ISOLATION FROM CASES OF INFANTILE DIARRHEA OF A FILTRABLE AGENT CAUSING DIARRHEA IN CALVES

BY JACOB S. LIGHT, M.D., AND HORACE L. HODES, M.D.

(From Sydenham Hospital, Baltimore City Health Department, and the Department of Pediatrics of the Johns Hopkins University School of Medicine, Baltimore)

(Received for publication, March 18, 1949)

Many species of bacteria have been shown to have the capacity to produce diarrhea with regularity in animals or man. Until comparatively recently, however, evidence for a possible viral etiology of diseases characterized chiefly by diarrhea has been scanty. Between 1928 and 1939 a number of investigators (1–5) described experiments which indicated that a natural disease of cats, sometimes called infectious panleukopenia, characterized by leukopenia or diarrhea or both, was probably caused by a virus. In 1942 and 1943 Macchiavello and associates (6–8) brought forward convincing experimental evidence that this was actually the case. In 1942 Baker (9, 10) isolated a virus from a natural disease of calves featured by diarrhea, pneumonia, and fever. This agent on passage regularly produced the disease in calves and on intranasal inoculation caused pneumonia in mice. In 1944 Buddingh and Dodd (11, 12) reported the isolation from infants ill with diarrhea and stomatitis of a filtrable agent that caused lesions on the cornea of rabbits, which were reproducible. In 1947 Gordon, Ingraham, and Korns (13) reported the isolation of a filtrable agent from persons suffering from an epidemic form of gastroenteritis. These workers were able to transmit the disease in series to human volunteers by oral administration of filtrates of fecal material and throat washings, but transmission to experimental animals and cultivation on embryonated hen's eggs was not accomplished. In 1948 Cheerer and Mueller (14) and Pappenheimer and Cheerer (15) described a diarrheal disease endemic in suckling mice bred for laboratory purposes. Cytoplasmic inclusions were found in the epithelial cells of the small intestine of the affected animals, and transmission of the disease by bacteria-free extracts of this organ was demonstrated.

In 1943 the present authors (16) briefly described studies carried out during the course of six hospital nursery outbreaks, in Baltimore and Washington, of diarrhea among newly born infants. At that time we reported the isolation of a filtrable agent, in connection with four of these outbreaks, which regularly produced diarrhea in calves. The present paper describes these studies in detail.

In all the outbreaks studied the disease appeared to be limited to infants in the newborn period. Older infants and adult attendants in the nursery had no apparent diarrhea or other symptoms of disease which could be connected with the illness of the newborn infants. In none of the outbreaks studied was stomatitis found to be a feature of the disease. In this way the disease studied by the

* This study was financed in part by a grant from the Evaporated Milk Association.
authors differed from that described by Buddingh and Dodd (11) referred to above.

EXPERIMENTAL

Earlier Outbreaks Studied.—The first two outbreaks studied occurred almost simultaneously in the nurseries for the newborn of two Baltimore hospitals, designated respectively as Hospital A and Hospital B, in the fall of 1941. Morbidity and case fatality rates were high in both instances. Stools, nasopharyngeal washings, and blood were obtained from a number of the babies in both outbreaks. All stools of infants used in these as in succeeding outbreaks, were cultured by the authors and no known diarrhea-producing pathogens were found. The stools were transferred directly from the diaper, by means of sterile tongue blades, to sterile normal saline in roughly a 1:10 suspension. Nasopharyngeal washings were obtained by maintaining suction on a catheter in the nasopharynx while dropping saline very slowly into the nose. Blood specimens were obtained by jugular puncture, and defibrinated. Material was sometimes used as soon as obtained, but more often was frozen at −70°C. and stored in carbon dioxide ice for a few days before use.

This material was inoculated intranasally into mice. Intraperitoneal and intracerebral inoculations were also made, after Seltz filtration of the nasopharyngeal washings and ether treatment (17) of the stools. Similar inoculations were made into hamsters, and in the case of the Hospital B outbreak also into guinea pigs and rabbits. No significant disease resulted in any of these animals.

Later Outbreaks Studied.—The third outbreak studied occurred at one of the hospitals mentioned above, Hospital B, in March, 1942. The morbidity rate in this outbreak was high and the case fatality rate low. Adult mice, hamsters, and cotton rats, a litter of 10-day-old mice, and a litter of 4-day-old kittens were injected as described above, with no definite result. At this time there came to our attention the description of the virus of pneumoenteritis of calves, isolated by Baker (9, 10), and mentioned above. Because of the presence of diarrhea as part of that disease it was decided to inoculate material from infants in this outbreak into a calf.

The infants from whom material was obtained were empirically limited to those who had not received sulfonamides. In this experiment, as in the studies described in the balance of this paper, no fresh material was used for inoculation. All specimens were maintained frozen for a few days (longer as specified) before use. Material obtained from four of the babies at the time they were in the 1st or 2nd days of their disease was pooled after thawing and given to a 3-week-old Guernsey calf as follows: Unfiltered saline stool suspension, 0.5 cc. nasally into one nostril; unfiltered nasal washings, 0.5 cc. into the other nostril; defibrinated blood, 0.25 cc. subcutaneously; Seltz-filtered nasal washings, 1.0 cc. subcutaneously. Two days following inoculation this calf had onset of bloody mucoid diarrhea from which he subsequently recovered (see Table I). The characteristics of this disease, and its transmission through further passages by use of unfiltered or bacteriologically sterile filtered stool given intranasally, will be discussed below. The agent propagated from the use of the stools of this calf is designated as strain I and was carried through twenty-nine successive passages, of which nine were accomplished with bacteriologically sterile filtrates (see Tables II and III).

The fourth outbreak studied took place, again in the nursery for newborn infants of Hospital B, during the latter part of July and the early part of August, 1942. The morbidity rate was high and the case fatality rate moderate. Material was obtained and frozen at the very end of the outbreak, from the baby who was the last to develop the disease, blood being taken on the 5th day of his disease and stool on the 5th and 6th. The stool suspensions, after thawing and pooling, were filtered through a Seltz filter, and 20 cc. of the bacteria-free filtrate was given intranasally to a 3-week-old Guernsey calf. Two days following inoculation this calf had onset of mucoid diarrhea which within a few days became also bloody (see Table I). On the 11th
TABLE I
Summary of Calf Inoculations from Four Hospital Outbreaks of Infantile Diarrhea

<table>
<thead>
<tr>
<th>Nursery outbreak</th>
<th>Date</th>
<th>Material used for calf inoculation</th>
<th>Method of inoculation</th>
<th>Calf inoculated Number, breed, age</th>
<th>Disease in calf</th>
<th>Subsequent passage of agent</th>
<th>Designation of strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Third, Hospital B, Baltimore</td>
<td>March 1942</td>
<td>Pooled nasal washings, blood, and stools from 4 infants, 1st or 2nd day of disease</td>
<td>Unfiltered stool, blood, and nasal washings intranasally</td>
<td>No. 1 Guernsey 3 wks.</td>
<td>Incubation 2 days. Severe diarrhea with much mucus and blood in stools. Recovered in 16 days</td>
<td>29 successive calf passages by intranasal inoculation of infected stool suspension, 9 of these passages by use of Seitz-filtered stool suspensions</td>
<td>I</td>
</tr>
<tr>
<td>Fourth, Hospital B, Baltimore</td>
<td>July</td>
<td>Stools from one infant obtained on 5th and 6th days of disease</td>
<td>Seitz-filtered stool suspension intranasally</td>
<td>No. 4-4 Guernsey 3 wks.</td>
<td>Incubation 2 days. Severe diarrhea with much mucus and blood in stools. Died on 18th day of disease</td>
<td>4 successive calf passages by intranasal inoculation of suspensions of infected stool; 2 of these passages by use of Seitz-filtered stool suspensions</td>
<td>II</td>
</tr>
<tr>
<td>Fifth, Hospital C, Washington, D. C.</td>
<td>August</td>
<td>Blood from one infant obtained on 5th day of disease</td>
<td>Unfiltered blood subcutaneously</td>
<td>No. 4-2 Guernsey 3 wks.</td>
<td>Incubation 3 days. Severe diarrhea with mucus and blood. Recovered in 32 days</td>
<td>8 successive calf passages by intranasal inoculation of suspensions of infected stool; 6 of these passages by use of Seitz-filtered stool suspensions</td>
<td>III</td>
</tr>
<tr>
<td>Sixth, Hospital C, Washington, D. C.</td>
<td>December</td>
<td>Pooled stools of 2 infants on 2nd and 4th days of disease</td>
<td>Seitz-filtered stool suspension intranasally</td>
<td>No. 5-6 Guernsey 2 wks.</td>
<td>Incubation 3 days. Severe diarrhea with mucus and blood. Recovered in 13 days</td>
<td>2 successive calf passages by intranasal inoculation of suspensions of infected stool; both of these passages by use of Seitz-filtered stool suspensions</td>
<td>IV</td>
</tr>
</tbody>
</table>
day of the disease he became prostrated, and on the 15th day died. The agent passaged by the use of his stools is designated as strain II, and was carried through four passages, of which two were with the use of filtered material. To a second calf was given subcutaneously 5.0 cc. de-

