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A POSSIBLE SOURCE OF SECONDARY INVADING STAPHYLOCOCCI IN SALMONELLA INFECTED MICE EXPOSED TO ACUTE COLD

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(Prepared under Contract AF41(657)-340 by
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ABSTRACT

In an effort to determine the origin of the staphylococci known to invade the deep tissues (liver, spleen, kidneys, lungs and heart) of mice exposed continuously to 5° C in individual compartments without bedding, the intestinal tract was freed of these organisms, as judged by absence of growth when fecal suspensions were inoculated into selective media. Substitution of 0.01 N hydrochloric acid for drinking water eliminated staphylococci within a few days, yet the incidence of tissue invasion was unaltered. The coagulase negative strains normally present in feces and in tissues persisted in tissues even though the intestine was seeded with a coagulase positive strain by feeding contaminated food. Cultures from the external nares continued unaltered. This suggests that the respiratory tract is a possible origin of the staphylococci found in tissues of the cold stressed mice.

PUBLICATION REVIEW

Horace F. Drury

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Director of Research

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SECTION 1. INTRODUCTION

It was reported earlier (Miraglia and Berry, 1962) that CF_1 female mice maintained at 5° C experience invasion of deep tissues by a coagulase negative staphylococcus. The incidence of this secondary involvement could be increased in a predictable manner by first infecting the animals with graded doses of Salmonella typhimurium, strain RIA (avirulent). The object of this report is to elucidate the source of this secondary invader in normal uninfected mice exposed to acute cold. Since coagulase negative staphylococci cannot be phage typed, the origin of the organism could be traced by this technique. Use was made of procedures by means of which new strains of known type were established in the experimental animals.

SECTION 2. SUMMARY

The feeding of 0.01 N hydrochloric acid in mice in lieu of drinking water is apparently harmless to the general well being of the animals under the conditions indicated, but rids the gut of all culturable staphylococci in five to seven days in experiments conducted in the winter.

The period of hydrochloric acid treatment must be extended to achieve comparable results in summer studies. Neither ridding the gut of the normally present coagulase negative staphylococci nor establishing a coagulase positive strain by the feeding of contaminated food altered the incidence of tissue invasion by coagulase negative organisms.

Hydrochloric acid treatment failed to alter the incidence of nasal staphylococcal carriers. Hence, the origin of the secondary invading staphylococci appears to be the upper respiratory tract and not the gut; however, coagulase positive strains artificially established in the nose by eating infected food could not be made to invade deep tissue.

SECTION 3. MATERIALS AND METHODS

The animals and housing conditions employed have been reported elsewhere (Previte and Berry, 1962). Stool cultures were made as described (Miraglia and Berry, 1962) in an effort to focus upon the source of the secondary invading organism. Tissues cultured at the termination of each experiment consisted of heart, lungs, kidneys, spleen and liver.

Nasal cultures were also desired but owing to the obvious difficulties in attempting to obtain inocula from the nasopharynx, only the external nares of the animals were cultured by the imprint method on appropriate medium for staphylococci.

In lieu of drinking water, 0.01 N hydrochloric acid (pH 2.0) modified after the method of Schaedler and Dubos (1962) was given to the mice to rid the gut of staphylococci. The absence of these organisms was confirmed by stool culture. Where recolonization of the intestine with a specific strain (in this case Staphylococcus aureus, strain Giorgio) was desired, hydrochloric acid treatment was terminated, and for a period of 12 hours the desired microorganism was fed to the mouse as a contaminant in the ration.

The coagulase test was conducted in Wassermann tubes using 0.5 ml of reconstituted coagulase plasma (Difco) to which was added two drops of a 16 hour brain-heart infusion broth culture (Difco) of the staphylococcus under test. Tubes were read after three hours incubation at 37° C.

SECTION 4. RESULTS

Animals have been maintained on hydrochloric acid drinking water for over 40 days without any obvious untoward effects. The appearance and behavior of the animals are normal. There is normal weight gain and growth, and the pH and character of the stools is indistinguishable from that of normal controls.

