Arch Virol (1988) 99: 163–172

Archives of Virology

Physicochemical properties of transmissible gastroenteritis virus hemagglutinin

M. Noda[1], **F. Koide**[2], **M. Asagi**[3], and **Y. Inaba**[4]

[1] Higashi-hiroshima Livestock Hygiene Service Center, Higashi-hiroshima, Hiroshima,
[2] Department of Veterinary Radiology, College of Agriculture and Veterinary Medicine, Nihon University, Fujisawa, Kanagawa,
[3] Nippon Vaccine Co., Ltd., Sakura, Chiba, and
[4] National Institute of Animal Health, Tsukuba, Ibaraki, Japan

Accepted January 14, 1988

Summary. Transmissible gastroenteritis virus was readily adsorbed onto chicken erythrocytes at 4 °C. The hemagglutinin thus adsorbed could be eluted from the erythrocytes by incubating in phosphate buffered saline at 37 °C. The receptor on chicken erythrocytes for the hemagglutinin was inactivated by neuraminidase and potassium periodate, but not by trypsin, 2-mercaptoethanol and formalin. The hemagglutinin was inactivated by trypsin, papain, pepsin, α-amylase, phospholipase C, neuraminidase, formalin, 2-mercaptoethanol, potassium periodate, ethylether, chloroform, Tween-80 and β-propiolactone, but not by sodium deoxycholate and trichlorotrifluoroethane, suggesting that the active component of the hemagglutinin involved glycoproteins. The hemagglutinin was stable at 37 °C or lower temperatures but not at 60 °C or higher temperatures. The hemagglutinin activity was resistant to ultraviolet irradiation, while the infectivity was very susceptible. The hemagglutinin and the infectivity were readily sedimented by ultracentrifugation at 45,000 × g for 60 minutes. In rate zonal centrifugation of the hemagglutinin preparation on a sucrose density gradient, the hemagglutinin activity showed a sharp peak at 1.19 g/ml coinciding with the peak of infectivity. The activity in the peak fraction seemed to be structually associated with virus particles.

Introduction

Transmissible gastroenteritis (TGE) virus, a member of the family *Coronaviridae*, causes a highly contagious disease of swine characterized by vomiting, profuse watery diarrhea, and high mortality in pigs less than 2 weeks of age. Hemagglutination (HA) by some coronaviruses, such as human coronavirus (HCV) [11, 12, 15, 19], bovine coronavirus (BCV) [15, 19, 21, 24], hemagglutinating encephalomyelitis virus (HEV) [7, 15, 16, 18, 19, 20], and infectious

bronchitis virus (IBV) [1, 2, 3], has been reported. We have also shown TGE virus to agglutinate erythrocytes from chickens, guinea pigs, and cattle [17].

In the present study we further investigated the physicochemical properties of the TGE virus hemagglutinin.

Materials and methods

Virus

Three strains of TGE virus were used: the TO strain [4] isolated in Japan, and the Miller [26], and Purdue strains [14] isolated in the U.S.A. All were supplied by Dr. S. Furuuchi, National Institute of Animal Health, Tsukuba, Japan. The TO strain was used in this study, unless otherwise stated. These strains were passaged in primary swine kidney (SK) cell cultures before use in this study.

Cell culture

CPK cells, a continuous cell line derived from pig kidney [13], were grown at 37 °C in Eagle's minimum essential medium (MEM) containing 10% inactivated calf serum 10 per cent tryptose phosphate broth (TPB), 1 per cent of 7 per cent $NaHCO_3$, 100 units/ml penicillin, 100 μg/ml streptomycin, 100 μg/ml kanamycin and 2 μg/ml fungizon. The maintenance medium (MM) was MEM containing 10 per cent of TPB, 2 per cent of 7 per cent $NaHCO_3$ and antibiotics as above.