**TABLE II**

*Summary of First Six Calf Passages, Strain I*

<table>
<thead>
<tr>
<th>Date of inoculation</th>
<th>No. of passage</th>
<th>Material used for intranasal calf inoculation</th>
<th>Calf inoculated Number, breed, age</th>
<th>Disease in calf</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-25-42</td>
<td>2</td>
<td>1.0 cc. unfiltered suspension of stools of calf 1, which had been used for primary isolation</td>
<td>No. 2 Guernsey 2 wks.</td>
<td>Incubation 2 days. Severe diarrhea with much mucus and some blood. Recovered in 12 days</td>
</tr>
<tr>
<td>3-30-42</td>
<td>3</td>
<td>1.0 cc. unfiltered suspension of stools of calf 2</td>
<td>No. 4 Guernsey 4 wks.</td>
<td>Incubation 2 days. Severe diarrhea with much mucus and little blood. Recovered in 21 days</td>
</tr>
<tr>
<td>4- 6-42</td>
<td>4</td>
<td>20 cc. Berkefeld V filtrate of stool suspension of calf 4</td>
<td>No. 5 Holstein 3 days</td>
<td>Incubation 5 days. Moderate diarrhea with some mucus, no blood. Recovered in 8 days</td>
</tr>
<tr>
<td>4-19-42</td>
<td>5</td>
<td>2.0 cc. unfiltered suspension of stools of calf 5</td>
<td>No. 8 Guernsey 4 days</td>
<td>Incubation 4 days. Very severe diarrhea, with much mucus, much blood, severe constitutional reaction. Sacrificed on 8th day for autopsy purposes</td>
</tr>
<tr>
<td>4-28-42</td>
<td>6</td>
<td>18 cc. Seitz filtrate of stool suspension of calf 8</td>
<td>No. 9 Guernsey 2 wks.</td>
<td>Incubation 3 days. Severe diarrhea with much mucus and little blood. Sacrificed on 9th day of disease</td>
</tr>
<tr>
<td>5- 6-42</td>
<td>7</td>
<td>12.5 cc. Seitz filtrate of stool suspension of calf 9</td>
<td>No. 1-4 Guernsey 4 wks.</td>
<td>Incubation 3 days. Moderate watery diarrhea with mucus and very little blood. Recovered in 14 days</td>
</tr>
</tbody>
</table>

fibrinated blood of the baby mentioned above. This calf remained well, and was later shown to be susceptible to strain II when exposed by pen contact.

The fifth outbreak occurred in the nursery for premature infants of a Washington, D. C. hospital, designated as Hospital C, in August, 1942. Morbidity and case fatality rates were high. Stools were gathered and frozen on two separate occasions within 1 week, from a total of five babies all of whom were in the 2nd day of the disease at the time. Four of these babies were premature, the fifth a full term infant who had apparently acquired the disease by cross-
infection from infected premature infants transferred to the pediatric ward. These specimens, after several days storage in carbon dioxide ice, were thawed and pooled and a bacteriologically sterile Seitz filtrate prepared of which 20 cc. was given intranasally to a 6-day-old Guernsey calf. Five days after injection this calf had onset of a bloody mucoid diarrhea from which he finally recovered (see Table I). The agent propagated from his stools is designated as strain III and underwent eight successive passages, of which six were with the use of filtrates.

**TABLE III**

*Summary of Four Successive Filtrate Passages, Strain I*

<table>
<thead>
<tr>
<th>Date</th>
<th>No. of passage</th>
<th>Material used for intranasal inoculation</th>
<th>Calf inoculated Number, breed, age</th>
<th>Disease in calf</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-28-42</td>
<td>20th</td>
<td>25 cc. Seitz filtrate of stool suspension of calf 5-2, which represented 19th passage with strain I</td>
<td>No. 5-4 Guernsey 15 days</td>
<td>Incubation 3 days. Severe diarrhea with much mucus and no blood. Recovered in 38 days</td>
</tr>
<tr>
<td>12-9-42</td>
<td>21st</td>
<td>20 cc. Seitz filtrate of stool suspension of calf 5-4</td>
<td>No. 5-5 Guernsey 11 days</td>
<td>Incubation 2 days. Severe diarrhea with much mucus and little blood. Recovered in 11 days</td>
</tr>
<tr>
<td>12-17-42</td>
<td>22nd</td>
<td>18 cc. Seitz filtrate of stool suspension of calf 5-5</td>
<td>No. 5-7 Guernsey 4 days</td>
<td>Incubation 5 days. Moderate diarrhea with much mucus and very little blood. Recovered in 10 days</td>
</tr>
<tr>
<td>1-1-43</td>
<td>23rd</td>
<td>18 cc. Seitz filtrate of stool suspension of calf 5-7</td>
<td>No. 6-0 Guernsey 19 days</td>
<td>Incubation 5 days. Severe diarrhea with mucus and little blood. Recovered in 16 days</td>
</tr>
</tbody>
</table>

The sixth outbreak took place also in the nursery for premature infants of Hospital C, in December of 1942, again with high morbidity rate. The case fatality rate, however, was low. Stool specimens were obtained from two infants, one in the 2nd day of the disease and the other in the 4th. This material was treated in the manner described in the case of the fifth epidemic, and 25 cc. of a bacteria-free Seitz filtrate was given intranasally to a 2-week-old Guernsey calf. Three days after inoculation this calf developed bloody mucoid diarrhea, with subsequent recovery (see Table I). The agent passaged from his stools is designated as strain IV and was carried through two passages, both with the use of filtrates.

**Procurement, Isolation, Care, and Feeding of Calves.**—In all, 101 calves were obtained for use in this study. All except 34 were males. Of the total number, five became ill of diarrhea and two of other causes during preliminary isolation and were discarded, while four were employed solely in experiments which did not involve the use of infectious material. Of the remaining 90 animals, 89 were infected with the agent in question during the course of the experiment. Twelve of these were of the Holstein breed, 5 Jersey, 5 Ayrshire, and 1 Hereford. The remainder, numbering 66, were Guernseys. The age range at the time of injection was from...
2 days to 2 months, the majority being between 7 and 21 days old (see Table IV). The limits
of the weight range were from 65 to 142 pounds, the great majority falling into the 80 to 100
pound group.

Guernsey calves were the most easily obtainable in the vicinity of Baltimore, and they
proved to be quite satisfactory for purposes of the present study. The disease produced in the
5 Jerseys and 1 Hereford was comparable to that of the Guernseys, but in the Ayrshires, though
typical, it was of less severity and shorter duration. In Holstein calves the manifestations did
not seem quite so typical as in the other breeds, and 1 Holstein calf was apparently insuscepti-
ble. Aside from this single animal the calves used were found uniformly susceptible.

