

The behaviour of porcine cytomegalovirus in commercial pig herds

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SUMMARY

A longitudinal, virological and serological study of pigs in two herds with respiratory disease showed that infection by porcine cytomegalovirus (PCMV) was universal in both.

Virus excretion usually began when piglets were between 3 and 6 weeks of age and reached a maximum between 5 and 8 weeks; it was usually no longer detectable at 11–12 weeks. Antibody demonstrable in indirect immunofluorescence (IIF) tests was present to moderate or high titre in all piglets at 2–3 weeks. This was presumed to be maternal in origin as it declined in titre between 2–3 and 5–6 weeks. After this fall the majority of piglets showed seroconversion as a result of virus infection. One group of 12 pigs in which infection occurred earlier than usual showed a very poor antibody response, which, nevertheless, persisted through to week 27.

The findings are discussed with relation to porcine atrophic rhinitis and cytomegalovirus infection in other species.

INTRODUCTION

Infection by porcine cytomegalovirus (PCMV) was first described as ‘inclusion-body rhinitis’ by Done (1955), who was investigating the aetiology of atrophic rhinitis of swine. The characteristic enlarged cells with intranuclear and, occasionally, intracytoplasmic inclusions were observed particularly in the mucous glands of the nasal mucosa but they occurred also in other situations, such as the salivary and Harderian glandular epithelium as well as in kidney tubules. This wider distribution was closely reminiscent of the pattern already established for symptomless cytomegalovirus (CMV) infections of the mouse, guinea-pig and man. (For reviews see Smith, 1959; Hanshaw, 1968; Plummer, 1973.)

Cytomegaloviruses may be excreted over periods of many months, without concomitant clinical signs, for example in mice (Brodsky & Rowe, 1958; Medearis, 1964*b*) and children (Benyesh-Melnick, Rosenberg & Watson, 1964; Stern, 1968; Levinsohn *et al.* 1969; Starr, Bart & Gold, 1970). Until recently the pathogenesis, pattern of excretion and serology of the infection in pigs could not be adequately investigated because of the lack of a technique for *in vitro* detection, assay and propagation of the virus. Such a method became available after the demonstration

of the susceptibility of pig lung macrophage (PLM) cultures (Watt, Plowright, Sabo & Edington, 1973).

This paper describes the results of a longitudinal study of PCMV in two commercial pig herds, in which respiratory disease was reported to be a problem.

MATERIALS AND METHODS

Herds investigated

Herd A, established 10 years previously, contained about 70 breeding females, all Large White-Landrace crosses; only boars were bought in occasionally. Each sow with its litter was 'isolated' for 3 weeks after farrowing; thereafter batches of four sows and their litters were mixed until the piglets were 5 weeks old, when they were weaned. At 12 weeks of age groups of 10 pigs were transferred to fattening pens.

Atrophic rhinitis had reached serious proportions in this herd in 1971 and prophylactic treatment with sulphonamides had been adopted since that time, with apparent success.

Herd B was recently established, comprising 270 sows of various breeds to which new stock was added frequently. The sows were held in farrowing houses, each of which accommodated 12 animals in separate, low-walled units. The management was similar to that in herd A until the piglets were 5 weeks old; thereafter they were moved gradually, in groups of 50, through two fattening houses, equipped with forced ventilation and dry feeding facilities.

Rhinitis was widespread in herd B, about 10% of animals showing distortion of the nasal bones.

On each farm 2 groups of 12 piglets were selected, 3 each from 4 separate litters farrowed in the same week. On both properties, one group (designated 's') was born in the summer, the other ('w') in winter. These animals were examined every 3 weeks, at which times nasal swabs and serum samples were collected. In herd B, pigs of group 's' were swabbed weekly from the third to tenth weeks in an attempt to define the period of virus excretion more accurately. At slaughter, after 19-30 weeks, the respiratory tract was examined macroscopically and portions of nasal mucosa, lung and bronchial lymph node were taken for virus isolation and histopathology.

