Numerous large foci of multinucleated syncitia could then be observed as early as 18 hours after inoculation of virus. These foci could be seen in unstained cultures, but were most obvious in infected cells that had been grown on cover slips and stained with haematoxylin and eosin. The virus suspension was found to directly haemagglutinate mouse, rat, chicken and turkey erythrocytes at room temperature, but not goose, duck, guinea pig, rabbit, sheep, horse, cow, pig or human type O erythrocytes.

Haemagglutinating encephalomyelitis virus is at present classified as a coronavirus (Greig et al 1971; Phillip et al 1971). Supernatant fluid from the 9th passage of the virus in LLC-PK1 cells was mixed with equal volumes of serial tenfold dilutions of pig serum collected from gilts that had HEV affected litters. The mixtures were allowed to react at 4°C overnight centrifuged at 10,000 g for 60 minutes and the supernatant discarded. The deposit was resuspended in 3 drops of sterile distilled water, the preparation negatively stained with potassium phosphotungstate at pH 6.4 and examined in a Hitachi H300 electron microscope. Aggregates of virus particles morphologically resembling coronavirus were observed at serum dilutions to 100.

The isolation of haemagglutinating agent with the morphological features of a coronavirus from the brains of pigs exhibiting a nervous disease suggested the virus was HEV of pigs. The virus was referred to the Central Veterinary Laboratory, Weybridge, Surrey, England for confirmatory tests, as no reference HEV antiserum was available in Australia. The virus isolated was identical, or closely related, to the Weybridge strain of HEV when tested by an indirect fluorescent antibody test (S.F. Cartwright, personal communication).

A serological survey of pig herds in the State of Victoria using a haemagglutination-inhibition (HI) test was subsequently undertaken. The HI test was performed in V-shaped microtitre trays using 8 haemagglutinating units of serum, viral twofold dilutions of serum inactivated at 56°C for 30 minutes and 0.75% suspension of chicken erythrocytes. All sera were treated with a 25% suspension of acid washed kaolin in borate saline at pH 9.0 and adsorbed against chicken erythrocytes prior to testing. The virus-serum mixtures were allowed to react for 60 minutes and the test was read at room temperature 45 minutes after the addition of erythrocytes. An HI titre of 1 in 16 and greater was considered to be indicative of HEV specific antibody. A total of 364 sera from 24 herds (a minimum of 10 sera per herd) were examined. Ten of the 24 herds were found to have specific antibody, with the HI titre of most sera from infected herds being 1:64 to 1:128. Retrospective examination of stored serum revealed the presence of specific antibody in serum collected from herds of pigs in 1972, 1973 and 1974. These herds had experienced outbreaks of a syndrome resembling Vomiting and Wasting Disease as described by Cartwright et al (1969) but the virus had not been isolated.

The results indicate that HEV is endemic in pigs in Victoria, and that it has been present since at least 1972. Clinical outbreaks of the disease, however, appear to be uncommon.

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HAEMATOLOGICAL VALUES IN VITAMIN B₁₂ RESPONSIVE CALVES

We have recently investigated haematological values in calves responding to vitamin B₁₂ supplementation. The investigation was carried out near Robe, South Australia with Hereford calves depastured on calcareous sands supporting largely strawberry clover and lucerne. In May-June 1978 the calves, aged between 1 and 12 weeks, were stratified by bodyweight within sex and allocated at random to treatment groups. One group of calves the 'B₁₂' group, received subcutaneous injections of vitamin B₁₂ at approximately 6-weekly intervals. In a second group of calves, the 'COP' group each calf received by mouth two 10g cobalt pellets ‡ when introduced to the trial and a third group, the 'Nil' group, were not treated. All calves grazed as one herd and were weighed and given anthelmintic † at 6-weekly intervals. Subcutaneous injections of copper § were given to all calves when introduced into the trial and then at 6, 13 and 32 weeks after the trial had commenced: serum copper of the calves, monitored at 6-weekly intervals, was maintained in the normal range of 8 to 22 umol/l.

