Veterinary Microbiology, 6 (1981)143—155
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

INHIBITORS OF BOVINE PARVOVIRUS, CORONAVIRUS AND ROTAVIRUS IN PRECOLOSTRAL AND FETAL BOVINE SERA

S. TOKUHISA, Y. INABA, K. SATO, Y. MIURA, H. AKASHI, K. SATODA and M. MATUMOTO*

National Institute of Animal Health, Tsukuba, Ibaraki (Japan)
**Kitasato Institute, Minato-ku, Tokyo (Japan)*

(Accepted 12 January 1981)

ABSTRACT

Tokuhisa, S., Inaba, Y., Sato, K., Miura, Y., Akashi, H., Satoda, K. and Matumoto, M., 1981. Inhibitors of bovine parvovirus, coronavirus and rotavirus in precolostral and fetal bovine sera. *Vet. Microbiol.*, 6: 143—155.

Eleven, 11 and 2 of 11 precolostral sera from normal calves and 13, 14 and 9 of 14 sera from normal fetuses, 7 to 10 months of gestation, neutralized bovine parvovirus, coronavirus and rotavirus, respectively.

When assayed by single radial immunodiffusion, all the sera contained IgG at 360 to 1400 mg/dl, and some of them had much smaller amounts of IgM or IgA. Most of the neutralizing activities against bovine coronavirus and rotavirus were readily inactivated by treatment with acetone or 2-mercaptoethanol. Some sera fractionated by Sephadex G-200 gel filtration or starch block electrophoresis had neutralizing activities against bovine parvovirus or coronavirus in fractions containing no detectable amounts of immunoglobulins. These observations seem to indicate the presence of substance(s), other than immunoglobulins, capable of inhibiting replication of bovine parvovirus, coronavirus or rotavirus. The chemical nature and the mode of action of the inhibitors await elucidation.

INTRODUCTION

Transplacental transfer of maternal immunoglobulins does not occur in cattle, and the newborn animal acquires maternal antibodies by ingesting colostrum (Brambell, 1970; Solomon, 1971). On the other hand, bovine fetuses develop the ability to produce antibodies upon antigenic stimulation relatively early in gestation (Solomon, 1971; Osburn, 1973; Schultz, 1973; Schultz et al., 1973). Many workers have reported the presence of antibodies against various viruses in sera from colostrum-deprived calves and fetuses (Kniazeff et al., 1967; Dunne et al., 1973; Horner et al., 1973; Hubbert et al., 1973; Schultz et al., 1973; Miura et al., 1974; Rossi and Kiesel, 1974; Kurogi et al., 1975). On the other hand, there have been reported studies suggesting the presence of virus-neutralizing substances, other than immunoglobulins, in

0378-1135/81/0000—0000/$ 02.50

bovine sera (McFerran, 1962 a, b; Kanamitsu et al., 1967; Doggett et al., 1968; Urasawa et al., 1968 a, b; 1969).

Recently we have reported that high percentages of normal precolostral and fetal calf sera neutralize bovine parvovirus, coronavirus and rotavirus (Sato et al., 1980). In the present study, experiments were carried out to relate these neutralizing activities to immunoglobulins in the sera, and the results obtained suggested that some precolostral and fetal calf sera might contain substances, other than immunoglobulins, capable of inhibiting the replication of these bovine viruses.

MATERIALS AND METHODS

Sera. Serum samples were obtained by jugular puncture from normal newborn calves before or after ingestion of colostrum and from some of their dams in 1973 and 1974, and by cardiac puncture from normal fetuses, 7 to 10 months of gestation, in 1979. The sera were stored at −20°C and inactivated at 56°C for 30 min before use.

Viruses. The K-71 strain of bovine parvovirus was originally isolated from an aborted fetus (Sugimura, et al., 1974) and had undergone 5 passages in primary cultures of bovine kidney (BK) cells when used in the present study. The Lincoln strain of bovine rotavirus (Mebus et al., 1971) was used at the 7th passage level in BK cell cultures. Calf diarrhea coronavirus (Mebus et al., 1973) was found in our laboratory to replicate with cytopathic effect in cultures of the BEK-1 cell line derived from bovine embryonic kidney (Inaba et al., 1976). In the present study the virus was used at the 7th BEK-1 passage.

Neutralization tests. Neutralization tests with bovine parvovirus and rotavirus were carried out in tube cultures of BK cells and those with bovine coronavirus in tube cultures of BEK-1 cells. The medium for cell growth was Eagle's minimum essential medium (MEM) containing 10% tryptose phosphate broth, 10% bovine serum and antibiotics, and the maintenance medium was MEM containing 10% tryptose phosphate broth, 0.5% sodium glutamate and 0.05% yeast extract. Cultures were prepared in 11 × 100 mm tubes by seeding 0.5-ml volumes of growth medium containing 2.5×10^5 cells/ml and incubating at 37°C for 4 days. Serial twofold dilutions of the serum or fractionated materials were made with maintenance medium, and each dilution was mixed with an equal volume of maintenance medium containing 200 $TCID_{50}$ per 0.1 ml of virus. The virus-serum mixtures were incubated at 37°C for 90 min, inoculated in 0.1-ml volumes into 2 tube cultures per serum dilution. The inoculated cultures were incubated at 37°C for 90 min for virus adsorption, fed with 0.5 ml of maintenance medium and incubated in a roller drum at 37°C for 8 to 9 days with parvovirus or for 6 or 7 days with coronavirus and rotavirus. The titer was expressed as the

reciprocal of the highest serum dilution showing neutralization in at least one of the two tubes.

