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Attempts to infect pigs with Coxsackie virus type B5

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SUMMARY

Despite the existence of a close serological relationship between the enteroviruses Swine Vesicular Disease (SVD) and Coxsackie type B5 (Cx B5), the administration of this Coxsackie virus type to susceptible pigs by various routes failed to produce clinical disease.

Viraemia was not detected after exposure but virus was recovered intermittently from faeces and buccal swabs. A mixed virus population was demonstrated in faecal cultures from some pigs, including Coxsackie virus type B5 and other agents, presumably native pig enteroviruses. The Coxsackie virus persisted in faeces in declining amounts for up to 8 days after primary exposure.

Serum neutralizing antibody showed a transient rise to Coxsackie virus, reaching a peak at 14 days and declining below demonstrable titres by 28 days after exposure. The antibody titres attained were proportional to the dose of virus administered and the degree of neutralization was very similar to both SVD and Cx B5 viruses.

On cross challenge by exposure to SVD virus 28 days after exposure to Cx B5 virus, most animals (5/6) succumbed with typical vesicular lesions, although the serum neutralizing antibody titres showed a characteristically anamnestic response to both viruses.

INTRODUCTION

Swine vesicular disease is recognized as an emerging problem in domestic pigs. The condition has been recorded in Italy (Nardelli *et al.* 1968), Hong Kong (Mowat, Darbyshire & Huntley, 1972), the United Kingdom (Dawe, Forman & Smale, 1973) and a number of European countries, including France (Dhennin & Dhennin, 1973), Austria and Poland (Report of F.A.O. Special Meeting, 1973).

Clinically the disease is characterized by vesiculation of the feet, snout, buccal cavity and skin in a manner indistinguishable from foot-and-mouth disease. Several aspects of the pathogenesis of the disease have been studied (Burrows, Greig & Goodridge, 1973; Burrows, Mann & Goodridge, 1974; Sellers & Herniman, 1974).

Recently American workers investigated the relation between SVD virus and forty-two human enterovirus immunotypes using Lim Benyesh-Melnick serum pools (Graves, 1973). SVD virus was tested with serum raised against poliovirus types 1–3, echovirus types 1–7, 9, 11–27, 29–33, Coxsackie virus types A 7, 9, 16 and types B 1–5. The tests revealed a highly significant neutralization of SVD

virus by antiserum to Coxsackie virus type B5 and a similar effect was observed in reciprocal tests using Cx B5 virus and antiserum to SVD virus.

The striking serological relation between the porcine pathogen, SVD virus, and the human pathogen, Cx B5 virus, raised the question of other relations including the possibility of a zoonosis. This communication describes attempts to determine the susceptibility of the domestic pig to Coxsackie virus type B5.

MATERIALS AND METHODS

Animals

Pigs. Large White pigs were used weighing 30–40 kg. at the outset of the experiment. The pigs were housed in an isolation unit on concrete with straw bedding and were fed on pig meal with water *ad libitum.*

Mice. One-day-old mice of the Pirbright P (Parkes) strain of albino mice were used for the isolation and assay of virus for certain samples.

Guinea-pigs. Dunkin–Hartley strain of guinea-pigs weighing at least 500 g. were used for production of antiserum to Cx B5 virus.

Viruses

Coxsackie virus type B5. This was supplied by courtesy of Dr D. R. Gamble of the Public Health Laboratory Service, Epsom, Surrey. The virus was a prototype strain (Faulkner) originally recovered from human faeces in the United States of America and had been maintained in monkey kidney tissue culture for some 20 years. The exact passage history of the strain was unknown. The virus was administered to pigs as received and also after two further passages in pig kidney cell cultures. A glycerinated filtrate of the third pig kidney passage was stored at $-20°$ C. and used as an antigen in serum neutralization tests.

Swine Vesicular Disease virus. Strain England UK- G 27/72, recovered from a British field outbreak in 1972, was given two passages in pig kidney cell cultures before being stored as a filtrate with 50 % glycerol at $-20°$ C. This stock was used as an antigen in serum neutralization tests and in the infection of donor pigs for cross-challenge purposes.

