B_{12} in sea water was tested with water collected in July 1957 from two stations in Long Island Sound and frozen within 5 hours after collection. For the assay, the sea-water samples were enriched with glucose, glutamate, lysine, thiamine, and agar at the level of the basal medium. Five- and 2.5-ml portions of each sample were diluted with full-strength basal medium (without bicarbonate) to a final volume of 10 ml. Undiluted samples were inhibitory to *Thraustochytrium globosum* in the presence or absence of added vitamin B_{12}. Consistent results and satisfactory recovery of added vitamin B_{12} (5 μμg/ml) were obtained with one sample at both dilutions; with the other, only at the greater dilution. Both samples assayed 16 to 20 mμg of vitamin B_{12} per liter—a level somewhat higher than that found for unfiltered ocean water at other locations and at various seasons by Cowey (*5*) and by Droop (*6*) (0.2 to 4.0 mμg/lit and 5 to 10 mμg/lit, respectively).

ELIZABETH J. ADAIR
HELEN S. VISHNIAC
Department of Microbiology, Yale University, New Haven, Connecticut

References and Notes

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Propagation of Infectious Canine Hepatitis Virus in Porcine Kidney Tissue Culture

Infectious canine hepatitis virus has generally been considered to be a host-specific agent (*1*). Numerous attempts have been made, in this laboratory and elsewhere, to adapt this virus to embryonated eggs, mice, rabbits, and other animals, with essentially negative results. The successful propagation of infectious canine hepatitis virus in dog-kidney tissue cultures with the production of a typical cytopathogenic effect has been reported (*2, 3*). By continuous passage in dog-kidney tissue culture, the virus was modified so that it did not produce disease when it was inoculated into susceptible dogs (*3*). Previous attempts in this laboratory to adapt virulent infectious canine hepatitis virus to pig-kidney explants were unsuccessful. This report shows that the virus can be successfully propagated in pig kidney epithelial cells after a series of passages in dog-kidney tissue cultures.

Tissue cultures were prepared from the kidney cortex of pigs of between 2 and 12 weeks of age by a trypsin digest method in which 0.33 percent trypsin was used. Two nutrient media were employed: (i) 0.5 percent solution of lactalbumin hydrolyzate in Earle's balanced salt solution, fortified with 10 percent inactivated horse or bovine serum (ELS medium); (ii) medium No. 199 (Parker) without serum. Each medium contained penicillin and streptomycin. Initial attempts to adapt infectious canine hepatitis virus to trypsinized-pig-kidney tissue cultures were made with virulent virus. From portions of liver from two dogs killed during the acute phase of infection, 20-percent suspensions in physiological saline were made, and the supernatants of these were inoculated into culture tubes containing ELS medium. Additional cultures were inoculated, in the same manner, with tissue culture fluid from the 14th dog-kidney tissue culture passage of infectious canine hepatitis virus. The cultures, incubated at 35°C, were observed for 7 days, after which time the fluids from each group of tubes were harvested and passed into additional tubes. Subpassages of each group of cultures were made three times, at 7-day intervals. At that time, the tissue culture fluids were inoculated into trypsinized-dog-kidney roller tubes, to test for the presence of virus. No virus could be detected.

Further efforts at adaptation were made with modified virus which had undergone 134 passages in dog-kidney cultures. Several pig-kidney tissue cultures in tubes containing ELS medium were inoculated in a manner similar to that described above, and subpassages were prepared. Commencing with the fifth tissue culture passage, a portion of the cells exhibited a cytopathogenic effect that resembled, to some degree, the changes previously observed in dog-kidney tissue cultures. This effect continued to occur, in an erratic manner, through the tenth passage. Beginning with the tenth passage, the nutrient medium was changed to No. 199. Thereafter, a complete cytologic change developed in the cells 3 to 6 days after inoculation and occurred regularly through 28 additional passages. The titer of the tenth pig-kidney passage was $10^{5.0}$, as was demonstrated by titrations in dog-kidney tissue culture. Titrations at intervals through the 38th passage ranged from $10^{4.5}$ to $10^{5.5}$. The affected cells became swollen, rounded-up, and highly refractile. Epithelial sheets broke up, and the cells formed small, grapelike clusters.

Serum neutralization tests to identify the cytopathogenic agent in pig-kidney tissue were performed at various passage levels. Infected tissue culture fluid prepared in tenfold dilutions was mixed with an equal amount of known infective-canine-hepatitis-positive canine antiserum. These mixtures were incubated for 2 hours in a 37°C water bath and inoculated, in 0.2-ml amounts, into each of several pig- and dog-kidney cultures. Up to 100,000 tissue culture infectious doses of virus were neutralized both in pig and dog cultures by the positive serum, while the usual cytopathogenic effect occurred in control cultures that contained the normal serum.

Tests were made in dogs to provide additional evidence that the cytopathogenic agent propagated in pig-kidney tissue culture was infectious canine hepatitis virus. Fourteen infectious-canine-hepatitis-susceptible dogs were used. Eight were inoculated subcutaneously with 19th-passage, and six with 34th-passage, material. Daily temperature recordings and observations for other signs of illness were made for 3 weeks, following which the animals were challenged with known virulent virus. No signs of illness were observed in the dogs inoculated with pig-adapted virus, and none of the dogs responded to challenge. Preinoculation serums were found to be negative (by a serum-neutralization test employing known infectious canine hepatitis virus that had been grown in dog-kidney culture); all dogs had developed neutralizing antibody titers of from 100 to 3200 three weeks later. The development of specific antibodies in the dogs further demonstrates that the cytopathogenic agent is infectious canine hepatitis virus. The lack of illness in the inoculated dogs indicates that the virus is of modified virulence and could be used for development of a vaccine.

JERRELL B. EMERY
CHARLES J. YORK
Research Division, Pitman-Moore Company, Indianapolis, Indiana

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Action of Hydrocortisone on Respiration and Aerobic Glycolysis of Cultured Cells

In previous reports, inhibition of growth of fibroblasts by a single high dose of hydrocortisone was demonstrated (*1*). Cells derived from a single fibroblast, which had been isolated from a culture of normal subcutaneous tissue of the mouse, Earle's strain L, have been