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AN INVESTIGATION AND CHARACTERIZATION OF ENTEROVIRUS STRAINS IN SWEDISH PIGS

I. ISOLATION, BIOLOGICAL, CHEMICAL AND PHYSICAL PROPERTIES[1])

By

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In the search for poliomyelitis virus in faecal material of man, using the tissue-culture technique, a number of apparently related viruses were isolated. Whereas poliomyelitis and coxsackie viruses were already known as causes of diseases, a great number of so-called ECHO-viruses had, to begin with, no association with any disease. In 1957 the Committee on Enteroviruses expressed the opinion that the poliomyelitis, coxsackie, and ECHO viruses should be classed as one entity under the heading Enteroviruses (14). They inhabit the digestive tract of man and are pathogenic to human and monkey tissue cultures. Neutralizing serum-antibodies are present in individuals from whom the viruses are isolated, as an evidence that infection does occur. They are resistent to ether. The particle-size does not exceed 35 mμ. The occurrence of the enteroviruses shows a seasonal prevalence and they have a characteristic epidemiological pattern (14, 25).

Soon after this, studies were published on the isolation from different animals of viruses which possessed properties similar to those of the Enterovirus group. In connection with the tissue-culture work using monkey tissues, a number of such agents were isolated from monkeys. In domestic animals they have so

[1]) Aided by grants from Fonden för främjande av forsknings- och försöksverksamheten på jordbrukets område.

far been found in cattle, swine, and, more seldom, birds and cats (25).

To the enteroviruses of pigs belongs the causal agent of the *Teschen disease,* which has been known since 1930. The *Talfan disease* (13, 15, 34) and the *benign enzootic paresis* in Denmark (2, 49) are considered to be mild forms of the Teschen disease. Teschen virus was first cultivated in tissue culture by *Larski* (29) and *Mayr & Schwöbel* who performed a more elaborated study (30, 31, 32).

In the last few years several enteroviruses have been demonstrated in healthy swine under the name of ECSO or ECPO (Enteric Cytopathogenic Swine/Porcine/Orphans). In 1958, and subsequently, *Beran et al.* reported the isolation of a great number of viruses belonging to one prototype (3, 4, 51). In 1958 *Lamont & Betts* (27) reported about 13 isolates of three immunologically distinct types. In 1959 *Webster* in New-Zeeland described 5 isolates of one prototype (50), *Moscovici et al.* in U.S.A. one isolate (39) and *Hancock et al.* 30 isolates distributed over five different serogroups (12, 20, 47). In 1960 *Betts et al.* (England) published reports concerning 3 isolates of two serotypes (5, 6, 7, 8, 9, 24) and *Sibalin & Lannek* (Sweden) reported 9 isolates of one established serotype (43, 44). In the following year *Mayr & Bögel* in Germany isolated 4 swine enteroviruses belonging to two serotypes (11, 33). *Szentiványi* in Hungary described several isolates (48), and *Betts et al.* 19 isolates belonging to nine serogroups (10). *Morimoto et al.* (38) in Japan isolated from faeces of swine two serotypes of enteroviruses. *Mitchell et al.* (37), *Greig et al.* (19) and *Richards & Savan* (42) in Canada isolated a serotype of porcine enterovirus from pigs with nervous symptoms. It was serologically distinct from the virus of Teschen disease.

In 1962 *Pette* in Germany described 15 enterovirus isolates grouped into five serotypes (41). *Izawa et al.* from the U.S.A. reported about two swine enterovirus serotypes. One of them was isolated from swine affected with diarrhoea and enteritis (22, 23).

Most isolates originated from the faeces of healthy swine. Exceptions were the isolates of *Moscovici* (39) and some isolates of *Hancock et al.* (20, 47), which originated from pigs with diarrhoea. Experimental evidence of disease in the host animal caused by enteroviruses, was given by *Moscovici* (39), and by *Betts et al.* (7) who produced encephalomyelitis in so-called antibody-free pigs using strain T80. *Sibalin & Lannek,* who used

strain S180/4, produced encephalomyelitis and pneumonitis in colostrum-deprived piglets. *Izawa et al.* produced gastroenteritis, paralysis and poliomyelitis-like lesions in the central nervous system of colostrum-deprived pigs, using enterovirus strains of one serotype originated from pigs affected with enteritis (23).

Little is known of the relationship between the enterovirus strains isolated by different workers. The presence of one serotype in different countries has been established, however (44). Thus, strains T80, T52A (England), S180/4 (Sweden) (44), and ES Tübingen 1 (Germany) belong to the same serotype (11, 33).

The definition of enteroviruses in domestic animals and their characterization follow the lines laid down by the Committee on human enteroviruses. According to *Mayr & Bögel* (11, 33) animal enteroviruses are characterized by cytopathogenicity to the tissue culture, strong affinity to the digestive tract, a particle-size of up to 35 mμ, and resistance to chloroform. *Betts et al.* have suggested that swine enteroviruses should fulfil the following four criteria: particle size not exceeding 30—40 mμ, resistance to 20 % ether during 18 hours at 4°C, elimination in the faeces for a considerable time, and production of cytopathogenic effect on swine tissue culture (10).

