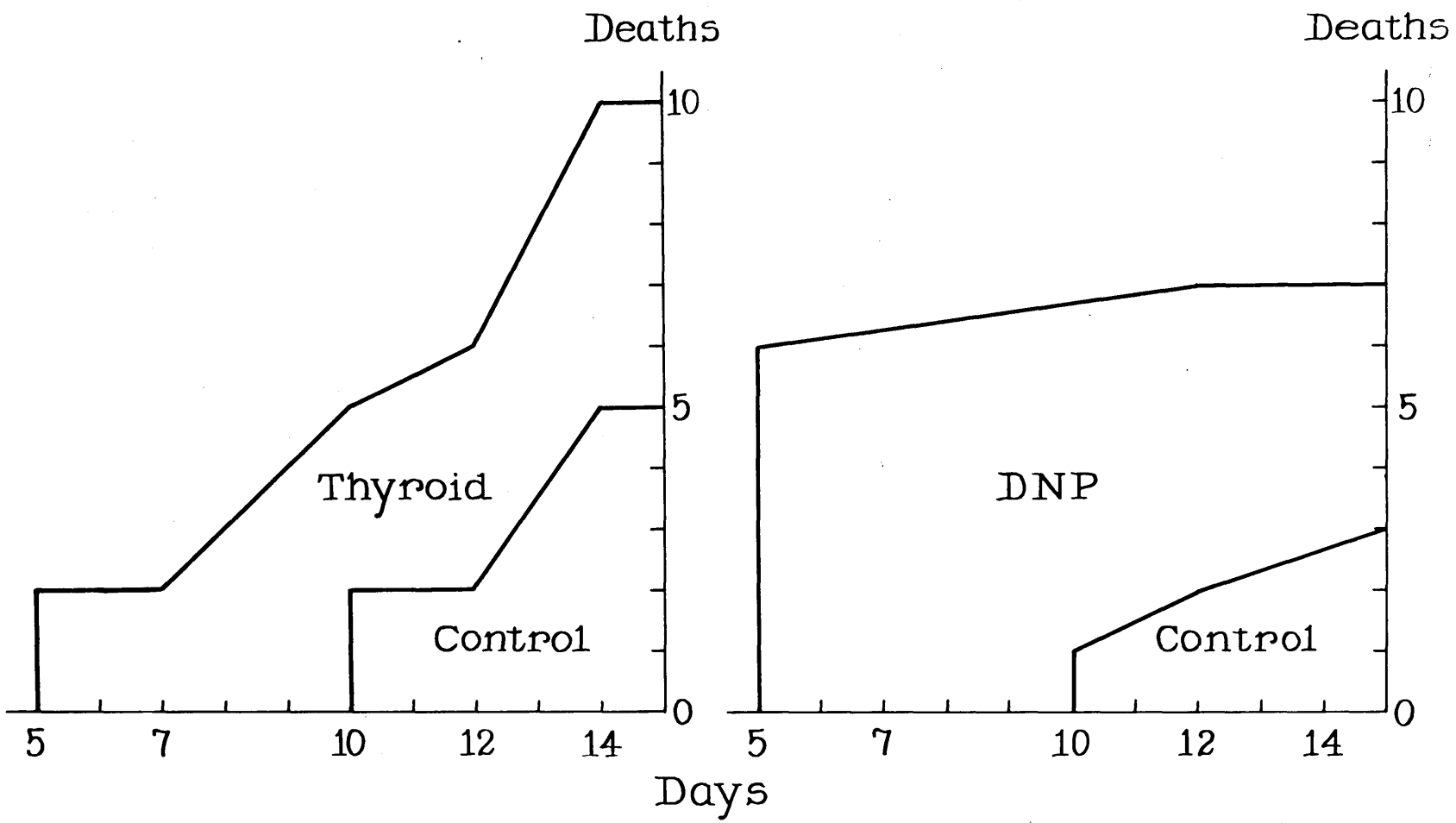
Cumulative Mortality from Staphylococcal Infection
Deaths
10
5
0
Thyroid
Control
5
7
10
12
14
Days
Deaths
10
5
0
DNP
Control
5
7
10
12
14

Figure 8.

mice similarly treated but receiving a control diet.

The animals were killed one, two , three and four days after infection and the liver, spleen, lung and kidneys were ground. The surviving cocci in these organs were titered as previously described. It will be seen from Table 30 that the initial distribution of cocci in the organs is the same for the two groups. The results in the rest of the early phase suggests that the clearing of the cocci is delayed in the thyroid treated animals. In the later phases of the experiment, however, there is no detectable difference in the two groups.

2. Weakly Coagulase Positive Staphylococci --- Strain MAM

It has been previously shown that these staphylococci given intravenously to normal mice do not cause the death of the animals even when they are given in large dose. Mice which had received control and thyroid diets for one week were given an intravenous dose of 0.1 cc. of overnight culture of Staph. M. A. M. Five deaths occurred in the treated animals compared with no deaths in the controls. (Table 31)

Distribution of Weakly Coagulase Positive Staphylococci in the Organs of Thyroid Treated Mice

The grinding technique has also been used to study this phenomenon. The situation found was indentical with the experiments with the Smith strain. Initial distribution was equal in both the treated and untreated group and although there was some evidence of a defect in clearance later multiplication did not appear obviously different in the two groups. (Table 32)

TABLE 30A Expt. 75

Course of Infection in Mice Infected with 0.1 ml. I.V. Culture (Staph Smith)
Tube Technique. Results with Control and Thyroid Diets.