Preparation of hemagglutinin

Confluent cultures of CPK cells prepared in 500 ml bottles were inoculated with 200 $TCID_{50}$ (see below) of virus, 30 ml of MM added, and incubated at 37 °C for 4 days or longer until the cytopathic effect was complete. The culture fluid was clarified by centrifugation and concentrated by ultracentrifugation as described previously [17] and a 100-fold concentrated suspension was made with Dulbecco's phosphate buffered saline (PBS) (pH 7.2). The hemagglutinin thus prepared was stored at 4 °C or −80 °C.

HA test

This was carried out by the microtiter method using chicken erythrocytes, unless otherwise stated, as described previously [17]. The diluent used was PBS. Blood was obtained in Alsever's solution and stored at 4 °C. Erythrocytes were used in a 0.5 per cent suspension for the HA test. Serial 2-fold dilutions of the hemagglutinin were prepared in 0.025 ml volumes, and mixed with 0.025 ml of erythrocyte suspension after adding 0.025 ml of PBS. The mixtures were incubated at 4 °C overnight before the results were read. The HA titer was expressed as the reciprocal of the highest hemagglutinin dilution showing complete HA.

Infectivity assay

In the infectivity assay of the TGE virus, CPK cell cultures were prepared in 11 × 100 mm tubes. Serial decimal dilutions of the virus suspension in MM were inoculated in 0.1 ml volume into 4 tube cultures per virus dilution and 0.5 ml of MM were added to each tube. The cultures were incubated at 37 °C in a roller drum for 5 days, examined for any cytopathic effect and the $TCID_{50}$ titer was calculated.

Adsorption and elution tests of the hemagglutinins prepared from the TO, Miller, and Purdue strains

Temperature and time required for adsorption

In each of test tubes 0.5 ml of hemagglutinin and 0.5 ml of a 5 per cent suspension of chicken erythrocytes were throughly mixed. The test tubes were divided into two groups. One group was placed in a refrigerator at 4 °C and the other in a water bath at 37 °C. The tubes were removed at various intervals, one from the group at each time. After low-speed centrifugation to sediment erythrocytes, the supernatant was titrated for unadsorbed hemagglutinin. Test tubes containing PBS, instead of chicken erytrhocytes were set up as the control for spontaneous degradation of hemagglutinin during the incubation.

Adsorption with various concentrations of erythrocytes

Mixtures of 0.5 ml of hemagglutinin and 0.5 ml of chicken erythrocyte suspension of various concentrations were incubated at 4 °C. At various intervals free hemagglutinin was measured in the supernatant after low-speed centrifugation in a refrigerated centrifuge.

Elution of adsorbed hemagglutinin

Hemagglutinin was delivered into test tubes in 0.5 ml amounts. The tubes, after receiving 0.5 ml each of 5, 10, or 20 per cent chicken erythrocyte suspension were incubated at 4 °C for 60 minutes and the erythrocytes were collected by low-speed centrifugation in a refrigerated centrifuge. The packed erythrocytes were resuspended in the initial volume of PBS and incubated at 37 °C for various lengths of time. At the end of each incubation one tube was taken out and the eluted hemagglutinin was titrated with the supernatant after low-speed centrifugation to sediment erythrocytes.

Chemical and enzyme treatments of erythrocytes

One volume of 1 per cent erythrocyte suspension was mixed with one volume of 0.02 per cent trypsin (type III, twice crystallized, 11,000 units/mg, Sigma, U.S.A.), 0.02 units/ml neuraminidase (type V, Clostridium perfringens, 1.8 units/mg, Sigma, U.S.A.), 0.5 per cent formalin (Nakarai, Japan), 0.01 per cent potassium periodate (Wako, Japan), 1.6% 2-mercaptoethanol (Wako, Japan) or 0.02 per cent sodium deoxycholate (Difco, U.S.A.) and allowed to stand at 22 °C for 60 minutes except for trypsin and neuraminidase with which the treatment was carried out at 37 °C. The erythrocytes were washed with PBS by centrifugation and used in a 0.5 per cent suspension in HA test. In general a concentration was used wich was as high as could be conveniently reached without causing lysis, nonspecific agglutination or other adverse effects.