In the autumn 1960 a series of isolations of ether-resistant viruses from healthy Swedish pigs were attempted. Altogether 16 viruses belonging to 8 different serogroups were isolated (46). One strain of each serogroup was more closely examined for properties in swine-kidney tissue culture, temperature sensitivity, resistance to ether and chloroform and the size of the infective particle.

MATERIAL AND METHODS

Collecting and handling of specimens. During August-September, 1960, faecal and blood specimens were collected from pigs in three slaughterhouses (Nyköping, Uppsala, and Västerås) in the central part of Sweden. The pigs were normal slaughter pigs at the age of 6—7 months. Each pig represented one separate herd, mostly from farms with a small number of pigs. Twenty faecal and corresponding blood specimens were taken at each slaughterhouse. The specimens were marked with a letter (the intial of the name of the place, N = Nyköping, U = Uppsala, and V = Västerås) and numbers 1—20 for each slaughterhouse.

The faecal specimens were taken at the processing of the pigs,

i. e. 10—20 minutes after they had been electrically executed and stuck. A formed faecal ball in the upper part of rectum or lower part of the colon was selected. The blood specimens were collected when the pigs were stuck.

The faecal specimens were stored at —20°C until they were prepared for inoculation of tissue-culture tubes. The specimens were then thawed at room temperature. A 10 % suspension was made in Hanks' buffered salt solution (Hanks' BSS) containing 0.5 % hydrolysed lactalbumin but without serum. After addition of 200 i. u. of penicillin and 100 μg. of streptomycin per ml., the suspension was centrifuged at 8,000 r.p.m. for 10 minutes at room temperature. Ten t. c. tubes were then inoculated with 0.2 ml. of the supernatant to each tube. During the next 10 days the tubes were screened daily for cytopathogenic changes. Non-inoculated tubes were examined at the same time. No blind passages were performed. The N and V strains were isolated in one batch of t. c. tubes, *i. e.* originating from one swine kidney, and the U strains in another.

Preparation of tissue cultures. Swine-kidney t. c. was prepared in tubes and in flasks by the method of *Dulbecco* as described previously (16). Four to five week old pigs were used. By the trypsinization isolated cells and clusters of cells were obtained. To 100 ml. of medium was added 0.7 ml. of packed cells or, to flasks, 0.9—1.0 ml., which resulted in monolayer cultures within 3—4 days. The growth medium contained 5—7.5 % of filtered active calf serum. No serum was added to the maintenance medium. Each tube contained 1 ml. of medium and was used within a week after the appearance of a confluent monolayer culture. The composition of the media and other details have been described previously (43).

Plaque technique. Monolayers of swine-kidney cells were prepared in flasks of neutral glass measuring 14×4.5×4.5 cm., the surface for t. c. amounting to about 50 sq.cm. On the whole, the technique of *Dulbecco* (17) was followed. Hanks' BSS containing 0.5 % lactalbumin and 2—5 % calf serum was substituted for Earle's BSS. After removal of the fluid medium the monolayers were washed three times with 10 ml. of PBS (17). Each flask was then inoculated with 0.1 ml. of the diluted virus suspension. At higher dilutions 4 flasks per dilution were used. The time of adsorption was 1 hour at 37°C. When pure progeny cultivation was intended, the flasks were washed three times with

10 ml. of PBS at 37°C. At plaque titrations (determination of plaque-forming units/PFU/), no washing was done after the adsorption.

The amounts of Hanks' A and B solutions were concentrated 10 times calculated so that the final agar-medium mixture would contain 5 % of A and B solution, respectively. The cell culture was then covered with 15 ml. of the final mixture.

For plaque purification, flasks containing one or two plaques with margins situated not less than 2 cm from each other were used.

Titration of virus suspensions. The fluid of the t. c. tubes which were used for isolation of the agent from faeces was pooled when the cytopathogenic changes comprised 70—90 % of the cell culture. The second passage was tested for virus content. From this passage one tube showing cytopathic changes was passaged at a dilution close to the upper dilution limit (limit-dilution method). This was done twice. The isolated strains were then transferred to t. c. flasks and this 5th passage was treated with ethyl ether (1). The titre was compared with that obtained without ether treatment.

Limit-dilution suspension of the ether-treated strains was inoculated on Roux flasks. Thus, the 6th passage of virus strains had been purified three times by limit dilution and treated once with ether.

The harvest of the flasks was centrifuged at 2.000 r.p.m. for 15 minutes. The supernatant was divided into amounts of 5 ml. and stored at —20°C. The 6th passage was the main store for further examination.

All subsequent propagation of virus was done in flasks. Before use the suspensions were thawed in a water-bath at 37°C.

