

# Studies on Epidemic Diarrhea of the New-born: Isolation of a Filtrable Agent Causing Diarrhea in Calves\*

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**D**URING the past two years we have had the opportunity of studying six separate epidemics of diarrhea of the new-born occurring in three hospitals in the Baltimore-Washington area. This study has been approached from the standpoint of a possible filtrable agent etiology of the disease.

The first two epidemics occurred almost simultaneously at two Baltimore hospitals in the early fall of 1941. Stools, blood, and nasal washings were obtained from a number of the babies and injected by various routes into a variety of the smaller animals. No definite disease resulted in any of the animals.

The subsequent four epidemics occurred during 1942, two of them in the full-term nursery of a Baltimore hospital and two in the premature nursery of a Washington hospital. All four were characterized by a high morbidity rate; in the last three of these epidemics the mortality was high, while in the first, occurring among full-term babies during the month of March, the mortality was low. Stool cultures were uniformly negative for known diarrhea-

producing organisms. In each of the four epidemics, stool of affected babies was given nasally to a calf. This material was Seitz filtered in three of the instances, unfiltered in the fourth. In all four instances there followed in the calf a bloody, mucoid diarrhea with comparable incubation period and clinical characteristics.

On the first occasion in which this result was obtained in the calf, the question arose as to whether attempt at further serial calf-to-calf passage would be worth while. The calf, like the human infant, is naturally prone to diarrheal episodes from a variety of causes, and the mere occurrence of diarrhea following injection in a calf would not necessarily signify that successful passage had been carried out. However, in view of the presence of blood and mucus in the stools of this first calf, it was hoped that if there were an agent here which could be passaged, the clinical picture in the calf might be distinctive enough to provide fairly reasonable assurance of successful passage. This hope has apparently been realized; successive calf passages were found readily possible with each of the four strains and the results of cross-immunity studies have indicated that the four strains represent a single agent.

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Up to the present time this disease has been produced in a total of 84 calves. In the case of the first strain there have so far been carried out 29 successive passages, 10 of which were with filtered material; with the second strain 4 successive passages, 2 of them filtered; with the third strain 8 successive passages, 6 filtered; and with the fourth strain 2 passages, both filtered.

The majority of the calves used have been of the Guernsey breed, and these were found quite satisfactory. In Holstein calves the disease did not seem quite so typical and one Holstein calf was apparently not susceptible. Aside from this single animal, the calves used were found uniformly susceptible. Ages of the animals ranged from 2 days to 2 months. Most of them were in the 1 to 3 week age period, and in calves older than this the clinical disease tended to be milder.

Passage material consisted of saline suspension of infected calf stools obtained during the acute stage of the disease. The material was frozen when obtained and stored in a dry ice container until ready for use, then thawed at 37° C. and given by simple nasal injection. The minimum dose uniformly successful in passage was 1.0 ml. With Seitz filtrate preparations of this material a larger dose was necessary, the smallest successful dose ever used being 8.0 ml. Material given nasally to the unanesthetized calf is swallowed and it may be that the portal of entry for the agent is actually within the gastrointestinal tract, as the one attempt made at passage through the use of a stomach tube was successfully accomplished.

The clinical disease in these calf-to-calf passages was quite similar to that which followed the injection of material from the babies in the four epidemics mentioned above. The incubation period regularly ranged from 2 to 5 days. The disease itself, though ex-

hibiting a certain amount of variability, was uniformly characterized by the occurrence of diarrhea with the production of mucus, usually in very large amounts. Almost every calf also showed blood in the stools at some time during the course of the disease. As a rule, constitutional reaction was minimal, and aside from mild dehydration and anorexia at the height of the process, the calves would usually not seem particularly ill. Temperatures were taken on a few of the calves and very little fever was found.

The disease proved to be of a peculiarly relapsing nature in the calf, and in rare animals the mucoid diarrhea actually became chronic. In those calves which survived, the total duration of the process varied from 8 to 54 days; in the average case the duration was in the neighborhood of 3 weeks. Most of the animals recovered, the mortality being about 13 per cent. Immunity was found to be present following recovery, large doses of active material then causing no disease.

On gross pathological examination both small and large intestine showed hyperemia, while the intestinal lymphoid tissue was swollen and the mesenteric lymph nodes appeared enlarged. Microscopically, there were found areas of infiltration of the intestinal wall with polymorphonuclear leukocytes and in one very severe case ulcerations of the mucosa, while the intestinal lymphoid tissue and the mesenteric lymph nodes appeared hyper-plastic.