During the earliest of the experiments the animals were obtained from a slaughter-house,
so that their age could only be estimated and their previous history was unknown. These
calves, except for the first three used in experiment with strain I, were maintained in isolation
for at least 5 days before use, so that those showing looseness of the stools or respiratory in-
fection or other apparent abnormality could be discarded. Because of the obvious disadvan-
tages entailed, this source of supply was discontinued in favor of animals obtained directly
from dairy farms, and was resorted to thereafter only occasionally, when the latter source
failed. It was early found unwise to obtain calves from large herds, where many calves were
raised, as calf scours (natural calf diarrhea) was found to be prevalent among such herds and
three such animals developed diarrhea while in the preliminary period of isolation. Though the
picture in these calves differed from that of the disease studied, in that mucus and blood were
not present in the stools, it was still considered advisable to discard such animals. The best
source of supply, and that most commonly employed, was found to be a group of small dairy
farms where very few or none of the animals were raised and scours could get no foothold in
the herd (calves used for initial isolation of strains II, III, and IV were from this source).
These herds were kept under frequent personal observation for freedom from scours, and calves
from them were found to be quite satisfactory. They were allowed to suckle the dam for at
least 2 days following birth, and were then given raw milk until they were removed by us from
the herd. The majority were thus removed at the age of 3 to 6 days, and were then given a pre-
liminary period of isolation before use, though occasionally such a calf was inoculated at once,
sometimes purposely from the standpoint of observing the disease in the calf at an early age.

Isolation was accomplished by placing calves individually in widely separated areas, mostly
farms, where no other calves were present. Such isolation was maintained throughout the
course of the disease in the case of some of the animals, while in other cases, following the
acute period of the disease, calves infected with the same strain of the agent were allowed to
convalesce together. Following removal of an infected calf, the area employed was cleaned up
as thoroughly as the particular local conditions would allow, then left idle for at least 1 week.
Following this interval, such locations were apparently not infectious, as demonstrated by 18
instances in which calves were left isolated in such areas for periods varying from 7 to 18 days
without showing symptoms. All these calves were later proved to be susceptible, either by in-
jection with active material or on exposure to an infected calf.

In the immediate surroundings of a calf adequate warmth, avoidance of moisture, and main-
tenance of cleanliness were, as expected, found essential. Young calves commonly nibble at
their bedding, and since the ingestion of shavings or straw seemed sometimes to lead to loose-
ess of the stools, hay was employed for this purpose, timothy being found the most satis-
factory. Loose or watery stools passed into the bedding are often lost to observation; for this
reason just enough hay was placed in one corner of the room to allow bedding down of the calf,
while the remainder of the floor was left bare. Concrete floors were found to be the best for
this purpose, providing for the most accurate observation of the number and characteristics
of the stools. A pail of tap water was left available to each calf, and the water changed daily.

Because of the difficulties involved in the distribution and refrigeration of fresh milk, ir-
radiated evaporated milk, in 13 ounce cans, was used in feeding, and proved to be satisfactory.
To each can of milk was added 1 to 2 volumes of water (depending on the appetite of the calf) which had previously been heated sufficiently to give a resulting warm mixture. Two feedings of equal size were given daily, the first at about 9:00 a.m. and the second at about 6:00 p.m. A somewhat low caloric intake was employed, a total of 39 ounces of evaporated milk daily being given under the age of 1 week, and 52 ounces subsequent to that. The latter feeding was usually not increased till convalescence. Feeding milk by means of an ordinary bucket was found to present difficulties for the calf, so that "calf feeding buckets" with attached nipples were employed until late in convalescence.

Method of Passage of Infectious Agent.—Material for passage consisted of saline suspension of infected calf stool, in roughly a 30 per cent dilution, obtained on at least 2 of the first 5 days of the disease, always including the 1st or the 2nd day or both. Freshly passed specimens were caught in a flask, either on standing by while the calf was fed, at which time defecation is likely to occur, or by stimulating the lower rectum by means of the gloved finger or a glass rod. In case of failure by these methods, the freshest stool seen was taken from the floor. Each specimen was divided into several containers, frozen at \(-70^\circ\text{C.}\), and stored in carbon dioxide ice until ready for use, at which time one container of each specimen from a given calf was thawed at \(37^\circ\text{C.}\) and these pooled and shaken up briefly before use. In no instance was fresh, non-frozen material used for passage. Occasionally material was used after two alternate freezings and thawsings, and activity was found to be maintained.

Inoculations were made by the nasal route without anesthesia. The animals were either thrown down or immobilized in the standing position, the head held up, and the material quickly injected into the nose with a syringe the barrel of which blocked the nostril so that there could be no important loss due to sneezing or blowing. Unless the injections were begun during inspiration, there was no significant aspiration or cough and the material was readily swallowed. Subsequent respiratory symptoms were limited to occasional mild nasal discharge for 1 or 2 days after injection. That the portal of entry for the agent is probably the gastrointestinal rather than the respiratory tract was suggested by the successful accomplishment of the one stomach tube passage that was attempted. Injections were usually made on an empty stomach and several hours before the time for the next feeding, although the two calves inoculated immediately after feeding developed typical disease.

Because of the possibility that Seitz filtration of infected material might reduce the concentration of agent to a point below that necessary to cause disease, all experiments in which filtered material was used were carried out with stool suspensions frozen no more than 15 days. This time limit was not observed when unfiltered stool suspension was employed since it was shown that such material remained infectious after 3 months in the carbon dioxide ice box. The dose employed with unfiltered saline calf stool suspension varied somewhat according to the age of the material. With material frozen for less than 15 days the dose usually ranged from 1.0 to 10 cc. (10 calves) but was sometimes larger (5 calves were given 15 to 30 cc.); with material frozen more than 15 days but less than 1 month the dose was 2.0 to 30 cc. (4 calves); and with material frozen 1 to 3 months, 10 to 30 cc. (11 calves). All attempted passages with such material were successful, with the single exception of the one Holstein calf mentioned above which is not included in this tabulation.

In certain instances material frozen not more than 15 days was shaken up well, following thawing and pooling, and then spun in the horizontal centrifuge for 30 minutes at approximately 3000 R.P.M., the supernatant being then pipetted off and used for inoculation. Fifteen attempts were made in this manner with doses ranging from 1.5 up to 5.0 cc., except in one instance in which 40 cc. was given. All these animals developed typical diarrheal disease. In one experiment only 0.15 cc. of such supernatant, diluted to 1.5 cc. with saline, was given to a calf and failed to bring about infection. This calf was later proven fully susceptible, by cross-infection, on exposure to the disease.

In the filtration experiments the specimens were first shaken up thoroughly and centri-
fuged (and if necessary recenrifuged) in an angle centrifuge at 3600 r.p.m. for periods ranging from 1 to 4 hours before filtration was attempted. The first two successful filtrate passages were carried out with Berkefeld V filtrates. The remainder were with Seitz filtrates, discs of size 3 being used. All filtrates were cultured on a blood agar plate and in two tubes of blood broth, one of which was layered with vaseline. In six instances cultures were also made in cooked meat, litmus milk, Brewer's medium, and a poured blood agar plate incubated in an anaerobic jar. With the exception of a rare contaminant limited to a single medium, all cultures were bacteriologically sterile. Of twenty-two attempts at passage with the use of filtrates, nineteen were successful. All calves used in the successful filtrate passages were under the age of 1 month at the time of inoculation. The disease produced by inoculation with filtrates differed in no way from that obtained with the use of unfiltered material. At least two filtrate passages were carried out with each of the four strains. Of the nineteen such passages carried out, two were in duplicate, so that 21 calves were infected in this manner. The doses ranged from 12.5 to 40 cc., the average dose being 25 cc. The longest series of successive Seitz filtrate passages ever attempted was four, each calf in the series receiving filtrate of the stools of the calf preceding him in the series. Each of these passages was successful.