Tissue culture

The IB-RS-2 pig kidney cell line (De Castro, 1964) was used exclusively. Virus stocks were grown in Roux bottles, recovery and assay of virus from pig samples was performed in tubes incubated on a roller apparatus and neutralization tests and some infectivity titrations were carried out in petri dish cultures.

Infection of animals

Exposure of pigs to Cx B5 virus. Eight pigs (numbers KH13-20) were divided into two groups of four animals housed in the same loose box. One group was given the Cx B5 virus as originally obtained and the other received Cx B5 virus after two further passages in pig kidney tissue culture. The two inocula contained $10^{6.5}$ and $10^{7.0}$ plaque forming units (p.f.u.) per ml. respectively and Table 1 shows the route and dose of virus administered to each animal.

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Table 1. *Protocol of inoculation of pigs with Cx B5 virus*

Virus	Animal number	Route of administration	Volume (ml.)	Total virus dose
Cx B5 prototype strain	KH13	Not inoculated	—	—
	KH14	Intradermal Bulbs of heel and coronary bands of all four feet	Approx 2·0	Approx 6·7*
	KH15	As for KH14		
	KH16	Subcutaneous	1·0	7·2
		Intramuscular	1·0	—
		Intravenous	1·0	—
		Intranasal	1·0	—
		Oral	2·0	—
Cx B5 prototype at 2nd IB-RS-2 passage	KH17	Not inoculated	—	—
	KH18	Intradermal Bulbs of heel and coronary bands of four feet	Approx 2·0	Approx 7·2
	KH19	As for KH18		
	KH20	Subcutaneous	1·0	8·5
		Intramuscular	1·0	—
		Intranasal	10·0	—
		Oral	20·0	—

* Log_{10} plaque-forming units.

Cross challenge of pigs with SVD virus. Twenty-five days after the first exposure of pigs to Cx B5 virus two fresh pigs (KH 56 and 57) housed in a different isolation unit were infected with SVD virus by intradermal inoculation of the coronary band and bulbs of heel using virus with a titre of $10^{6\cdot1}$ p.f.u. per ml. These donor pigs were used to contaminate two loose boxes which were not cleaned out after their introduction. Twenty-eight days after exposure to Cx B5 virus the eight pigs were transferred to the second isolation unit and there segregated into two groups of four, KH 13–16 and KH 17–20. Four fresh, non-inoculated pigs (KH 58–61) were added, two to each group, as controls for contact infection. The SVD donor animals were removed and the two groups of six pigs were then placed in the contaminated boxes.

Inoculation of mice. Mice were inoculated with buccal, pharyngeal and faecal samples from pigs by the intracerebral route with a dose of 0·03 ml. and were examined daily for the following 12 days.

Inoculation of guinea-pigs. Guinea-pigs were given 1·0 ml. of an equal mixture of Cx B5 virus at the third pig kidney cell passage and Freund's complete adjuvant. The dose was divided and inoculated intramuscularly in both hind legs. Twenty-eight days later a further 0·5 ml. of Cx B5 virus was administered intraperitoneally and after 10 days the animals were exsanguinated to provide antiserum.

Clinical examination, sampling and processing of samples

Pigs were examined clinically, their rectal temperatures recorded and samples collected from the blood, faeces, buccal cavity and occasionally the pharynx

before exposure to infection, daily for 10 days after infection and then every second or third day up to 28 days after infection with Cx B5 virus. Serum samples were collected at intervals for 22 days after cross challenge with SVD virus. Buccal swabs were taken by vigorously rubbing cotton wool buds over the oral mucosa. The swabs were shaken mechanically for 10 min. in 2·0 ml. of phosphate buffered saline (PBS) with antibiotics to give an approximately 1/10 dilution of the original sample for assay. The pharyngeal region was sampled by means of the probang cup described by Burrows (1968), the instrument being rinsed in 2·0 ml. of PBS resulting in a dilution of about 1/10. Faeces samples were taken from the rectum. One gram of faeces was shaken in 9·0 ml. of PBS as described above, the suspension was clarified by centrifugation at 3000 rev./min. for 10 min. and the supernatant was examined for the presence of virus. Blood was obtained by venupuncture from the anterior vena cava and was processed for serum in the normal manner. The majority of samples were processed and inoculated onto tissue cultures within three hours of collection. Samples were subsequently stored at 4° C. for up to 7 days or at −20° C. for longer periods.