At titrations of virus suspensions ten-fold dilutions were prepared in PBS using 3—5 t. c. tubes per dilution. At titrations in the resistance experiments and in studies of growth curves 5 tubes were always used. The volume for inoculation was 0.1 ml. per tube. Thereafter the medium was not changed. The tubes were observed microscopically several times until the 7th day after the inoculation, when $\log_{10}TCD_{50}$/ml. was determined by *Kärber*'s method (26).

Viruses used. In addition to the new isolates described here, some other viruses were used for control purposes, namely the swine-enterovirus strains S180/4 and S159 (43, 44), the

Teschen strain U6 isolated in Sweden (45), and strain Tirol. The latter was kindly provided by Dr. *A. Mayr,* Federal Institute for Animal Virus Diseases, Tübingen, Germany.

Growth experiments. A virus amount of 1.000 TCD_{50} contained in a volume of 0.1 ml. was inoculated into each of a sufficient number of tubes. The fluid medium of 5 tubes was pooled for titration after 0, 6, 12 and 24 hours and then daily for 5 days. All titrations were done in t. c. tubes prepared from the same kidney.

Resistance experiments. The 6th virus passage, stored at —20°C, was titrated at convenient intervals. Suspensions of the same virus passage, stored at 4°C, was titrated after three months. The resistance against higher temperatures, 37°C and 56°C, was examined by keeping the virus suspension in sealed Vidal tubes in a water-bath. The virus kept at 37°C was titrated at 0 and after 24 hours, and the virus kept at 56°C at 0 and after 60 minutes.

Ether and chloroform sensitivity tests. Ether-sensitivity tests were done by the method of *Andrewes & Horstmann* (1). Virus suspension of the 5th passage was centrifuged at 2.000 r.p.m. and transferred to flasks. Ethyl ether was added to make 20 % by volume. The flasks were shaken for 1 hour, then kept at 4°C for 18 hours, and finally at 37°C until all ether had evaporated. Titration was then done. A corresponding amount of virus suspension was carried through the same procedure but without the addition of ether.

Chloroform sensitivity was determined by the method of *Mayr & Bögel* (33). After a passage for reactivation, chloroform was added to make 10 % by volume. After shaking for an hour the mixture was kept at 4°C for 18 hours, then centrifuged at 2.000 r.p.m. for 10 minutes and titrated.

The sedimentation constants (s_{20}) were determined by gradient centrifugation in sucrose solution.[1])

Histological examination. With appropriate intervals tubes inoculated with 1.000 TCD_{50} were fixed in Bouin's solution for 2 hours, embedded in 4 % collodium, and stained with haematoxylin and eosin for microscopical observation.

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[1]) Kindly performed by Dr. *K. Strohmaier,* Federal Institute for Animal Virus Diseases, Tübingen, Germany. The technical procedure is to be published.

RESULTS

The frequency of cytopathogenic agents isolated from the examined pigs is shown in Table 1. Out of the 60 specimens ether-resistant strains were isolated from 16 (26.6 %). In 4 specimens (6.6 %) agents were observed which were ether sensitive, while 39 specimens (65 %) gave wholly negative results. There were no obvious differences in frequency between the three slaughter-house materials, which speaks in favour of a fairly equal occurrence of the viral agents under discussion in the eastern part of central Sweden during this time of the year.

Table 1. Results of isolations of viral agents from faecal specimens of pigs in central Sweden during August-September, 1960.

Area	Date for collection of specimens	Number of examined pigs (herds) (%)	Results of the isolation attempts (1st passage)		
			Ether-resistant %	Ether-sensitive (%)	Negative (%)
Nyköping (N)	Sept. 1960	20 (100)	5 (25)	2 (10)	13 (65)
Uppsala (U)	Aug. 1960	20 (100)	4 (20)	1 (5)	15 (75)
Västerås (V)	Sept. 1960	20 (100)	7 (35)	1 (5)	12 (60)
	Total:	60 (100)	16 (26.6)	4 (6.6)	40 (66.6)

The characteristic data for the virus strains in t. c. are given in Table 2. The first cytopathic changes at an inoculation dose of 1.000 TCD_{50} are observed after 24—36 hours at the earliest, or, on the average, 48—72 hours after the inoculation. The focal appearance of the changes was evident for all strains.

Two different forms of cytopathic pattern were observed. In one type there appear, at the beginning, rounded cells with hyperchromatic nuclei and expanding outflowing cytoplasm (Figs. 7, 10). In the subsequent stage the cells, which now have markedly hyperchromatic and pyknotic nuclei, detach themselves from the glass surface. The foci that appear first as well as later ones, increase, so that in an advanced phase of the degeneration only a net-like pattern of monolayer remains (Figs. 8, 9). A complete destruction has generally occurred after 96—120 hours. At an inoculation dose larger than 1.000 TCD_{50} the foci appear faster and the net-like pattern develops earlier.

Table 2. Properties of the isolated virus strains in swine kidney t. c. including resistance to ether and chloroform. (Titres are given as $\log_{10}$ TCD_{50}/ml).