Of the three unsuccessful attempts at filtrate passage, the first was in the Holstein calf described above as probably susceptible. This animal was given 10 cc. of a Berkefeld V filtrate, with no apparent response; on later test with active unfiltered material it remained well. The second was in a calf given 7.0 cc. of Seitz filtrate; when later exposed to cross-infection it developed the "modified" type of the disease (see below). It is possible that the failure in this latter animal may have been due to inadequate dosage. The third unsuccessful attempt was in the oldest calf ever used in an attempt at filtrate passage, 5 weeks, the dose being 23 cc. of Seitz filtrate; when later tested with unfiltered material this calf developed the typical diarrheal disease in mild fashion.

Three attempts, each with a different strain of the agent, were made at passage with material which had been dried from the frozen state. Material selected for drying consisted of the supernatant withdrawn after 30 minutes' centrifugation in the horizontal centrifuge at 3000 r.p.m. The period between the time of drying and the time of use was 25 days in one case, 5 months in the second, and 6½ months in the third. The dose given was 6.0 cc. in two of the instances, 12 cc. in the third. There resulted uniformly in the inoculated animals "modified" disease, with prolonged incubation period and quite mild diarrhea. Further serial passages, as described below, resulted in eventual production of the typical disease.

Attempts to Produce Diarrhea by Inoculation of Blood of Infected Calves.—Three attempts were made to produce the disease by the use of defibrinated blood of infected calves. In the first attempt the blood was taken from a prostrated animal severely ill with diarrhea, on the 6th day of disease, about 12 hours before its death. Five cc. was given intranasally and 2.0 cc. subcutaneously to a single calf. The result of this experiment was not clear. The test animal was exposed to a heavy, cold rain on the day following inoculation. Next day, bloody mucoid diarrhea was present, but at this time there was manifest evidence of pneumonia which proved fatal within 48 hours.

The second attempt was made with a pool of blood obtained from two calves, one of which was in the 1st day of the disease and one in the 2nd day. Ten cc. of blood from the pool was inoculated intranasally into one calf and the same amount subcutaneously into another. The result of this experiment was not clear. The test animal was exposed to a heavy, cold rain on the day following inoculation. Next day, bloody mucoid diarrhea was present, but at this time there was manifest evidence of pneumonia which proved fatal within 48 hours.

The second attempt was made with a pool of blood obtained from two calves, one of which was in the 1st day of the disease and one in the 2nd day. Ten cc. of blood from the pool was inoculated intranasally into one calf and the same amount subcutaneously into another. Both remained well for 5 days following inoculation. At this time, employing the same two calves as test animals, this procedure was repeated using specimens of blood obtained from two calves in the 4th day of the disease. Both these latter animals were ill with severe diarrhea, and one eventually succumbed. Two days later both of the inoculated animals developed the typical diarrheal disease, and one subsequently died. All blood specimens used for inoculation in the above experiments were found to be sterile on aerobic and anaerobic culture.
Features of the Disease in the Calf

The following data on the obvious features of the disease produced in the calf by inoculation with infected stool are based on the 67 animals which were given fully active material. Of these 67 animals, 21 were given Seitz or Berkefeld-filtered material, and 46 unfiltered material. Forty-four were infected with strain I, 2 with strain II, 19 with strain III, and 2 with strain IV. No distinction as to symptoms is made between the strains since the picture was the same with each and since they were found to cross-immunize (see below). The disease produced in these calf-to-calf passages was quite comparable to that initially obtained in the four calves given human material and described above, and those four calves are included in the group of 67.

The incubation period ranged between 2 and 5 days (see Table IV). The picture itself showed considerable variability in severity and manifestations, although all the calves displayed in common diarrhea with production of mucus, usually in very large amounts, and in addition almost all of them showed some blood at some time during the course of the disease. The mucus appeared most often in the form of numerous long ropes and strands, but also as masses, blobs, discs, fibrinous sheets, and even as several inch long casts of the intestinal lumen. It was usually seen at the onset of the disease and was rarely delayed in appearance for more than 24 hours after the onset. Microscopically these stools usually showed a definite increase in the number of white blood cells, but no actual pus. Blood, when present, was in the form of irregular spots or streaks, clotted and on the surface of the mucus, but almost never in the in-

<table>
<thead>
<tr>
<th>Incubation period</th>
<th>Passages with unfiltered material</th>
<th>Passages with filtered material</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of calves in each age period</td>
<td>No. of calves in each age period</td>
</tr>
<tr>
<td></td>
<td>2-6 days</td>
<td>7-14 days</td>
</tr>
<tr>
<td>2 days</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>3 days</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>4 days</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>5 days</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Total calves</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Average incubation</td>
<td>4.5</td>
<td>3.5</td>
</tr>
</tbody>
</table>
mate jelly-like relation seen in human dysentery. Red blood cells were sometimes seen microscopically when no gross blood was present.

The color of the stools was most commonly yellow, sometimes white, but varied to a certain extent from animal to animal and even in the same animal at different times, so that brown, gray, or light green stools were not unusual. Rarely the normal dark green color would be retained throughout the course of the disease, even though the stools were very loose. Watery stools were common, but sufficient stickiness and sediment remained to give them some minimal consistency.

Constitutional symptoms were usually minor and restricted to slight anorexia and mild dehydration during the first few days of the illness, although an occasional calf became prostrated. Daily temperature readings were taken on seven of the calves, and rises to a level of between 103.2 and 104.2°F. commonly found at some time during the first 3 days of the disease. These rises usually coincided with an appearance of dehydration, and as the normal upper limit was considered to be in the neighborhood of 103.4°F, they represented little in the way of fever. Tenesmus and distension were occasional symptoms. In general, the older or larger the calf, the less severe was the diarrhea.

The disease proved to be a peculiarly relapsing one, the relapse constituting an almost universal feature. Commonly it followed by a few days what seemed at first to be significant improvement. In the milder cases it was usually evidenced only by the passing of a small amount of mucus, with or without a blood streak, on one of the stools, while in the severe cases it often approached the initial acute stage in intensity and duration. The occurrence of more than one relapse was seen several times. The total duration of the disease, from onset until the stools became and remained normal both in color and consistency, varied from 8 to 54 days, although in the average case it was from 17 to 21 days. No special treatment was given aside from the administration of an additional supply of water, and rarely saline, by mouth. Most of the calves recovered and could then be successfully raised, though they were usually thin and rather debilitated for a short time following recovery. The over-all case fatality rate was approximately 13 per cent, all the deaths occurring in animals which were less than a month old at the time of inoculation.

Animals fell roughly into several groups so far as the nature of the stools and severity of the diarrhea were concerned. In six calves the diarrhea was very severe, with very large amounts of mucus and blood, and all six died of diarrhea and prostration in the 1st or 2nd week of the disease. In thirty-two calves the diarrhea was moderately severe, stools numbering in the neighborhood of six to ten a day for a week or more, with large amounts of mucus and variable amounts of blood (see Fig. 1 for an example of a calf in this group). In six of these 32 calves the course of the disease was protracted; two animals in this category died, one from marasmus and one from complicating pneumonia.
In twenty-one animals the disease was comparable to the foregoing group except that the diarrhea was moderate, of shorter duration, and characterized by the production of smaller quantities of blood and mucus in the stools. In five others the diarrhea was quite mild, stools not exceeding five per 24 hours, mucus and blood production minor. None of these animals succumbed. Finally, in the case of three animals, of which two were Holsteins, the diarrhea was watery with occasional appearance of small fibrous disc-like sheets of bloody mucus. One of these three died of marasmus consequent on chronic diarrhea. The various types of disease were not clearly defined, readily merging into one another;

![Graph showing temperature, blood, mucus, and diarrhea over days](image)

Fig. 1. Result of experiment with calf 100, which was given an inoculation of normal calf stool without resulting disease. A second inoculation, of infected calf stool, resulted in typical diarrheal disease with the production of blood and mucus. The dotted horizontal line represents temperature of 103.4°F., taken as upper limit of normal for the calf. NCS indicates normal calf stool; ICS, infected calf stool.

sometimes the type would change during the course of the disease in a given calf.