1 Hour	~~Day~~	1 Day		2 Days	
Con.	Thy.	Con.	Thy.	Con.	Thy.
		Liver			
7.60	8.77	6.97	5.93	5.15	4.08
6.50	7.15	5.26	5.15	4.56	5.60
8.73	7.12	5.53	5.15	5.85	3.68
7.75	7.08	5.71	6.50	4.15	5.15
7.35	7.30	5.73	7.05	3.90	5.06
8.35	7.50	4.93	6.05	5.15	4.75
7.75	7.34	4.68	5.93	4.05	4.40
7.75	8.10	5.02	5.05	5.15	5.56
7.62	8.05	5.15	4.45	5.05	4.93
7.62	7.97	4.60	5.15	4.45	5.15
		Spleen			
6.62	7.40	5.45	5.50	3.97	5.45
6.62	7.40	5.91	5.60	5.04	5.23
6.85	8.05	5.62	6.05	4.83	4.53
8.20	7.77	6.62	5.53	4.01	5.68
7.20	7.75	6.19	5.05	3.53	4.88
7.02	6.60	6.45	4.75	3.30	4.77
7.11	7.80	5.93	5.40	3.15	4.79
9.71	7.35	5.88	5.35	4.62	5.93
8.10	7.75	4.93	5.40	3.94	5.62
	7.40	5.91	5.62	4.35	5.45

TABLE 30B

Course of Infection in Mice Infected with 0.1 ml. I. V. Culture (Staph Smith)
Results with Thyroid and Control Diets

1 Day		2 Day		3 Day		4 Day	
Con	Thy	Con	Thy	Con	Thy	Con	Thy
			Liver				
5.51	5.55	4.77	5.91	5.49	5.88	3.53	6.17
5.86	5.53	4.38	6.43	3.93	5.88	4.85	3.23
5.57	6.84	4.95	5.46	4.79	5.88	3.23	D
			Spleen				
5.94	5.66	3.71	5.06	3.23	5.51	0	3.83
6.14	5.51	4.28	5.49	3.83	5.40	3.71	0
5.53	5.75	4.08	5.51	3.71	5.87	3.23	D
			Lung				
4.87	0	3.83	4.80	4.28	4.61	4.87	7.31
4.40	3.71	3.71	5.43	5.23	4.75	5.01	3.53
4.23	5.02	5.83	6.28	5.34		4.43	D
			Kidney				
7.08	6.16	3.93	8.17	9.99	8.63	9.28	9.88
7.14	6.49	8.09	9.83	8.55	12.23	8.49	7.95
6.34	7.59	8.18	8.31	8.81	5.53	10.34	D

TABLE 30C

Course of Infection in Mice Infected with 0.1 ml. I.V. Culture (Staph Smith) Results with Control and Thyroid Diet

1 day		2 days		6 days	
Con	Thy	Con	Thy	Con	Thy
		Liver			
5.82	5.81	5.01	5.77	0	3.93
6.08	6.11	4.68	5.49	3.23	4.13
5.55	5.57	5.04	6.01	3.71	3.23
5.68	6.15	5.23	5.91	D	3.23
		Spleen			
6.31	6.20	5.13	5.55	0	
6.34	6.00	4.01	6.16	0	3.23
6.49	5.31	4.75	5.40	0	0
5.63	5.98	3.23	5.57	D	0
		Lung			
4.38	4.28	0	4.55	0	
3.83	3.83	4.01	4.13	5.53	5.40
4.38	0	4.34	0	0	0
4.08	4.69	4.01	4.49	D	0
		Kidney			
5.86	5.13	3.71	8.06	0	10.38
5.16	4.55	6.01	8.31	10.01	9.23
5.04	6.40	8.12	4.01	9.90	9.02
5.57	5.49	5.43	7.90	D	0

TABLE 31

Effect of Thyroid Feeding on Mortality in Mice Following Infection with Staphylococcus MAM

Thyroid mgm/kilo	Days of death after infection.	Total Deaths
0	S S S S S S S S S S	0
300	S S S S 6 8 12 14 14	5

TABLE 32B

Course of Infection in Mice Infected with 0.1 ml. I.V. Culture (Staph MAM) Results with Thyroid and Control Diets.

1 Hour		1 Day		2 Day		4 Day	
Con	Thy	Con	Thy	Con	Thy	Con	Thy
			Liver				
7.49	7.28	4.38	4.55	3.53	3.23	0	0
7.20	7.40	0	4.92		4.63	0	3.23
8.00	7.43	3.53	4.76	4.08	4.69	0	D
			Spleen				
6.03	5.49	3.83	3.71	3.23	3.23	0	3.23
6.66	6.31	3.53	5.13	0	3.71	3.23	3.23
6.59	6.72	3.23	4.49	3.23	0	3.23	D
			Lung				
5.28	4.59	0	3.71	0	0	0	0
4.66	4.71	0	0	5.81	3.53	0	3.23
5.00	4.68	0	5.46	5.61	6.13	0	D
			Kidney				
4.97	3.53	5.40	4.73	0	0	4.88	3.53
4.98	4.57	4.34	0	8.46	5.89	5.98	0
4.49	3.71	3.23	3.23	0	6.69	5.02	D

TABLE 32A

Course of Infection in Mice Infected with 0.1 ml. I. V. Culture (Staph MAM) Results with Control and Thyroid Diets.