Preparation of materials

Nasal secretions were collected on cotton swabs which were inserted about 3-6 cm into one of the nares. The swab was broken off immediately into 2 ml. of a virus-transport medium containing PBS (Dulbecco & Vogt, 1954), with 10% fetal bovine serum and a high concentration of antibiotics (penicillin 1000 i.u./ml., streptomycin 1000 µg./ml., kanamycin 1000 units/ml. and amphotericin B, 25 µg./ml.).

Samples were transported on ice and the swabs then expressed with the aid of forceps, prior to storing the fluids at -70° C. Before use they were thawed rapidly at 37° C. and inoculated into cultures without clarification.

Lung and turbinate mucosal tissue from pigs at slaughter was stored in solid

form at -70°C . Cultures of macrophages were also prepared at this time from fresh lung tissue by methods described below. For virus recovery, solid tissues were thawed and used to prepare 10% (w/v) suspensions in culture maintenance medium. Ten Broeck grinders were employed for this purpose.

Cell cultures

Suspensions of lung macrophages from gnotobiotic or 'minimal disease' piglets, 1-6 weeks old, were prepared as already described (Watt *et al.* 1973), except that, for consistent quality, 2×10^6 cells/ml. were found to be necessary, instead of 0.8 to 1.3×10^6 . The growth and maintenance medium was also modified by the addition of a 10% cell-free extract of minced lung from germ-free piglets and 3 mg./ml. of L-glutamine; this appeared to enhance the quality and longevity of some batches of cells.

For virus isolation cultures were grown in stationary tubes (16 \times 125 mm.) with flying cover-slips, 22 \times 6 mm. For immunofluorescence tests, 25 similar cover-slip cultures were grown and infected in disposable (plastic) Petri dishes, of 9 cm. diameter, incubated in an atmosphere of 10% CO_2 in air. These were seeded with 2×10^7 cells in 10 ml. of medium.

Inoculation of cultures

Nasal swab extracts were inoculated into three tube cultures of PLM cells in a dose of 0.1 ml. After 1 hr. at 37°C . the inoculum was washed off with PBS and fresh medium was added. Further changes of medium were carried out at 3- to 4-day intervals, cover-slips being finally harvested for cytological examination after 9-12 days incubation at $36.5 \pm 0.5^{\circ}\text{C}$. Suspensions of tissues were adsorbed in a similar manner to swab extracts.

Cover-slips for immunofluorescence were inoculated with about 10^3 TCD₅₀ per ml. of PCMV which had been passaged 8-10 times in PLM cells. After 9-11 days incubation they were rinsed twice in PBS, fixed in cold acetone, dried in air and stored at -20°C .

Cultures for cytological examination were fixed and stained as described previously (Watt *et al.* 1973). They were scanned systematically over their whole extent for intranuclear inclusion-bodies typical of PCMV; a magnification of $\times 250$ was adequate. The proportion of inclusion-bearing cells was graded arbitrarily from - to + + + +, these symbols giving an approximate indication of the amount of infectious virus in the inoculum. Thus, + was used for minimal numbers, + + for multiple foci of inclusions, and + + + + for $> 80\%$ of cells specifically affected.

Immunofluorescence tests

Cover-slip cultures with numerous, well-distributed cells containing inclusion bodies were employed in the indirect method. They were rehydrated in PBS, pH 7.2, and flooded with about 0.05 ml. of twofold dilutions of serum, usually in the range 1/4 to 1/1024, prepared with Takatsy loops in microtitre plates.* After

* Cooke Manufacturing Co.

Table 1. *The prevalence of atrophic rhinitis in pigs at slaughter*

Herd	Age (weeks)	Proportion of pigs with turbinate atrophy	
		Group 'w'	Group 's'
A	23-24	5/10	2/10
B	27-30	5/6*	4/4*

* All pigs from herd B were not available for examination.

1 hr. at 37° C. in a humidified container, non-adsorbed antibody was removed by six serial washes with PBS and the cover-slips were flooded with an appropriate dilution of a commercial antipig γ -globulin, prepared in goats or rabbits and conjugated with fluorescein isothiocyanate. Following further incubation at 37° C. for 30 min. and repeated washing, the cultures were mounted in 10 % glycerol in PBS and examined by ultraviolet trans-illumination in a Zeiss RA microscope.

