Susceptibility of Pig Kidney Tissue Cultures to Certain Viruses. (23466)

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During attempts to replace the costly monkey kidney tissue cultures used for the diagnosis of various virus diseases, it was found that pig (porcine) kidney tissue cultures were susceptible to the Coxsackie viruses of Group B but were unaffected by the Echo virus group. A differential diagnostic test is thus suggested for these 2 groups of viruses. Although we could find no record of previous use of pig kidney tissue cultures for the propagation of viruses pathogenic for humans, the viruses of vesicular exanthema(1), vesicular stomatitis(2) and foot and mouth disease (2) have been shown to reproduce in such cultures.

Materials and methods. Kidneys were obtained either from suckling pigs, 3 to 6 weeks of age, in the laboratory or from adult pigs at an abattoir. In the latter case, kidneys were removed as aseptically as possible within 30 minutes of death of the animal, placed immediately into synthetic medium 150(3,4) to which 100 units/ml of penicillin, 100 μg/ml of streptomycin and 40 units/ml of mycostatin had been added. They were removed to the laboratory and immediately trypsinized at 4°C by the method of Bodian(5). For the initial cell growth period, 2% calf serum was added to the cells which were suspended in either Bodian's medium(5) or medium 150. Kidney cultures prepared from suckling pigs grew rapidly but had a shorter survival time than those prepared from adult pig kidneys. Since virus susceptibility was the same in both cases, adult pig kidneys were chosen for the work reported here. All of the established virus strains studied had been maintained in this laboratory in monkey kidney tissue cultures prior to the tests except the Coxsackie Types A1-A8 and A10-A19 which had been maintained by serial suckling mouse brain passage. Tissue cultures were observed for 7 days after inoculation at which time complete cell degeneration had taken place in those inoculated with cytopathogenic strains.

Results. A summary of the susceptibility of monkey kidney and pig kidney tissue cultures to the viruses studied is presented in Table I. The type of degeneration observed in pig kidney cultures was similar to that seen in monkey kidney cultures, that is, the appearance of round, refractile cells which finally fall away from the wall of the culture flask.

Echo virus Types 1-11 and 14-19, inclusive, have been studied in this work. With the exception of Type 10, which did cause cytopathic effects to some degree, these agents did not induce cytopathic changes in pig kidney tissue cultures.

Two to 4 serial passages were carried out with all strains and serum-virus neutralization tests with standard immune sera indicated that the specificity of the cytopathogenic strains had not been altered. Essentially the same virus titers were obtained with both monkey kidney and pig kidney infected culture fluids, varying from titers of 10^3 TCD_{50}

TABLE I. Cytopathogenicity of Various Viruses in Pig Kidney and Monkey Kidney Tissue Cultures.

Virus	Type or strain	Pig kidney	Monkey kidney
Coxsackie:			
Group B	B-1 to B-5 incl.	+	+
" A	A-1 to A-8; A-10 to A-19 incl.	—	—
	A-9	—	+
Echo*	Types 1 to 9; 11; 14 to 19 incl.	—	+
Poliomyelitis:			
Type I	Mahoney, Brunhilde	—	+
" II	MEF1, YSK, MEF1-mouse adapted	—	+
" III	Saukett, Leon	—	+
Virus B		+	+
Adenovirus†	Types 1 to 7 incl.	+	+

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(tissue culture infective doses) for the adenoviruses to 10^7 TCD_{50} for the Coxsackie B viruses.

Several strains of Coxsackie virus Type B which had been isolated from human stool specimens in monkey kidney tissue cultures were also isolated in pig kidney cultures from the same specimens. On the other hand, poliomyelitis Types I, II and III viruses which had been isolated in monkey kidney cultures from similar specimens could not be isolated in pig kidney cultures.

Summary. 1) Susceptibility of pig kidney tissue cultures to the following viruses has been described: Coxsackie A and B, Echo, poliomyelitis, adenoviruses, and virus B. 2) Susceptibility of these cultures to Coxsackie group B viruses, virus B and the adenovirus group has been established. From the results it is apparent that pig kidney cultures may be utilized as an additional tool in the diagnostic differentiation between Coxsackie Group B viruses and the Echo virus group.

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Some Factors Affecting Differential Excretion of D-Phenylalanine in Man.* (23467)

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Marked variability between individuals in urinary excretion and utilization of D-phenylalanine by man has been noted by several investigators(1,2). It has also been recently observed through limited twin studies that some of this variability may have a genetic basis(2). Since the physiological basis of this differential excretion of D-phenylalanine could be quite complex, we felt it would be worth while to investigate various physiological aspects of this problem before going on with further genetic studies. Therefore, the present study of the role of the following physiological variables in the differential excretion of D-phenylalanine was undertaken: (1) absorption of D-phenylalanine into the blood, (2) metabolism of D-phenylalanine, and (3) renal excretion of D-phenylalanine.

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Methods and materials. The subjects were normal, healthy adults and were tested after an overnight fast. At this time, blood and urine samples were obtained and the subjects were then given orally 0.5 g of D-phenylalanine (Mann Laboratories) dissolved in 200 ml of water. Blood was taken from the anticubital vein into heparinized tubes and the plasma drawn off. The plasma and urine samples were then stored at −10°C until ready for analysis. On 2 of the 4 occasions where experiments were repeated on the same individuals, 1.0 g of D-phenylalanine was given instead of 0.5 g for the second trial. One hour after feeding, blood samples were again taken and the urine flow for the one hour test period collected. L-phenylalanine determinations were carried out on all plasma and urine samples by a modification(3) of the Udenfriend and Cooper decarboxylase method(4). D-phenylalanine was estimated by the difference between L-phenylalanine determinations and paper chromatographic estimations of DL-phenylalanine on the second blood and urine samples. Phenylpyruvic acid determinations