Treatment of serum with acetone. One volume of test serum was mixed with 20 volumes of chilled acetone, shaken vigorously for 5 min, and centrifuged at 500 × *g* for 5 min at 4° C. The supernatant fluid was carefully aspirated from the sediments, which were again treated with acetone in the same manner. The resulting sediments were dried under vacuum at room temperature and resuspended in sufficient PBS, pH 7.2, to make a 1:4 dilution based on the volume of serum initially introduced.

Treatment of serum with 2-ME. One volume of test serum was mixed with one volume of 0.1 M 2-ME (2-mercaptoethanol) in PBS, pH 7.2. After standing at 4° C for 24 h, the mixture was dialyzed overnight against 0.02 M iodoacetamide in PBS at 4° C. It was further dialyzed against PBS for 2 days at 4° C. A sufficient amount of PBS was added to the resulting material to make a 1:4 dilution based on the volume of serum initially introduced.

Assay and identification of bovine immunoglobulins. Quantitation of immunoglobulins was made by single radial immunodiffusion according to the method of Kniazeff et al. (1967) using rabbit anti-bovine IgG, IgM and IgA (Miles Laboratories, Elkhard, Indiana). Of each test serum, 0.005 or 0.01 ml was transferred to a well and tests were run in parallel with a standard serum having known concentrations of immunoglobulins. The plates were incubated at room temperature for 22 h, and then examined for the appearance of precipitation rings. Ring diameters were measured to determine Ig concentrations in relation to controls. The lower limit for the detection of IgG, IgM and IgA was 1.6, 4.0 and 5.0 mg/dl, respectively.

Immunoglobulins were identified by the slide microgel diffusion precipitin test of Mansi (1958) using rabbit anti-bovine immunoglobulins, IgG_1, IgG_2, IgM, and IgA.

Immunoelectrophoresis. A well of 3-mm diameter was punched in the center of a 80 × 120 mm agar gel plate prepared with 1% Noble agar in veronal buffer (pH 8.6, ionic strength 0.05). Test serum (0.03 ml) was placed in the well and run by conducting an electric current at a rate of 5 mA/cm for 150 min. After completion of the electrophoresis, rabbit antiserum against bovine whole serum was poured into troughs cut on both sides of the well parallel with the electric current and allowed to react for 48 h in a moist chamber at room temperature with the serum components separated electrophoretically.

Starch block electrophoresis. The method of Miller and Bale (1954) was employed. Potato starch in veronal buffer (pH 8.6, ionic strength 0.05) was packed in a 36 × 5 × 1 cm cell, and a trough, 1.5 cm wide, was cut and filled

with starch in 3 ml of the test serum and an electric current was conducted at a potential gradient of 5 V/cm for 28 h at 4°C. After completion of the electrophoresis, the starch layer was cut into 12 strips, each of which was extracted with PBS, pH 7.2. The fractions were concentrated by ultrafiltration in collodion bags and 1:2 dilutions were made with PBS based on the volume of serum initially introduced.

Sephadex G-200 gel filtration. This was carried out by the method of Flodin and Killander (1962). A 2.5 × 70 cm column of Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala) was equilibrated with a solution containing 0.15 M NaCl and 0.01 M phosphate buffer of pH 7.2, and 3 ml of test serum diluted 1:2 with PBS was applied thereupon. Gel filtration was performed using the above buffer at a flow rate of 18 ml per hour at 4°C and filtrates were collected in 3-ml amounts. The protein content of the fractions was measured as the extinction at 280 nm. The presence of IgG_1, IgG_2, IgA and IgM in the fractions was tested for by the slide microgel diffusion precipitin test (see above). The fractions were also tested for neutralizing titers against parvovirus, coronavirus and rotavirus.

RESULTS

Immunoglobulin contents

Serum samples from 11 normal precolostral calves and 14 normal fetuses, 7 to 10 months of gestation, were subjected to single radial immunodiffusion to determine immunoglobulin contents. Serum samples from 3 pairs of cows and their calves were also included in the tests. The results are shown in Table I. The serum samples from the cows and their colostrum-fed calves had immunoglobulins, IgG contents being highest, followed by IgM and then IgA. The colostrum-fed calves (C-1, C-2, C-3) showed higher Ig contents than their dams (M-1, M-2, M-3), although they had smaller or similar amounts of IgG and only trace amounts or no IgM and IgA before ingestion of colostrum (P-1, P-2, P-3). Of the 11 colostrum-deprived calves (P-1, to P-11), all had IgG, 360 to 1400 mg/dl and 7 calves, or 64%, each had much smaller amounts of IgM, trace to 42 mg/dl, or IgA, trace to 14 mg/dl. Of the 14 fetuses (F-1 to F-14) all had IgG, 360 to 1400 mg/dl, 10 fetuses, or 71%, had trace to 20 mg/dl of IgM, and 9 fetuses, or 64%, had a trace only of IgA.