Specific fluorescence was regarded as present in cells with a bright, rounded, paranuclear body and sometimes nuclear membrane or intranuclear fluorescence (Pl. 1, Figs. 1, 2). The 'paranuclear body' corresponded with the area of the cytoplasmic 'inclusion' and was often visible when no distinct intranuclear or membrane fluorescence was present. The end-point serum dilution was regarded as the highest which produced readily detectable, green-yellow globules or granules in infected cells. The minimal dilution of serum tested was 1/4.

A standard antiserum was included with each series of tests and one cover-slip only was used per serum dilution. All sera from one pig were tested simultaneously, using the same batch of cultures. No test was accepted as valid unless the titre of the standard serum fell within the range 1/256 to 1/1024.

RESULTS

Clinical observations

In herd A sneezing was noticed at 5-8 weeks of age in a few pigs of both groups 's' and 'w', particularly in the latter. A conjunctival exudate gave rise to a ring of black discoloration around the eyes of affected pigs but no facial distortion was observed. Infrequent coughing tended to replace the sneezing at about 3 months of age.

In herd B growth of piglets was uneven, deviations of the snout were apparent by 8 weeks and handling caused acute respiratory embarrassment. Atrophy of the turbinate bones was detectable during the swabbing procedure, as was also a mucopurulent and often haemorrhagic nasal exudate. In the fattening units the dry feeding and forced ventilation were associated with frequent coughing.

Post-mortem observations

The prevalence of atrophic rhinitis at slaughter, as judged by the criteria of Done, Richardson & Herbert (1964), is given for both herds in Table 1. The age at slaughter was 23-24 weeks for both groups in herd A but extended to 27-30 weeks

Table 2. Recovery of virus and antibody to PCMV in pigs of group 'w', herd A

Sow no.	Pig no.	Age (weeks)													
		2		5		8		11		14		18		23	
		V*	A†	V	A	V	A	V	A	V	A	V	A	V	A
5184	778	-	16	+	8	-	16	-	32	-	16	-	16	-	16
	782	-	32	-	16	-	64	-	64	-	64	-	32	-	32
	784	-	64	++	64	-	128	-	128	-	128	-	64	-	64
6154	768	-	32	-	16	++	16	-	4	-	16	-	32	-	NT†
	769	-	NT	+	16	+++	8	-	8	-	32	-	32	-	64
	773	-	4	-	4	+++	4	-	8	-	8	-	16	-	16
6197	791	-	64	++	16	++	4	-	4	-	4	-	8	-	16
	793	-	64	++	16	++	32	-	4	-	4	-	8	-	8
	794	-	32	-	16	++	8	-	8	-	4	-	16	-	16
7002	751	-	64	-	8	-	8	-	4	-	32	-	64	-	32
	753	-	64	-	16	+++	4	-	8	-	8	-	64	-	32
	754	-	32	-	16	-	8	-	8	-	8	-	NT	-	NT
Total virus recoveries		0/12	.	5/12	.	9/12	.	0/12	.	1/12	.	0/12	.	0/12	.
Mean antibody titre		.	43	.	11	.	15	.	22	.	29	.	31	.	30

* V = presence of virus in nasal secretions.

† A = reciprocal of antibody titre by IIF technique.

‡ NT = not tested.

Table 3. Recovery of virus and antibody to PCMV in pigs of group 's', herd A

Sow no.	Pig no.	Age (weeks)											
		2		5		8		11		19		23	
		V*	A†	V	A	V	A	V	A	V	A	V	A
4565	144	—	8	++	32	—	256	—	128	—	128	—	128
	145	—	16	—	4	—	8	—	8	—	16	—	NT‡
	146	—	32	—	NT	—	NT	—	NT	—	NT	—	NT
6456	147	—	16	—	8	—	256	—	64	—	64	—	NT
	148	—	64	—	16	+++	8	—	32	—	128	—	64
	149	—	64	—	32	—	8	—	32	—	64	—	64
6255	150	—	128	—	32	—	32	—	32	—	256	—	256
	151	—	64	—	16	+	8	—	32	—	32	—	32
	152	—	32	—	4	—	8	—	32	—	64	—	64
3163	153	—	64	—	4	+	8	—	128	—	128	—	256
	154	—	16	—	8	++	16	—	128	—	32	—	32
	155	—	64	—	16	++	16	—	64	—	NT	—	512
Total virus recoveries	0/12	.	1/12	.	5/12	.	0/12	.	0/12	.	0/12	.	
Mean antibody titre	.	47	.	16	.	57	.	62	.	91	.	156	

