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Relationships of V1030, V1880 and Pett Viruses to the Prototype A24 Coxsackievirus.* (31469)

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V1030 and V1880 viruses were isolated in 1961 from fecal specimens of 2 children vaccinated with inactivated polio-diphtheria-pertussis-tetanus vaccine. The two viruses possessed properties of picornaviruses, but no definite relationship between them and the known enteroviruses tested could be established(1). Subsequently they were submitted to this laboratory as potentially new enterovirus prototypes. Our preliminary study of these 2 viruses and reference antisera indicated a relationship between V1030 and coxsackievirus A24. Further study showed that V1030 and V1880 viruses were related to each other and that both were related to the prototype strain of coxsackievirus A24. Another unassigned virus, named Pett, was included in this study because of some evidence of antigenic relationship between it and coxsackievirus A24(2). The Pett virus was isolated during the summer of 1956; it is the prototype of 8 agents obtained from fecal specimens of 10 children during an outbreak of respiratory illness(3).

Materials and methods. Viruses. V1030 and V1880 were received from Dr. Wolf Henigst of Munich, Germany, as infected fluid overlays of the sixth passage in primary human amnion cultures. Coxsackievirus A24 was the National Institutes of Health (NIH) reference virus prepared at this laboratory. The Pett virus was received from Dr. Julius A. Kasel of the NIH as infected HeLa cell culture fluid. All 4 viruses were passed in primary human amnion cells and purified by 3 terminal dilution passages. For the immunization of monkeys and performance of serologic tests a stock of each virus was prepared in Roux bottles containing human amnion cells.

Antisera. Three monkeys were inoculated using the schedule described for enteroviruses (4). One or two additional booster doses were given in order to obtain satisfactory homologous antisera.

Cell cultures. Human amnion cell cultures were used for (a) propagation of all 4 viruses, (b) performance of serum neutralization tests and (c) preparation of both hemagglutinating and complement fixing antigens. For determining spectra of cell susceptibilities of V1030 and V1880 viruses, cell cultures of WI-38, rhesus and green monkey kidney and BHK-21 were used. Growth and maintenance of these cells were according to the procedures described by Schmidt(5).

Newborn mice. One-day-old white Swiss mice were inoculated either intracerebrally and intraperitoneally or intracerebrally and subcutaneously. When no symptoms appeared, mice were sacrificed after 14 days and a 20%

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TABLE I. Cross Serum Neutralization Tests with Coxsackie A24, V1030, V1880 and Pett Viruses and Their Antisera.

Virus	Antisera			
	Cox. A24	V1030	V1880	Pett
Cox. A24	250* (316)†	75 (32)	50 (176)	<25 (176)
V1030	25 (55)	50 (176)	200 (176)	<25 (176)
V1880	<25 (176)	<25 (176)	400 (550)	<25 (176)
Pett	<25 (176)	<25 (176)	<25 (176)	1600 (316)

* Reciprocal of serum dilution endpoint.
† TCD_{50} in parentheses.

suspension of torso and brain was made for the next passage.

Serum neutralization (SN) tests. These tests were done with 100-500 TCD_{50} of virus and 4-fold dilutions (1:25-1:1600) of antisera inactivated at 56°C for 30 minutes. Serum endpoints were determined by the method of Reed and Muench(6).

Hemagglutination-inhibition (HI) and complement fixation (CF) tests. All 4 viruses were tested for hemagglutination (HA) with human O erythrocytes at pH 5.8, 6.9 and 7.4 and at temperatures 4°, 25° and 37°C. Sera were treated with kaolin and absorbed with erythrocytes for HI tests and heated at 56°C for 30 minutes for CF tests. All tests were performed by the microtiter system. Freon TF obtained from DuPont and Co., Chicago, Ill., was used to purify virus stocks for HA activity (1:1 ratio extraction in the cold for 10-20 minutes).

