New Types of Coxsackie Virus, Group A. Cytopathogenicity in Tissue Culture. (25383)

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Because of increasing concern about the role of Coxsackie Group A viruses in infection and as an aid in classification of the enteric viruses now so readily isolated, type numbers have been assigned to representative strains of 5 new types and their activity in tissue culture is described. Identification as Coxsackie virus, Group A, rests on infectivity for suckling mice, together with characteristic histopathology and signs of disease. Type determinations were made in suckling mice with hamster and mouse immune sera(1). *Type 20, 20(a), 20(b).* Serum prepared with each of the 3 representative strains neutralizes the others but the heterologous titer is in each case only approximately one-fourth of the homologous. Further experience is necessary to indicate whether all new isolations will show a degree of strain specificity or whether they will tend to be more like one or other of the representative strains. The representative strain for Type 20, No. 55166, was isolated in 1955 in this laboratory on human embryonic cell cultures and reisolated on HeLa cells by Dr. Sonja Buckley from a pool of fecal specimens from patients with infectious hepatitis. The Type 20(a) representative strain, No. 5536, originally isolated in 1951 in the laboratory of Dr. John Fox on human skin-muscle tissue culture from stool specimen of a suspected poliomyelitis patient, was received by us in 1955 from Drs. Edwin D. Kilbourne and Martin Goldfield of Tulane Univ. School of Medicine. Type 20(b) No. 5721 was isolated in 1951 in the laboratory of Dr. J. H. S. Gear, South Africa, from feces of a child living in Germiston Native Location. The representative strain of Type 21, No. 55161, was received in 1955 as TC_4 SM_7 from Dr. Edwin Lennette, Berkeley, Calif., with a history of isolation in human fetal tissue from stool material. This strain has occasionally induced paralysis in 10-12 g mice(2). Type 22 representative strain, No. 5630, was isolated in this laboratory directly in suckling mice from a stool specimen. Type 23 is the designation given to the virus at present generally known as ECHO 9. The first strain studied by us, No. 55222, was received from Dr. Italo Archetti, Rome, Italy, in Dec. 1955(3). Epidemic strains of this type do not usually infect suckling mice directly from the fecal specimen; tissue cultures do so in many cases but not in all(4,5,6). The virus chosen as the representative strain, No. 5844, however, was isolated in this laboratory directly in suckling mice from a stool specimen. The representative strain of Type 24, No. 5720, was isolated in 1952 from feces of a Germiston native baby in the laboratory of Dr. J. H. S. Gear, South Africa.

Methods. Suckling mice. Infectivity, type determination and cross reactivity tests were made in suckling mice. Typing sera were prepared in hamsters or large mice, using as antigen muscle suspension of suckling animals of the same species. Type 20 No. 55166, when first tested had little pathogenicity for suckling hamsters and a mouse serum was prepared with this strain. For the other strains, antisera were readily prepared in hamsters. *Tissue culture.* Human uterine tissue in plasma clot and trypsinized monkey kidney tissue were prepared as previously described (7). Medium 199 was used as maintenance medium for both types of culture. Two lines of HeLa cells were used, one received originally from Tuskegee Inst., the other from Dr. William Scherer. Both had been maintained in this laboratory for some time. For growth, 10% of human serum was added to a nutrient fluid prepared by adding 0.1% glucose, 0.1% yeast extract and 0.25% tryptose to Hanks' solution. This was replaced at time of virus inoculation by the yeast extract medium of Syverton and McLaren(8) containing 10% serum of which 2 parts were monkey

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TABLE I. Cytopathogenicity of Coxsackie Virus.

Type	Tissue cultures				
	Monkey kidney	Human uterine	HeLa	Human amnion	Trypsinized chick tissue
20	—	—	+*	—	—
20(a)	—	—	+*		—
20(b)	—	—	+*		—
21	—	+	+	+	—
22	—	—	—	—	—
23	+†	—	—	+†	
24	—	—	—		—

The inoculum for all cultures was a suspension of infected suckling mouse tissue.

* Types 20, 20(a), and 20(b) are cytopathogenic for both cell lines of HeLa used, but were more active on the line originally received from Tuskegee Inst.

† Type 23 is markedly cytopathogenic for monkey kidney, less so for human amnion.

serum and one part calf serum. Human amnion cell cultures prepared by trypsinization were grown in Bodian's medium (9) modified by omission of liquid bovine albumin and with addition of 20% human serum. For maintenance, the modified Bodian medium plus 2% calf serum was used. Chick embryo tissue cultures were prepared by trypsinizing 7 or 8 day embryos. Medium 199 plus 2% horse serum was used for growth; for maintenance the serum was omitted. Sterile sodium bicarbonate was used to adjust the reaction. Penicillin and streptomycin were added at time of use. The cytopathogenicity of virus strains was studied on various tissues but with the exception of Type 20 strains on HeLa cells, no attempt was made to adapt them.

Results. All strains induced flaccid paralysis in newborn mice with marked myositis but no significant lesions in the central nervous system. They caused no apparent disease in 10-12 g mice, with the exception of Types 21 and 24 which occasionally caused paralysis. On subsequent passages no paralysis was induced. Type 23 virus was not neutralized by Coxsackie A-9 hamster serum in neutralization tests in mice. Tyrrell and coworkers (10) noted that in complement-fixation tests the 2 types, while distinct, have some antigenic relationship.

The cytopathogenicity of the strains for various tissues is shown in Table I.

Summary. Five additional types of Coxsackie virus Group A have been assigned numbers, Types 20-24. Strains of all types with the exception of Type 23 are isolated readily in suckling mice. Type 23, which is the infectious agent presently known as ECHO 9, may be more easily isolated from the fecal specimen when tissue cultures are used for isolation. Types 20, 21 and 23 were cytopathogenic for one or more types of tissue in cultures; Types 22 and 24 did not induce damage on any of the cell types used.

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Received October 8, 1959. P.S.E.B.M., 1959, v102.