At least one stool culture was carried out during the acute stage of the disease in the case of each calf. These were streaked on plates of eosin-methylene blue, desoxycholate, and desoxycholate-citrate media. Some of these cultures were examined by Miss Helen Zepp at the Johns Hopkins Hospital, others by Mrs. Alice Meyers at Sydenham Hospital, and others by the authors. All were found negative for known diarrhea-producing bacteria. Single blood cultures were taken from eight calves, all of whom were suffering from the severer types of the disease, at times ranging from the 1st to the 10th day after onset of illness. One of these yielded *Staphylococcus albus*, which may have represented a skin contaminant, while the remainder were negative. We are indebted to Dr. E. L. Burky for testing the sera of three calves, 2 months after recovery, for agglutinins against *B. abortus* and *B. suis*. These were found to be negative. The same three animals were also skin-tested with brucellin (*suis*) and these tests too were negative.
The three calves which had been given material which had been dried from the frozen state developed a "modified" form of the disease. After an incubation period of 7 to 8 days there was onset of very mild diarrhea, characterized by softness rather than looseness of the stools, with little or no increase in stool frequency. There was minimal production of mucus and in two of the calves small quantities of blood were noted. The total duration of the illness ranged from 2 to 5 days, and no relapse occurred. Following recovery all three calves were given large doses of fully infectious stool suspension and proved to be immune. Suspensions of the stools from two of the animals exhibiting this very mild disease induced by dried material were further passaged, unfiltered, in doses of 30 cc., one calf being used for each passage. In each of the inoculated calves the typical diarrheal disease of mild nature occurred following an incubation period of 4 days in one case and 6 days in the other. Passage of Seitz filtrate of the stools of each of these latter calves to a respective third calf then gave rise to the typical disease with full virulence in each case. In one of these, further passages were carried out and the disease produced remained quite typical.

Cross-Infections.—A total of eleven calves, all under the age of 1 month, were exposed to cross-infection. Seven of these were exposed to animals in the early acute stage of the disease, four to animals in a relapse. In one instance exposure was by direct contact within the same pen; in eight instances by contact in an adjoining pen, or a non-communicating pen or room some yards away in the same area, the attendant being allowed to go back and forth as necessary with only simple precautions aside from the fact that individual feeding pails were used. In the remaining two instances indirect contact was effected by means of placing the calf in the same pen in an area from which an infected calf had been removed and no cleanup enforced; in one of these instances the infected calf had been removed an hour before, in the other 3 days before.

All eleven exposed calves developed the disease. Of these eleven cross-infections seven were typical in every way except that the incubation periods ranged from 3 to 6 days rather than from 2 to 5. In the remaining four animals the incubation period was 7 to 8 days and the disease itself was very mild and of short duration, comparable to the modified disease obtained by inoculation of material which had been dried (see above). This modified disease was found to confer immunity, as later injection with fully infectious material was carried out in two of the calves and caused no illness. Analysis of the data did not allow of any definite conclusion as to the factors operative in leading to the production of the modified, rather than the full-blown disease, in these four animals. No cross-infections occurred among a group of thirty very young calves who were exposed by a farmer to thirteen recovered calves, all of whom had been well for at least 1 month.
Pathology in the Calf.—

Complete autopsy was carried out on one calf which died on the 7th day of the disease, and on four additional calves which were sacrificed for this purpose at periods varying from the 1st to the 7th day of the disease. Blocks were cut, in each case, at various levels of the gastrointestinal tract, as well as of heart, lung, liver, spleen, kidney, and mesenteric lymph node, in two cases also of the brain, and in one case of the gall bladder. These were fixed in formalin, and the sections stained with hematoxylin and eosin.¹

The animal killed on the 1st day of the disease showed only patchy hyperemia of the mucosa of both small and large intestine, without demonstrable microscopic change. In the later cases the hyperemia became extensive and largely confluent and was accompanied by obvious edema of the intestinal wall as well as by swelling of Peyer's patches, which became raised from the surface of the mucosa and took on a "tigroid" appearance. The summits of the folds in the large intestine were commonly so hyperemic as to appear hemorrhagic, while mucus was found both in the small and large intestine. The mesenteric lymph nodes appeared enlarged in three of these four calves.

Microscopically, in the large intestine, polymorphonuclear cells were seen at the bases of the glands and infiltrating the intestinal wall. In one case these were seen at the glands in the small intestine also, and in another case were found infiltrating the submucosal lymphoid tissue. Definite ulcerations of the large intestinal mucosa were found in one calf. No inclusion bodies were seen. Sections of mesenteric lymph nodes showed varying degrees of hyperplasia.

The animal which died on the 7th day of the disease had been prostrated and was unable to get up off the floor for 2 days prior to death. The lung lappets gave the gross appearance of partial atelectasis, and the sections through them revealed bronchopneumonia. Another calf showed some microscopic evidence of purulent bronchitis. In the case of one animal, which had been severely ill and prostrated before being sacrificed, a few small subacute abscesses were found in the kidney cortex.

Immunity in the Calf Following Recovery.—Six animals, following recovery, while in the age period from 4 to 7 weeks, were tested for immunity by the nasal administration of a large dose of unfiltered material, proven infectious, of the same strain as that with which they had been originally infected. Two additional animals were tested in the same way, with the use of stool suspensions of infected calves, except that the infectiousness of the material was not tested by inoculations into non-immune animals. Following the test injection each animal was carefully watched for a period of at least 10 days, and all eight remained well (see Table V).