Neutralizing activity to bovine parvovirus

As shown in Table I, the dams (M-1, M-2, M-3) and their colostrum-fed calves (C-1, C-2, C-3) had high titers of neutralization against bovine parvovirus. Most of the 11 colostrum-deprived calves (P-1 to P-11) also had high titers of neutralizing activity, while of the 14 fetuses (F-1 to F-14) 3 had high titers, but the others had lower titers; all but one (F-1) were positive.

TABLE I

Immunoglobulins and neutralizing activies to parvovirus, coronavirus and rotavirus in serum samples from cows, calves and fetuses

Serum sample[a]		Immunoglobulin content (mg/dl)			Neutralizing titer		
		IgG	IgM	IgA	Parvo	Corona	Rota
Dams	M-1	1200	450	33	⩾512	<2	64
	2	800	350	26	⩾512	128	8
	3	1400	130	50	512	⩾128	32
Calves	C-1	2800	780	120	⩾512	32	64
	2	3400	520	250	⩾512	128	32
	3	3400	200	540	⩾512	⩾128	32
Calves[b]	P-1	800	T	T	⩾512	64	<2
	2	1000	T	0	256	32	<2
	3	360	0	T	⩾512	32	32
	4	900	20	T	⩾512	64	<2
	5	1400	42	14	32	8	<2
	6	1400	14	0	2	8	<2
	7	1000	T	0	256	32	<2
	8	1400	0	0	⩾512	16	2
	9	640	0	T	⩾512	64	<2
	10	640	0	T	⩾512	64	<2
	11	500	T	T	⩾512	16	<2
Positive rate		11/11	7/11	7/11	11/11	11/11	2/11
Fetuses[c]	F-1	800	0	T	<2	⩾128	32
	2	800	0	T	32	128	128
	3	500	T	T	16	32	2
	4	640	0	T	16	32	<2
	5	800	20	T	128	32	<2
	6	640	T	0	⩾512	8	8
	7	360	18	0	2	32	128
	8	640	T	0	⩾512	⩾128	32
	9	800	14	0	32	⩾128	8
	10	800	0	T	2	32	<2
	11	800	14	T	8	32	2
	12	800	T	T	2	⩾128	2
	13	640	T	T	32	4	<2
	14	1400	18	0	16	8	<2
Positive rate		14/14	10/14	9/14	13/14	14/14	9/14

[a]Serum samples, C-1, C-2 and C-3 were obtained after ingestion of colostrum from calves of dams, M-1, M-2 and M-3, respectively; P-1, P-2 and P-3 were obtained from the same calves before ingestion of colostrum.
[b]Serum samples from colostrum-deprived calves.
[c]Serum samples from fetuses, 7 to 10 months of gestation.

The neutralizing activity shown in these sera was little affected by treatment with acetone or 2-ME, although some sera with low titers became negative after these treatments (Table II).

TABLE II

Effects of treatment of sera with acetone and 2-mercaptoethanol on neutralizing activities to parvovirus, coronavirus and rotovirus

Animal	No. tested	No. of sera having neutralizing activity to								
		Parvovirus			Coronavirus			Rotavirus		
		Un[d]	Ac[d]	ME[d]	Un	Ac	ME	Un	Ac	ME
Dams	3	3	3	3	2	2	2	3	2	2
Calves[a]	3	3	3	3	3	3	2	3	3	3
Calves[b]	11	11	10	10	11	6	1	2	1	0
Fetuses[c]	14	13	7	7	14	2	0	9	0	0

[a]Colostrum-fed calves.
[b]Colostrum-deprived calves.
[c]Fetuses, 7 to 10 months of gestation.
[d]Un: Untreated. Ac: Acetone treated. ME: 2-mercaptoethanol treated.

Neutralizing activity to bovine coronavirus

As shown in Table I, one (M-1) of the 3 cows had no detectable neutralizing activity and the colostrum-fed calves (C-1, C-2, C-3) all had positive titers. The activity of these sera was little affected by acetone or 2-ME treatment, excepting the serum (C-1) from one of the calves which had a low titer and became negative after 2-ME treatment (Table II). All the serum samples from the colostrum-deprived calves (P-1 to P-11) and the fetuses (F-1 to F-14) showed this activity, which was reduced by acetone treatment, and particularly by 2-ME treatment (Tables I, II).

Neutralizing activity to bovine rotavirus

Serum samples from the cows (M-1, M-2, M-3) and their colostrum-fed calves (C-1, C-2, C-3) had neutralizing titers ranging from 8 to 64 (Table I). The activity of these sera was not much affected by acetone or 2-ME treatment (Table II). Only 2 of the 11 colostrum-deprived calves (P-1 to P-11) were positive with low titers of 2 and 32, while 9 of the 14 fetuses (64%) had titers ranging from 2 to 128 (Table I). After acetone or 2-ME treatment all the sera became negative excepting one precolostral serum (P-3) which showed a titer reduction from 32 to 8 following acetone treatment (Table II).