Detection of virus and assay of infectivity

Samples were inoculated onto IB-RS-2 monolayers in tubes or Petri dishes using two cultures per dilution. Tubes were examined microscopically each day for five days. Typical enterovirus cytopathic effect (CPE) developed at about 48 hr. and increased up to 96 hr. after infection and tubes showing CPE were harvested to provide antigen for subsequent tests of viral identity. Plaque counts were made after 4 days of incubation under agar, the monolayers having been stained with methylene blue or neutral red solution.

Serological methods

Serum neutralizing antibody. Serum neutralizing antibody titre was determined by means of a plaque reduction test in IB-RS-2 monolayers using a constant virus/variable serum method. A 1/10 dilution of serum was heated at 56° C. for 30 min. before dilution in ten-fold steps from starting dilutions of 1/10 and 1/30, and 1·0 ml of each serum dilution was added to an equal volume of PBS containing approximately 300 p.f.u. of Cx B5 or SVD virus. The mixtures were incubated for 90 min. at 37° C. before inoculation in 0·2 ml. volumes onto cell monolayers. After 60 min. adsorption at 37° C., the cell sheets were overlaid with agar and returned to incubate at 37° C. for 4 days at which time the monolayers were stained and the plaques enumerated. The neutralizing antibody titre was expressed as the log reciprocal of that serum dilution which neutralized 90 % of the test virus.

Identification of porcine viral strains. All viral strains isolated from faeces and buccal swabs were examined in neutralization tests with guinea-pig antiserum to Cx B5 virus. The strains were tested as first or second tissue culture passage harvests when titration had shown that the passaged material contained $10^{6\cdot0}$ or greater p.f.u. per ml. A constant serum/varying virus dilution method was employed with 1·0 ml. of a 1/20 dilution of pooled guinea-pig antiserum heated

Table 2. *Virus content of samples derived from pigs after challenge with Cx B5 virus*

Animal number	Sample	Days after inoculation/exposure 0	1	2	3	4	5	6	7	8	9	10	12	14	16	18	21	25	28
KH13	BS*	0	2·0	0	2·0	0	1·5	0	0	0	0	0	0	0	1·5	0	1·0	0	0
	PH†	—	—	—	—	—	—	—	0	—	—	—	—	0	—	—	0	—	—
	F‡	0	0	1·0	2·5	4·0	0	4·5	4·5	0	2·0	2·5	0	2·5	2·0	0	0	2·0	1·5
KH14	BS	0	2·0	0	1·5	0	0	0	1·5	0	0	0	0	0	0	0	0	0	0
	PH	—	—	—	—	—	—	—	2·0	—	—	—	—	0	—	—	0	—	—
	F	0	4·0	2·0	3·0	3·0	2·5	3·0	2·5	0	0	0	0	0	0	0	0	0	0
KH15	BS	0	1·5	1·0	0	1·5	1·0	1·5	2·0	2·0	0	0	1·5	0	0	1·0	0	0	0
	PH	—	—	—	—	—	—	—	2·0	—	—	—	—	2·0	—	—	0	—	—
	F	0	3·5	0	2·0	0	2·5	2·0	2·5	2·0	1·5	0	1·5	0	0	0	0	2·5	0
KH16	BS	0	1·5	0	2·0	1·5	0	1·5	1·0	3·0	0	0	2·0	0	0	0	0	1·5	0
	PH	—	—	—	—	—	—	—	1·0	—	—	—	—	1·5	—	—	1·5	—	—
	F	0	3·0	0	4·5	3·5	3·5	3·0	2·0	3·0	0	0	2·5	0	1·0	0	1·0	0	0
KH17	BS	0	2·0	1·0	1·5	0	0	1·0	0	0	0	0	0	0	0	0	0	0	0
	PH	—	—	—	—	—	—	—	0	—	—	—	—	0	—	—	0	—	—
	F	0	3·0	3·5	3·0	3·0	0	2·0	0	2·0	0	0	0	0	0	0	0	0	0
KH18	BS	0	1·0	0	1·5	0	1·0	0	0	0	0	0	0	0	1·5	0	1·0	0	0
	PH	—	—	—	—	—	—	—	0	—	—	—	—	0	—	—	0	—	—
	F	0	0	2·5	2·2	0	2·0	0	1·5	2·0	0	0	0	1·0	0	0	1·5	0	0
KH19	BS	0	1·5	1·0	1·0	1·5	0	0	0	0	0	0	0	0	0	0	0	0	0
	PH	—	—	—	—	—	—	—	0	—	—	—	—	0	—	—	0	—	—
	F	0	3·5	3·0	3·0	0	2·0	2·0	0	1·5	0	0	0	0	0	0	0	0	0
KH20	BS	0	4·0	2·5	3·5	2·0	0	3·0	3·0	0	2·5	0	1·0	0	1·5	0	0	0	1·5
	PH	—	—	—	—	—	—	—	4·0	—	—	—	—	0	—	—	2·0	—	—
	F	3·5	5·5	5·0	3·0	1·5	4·5	0	3·0	2·5	0	1·5	0	0	2·5	0	2·5	0	0

