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# Mucin Ineffective in Establishing Salmonella Infections in Mink and Foxes

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PREVIOUS ATTEMPTS to infect mink by feeding virulent Salmonella cultures grown in tryptose phosphate broth (Difco) have not been successful.[1] According to available literature, no satisfactory method has been found to produce a Salmonella infection in foxes by *per os* exposures.

The coating effect of mucin on bacteria, the subsequent inhibition of phagocytosis, and the inhibition of intraphagocytic digestion have been described.[2,3,4,5] Therefore, it was thought desirable to suspend virulent Salmonella in mucin and feed the suspension to mink and foxes. Exposure trials were conducted on healthy and depleted groups.

## MATERIALS AND METHODS

*Bacteria.*—In this investigation, *Salmonella typhimurium* (IV, V, XII...: i- 1, 2, 3...) was selected as the test pathogen. The culture was obtained through the courtesy of Dr. A. H. Kennedy, Ontario Veterinary College. This particular strain was isolated from an outbreak of salmonellosis in mink.*

*Preparation of Mucin.*—Fifty grams of granular mucin (Wilson 1701-W)** were mixed with 1,000 cc. of distilled water and allowed to stand approximately forty-five minutes with occasional stirring. Portions of the mixture were poured into a Waring blendor and agitated for two minutes. This produced a homogeneous, foamy suspension which was then sterilized for twenty minutes at 15 lb. of pressure. The foam disappeared during the heating. The pH of the mucin was adjusted to 7.3 just prior to use.

*Preparation and Suspension of Cultures.*—To increase the virulence of the organism, mice were used without intervening growth on mediums, *i.e.*, mouse-to-mouse passage by heart blood inoculation. When mice were killed in eighteen hours, the Salmonella was considered virulent.† Purity checks on each transfer were made; if contamination was found, the series was not used. To prepare the cultures, heart blood was aseptically withdrawn from moribund mice and inoculated into a flask containing 1,000 cc. of prewarmed tryptose phosphate broth (Difco). The flask was incubated at 37.5 C. for twenty hours. The broth culture was then transferred to large centrifuge tubes in approximately 150-cc. quantities and centrifuged at 2,000 r.p.m. for twenty minutes. Following removal of the supernatant, the organisms in each tube were resuspended in a small quantity of sterile physiologic saline. The combined suspensions from all the tubes totaled approximately 150 cc. This suspension was immediately mixed in 1,000 cc. of the mucin preparation, prewarmed to 37.5 C., and fed to the test animals.

*Isolation of Cultures.*—The procedure used to isolate Salmonella from the feces of the exposed test animals was as follows: One gram of feces was suspended in 10 cc. of Kauffmann's[6] tetrathionate broth and incubated overnight at 37.5 C. On the second day, a plate of Kauffmann's[6] brilliant green agar was streaked from the broth and incubated. On the following day, two representative, translucent pinkish white colonies from the plate were cultured on Kligler slants, followed by culturing in urease and Felsenfeld's sucrose, lactose, and salicin mediums to differentiate Proteus species and paracolons. Slide-agglutination tests with *S. typhimurium* antiserum were conducted on cultures which produced no change in the urease and Felsenfeld mediums. Final serologic confirmation was made through the courtesy of Dr. P. R. Edwards.

*Animals.*—Stock mink and foxes from the Fur Animal Disease Research Laboratory were used. The mink were standard dark and the foxes were Silver Foxes. The feces of the animals selected were cultured and none were

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*The culture was stored on stock culture mediums at −25 C. for approximately a year. It is very possible that Dr. Kennedy also had the culture on artificial mediums for some length of time.

**Obtained from the Wilson Laboratories, Chicago, Ill.

†We have no evidence that virulence for mink and foxes would be raised by passage of the organism through mice. We only assumed this point, although it is conceivable that the passage of the organisms through mice only increased the virulence of the Salmonella for mice and not mink and foxes. If we had sufficient animals at the present time, we could inoculate some mink and foxes intraperitoneally with Salmonella that had been passaged through mice and Salmonella passed on culture mediums to determine the minimum lethal dose of both groups. When we ran the original experiment, we did not consider this particular angle, although admittedly it is an important one. The literature here is rather scanty.

found to be carriers of any Salmonella organisms.

## EXPERIMENTAL

*Experiment 1.*—Nine mink and 6 foxes were used to test the *per os* effect of Salmonella suspension in mucin on healthy mink and foxes.

On Oct. 16, 1948, 9 mink each received approximately 50 cc. of the suspension mixed in their regular ration; similarly, 6 foxes were given approximately 100 cc. each. Feed was withheld on the following day. On October 18, the same animals were given a feeding similar to the preceding. All animals consumed the mixture of feed and mucin promptly.

All of the mink and foxes remained normal after both feedings and have never shown any symptoms suggestive of a Salmonella infection. The feces of each animal were cultured on the day following the last feeding (Oct. 19), and all specimens were found to contain *S. typhimurium*.

*Experiment 2.*—Since the feeding of cultures in mucin failed to cause an infection in healthy mink and foxes, an attempt was made to lower the animals' resistance by halving the daily food intake.

A new group of animals consisting of 9 mink and 6 foxes was selected and placed on the reduced ration on October 21. Each mink received approximately 3 oz. of regular ration a day, and each fox received approximately 6 oz. Feed was withheld every seventh day. Water was available at all times. On November 23, both mink and foxes showed evidence of emaciation and nervousness, and had developed rough, unkempt coats.

On November 24, the 9 mink and 6 foxes were given the Salmonella-mucin suspension. Each mink received approximately 50 cc. of the material in the ration; similarly, each fox was given approximately 100 cc. The animals ate the mixture promptly. A similar feeding was administered on November 26.

No symptoms were recorded in either the mink or foxes. The feces were cultured following the last feeding, and in all cases were found to contain *S. typhimurium*.

## DISCUSSION

It is comparatively simple to expose mink and foxes experimentally with Salmonella organisms *per os* and subsequently recover the organisms from the intestinal contents.

The experimental production of Salmonella infection in mink and foxes appears to be difficult. Previous attempts made at this station to produce infection by feeding cultures of Salmonella and Salmonella-infected carcasses to mink have not been successful.[1]

The use of virulent Salmonella organisms suspended in mucin and fed to normal and depleted mink and foxes offered no advantages over the feeding of virulent cultures alone. It is evident that factors occurring in field outbreaks were not present.

Reports in the fur trade publications have greatly overemphasized the relative importance of salmonellosis in mink. Animals exhibiting a diarrhea are often diagnosed as having a Salmonella infection, apparently without cultural and serologic confirmation. The agents or factors which predispose to or cause a great percentage of the enteritides that commonly occur in mink have been unknown.

Recently, Schofield[7] has demonstrated a virus as being a cause of enteritis in mink in Ontario. Attempts should be made by other investigators in the mink-raising sections of Canada and the United States to determine the presence of this agent.

There have been field outbreaks of salmonellosis in which the etiologic agent was subjected to antigenic analysis and positively identified.[1] The percentage of Salmonella isolation in cases of diarrhea is very low. To illustrate this contention, Momberg-Jorgensen and Sompolinsky in Denmark found only 0.6 per cent infection in approximately 3,000 necropsies on mink.[8] In approximately 1,000 studies on mink at this station, the authors have never isolated a Salmonella species.

In contrast to mink, field outbreaks of salmonellosis in foxes have been frequently reported in North America and Europe. In these cases, the agent has been adequately identified.

## SUMMARY

*Salmonella typhimurium* organisms suspended in mucin and fed to normal and depleted mink and foxes failed to cause an infection.

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