Starch block electrophoresis

Precolostral calf serum P-8 was subjected to starch block electrophoresis to relate the immunoglobulins contained in the serum to the neutralizing activities for the viruses. The original serum sample contained 1400 mg/dl of IgG, but no IgM or IgA. Of the 12 fractions obtained, only the 4th and 5th fractions were found to contain IgG at 640 and 280 mg/dl, respectively. The original sample had neutralizing titers of $\geqslant$512, 16 and 2 for parvovirus, coronavirus and rotavirus, respectively. Neutralizing activity to parvovirus was shown in the 8th and 9th fractions in titers of 128 and 32, respectively, while it was not shown in the other fractions excepting the fraction of origin (the 6th) which had a low titer of 8. None of the 12 fractions showed any neutralizing activities for coronavirus or rotavirus. Immunoelectrophoresis demonstrated gamma-globulin in the 4th fraction, alpha- and beta-globulin in the 8th fraction and alpha-globulin and alubumin in the 9th fraction.

Sephadex G-200 gel filtration

The serum samples examined were those from a cow (M-3) and her calf, colostrum-fed (C-3) and colostrum-deprived (P-3). In addition 2 samples each from precolostral calves (P-2, P-8) and from fetuses (F-3, F-4) were examined. Each fraction obtained by gel filtration was tested for the presence of IgG_1, IgG_2, IgM and IgA and for virus neutralizing activities. The results are shown in Figs. 1 to 4.

As shown in Fig. 1, fractions of the cow serum M-3 had neutralizing activity against parvovirus and contained IgG_1 and IgG_2 and some early fractions also contained IgM. Sample C-3 from the colostrum-fed calf of this dam showed a similar pattern of parvovirus neutralizing activity, although the activity began to appear in somewhat earlier fractions than in the case of M-3 serum (Fig. 2). Precolostral serum P-3 of this calf contained only IgG_1, but no IgG_2, IgM or IgA. Neutralizing activity against parvovirus began to appear before the appearance of IgG_1 and some early fractions containing IgG_1 showed neutralizing activity (Fig. 3). M-3, C-3 and P-3 serum samples showed neutralizing activities against coronavirus and rotavirus in fractions of the IgG elution region (Figs. 1–3). The other precolostral serum samples, P-2 and P-8, contained eluted neutralizing activities against parvovirus and coronavirus in fractions before IgG_1 eluted (Figs. 3). No fraction obtained from P-2 and P-8 showed neutralizing activities against parvovirus or rotavirus. Fetal serum F-3 contained IgG_1, but neutralizing activity against coronavirus was shown in fractions containing no immunoglobulin. No fraction from F-3 showed neutralizing activity against parvovirus or rotavirus (Fig. 4). On the other hand, fetal serum F-4 contained IgG_1, and neutralizing activity against coronavirus was restricted to fractions containing IgG_1. Neutralizing activities against parvovirus and rotavirus were not detected in any of the fractions (Fig. 4).

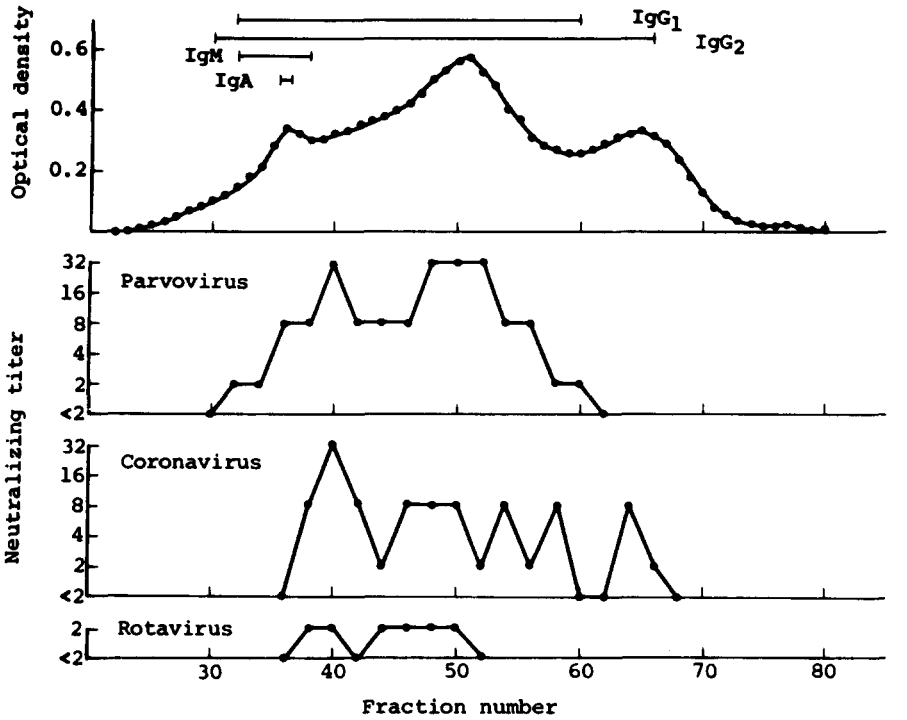


Fig. 1. Sephadex G-200 gel filtration: cow serum M-3.

