Identification of Thai C-18 Virus as a Member of the Coxsackie Virus A20 Subgroup.* (29336)

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Thai C-18 virus was isolated by Abraham and Cheever in the FL line from a patient who became ill during the 1958-59 cholera outbreak in Bangkok, Thailand(1). The virus did not grow in monkey kidney cell cultures but was pathogenic for suckling mice and possessed the general characteristics of group A Coxsackie viruses. Because these workers were unable to show a relationship between this virus and the known enteroviruses tested, they submitted it to this laboratory as a new prototype. In this communication we are reporting that the virus has been identified as a new and distinct member of the Coxsackie virus A20 subgroup. Sickles *et al*(2) have referred to the existence of this subgroup, but as nothing further has been reported, information about them is scanty.

Materials and methods. The virus was received in molar $MgCl_2$ and was successfully grown in both human embryonic kidney and human diploid (WI-38) cell cultures. No propagation occurred in either rhesus or green monkey kidney cells. Preliminary typing with combination serum pools(3,4) containing antibodies to all the Coxsackie viruses that have been adapted to tissue culture indicated an antigenic relationship only with Coxsackie virus A20. The Thai virus as well as the 3 prototype strains of the subgroup (A20, A20A, A20B) were grown in human diploid cell cultures and cross neutralization tests with respective antisera‡ and $10^{2\pm0.5}$ TCD_{50} of each virus were performed using the same cell system. The tests were carried out as recommended by the Committee on Enteroviruses(5).

Results. The results of 3 tests were averaged; they are tabulated in Table I. They indicate that Thai C-18 is a new subtype of the A20 virus, sharing antigens with all 3 known serotypes. It is sufficiently distinct to warrant its being classified as group A Coxsackie virus type 20C.

In the course of these cross tests, it was observed that Coxsackie virus A20 serum readily neutralizes other related subtypes, but the A20 virus is not readily neutralized by heterologous antisera of strains within the subgroup. The best indicator strain within this enterovirus subgroup proved to be A20A as it is readily neutralized by antisera of all 4 subtypes, similar to the manner in which the DuToit strain serves as the indicator strain of echovirus type 4.

Discussion. Enteroviruses are known to show antigenic variation within types(6). Prime strains exist which can hardly be neutralized by antiserum to related strains. In such cases the relationship is established by strong neutralization of the related strain by antiserum against the prime strain. Prime strains are known for echoviruses 1, 4, 6, 9, and 30, and for Coxsackie viruses B2 and B3, as well as for Coxsackie virus A20. In this report another example of this antigenic variability is shown. It would appear from

TABLE I. Cross Neutralization Tests with Subgroup A20 Coxsackie Viruses and Thai C-18 Virus.

	Antisera titers (mean of 50% serum dilution endpoints)			
	Antisera			
Viruses	A20	A20A	A20B	Thai C-18
A20	3100	<25	60	<25
A20A	1400	300	1400	600
A20B	3100	125	3100	<25
Thai C-18 (A20C)	125	<25	300	3100

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‡ These antisera were prepared in monkeys in Dr. Herbert Wenner's laboratory, Univ. of Kansas, under the auspices of the Panel for Picornaviruses, Nat. Inst. of Allergy and Infect. Dis., N.I.H.

the data presented that if only antisera to single strains are used at the dilutions recommended for typing purposes, certain isolates only partially related to the prototypes might be missed, and considered as new types. By subgrouping such partially related types, it may be possible to limit the expansion of picornavirus types, which now number close to 100.

The A20 Coxsackie viruses are global in distribution(2). The first member of the group was isolated from patients with hepatitis in New York State. The A20A prototype was recovered from a patient with suspected poliomyelitis in New Orleans, A20B from a normal child in South Africa, and A20C from a cholera patient in Bangkok(1).

Summary. Thai C-18 virus, previously believed to be a new enterovirus type, has been shown to belong to the Coxsackie virus A20 subgroup. Four antigenic variants are now known for this subgroup. The indicator strain of choice within the subgroup is A20A, as it is most readily neutralized by antisera against all 4 variants.

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In vivo Inhibition of Lipogenesis by Biotin Deficiency.* (29337)

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Biotin deficientcy has been shown to inhibit lipogenesis *in vitro*(1,2,3). It has been suggested that the block in lipogenesis that results from feeding fat occurs between acetyl CoA and malonyl CoA(4), a reaction which requires biotin. Attempts to produce biotin effects on lipogenesis *in vivo* have not been successful perhaps because the diets employed contained 5-12% added fat which itself could have depressed lipogenesis(5,6). This paper reports the effects of biotin deficiency in intact chicks when fat was not included in the control or deficient diets.

Methods. Day-old White Rock × Cornish female chicks were reared in electrically-heated batteries with raised-wire floors and were fed the diet shown in Table I, with or without 2 mg of biotin/kg. Chicks fed the deficient diet exhibited retarded growth and severe dermatitis of the feet and legs at 21 days of age. At 25 days, 12 chicks were selected according to body weight from each group. Six chicks fed each diet were injected intraperitoneally with 2 μc of sodium acetate-

TABLE I. Composition of Experimental Diets.

Ingredient	% of diet
Sucrose	62.8
"Vitamin-free" casein*	25.0
DL-methionine	.3
Glycine	1.0
L-arginine	.5
Cellulose†	4.0
Vitamin mix‡	.4
Mineral mix§	6.0

* Nutritional Biochemicals Corp., Cleveland, Ohio.

† Solka-floc, Brown Co., Boston, Mass.

‡ Same mix less biotin as used by Donaldson, W. E., *J. Nutrition,* 1964, v82, 115. Control diet contained 2 mg of biotin/kg.

§ Fox, M. R. S., Briggs, G. M., *J. Nutrition,* 1960, v72, 243.

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