Cross-immunity studies among the four strains were carried out in a similar

¹ For preparation and interpretation of the microscopic sections we are indebted to Dr. Richard H. Follis, of the Pathology Department of the Johns Hopkins Hospital.
<table>
<thead>
<tr>
<th>Calf No.</th>
<th>Date and age at first inoculation</th>
<th>Material used for first inoculation</th>
<th>Method of inoculation</th>
<th>Result</th>
<th>Date of challenge inoculation</th>
<th>Material used for challenge inoculation, given intranasally</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3-17-42 3 wks.</td>
<td>Pooled nasal washings, blood, and stools from 4 infants of 3rd epidemic, on 1st or 2nd day of disease. Origin of strain I</td>
<td>Unfiltered stool, blood, and nasal washings intranasally. Blood and filtered nasal washings subcutaneously</td>
<td>Incubation 2 days. Severe diarrhea with blood and mucus. Recovered in 16 days</td>
<td>4-5-42</td>
<td>Strain I 1.0 cc. unfiltered stool suspension from call 6, which represented a later strain I passage (see Table II)</td>
<td>Remained well. Observed 10 days</td>
</tr>
<tr>
<td>2-0</td>
<td>5-20-42 2 wks.</td>
<td>Strain I 1.5 cc. supernatant of unfiltered suspension of infected calf stool</td>
<td>Intranasally</td>
<td>Incubation 2 days. Severe diarrhea with much mucus and little blood. Recovered in 16 days</td>
<td>6-7-42</td>
<td>Strain I 3 cc. unfiltered suspension of infected calf stool</td>
<td>Remained well. Observed 2 mos.</td>
</tr>
<tr>
<td>2-9</td>
<td>6-17-42 4 wks.</td>
<td>Strain I 2.0 cc. supernatant of unfiltered suspension of infected calf stool</td>
<td>Intranasally</td>
<td>Incubation 5 days. Mild diarrhea with mucus. Recovered in 6 days</td>
<td>6-29-42</td>
<td>Strain I 3 cc. unfiltered suspension of infected calf stool</td>
<td>Remained well. Observed 5 wks.</td>
</tr>
<tr>
<td>3-0</td>
<td>6-16-42 1 wk.</td>
<td>Strain I 20 cc. unfiltered suspension of infected calf stool</td>
<td>Intranasally</td>
<td>Incubation 2 days. Severe diarrhea with much mucus. Recovered in 9 days</td>
<td>6-28-42</td>
<td>Strain I 3 cc. unfiltered suspension of infected calf stool</td>
<td>Remained well. Observed 2 wks.</td>
</tr>
<tr>
<td>3-7</td>
<td>7-12-42 2½ wks.</td>
<td>Strain I 1.75 cc. supernatant of unfiltered suspension of infected calf stool</td>
<td>Intranasally</td>
<td>Incubation 3 days. Severe diarrhea with much mucus and little blood. Recovered in 12 days</td>
<td>7-29-42</td>
<td>Strain I 10 cc. unfiltered suspension of infected calf stool</td>
<td>Remained well. Observed 10 days</td>
</tr>
<tr>
<td>6-7</td>
<td>3-1-43 3 wks.</td>
<td>Strain I 6 cc. unfiltered suspension of infected calf stool</td>
<td>Stomach tube</td>
<td>Incubation 5 days. Moderate diarrhea with mucus and blood. Recovered in 13 days</td>
<td>3-27-43</td>
<td>Strain I 30 cc. unfiltered suspension of infected calf stool</td>
<td>Remained well. Observed 2½ wks.</td>
</tr>
<tr>
<td>7-2</td>
<td>3-11-43 1½ wks.</td>
<td>Strain I 27 cc. Seitz filtrate of infected calf stool suspension</td>
<td>Intranasally</td>
<td>Incubation 3 days. Mild diarrhea with mucus. Recovered in 7 days</td>
<td>3-24-43</td>
<td>Strain I 30 cc. unfiltered suspension of infected calf stool</td>
<td>Remained well. Observed 5 wks.</td>
</tr>
<tr>
<td>7-8</td>
<td>4-0-43 1 wk.</td>
<td>Strain III 27 cc. Seitz filtrate of infected calf stool suspension</td>
<td>Intranasally</td>
<td>Incubation 3 days. Severe diarrhea with much mucus and little blood. Recovered in 16 days</td>
<td>5-2-43</td>
<td>Strain III 10 cc. supernatant of unfiltered suspension of infected calf stool</td>
<td>Remained well. Observed 5 wks.</td>
</tr>
</tbody>
</table>
manner except that some of the animals were given filtrates rather than unfiltered material (see Table VI). Such tests were rechecked later (6 to 10 days after the inoculation of filtrate) by the administration of unfiltered material, on the chance that the dose contained in the filtrate might have been too small for the purpose. Two different calves recovered from infection with strain I were tested for immunity against each of the other three strains, making six calves in all. All appeared to be immune. In addition, three calves recovered from infection with strain II, five recovered from strain III, and two recovered from strain IV were tested for immunity against strain I. All these animals remained well, with the exception of one of the calves recovered from strain IV, which on the 5th day following injection developed a moderate form of the disease.

Results of Inoculation of Calves with Normal Infant or Calf Stool

The question naturally arose as to whether the agent discussed in this paper might represent a normal constituent of infant or of calf stool. With this question in mind, stools were gathered from eight normal newborn infants in the nurseries of the Johns Hopkins Hospital, ranging in age from 2 to 20 days. These specimens were made up in saline suspension in the manner described and pooled in groups of two, one member of each group being stool from a breast-fed baby and one from an artificially fed baby. The pools were maintained frozen for several days, then each was given nasally to a calf, unfiltered, in a dose of 5.0 cc. The four animals receiving this material remained well. Three of them were later tested for susceptibility by the administration of infectious material; all developed the typical diarrheal disease. The stools of five normal calves ranging in age from 11 days to 1 month were tested in similar fashion, 30 cc. of each, unfiltered, being given to one calf (one test animal was used twice), again with negative results. Three of these four animals were later given stool suspensions from infected calves, and proved to be fully susceptible (see Table VII).

Results of Attempts to Inactivate the Agent with Heat

For heat inactivation tests Seitz filtrates were prepared and divided into sealed pyrex glass containers in amounts not exceeding 8.0 cc. to the container, then submerged in water baths at the desired temperature. One minute was empirically allowed for the material to reach the given temperature before time was counted. This procedure was carried out in all tests except one in which the material was boiled for 5 minutes directly over a flame in an open Erlenmeyer flask. One animal was used for each category of material at a test. All heat inactivation tests were controlled with the same batch of pooled filtrate as the test material, and this was allowed to stand at room temperature during the time the test material was being subjected to heat. Seven control animals were employed in all. Each developed typical diarrheal disease.

The calf which received the material that had been boiled directly over a flame for 5 minutes remained well. When later injected with infectious material this animal displayed no immunity, developing typical disease. One experiment was carried out by submerging filtrate in sealed containers in boiling water in a water bath for 5 minutes. The agent was apparently not inactivated; the calf receiving this material developed typical diarrheal disease and an injection with infectious material following recovery showed him to be immune.

Three experiments were carried out with stool filtrate in sealed ampoules submerged in boiling water for 10 minutes, and this apparently inactivated the agent, since the three test calves remained well and when later given infectious material showed typical disease. Two experiments were made with filtrate maintained at 70°C. for an hour; this heating apparently
**TABLE VI**

Summary of Typical Experiments Illustrating Cross-Immunity Following Infection with the Four Strains of Filtrable Agent

<table>
<thead>
<tr>
<th>Calf number and age</th>
<th>Date of initial inoculation</th>
<th>Material used in initial intranasal inoculation</th>
<th>Result following initial inoculation</th>
<th>Date of challenge inoculation</th>
<th>Material used in challenge intranasal inoculation</th>
<th>Result following challenge inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-8 1 wk.</td>
<td>2-19-43</td>
<td>Strain I 20 cc. filtrate of suspension of infected calf stool</td>
<td>Incubation 3 days. Moderate diarrhea with mucus and little blood. Recovered in 13 days</td>
<td>1- 8-43</td>
<td>Strain II 30 cc. unfiltered suspension of infected calf stool</td>
<td>Remained well. Observed 3 wks.</td>
</tr>
<tr>
<td>8-4 3 days</td>
<td>5-6-43</td>
<td>Strain I 30 cc. unfiltered suspension of infected calf stool</td>
<td>Incubation 4 days. Severe diarrhea with much mucus and no blood. Recovered in 15 days</td>
<td>6- 2-43</td>
<td>Strain III 30 cc. unfiltered suspension of infected calf stool</td>
<td>Remained well. Observed 2 wks.</td>
</tr>
<tr>
<td>5-5 1½ wks.</td>
<td>12-9-42</td>
<td>Strain I 20 cc. filtrate of suspension of infected calf stool</td>
<td>Incubation 2 days. Severe diarrhea with much mucus and little blood. Recovered in 11 days</td>
<td>12-24-42</td>
<td>Strain IV 10 cc. unfiltered suspension of infected calf stool</td>
<td>Remained well. Observed 9 days</td>
</tr>
<tr>
<td>6-5 1½ wks.</td>
<td>2-9-43</td>
<td>Strain II 30 cc. filtrate of suspension of infected calf stool</td>
<td>Incubation 3 days. Moderate diarrhea with mucus and no blood. Recovered in 13 days</td>
<td>2-27-43</td>
<td>Strain I 6 cc. unfiltered suspension of infected calf stool</td>
<td>Remained well. Observed 2 wks.</td>
</tr>
<tr>
<td>4-6 6 days</td>
<td>8-20-42</td>
<td>Strain III 30 cc. filtrate of pooled stools of 5 infants of 5th epidemic, origin of strain III</td>
<td>Incubation 5 days. Severe diarrhea with much mucus and some blood. Recovered in 19 days</td>
<td>10-7-42</td>
<td>Strain I 10 cc. unfiltered suspension of infected calf stool</td>
<td>Remained well. Observed 2 wks.</td>
</tr>
<tr>
<td>3-6 1½ wks.</td>
<td>12-12-42</td>
<td>Strain IV 25 cc. filtrate of pooled stools of 2 infants of 6th epidemic, origin of Strain IV</td>
<td>Incubation 3 days. Severe diarrhea with much mucus and blood. Recovered in 13 days</td>
<td>12-30-42</td>
<td>Strain I 25 cc. filtrate of suspension of infected calf stool</td>
<td>Remained well*</td>
</tr>
<tr>
<td>5-9 1 wk.</td>
<td>12-20-42</td>
<td>Strain IV 24 cc. filtrate of suspension of infected calf stool</td>
<td>Incubation 4 days. Moderate diarrhea with much mucus and no blood. Recovered in 10 days</td>
<td>1-3-43</td>
<td>Strain I 30 cc. unfiltered suspension of infected calf stool</td>
<td>Remained well. Observed 1½ mos.</td>
</tr>
</tbody>
</table>