* V = presence of virus in nasal secretions.

† A = reciprocal of antibody titre by IIF technique.

‡ NT = not tested.

for 4 of the 12 pigs in group 'w' and 6 of 12 animals in group 's' of herd B. Atrophy of the turbinate bones was more commonly encountered in winter-born than summer-born pigs and more frequent, in fact almost invariable, in herd B.

Virus recovery

The results of virus excretion tests are detailed in Tables 2-5.

In herd A no virus was recovered from piglets 2 weeks of age, i.e. a few days before sows and litters were pooled for multisuckling. By 5 weeks 1/12 animals in group 's' and 5/12 in group 'w' were excreting PCMV. The excretion rate was maximal at 8 weeks, when 5/12 and 9/12 piglets were positive in groups 's' and 'w' respectively; this was also the time when virus was present in greatest quantity. As was probably to be expected, virus excretion was recorded in some or all of the offspring of every sow. Shedding of virus was detected on one or more occasions in 6/12 pigs of group 's' and 11/12 in group 'w'. The duration of virus excretion by the nasal route was at least 3 weeks in 4/12 pigs of group 'w' but, in the 13 other confirmed infections in herd A, swabs were positive on one occasion only. The latest sample in which virus was identified was taken at 14 weeks from pig 754 (group 'w').

In herd B, the animals of group 'w' behaved in a similar manner to those of herd A. Virus was absent from nasal secretions at 3 weeks but detected in 9/12 pigs at 6 weeks; it had disappeared from the nasal secretions by 9 weeks, except for one additional animal (No. 127) which became strongly positive at that time.

Table 4. Recovery of virus and antibody to PCMV in pigs of group 'w', herd B

Sow no.	Pig no.	Age (weeks)											
		3		6		9		12		15		19	
		V*	A†	V	A	V	A	V	A	V	A	V	A
455	120	-	8	+	8	-	4	-	4	-	4	NT‡	NT
	123	-	16	-	8	-	8	-	8	-	8	NT	NT
	124	-	8	++++	8	-	32	-	32	-	32	NT	NT
613	125	-	16	++	< 4	-	16	-	32	-	32	-	32
	126	-	16	++	NT	-	4	-	4	-	4	NT	NT
	127	-	32	-	8	+++	8	-	8	-	32	NT	NT
246	128	-	16	+	8	-	32	-	32	-	16	-	32
	129	-	8	++++	4	-	32	-	32	-	64	-	64
	130	-	16	+++	8	-	32	-	32	-	16	NT	NT
580	132	-	32	++	8	-	32	-	32	-	32	NT	NT
	134	-	64	++	16	-	32	-	32	-	32	-	16
	135	-	16	-	8	-	8	-	16	-	64	NT	NT
Total virus recoveries		0/12	.	9/12	.	1/12	.	0/12	.	0/12	.	0/4	.
Mean anti-body titre		.	21	.	8	.	20	.	22	.	28	.	38

* V = presence of virus in nasal secretions.
 † A = reciprocal of antibody titre by IIF technique.
 ‡ NT = not tested.