Blood samples for haematology were taken into EDTA and values determined using a Coulter S blood cell counter. The haematocrit values obtained by this method were checked manually using a micro-haematocrit centrifuge. When the values did not agree, the manual value was substituted to establish the true red blood cell count. This procedure was undertaken because the more microcytic cells may have fallen

* 2 mg hydroxocobalamin/50 kg bodyweight; 'Coalex', V.R. Laboratories, Sydney, New South Wales.
† 1IC Australia Ltd, Melbourne, Victoria.
‡ Supplied by the Attwood Veterinary Research Laboratory, Department of Agriculture, Mickleham Road, Westmeadows, Victoria.
§ 60 mg/calf, 'Cuprate', Phillips-Duphar Pty Ltd, Sydney, New South Wales.
ǁ Coulter Electronics Inc, Harpenden, Herts, England.

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Differences in bodyweights of calves were associated with differences in haematological values (Table 1). At 32 weeks the 'Nil' calves were similar in haematological values to those observed in the 'Nil' calves (Table 1). At the 32nd week of the trial, the bodyweights of the 'B12' calves were heavier than the 'Nil' calves. 'Nil' calves were then given subcutaneous injections of vitamin B12 to prevent bodyweight losses and at the 38th week of the trial the 'Nil' calves were similar in bodyweight to the 'CoP' calves over the last 6 weeks of the trial although the effect of other factors such as age cannot be excluded. Schalm et al. (1975) in summarising haematological values of cattle reported that red blood cells initially decrease in size over the first 3 to 4 months of life of the calf and then gradually increase in parallel with a gradual decrease in red cell numbers.

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### TABLE 1

| Weeks after start of Trial | Experimental Group | Bodyweight** (kg) | Leucocyte Count No x 10^9/l | Red Cell Count No x 10^12/l | Haemoglobin Value g/l | Haematocrit Value | Meal Cell Volume fl | Mean Cell Haemoglobin Concentration g/l |
|---------------------------|--------------------|-------------------|-----------------------------|----------------------------|-----------------------|-------------------|-------------------|----------------------------------------|
| 20                        | Nil                | 114               | 10.0±2.1                    | 8.53±1.57                  | 103±10               | 0.33±0.03        | 40±4.9            | 309±9                                  |
|                           | B12                | 121               | 10.5±1.1                    | 7.54±0.61a                 | 100±10               | 0.34±0.03        | 45±4.0a           | 296±10                                 |
|                           | CoP                | 119               | 11.9±2.8                    | 9.67±0.86a                 | 111±7                | 0.35±0.02        | 36±2.3a           | 314±18                                 |
| 32                        | Nil                | 160±19.9c         | 10.3±1.9c                   | 8.44±0.94a                 | 121±12               | 0.34±0.03        | 40±2.3a           | 357±10                                 |
|                           | B12                | 205±5             | 12.8±1.7                    | 6.38±0.96b                 | 110±10c              | 0.32±0.03c       | 51±4.6ac           | 345±14                                 |
|                           | CoP                | 190±8             | 16.6±3.9c                   | 8.93±1.12b                 | 132±11c              | 0.37±0.02c       | 42±3.6c           | 353±8                                   |
| 38                        | Nil                | 200±9             | 9.9±1.2                     | 6.64±0.41                  | 110±6                | 0.32±0.02        | 48±3.1            | 344±18                                 |
|                           | B12                | 237±10            | 10.1±1.5                    | 7.46±0.72                  | 120±8                | 0.35±0.02        | 48±2.5            | 339±5                                   |
|                           | CoP                | 214±8             | 9.1±2.9                     | 8.32±1.81                  | 124±17               | 0.38±0.05        | 46±5.5            | 327±17                                 |

* Five calves in the 'Nil' group and their counterparts (determined by bodyweight at the start of the trial) in each of the other groups were bled. The same calves were bled on each occasion. Bodyweight responses were tested by two-way analysis of variance and haematological differences by Students' 't' test. For each stage of the trial and for each column, values with the same superscript 'a' or 'b' differed at P < 0.01 and with the superscript 'c' differed at P < 0.05.

** Mean values for 16 calves.

† 'Nil' calves were each given a single injection of vitamin B12 at the 32nd week.