Designation of isolates	First appearance of CPE in 1st passage	Number of CP titre tubes with CPE in 1st passage[1])				Resistance to					
		Days		Passage		Ether (20% by volume)			Chloroform (10% by volume[2])		
		3	5	2	3	Not treated	Treated	Difference	Not treated	Treated	Difference
N4	3	5	7	5.9	6.5	6.1	1.5	— 4.6 (99 %)			
N5	4	0	4	5.5	5.3	5.5	5.7	+ 0.2			
N7	1	8	10	6.9	7.7	7.5	7.5	— 0.2	7.1	6.7	— 0.4
N8	2	3	8	6.1	7.1	6.9	7.3	+ 0.4			
N10	3	4	10	6.5	6.9	6.9	6.9	0.0			
N11	4	0	6	5.7	6.1	6.3	6.5	+ 0.2	6.5	6.3	— 0.2
N15	3	4	8	7.1	7.7	8.1	3.5	— 4.6 (99 %)			
U1	2	7	9	6.5	7.1	7.1	6.9	— 0.2	6.5	6.1	— 0.4
U4	2	8	10	6.9	7.7	7.3	7.7	+ 0.4			
U6	2	6	9	7.5	7.7	7.3	7.1	+ 0.2	7.5	7.5	0.0
U8	1	7	10	6.1	6.9	7.3	4.9	— 3.4 (99 %)			
U10	3	4	8	5.7	7.5	6.9	7.3	+ 0.4	7.1	7.3	+ 0.2
V4	3	6	10	6.9	7.3	6.7	7.1	+ 0.4	6.9	7.1	+ 0.2
V5	2	8	10	5.5	5.7	5.7	5.5	— 0.2			
V10	3	4	10	6.1	6.3	6.3	6.5	+ 0.2			
V11	3	7	10	6.1	6.9	6.9	0.0	— 6.9 (100 %)			
V13	4	0	6	4.1	6.1	6.3	6.7	+ 0.4			
V14	2	8	10	6.1	6.9	6.7	7.1	— 0.4			
V16	3	4	8	6.9	7.1	6.5	6.1	— 0.4			
V18	4	0	4	4.9	6.9	7.1	7.3	— 0.2			
S180/4	—	—	—	—	—	7.1	6.9	— 0.2			
S159	—	—	—	—	—	6.5	6.5	0.0			

[1]) Total number of tubes = 10. Volume inoculated 0.2 ml.

[2]) The tests on resistance to ether and chloroform were not done simultaneously.

Acidophilic cytoplasmatic inclusion bodies are rare.

The strains which are characterized by this form of changes are N11, U6, V4 and S180/4.

The second type of cytopathic pattern also starts with degenerating foci, but these expand more diffusely than in the first type, so that no net-like picture develops (Fig. 5). It is easy to separate these two types from each other in the microscope at low magnification.

The second type is also characterized by hyperchromatic nuclei, but expanding cytoplasm is not observed. A typical phenomenon ih pericellular drop-like protrusions, apparently originating from the cytoplasm (Fig. 6), often forming a corona adjacent to the cell. In subsequent stages the nucleus will be denser and covered by retracting cytoplasm. The cell detaches itself from the glass.

The second type of cytopathic picture is seen with the N7, U1, U10, and S159 strains. With these strains the first changes appear after about 24 hours and are completed after 72—120 hours. S159 is especially fast. Here the 26th passage caused the first visible foci after 16 hours and a complete destruction after 72—80 hours.

In the cultivation of all the strains it was apparent that the cytopathic changes appeared and were completed somewhat earlier in the later passages than in the first ones after the isolation.

The fluid of the inoculated tubes was harvested between 3 and 5 days after the inoculation, or when 50—80 % of the cells showed degeneration. If a tube was left for observation without harvesting the fluid, the destruction of the cells generally comprised about 95 % of the cells. The non-inoculated control tubes showed no cytopathic changes during an observation time of 14 days. In the first two or three passages after the isolation the virus titre increased, probably owing to a certain adaptation to the t. c. conditions.

The microscopical appearance of the tissue-lesions of the isolated strains, starting as degenerative foci, was well developed in the 2nd passage. Most strains had cytopathic changes similar to those of S180/4. No difference in this appearance was brought about by the limit dilution or ether treatment.

The ether-sensitive strains N4, U8, and V11 also caused focal cytopathic changes, which rapidly spread to nearly the whole monolayer.

The remaining cells in the t. c. tubes, which do not become degenerated and necrotic, vary in number from one t. c. batch to

Table 3. Plaque types and titration of the strains.

