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# The Polypeptide Structure of Canine Coronavirus and its Relationship to Porcine Transmissible Gastroenteritis Virus

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SUMMARY

Canine coronavirus (CCV) isolate 1-71 was grown in secondary dog kidney cells and purified by rate zonal centrifugation. Polyacrylamide gel electrophoresis revealed four major structural polypeptides with apparent mol. wt. of 203 800 (gp204), 49 800 (p50), 31 800 (gp32) and 21 600 (gp22). Incorporation of $^{3}$H-glucosamine into gp204, gp32 and gp22 indicated that these were glycopolypeptides. Comparison of the structural polypeptides of CCV and porcine transmissible gastroenteritis virus (TGEV) by co-electrophoresis demonstrated that TGEV polypeptides corresponded closely, but not identically, with gp204, p50 and gp32 of CCV and confirmed that gp22 was a major structural component only in the canine virus. The close similarities in structure of the two coronaviruses augments the relationship established by serology.

The first suggestion that a virus antigenically related to porcine transmissible gastroenteritis virus (TGEV) was capable of infecting dogs came from Norman *et al.* (1970). Their survey of canine sera revealed antibody to TGEV in American dogs that had never been in contact with pigs and would not have been infected with TGEV. A subsequent report from the U.K. (Cartwright & Lucas, 1972) identified rising titres of antibody to TGEV in a kennel of 40 dogs in which an outbreak of vomiting and diarrhoea had occurred. Once again there was no known contact of the dogs with pigs.

The possibility that TGEV-neutralizing antibodies resulted from exposure of dogs to TGEV, with transmission from dog to dog, could not be excluded, however, since Haelterman (1962) had clearly demonstrated that dogs and foxes could be infected with the porcine virus. There were no clinical signs but TGEV-neutralizing antibodies were produced in the serum and virus was isolated from faeces of the infected animals. The subject was partially resolved, however, when Binn *et al.* (1975) reported the isolation of a canine enteric coronavirus from American military dogs in 1971. The virus, designated 1-71, could be transmitted experimentally to puppies, resulting in acute gastroenteritis and dehydration, but attempts to infect newborn piglets with the isolate were unsuccessful. The 1-71 isolate, which grew in cell cultures, was tested with antisera to several coronaviruses in a neutralization test and reacted positively only with antiserum to TGEV, but to a lesser extent than did the homologous TGEV.

We have studied the canine coronavirus (CCV) because of the epizootiological importance of *Canidae* as potential carriers of TGEV between pig herds, a situation that is confused by the presence of an antigenically related virus that is not pathogenic for pigs. Our objective was to determine ways to differentiate the two viruses and we report elsewhere (Reynolds *et al.*, 1980) the results of a serological study. This paper describes the polypeptide structure of CCV and shows it to be similar but not identical to that of TGEV.

The 1-71 isolate of CCV (Binn *et al.*, 1975) was cloned by three cycles of plaque isolation from secondary dog kidney (DK/2) cell cultures. Radioactive CCV was grown in DK/2 cell monolayers. Virus inoculum was allowed to adsorb to confluent monolayers for 1 h at 37 °C

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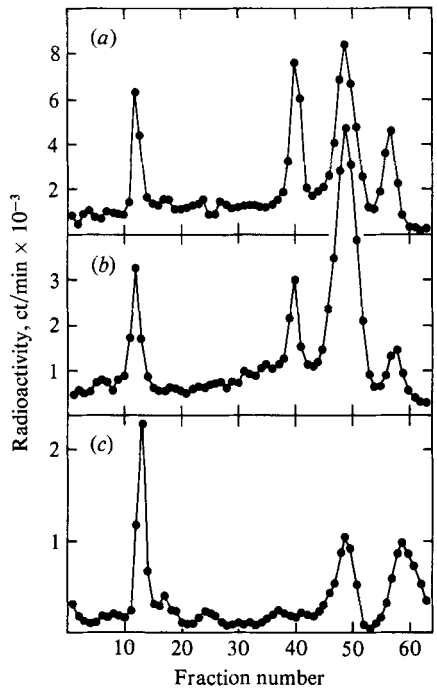