Table 5. Recovery of virus and antibody to PCMV in pigs of group 's', herd B

Sow no.	Pig no.	Age (weeks)											
		3		4	5	6		7	8	9	10		27
		V*	A†	V	V	V	A	V	V	V	V	A	A
A70	1	-	64	++	-	-	8	-	-	-	-	4	8
	2	-	64	-	-	-	8	-	-	-	-	8	8
	3	+	32	+	+	-	8	-	-	-	-	8	NT‡
A58	4	++	32	+++	-	-	8	-	-	-	-	8	NT
	5	++++	16	-	+	-	16	-	-	-	-	8	NT
	6	+++	8	-	-	-	8	-	-	-	-	4	NT
30	7	+++	16	++	-	-	8	-	-	-	-	8	NT
	8	++	32	-	-	-	8	-	-	-	-	8	4
	9	+++	16	+++	-	-	16	-	-	-	-	8	4
A74	10	-	16	+	+	-	< 4	-	-	-	-	< 4	< 4
	11	-	32	-	-	-	8	-	-	-	-	4	NT
	12	+	32	+	-	-	8	-	-	-	-	8	4
Total virus recoveries		8/12	.	7/12	3/12	0/12	.	0/12	0/12	0/12	0/12	.	.
Mean anti-body titre		.	30	.	.	.	9	6	5

* V = presence of virus in nasal secretions.
 † A = reciprocal of antibody titre by IIF technique.
 ‡ NT = not tested.

In group 's' of this herd, which was examined at weekly intervals, a very different pattern of virus excretion was observed; 8/12 pigs were positive at 3 weeks and the quantity of virus present made it conceivable that infection had occurred congenitally or in the immediate post-natal period. A further two piglets had become positive by 4 weeks but no shedding was detected at any time in the remaining 2 animals (nos. 2 and 11).

The duration of excretion in this group ('s') was at least 2 weeks in 7/10 positive piglets and 3 weeks in 2 of them (nos. 3 and 5). No virus was detected during or after the 6th week in any of the 12 pigs.

Virus recovery at slaughter

No PCMV was isolated from lung or turbinate mucosal tissues taken at slaughter.

Antibody development

The results of indirect immunofluorescence (IIF) tests are given in detail in Tables 2-5.

In herd A every piglet at 2 weeks of age had antibody varying in titre from 1/8 to 1/128; the mean titre was 1/47 in group 's' and 1/43 in group 'w'. This antibody was presumably derived passively through the colostrum, since by 5 weeks the mean titres had fallen to 1/16 and 1/11 respectively. All piglets in group 's' showed a decline in antibody at this time, except no. 144 which was an early virus excretor and exhibited a four-fold increase of titre; in group 'w' only no. 773 failed to exhibit a decline.

In herd A, group 's', the majority (7/11) of serological conversions, i.e. four-fold or greater increases in antibody titre, above basic levels which were partly, at least, passively acquired, occurred between the 8th and 11th weeks. This corresponded closely with what could have been predicted from the time of active infection, as judged by virus excretion. No piglet failed to develop serological evidence of PCMV infection. In group 'w' the pattern was less distinct, two conversions occurring between 5 and 8 weeks, one only between 8 and 11 weeks, the remainder being deferred still later.

Maximal antibody titres (1/32 to > 1/512) were reached as early as the 8th week in individual pigs (e.g. nos. 144 and 147 in group 's') but were sometimes delayed as late as the 18th or 23rd weeks (e.g. pigs 150 and 155 in group 's'). A significant decline from peak titres was infrequent but a four-fold decrease was recorded in a few animals (e.g. nos. 147 and 154 in group 's' and no. 793 in group 'w'). The stability of antibody titres in general is shown by the mean figures up to 23 weeks (Tables 2, 3).

In herd B all 12 piglets from 4 litters in group 'w' had antibody at 3 weeks of age, which was presumed to be passively acquired since it subsequently declined in 11 instances and 10/12 animals excreted virus later. The mean titre declined in this group from 1/21 to 1/8 between weeks 3 and 6. The majority of later sero-conversions (6/9) occurred between weeks 6 and 9 with a rise of mean titre from 1/8 to 1/20. However, there were three piglets, nos. 120, 123 and 126, in which no

seroconversion occurred, although two (nos. 120, 126) were shown to excrete virus and all maintained low titres of antibody (1/4 or 1/8) at least to week 15.