1 hour		1 day		2 days		3 days	
Con	Thy	Con	Thy	Con	Thy	Con	Thy
			Liver				
7.49	7.28	4.38	4.55	3.53	3.23	0	0
7.20	7.40	0	4.91		4.63	0	3.23
8.00	7.43	3.53	4.95	4.08	4.69	0	D
			Spleen				
6.03	5.49	3.83	3.71	3.23	3.23	0	3.23
6.66	6.31	3.53	5.13	0	3.71	3.23	3.23
6.59	6.72	3.23	4.49	3.23	0	3.23	D
			Lung				
5.28	4.59	0	3.71	0	0	0	0
4.66	0	0	0		3.53	0	3.23
5.00	4.68	0	3.23	5.61	6.13	0	D
			Kidney				
4.97	3.53	5.40	4.73	0	0	4.88	3.53
4.98	4.57	4.34	0		5.89	5.98	0
4.49	3.71	3.23	3.23	5.61	6.69	5.02	D

TABLE 32 C

Course of Infection in Mice Infected with 0.1 ml. I.V. Culture (Staph MAM) Tube Technique Results with Control and Thyroid Diets

1 min		7 days		14 days		28 days	
Con	Thy	Con	Thy	Con	Thy	Con	Thy
			Liver				
7.75	7.65	0	0	1.45	D	0	D
7.62	7.85	0	4.45	4.53	D	0	D
7.53	7.85	0	0	4.60	D	0	D
			Spleen				
5.98	6.56	2.40	0	0		0	D
6.15	5.98		2.91	0	D	0	D
6.15	6.60	1.15	0.45	0	D	0	D
			Lung				
6.53	6.53	2.93	0	1.75	D	3.97	D
6.73	6.75	0	3.45	3.30	D	4.81	D
6.35	6.65	0	0	3.05	D	1.45	D
			Kidney				
6.23	6.23	4.93	1.75	0		0	D
6.20	6.15	4.93	6.93	4.68	D	4.85	D
6.15	6.53	5.85		4.15	D	0	D

Histological Sections

Studies of sections of the liver, spleen, kidney and lung of animals receiving thyroid diet were made. One day and seven days after infection these were compared with sections made from animals on control diet. All the lesions found in the control animals were also found in the thyroid animals, but they were present in greater abundance. No new lesions were detected.

Effect is due to thyroxin

The fact that this effect is due to thyroxin and not to other materials present in dessicated thyroid can be shown by feeding the pure hormone. Mice received thyroxin containing diets for seven days before being infected with staphylococci (Staph. Smith) in the usual manner. Mortality data are shown in Table 33. Furthermore, it was possible to show that the precursor of thyroxin--di-iodotyrosine--did not produce the same effect (Table 34) nor did iodine (Table 35).

TABLE 33 Expt. 21

Effect of Oral Thyroxine in Staphylococcal Infection in Mice

Thyroxine:	Days of Death	Deaths/10 mice
1mgm/kilo	1 5 5 5 5 5 5 5 6 11	10
0.5 mgm/kilo	5 5 6 6 6 11 11 11 S S	8
0.25 mgm/kilo	5 6 11 S S S S S S S	3
0.125 mgm/kilo	1 11 S S S S S S S S	2

TABLE 34 Expt.

DIET	Deaths/ Total Mice
Basic	1/9
Basic plus D.I.T. 4 mgm/kilo	2/8
Basic plus D.I.T. 1 mgm/kilo	0/10
Basic plus D.I.T. 0.25 mgm/ kilo	2/10

TABLE 35 Expt.

DIET	DEATHS
Basic	1/9
Basic plus KI 3,000 mgm/kilo	1/10
Basic plus KI 100 mgm/kilo	1/10
Basic plus KI 1 mgm/kilo	0/10

One of the main actions of thyroid hormone is to increase the oxygen consumption of tissues. This can also be accomplished by 2:4 dinitrophenol. Accordingly, this chemical was also used in diet experiments with mice infected with staphylococci.

Methods

2:4 Dinitrophenol was added to the solid phase of the basic diet previously used. This was thoroughly mixed and 7.5% gelatin was added. In most of the experiments 500 mgm/kilo was used. Other strengths are indicated in the text.

The Effect of Dinitrophenol on normal mice.

This chemical was incorporated in varying concentrations in the basic diet. This diet was then fed to groups of ten mice for a period of three weeks. The results of this experiment are shown in table 36. It will be seen that no deaths occurred in the group receiving 500 mgm/kilo.

The Effect of D. N. P. on Infected Mice.

When this dose is used added to the basic diet more mice die and these deaths occur earlier than in the controls. This is tabulated in table 37 and shown graphically in figure 8. Lower doses are not effective (table 38).

By use of the grinding technic the fate of the cocci in DNP treated animals was followed over a six day period. There is no obvious difference between the DNP animals and the controls. (Table 39).

Effect of DNP on Staphylococci in vitro

This chemical has an opposite or inhibitory action on the growth of staphylococci in vitro. Half a cc. of an 18 hour culture of Staph. Smith was added to 5 cc. volumes of Pf broth with or without dinitrophenol as shown in table 40. The tubes were incubated overnight and plated on Pf agar, colony

TABLE 36

The Effect of Dinitrophenol on Normal Mice

Content of DNP per Kilo of Diet MGM.	Approx. Dose /mouse/day MGM.	Day of Death*
500	4	S S S S S S S S S S
1000	8	6 13 S S S S S S S S
2000	16	1 5 5 5 5 6 6 7 7 9
4000	32	1 5 5 5 5 5 5 6 6 8

*S Survived three weeks after start of experiement.