Strain	First appearance of plaques (days after inoculation) [1]	Maximal number of plaques at highest virus dilution on days	Description of plaques	Size of individual plaques on days [2]				CP titre in t. c. tubes ($log_{10}TCD_{50}$/ml.)	PFU/ml. log_{10}
				1	3	5	8		
N7	2—3	4—5	Distinct boundaries, large	3—5	8—10	16—20	20—26	7.1	7.0
N11	4—5	6—8	Indistinct boundaries, small	< 1—1	1—2	2—3	3—4	6.5	6.0
U1	2—3	5—6	as N7	3—5	8—10	13—16	16—20	7.1	7.5
U6	4—5	6—8	as N11	< 1	1	2—3	3—4	7.3	7.0
U10	3	4—5	as N7	2—4	5—7	11—15	15—18	6.5	7.0
V4	2—3	5—6	Indistinct boundaries, medium-sized	1—2	2—5	6—8	8—10	7.1	7.0
Tirol	4	6	as N11	1	2	2—3	4—5	6.7	7.0
S180/4	2—3	5—6	as V4	1—2	2—5	6—8	8—10	6.3	6.6
S159	2	3—5	as N7	3—5	9—12	16—24	20—30	5.9	6.4

[1]) At dilution 10^{-1} of the corresponding virus suspension.
[2]) At dilution 10^{-4}—10^{-6} of the corresponding virus suspension.

another. In some batches all the strains have caused a complete destruction. Generally, in older t. c. tubes, for instance more than 1 week old when inoculated, a greater part of cells will be left than in new ones.

For all the isolates that could be propagated the limit dilution method was used for the 2nd and 3rd passage. The 5th passage was tested against ethyl ether (Table 2). All the strains except N4, N16, U8, and U11 were resistant. Out of 18 other strains there was a slight titre decrease ($\leqq 0.4 \log_{10}$) in 6, a slight titre increase ($\leqq 0.4 \log_{10}$) in 10, and no change in 2 strains. There seems to be no reason to expect that the titre changes observed were an effect of the ether treatment. The sensitive strains lost more than 99 % of their infectivity.

As is shown in Table 2 the tested strains are also resistant to chloroform. Only one strain representative of each serological group was tested, however.

In Table 3 the plaque characteristics of the examined strains

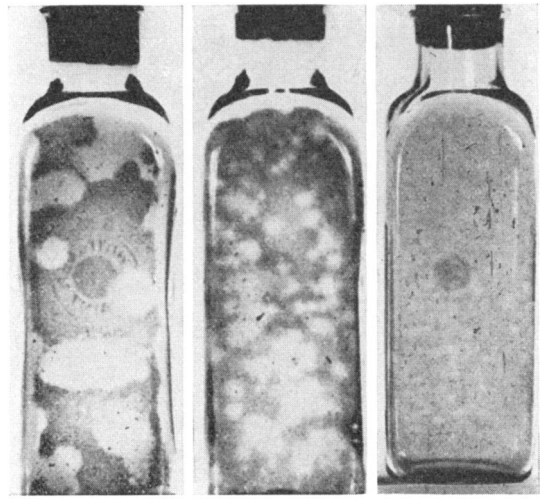


Fig. 1. Three different plaque types (cf. the text). No washing done after adsorption.

1 a. Large plaques with distinct boundaries, strain N7, 7 days after appearance.

1 b. Medium-sized plaques with indistinct boundaries, strain V4, 7 days after appearance.

1 c. Small plaques with indistinct boundaries, strain U6, 7 days after appearance.

Table 4. Influence of storage and heat on the enterovirus strains N7, N11, U1, U6, U10, V4, S180/4, and S159, mean titres ± standard error of the mean are expressed as neg $\log_{10}TCD_{50}$/ml.

Storage at				Exposure to heat at			
—20°C		4°C		37°C		56°C	
t. c. titre after months				t. c. titre after hours		t. c. titre after minutes	
0	6	0	3	0	24	0	60
6.8 ± 0.19	5.8 ± 0.19	6.8 ± 0.19	4.8 ± 0.26	6.4 ± 0.24	4.8 ± 0.19	6.4 ± 0.14	1.3 ± 0.

are shown. It will be seen that three different plaque forms are produced. In the first form (Fig. 1 a) the plaques develop fast and have sharp borderlines against non-affected tissue. This form is produced by strains N7, U1, U10, and S159.

The second form (Fig. 1 b) which is produced by strains V4 and S180/4, has less distinct borderlines and grows more slowly. At the same time after the inoculation these plaques are therefore, on an average, smaller than in the first form.

The smallest plaques (3rd plaque form) is produced by the Teschen strain Tirol and by U6 and N11. The borderline against surrounding tissue is indistinct (Fig. 1 c). Determination of PFU for strains N11 and U6 often required the use of the microscope in order to exclude the possibility of artefacts.

For most strains the number of PFU gave a higher titre than did titration using the cytopathic effect in t. c. tubes. The data in

Table 5. Data from determination of sedimentation constants (s_{20}).