DISCUSSION

The present study has confirmed the previous report (Sato et al., 1980) that high percentages of normal precolostral and fetal calf sera neutralize bovine parvovirus, coronavirus or rotavirus (Table I). Furthermore, all of the 11 precolostral sera and the 14 fetal sera examined in the present study were shown to contain immunoglobulins; all of them contained IgG, 360 to 1400 mg/dl, and some contained much smaller amounts of IgM or IgA (Table I). These findings were taken to indicate that these calves and fetuses had produced neutralizing antibodies to these viruses as the result of intrauterine infection. Several reports are available on the occurrence of immunoglobulins in sera of precolostral calves and fetuses not overtly stimulated by antigens (Pierce, 1955; Kniazeff et al., 1967; Klaus et al., 1969; Schultz et al., 1971; Sawyer et al., 1973; Schultz et al., 1973).

On the other hand, most of the neutralizing activities against bovine coronavirus and rotavirus in the precolostral and fetal sera were lost by treatment with acetone or 2-mercaptoethanol, whereas the neutralizing activity against bovine parvovirus in these sera was not much affected by these treatments. Since immunoglobulins are not affected by acetone treat-

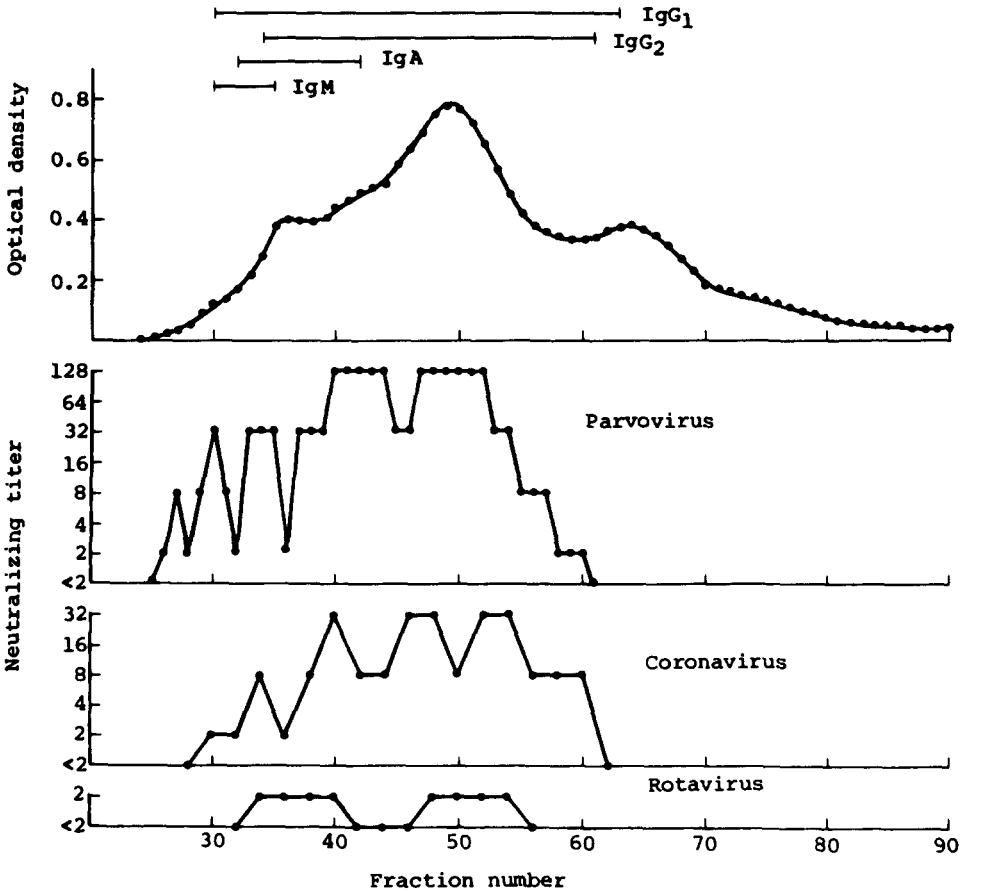
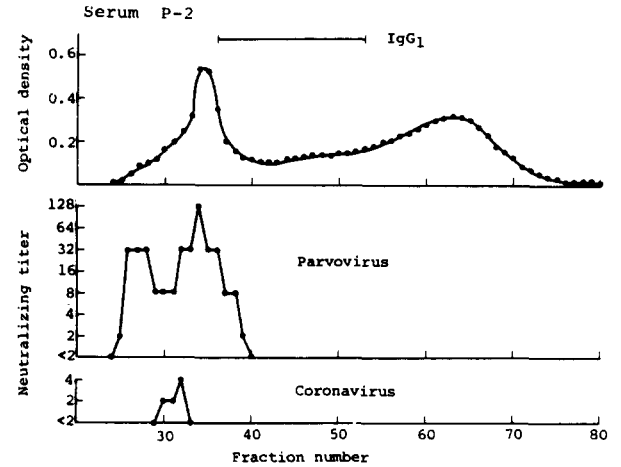
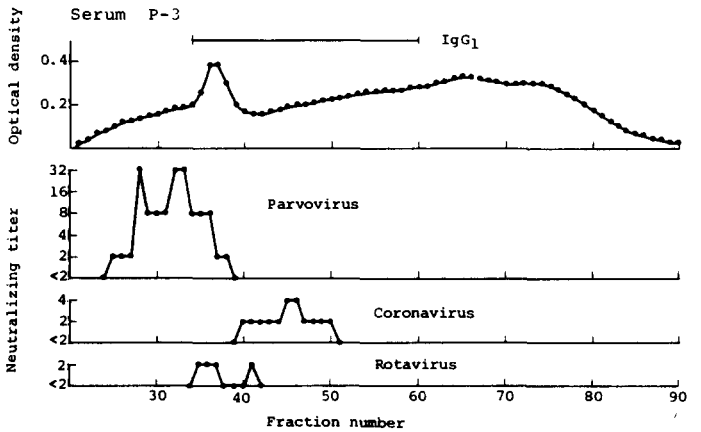