Effect of Acid Treatment on the Per Cent of Mice with Feces Positive for Staphylococci

In most experiments five to seven days on acid treatment sufficed to rid the gut completely of culturable staphylococci. In this connection, however, a seasonal effect was noted in experiments conducted during the summer months even though the animals had air conditioned quarters. At this time

of year, additional time on acid treatment (Table I) was required to free the intestine of staphylococci. Moreover, while studies conducted during the winter consistently yielded coagulase negative staphylococci from the feces, summer studies revealed a low percentage of coagulase positive strains as well as a high percentage of coagulase negative strains. When no staphylococci could be cultured from the feces after a period of acid treatment, the digestive tract was then recolonized with strain Giorgio, a coagulase positive staphylococcus, and for about a week thereafter the stool cultures contained only coagulase positive strains. While mice continued to shed these strains for at least several additional weeks, they also began to discharge coagulase negative strains by the 14th day. This occurred in animals maintained at both 25° and 5° C.

TABLE I

Staphylococci Recovered from the Feces of Mice After Various Periods of Hydrochloric Acid Treatment

Duration of HCl treatment (Days)	Per cent of mice with fecal staphylococci		Per cent coagulase negative	Per cent coagulase positive
0	95	(308)	95	0
6	67	(15)	67	0
10	11	(27)	11	0
12	5	(20)	5	0
28	40	(20)	30	10

Number of mice tested shown in parentheses.

Although the gut can be made free of staphylococci by treatment with hydrochloric acid, these organisms became reestablished in approximately 50 per cent of the animals within several days after tap water was substituted for the acid. In fact, in animals autopsied after the usual experimental

period of 14 days, coagulase negative staphylococci were found in nearly all stool cultures. This too was independent of the environmental temperature at which the mice were kept.

Effect of Acid Treatment on the Per Cent of Mice with Tissues Positive for Staphylococci

Deep tissue invasion, with only a few exceptions, has consisted of coagulase negative strains. Table II summarizes the results. Therefore, the intestine when colonized with the coagulase positive Giorgio strain could not be the origin of the secondary invader. But even more revealing is the fact that staphylococci were still isolated from the tissues of cold exposed mice with the anticipated frequency in individuals whose feces contained no culturable staphylococci.

TABLE II

Staphylococci Recovered from Tissues of Mice
After Various Treatments

Treatment	Per cent of tissues positive for staphylococci*	
1 day at 5° C	0	(5)
5 days at 5° C	0	(5)
8 days at 5° C	40	(5)
14 days at 5° C	50	(18)
21 days at 5° C	40	(5)
14 days at 25° C	0	(10)
10 days HCl + 5 days at 5° C	33	(3)
10 days HCl + 14 days at 5° C	56	(9)
10 days HCl + 14 days at 25° C	10	(10)

All isolates were coagulase negative
Number of mice tested shown in parentheses.
* Tissues tested: liver, kidney, spleen, lung and heart.

Reestablishment of coagulase negative staphylococci in the gut of mice rendered free of staphylococci by acid treatment did not alter the frequency of tissue invasion by coagulase negative organisms. This too is evidence against the possibility that the invaders are intestinal in origin.

In tissues of acid treated, cold exposed mice, a few cases were recorded in which both coagulase positive and coagulase negative strains were found in the same individual but only one type was present in any particular organ. In cases where coagulase positive staphylococci were isolated from the tissues, the nasal flora likewise consisted of coagulase positive strains. These data, in conjunction with those above, implicate the respiratory tract (nares) as a possible focus from which secondary invaders arise.

The data of Table II demonstrate that a secondary invasion by staphylococci in normal mice exposed to cold requires approximately a week and appears to reach a maximum in 14 days. Moreover, prior treatment of the mice with hydrochloric acid drinking water did not alter significantly either the incidence or timing of staphylococcal involvement of deep tissue.