* A second challenge inoculation was given on 1-5-43, by use of 30 cc. of unfiltered suspension of infected calf stool (strain I). After an incubation period of 5 days, there occurred mild diarrhea with mucus and no blood, of 7 days' duration. The characteristics were those of a new attack of the disease. This was the only instance in 16 tests in which an animal recovered from disease with one of the strains succumbed to infection from another.
### TABLE VII

<table>
<thead>
<tr>
<th>Calf No.</th>
<th>Age at first inoculation</th>
<th>Material used in first inoculation</th>
<th>Result</th>
<th>Age at second inoculation</th>
<th>Material used in second inoculation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-9</td>
<td>18 days</td>
<td>30 cc. unfiltered stool suspension of a normal 1 mo. old calf</td>
<td>Remained well</td>
<td>27 days</td>
<td>30 cc. unfiltered stool suspension of a normal 3 wk. old calf</td>
<td>Remained well. Observed for 8 days</td>
</tr>
<tr>
<td>9-4</td>
<td>10 days</td>
<td>30 cc. unfiltered stool suspension of a normal 1 mo. old calf</td>
<td>Remained well. Stool soft on 4th day, within normal</td>
<td>20 days</td>
<td>10 cc. unfiltered suspension of infected calf stool, strain III</td>
<td>Incubation 4 days. Severe diarrhea with mucus and very little blood. Recovered in 21 days</td>
</tr>
<tr>
<td>9-9</td>
<td>11 days</td>
<td>30 cc. unfiltered stool suspension of a normal 2 wk. old calf</td>
<td>Remained well. Stool soft on 6th and 7th days, within normal</td>
<td>19 days</td>
<td>30 cc. unfiltered suspension of infected calf stool, strain III</td>
<td>Incubation 4 days. Severe diarrhea with much mucus, little blood. Recovered in 21 days</td>
</tr>
<tr>
<td>1-00</td>
<td>10 days</td>
<td>30 cc. unfiltered stool suspension of a normal 11 day old calf</td>
<td>Remained well</td>
<td>18 days</td>
<td>30 cc. unfiltered suspension of infected calf stool, strain III</td>
<td>Incubation 3 days. Severe diarrhea with much mucus and blood. Recovered in 31 days. (See Fig. 1.)</td>
</tr>
<tr>
<td>5-1</td>
<td>2 wks.</td>
<td>5 cc. unfiltered suspension of pool of stools of 2 normal infants, 14 years and 20 days respectively</td>
<td>Remained well. Observed 17 days</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5-3</td>
<td>2 wks.</td>
<td>5 cc. unfiltered suspension of pool of stools of 2 normal infants, age 8 days and 9 days respectively</td>
<td>Remained well</td>
<td>5 wks.</td>
<td>30 cc. unfiltered suspension of infected calf stool, strain I</td>
<td>Incubation 3 days. Moderate diarrhea with mucus and blood. Recovered in 14 days</td>
</tr>
<tr>
<td>9-1</td>
<td>2 wks.</td>
<td>5 cc. unfiltered suspension of pool of stools of 2 normal infants, age 2 days and 9 days respectively</td>
<td>Remained well</td>
<td>4 wks.</td>
<td>30 cc. unfiltered suspension of infected calf stool, strain I</td>
<td>Incubation 5 days. Mild diarrhea with mucus and no blood. Recovered in 11 days</td>
</tr>
<tr>
<td>9-2</td>
<td>1 wk.</td>
<td>6 cc. unfiltered suspension of pool of stools of 2 normal infants, age 1 and 7 days respectively</td>
<td>Remained well</td>
<td>3 wks.</td>
<td>30 cc. unfiltered suspension of infected calf stool, strain III</td>
<td>Incubation 3 days. Severe diarrhea with much mucus and blood. Recovered in 10 days</td>
</tr>
</tbody>
</table>
failed to inactivate the agent, as both calves developed the typical disease and the infection caused death in one of them. Two calves were similarly given material maintained at 80°C for 1 hour, in two separate experiments; one of these animals remained well and on later injection with active material showed no immunity, while the other calf underwent the same type of modified disease, with prolonged incubation period, as described above in the case of passage with dried material. When tested with active material following recovery, this calf was then found immune.

These data indicate that in one case the agent had been inactivated by heating at 80°C. for 1 hour and that in the other case it had been attenuated. A single experiment was carried out with material heated at 90°C. for 1 hour; the calf that received this remained well, and later was proven susceptible by the giving of active material.

**Protection Tests**

Protection tests were attempted with sera obtained from babies recovered from the outbreak in connection with which strain I was isolated.

The source of infectious agent for these tests consisted of the supernatant of infected calf stool suspension prepared as described above. The sera were obtained from the infants at times varying from 1½ to 3 months following recovery. Amounts varying from 1.5 to 2.0 cc. of supernatant were mixed with 1.5 to 2.5 cc. of serum in the various tests, and the resulting mixture allowed to stand at room temperature, with frequent gentle agitation, for 2 hours. One calf was used for each serum.

The sera of six infants were tested in all. Four controls were set up with the use of sera of normal babies of age similar to the tested babies.

The control sera showed no apparent protection, the four respective calves developing typical disease. Two were tested, following recovery, with infectious material, and found then to be immune. Of the six calves receiving the sera from the infants who had recovered from the disease, two remained entirely well. One of these calves was later given active material and proven susceptible. Two calves underwent the same sort of modified disease with prolonged incubation period as described above in connection with inoculation of dried material; one of these was tested, following recovery, with infectious material, and was found to have become immune. In the remaining two animals, because of intercurrent illness, the issue was not clear and no definite conclusions could be drawn regarding the sera tested.

One protection test was carried out in the manner described above with the serum of a recovered calf, controlled with serum from a normal calf. The serum of the recovered calf apparently yielded protection, as the animal receiving it remained well, while the control developed typical diarrheal disease.

**Immunization of Rabbit against the Filtrable Agent**

A 4½ month old rabbit was given a total of 26.5 cc. of Seitz filtrate of infected calf stool, in eighteen small intravenous injections, over a period of 2 months. Two weeks after the last
injection a protection test was set up with serum from the animal, using the technique described above except that 40 cc. of Seitz filtrate was used as infectious agent and this was mixed with 15 cc. of serum. The serum of a non-injected litter mate was used as a control. The control calf developed typical disease, while the test calf remained well and on later injection with active material was found fully susceptible.