Pigs in group 's' of herd B, which had possibly undergone congenital or early neonatal infection, with proved virus excretion, were all remarkable for their poor immunological response. The mean titre at 3 weeks was 1/30 with a range of 1/8 to 1/64 but this, probably at least partly colostral in origin, had declined to 1/9 at 10 weeks and 1/5 at 27 weeks; no animal, however, became serologically negative.

DISCUSSION

The present study showed unequivocally that infection with PCMV was universally present in piglets in 2 commercial herds which reported respiratory disease. The same virus has also been isolated by us from 7 or 8 additional herds investigated, including 3 in which no respiratory disease was recorded. In a 'minimal disease' herd from which experimental animals are regularly purchased only 10/102 pigs yielded virus in direct PLM cultures at 4-6 weeks of age; there may, therefore, be considerable difficulties in demonstrating infection by recovery of the agent from herds with enzootic infection. Our experience, however, has shown that it is difficult to find herds in which there is not widespread or universal infection by PCMV as shown by antibody detectable in indirect immunofluorescence (IIF) tests.

On presently available evidence we conclude that all infected animals do develop antibody detectable in IIF tests, even if infection occurs very soon after birth or, perhaps, congenitally as in group 's' of herd B. Moreover, one herd in which frequent, direct examination of PLM cultures prepared from piglets 4-6 weeks of age did not yield virus also showed no evidence of IIF antibody at a 1/4 dilution of serum. The IIF antibody is much more easily demonstrated than virus-neutralizing activity; thus it was found that a 'hyperimmune' serum prepared in a gnotobiotic pig completely neutralized a standard dose of virus (*ca.* 10^2 TCD₅₀) to a dilution of 1/16 only, whereas its titre in IIF tests was 1/256 to 1/1024. Furthermore, neutralization tests which require quantitative assays and detection of minimal amounts of virus are extremely laborious, as each cover-slip culture must be examined microscopically for PCMV inclusion-bodies; direct microscopy of living PLM culture gives no detectable indication of virus-induced cytopathology.

Our attempts to recover virus from pigs in the field would undoubtedly have been successful more frequently if a better system for virus isolation had been available. The main drawbacks to the present system include the following:

(i) Batches of PLM cultures vary considerably in their quality and sensitivity to detect virus, even with an identical preparative technique and medium constituents.

(ii) Cultures of PLM cells often develop cytotoxic changes when inoculated with nasal-swab extracts and their sensitivity is thereby lowered.

(iii) The maximum period of retention of PLM cultures is commonly 11-12 days because of progressive detachment of cells. After such a short incubation period,

minimal quantities of PCMV produced extremely few cells with inclusion-bodies and these were easily missed during microscopical examination.

