fulvin catabolism(9), the finding was pertinent that both in rats(3) and humans(4) pretreatment with phenobarbital was followed by lower than control blood levels of griseofulvin.

In the present study pretreatment with primidone, another frequently used anticonvulsant, was found to cause a significant fall in the griseofulvin content in rat serum and livers. The effect was virtually the same as that with phenobarbital(3). It is, therefore, tempting to suggest that, as with phenobarbital, pretreatment with the structurally related primidone may accelerate the enzymatic breakdown of griseofulvin. Our data are compatible with the reports that primidone is metabolized to phenobarbital(10,11). However, they do not exclude the possibility that, like phenobarbital, primidone may affect *per se* the hepatic enzymes involved in drug metabolism.

In view of the similarity of effects of primidone and phenobarbital in rats and, since the depressing effect of phenobarbital on blood levels of griseofulvin has already been observed in human patients, it is likely that in humans primidone may cause the same.

Summary. Following oral administration significantly lower levels of griseofulvin were found in the serum and livers of rats pretreated with primidone. The fall was similar to that produced by phenobarbital. It is likely that primidone, like phenobarbital, accelerates the catabolism of griseofulvin.

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Experimental Infection of Calves with a Bovine Adenovirus.* (30623)

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Adenoviruses, as a group, are often found as the cause of acute respiratory illness in man. These viruses have been demonstrated in the eye, nasopharynx and intestinal tract of man and some animals. Neutralizing antibodies to various types of adenoviruses have been demonstrated in bovine sera(1,2). Two strains of adenovirus which are antigenically related to human adenoviruses(3,4), have been isolated from the feces of apparently healthy cows. This report is concerned with clinical and serological response of calves after exposure to a strain of bovine adenovirus by various routes of inoculation.

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Materials and methods. Virus. A bovine adenovirus (strain 10088) was obtained from Dr. M. Klein, Dept. of Microbiology, School of Medicine, Temple University, Philadelphia, Pa. Identifying properties of this virus as

described by Klein *et al*(3) are characteristic intranuclear inclusions and growth in calf kidney cell cultures as manifested by cytopathic effects at a low titer. Antigenic relation of the virus to human adenoviruses has been established by serologic analysis using human gamma globulin and adult human serum in neutralization tests. Furthermore, a group-reactive complement-fixing antigen similar to that present in human adenoviruses was found.

In our laboratory, the virus agglutinated bovine erythrocytes after several passages in bovine embryonic kidney (BEK) cell cultures. The virus was routinely propagated in BEK cell cultures. The inoculum for each calf contained approximately $5 \times 10^{6.5}$ $TCID_{50}$ of virus.

Test animals and inoculation procedure. Nine calves, 6-12 weeks of age and seronegative to the strain 10088 virus, were selected for this experiment. Heifer and bull calves of different breeds were divided into 4 groups, each group being housed in an isolation stall. The first group (2 calves) served as controls, one of which was inoculated intranasally with 5 ml of Hanks' balanced salt solution (HBSS) and the other, uninoculated. The second group (3 calves) received virus *via* intranasal inoculation as previously described (5). The third group (2 calves) was inoculated intratracheally. The virus was instilled into each eye of the remaining 2 calves by dropping the inoculum onto the conjunctiva.

Specimens. Specimens for virus isolation were collected in HBSS to which antibiotics had been added. Nasal and rectal swab specimens were collected from calves receiving inoculum *via* the intratracheal and intranasal routes while specimens from calves inoculated by eye instillation were obtained by ocular and nasal swabbing. Prior to inoculation into BEK cultures, the material was centrifuged at low speed. Swab materials were "blind passed" 3 times before they were considered negative. Identification of isolates by standard serum neutralization (SN) and hemagglutination inhibition (HI) tests, collection of sera, titration of serum antibody and clinical observations of calves before and after infection were the same as for the SF-4 virus study previously described(5).

Results. Clinical observations. Control calves No. 1 and 2 remained normal throughout the experiment. Average daily temperatures of these calves never exceeded 102.2°F and respiratory rates of 25 to 35 per minute were recorded from postinoculation day 1 through 18.

