

VIBRIOS FROM THE INTESTINAL TRACT OF THE GRAY RAT

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PLATE 35

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INTRODUCTION

We have described a diarrhea of cattle and shown that it is caused by a vibrio that multiplies in the small intestine but does not penetrate the mucosa. This disease has been recognized in epidemic form only in the late fall and winter months. Because of the seasonal distribution and relatively mild character of the disease, it may be argued that the vibrio is not a true parasite of the cow and that it originates from some other animal. Rodents, and especially rats, would be the first animals suspected as a source of infection since they usually migrate to cow barns when cold weather appears and while there are in intimate contact with the cows and their food. In order to test the hypothesis that rodents might be carriers of this disease, we have examined a number of rats and mice for vibrios and it is the purpose of this paper to report our results.

The diarrhea in cows to which we have called attention is associated with an infection of the small intestine with vibrios. The vibrios apparently possess little pathogenicity for the cow but when they lodge in the small intestine are capable of rapid multiplication and thus incite an inflammatory reaction frequently accompanied by severe diarrhea. It might be considered that the etiological factor is not a true parasite of the cow and that it originates in other species. This, coupled with the fact that rodents are apt to migrate to the barns in the colder months, suggested that they might be the true source of infection. Our investigations were primarily directed toward this phase of the problem and, although we were unable to show that

vibrios of rodent origin were identical with those obtained from cows, the fact that vibrios, frequently morphologically identical with those from the cow, inhabit the small intestine and cecum of the rat seems worth while reporting.

Methods and Materials

The rodents were obtained from farms where diarrhea existed and brought to the laboratory alive. They were chloroformed and utilized as rapidly as possible.

In general the methods employed for the cultivation of vibrios from the intestinal tracts of rats and mice were the same as those previously reported.¹ After the animals had been chloroformed pieces of mucosa from various levels of the small intestine were washed in six changes of salt solution, ground, and resuspended in NaCl solution. The suspensions were added to the condensation fluid of agar slants containing 0.5 cc. of defibrinated horse blood. The content of the lower portion of the ileum of the rat frequently contained large numbers of actively motile vibrios. When motile vibrios could be readily demonstrated by microscopic examination another procedure was used. It had been noted that these organisms, suspended in liquid and examined in the hanging drop, tended to migrate toward the edge of the drop so that after an hour or more the greatest number of them was observed along the shallow borders. A method whereby this migratory property was utilized proved of great advantage in obtaining pure cultures from the intestinal content, fecal mucus, and from mixed cultures.

The procedure was as follows: A circular chamber, 4 or 5 cm. in diameter was made in the center of a sterile Petri dish by means of a wall of sterile paraffin. The dish was then placed on a warm stage (38°C.) and the chamber filled with calf serum water or sterile horse serum (5 per cent) diluted with NaCl. After the medium had become warm the intestinal suspension or mixed culture was added to the center of the liquid as gently as possible. At intervals subcultures were made from the borders of the liquid into the condensation fluid of an agar slant containing 0.5 cc. defibrinated horse blood. Finely drawn capillary pipettes proved admirable for the purpose since capillary traction is sufficient for the collection of a drop and the procedure creates little disturbance in the medium. It was found advisable to begin to subculture after 5 minutes and to continue at 5 minute intervals for $\frac{3}{4}$ hour. Frequently all tubes inoculated during this period contained vibrios in pure culture but after longer intervals other bacteria reached the borders of the chamber and mixed cultures developed.

In all, 19 rats and 12 mice were examined. As mentioned before, the lower ileum and cecum of the rat contained actively motile vibrios of which there were two recognizable types. (Although extensive

¹ Jones, F. S., and Little, R. B., *J. Exp. Med.*, 1931, 53, 835.

studies have not been made, similar vibrios may be demonstrated in the content of the lower ileum of the white rat.) The larger type with shallow undulations and active motility (Fig. 1) was readily cultivated. A more delicate form was often seen but was never cultivated. In the stained preparations from the intestines the larger type (Fig. 1) appeared much thicker than the organisms from cattle, but in culture it was indistinguishable from many of the bovine strains (Figs. 2, 3). In all, twenty strains of vibrios were cultivated from the intestinal tract and one from the spleen of rats. Mice yielded only two strains, one from the cecum and the other from the spleen. Neither resembled the vibrios from the cow.

It has been stated that the rat cultures closely resembled those from the cow in general morphology. The rat vibrios usually have flagella at both ends of the body. In the original stained material from the intestine the larger vibrios measured from 7 to 10μ in length. The number of coils varied from two to four with a distance between coils of 1.5μ . In culture there was a greater variability in length. S forms as short as 1.5μ were not uncommon and undulated forms as long as 9.4μ were noted.

The vibrios possess little pathogenicity for rabbits or guinea pigs since intramuscular or subcutaneous injections of culture or material from the intestinal tract containing vibrios fails to cause marked disturbances. Three actively growing, freshly obtained, strains were fed to a calf 17 days old. The calf failed to show suggestive symptoms and when slaughtered 4 days later, vibrios could not be demonstrated in the normal appearing small intestine.