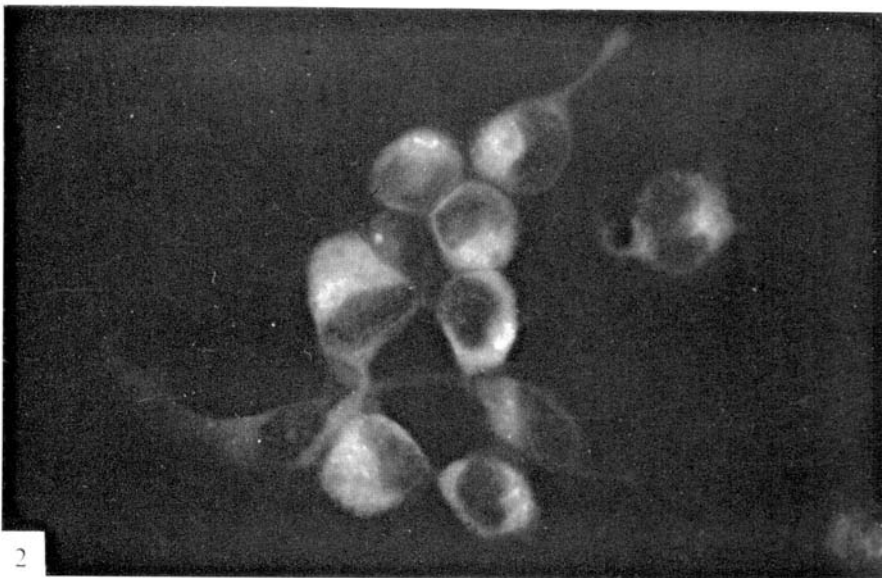
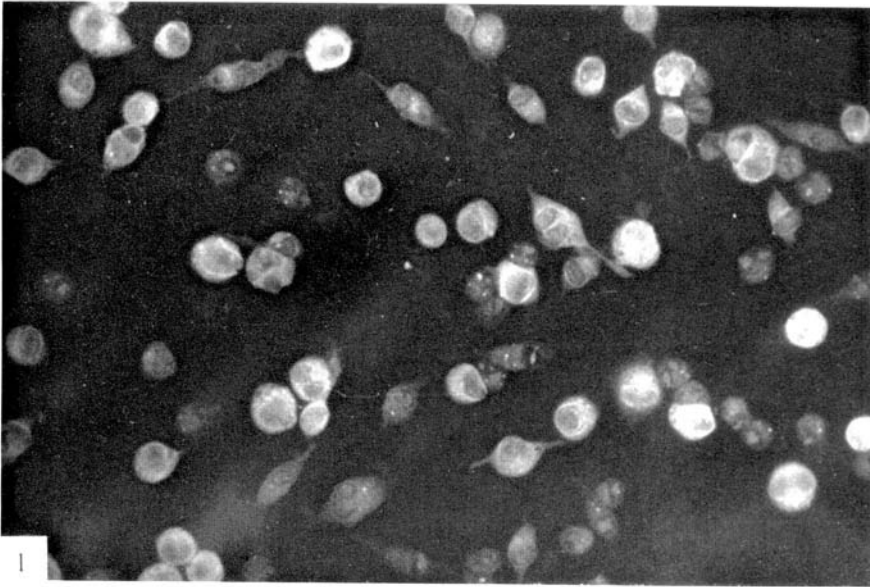
In spite of these drawbacks the pattern of PCMV excretion was clearly demonstrated. Infection usually became most active between 5 and 8 weeks and caused the appearance of autogenous antibody between 8 and 11 weeks. This pattern was probably associated both with the practice of mixing together several litters at the age of 3 weeks and with the progressive decline of maternal antibody. The source of virus for the piglets was not determined; it could have been a minority of congenitally infected animals (L'Ecuyer, Corner & Randall, 1972; N. Edington *et al.* 1975, to be published), or the excretions of recently infected breeding stock; in this connexion it may be significant that some gilts had recently been bought into herd B.

The major route of excretion of PCMV by young pigs is undoubtedly through the nasal secretions, in which it was shown that large quantities of virus were present. The speed and extent of the cytopathic effects in PBM cultures suggested that nasal swabs contained up to about 10^5 TCD₅₀ of PCMV, and such amounts would be readily disseminated by sneezing. We have detected virus in the oropharyngeal secretions and urine of experimental gnotobiotics but always in relatively small quantities; newly infected adults or carrier pigs could excrete more virus by these routes, which are probably the main media for natural transmission of human cytomegalovirus (e.g. Benyesh-Melnick *et al.* 1964; Stern, 1968; Cox & Hughes, 1974).

The duration of detectable PCMV excretion by naturally infected pigs was much shorter than that of CMV in infected mice and children; periods of 15–24 months or more have been recorded in the latter (Rowe, Hartley, Cramblett & Mastrota, 1958; Weller & Hanshaw, 1962; Medearis, 1964*a*) and 6–12 months in the former (Brodsky & Rowe, 1958; Medearis, 1964*b*). We have found that nasal excretion of PCMV by gnotobiotic pigs can persist for at least 4 weeks. In this connexion it is possible that swabs collected later in the course of infection in the field may have contained sufficient antibody to neutralize any infectious virus during transit and before the samples could be inoculated into cultures or frozen at -70° C.

Whilst PCMV is not admittedly a direct cause of atrophic rhinitis, whether experimentally or in the field, there is little doubt that such a universal virus could be an important contributory factor in lowering normal resistance mechanisms to bacteria such as *Bordetella bronchiseptica*. The destruction of acini in the mucous glands of the nasal mucosa, together with metaplasia of the overlying respiratory epithelium, must severely prejudice the proper functioning of the muco-ciliary apparatus in the upper respiratory tract.