* BS, buccal swab: infectivity expressed as TCID 50 per sample.
† PH, pharyngeal sample: infectivity expressed as TCID 50 per sample.
‡ F, faeces: infectivity expressed as TCID 50 per sample.
—, not sampled.
0, no virus detected at the lowest dilution examined.

at 56° C. for 30 min. being added to an equal volume of serial ten-fold dilutions of virus. A parallel titration was carried out with normal heated guinea-pig serum for each virus strain and infectivity was measured by the plaque technique as detailed above, a neutralization index being derived from the difference in infectivity titre of viral strains incubated with normal and immune sera.

RESULTS

Attempts to infect pigs with Cx B5 virus

In daily examinations of pigs after exposure to Cx B5 virus, the rectal temperatures remained within normal ranges and no clinical abnormalities whatsoever were detected.

Viraemia was consistently absent. Table 2 gives the virus content of faecal, oral and pharyngeal samples and shows that virus was recovered intermittently from all sites at concentrations of up to $10^{5.5}$ TCID50 per g. in faeces and $10^{4.0}$ TCID50 per sample in buccal and pharyngeal samples. The frequency and titre of viral recovery declined with time after exposure to virus. Attempts to isolate virus from porcine samples by the inoculation of one day old mice proved unsuccessful.

All buccal swab samples were free of detectable virus before the introduction of Cx B5 virus but virus was recovered from the faeces of one pig at that time. The presence of pre-existing faecal virus and the rather rapid appearance of relatively high titres of virus in faecal and oral samples derived from non-inoculated contact pigs after the introduction of experimental infection led to concern about the identity of the virus which was being recovered. Neutralization tests were carried out to determine the extent to which Cx B5 virus was involved in the recoveries and Table 3 shows the degree to which various virus strains were neutralized by guinea-pig antiserum to Cx B5 strain. The results indicated that Cx B5 virus was excreted at declining titres for up to eight days after exposure and that a considerable proportion of isolations were wholly or partly composed of other virus or viruses which were not serologically related to Cx B5 virus.

The serum neutralizing antibody response to both Cx B5 and SVD viruses is shown in Table 4. All sera were free of detectable antibody at the outset of the experiment and a transient rise in serum antibody was detected reaching a peak at 14 days and declining below demonstrable titres by 28 days after exposure to Cx B5 virus. There was some suggestion of a serum antibody response in the contact control animals which was confirmed after cross challenge.

In general the antibody titres attained were proportional to the dose of virus administered and the degree of neutralization was very similar with both Cx B5 and SVD viruses, thereby confirming the work of Graves (1973).