Chemical and enzyme treatments of hemagglutinin

The chemical substances used were listed in Table 2. Trypsin (see above), trypsin inhibitor (type I-S, Sigma, U.S.A.), papain (type III, twice crystallized, 25 units/mg, Sigma, U.S.A.), pepsin (1 : 2,500 powder, 450 units/mg, Sigma, U.S.A.), α-amylase (type XI-A, 1,000 units/mg, Sigma, U.S.A.), phospholipase C (type I, 12 units/mg, Sigma, U.S.A.), potassium periodate (see above), sodium deoxycholate (see above), formalin (see above), 2-mercaptoethanol (see above), ethylether (Wako, Japan), chloroform (Wako, Japan), Tween-80 (Wako, Japan), β-propiolacetone (Sigma, U.S.A.), neuraminidase (see above), and trichlorotrifluoroethane (Daifron-S3) (Daikin, Japan) were used. Each was dissolved to make a solution of double strength of the final concentration. After adjusting to pH 7.2 with PBS, each solution was mixed with an equal volume of hemagglutinin, incubated at 37 °C for 5 or 60 minutes and tested for HA activity.

Physical treatments of hemagglutinin

The hemagglutinin was incubated in a water bath at 4, 22, 37, 60, 70, or 100 °C for 5, 10, 20, 30, 60, or 180 minutes and tested for HA activity and infectivity. For ultraviolet irradiation, hemagglutinin delivered in 0.5 ml volumes into petri dishes, 14 mm in diameter, was irradiated for 5, 10, 20, or 30 minutes at a distance of 34 cm with a 15W ultraviolet lamp (Toshiba Co., Tokyo).

Ultracentrifugation

In a type 410 rotor (Damon/IEC, B-60, U.S.A.), 200 ml of infectious culture fluid, after clarified by low-speed centrifugation to remove cell debris, was centrifuged at 45,000 × g for 60 minutes in a Damon/IEC B-60 centrifuge. The sediment was resuspended in 2 ml of PBS. The HA and infective titers were determined for each fraction.

Buoyant density of hemagglutinin

This was determined by rate zonal centrifugation in a linear 10–60 per cent sucrose density gradient. Upon an 11 ml gradient prepared in a 13 ml centrifuge tube, 2 ml of hemagglutinin (128 HA units) was layered and centrifuged in a RPS40T rotor (Hitachi Koki, Co, Japan) at 100,000 × g for 150 minutes in a Hitachi SCP70H centrifuge. Fractions were collected in 0.4 ml volumes by tube bottom puncture and tested for density, infectivity titer and HA titer. The HA titers were obtained by using bovine erythrocyte suspension.

Results

Adsorption and elution of hemagglutinin

When hemagglutinin was mixed with 0.5% or greater concentrations of chicken erythrocytes it was rapidly adsorbed (>95% within 10 minutes) at 4 °C, but not at all at 37 °C. Moreover, hemagglutinin adsorbed at 4 °C to any of several concentrations of chicken erythrocytes (5, 10, 20%) was completely eluted within 20 to 30 minutes by subsequent incubation at 37 °C.

Effect on HA reaction of chemical and enzyme treatments of erythrocytes

As shown in Table 1, erythrocytes treated with potassium periodate gave a much reduced HA titer as compared with untreated erythrocytes. Treatment with the other reagents tested, gave little (neuraminidase) or no change (trypsin, 2-mercaptoethanol, sodium deoxycholate and formalin) in the agglutinability of erythrocytes.

Effect of chemical and enzyme treatments on hemagglutinin

The hemagglutinin was readily inactivated by treatment with trypsin, potassium periodate, chloroform, and β-propiolactone and its activity was considerably reduced with papain, pepsin, α-amylase, phospholipase C, neuraminidase, formalin, 2-mercaptoethanol, ethylether and Tween-80 (Table 2).

In contrast, the hemagglutinin was shown to be resistant to sodium deoxycholate and trichlorotrifluoroethane.