Strain	Titre virus suspension		Centrifugation			Evaluation		
	initial dilution	titre	r.p.m.×1000	Time minutes	G 10^{10} sec^{-1}	mean temperature °C	Median position	s_{20}
N7	1:100	$10^{-3.7}$	23.7	60	2.14	20.6	9.00	143
V4	1:100	x	24	60		20.2	x	x
U10	1:10	$10^{-4.5}$	23.8	60	2.16	2x.2	8.98	140
N11	1:100	$10^{-2.0}$	23.8	60	2.17	21.3	9.10	146
V4	1:10	$10^{-5.7}$ $10^{-5.0}$	23.9	60	2.17	21.8	8.91	129
U1	1:10	$10^{-4.5}$	23.8	60	2.17	20.4	9.04	143
V4	1:100	$10^{-2.7}$	23.8	60	2.14	20.5	8.94	136
SL 24	1:10	$10^{-3.7}$	23.9	60	2.17	20.0	8.98	139

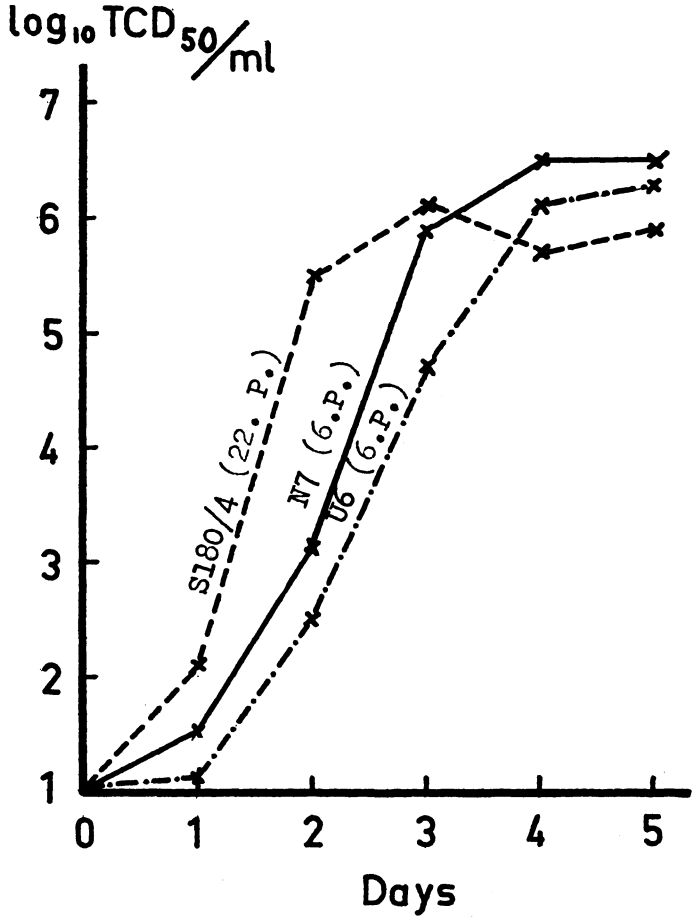


Fig. 2. Release of virus.

Table 3 on the number of PFU per ml. and titre determined in tubes were taken from the third plaque passage.

For estimation of plaque-size all three plaque passages were considered. The consistency of plaques, especially the large ones, seem to be especially dependent on the quality of the tissue culture. For growth-curve studies three strains were selected. Strain S180/4, which had undergone the most numerous passages, showed the most rapid release of virus into the medium. With an inoculation dose of 1.000 TCD_{50} it reached a maximum titre on the 3rd day, whereas the two other strains, N7 and N11, reached corresponding maxima after 4—5 days (Fig. 2).

The influence of storage and temperature is shown in Table 4.

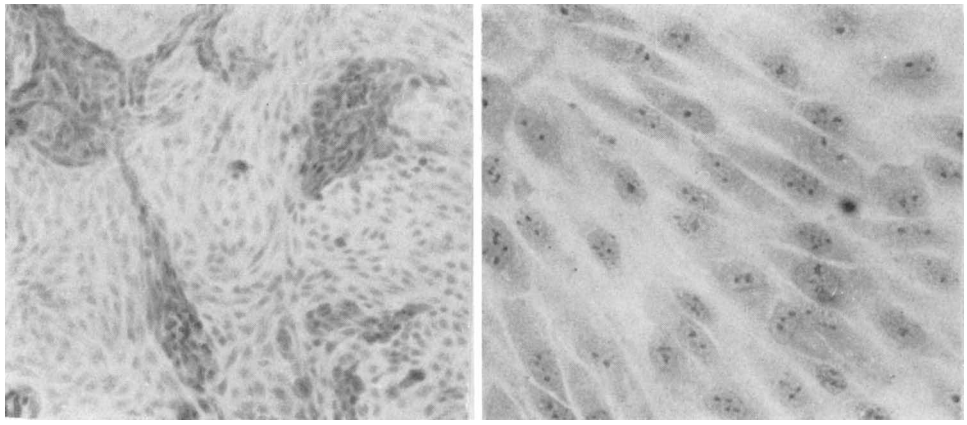
Determination of the sedimentation constant for S159 by *Mayr & Bögel* (10, 11) gave a sedimentation constant of 157.7. The sedimentation constants of the other strains, excluding U6, are shown in Table 5.