Fig. 1. Polyacrylamide gel electropherograms of CCV polypeptides labelled with (*a*) $^{3}$H-leucine, (*b*) $^{35}$S-methionine and (*c*) $^{3}$H-glucosamine. Migration was from left to right.

after which it was removed and the cells were washed twice with buffered isotonic saline to remove amino acids. Infected cells were incubated with serum-free EG + AA medium (Garwes & Pocock, 1975) buffered with 16·7 mM-sodium bicarbonate and 25 mM-HEPES at pH 6·8. The levels of leucine and methionine in the medium were adjusted to 50 $\mu$M and 10 $\mu$M respectively and L-4,5-$^{3}$H-leucine (sp. act. 55 Ci/mmol), L-$^{35}$S-methionine (sp. act. 1170 Ci/mmol) or D-6-$^{3}$H-glucosamine hydrochloride (sp. act. 38 Ci/mmol) were included at 10 $\mu$Ci/ml. Radiochemicals were obtained from The Radiochemical Centre, Amersham, U.K. CCV reached an infectivity titre of between $10^{6}$ and $10^{7}$ $TCID_{50}$/ml after 42 h incubation in DK/2 cells. The infected cells became unusually refractile but there was little loss of cells into the supernatant medium.

The cultures were frozen after 42 h incubation at 37 °C and the viruses were purified by rate zonal centrifugation as previously described for TGEV (Garwes & Pocock, 1975). Polyacrylamide gel electrophoresis was carried out in 5% acrylamide rod gels cross-linked with 0·135% ethylene diacrylate and containing 1% SDS (Garwes & Pocock, 1975). After electrophoresis at 12·5 mA/gel, the rods were sliced into 1 mm sections, solubilized with 1 M-piperidine and the radioactivity determined. Mol. wt. were estimated by comparison with the migration of bovine haemoglobin (17000 mol. wt.), porcine pancreatic trypsin (24000 mol. wt.) and bovine serum albumin, monomer (69000 mol. wt.) and dimer (138000 mol. wt.).

Electrophoresis of CCV grown in the presence of $^{3}$H-leucine or $^{35}$S-methionine revealed four major structural polypeptides (Fig. 1*a*, *b*) of which three were labelled with $^{3}$H-glucosamine (Fig. 1*c*), indicating that they were glycopolypeptides. The polypeptides are designated gp204, p50, gp32 and gp22, following the convention suggested by August *et al.* (1974) and their apparent mol. wt., estimated from 18 determinations with 13 batches of virus, were 203800 ± 6400 (gp204), 49800 ± 900 (p50), 31800 ± 1100 (gp32) and 21600

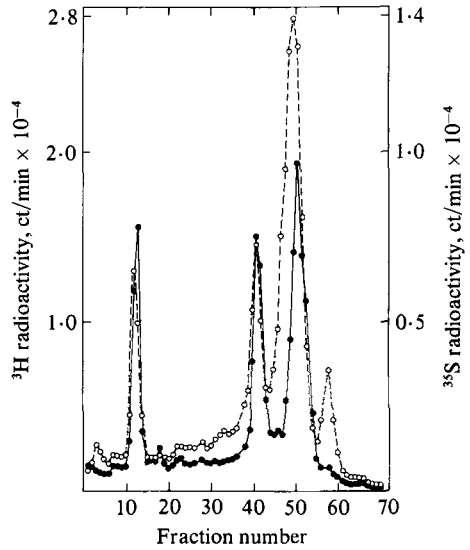


Fig. 2. Polyacrylamide gel electropherogram of the structural polypeptides of CCV labelled with $^{35}$S-methionine (O———O), and TGEV labelled with $^{3}$H-leucine (●———●). Migration was from left to right.

± 1500 (gp22). The proportion of gp32 in the $^{35}$S-methionine-labelled virus appeared to be higher than in the preparations labelled with $^{3}$H-leucine and this is similar to the observation of Sturman (1977) for the membrane glycoprotein of mouse hepatitis virus A59.