**Summary of Number of Animals Infected during the Study**

Including all procedures, the disease had been produced in a total of 89 calves during the course of the study. Tabulation is as follows: Unfiltered stool suspension given nasally, 30 animals; same material given by stomach tube, 1 animal; supernatant of unfiltered stool suspension given nasally, 15 animals; bacteriologically sterile filtrates of stool suspension given nasally, 21 animals; material dried from the frozen state given nasally, 3 animals; serial passage from animals given dried material, 2 animals; stool material the activity of which was apparently diminished by mixture with convalescent serum in protection tests, given nasally, 2 animals; stool material the activity of which was apparently diminished by heat treatment, given nasally, 1 animal; cross-infection, 11 animals; passages with the use of blood of infected calves, 3 animals. Total 89.

**Attempts at Establishment of the Agent in Smaller Animals**

An attempt was made to establish the agent in 4- to 6-week-old mice, by various methods of inoculation, through serial passage. The supernatant after horizontal centrifugation of infected calf stool suspension was given nasally and serial passages carried out in two groups, the first lung to nose and the second intestine to nose. In addition, Seitz filtrate was given intracerebrally and further passages attempted by brain to brain inoculation. Filtrate was also given intraabdominally and serial passages done by the use of a pool of liver and spleen. Six attempted passages, at 5 day intervals, were carried out with each of the four groups. No significant disease developed.

To four 5-day-old rabbits, 0.25 cc. of filtrate was given by mouth. No symptoms attributable to this material appeared. Two ferrets were given 1.0 cc. of filtrate by mouth; this experiment too had negative results.

**Experience with Calf Scours in the Vicinity of Baltimore**

During the course of this study the opportunity arose to observe natural calf scours as it occurred in various dairy herds in the vicinity of Baltimore. In all but one of these herds the disease picture differed substantially from that displayed by the calves infected with the agent discussed in this paper. In this herd, however, there were certain similarities, particularly with regard to the production of mucus, though blood was only rarely seen. In an attempt to determine whether the causative agents were identical, stools were obtained from a severely ill calf in this herd showing a picture indistinguishable from the severer types of bloody diarrhea produced by the agent studied. An unfiltered suspension of these stools was given to two young healthy calves. No disease resulted in these calves, and they later proved susceptible to the agent reported in this paper when given infectious stool preparations. Another
calf in the infected herd, with mucoid diarrhea, was allowed to recover and then tested for immunity through the administration of Seitz filtrate of the agent studied. He showed no apparent immunity, developing the typical diarrheal disease. These data seemed to indicate that the cause of the diarrhea in this herd was not the same as of that discussed in this paper. However, the discovery that diarrhea with similar features sometimes occurs naturally among calves served further to emphasize the importance of the greatest care in the selection and isolation of experimental animals.

DISCUSSION

It is clear that diarrhea may be caused by a number of infectious agents and the evidence is quite strong that in the case of certain natural animal diseases in which diarrhea is an important feature of the illness, viruses may be the etiologic agents. As we have indicated above, this is the case in the pneunoma-enteritis of calves described by Baker (9, 10), the panleukopenia and diarrhea of cats (6–8), and the diarrhea of suckling mice (14, 15). In man the virus described by Buddingh and Dodd (11, 12) in 1944 appears to be the cause of a disease characterized by diarrhea and stomatitis in infants and young children. The virus described by Gordon and associates (13) was the causative agent involved in the New York State institutional epidemics of gastroenteritis in 1946 and 1947.

As we have stated above the disease studied by us in 1941 and 1942, which is the subject of the present report, appeared to be a single clinical entity and differed from those described in human beings by the aforementioned workers. The features of the disease common to all the six outbreaks which we studied were a high morbidity rate, absence of known pathogenic bacteria as causative agents, and limitation of obvious symptoms of the disease to infants under the age of 6 weeks. Stomatitis was not seen in any of our patients. The disease appeared regularly in both breast and artificially fed infants, and premature infants seemed more susceptible than full term babies. Because of these characteristics, we have considered that we were dealing with outbreaks of “epidemic diarrhea of the newborn,” as the disease was first designated by Rice and co-workers (18–21). Although this disease appears to be a single clinical entity, it is realized that a variety of agents might produce the same clinical picture and it is not supposed that the filtrable agent described in the present report is the cause of all outbreaks of “epidemic diarrhea of the newborn.”

We have shown that the agent described in this paper is capable of producing diarrhea with great regularity in the calf. Seventy-two of 75 animals which were used for passage developed diarrheal disease. The filtrable agent was apparently self-perpetuating since four successive passages with filtered material were accomplished. For each passage less than 10 times the minimal infective dose
(contained in 18 to 25 cc.) was employed. Because of the dilution factors involved, it is evident that multiplication of the virus in the passage animals occurred.

The virus described in the present report appears to be different from that isolated by Baker (9, 10) as the cause of pneumoenteritis of calves. Baker's virus produced pneumonia and high fever as well as diarrhea in the calf, and in mice caused pneumonia after intranasal inoculation. The agent isolated by us caused very little fever and never seemed to be the primary cause of pneumonia in calves, while attempts to cause pneumonia in mice with it were unsuccessful.

The question arises as to whether or not the virus described can be found in the stools of normal calves. We cannot state categorically that this possibility has been excluded, but evidence is at hand that it was at least not regularly present among the stock animals used in our experiments. On five separate occasions we attempted to produce the disease with stool from a normal calf but in no instance did diarrhea result. As stated above, scours, a naturally occurring disease of calves characterized by diarrhea, is found among herds of cattle in the vicinity of Baltimore. We have already mentioned that the available evidence indicates that the scours observed by us was not the same disease as the experimental disease produced by the virus we have described. This belief is supported by the fact that stools from a calf severely ill from scours did not produce diarrhea when inoculated into two young calves which were later proved quite susceptible to the virus which we have isolated. Furthermore, a calf which had recovered from an attack of scours was found to be susceptible on subsequent inoculation to infection with the virus.

We believe the evidence is clear that a virus has been isolated which causes a regularly reproducible disease in calves, which is characterized by the occurrence of diarrhea. The evidence is not so convincing that this agent actually came from the infants suffering from epidemic diarrhea of the newborn. However, certain findings bolster the belief that the affected infants may have been the source of the virus isolated. The fact that in studies involving infants ill in four different outbreaks filtrable agents were obtained which caused the same type of disease in calves and which were apparently identical, as evidenced by cross-immunity studies, lends support to this view. On the other hand it is well known that animals may harbor a virus which causes no harm until some interference with their normal existence occurs. It is possible that activation of a latent virus in the calves was brought about by inoculation with the excreta of the infants. Additional evidence that the virus described was the cause of the disease in the infants is furnished by neutralization tests carried out with sera from four infants who had recovered from epidemic diarrhea of the newborn. The sera of two of these infants completely protected two calves against infection with the virus, while the sera of two others showed partial protection.
The question arises as to whether the agent in question might be present in the stools of normal infants. No final answer is possible, but the stools of the eight normal infants studied caused no disease in calves.

It might be mentioned at this point that Cummings (22), employing techniques described by us, has recently reported the production of diarrhea in calves by the inoculation of stools of newborn infants ill with epidemic diarrhea. He obtained a filtrable causative agent and carried it through five passages before it was lost. Cummings believed this agent similar to that now described.

SUMMARY

From instances of diarrhea of the newborn in four separate hospital outbreaks a filtrable agent was isolated which regularly produced diarrhea in calves. This agent appeared to have the characteristics of a filtrable virus.

The four strains of virus isolated in the outbreaks studied appeared to be identical or very closely related.

The virus was not found present in the stools of any of eight normal newborn infants or five normal calves.

Evidence is presented that the virus may be one of the causes of epidemic diarrhea of the newborn.

The authors are indebted to a number of people for cooperation and assistance of various kinds which have greatly aided in the prosecution of this study. Among them are the following, to whom acknowledgment has not been made above: Dr. Lewis K. Sweet, Dr. Joseph M. Cordi, Dr. Ethel C. Dunham, Dr. Fletcher L. Vinson, Mr. Bartlett F. Johnston, Jr., Mr. G. H. Hibberd, and Mr. Edwin Clay.

BIBLIOGRAPHY