Inasmuch as the morphology and growth requirements of the rat vibrios resembled those from the cow it seemed advisable to test their immunological relationship by means of agglutination. Sera were prepared from all strains of vibrios obtained from cows. In general it was true that the rat cultures failed to react with such sera. However, cross-agglutination occurred in certain instances as brought out in Table I.

The results given in Table I are selected as the best examples of many tests which might be regarded as indicating immunological relationship between the rat vibrios and those obtained from cattle. The protocols relating to strains from Rat 10 and Rat 11 indicate a partial

TABLE I
The Results of the Cross-Agglutination Tests between Cow and Calf Vibrio and Two Rat Vibrios

Cultures	Serum—Calf Vibrio 1629										Serum—Cow Vibrio B ₄									
	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/70	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	C	
1629 (bovine)	C	C	C	C	C	C	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	-
B ₄ (bovine)	++	+++	+++	+++	++	++	+	-	-	C	C	C	C	C	C	+	+	+	-	-
Rat 11 Intestine 410	+++	+++	++	++	++	++	+	-	-	++	++	++	++	++	++	++	++	++	+	-
Rat 10 Cecum 15	++	++	++	+	+	+	-	-	-	+++	+++	+++	+	+	+	+	+	-	-	-
	Serum—Cow Vibrio 1763										Serum—Rat Vibrio 1									
1763	C	C	C	+++	+++	+++	++	++	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	-
Rat 7	C	C	+++	+++	+++	++	++	-	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+
Rat 1	++	+	+	+	-	-	-	-	-	C	C	C	C	+	+	+	+	+	+	-

antigenic relationship between these and Bovine Cultures 1629 and B₄ but when the cattle vibrio sera were absorbed with the rat cultures there was relatively little loss of titre. There seemed to be greater antigenic similarity between Cow Vibrio 1763 and the Rat 7 organism, especially as Rat 1 vibrio antiserum, which agglutinated all the rat strains, reacted with Vibrio 1763. It was possible to show by absorption of 1763 antiserum with Rat 7 culture that the titre of the serum from the homologous organism was reduced. When Rat 1 vibrio serum was absorbed with Vibrio 1763, relatively little of the antibody was removed. It can be argued then that Vibrio 1763 and Rat 7 possessed certain antigens in common but that the evidence indicated an incomplete antigenic relationship.

On the other hand it is remarkable that all the rat strains isolated from the intestine agglutinated well with two typical rat vibrio antisera.

DISCUSSION

We have shown that the rat carries in the lower ileum and cecum at least two types of vibrios. One of these we have not cultivated, while the other has been grown on culture media and found to resemble in its growth characters and in its morphology the vibrio that is associated with the infectious diarrhea of cattle. Serologically this latter vibrio differs from the cow organism, and in one test we were unable to produce diarrhea or other disturbances by feeding it to a calf. It is of course possible that when transferred from the rat to the cow the serological characters of the vibrio might have been altered, but we have no evidence to support such a notion and our results do not explain the source of the cattle infection.

SUMMARY

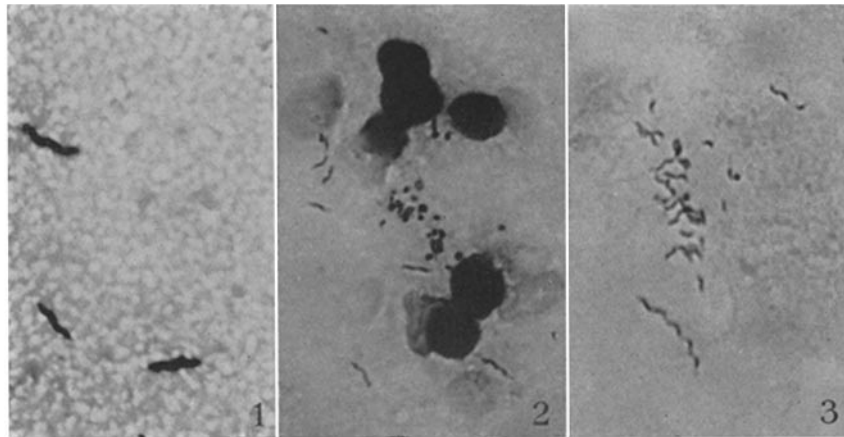
Vibrios obtained from the intestinal tracts of wild rats are described. The organism is a normal inhabitant of the lower ileum and cecum of the rat and may be cultivated from these regions. While these rat organisms are in their growth characters and morphology like those found in an intestinal disease of cattle they differ serologically from the cow organisms.

EXPLANATION OF PLATE 35

FIG. 1. Film from the mucosa of the ileum of a gray rat after prolonged staining with dilute carbol-fuchsin. Thick vibrios with shallow undulations are shown. $\times 1640$.

FIG. 2. Original blood agar culture from the material referred to in Fig. 1. Note the difference in thickness between the forms in Figs. 1 and 2. Fragments of vibrios are illustrated. Giemsa stain. $\times 1640$.

FIG. 3. The same culture after thirty transfers on blood agar. A long undulated form as well as shorter types are shown. Giemsa stain. $\times 1640$.



(Jones *et al.*: Vibrios from intestinal tract of gray rat)