Chicken erythrocytes were also agglutinated by trypsin, neuraminidase, po-

Table 1. Effect on HA reaction of chemical and enzyme treatments of erythrocytes

Treated with[a]	Final concentration	HA titer
Trypsin	0.01%	256
Neuraminidase	0.01 unit/ml	64
Formalin	0.25%	256
Potassium periodate	0.005%	4
2-mercaptoethanol	0.8%	256
Sodium deoxycholate	0.01%	256
Control (PBS)		256

[a] Treated at 22 °C for 60 minutes except for trypsin and neuraminidase with which the treatment was carried out at 37 °C

Table 2. Effect of chemical treatments on hemagglutinin

Treated with[a]	Final concentration	Length of treatment	
		5 min	60 min
Trypsin	0.25%	<4[b]	<4
Papain	0.25%	32	8
Pepsin	0.25%	16	16
α-amylase	0.25%	8	8
Phospholipase C	0.02%	64	64
Neuraminidase	0.1 unit/ml	128	16
Sodium deoxycholate	0.25%	256	256
Formalin	1%	128	64
	0.25%	256	16
2-mercaptoethanol	0.8%	8	2
Potassium periodate	0.25%	<2	<2
Ethylether	50%	64	16
Chloroform	50%	4	2
Tween-80	0.25%	16	16
Trichlorotrifluoroethane	50%	256	256
β-propiolactone[c]	0.2%	4	<2
Control (PBS)	50%	256	256

[a] Treated at 37 °C except for ether, chloroform and trichlorotrifluoroethane with which the treatment was carried out at 22 °C. Trypsin activity was stopped by adding an equal volume of trypsin inhibitor (0.25%) to the mixture of trypsin and hemagglutinin
[b] HA titer after treatment
[c] Chilled at −20 °C before use

tassium periodate and sodium deoxycholate, and lysed by potassium periodate and sodium deoxycholate at the higher concentration than that used in this experiment. The pH of hemagglutinin treated with β-propiolactone declined rather rapidly with the lapse of time.

Effect of physical treatments of hemagglutinin

When the stability of HA activity (256 units) was tested at several temperatures for various intervals of up to 180 minutes, we found that such activity was unchanged at 4, 22, and 37 °C, whereas, at 60 °C it was reached to 2 and <2 HA units in 5 and 10 minutes, respectively, and at 70 and 100 °C it was reduced to <2 HA units in 5 minutes. Storage at −20 or −80 °C gave better results than that at 4 °C. At 4 °C a 2-fold reduction in titer occurred in 7 days and a 4-fold reduction in 30 and 60 days. Storage at −20 °C for 60 days resulted in only a 2-fold reduction in titer. There was no loss of either HA activity or infectivity during 3 months storage at −80 °C.

When the stability of HA activity and infectivity to ultraviolet irradiation was examined for various intervals of up to 30 minutes, the HA activity was unchanged in 5 and 10 minutes, whereas, it was reduced to 1/2 and 1/4 of the original titer in 20 and 30 minutes, respectively. In contrast, the infectivity was rapidly declined, and no titer left after irradiation for 5 minutes (Table 3).

When an infectious culture fluid with an HA titer of 4 was concentrated by ultracentrifugation at 45,000 × g for 60 minutes, a preparation having an HA titer of 1,024 was obtained, whereas no hemagglutinin was detected in the supernatant fluid. In this experiment, the infectivities for the starting material, the supernatant fluid and the sediment resuspended in 1/100 the original volume of PBS, were $10^{5.5}$, $10^{3.5}$, and $10^{6.75}$ $TCID_{50}$/ml, respectively (Table 4).