Calves No. 3, 4 and 5, inoculated intranasally, developed respiratory illness of varying severity characterized by coughing, nasal discharge, anorexia, depression and hyperpnea. In addition, several had diarrhea. Body temperatures of 103.0°F to 104°F were recorded for calf No. 3 on days 4 to 8 with nasal discharge, hyperpnea, coughing, and diarrhea with return to normal by day 10. Calf No. 4 had a marked febrile response, first noticed on day 3, and reaching a peak of 106.0°F on day 7. Its temperature dropped to normal on days 9 and 10, but again was elevated on days 11 and 15 (103.0°F to 106.4°F). The calf had mucoid nasal discharge, soft cough, anorexia, and dyspnea on days 4 through 9 and had returned to normal by day 17. Calf No. 5 experienced a very mild illness. Its average daily temperature ranged from 103.0°F to 103.8°F on days 4 through 9 with slight nasal discharge, hyperpnea and diarrhea on days 5 to 7. By day 10, the calf had returned to normal.

Calves No. 6 and 7, inoculated intratracheally, had the most severe response. Clinical symptoms consisted of nasal discharge, soft cough, anorexia, depression and hyperpnea with respiratory rates as high as 65, and occasionally 75, per minute. The average daily temperature of calf No. 6 varied from 103.0°F to 103.8°F (days 1 to 6). It had watery nasal discharge and dyspnea on days 2 and 3, cough on days 6 and 7 and hyperpnea from day 6 through day 13. Its temperature dropped to normal on days 7 to 9, but again varied from 103.0°F to 104.6°F on days 10 through 15. The fever had lessened by day 16 and the calf returned to normal by day 18. Calf No. 7 had an elevated temperature of 104.0°F only on days 4 and 5. Heavy nasal discharge, dyspnea, anorexia and depression were noticed on days 3 to 5, with

TABLE I. Recovery of Virus from Calves Following Their Exposure to a Bovine Adenovirus (Strain 10088).

Calf No.	Route of virus inoculation	Postinoculation day 0	1	3	5	7	9	11
1	Control	—	—	—	—	—	—	—
2	"	—	—	—	—	—	—	—
3	Intranasal	—	N	N	R	—	—	—
4	"	—	NR	NR	—	N	—	—
5	"	—	N	NR	—	—	—	—
6	Intratracheal	N	R	R	R	R	—	—
7	"	—	R	R	—	—	—	—
8	Ocular	—	E	—	—	—	—	—
9	"	E	EN	—	—	—	—	—

—, no recovery; N, recovery of virus from nasal swab; R, recovery of virus from rectal swab; E, recovery of virus from ocular swab.

soft cough and hyperpnea from days 5 through 13. The calf returned to normal by day 15.

Calves No. 8 and 9, given virus by eye instillation, had no clinical response. Conjunctivitis was not noticed in either of these calves and there was no rise of temperature.

Virus recovery. Recovery of virus from nasal, ocular and rectal swabs is shown in Table I. Virus in trace amounts was isolated on single occasions from the preinoculation nasal and ocular swabs of calves No. 6 and 9, respectively. The virus was isolated in high titers ($10^{3.0}$-$10^{3.5}$ $TCID_{50}$ per 0.1 ml swab culture) from nasal and rectal swabs of calves which had been inoculated intranasally. Virus was isolated only from rectal swabs of calves inoculated intratracheally. These calves also excreted the virus in high titer ($10^{3.5}$-$10^{4.0}$ $TCID_{50}$/0.1 ml). Calf No. 6 excreted the virus regularly through day 7; but calf No. 7, only through day 3. The virus was recovered on day 1 only from calves which received eye instillation of the virus. Virus was not isolated from control calves.

Serologic response. Serologic response of calves to bovine adenovirus is shown in Table II. All calves were seronegative to strain 10088 virus prior to inoculation. Control calves remained negative throughout the experiment although HI titer of 1:10 was noted for calf No. 1 on day 21 and a titer of 1:10 for calf No. 2 on days 21 and 28.