Cross challenge with SVD virus

The two donor pigs developed generalized disease within 48 hr. of inoculation and the amount of virus in the loose boxes was such that the non inoculated recipient control animals first showed lesions on the second and fourth day after

Table 3. *Neutralization of virus strains from pigs by antiserum to Cx B5 virus*

Animal number	Origin	Day after inoculation/exposure to Cx B5 virus on which the original isolation was made													
		0	1	2	3	4	5	6	7	8	9	10	12	14	16
KH13	BS	—	2·6*	—	2·0	—	1·7	—	—	—	—	—	—	—	0·4
	F	—	—	2·7	2·5	1·5	—	0·9	0·4	—	0·0	0·2	—	0·0	0·0
KH14	BS	—	5·0	—	⩾ 6·0	—	—	—	⩾ 6·0	—	—	—	—	—	—
	F	—	5·7	⩾ 6·0	5·9	5·7	⩾ 6·0	5·7	⩾ 6·0	—	—	—	—	—	—
KH15	BS	—	3·2	2·8	—	2·5	2·1	1·3	0·2	0·4	—	—	0·3	—	—
	F	—	2·7	—	2·0	—	2·4	0·6	0·0	0·5	0·0	0·0	—	0·3	0·2
KH16	BS	—	2·2	—	1·8	2·5	—	1·8	1·5	0·8	—	—	0·0	0·0	0·0
	F	—	3·0	—	2·4	3·6	2·0	2·5	1·0	1·0	—	—	0·2	—	0·3
KH17	BS	—	⩾ 6·0	5·7	⩾ 6·0	—	—	5·7	—	—	—	—	—	—	—
	F	—	5·7	5·9	⩾ 6·0	⩾ 6·0	—	⩾ 6·0	—	5·5	—	—	—	—	—
KH18	BS	—	1·6	—	2·0	—	0·5	—	—	—	—	—	—	—	0·4
	F	—	—	2·5	1·8	—	0·7	—	0·2	0·0	—	—	—	0·5	—
KH19	BS	—	5·7	⩾ 6·0	⩾ 6·0	5·9	—	—	—	—	—	—	—	—	—
	F	—	5·7	5·9	5·7	—	⩾ 6·0	5·6	—	⩾ 6·0	—	—	—	—	—
KH20	BS	—	3·0	3·6	3·2	2·8	—	1·7	2·5	—	0·2	—	0·4	—	0·2
	F	0·5	3·5	4·0	2·8	1·3	2·0	—	3·0	3·2	—	0·0	—	—	0·5

* Log reduction in titre of virus strain containing ⩾ 10^6 p.f.u./ml. effected by antiserum to Cx B5 virus.
BS, buccal swab. F, faeces. —, no virus isolated.

Table 4. *Results of neutralization test on the sera of pigs after challenge with Cx B5 virus*

Animal number	Days after inoculation/exposure				
	0	7	14	21	28
KH13	—/—	—/—	1·0/1·0	—/—	—/—
KH14	—/—	1·0/1·5	1·5/2·0	—/1·0	—/—
KH15	—/—	1·5/1·0	2·0/2·0	1·0/1·0	—/—
KH16	—/—	1·0/1·0	2·5/2·0	1·5/1·0	—/—
KH17	—/—	—/1·0	1·0/1·0	1·0/—	—/—
KH18	—/—	1·0/1·5	2·0/1·5	1·0/1·0	—/—
KH19	—/—	1·5/1·5	2·5/2·5	2·0/1·5	—/—
KH20	—/—	1·5/2·0	3·0/3·5	2·5/2·0	—/—

Numerator/denominator = serum neutralization titre against SVD virus/serum neutralization titre against Cx B5 virus. Results expressed as the log reciprocal of the serum dilution with neutralized 90 % of the test virus.

— = < 1·0.

Table 5. *Development of clinical disease in pigs cross-challenged with SVD virus*

Box number	Animal number	Status	Days after exposure												
			0	1	2	3	4	5	6	7	8	9	12	14	22
12	KH13	Prior exposure to Cx B5 virus	†	—	—	—	—	—	—	—	—	4*	4	4	4
	KH14		†	—	—	—	—	—	—	—	—	2	4	4	4
	KH15		†	—	—	—	—	—	—	—	—	—	—	—	—
	KH16		†	—	—	—	—	—	—	—	—	—	3	4	4
	KH58	No prior exposure	†	—	—	—	—	—	—	—	—	—	—	—	—
	KH59		†	—	—	?	1	1	1	2	2	4	4	4	4
13	KH17	Prior exposure to Cx B5 virus	†	—	—	—	—	1	1	1	1	1	1	1	1
	KH18		†	—	—	—	1	4	4	4	4	4	4	4	4
	KH19		†	—	—	1	2	2	4	4	4	4	4	4	4
	KH20		†	—	—	—	—	—	1	1	1	1	1	1	1
	KH60	No prior exposure	†	—	—	2	4	4	4	4	4	4	4	4	4
	KH61		†	—	2	4	4	4	4	4	4	4	4	4	4