Table 3. Effect of ultraviolet irradiation on hemagglutinin

Irradiation	HA titer	Infectivity
Before irradiation	256	6.25[a]
5 minutes	256	<0.5
10 minutes	256	<0.5
20 minutes	128	<0.5
30 minutes	64	<0.5

[a] Log $TCID_{50}$/ml

Table 4. Effect of ultracentrifugation on hemagglutinin

Material	HA titer	Infectivity
Starting material	4	5.5[a]
Supernatant[b]	<2	3.5
Sediment[b] (concentrated 100 times)	1,024	6.75

[a] Log $TCID_{50}$/0.05 ml
[b] After centrifugation at 45,000 × g for 60 minutes

Rate zonal centrifugation of hemagglutinin in a sucrose density gradient

Figure 1 illustrates the HA titer, infectivity titer and density of each fraction obtained. The HA activity showed a sharp peak at a density of 1.19 g/ml coinciding with a peak of the infectivity.

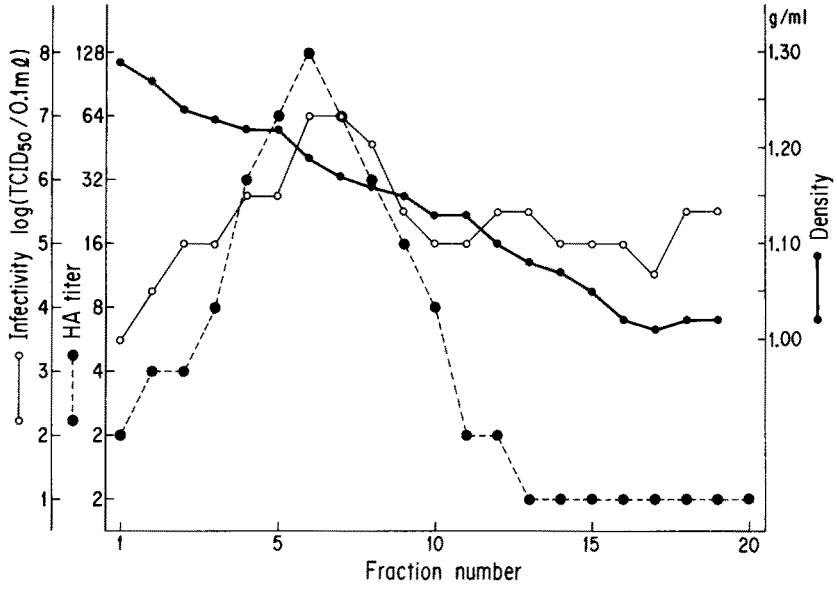

Fig. 1. Sucrose linear density gradient centrifugation of TGE virus hemagglutinin

Discussion

In the present study some physical and chemical properties of TGE virus hemagglutinin were investigated. As shown in the previous paper [17] the HA reaction of TGE virus is dependent on the incubation temperature. A Japanese strain and 2 American strains of TGE virus were readily adsorbed onto chicken erythrocytes at 4 °C but not at 37 °C. The hemagglutinin thus adsorbed onto erythrocytes was readily eluted when the erythrocytes was resuspended in PBS and incubated at 37 °C. Elution of hemagglutinins of HEV and BCV from erythrocytes has not been observed [7, 16, 19, 24], but on the other hand those of HCV, MECV and IBV have been demonstrated to elute from erythrocytes [1, 3, 11, 12, 27].

The chicken erythrocyte receptor for the hemagglutinin of TGE virus was inactivated by treatment with neuraminidase and potassium periodate, but was not inactivated with trypsin, formalin, 2-mercaptoethanol and sodium deoxycholate. The fact that the receptor was destroyed by neuraminidase, together with the results of elution of hte hemagglutinin from erythrocytes, suggests the presence of a neuraminidase-like enzyme in the hemagglutinin of both TGE virus as well as IBV [2], although no neuraminidase activity was detectable. On the other hand, the elution of the hemagglutinin of HCV and MECV is not

neuraminidase mediated as erythrocyte receptors are not lost and neuraminidase assays have remained negative [11, 19, 27]. Sentsui and his associates reported that neuraminidase rendered horse, cat, and guinea pig erythrocytes more susceptible to agglutination by equine infectious anemia virus [22] and mouse erythrocytes more susceptible to agglutination by bovine leukemia virus [23]. Further orthomyxoviruses are well known to elute from erythrocytes at 37 °C by neuraminidase activity possessed by the virus particle [6, 10]. The TGE virus used in this study did not show any cross reaction with the swine influenza virus A (Wisconsin strain), HEV, BCV, and porcine parvovirus by hemagglutination inhibition and neutralization tests in our laboratory (data not shown).

