THE EFFECT OF NUTRITIONAL DISTURBANCES ON THE SUSCEPTIBILITY OF MICE TO STAPHYLOCOCCAL INFECTIONS*

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Recent studies have revealed that it is possible to increase at will the susceptibility of mice to experimental infections with tubercle bacilli or with various Gram-negative bacilli by subjecting the infected animals to certain procedures designed to upset their metabolism (1, 2). It will be shown in the present paper that the course of staphylococcal infections in mice is likewise dependent upon the nutritional state of the animals at the time of exposure to the infective dose.

EXPERIMENTAL

The culture of staphylococcus used in the present study was the coagulase-positive strain "Smith" described in the preceding paper of this series. The infective dose was in all cases 0.1 ml. of an 18 hour old culture diluted with 0.1 ml. saline and injected by the intravenous route. The techniques used for following the course of the infectious process have also been described in the preceding paper. (3)

Effect of Fasting on Survival of Mice Infected with Staphylococci.—It has been shown elsewhere that the life expectancy of tuberculous mice can be markedly shortened by depriving the animals of food for periods of 36 to 48 hours during the course of their disease (1, 2). The following experiments demonstrate that it is possible to increase the susceptibility of mice to staphylococci by fasting the animals for similar periods of time immediately prior to infection.

Mice were fed pellets and water *ad lib.* for 1 week after weaning. The food was then removed for 36 hours from lots of ten animals, water remaining available *ad lib.* The animals were infected at the end of the period of fasting, and again provided with pellets and water *ad lib.* immediately after infection.

In a second experiment, the animals were deprived of food twice, once immediately before infection and a second time for 36 hours 1 week after infection. The control groups consisted of mice kept under the same conditions and also fasted either once or twice for 36 hours, but not infected. The animals of a third group were infected at the same time as those of the fasted infected group, but were not fasted and received pellets *ad lib.* throughout the period of experimentation. The mice were observed for 1 month and the time of death recorded.

* The experiments described in this paper have been presented by Dr. J. Maclean Smith as part of a thesis for the degree of M.D. in the University of Glasgow.

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TABLE I

Effect of Fasting on Survival Time of Mice Infected with Coagulase-Positive Staphylococci

I A

Fasting	Days of death									
0	6*	23	—*	—	—	—	—	—	—	—
Once 36 hrs.‡	6	7	7	12	14	18	19	—	—	—
0	5	9	9	12	14	27	28	—	—	—
Twice 36 hrs.‡	5	5	5	5	5	5	7	7	9	11

* The figures indicate the number of days after infection at which death occurred. The sign (—) indicates survival; experiment discontinued 30 days after infection.

‡ In the first experiment, the mice were deprived of food for 36 hours immediately prior to infection; in the second experiment the animals were deprived of food for 36 hours on two occasions: once before infection, and again one week later. All fasted, but non-infected animals were alive and well when the experiments were terminated.

I B

Fasting	Cumulative No. of deaths on day			Survivors on day 14
	7	10	14	
hrs.				
0	1	1	1	9 out of 10
36	3	3	5	5 " " 10
0	1	3	5	5 " " 10
36	8	9	10	0 " " 10
0	0	0	0	10 " " 10
48	1	4	4	6 " " 10
0	0	0	0	8 " " 8
48	2	5	5	3 " " 8
0	1	1	1	5 " " 6
36	2	2	3	3 " " 6
0	1	4	4	6 " " 10
36 + 12*	3	5	5	5 " " 10
0	2	4	4	5 " " 9
36 + 12*	6	7	8	2 " " 10

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

The results of these two experiments are presented in Table I A. Table I B reports in an abbreviated form the deaths of mice occurring within 14 days after infection in seven similar consecutive experiments.