DISCUSSION

Viruses which have properties in common with the enteroviruses have been isolated from the faeces of pigs in many countries. Most papers on this subject deal with the characterization of the swine enteroviruses, aiming at a classification similar to that of human enteroviruses. Although the interest had been focussed on the relations to the human enteroviruses, the association between the recently isolated swine enteroviruses and the cause of the Teschen disease has not attracted the same attention. It has been demonstrated (34) that the Teschen disease and poliomyelitis in man have similar epidemiological patterns. Because of the sporadic or enzootic occurrence and the marked variation in the severity of the disease, the causal agents of the Talfan disease and benign enzootic paresis have only recently been accepted as members of the Teschen group. In this paper the name enterovirus has been used regardless of the disease-producing capacity of the strain. Eight selected swine-enterovirus strains belonging to antigenically different groups fulfil the criteria established by the Committee on Enteroviruses of 1957. Accordingly, they are resistant to ether, have a particle-size of about 35 mμ, produce CPE in swine-kidney t. c., are eliminated via the faeces for a long time, and are separated from other viruses.

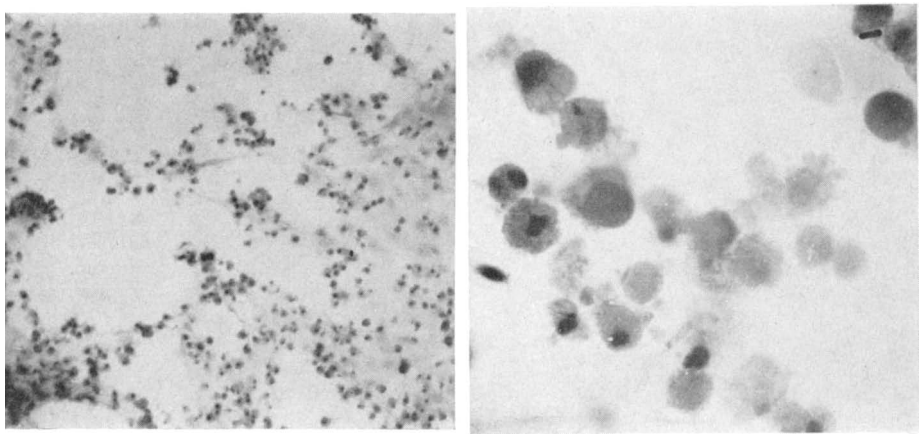
The CPE of strains N11, U6, V4, and S180/4 is similar to that which is described for poliomyelitis in monkey-kidney t. c., Teschen virus (30), and swine-enterovirus strains T80 and T52A (5, 24). The strains N7, U1, U10, and S159 have a CPE which is similar to that described for swine enterovirus V13, and for human poliomyelitis virus in human amniotic t. c. (18).

Different strains produce different types of plaques. U6 and the Teschen Tirol strain, which belong to the same serological group (45), but also the serologically different strain N11, produce similar plaques. Among 5 antigenically different swine enterovirus, *Bohl et al.* (12) described the mentioned types of plaque and an additional one, which was very indistinct. Four



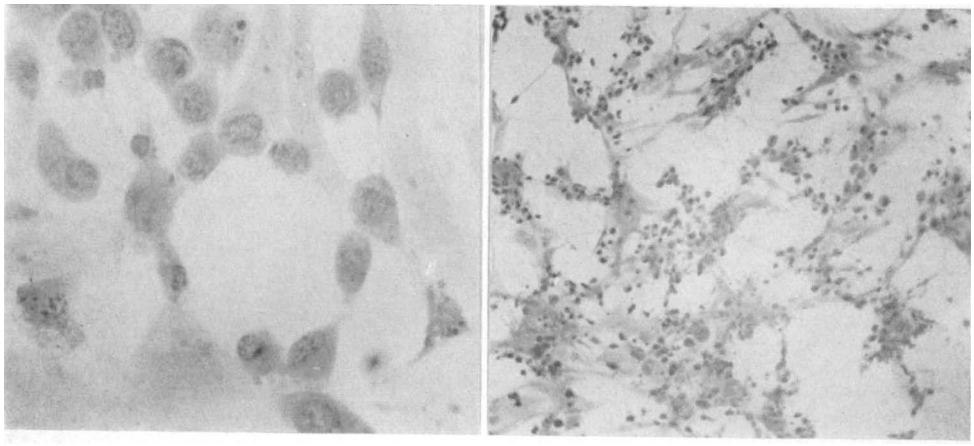
F i g. 3. Normal pig kidney t. c. monolayer, 9 days old (about 70 ×). Stained H. E.

F i g. 4. Normal pig kidney cells in t. c., 9 days old (about 350 ×). Stained H. E.