Fig. 2. Sephadex G-200 gel filtration: colostrum-fed calf serum C-3.

ment, and IgG is resistant to 2-mercaptoethanol, these findings seem to indicate the possibility that some substance(s) other than immunoglobulins may be responsible for most of the neutralizing activities against bovine coronavirus and rotavirus, whereas the neutralizing activity against bovine parvovirus may be due to specific immunoglobulin.

The results of studies on some precolostral and fetal calf sera with Sephadex G-200 gel filtration and starch block electrophoresis seem to indicate the presence of substance(s), other than immunoglobulins, capable of inhibiting the replication of bovine parvovirus or bovine coronavirus. Thus, precolostral serum P-8, fractionated by starch block electrophoresis, had neutralizing activity against bovine parvovirus in fractions in which no immunoglobulin was detected by single radial immunodiffusion and immunoelectrophoresis. Sephadex G-200 gel filtration of P-8 sample also demonstrated neutralizing activity against bovine parvovirus in fractions con-





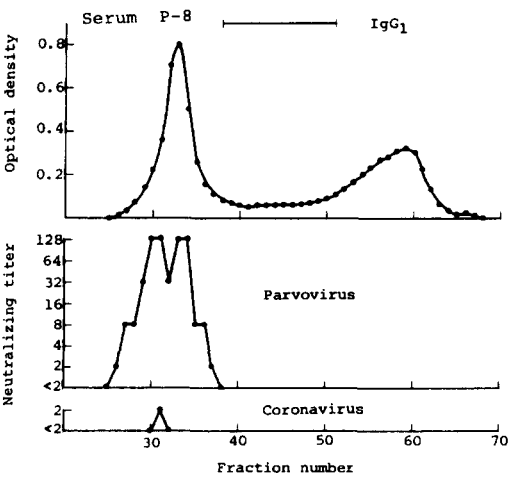


Fig. 3. Sephadex G-200 gel filtration: colostrum-deprived calf serum P-2, P-3 and P-8.