TABLE 37

Effect of D. N. P. Feeding on Mortality of Mice Infected with Staphylococci

DNP Mgm/Klo of diet	Cumulative No. of Deaths at Day 5	7	10	12	14	Survivors
500	2	2	5	5	5	5/10
--	0	4	5	5	5	5/10
500	1	4	4	4	5	4/9
--	2	3	3	3	3	6/9
500	6	6	6	7	7	5/10
--	0	0	1	2	3	10/10

TABLE 38 Expt. 30

Dose Response of D. N. P. in Infected Mice

Diet	Deaths
Basic	3/9
Basic plus 500 mgm. per kilo	5/9
Basic plus 250 mgm. per kilo	2/10
Basic plus 125 mgm. per kilo	2/10
Basic plus 62 mgm. per kilo	2/10
Basic plus 30 mgm. per kilo	2/10

TABLE 39

Course of Infection in Mice Infected with 0.1 ml. I. V. Culture (Staph Smith) Results with Control and DNP diets.

1 day		2 days		6 days	
Con	DNP	Con	DNP	Con	DNP
		Liver			
5.82	5.79	5.01	5.31	0	0
6.08	5.40	4.68	5.41	3.23	3.23
5.55	5.38	5.04	4.31	3.71	3.83
5.68	6.31	5.23	5.38	D	D
		Spleen			
6.31	6.05	5.13	4.95	0	
6.34	5.69	4.01	4.93	0	0
6.49	5.65	4.75	0	0	3.71
5.63	5.53	3.23	5.38	D	D
		Lung			
4.38	5.22	0	0	0	4.40
3.83	4.18	4.01	5.76	5.53	3.53
4.38	3.23	4.34	3.23	0	4.01
4.08	5.71	4.01	3.71	D	D
		Kidney			
5.86	8.23	3.71	7.43	0	9.55
5.16	5.01	6.01	7.86	10.01	0
5.04	5.63	8.12	0	9.90	9.04
5.57	6.40	5.43	6.68	D	D

counts were made after a further overnight incubation. A marked inhibition of growth is noted.

It is therefore evident that D. N. P. acts the best and as its infection enhancing action is common to both thyroid and this compound, it is probable that it is brought about by the property of increasing oxygen consumption common to the two compounds.

The Effect of an anti--thyroid compound

Normal mice were fed methyl thiouracil in amounts ranging from 50 to 1600 mgm/kilo in basic diet without deleterious effect. (Table 41)

Animals that were fed 800 mgms/kilo diet for seven days prior to infection were compared with normal mice regarding their susceptibility to I. V. Staph. Smith infection. (Table 42 A and B)

The Effect of Thyroid and DNP Feeding on the Susceptibility of Mice to other Infections.

Groups of animals were infected with various titers of overnight cultures either intravenously or intraperitoneally. L. D. 50% titers were calculated using the Reed and Muench method (41), and these are recorded as logarithms to the base 10 in Table 43. From this information a suitable titer to inject I. V. was chosen and given to groups of control mice and groups of mice that had received the standard dose of thyroid or of D. N. P. for seven days before infection. The enhancing effect of these chemicals is shown by the mortality data in Table 44.

DISCUSSION

Perla and Marmorston (42) discuss the relationship of the thyroid to resistance. They conclude that a moderate increase in resistance

TABLE 40

The Growth of Staphylococci in Vitro with D. N. P.

D. N. P. added	Media autoclaved Titer/cc.	Not autoclaved Titer/cc.
NIL.		10.37
Saturated Approx 0.5%	0.00	0.00
0.25%	0.00	0.00
0.125%	0.00	7.31
0.06%	8.83	8.70
0.03%	8.53	8.87
0.015%	8.83	8.83

TABLE 41 Expt. 59

Toxicity of Methyl Thiouracil in Normal Mice

Methyl Thiouracil in mgm/kilo diet.	Deaths after 21 days of diet
1600	0
800	0
200	0
50	0

TABLE 42A Expt. 107

		DAYS OF DEATH								
Control Diet	10	S*	S	S	S	S	S	S	S	S
Methyl Thiouracil Diet	10	13	S	S	S	S	S	S	S	S

S* Sacrificed 14 days after infection.

TABLE 42B

Course of Infection in Mice Infected with 0.1 ml. I.V. Culture (Staph Smith) Results with Control and Methyl Thiouracil Diets

1 Day		2 Days	
Con	M. T.	Con.	M. T.
		Liver	
6.69	7.11	5.61	4.59
6.79	6.80	5.85	5.63
6.23	6.63	6.02	5.53
7.08	6.28	5.68	5.01
7.08	6.72	5.89	6.23
		Spleen	
5.99	5.55	5.11	4.13
5.81	6.51	5.06	5.05
6.16	6.22	4.43	4.61
5.91	5.85	5.28	4.38
6.13	5.73	4.43	5.23

TABLE 43

Standardisation of Miscellaneous Infections

Organism	I. V. L. D. 50	I. P. L. D. 50
2. Pneumococcus	-1.00	-3.50
1. Friedlander C.	-2.75	-3.00
2. Streptococcus C.	-5.50	--
3. Candida	-1.74	undil.
1. Shigella Dys. sonne	-0.25	-1.25
4. Streptobacillus and Pleuropneumonia like organisms L.I.	-2.00	-0.37
1. Corynebacterium pseudotb.	-3.00	-1.00
1. Proteus	undil.	undil.