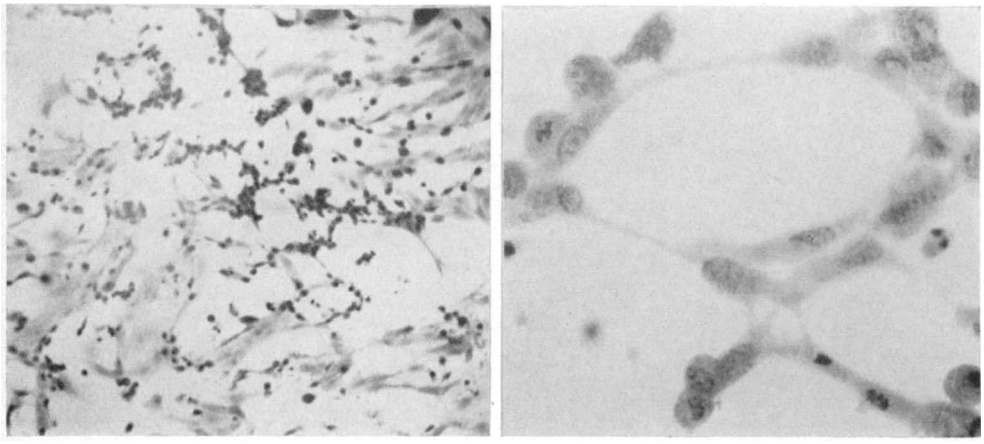
F i g. 5. CPE of N7 in swine kidney t. c. 30 hours p. i. (about 70 ×). Stained H. E.

F i g. 6. CPE of N7 in swine kidney t. c. 30 hours p. i. The nodules surrounding the cells and the cytoplasmatic protrusions are evident (about 350 ×). Stained H. E.



F i g. 7. CP focus caused by the V4 strain, 24 hours p.i. (about 350 ×). Stained H. E.

F i g. 8. Reticular pattern 72 hours p. i., V4 strain (about 70 ×). Stained H. E.



F i g. 9. Reticular pattern 72 hours p. i., U6 strain (about 70 ×). Stained H. E.

F i g. 1 0. Cytoplasmatic protrusions in t. c. inoculated with strain V4, 72 hours p. i. (about 350 ×). Stained H. E.

antigenically different, although related, strains produced indistinguishable plaques.

Like other enteroviruses the examined viruses are resistant to storing and heat. End-points of the termal inactivation were not determined, as the intention was to give a general idea of the heat stability. Swine enteroviruses, like enteroviruses in general, are not type-specific but strain-specific with regard to heat resistance (34). The whole enterovirus group is characterized by a relatively high resistance to heat.

Those few results of examinations on the heat resistance of swine enteroviruses which have been published were made under different experimental conditions, which makes a closer comparison uncertain.

The pathogenic capacity of the isolated strains in piglets and other experimental animals, the duration of elimination via the faeces, and the serological relationship will be described in a subsequent paper.

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SUMMARY

From 60 swine herds in central Sweden 16 ether-resistant viruses were isolated in swine kidney tissue-culture. Altogether 8 different serological groups were obtained. They produced two different cytopathic pictures. Antigenically different strains produced the same type of plaques. The strains possessed properties that are characteristic of enteroviruses, e. g. cytopathic effect in swine-kidney tissue-culture, resistance to ether and chloroform, relative resistance to heat, and sedimentation constants of the same order of size as Teschen control virus.

ZUSAMMENFASSUNG

Untersuchungen und Charakterisierung der Enterovirusstämme bei schwedischen Schweinen. I. Isolierung, biologische, chemische und physikalische Eigenschaften.

Von 60 Schweinebestände aus Mittel-Schweden, wurden in der Schweinenierengewebekultur 16 ätherresistente Stämme isoliert. Bei den so erhaltenen acht antigen verschiedenen Stämmen, konnte man zwei unterschiedbare zytopathische Effekte beobachten. Dieselbe Plaque-Form wurde bei antigen verschiedenen Stämmen erhalten. Ausserdem wurden bei den untersuchten Stämmen folgende charakteristische Eigenschaften der Enteroviren festgestellt: die cytopathische Veränderungen in der Schweinenierengewebekultur, die Äther- bzw. Chloroform-Resistenz, eine relative Wärmeresistenz und Sedimentationskonstanten von derselben Grösse als der Teschen Kontrollstamm.

SAMMANFATTNING

Undersökningar och karakterisering av enterovirusstammar hos svenska grisar. I. Isolering, biologiska, kemiska och fysikaliska egenskaper.

Från svin tillhärande 60 besättningar isolerades 16 eterresistenta virusstammar på vävnadskultur av svinnjure. Av dessa erhölls 8 serologiskt skilda grupper. De gav upphov till två olika degenerationsbilder. Antigent skilda stammar gav samma typ av plaques. Stammarna hade egenskaper, vilka är typiska för enterovirus, såsom cytopatogen effekt i vävnadskultur av svinnjure, relativ resistens mot eter, kloroform och värme samt sedimentationskonstanter av samma storleksordning som Teschen kontrollvirus.

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