As appears from the results presented in Tables I A and I B, the percentage of deaths in the control animals differed from one experiment to another, even though all the mice used were of approximately the same age and weight, and the infective dose always consisted of 0.1 ml. of an 18 hour old culture. It is certain that individual animals differ markedly in their resistance to staphylococcal infection, as appears clearly from the bacteriologic studies reported in the preceding paper (3). It is probable furthermore that differences in the bacterial population (due to unrecognized differences in the composition of the beef heart infusion–peptone broth, or in the conditions of incubation of the culture, or in both) accounted in part for the irregularities in the results. Despite the variability in the control groups, it is clear nevertheless that animals deprived of food for periods of 36 hours or longer proved more susceptible to infection that the non-fasted animals in all experiments reported in Tables I A and I B. This fact has been confirmed without exception in many other experiments not described here.

Effect of Fasting on the Fate of Staphylococci in the Organs of Infected Mice.— As shown in the preceding paper, staphylococci of the Smith strain injected intravenously into normal mice disappear progressively from the blood, lungs, liver, and spleen but eventually give rise to destructive abscesses in the kidneys. The following experiments were carried out to determine whether the fate of the staphylococci in the liver and spleen could be modified by depriving the animals of food just before infection.

Mice were fed pellets and water *ad lib.* for 1 week after weaning. All food, but not water, was then withdrawn from one group for 36 hours. These animals were infected during the period of fasting and were further left without food for 12 hours after infection. They were then given pellets *ad lib.* at the end of the 48 hours' fasting period. Water was provided *ad lib.* throughout the experiment. Other mice were similarly infected at the same time, but without any period of fasting. Several animals of each group were sacrificed at various periods of time after infection, their organs ground, and the numbers of living staphylococci in the organ homogenates determined by quantitative bacteriologic techniques. The results obtained for the liver and spleen in three consecutive experiments are presented in Table II.

The results presented in Table II show considerable differences in the numbers of colonies of staphylococci recovered from the organs of individual mice at any given time after infection. The results of the three experiments show clearly nevertheless that many more colonies were recovered from animals fasted immediately before and after infection than from those fed a normal diet continuously *ad lib.* This was particularly striking in the liver; in this organ the difference between the fasted and non-fasted group was approximately tenfold at any given day after infection.

The results of other similar experiments indicate that the minimum period of fasting required to produce the infection-enhancing effect differs depending upon the age of the animals and the type of diet fed them prior to the with-

TABLE II

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

Time after infection	Organ	Log Nos.* of staphylococcus colonies recovered from individual mice											
		Fed continuously *ad lib.*						Fasted‡ for 36 + 12 hrs.					
days													
2	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	"	3.71	?	4.34	0	4.51	3.23	5.53	3.23	5.16	4.73	5.17	4.13
2	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	"	0	?	3.23	3.23	4.08	0	3.53	0	3.53	3.23	0	0
1	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	"	?	3.23	4.34	3.93	4.28	4.91	5.92	4.59	5.51	5.38	5.34	4.63
1	Spleen	5.77	5.43	5.21	6.18	5.31	6.04	5.40	5.97	5.49	5.49	4.63	4.23
3	"	4.28	4.08	3.93	0	4.75	3.93	5.68	3.53	4.95	3.83	5.01	4.53
1	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	"	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	Spleen	6.34	6.02	6.15	6.28	6.83	5.93	6.28	5.77	6.23	6.12	6.53	5.80
3	"	5.15	4.93	4.75	5.51	4.08	4.57	5.09	6.09	5.12	5.00	4.38	5.38

* The figures are the logarithms to base 10 of calculated numbers of colonies recovered per whole organ. Because of limitations in the enumeration technique the figure 0 corresponds in reality to 3.23, or less. The sign ? indicates that the culture was lost by accident or contamination. The results are entered in the order in which the mice were sacrificed; in any given series, the results for the liver and spleen of an individual animal occupy the same relative position in the table.