Cultures made in:
1. Pf Broth
2. 2% blood Pf broth
3. Sabouraud's liquid medium
4. 20% serum Pf broth.

TABLE 44

Thyroid & DNP Feeding with Miscellaneous Infections

	Titer I. V.	Death per 10 mice		
		Control	Thyroid	DNP
Pnemo III	-5	1	1	1
Fried. C.	-3	1	1	7
Strep. C.	-7	1	10	1
Candida	-3	0	7	1
Shigella	-1	0	10*	3
Coryneb. pseudo.	-4	0	2	1
Proteus	Neat	1	4	0

*All on day 1.

to infection and to anaphylaxis results from the removal of the thyroid whereas thyroid therapy in animals, and in Graves disease in man, if frequently associated with an impairment of the defense mechanism to toxins, drugs, anesthetics and to certain infectious and operative procedures.

Removal of the thyroid in chicks has no effect on infection with Spirochoata gallincown (43, 44). In rabbits, however, enhanced resistance to Bacterium dysenteria (Shiga) is present after thyroidectomy. (45, 46) A difference of opinion exists on the effect of decreased thyroid function on infection with Mycobacterium tuberculosis. Webb (47) found in guinea pigs that 3/4 or total thyroidectomy prior to infection had no effect on the infection whereas earlier death and greater disease than in controls resulted if the operation was done after infection. Working with albino rats which had been thyroidparathyroidectomised, Steinbach (48) found more disease in the treated animals than in the controls both with bovine and human bacilli. Koch and Schafer used thyroxine and methyl thiouracil in guinea pigs with experimental tuberculosis. (49) They found thyroxine had a slightly beneficial effect and methyl thiouracil, an unfavorable effect on guinea pigs infected with tubercle bacilli. Godeke and Jacobs showed that Methyl thiouracil given to guinea pigs induced worse disease and caused earlier death from tuberculosis than in controls. These authors noted longer survival both in the control animals and in the tested ones in the winter compared with the summer (50)

Thyroid feeding in guinea pigs caused fatal results from intraperitoneal injections of typhoid bacilli in amounts not lethal to controls (51). This was confirmed by Rocchini (42). Also thyroid feeding prevented the curative effect of antiserum against Pasturella pestis in guinea pigs (52).

Injections of thyroxine unfavorably influenced the resistance of mice and rabbits to the toxin of the Shiga dysentery bacillus (42). From very inadequate data Hanns (53) concluded that thyroid had no effect on experimental tuberculosis in guinea pigs.

In young and adult mice injected thyroxin and thiouracil had no effect on the L. D.^{50} following intracerebral meningo-pneumonitis virus. Likewise, in rats no pronounced effect on mortality was noted in animals treated with these drugs when compared with controls with the exception of a possible effect when thyroxin was used at near toxic levels in young rats. Susceptibility of young rats was increased by the administration of 2. 4 dinitrophenol but this was ineffective with adults (54).

The phagocytic activity of leukocytes and the opsonic index of the serum are stated to be lowered in patients with Graves disease (55).

The fact that patients with thyrotoxicosis are more susceptible to infections is well appreciated by clinicians (3, 56), but a review of the literature shows that this has not been well documented. Also it is well known that early in many infections a swelling of the thyroid occurs and may be accompanied by symptoms of thyrotoxicosis.

The effects of thyrotoxicosis in man are considered to be due entirely or in large part to an over production of thyroid hormone. It is known that severe wasting follows excessive destruction of protein with a more or less constant loss of nitrogen, that there is a negative calcium and phosphorus balance leading in severe cases to a demineralisation of the skeleton that there is a spontaneous creatinuria on a creatine-free diet, that there may

be a minor reduction in the blood cholesterol and a spontaneous glycosuria with variable degrees of hyperglycemia in many cases. Other findings of interest are fatty infiltration of muscle and liver, pigmentary atrophy of the liver or hepatitis, diffuse lymphoid hyperplasia with enlargement of the thymus, and increase in the mononuclear cells of the blood. Probably the most obvious effect is the elevation in the basal metabolic rate.

TABLE 45.

The Relationship of System Involvement to Fatality in Staphylococcal Septicemia

System Involved	Number of cases	Percent Fatal
C. V. S.	13	85
R. S.	31	77
GI. S.	13	77
GU. S.	30	73
C. N. S.	13	69
SKIN	29	41
BONE	34	29

TABLE 46.

Primary Focus and Fatality in Staphylococcal Septicemia

Primary Focus	Cases	Fatal %
Neuro	4	75
Respiratory	3	66
GU	25	56
Skin	35	46
Surgery	4	50
Throat	5	20
Bone	15	20
GI	3	0
ENT	2	0
Unknown	4	0

PART IV.

At this point in the investigation it was decided to re-evaluate the human disease with regard to predisposing factors and also in regard to the possibility that the growth of staphylococci in the kidney might be related to virulence in the human patient.

METHODS

The records of the first 100 patients diagnosed as having staphylococcal septicemia during the years 1936 to 1951 inclusive have been examined. All the patients were seen in this hospital and all were native Iowans. Additional information was obtained from the autopsy records.

The salient points of the case were recorded on marginal punch cards and this sorting method was used for the subsequent analysis. (58)

Age and Sex Distribution

The youngest two cases were impetigenous new born infants, one the child of a diabetic mother and the oldest case was an 85 year old white male who died after a prostatectomy. Most cases occur in the first two decades the second decade in males being particularly common. The third most common group is males aged 70 to 80. The disease occurs in males more than females: the ratio being more than two to one.