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REFERENCES

- BENYESH-MELNICK, M., ROSENBERG, H. S. & WATSON, B. (1964). Viruses in cell cultures of children with congenital heart malformations and other diseases. *Proceedings of the Society for Experimental Biology and Medicine* **117**, 452.
- BRODSKY, I. & ROWE, W. P. (1958). Chronic subclinical infection with mouse salivary gland virus. *Proceedings of the Society for Experimental Biology and Medicine* **99**, 654.
- COX, F. & HUGHES, W. T. (1974). Fecal excretion of cytomegalovirus in disseminated cytomegalic inclusion disease. *Journal of Infectious Diseases* **129**, 732.
- DONE, J. T. (1955). An 'inclusion-body' rhinitis of pigs. *Veterinary Record* **67**, 525.
- DONE, J. T., RICHARDSON, M. D. & HERBERT, C. N. (1964). Rhinitis of swine: a survey carried out in Britain in 1956-57. *Animal Disease Surveys*. Report no. 3, p. 29. H.M.S.O.
- DULBECCO, R. & VOGT, M. (1954). Plaque formation and isolation of pure lines with poliomyelitis viruses. *Journal of Experimental Medicine* **99**, 167.
- L'ECUYER, C., CORNER, A. H. & RANDALL, G. C. B. (1972). Porcine cytomegalic inclusion disease: transplacental transmission. *Proceedings of the International Pig Veterinary Society Congress, Hannover*, p. 99.
- HANSHAW, J. B. (1968). Cytomegaloviruses. *Virology Monographs* **3**, 1. New York: Springer-Verlag.
- LEVINSOHN, E. M., FOY, H. M., KENNY, G. E., WENTWORTH, B. B. & GRAYSTON, J. T. (1969). Isolation of cytomegalovirus from 100 infants throughout the first year of life. *Proceedings of the Society for Experimental Biology and Medicine* **132**, 95.
- MEDEARIS, D. N. (1964a). Observations concerning human cytomegalovirus infection and disease. *Bulletin of Johns Hopkins Hospital* **114**, 181.
- MEDEARIS, D. N. (1964b). Mouse cytomegalovirus infection, II. Observations during prolonged infections. *American Journal of Hygiene* **80**, 103.
- PLUMMER, G. (1973). Cytomegaloviruses. *Progress in Medical Virology* **15**, 92-125.
- ROWE, W. P., HARTLEY, J. W., CRAMBLETT, H. G. & MASTROTA, F. M. (1958). Detection of human salivary gland virus in mouth and urine of children. *American Journal of Hygiene* **67**, 57.
- SMITH, M. G. (1959). The salivary gland viruses of man and animals. *Progress in Medical Virology* **2**, 171.
- STARR, J. G., BART, R. D. & GOLD, E. (1970). Prevalence and duration of postnatally acquired cytomegalovirus. *Journal of Chronic Diseases* **22**, 603.
- STERN, H. (1968). Isolation of cytomegalovirus and clinical manifestations of infection at different ages. *British Medical Journal* **i**, 665.
- WATT, R. W., PLOWRIGHT, W., SABO, A. & EDINGTON, N. (1973). A sensitive cell culture system for the virus of porcine inclusion body rhinitis (cytomegalic inclusion disease). *Research in Veterinary Science* **14**, 119.
- WELLER, T. H. & HANSHAW, J. B. (1962). Virologic and clinical observations on cytomegalic inclusion disease. *New England Journal of Medicine* **266**, 1233.

EXPLANATION OF PLATE

PLATE 1

Fig. 1. Pig lung macrophage (PLM) culture showing specific fluorescence with IIF technique for PCMV antibody. Staining is most intense at and near nuclear membranes; intranuclear inclusions are visible but less bright. $\times 380$.

Fig. 2. PLM culture showing intense fluorescence of 'paranuclear bodies' and absence of intranuclear staining. $\times 960$.