In view of the above and since it is well known (Taylor and Dyrenforth, 1938) that acute cold adversely affects the upper nasal passages, this area was studied to determine if it might serve as a possible portal for deep tissue invasion.

Effect of Acid Treatment on the Per Cent of Mice with Noses Positive for Staphylococci

Table III shows that the percentage of mice with culturable staphylococci from the nose remains essentially unaltered regardless of the experimental procedures to which the animals are subjected. For example, hydrochloric acid drinking water given for various periods up through 28 days did not lower the per cent of staphylococcal nasal carriers among normal or Giorgio fed mice. This is contrary to what was noted in the gut since it could be freed of staphylococci following acid water treatment and then recolonized with the Giorgio strain with great facility. Thus, nasal staphylococci which are mainly coagulase negative were found to persist unabated in all 228 mice studied.

Nasal cultures also revealed that in the winter only coagulase negative strains were harbored, but that in the summer a low incidence of positive strains was also evident. In a group of mice from which coagulase positive strains were isolated from the tissues for the first time, it was found that the stools contained only coagulase negative staphylococci, whereas also for the first time coagulase positive organisms were isolated from the nose. Coagulase positive strains have never been isolated from the tissues of mice which

had coagulase negative nasal flora. Thus, a correlation may exist between the nasal flora and the organisms isolated from deep tissue as secondary invaders in cold stressed mice.

TABLE III

Staphylococci Recovered from the Noses of Mice After Various Periods of Hydrochloric Acid Treatment

Duration of HCl treatment (Days)	Per cent of mice with nasal staphylococci		Per cent coagulase negative	Per cent coagulase positive
0	100	(60)	100	0
10	100	(78)	95	5
12	100	(80)	100	0
28*	100	(10)	90	10

All mice were nasal carriers of staphylococci before acid treatment
Number of mice tested shown in parentheses
* Summer mice

Effect of Acid Treatment on the Per Cent of Salmonella Carrier Mice with Feces Positive for Salmonella

It is reasonable to assume that since hydrochloric acid treatment eradicates staphylococci from the gut, the population of other members of the intestinal microflora may be likewise altered. This phase of the study has not been actively pursued, but results from a preliminary experiment using 40 mice show that the carrier rate for salmonella was reduced from 80% to 5%, $P<0.008$ (Wilcoxon, 1949), in 24 hours by using the acid treatment. This indeed appears to be a dramatic reduction, but owing to the inherent shortcomings of the sampling method and the obvious danger of generalizations based on a single experiment, a more definite statement concerning the efficacy of this treatment for carrier mice must await more intensive studies.

SECTION 5. DISCUSSION

There still remain to be answered several perplexing questions. Not the least of these is the observation that while by their very nature staphylococcal infections tend to localize and form well defined foci of infection, this has never been observed in the hundreds of animals autopsied during the course of these investigations. Gross examination of the nasal passages and sinuses failed to show a localized pathology in 14 days, the usual term of these studies. The invasion involves lungs, heart, kidneys, spleen and liver in an unpredictable manner and without any apparent preference for any specific tissues, this in the face of the usual course of events in which the staphylococci frequently invade the kidneys with subsequent overt signs.

Paradoxical also has been the observation that while mice fed the coagulase positive Staphylococcus aureus, strain Giorgio, as a contaminant in their ration following acid treatment become intestinal carriers of this strain, they also become nasal carriers of the same strain, perhaps by the manner in which they eat. This state rarely lasts for more than 48 hours, nor are more than 30 to 40 per cent of the mice such transient carriers. Even so, these individuals never yielded coagulase positive isolates from deep tissue.

Thus it would appear that while a correlation seems to exist between the flora of the nose and that of deep tissue, under the conditions of these experiments this cannot be altered experimentally so that a coagulase positive strain established artificially in the nose by eating infected food could subsequently be made to invade deep tissue.

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2. Staphylococcus
3. Salmonella
4. Diseases
5. Mice
6. Exposure
7. Hydrochloric Acid

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