The mortality of the whole group was 41% and it was identical for the two sexes. The untreated cases showed a mortality of 48%. The age group 30-50 was notable because of the small number of cases and because of a low mortality 25%. The highest mortality of 80% was in the males over 70.

All the cases were white. This is not surprising as this hospital only treats a small number of colored patients.

Autopsies

Autopsies were carried out on 14 males and 8 females. The lungs and kidneys were most often affected. In women the respiratory disease was commoner and in men kidney disease was most frequent. In most cases abscesses were present in the lung in some lobular pneumonia and some had septic infarcts. In most cases the kidneys were peppered with abscesses. Some showed perinephric abscess and others acute pyelonephritis. There were four cases of myocardial abscesses, three of endocarditis and three of pericarditis. The central nervous system was involved in only 5 but this may be the result of autopsy procedure as some autopsies were resistricted in extent. A septic spleen was common.

The Cause of Death

A comparison has been made of the involvement of the different systems of the body in fatal and non-fatal cases. It was found that lesions of the cardiovascular system, lungs, GI tract and kidneys were most often associated with a fatal end. (Table 45) The spleen is open to doubt as it may, due to its softness, only be discovered to be affected at autopsy.

I n an attempt to investigate the possible presence of renal failure, creatinines were examined. In 12 surviving cases 2 or 17% were abnormal whereas in 25 fatal cases, 10 or 40% were abnormal. Similarly the blood urea was abnormal in 20% of surviving cases and it was abnormal in 61% of the fatal cases. It is probable that kidney malfunction contributes to death.

From the previously presented autopsy findings, it is possible that respiratory embarrassment is an additional cause of death but we have no way of measuring this. The staphylococcus produces several potent exotosins.

These toxins must influence the lethal effect of staphylococcal septicemia, but exactly how is not yet certain.

Some Unusual Occurrences

The association of scarlet fever with staphylococcal disease is decidedly rare. There are only three reports in the literature. A 3-$\frac{1}{2}$ year old female had vomiting and diarrhea 15 days before admission. Ten days prior to admission she developed convulsions, a fever, a sore thorat and a scarlatina-form rash. She went into coma and later had a local skin abscess. She was treated with both sulphadiazine and penicillin and made a good recovery. An 11 year old schoolboy complained of pain below his left knee. The next day he developed a rash and two days later he was admitted to the S. U. I. Hospital. At this time he was acutely ill and had on his trunk and extremities a fine, bright red, confluent rash which spared his face. He had anterior and posterior cervical lymphadenopathy and a strawberry tongue. He also had acute osteomyelitis and was treated with sulphathiozole convalescent serum, blood transfusion and surgery. He had hemolytic staphylococcus aureus in his blood and in the pus from his osteo but unfortunately he had a B hemolytic streptococcus in his throat! This illustrates the difficulty of attributing scarlet fever to the staphylococcus.

There are three cases of staphylococcus albus septicemia. In one fatal case in a young girl it followed a frontal lobe abscess and the organism was isolated both from the pus and the blood. In a second case, a male of 68 who survived, the organism was isolated twice from the blood with 17 days between each isolation. The patient was the one who later developed diabetes. The septicemia followed drainage of a gallbladder abscess. There

was a third staph albus case, in a 58 year old male with complex GI problems who developed a chill after IV fluids. The same organism was again isolated five years later after a pneumonic illness.

Jaundice occurred in two fatal cases. In a 41 year old prostitute the liver was enlarged 4 fingerbreadths but at autopsy no abscesses were found. The van den Bergh direct reaction was 26 units. This was associated with an extraordinary heavy growth in the blood culture (3,500 organisms/cc.) A 12 year old white female became jaundiced after an operation for osteomyelitis. At autopsy multiple septic infarcts of the lungs were found. The liver was normal. The van den Bergh was 25. A 3 months old baby with multiple abscesses, staphylococcal pneumonia and osteomyelitis was also jaundiced with a bilirubin of 1.5 mg. %, an icteris index of 25 units.

Agranulocytosis occurred in a 62 year old female who also--poor woman--had a bladder tumor, multiple GU operations and diabetes. The agranulocytosis may have been secondary to sulfadiazine.

An 18 year old boy developed osteomyelitis involving his left femur and right foot in 1935. In 1938 he had bilateral empyema and further involvement of the left ulna, the right radius and the dorsal spine. When he died in 1940 after a wound hemorrhage he had amyloid disease present in the liver, spleen, kidneys and intestines.

One child had vaccinia complicating staphylococcal skin disease and septicemia and another possibly had diphtheria as well as staphylococcal septicemia and acute lymphatic leukemia.

Predisposing Factors

There were 9% diabetics in this series and five of these nine

cases died. Another case developed diabetes four years after his septicemia. Five additional cases had one parent who was a diabetic.

Six cases had cancer or leukemia.

No less than 18 of these cases followed a prostatectomy operation. It accounts for over 70% of the male cases over 60 years of age. This brings up the question as to whether staphylococci are common in old men or in the noses of urologists.

A nineteen year old girl who died had nursed her father for pneumonia and developed pneumonia herself the day after his funeral. The only other possible case of contact with infection was in two male cases which were on the urology ward at the same time.

In eight cases trauma preceded the onset and five of these developed osteomyelitis.