‡ 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 III

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

Fasting before infection	Log Nos. * of staphylococcus colonies recovered from individual livers 3 days after infection					
hrs.						
0	3.23	3.83	4.28	4.31	4.38	4.73
3	3.83	4.08	4.18	4.34	4.51	4.89
6	3.83	4.08	4.46	4.55	4.80	4.81
18	3.53	3.93	4.01	4.01	4.43	4.82
36	4.01	4.43	4.57	5.14	5.23	5.89

* Legend as in Table II.

drawal of food. With mice 4 weeks old, fed either Sherman diet or commercial pellets, this period is of the order of 36 to 48 hours (Table III). In other preliminary experiments, it has been found that the infection-enhancing effect of withdrawal of food is no longer detectable when the fasted mice are tested 48 to 72 hours after a complete diet has been restored to them *ad lib.*

Effect of Feeding Glucose or Lactate to Fasting Mice on Their Resistance to Staphylococcal Infection.—In the preceding experiments the animals received only water during the fasting period. The effect of providing a caloric source in the form of glucose or sodium lactate was tested as follows.

Mice were deprived of solid food for 48 hours. During the fasting period they received as drinking fluid either one of the following aqueous solutions *ad lib.*: (*a*) 1 per cent sodium chloride; (*b*) 5 per cent glucose in 1 per cent NaCl; (*c*) 1 per cent sodium lactate at pH 6.5. The animals were infected at the end of the fasting period and were then immediately placed on a diet of pellets *ad lib.*; from then on they continued to receive as drinking fluids either solution (*a*), (*b*), or (*c*) respectively as during fasting. The time of death of the animals, in comparison with that of mice similarly infected but receiving pellets *ad lib.* throughout the period of experimentation, is shown in Table IV.

TABLE IV

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

Fasting	Drinking fluid	Time of death after infection								Survivors at 14 days
hrs.		*days*								
0	Saline	6*	—*	—	—	—	—			5 out of 6
36	"	6	7	12	14	—	—			2 " " 6
"	5 per cent glucose	4	6	9	13	—	—			2 " " 6
"	1 per cent lactate	6	10	13	—	—	—			3 " " 6
0	Saline	—	—	—	—	—	—	—	—	8 " " 8
36	"	6	7	8	9	10	—	—	—	3 " " 8
"	5 per cent glucose	2	7	7	8	9	10	11	—	1 " " 8
"	1 per cent lactate	8	9	—	—	—	—	—	—	6 " " 8
0	Saline	—	—	—	—	—	—	—	—	8 " " 8
48	"	7	7	10	10	10	—	—	—	3 " " 8
"	5 per cent glucose	5	7	7	10	10	10	12	—	1 " " 8

* Legend as in Table I.

As appears from the results presented in Table IV, administration of glucose in the drinking fluid failed to correct the infection-enhancing effect of fasting, and in fact increased it consistently. In contrast, lactate in the drinking fluid appeared to have some protective effect. In a number of preliminary experiments not to be described here, it was found that the sodium salts of other organic acids (butyric, glutaric, and pyruvic acids for example) failed to have any protective effect and indeed enhanced the severity of the infection. Table V, which shows the results of two consecutive experiments, makes it clear that the aggravation of the infectious process caused by giving glucose solution to fasting animals also manifested itself in the numbers of colonies of

TABLE V

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

Time after infection	Organ	Log Nos.* of staphylococcus colonies recovered from mice																	
		Fed continuously *ad lib.*						Fasted‡						Fasted but receiving glucose‡					
days																			
1	Liver	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	Spleen	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	Liver	?	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	Spleen	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	Liver	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	Spleen	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	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	6.53	5.51	5.73	5.55	5.06	4.46
3	Spleen	5.15	4.97	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	3.21

* Legend as in Table II.

‡ Animals fasted for 36 hours before and 12 hours after infection: they received either physiologic saline or 5 per cent glucose as drinking fluid during the fasting period. The results are those of two consecutive experiments.

staphylococci that could be recovered from the liver and spleen 1 day after infection.

It has been found in several experiments that the aggravating effect of glucose is transient and can be detected only during the initial phase of the infection. Indeed, among fasted animals that survived infection, those receiving glucose during the fasting period seemed better able to destroy the staphylococci than did those receiving only saline as drinking fluid. This finding will be more fully documented in a later publication. It may be mentioned in passing that the survival time following infection, and the fate of staphylococci in the organs, was not affected in any detectable manner by giving glucose solution instead of saline as drinking fluid to non-fasted animals.