Results. SN tests. The results of cross SN tests with all 4 viruses and monkey antisera prepared against them are presented in Table I. Data entered therein indicate that coxsackie virus A24 antiserum neutralized the homotypic virus at a dilution of 1:250, but showed little neutralization for V1030 and none for V1880 and Pett viruses. V1030 antiserum neutralized the homotypic and coxsackie A24 viruses at approximately the same titer but did not react with V1880 virus. V1880 antiserum, however, neutralized the homotypic as well as coxsackie A24 and V1030 viruses. Pett virus was not neutralized by any of the antisera, except the homologous, and Pett antiserum did not neutralize any of the 3 other viruses.

TABLE II. Cross Complement Fixation Tests with Coxsackie A24, V1030, V1880 and Pett Viruses and Their Antisera.

Antigen	Antisera			
	Cox. A24	V1030	V1880	Pett
Cox. A24	64*	<8	<8	<8
V1030	16	32	32	<8
V1880	16	32	32	<8
Pett	<8	<8	<8	16
Normal HuAm	<8	<8	<8	<8

* Reciprocal of serum dilution endpoint.

CF tests. Cross CF tests are recorded in Table II. The results indicate that coxsackievirus A24 antiserum is not only reactive with the homotypic virus but with V1030 and V1880 viruses as well. On the other hand, V1030 and V1880 show only mutual relationships by this test. No crossing between Pett and the other 3 viruses was observed.

TABLE III. Cross Hemagglutination-Inhibition Tests with Coxsackie A24 and V1030 Viruses and Antisera to Coxsackie A24, V1030 and Pett Viruses.

Virus	Antisera			
	Cox. A24	V1030	V1880	Pett
Cox. A24	160*	40	40	<10
V1030	10	80	40	<10

* Reciprocal of serum dilution endpoint.

HI tests. Stock preparation of coxsackievirus A24 showed good HA activity at 4°C and pH 5.8. HA activity could not be demonstrated for stock preparations of the other 3 viruses at various temperatures (4°, 25° and 37°C) and pH (5.8, 6.9 and 7.4). Additional preparations of these 3 viruses were made in human amnion tubes inoculated with only 10 TCD_{50} and rolled at 37°C and then harvested when 4+ CPE was present or 1-3 days after the appearance of 4+ CPE. No HA could be demonstrated with the above harvests. However, extraction of stock viruses with Freon TF by shaking the virus with equal amount of the fluorocarbon for 10-20 minutes in an ice bath produced HA activity (1:16) for V1030 virus. Hence, HI tests were performed with coxsackievirus A24 and V1030 virus and all 4 antisera. The results are presented in Table III. The interrelationships among coxsackievirus A24, V1030 and V1880 are well defined. Here again, Pett

virus failed to show any antigenic relationship with the other 3 viruses.

Mouse pathogenicity. It had been reported that intracerebral and subcutaneous inoculation of one-day-old mice with V1030 and V1880 viruses did not produce any illness; however, additional blind passages and histological study of inoculated mice were not attempted(1). These two viruses, as harvests of the eighth passage in human amnion cells (6 elsewhere and 2 at this laboratory), were tested for mouse pathogenicity by 3 blind passages in one-day-old mice. No symptoms could be observed in inoculated mice. Nonpathogenicity of Pett virus for suckling mice had already been established(3).

Host-cell range. Previous work(1) had indicated that V1030 and V1880 viruses do not grow in rhesus monkey kidney, KB and HEp-2 cell cultures. Further study of the host cell range for these 2 viruses showed that in addition to human amnion cells both viruses produced CPE in WI-38, but not in green monkey kidney or BHK-21 (continuous baby hamster kidney) cells. The TCD_{50} of both viruses in WI-38 and human amnion cells were $10^{4.75}$ and $10^{5.5}$ per ml respectively.