*, number of feet showing lesions.
†, pigs exposed to boxes contaminated with SVD virus.
—, no lesions detected.
?, presence of an early lesion queried.
Note: on day 5, KH61 was transferred to Box 12.

being introduced to the infected boxes. The challenge was therefore adequate and the initial exposure of pigs to a contaminated environment was quickly reinforced by exposure to additional virus excreted from the recipient control animals.

Some inequality of challenge may have occurred however, since only three of the four recipient control pigs developed overt disease and lesions developed rather slowly in the susceptible pig which was paired with the clinically resistant animal. One recipient control pig from the pair showing fully generalized disease was therefore transferred into the other loose box 5 days after the initiation of challenge to ensure a more evenly matched exposure for both groups of pigs.

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Table 6. *Results of neutralization tests on the sera of pigs after cross-challenge with SVD virus*

Status	Animal number	Days after exposure 0	3	7	14	22
Prior exposure to Cx B5 virus	KH13	—/—	1·0/1·5	1·5/1·5	3·5/2·0	4·0/3·0
	KH14	—/—	1·5/—	1·5/1·0	4·0/3·0	4·0/3·5
	KH15	—/—	1·5/1·0	2·0/1·0	3·0/2·5	3·5/2·0
	KH16	—/—	1·0/1·0	2·0/1·0	3·5/2·5	4·0/3·0
	KH17	—/—	1·0/1·0	1·5/1·0	3·0/2·5	3·0/2·5
	KH18	—/—	1·5/1·0	1·5/1·5	4·0/3·0	3·5/3·0
	KH19	—/—	1·0/—	1·5/1·0	3·5/3·0	4·5/3·0
	KH20	—/—	1·5/1·0	2·0/1·0	4·5/3·0	3·5/3·5
No prior exposure	KH58	—/—	—/—	—/—	2·5/2·0	2·5/2·0
	KH59	—/—	—/—	1·0/—	2·0/2·0	2·5/3·0
	KH60	—/—	—/—	1·0/1·5	2·0/2·0	2·5/3·0
	KH61	—/—	—/—	—/1·0	2·5/2·0	3·0/2·5

Numerator/denominator = serum neutralization titre against SVD virus/serum neutralization titre against Cx B5 virus. Results expressed as the log reciprocal of the serum dilution which neutralized 90 % of the test virus.

— = < 1·0.

The clinical results of the cross challenge are summarized in Table 5. Of the six pigs which were originally inoculated with Cx B5 virus, four developed typical severe vesicular lesions of all four feet, one showed mild lesions confined to a single foot and one remained free from clinical disease. Of the two non inoculated animals which had been in contact with the 6 pigs originally given Cx B5 virus, one developed fully generalized disease while the other showed lesions on one foot. As has been mentioned, one of the 4 recipient control animals in the SVD virus challenge remained free from clinical disease. The occurrence and extent of lesions was therefore variable in both control and experimental pigs but the failure of prior exposure to Cx B5 virus to confer protection against infection with SVD virus was clearly demonstrated.

The results of serum neutralizing antibody assays against both Cx B5 and SVD viruses after cross challenge are given in Table 6. Prior exposure to Cx B5 virus had sensitized both inoculated and in-contact pigs so that subsequent exposure to SVD virus resulted in a typically anamnestic response, the neutralizing antibody increasing more rapidly and to higher titres in these animals than in the control pigs which were exposed to SVD virus alone. High titre antibody was found to both viruses after cross challenge but the serum antibody response to SVD virus was higher than that to Cx B5 virus.

DISCUSSION

The failure of Cx B5 virus to produce clinical disease in pigs must be qualified by reference to a number of host and virus factors which may have influenced the outcome of the experiment.

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Concurrent infection with other viruses, presumably the commonly encountered porcine enteroviruses, was demonstrated in some of the experimental animals so that interference between viruses cannot be excluded. Similarly the insusceptibility of the pigs may have been related to local or secretory defence mechanisms which were not investigated.