Three attempts were made at passage with the use of the blood of infected calves. The first attempt failed. The other two, made with blood taken from exceptionally sick calves during the latter part of the first week of the disease, were successful, and it is interesting that one of the calves that came down from this material had been inoculated by the subcutaneous rather than the nasal route.

Susceptible calves exposed to infected calves were found to acquire the disease by cross-infection. In a few of the instances the disease thus acquired was clinically very mild, with relatively prolonged incubation period, but in most of the cases the picture produced was quite typical.

In view of the fact that cross-infections could occur, it was obviously necessary to enforce strict isolation of animals used in this study. This was accomplished by isolating calves singly in a number of widely separated areas such as farms where there were no other calves present.

In a consideration of the probable nature of the agent discussed in this paper, one of the first questions that comes up refers to the possibility that it is present in normal infant or calf stools. In this connection, the stools of 4 normal calves were given nasally in large dose respectively to 4 different calves. No disease resulted in these animals. The stools of 8 normal newborn infants were similarly tested, a pool of stools from 2 separate babies being given to each of 4 calves. Again no disease resulted.

As to the properties of the agent, it was found to be readily filtrable through Seitz filters, and cultures of filtrates on various aerobic and anaerobic media have been uniformly negative. The longest series of successive Seitz filtrate passages that was attempted was 4, and this was successfully completed, each calf in the series receiving Seitz filtrate of the stools of the calf that preceded him in the series. In view of the fact that the agent was not found in the stools of normal calves, this would seem to indicate that it is self-perpetuating.

Activity is maintained for at least 2 months when the material is frozen at  $-70^{\circ}$  C. and stored in dry ice. Material which has been dried from the frozen state seems to undergo attenuation, and when used in passage results in the pro-

duction of a very mild disease of short duration, with prolonged incubation period. This very mild disease produces immunity, as a subsequent inoculation with fully active material results in no observable disease. When the stools from this very mild disease are further passaged, 2 serial passages result in the reestablishment of full virulence.

Heat inactivation experiments, carried out by the submerged sealed tubes method, are as yet incomplete. The data so far available seem to indicate that the agent is not regularly inactivated by boiling for 5 minutes, but is regularly inactivated by boiling for 10 minutes. Heating at  $70^{\circ}$  C. for 1 hour apparently fails to inactivate the agent, but heating at  $80^{\circ}$  C. for 1 hour results either in complete inactivation or in attenuation.

Protection tests were carried out with the use of the sera of 6 babies recovered from the epidemic in connection with which the first strain of the agent was obtained. The data on these tests would require discussion too lengthy for the present occasion, but, in brief, it appeared that two of the sera showed complete protection, two showed probable protection, and two showed partial protection. In this connection, with reference to the antigenicity of the agent, a rabbit was given repeated small intravenous injections of Seitz filtrate of infected calf stool. Following this his serum showed protective antibodies. This experiment was controlled by the use of serum from a non-injected litter mate.

The question arises as to whether this disease occurs naturally among calves. The opportunity came up to study two herds of dairy cattle more or less chronically infected with calf scours. In one of these herds a calf was found whose clinical picture was quite similar to that of the calves infected with the agent described above.

The studies which were carried out, however, indicated that the cause of his disease was not this same agent, and at the present time it is impossible to say whether or not this disease occurs naturally among calves.

In this connection, another question comes up which refers to the possibility that this agent might actually be identical with the pneumoenteritis virus, isolated by Baker<sup>1</sup> from a natural disease of calves characterized clinically by pneumonia and diarrhea. Though samples of this virus were not available for experimental comparison, it seems unlikely on the basis of the evidence at hand that the two agents are the same, first because of the absence of

pneumonia in the calves affected by the agent discussed in this paper, and second because of failure with it of transmission to mice even after a number of serial mouse passages.

The conclusions which seem justified from the data presented are:

1. In connection with four separate epidemics of diarrhea of the new-born a filtrable agent has been isolated which regularly produces diarrhea in calves.

2. In the attempts so far made, this agent has not been isolated from the stools of normal infants or normal calves.

3. The evidence suggests, though it is not conclusive, that the agent may be a cause of epidemic diarrhea of the new-born.

#### REFERENCE

1. Baker, J. A. *Cornell Vet.*, 30:202-204, 1942.