The hemagglutinin of TGE virus was inactivated by trypsin, papain, pepsin, α-amylase, phospholipase C, neuraminidase, formalin, 2-mercaptoethanol, potassium periodate, ethylether, chloroform, Tween-80 and β-propiolactone, but not by sodium deoxycholate or trichlorotrifluoroethane, suggesting that the active component of the hemagglutinin might involve glycoproteins. The hemagglutinating activities of HCV, HEV, and IBV were also destroyed by trypsin, but not in case of MECV [2, 3, 7, 11, 12, 19, 27]. On the other hand, treatment with trypsin, ether, and Tween-80 and ether often enhances the activity of HA of certain paramyxoviruses [9, 25].

The hemagglutinin of TGE virus was stable at 37 °C or lower temperatures, whereas it was labile at 60 °C. The hemaglutinating activity was relatively resistant to ultraviolet irradiation, while the infectivity was very susceptible. Similar results were obtained with the hemagglutinins of HEV, BCV, HCV, and IBV [3, 7, 11, 12, 15, 16, 18, 19, 24].

Two observations suggested that the hemagglutinin is an integral part of the virion. First, infectivity and HA activity were cosedimented by ultracentrifugation. Second, both infectivity and HA activity had a buoyant density of 1.19 g/ml in a linea sucrose gradient. A virion density of 1.19 g/ml is in agreement with the previous results obtained with Minnesota and Illinois strains of TGE virus [26]. Several coronaviruses, HCV, HEV, BCV, MECV, and IBV, have been demonstrated to possess a virion associated hemagglutinin [3, 7, 12, 20, 24, 27]. Mengeling et al. [16] observed by electron microscopy that when HEV attached to chicken erythrocytes, the main body of the virus was located 10 to 15 nm from the erythrocyte membrane suggesting that the hemagglutinin was associated with the virus surface projections. Likewise HA with IBV has been confined to the strains which have projections, and treatment of HCV, HEV, MECV, and IBV with bromelain removes the hemagglutinating activity together with the projection [2, 3, 8, 20, 27]. Moreover, subunits removed from HEV virions by detergent treatment probably represent surface projections and also exhibit hemagglutinating activity [20]. In HCV, it has been suggested that the hemagglutinating activity is carried by one or both the glycopeptides of the projections [8], and the glycopeptide of TGE virus has been demonstrated as the major structural component of the projection [5].

Further detailed studies will be necessary to compare the hemagglutinin of TGE virus with that of coronaviruses from other species.