The Primary Focus

The normal habitat of the staphylococcus is the human nose. It is transferred from there to other places by the hands or possibly in a few cases by direct extension in the respiratory tract. If it leaves one individual it may persist in another member of the family and "entrenched" family infections are not uncommon.

An attempt has been made to define the "portal of entry" or more correctly the area of origin of the disseminated disease. A mortality figure has been calculated for each group and is shown in the next table, number 46.

Diagnosis

All the cases chosen here had a positive blood culture but it is

important to remember that in a number the culture was only intermittently positive. In the present period many cases are treated before entering the hospital. In these cases it may be hard to find the organism in the blood and it is wise to take a nasal smear and test any staphylococci found their for their antibiotic sensitivities.

As the lung is a common focus, a chest film should be taken. In five of our cases I. V. P. 's showed renal abscesses. This test should be considered particularly if the BUN or creatinine is elevated.

There are unfortunately no available serological tests for staphylococcal infection.

Prognostic Signs

I n eight cases where the blood culture was stated to show many staphylococci seven died. It has already been stated that the prognosis is very poor in the older age group of males.

Anemia was equally present in fatal and surviving cases--about 20% of each. Information regarding the maximum white cell count was available in 83 cases. The counts were divided into those over 20,000 those between 10 and 20,000 and those under 10,000. A low count was more often associated with a fatal outcome than was a normal or high count. 18% of fatal cases as agains 2% of non-fatal cases had a white cell count less than 10,000 per cubic mm. This was not confined to any age group. The maximum temperature was available in 89 cases. A temperature of less than 100^{o} is a good prognostic sign.

Treatment

This is naturally dependent on early diagnosis. As for most

septicemia six blood cultures in two days offers the best hope of success. The patient has to be in bed. Small blood transfusions are thought to be helpful. Surgery is certainly helpful in osteomyelitis. It may well be helpful if a perinephric abscess is suspected. Other abscesses should be drained where practical. Every effort must be made to find foci of pus as the septicemia cannot be maintained without such a focus.

Table 47 shows the effects of treatment.

Penicillin should be given in large doses whether the organism is sensitive in vitro or not. Fisher (59) has shown that the only single factor related to survival in staphylococcal endocarditis is the use of massive doses of penicillin whether the organism is sensitive or not. McCune has shown that mice can be protected against a lethal dose of penicillin resistant staphylococci by the use of penicillin.

METHODS

Organisms* were collected from the skin, sputum or lung, blood, throat, bone and ear. They were grown in a way similar to the other experiments and also injected in 0.1 cc. volume of culture. In each case 15 four week old mice were injected: 10 for mortality studies and 5 for the kidney titer.

Figure 9 shows the effects seen with these different organisms. It is at once evident that although this dose is suitable for laboratory cultures, it appears to be too high for the cultures newly isolated from patients. In a large number of cases all the ten animals injected for mortality studies died. The organisms isolated from osteomyelitis cases do not kill as many mice as do organisms from other sources. It is of interest in this connection that in surveys of human cases of septicemia the death rate in cases following osteomyelitis is lower than the death rate in cases arising from other foci. Due to the high mortality there are rather few survivors in which to test the kidney titer. Those which we have been able to test are shown in the bottom section of the slide. It is possible that this test may be useful in differentiating types of staphylococci. However, in its present form it is not as useful as it may yet be.

*Kindly collected by members of the Department of Bacteriology, State University of Iowa, from routine cultures.

TABLE 47.

The Effect of Treatment in Staphylococcal Septicemia

	Cases	Fatality %
None	21	48
Sulfa	47	53
Penic. & Sulfa.	23	13
Penic.	5	20
Penic. & Sulfa. & Other	4	50