The prototype virus used had been maintained in tissue cultures for many years and could have altered in pathogenicity for animals during serial passage. Despite its history, however, the virus readily grew to high titre in a variety of pig organ cultures, in primary pig kidney cells and in the IB-RS-2 cell line, proving that both differentiated and de-differentiated cells of porcine origin would support the growth of Cx B5 virus *in vitro*. Moreover, cultivation of the virus in pig monolayer cells through 15 serial passages did not result in any noticeable increase in virus yield which indicated that the virus strain was already well adapted to growth in this system (A. J. M. Garland, unpublished data). The failure to recover virus from pig samples by the inoculation of one day old mice (which had been used in the hope of differentiating between Cx B5 and porcine enteroviruses) may also be explained by the high degree of tissue culture adaptation of the virus strain and indeed, in comparative titrations of Cx B5 virus infectivity, IB-RS-2 cells proved to be more sensitive than mice to the extent of $10^{3\cdot8}$ ID50.

That pigs were in intimate contact with the agent was shown by the recovery of Cx B5 virus from the alimentary tract and by the primary and secondary serum antibody responses but the virus behaved as a non-replicating antigen, being eliminated from the gut in 8 days or less and eliciting only a transient rise in serum antibody. In this context Burrows *et al.* (1973, 1974) have drawn attention to the low titres of serological response in pigs experiencing subclinical infection with SVD virus.

In a preliminary experiment Graves (1973) also found that pigs were not susceptible to Cx B5 virus. The serological results differ, however, since he found that serum antibody continued to rise for at least 21 days after exposure and attained high titre. This difference may be explained by the method of repeated instillation of virus which he employed.

In recent years there has been an increasing interest in the inter-species transfer of viral agents. Many examples are known of serological relations between viruses from different hosts and in some instances the inter-relations extend to pathogenicity. There is good evidence for the production of natural infections in man and pigs by a single antigenic subtype of influenza virus A and it has been suggested that dogs, horses and birds may also play a part in the epidemiology of the disease as disseminators of virus or as hosts in which new virus might be produced by the hybridization of human and animal strains (various authors in the *Bulletin of the World Health Organization*, 1972). Similarly, Coxsackie virus type A5 has been associated with a concurrent outbreak of virus pneumonia in pigs and acute febrile illness in children (Verlinde & Versteeg, 1958). Parainfluenza type 3 viruses isolated from a variety of animals including man, sheep and cattle are also related serologically although their different origins may be determined

by a number of serological techniques (Fischman & Bang, 1966). Each strain has been associated with respiratory disease in its respective host, but in some cases pathogenic effects have been demonstrated in other species. Thus a human strain of Parainfluenza type 3 produced pulmonary lesions in hysterectomy-produced colostrum-deprived piglets (Betts & Jennings, 1966) and bovine strains caused disease in lambs although ovine strains failed to produce disease in calves (Stevenson & Hore, 1970). In the measles–distemper–rinderpest triad, the viruses exhibit a close serological relation but in terms of natural pathogenicity they appear to be strictly species specific for their respective hosts (Imagawa, 1968).

The close serological relation between Cx B5 and SVD virus is now well established. Clinical and serological evidence indicated that SVD virus is a human pathogen since a number of workers in this laboratory became ill after exposure to the virus with symptoms which ranged from vague malaise to classical aseptic meningitis and their convalescent sera neutralized both SVD and Cx B5 viruses to high titre. Immunodiffusion studies indicated that the infection had been associated with SVD rather than Cx B5 virus (Brown, Talbot & Burrows, 1973). The zoonosis aspect must not be overemphasized since there have been no reports from the field of association between human and porcine disease during the recent widespread British SVD epidemic and there was a very low incidence of both clinical disease and serological conversion among laboratory staff directly exposed to the virus.

The present study showed that one strain of Cx B5 virus was not pathogenic for pigs, since it failed to replicate, cause disease or produce cross immunity to SVD virus when administered by a number of routes. It is possible, however, that other Cx B5 strains, recently isolated or from pig organ culture, might cause clinical disease in pigs. The use of gnotobiotic animals might facilitate such investigations by eliminating possible interference effects from other porcine enteroviruses.

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