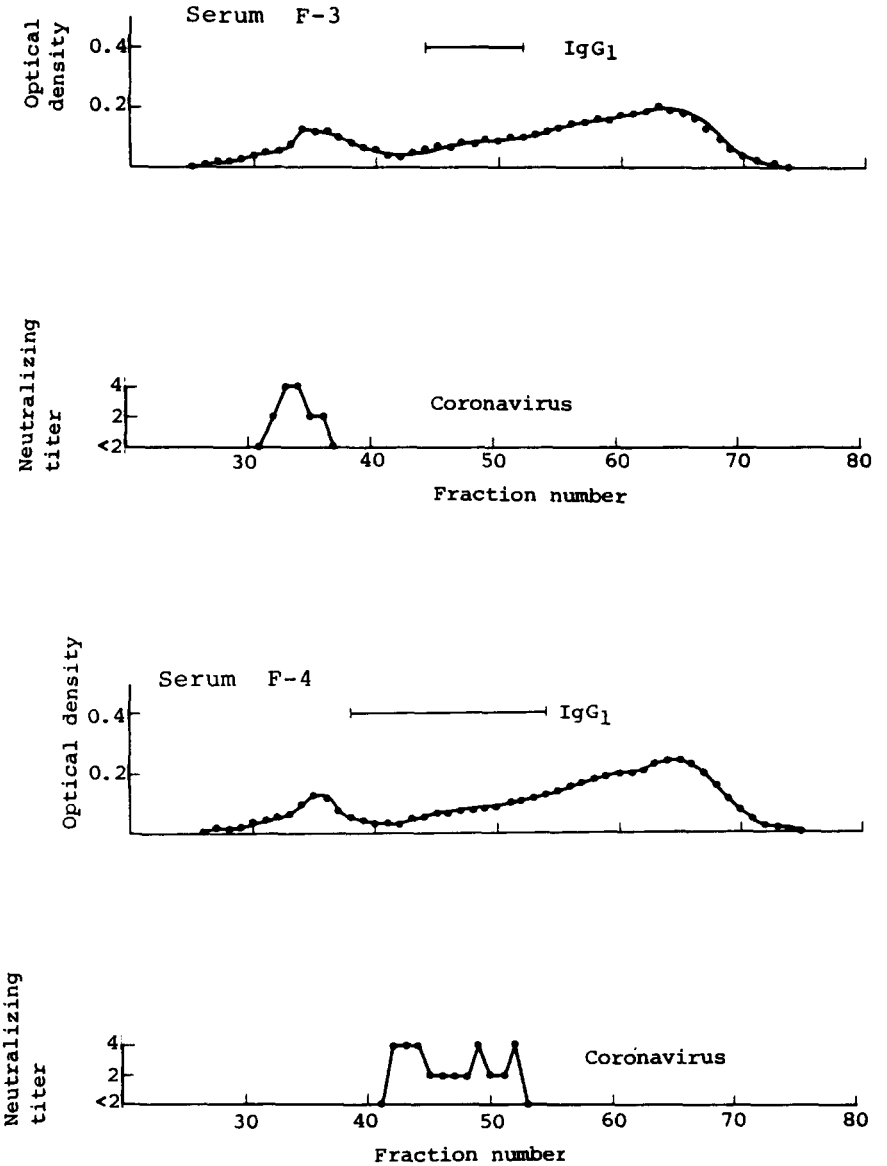


Fig. 4. Sephadex G-200 gel filtration: fetal serum F-3 and F-4.

taining no detectable amounts of immunoglobulins (Fig. 3). These findings suggest that the neutralizing activity against bovine parvovirus shown in P-8 serum may be due to some substance(s) other than immunoglobulins.

Precolostral sera, P-2 and P-3, were also studied by Sephadex G-200 gel filtration. Neutralizing activity against bovine parvovirus was detected in fractions before the elution of IgG_1 and in a few early fractions containing

IgG_1 (Fig. 3). This finding seems also to support the view that the sera may contain some substances, other than immunoglobulins, capable of inhibiting the growth of bovine parvovirus. With P-2 serum, neutralizing activity against bovine coronavirus was also detected in fractions containing no immunoglobulin (Fig. 3), while P-3 serum had neutralizing activities against bovine coronavirus and rotavirus in fractions containing IgG_1 (Fig. 3).

Sephadex G-200 gel filtration of fetal serum F-3 gave results suggesting the presence of some substance(s) other than immunoglobulins capable of inhibiting the replication of bovine coronavirus (Fig. 4). On the contrary, fetal serum F-4 showed neutralizing activity against coronavirus in fractions containing IgG_1, suggesting that the activity might be due to immunoglobulin (Fig. 4).

These discussions seem to indicate that some precolostral and fetal calf sera may contain substance(s), other than immunoglobulins, capable of inhibiting the replication of bovine parvovirus, coronavirus and rotavirus.

Further studies are needed to elucidate the chemical nature and the mode of action of the inhibitors. The possible presence of nonspecific inhibitors should be kept in mind in testing sera for neutralizing antibodies to these bovine viruses.

REFERENCES

Brambell, F.W.R., 1970. The transmission of passive immunity from mother to young, Frontiers of Biology, Vol. 18. North-Holland Publ. Co., Amsterdam, pp. 201—233.

Doggett, J.E., Robinson, D.T. and Gallop, R.G.C., 1968. A study of an inhibitor in bovine serum active against respiratory syncytial virus. Arch. Ges. Virusforsch., 23: 126—137.

Dunne, H.W., Ajinkya, S.M., Bubash, G.R. and Griel, L.C., 1973. Parainfluenza-3 and bovine enteroviruses as possible important causative factors in bovine abortion. Am. J. Vet. Res., 34: 1121—1126.

Flodin, P. and Killander, J., 1962. Fractionation of human serum proteins by gel filtration. Biochim. Biophys. Acta, 63: 403—410.

Horner, G.W., Johnson, R.H., Dennett, D.P. and Lane, W.R., 1973. A serological study of bovine fetal immunoglobulins. Aust. Vet. J., 49: 325—329.

Hubbert, W.T., Bryner, J.H., Fernelius, A.L., Frank, G.H. and Estes, P.C., 1973. Viral infection of the bovine fetus and its environment. Arch. Ges. Virusforsch., 41: 86—98.

Inaba, Y., Sato, K., Kurogi, H., Takahashi, E., Ito, Y., Omori, T., Goto, Y. and Matumoto, M., 1976. Replication of bovine coronavirus in cell line BEK—1 culture. Arch. Virol., 50: 339—342.

Kanamitsu, M., Hashimoto, N., Urasawa, S. and Chiba, S., 1967. Studies on poliovirus inhibitors in sera of domestic animals. I. Distributions and properties of poliovirus inhibitors in bovine and equine sera. Japan. J. Med. Sci. Biol., 20: 471—482.