All infected calves had HI antibody response on day 7. The maximum HI titer obtained was 1:320 on day 14, the maximum SN titer being 1:64 on days 21 and 28. Calves which received eye instillation of virus had the poorest serologic response. Calves No. 8 and 9 had maximum titers of 1:20 and 1:40, respectively. Both of these titers were attained at 21 days postinoculation, but they had dropped by day 28.

Discussion. Numerous viral agents have been isolated from nasal passages and intestinal tracts of cattle. Several recent isolates, including adenoviruses, are of public health interest. The study of bovine respiratory diseases as they occur naturally and experimentally presents an excellent opportunity to compare them with epidemiological patterns of similar human diseases. Experimental infections of dogs and chickens with adenoviruses without clinical response have been reported(6,7).

TABLE II. Serologic Response of Calves to a Bovine Adenovirus (Strain 10088).

Calf No.	Route of virus inoculation	Preinoc. HI*	Preinoc. SN†	Day postinoculation 7 HI	7 SN	14 HI	14 SN	21 HI	21 SN	28 HI	28 SN	42 HI	42 SN
1	Control	<10‡	<4§	<10	<4	<10	<4	10	<4	<10	<4	<10	<4
2	"	<10	<4	<10	<4	<10	<4	10	<4	10	<4	<10	<4
3	Intranasal	<10	<4	10	4	80	8	40	16	40	16	<10	16
4	"	<10	<4	10	<4	10	<4	40	<4	40	4	10	8
5	"	<10	<4	10	<4	20	4	40	8	20	8	20	8
6	Intratracheal	<10	<4	40	<4	160	8	40	16	40	64	20	8
7	"	<10	<4	≧80	<4	320	16	160	64	160	32	80	16
8	Ocular	<10	<4	10	<4	10	<4	20	<4	<10	<4	<10	<4
9	"	<10	<4	20	<4	20	<4	40	<4	10	<4	<10	4

* Hemagglutination inhibition test.
† Serum neutralization test.
‡ Reciprocal of the highest serum dilution showing complete inhibition of hemagglutination.
§ " " " " " " which completely neutralized 100 $TCID_{50}$ of virus.

It has already been shown that the bovine adenovirus isolates are antigenically related to human adenoviruses(3,4). The results of this study show that either intranasal or intratracheal inoculation of bovine adenovirus causes a respiratory illness of varying severity in young calves. The intranasal route is apparently the natural route of infection. The intratracheal route was chosen to determine the severity of infection.

Calves inoculated intranasally had a relatively milder response with symptoms of short duration. On the other hand, calves inoculated intratracheally had a more severe response with clinical manifestations of longer duration. It has been reported that adenoviruses produce conjunctivitis in man(8). In this study, the strain 10088 virus failed to produce conjunctivitis or any other clinical reaction in calves when inoculated by eye instillation.

Isolation of the virus from preinoculation swab cultures of 2 calves indicates that the virus is widespread in cattle populations. It is interesting that the virus was recovered more often from rectal swabs. It appears that the virus may be disseminated throughout a herd in feces and could account for the high percentage of cattle which have antibody to adenovirus. Recovery of the virus from rectal swabs of those calves inoculated intranasally indicates that the virus can withstand the acidic conditions of the stomach and gain entrance to the intestinal tract where its multiplication is presumably extensive. It may be assumed that this viral strain does not multiply in the eye of the calf since no virus recovery was made from ocular swabs beyond day 1 postinoculation.

Immunologic response of the calves generally was poor considering the dose of virus administered. Calves infected *via* the eye had the poorest serologic response. It is evident from this study that the strain 10088 bovine adenovirus could produce illness in calves. These findings appear to be significant from the public health standpoint since cattle may harbor adenoviruses and thus play a key role in transmission of these infections to man. Although there is yet no report that these infections are communicated to man from cattle, or *vice versa,* these findings seem to warrant further study.

Summary. Calves were experimentally infected with a bovine adenovirus using various routes of inoculation. Respiratory illness was observed in calves inoculated either intranasally or intratracheally. The virus had no effect on calves infected *via* the eye. Control calves remained normal throughout the experiment. The virus was recovered principally from rectal swabs of infected calves for 7 days. All infected calves responded serologically to the virus.

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