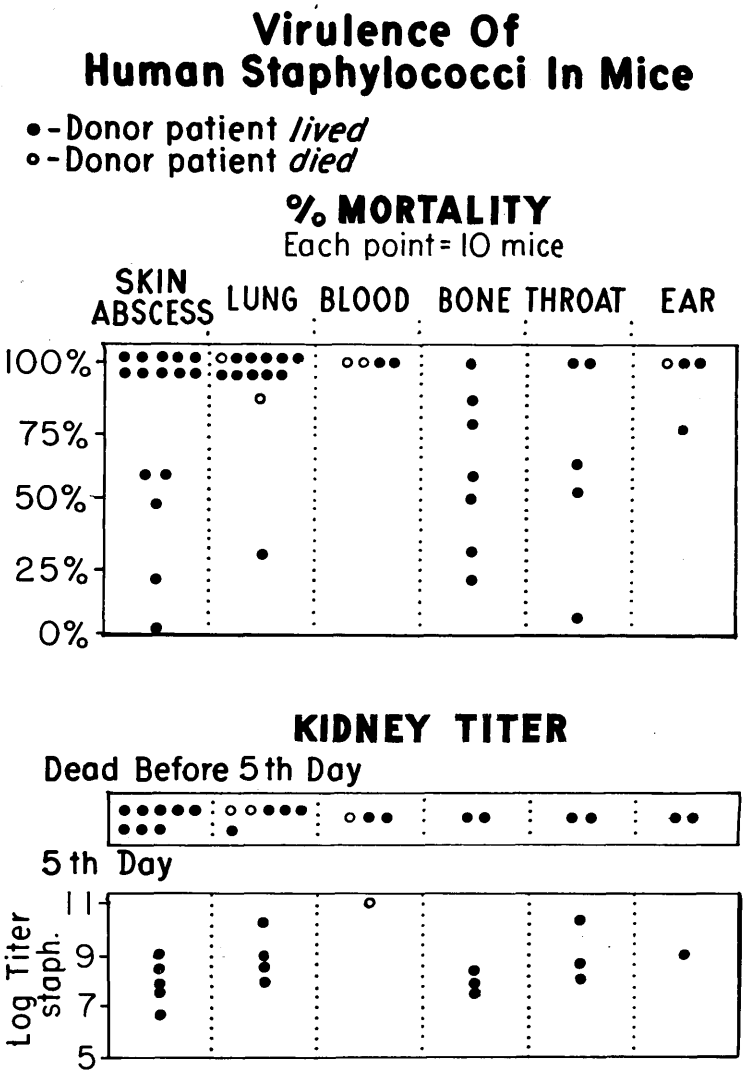

Figure 9. The Mouse Virulence of Human Staphylococci.

DISCUSSION

In all infectious diseases there is a balance between the destructive power of the invader and the resistance of the host. A very destructive organism is self destructive as the host does not survive. In staphylococcal disease we have a nice balance. This balance is easily upset in decreasing the resistance of the host. This will happen more often in the future due to the aging of the population and the longer survival of patients with chronic disease. Some of these problems have been illustrated here experimentally.

I n the future it is thought that treatment of staphylococcal disease will be improved by adherence to massive penicillin treatment with added antibiotics if indicated by the maintenance of renal function by all available means and by the judicious use of surgery. Experimentally more must be known about the growth of the organisms in the kidney. We have started to study this in human kidney tissue culture. More must be found out about the differences between the human and experimental diseases. Why is the lung relatively spared in mice? We are trying the effect of super added virus infections with P. V. M, P. R. 8 and vaccinia viruses. What happens in the first hour when there is rapid killing of the injected bacteria? This is being studied by others in relation to the liver and we are studying it in relation to living preparations of human polymorphs which we can study under the microscope. Are there any intracellular chemicals involved?

The next advance in the control of infections is unlikely to

occur in the antibiotic field. We feel that the next advance will concern new host mechanisms of protection and this may well develop out of studies such as this.

"Je pense que les recherches de l'avenir en matière d'infection à staphylocoques doivent s'orienter plus du côté de l'homme que du côté du microbe et de ses soi-disant variations de virulence, que nous ne pouvons guère analyser.....Nous ne progresserons, je crois, au sujet des maladies staphylococciques que quand nous aurons su définir les déficiences tissulaires ou humorales qui conditionnent l'immunité ou l'inverse..

Et, si puissante que devienne la chimiothérapie, il est a craindre que si nous negligeons l'etude du terrain, nous aurons, là encore, quelques déboires."

René Leviche (57)

Conclusions

1. It has been confirmed that normal mice die following the intravenous injection of coagulase positive staphylococci and do not die after the injection of coagulase negative organisms.

2. Death follows the progressive development of abscesses in the kidneys.

3. Histological studies have been made of the staphylococcal infection and confirm that the damage due to infection is largely confined to the kidney.

4. A new bacteriological method of following the fate of bacteria in the tissues has been successfully used to study staphylococcal infections in mice.

5. It is apparent, contrary to what might have been expected from invitro studies, that the mechanism of clearing coagulase positive and negative organisms from the tissues is very similar.

6. Differences in subsequent multiplication after the early clearing phase account for the different ultimate effects of coagulase positive and negative organisms. This difference is seen in the kidney where the coagulase positive cocci do so to a greater extent. It may be stated that the difference between coagulase positive and negative staphylococci in-vivo is quantitative rather than qualitative. Furthermore, within these two groups further quantitative differences can be defined so that a complete spectrum of virulence or pathogenicity can be demonstrated.

7. Again in distinction to earlier in-vitro studies it can be shown that coagulase negative organisms can survive over a long period in mouse tissues.

8. Short periods of fasting have an enhancing effect on staphylococcal infection in the mouse. The amount of fasting required and the amount of refeeding necessary to abolish this effect have been studied.

9. This infection enhancing effect of fasting has been shown to cause greater multiplication of staphylococci in the tissues particularly in the liver. Liver function as measured by B. S. P. excretion is reduced in fasting.

10. Glucose in the drinking water of mice during the fasting period causes a further increase in susceptibility to infection whereas lactate partially alleviates the fasting effect. Other substances were studied and found to be inactive. Increase in cocci in the tissues can again be shown but it is found at an earlier stage than in the case of fasting without glucose to drink.

11. By two different methods it was possible to prevent mice gaining weight and produce a state of undernutrition. These animals were not more susceptible to the lethal effects of staphylococcal infection than were corresponding controls. The fate and multiplication of staphylococci in the tissues was not significantly different in the two groups.

12. Animals given thyroid or dinitrophenol in their drinking water or in their food did not gain weight. These animals were found

to show earlier deaths and increased numbers of deaths from staphylococcal infection when compared with controls. There was no clear cut difference in the survival and multiplication of the cocci in these animals from that in normal animals.

13. This effect was not produced by iodine or di-iodotyrosine. It was caused by the feeding of thyroxine.

14. A series of human cases of staphylococcal septicemia have been examined from the point of view of host resistance and the nature of staphylococcal virulence.

15. Staphylococci of human origin have been studied for their virulence in mice.

16. These findings have been discussed in their relationship to clinical medicine. Certain areas of further investigation have been defined.

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Curriculum Vitae

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