Discussion. The prototype of coxsackievirus A24, strain Joseph, P3305 was isolated in suckling mice by Gear in 1952 from feces of a healthy 3-year-old male residing in Germiston Location, South Africa(7). In recent years, however, the virus has been isolated infrequently(2,8). V1030 and V1880 viruses were isolated by Henigst in human amnion cells from feces of 2 German children vaccinated with inactivated polio-diphtheria-pertussis-tetanus vaccine; they were subsequently characterized as human enteroviruses. Henigst observed a variable neutralization of these viruses with reference coxsackie A24 antiserum, but was unable to establish an antigenic relationship because of unavailability of satisfactory antisera to V1030 and V1880 viruses. In the study reported here, the 2 viruses were purified by 3 terminal dilution passages in human amnion cells and workable antisera similarly prepared in rhesus monkeys against coxsackie A24 and Pett viruses (also purified by 3 terminal dilutions) were cross tested by SN, HI and CF tests. The results indicate an antigenic relationship between coxsackie A24, V1030 and V1880 viruses. However, while V1880 serum neutralized itself as well as coxsackie A24 and V1030 viruses, V1030 serum neutralized only the homotypic and coxsackie A24 viruses. On the other hand, coxsackie A24 serum neutralized only the homotypic strain. Similarly, Henigst also observed that the infant excreting V1030 developed neutralizing antibodies against the infecting virus only while the infant who yielded V1880 showed neutralizing antibodies to both V1030 and V1880 viruses. Moreover, V1030 virus appears to differ from the other two strains in that it is a poor antigen since high titered antiserum against it could not be produced in monkeys even after one priming and 6 boostering doses of antigen. On the other hand, V1880 virus also differs inasmuch as it does not possess the hemagglutination property manifested by coxsackie A24 and V1030 viruses. The relationship observed here is similar to that reported for strains of echovirus type 6 in which the prime strains can hardly be neutralized by antiserum to the prototype(9). Another antigenic variation within one type of coxsackievirus is exemplified by coxsackievirus A20(10). Since mouse pathogenicity is an essential property of the coxsackievirus group, failure to establish such pathogenicity for V1030 and V1880 would seem to remove these two viruses from this group. However, it has been demonstrated that virulence for mice can be lost during passage in cell culture(11). Moreover, some strains of coxsackievirus possess such a low virulence for mice on primary isolation that several passages are required before typical signs of infection appear in these animals(12). As pointed out above, repeated blind passages of V1030 and V1880 viruses in suckling mice were not attempted. It is, thus, probable that lack of mouse pathogenicity observed at this laboratory for V1030 and V1880 was due to excessive passage in cell culture and that had the original clinical specimens been repeatedly passaged in suckling mice, the property of mouse pathogenicity could have been established for these 2 strains. It is also conceivable that these 2 viruses represent certain strains that are antigenically

related to members of coxsackievirus group but lack the property of mouse pathogenicity (intermediate strains). Cross serum neutralization, CF and HI tests failed to reveal any antigenic relationship between Pett virus and coxsackievirus A24 as well as V1030 and V1880 viruses. The basis for the suggested antigenic relationship between Pett and coxsackie A24 viruses is not clear. To our knowledge, no convincing data indicating such a relationship have been documented.

Summary. Two viruses isolated from children vaccinated with inactivated polio-diphtheria-pertussis-tetanus vaccine and designated V1030 and V1880 were submitted to this laboratory as possible new enterovirus prototypes. Cross serum neutralization, complement fixation and hemagglutination inhibition tests indicated that V1030 and V1880 viruses are antigenically related to the prototype coxsackievirus A24. However, both viruses, when tested after several passages in human amnion cells, failed to show mouse pathogenicity. These two viruses appear to be prime strains of coxsackievirus A type 24 that are either devoid of mouse pathogenicity or have lost this property due to excessive passage in cell culture. No antigenic relationship between Pett virus and coxsackievirus A24 could be detected.

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Dithizone Induced Changes in Carbonic Anhydrase and Alkaline Phosphatase of Rat Dorsolateral Prostates.* (31470)

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The dorsolateral prostate of the rat contains an unusually high content of zinc and carbonic anhydrase. The lateral lobes are much richer in these two components than are the dorsal lobes(1). The ventral prostate on the other hand is not distinguished by its high content of either component. Dithizone (diphenylthiocarbazone) is a zinc chelating agent and has been utilized for the *in vivo* and *in vitro* demonstration of zinc in the prostates of various species(2,3,4). Injection of dithizone into rats produces rapid and selective damage to the lateral lobe only(2,4,5). The sequence of changes in the lateral lobe of the rat prostate resulting from dithizone injection has been described by light and electron microscopy. In this presentation we report on the sequential changes in the weight

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