This finding is consistent with the general finding for coronaviruses of one, or very occasionally two, non-glycosylated proteins with mol. wt. between 50000 and 60000, glycopolypeptides of >60000 associated with the surface projections and glycopolypeptides of <50000 in the lipid envelope (Garwes, 1980). The present study was not concerned with the location of the CCV structural polypeptides in the virion but some preliminary data suggested that the detergent Nonidet P40 (NP40) removed gp204 from a subviral particle comprising p50, gp32 and gp22 (D. J. Garwes, unpublished results). This indicates that gp204 is associated with the surface projection, as shown for TGEV gp200 (Garwes *et al.*, 1976).

The polypeptide structure of CCV closely resembled that demonstrated for TGEV (Garwes & Pocock, 1975) and a comparative study involving co-electrophoresis was undertaken. The FS772/70 isolate of TGEV was grown in DK/2 cells under similar conditions to those used for CCV. Unlike CCV, however, TGEV produced extensive cytopathic changes including detachment of cells from the glass surface. The yield of virus at 42 h post-infection was $10^{6}$ to $10^{7}$ p.f.u./ml, determined in secondary adult pig thyroid cells (Pocock & Garwes, 1975). Radio-labelled TGEV was purified by the procedure used for CCV.

Preparations of purified CCV and TGEV, each labelled with either $^{3}$H-leucine or $^{35}$S-methionine, were mixed in the combinations $^{3}$H-leu-CCV plus $^{35}$S-met-TGEV, $^{3}$H-leu-CCV plus $^{35}$S-met-CCV and $^{35}$S-met-CCV plus $^{3}$H-leu-TGEV and subjected to SDS–polyacrylamide gel electrophoresis. There was coincidence of the four polypeptides in the double-labelled CCV gels, demonstrating that the substitution of one labelled amino acid for another did not affect the apparent mol. wt., but there were clear differences between CCV and TGEV seen with both conditions of isotopic label. Fig. 2 illustrates the condition $^{35}$S-met-CCV plus $^{3}$H-leu-TGEV. Whereas p50 was coincident for the two viruses, gp204 and gp32 of CCV were reproducibly larger than the equivalent polypeptides in TGEV, migrating approx. 1 to 2 mm slower in each of the three experimental determinations. The gp22

glycopolypeptide of CCV was not found in the TGEV preparations to any great extent although its presence as a minor component cannot be excluded.

The small differences in migration between CCV gp204/TGEV gp200 and CCV gp32/TGEV gp30/28·5 may have resulted from differences in mol. wt., amino acid composition or carbohydrate sequence. Since both viruses were grown in the same cell type under identical conditions, these differences did not result from differences in cultural conditions. However, analysis of only one isolate of each of the two viruses may not give a true representation of the similarities and differences which may exist between the viruses.

The most striking difference between the two viruses is the presence of gp22 as a major component of CCV. This polypeptide appeared in all of the CCV preparations which were examined and is therefore unlikely to represent an artefact. It might have arisen by proteolysis of one of the other structural proteins but this is unlikely as it is glycosylated, eliminating p50 as a possible precursor, does not appear to be removed by NP40, eliminating gp204, and is not rich in methionine as is gp32. It is probable that CCV has two membrane glycoproteins, gp32 and gp22, a characteristic it shares with many other coronaviruses.

The isolates of CCV and TGEV that were examined may, therefore, be distinguished. Together with the differences revealed by neutralization tests (Reynolds *et al.*, 1980) and the inability of CCV to grow in secondary pig thyroid cells (D. J. Reynolds, unpublished results) it should be possible to determine whether a dog is infected with the canine virus or is carrying TGEV, thus involving it in the epidemiology of the porcine disease. How the structural differences described above relate to the antigenic differences between the two viruses is not clear. The surface projection, gp200, is responsible for stimulating TGEV-neutralizing antibodies (Garwes *et al.*, 1978/79) so it would appear that at least one different antigenic determinant is located on the CCV gp204 compared with TGEV gp200.

In the context of virus evolution, it is clear that TGEV and CCV are closer than are the two porcine coronaviruses TGEV and haemagglutinating encephalomyelitis virus which share neither common antigens nor similar polypeptide profiles (Pocock & Garwes, 1977) and cause diseases with completely different pathogenesis. Whether TGEV and CCV were derived one from the other or both were derived from a common parent is a matter for speculation but as more members of the family *Coronaviridae* are investigated these lines of evolution may become clearer.

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