References

1. Alexander DJ, Chettle NJ (1977) Procedures for the haemagglutination and the haemagglutination inhibition tests for avian infectious bronchitis virus. Avian Pathol 6: 9–17
2. Berry DM, Cruickshank JG, Chu HP, Wells RJH (1964) The structure of infectious bronchitis virus. Virology 23: 403–407
3. Bingham RW, Madge MH, Tyrrell DAJ (1975) Haemagglutination by avian infectious bronchitis virus—a coronavirus. J Gen Virol 28: 381–390
4. Furuuchi S, Shimizu Y, Kumagai T (1975) Comparison of properties between virulent and attenuated strains of transmissible gastroenteritis virus. Natl Inst Anim Health Q (Jpn) 15: 159–164
5. Garwes DJ, Pocock DH, Pike BV (1976) Isolation of subviral components from transmissible gastroenteritis virus. J Gen Virol 32: 283–294
6. Gottschalk A (1965) The significance of neuraminic acid, serine and threonine for a glycoprotein inhibiting influenza virus haemagglutination. Aust J Exp Biol Med Sci 43: 391–393
7. Greig AS, Bouillant AMP (1972) Studies on the hemagglutination phenomenon of hemagglutinating encephalomyelitis virus (HEV) of pigs. Can J Comp Med 36: 366–370
8. Hierholzer JC, Palmer EL, Whitfield SG, Kaye HS, Dowdle WR (1972) Protein composition of coronavirus OC43. Virology 48: 516–527
9. John TJ, Fulginiti VA (1966) Parainfluenza 2 virus: increase in hemagglutinin titer on treatment with Tween-80 and ether. Proc Soc Exp Biol Med 121: 109–111
10. Kathan RH, Winzler RJ (1963) Structure studies on the myxovirus hemagglutination inhibitor of human erythrocytes. J Biol Chem 238: 21–25
11. Kaye HS, Dowdle WR (1969) Some characteristics of hemagglutination of certain strains of "IBV-like" virus. J Infect Dis 120: 576–581
12. Kaye HS, Hierholzer JC, Dowdle WR (1970) Purification and further characterization of an "IBV-lik" virus (Coronavirus). Proc Soc Exp Biol Med 135: 457–463
13. Komaniwa H, Fukusho A, Shimizu Y (1981) Micro method for performing titration and neutralization test of hog cholera virus using established porcine cell strain. Natl Inst Anim Health Q (Jpn) 21: 153–158
14. McClurkin AW, Norman JO (1965) Studies on transmissible gastroenteritis of swine I. The isolation and identification of a cytopathogenic virus of transmissible gastroenteritis in primary swine kidney cell cultures. Can J Comp Med Vet Sci 29: 46–53
15. McIntosh K (1974) Coronaviruses: a comparative review. Curr Top Microbiol Immunol 63: 85–129
16. Mengeling WL, Boothe AD, Ritchie AE (1972) Characteristics of a coronavirus (strain 67N) of pigs. Am J Vet Res 33: 297–307
17. Noda M, Yamashita H, Koide F, Kadoi K, Omori T, Asagi M, Inaba Y (1987) Hemagglutination with transmissible gastroenteritis virus. Arch Virol 96: 109–115
18. Pensaert MB, Callebaut PE (1974) Characteristics of a coronavirus causing vomition and wasting in pigs. Arch Ges Virusforsch 44: 35–50
19. Pensaert M, Callebaut PE (1978) The coronaviruses: clinical and structural aspects with some practical implications. Ann Méd Vét 122: 301–322
20. Pocock DH, Garwes DJ (1977) The polypeptides of haemagglutinating encephalomyelitis virus and isolated subviral particles. J Gen Virol 37: 487–499
21. Sato K, Inaba Y, Kurogi H, Takahashi E, Ito Y, Goto Y, Omori T, Matumoto M (1977) Hemagglutination by calf diarrheal coronavirus. Vet Microbiol 2: 83–87
22. Sentsui H, Kono K (1981) Hemagglutination by several strains of equine infectious anemia virus. Arch Virol 67: 75–85

23. Sentsui H, Thorn RM, Kono Y, Ferrer FJ (1982) Haemagglutination by bovine leukaemia virus. J Gen Virol 59: 83–89
24. Sharpee RL, Mebus CA, Bass EP (1976) Characterization of a calf diarrheal coronavirus. Am J Vet Res 37: 1031–1034
25. Sokol F, Blaskovic D, Rosenberg M (1961) Subunits of myxoviruses. I. Treatment of Newcastle disease, parainfluenza 1, and mumps viruses by ether. Acta Virol 5: 64–77
26. Stone SS, Kemeny LJ, Jensen MT (1976) Partial characterization of the principal soluble antigens associated with the coronavirus of transmissible gastroenteritis by complement fixation and immunodiffusion. Infect Immun 13: 521–526
27. Sugiyama K, Amano Y (1980) Hemagglutination and structural polypeptides of a new coronavirus associated with diarrhea in infant mice. Arch Virol 66: 95–105

Authors' address: Y. Inaba, National Institute of Animal Health, Tsukuba, Ibaraki, 305 Japan.

Received November 10, 1987