Klaus, G.G.B., Bennett, A. and Jones, E.W., 1969. A quantitative study of the transfer of colostral immunoglobulins to the newborn calf. Immunology, 16: 293—299.

Kniazeff, A.J., Rimer, V. and Gaeta, L., 1967. Gammaglobulin in foetal bovine sera: significance in virology. Nature, 214: 805—806.

Kurogi, H., Inaba, Y., Goto, Y., Miura, Y., Takahashi, H., Sato, K., Omori, T. and Matumoto, M., 1975. Serologic evidence for etiologic role of Akabane virus in epizootic abortion-arthrogryposis-hydranencephaly in cattle in Japan, 1972—1974. Arch. Virol., 47: 71—83.

Mansi, W., 1958. Slide gel diffusion precipitin test. Nature, 181: 1289—1290.
McFerran, J.B., 1962 a. Poliovirus-neutralizing substances in cattle sera. J. Pathol. Bacteriol., 83: 73—82.
McFerran, J.B., 1962 b. A substance in the sera of man and animals neutralizing a bovine enterovirus. J. Pathol. Bacteriol., 83 83—88.
Mebus, C.A., Kono, M., Underdahl, N.R. and Twiehaus, M.J., 1971. Cell culture propagation of neonatal calf diarrhea (scours) virus. Can. Vet. J., 12: 69—72.
Mebus, C.A., Stair, E.L., Rhodes, M.B. and Twiehaus, M.J., 1973. Neonatal calf diarrhea: propagation, attenuation and characteristics of a coronavirus-like agent. Am. J. Vet. Res., 34: 145—150.
Miller, L.L. and Bale, W.F., 1954. Synthesis of all plasma protein fractions except gamma globulins by the liver. The use of zone electrophoresis and lysine-C^{14} to define the plasma proteins synthesized by the isolated perfused liver. J. Exp. Med., 99: 125—132.
Miura, Y., Hayashi, S., Ishihara, T., Inaba, Y., Omori, T. and Matumoto, M., 1974. Neutralizing antibody against Akabane virus in precolostral sera from calves with congenital arthrogryposis-hydranencephaly syndrome. Arch. Ges. Virusforsch., 46: 377—380.
Osburn, B.I., 1973. Immune responsiveness of the fetus and neonate. J. Am. Vet. Med. Assoc., 163: 801—803.
Pierce, A.E., 1955. Electrophoretic and immunological studies on sera from calves from birth to weaning. I. Electrophoretic studies. J. Hyg., 53: 247—260.
Rossi, C.R. and Kiesel, G.K., 1974. Antibody to viruses affecting cattle in commercial tissue culture grade fetal calf serum. Appl. Microbiol., 27: 114—117.
Sato, K., Inaba, Y., Tokuhisa, S., Miura, Y., Akashi, H. and Tanaka, Y., 1980. Antibodies against several viruses in sera from normal bovine fetuses and precolostral calves. Nat. Inst. Anim. Health Q., 20: 77—78.
Sawyer, M., Osburn, B.I., Knight, H.D. and Kendrick, J.W., 1973. A quantitative serologic assay for diagnosing congenital infections of cattle. Am. J. Vet. Res., 34: 1281—1284.
Schultz, R.D., 1973. Developmental aspects of the fetal bovine immune response. A review. Cornell Vet., 63: 507—535.
Schultz, R.D., Confer, F. and Dunne, H.W., 1971. Occurrence of blood cells and serum proteins in bovine fetuses and calves. Can. J. Comp. Med., 35: 93—98.
Schultz, R.D., Dunne, H.W., and Heist, C.E., 1973. Ontogeny of the bovine immune response. Infect. Immun., 7: 981—991.
Solomon, J.B., 1971. Foetal and Neonatal Immunology, Frontiers of Biology, Vol. 20. North-Holland Publ. Co., Amsterdam, pp. 96—133, 190—201.
Sugimura, T., Tanaka, Y., Kita, E. and Nakahara, T., 1974. Isolation of bacteria and viruses from aborted bovine fetuses. Nat. Inst. Anim. Health Q., 14: 42—43.
Urasawa, S., Urasawa, T., Chiba, S., and Kanamitsu, M., 1968a. Studies on poliovirus inhibitors in sera of domestic animals. II. Determination of poliovirus inhibitors by the precipitation-in-agar-gel method. Japan. J. Med. Sci. Biol., 21: 155—166.
Urasawa, S., Urasawa, T., Chiba, S., and Kanamitsu, M., 1968b. Studies on poliovirus inhibitors in sera of domestic animals. III. A comparison of physico-chemical properties of poliovirus inhibitors and specific antibodies. Japan. J. Med. Sci. Biol., 21: 173—183.
Urasawa, S., Urasawa, T., Akahonai, Y., and Kanamitsu, M., 1969. Studies on poliovirus inhibitors in sera of domestic animals. IV. Fractionation and identification of poliovirus inhibitors. Japan. J. Med. Sci. Biol., 22: 87—99.