Lack of Correlation between Weight of Mice and Susceptibility to Staphylococcus Infection.—It was observed in the preceding experiments that mice lost approximately 10 to 20 per cent of their original body weight during the 36 to 48 hours periods of fasting. The loss of weight was as great when the drinking fluid consisted of 5 per cent glucose solution instead of water or saline. It appeared possible, therefore, that the increased susceptibility to staphylococci exhibited by mice placed on these regimens was merely an expression of the small size of the animals at the time of infection. To test this hypothesis, mice were placed on other types of regimen designed to interfere with their growth; they were then infected with staphylococci, and their resistance to infection studied in comparison with that of mice fed diets that allowed a rapid gain in weight.

In one experiment, mice were fed *ad lib.* either one of the two following diets: (*a*) wheat flour 66 per cent, skim milk 33 per cent, salts[1] 1 per cent; (*b*) wheat flour 45 per cent, cerelose 30 per cent, peanut oil 20 per cent, skim milk 4 per cent, salts 1 per cent. These two diets were resuspended in an equal weight of 8 per cent gelatin solution which was then allowed to gel in the ice box. The diets in the form of gelatin cakes and water were given *ad lib.* The animals were infected 32 days after being placed on their respective nutritional regimens and were kept on these regimens from then on. The infective dose was the same (0.1 ml.) irrespective of the weight of the animals. The fate of staphylococci in the liver and spleen was determined 48 hours after infection, the individual weight of each one of the animals used for the quantitative bacteriological studies being determined at the time of sacrifice. The results are presented in Table VI, which gives also the number of survivors in groups of 10 animals observed for 2 weeks after infection.

In another experiment, two groups of mice 4 weeks old were fed a diet consisting of: wheat flour 66 per cent; skim milk 33 per cent; salts 1 per cent. These materials were resuspended in a solution of 8 per cent gelatin at 37°C. which was then allowed to gel in the icebox. The mice of one group received the diet *ad lib.* Each of the animals in the other group received daily only 3.8 gm. of the gelatin cakes, an amount known from previous experience to be too small to allow uninfected mice of the same age to gain weight. The 3.8 gm. of food was given every morning and it was observed that the total amount was consumed within 1 hour after being given to the animals. Ten animals in each group were kept as uninfected controls in order to follow their weight curve. All others were infected on the 32nd day of the experi-

[1] Wesson modification of Osborne and Mendels salt mixture: *Science*, 1932, **75**, 339.

ment, with the same amount of culture (0.1 ml.) irrespective of their weight. Five animals of each infected group were sacrificed 48 and 72 hours after infection and the numbers of living staphylococci in their organs determined by quantitative bacteriological techniques. Ten others of each infected group were maintained on their respective regimens for 14 days and the time of death recorded (Table VII).

The results presented in Tables VI and VII make clear that mice prevented from gaining weight either by feeding them a diet low in skim milk or by re-

TABLE VI

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

	Mice fed *ad lib.* diets containing							
	33 per cent skim milk				4 per cent skim milk			
Weight 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	5.56
" " 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 VII

The Effect of Food Restriction on the Susceptibility of Mice to Staphylococcus Infection

	Mice fed *ad lib.*					Mice fed 3.8 gm. daily				
Weight change*.....	+6.8 gm.					+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 at 14 days..	6 out of 10					5 out of 10				

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

‡ Logarithm to base 10 of calculated number of colonies recovered per whole organ 2 days after infection. Because of limitations in the enumeration technique the figure 0 corresponds in reality to 3.23, or less.

stricting their daily food intake, survived infection at least as long as those fed *ad lib.* a diet permitting them a normal weight gain. Morover, no significant difference could be detected in the numbers of colonies of staphylococci that could be recovered from the liver and spleen in the various groups of animals.

DISCUSSION

Two criteria have been used in the present study to estimate the resistance of mice to staphylococcal infection: (*a*) the survival time of animals infected intravenously with 0.1 ml. of culture of a coagulase-positive hemolytic strain;

(*b*) the numbers of colonies of staphylococci that could be recovered from the liver and spleen at various intervals of time after infection. The validity of the second criterion can be questioned on the following grounds:—

It is known that the tissues of normal mice possess a high bactericidal power against staphylococci, the numbers of colonies of these organisms recoverable from the various organs beginning to fall precipitously within a short time after infection. If the infective dose is large enough, on the other hand, the cocci eventually multiply in the kidneys of most animals, forming large abscesses which can bring about the destruction of renal tissue and thereby cause death. In order to study the bacteriological aspects of resistance of the mouse to staphylococci, it would seem logical therefore to focus attention on the fate of the organisms in the kidneys. Unfortunately, quantitative determinations of the numbers of staphylococci in this organ always reveal wide differences from animal to animal. This is probably due to the fact that the size of the abscesses is greatly influenced in an erratic manner by accidents in the local conditions of lodgment of the cocci. Moreover, abscess production usually requires a few days before becoming evident and consequently this pathologic manifestation is not a convenient index for the study of the very early phase of the infectious process. For these reasons, it has seemed best to follow bacteriologically the course of the infection in the liver and spleen, in the hope of detecting the effect of various experimental procedures on the bactericidal power of the tissues. Although the lungs might have served as well for this purpose, they presented greater experimental difficulties, and were not uncommonly contaminated with bacterial species other than the staphylococcus.

The experiments described in the present paper have revealed that mice deprived of food for 36 to 48 hours before infection were more susceptible than mice fed a complete diet *ad lib.*; their average expectancy of life was reduced following intravenous injection of 0.1 ml. of culture, and larger numbers of cocci could be recovered from their liver and spleen 24 to 72 hours after infection. Animals given glucose solution *ad lib.* (with or without sodium chloride), during and after the fasting period, proved even more susceptible to infection than those receiving only water or saline—at least during the initial period of the nutritional disturbance. Experiments in progress indicate that susceptibility to infection can be still further increased by feeding the animals *ad lib.* a diet low in protein and fat but containing flour, amino acids, and a full complement of the known vitamins.

It has been found on the other hand that animals prevented from gaining weight for periods of 4 weeks, either by restricting their daily food intake to a low but constant level, or by feeding them *ad lib.* an inadequate diet (4 per cent skim milk) were just as resistant to staphylococcal infection as animals gaining weight rapidly on an unrestricted complete diet. Thus, it is unlikely that the weight of the animals at the time of infection was, *per se*, an important

factor in determining their resistance to staphylococci. It is possible, of course, that the sudden loss of weight just prior to or simultaneously with infection was more significant than the actual weight at the time of infection in determining the manner of response of the tissues to the staphylococci. In fact, experiments to be reported later indicate that animals fed a very inadequate diet can exhibit a high resistance to infection provided they come into contact with the infectious agent only after having been maintained on this diet continuously and exclusively for several weeks. This contrast between the effects exerted by temporary but complete withdrawal of food, and by chronic undernutrition has also been observed in experimental tuberculosis of mice (1, 2). The possibility seems worth considering that the ketosis which occurs during acute starvation and during uncontrolled diabetes may render the *in vivo* environment favorable for the survival and proliferation of staphylococci and tubercle bacilli, as well probably as of other microbial agents.

SUMMARY

The susceptibility of mice to intravenous injection of coagulase-positive hemolytic staphylococci was estimated by (*a*) observing the extent and time of mortality of infected animals; (*b*) determining the number of colonies of cocci that could be recovered from the liver and spleen at various intervals of time after infection.

Complete deprival of food for 36 to 48 hours immediately before infection was found to increase susceptibility. This infection-enhancing effect was further increased by allowing the animals to drink a 5 per cent glucose solution instead of water or saline during the fasting period. In contrast, sodium lactate partially corrected the effect of fasting. The infection-enhancing effect of fasting was reversible.

Mice prevented from gaining weight for several weeks either by restricting their daily food intake, or by feeding them *ad lib.* an inadequate diet, appeared just as resistant to staphylococcal infection as did mice that gained weight rapidly on an unrestricted, complete diet.

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