Toluca-3, a Newly Recognized Enterovirus. (29852)

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During a study of the hemagglutinating properties of a random group of enterovirus isolates a previously unrecognized enterovirus serotype was encountered. This report describes some of the characteristics of the prototype strain which has been given the designation, "Toluca-3." The laboratory procedures employed were the same as those previously described(1,2) unless otherwise noted.

Results. Toluca-3 virus and 3 other strains of the same serotype were isolated in Toluca, Mexico during a study involving the mass administration of live poliomyelitis vaccine to a large group of children(3). The prototype strain was obtained from a rectal swab taken from a 2-year-old child in November, 1959. The original rectal swab fluid failed to produce a cytopathic effect (CPE) in any of 3 rhesus kidney cell culture tubes, but did so in each of 3 HEP-2 cell culture tubes. The virus could be passed in rhesus kidney cell cultures, however, and produced a CPE characteristic of enteroviruses in these cells as well as in human kidney cell cultures. Virus pools with titers as high as $10^{7.0}$ $TCID_{50}$ per 1.0 ml have been obtained in rhesus kidney cell cultures.

Toluca-3 virus at a dosage of 100 $TCID_{50}$ or less was not neutralized by 20 or more units of antiserum for each of the following viruses: poliovirus types 1 through 3; Coxsackie A types 1 through 22 and 24; Coxsackie B types 1 through 6; ECHO types 1 through 6, 6′, 6″, 7, 8, 9, 11 through 32; and unclassified picornaviruses (U.S.) types 353, 1059, 1734, 11757, and 33342. The source, homologous titer, and dilution of the sera employed are shown in Table I. All sera from NIH were produced and tested with prototype strains of virus except for poliovirus type 1 and Coxsackie B types 3 and 4. The poliovirus type 1 serum was made with the "Mahoney" strain and tested with the "P2226" strain (obtained from Dr. A. B. Sabin). The Coxsackie B type 3 and 4 sera were made and tested with the "Texas pleurodynia" and "Powers" strains respectively.

A Toluca-3 antiserum, with a homologous titer of $\geq$1:2500, when used at a dilution of 1:50 failed to neutralize 100 ID_{50} or 100 PD_{50} of each of the recognized enteroviruses and unclassified picornavirus types 353, 1059, 1734, 11757, and 33342. Prototype strains of virus were employed except as follows: Poliovirus type 1 ("P2226" strain); Coxsackie A types 1, 2, 5, 6, 8, and 10 (strains isolated at NIH in studies on herpangina); Coxsackie A type 3 (a strain obtained from Dr. G. Dalldorf—it is not known whether this is the same as the strain now employed as the prototype); Coxsackie A type 7 ("AB-IV" strain); Coxsackie B types 3 and 4 ("Texas pleurodynia" and "Powers" strains respectively). The tests with the Coxsackie A viruses, with the exception of types 7 and 9 were done in suckling mice. Tests with all other viruses and Coxsackie A types 7 and 9 were done in cell cultures.

Since Toluca-3 virus agglutinated human erythrocytes with optimum hemagglutinin (HA) titers obtained at a temperature of 37°C and a pH of approximately 7.3, most hemagglutination-inhibition (HI) tests were carried out under these conditions.

A Toluca-3 antiserum with a hemologous HI titer of 1:640, had an HI titer of less than 1:10 against 4 to 8 HA units of the following viruses: ECHO types 3, 6, 7, 11, 12, 13, 19, 20, 21, 24, 25, 29, and 30; Coxsackie A type 21, and Coxsackie B types 1, 3, 5, and 6. Prototype strains were used to prepare the HA antigens except for ECHO types 6, 20, 21, 24, 25, and 30, and all the Coxsackie B types. Conversely, 4 HA units of Toluca-3 virus were not inhibited by the following sera prepared in Dr. Wenner's laboratory when they were used at a dilution of 1:20; ECHO types 1 through 6, 6′, 6″, 7, 8, 9, 11 through 28, and 30; Coxsackie B

TABLE I. Source, Homologous Titer, and Dilution of Antisera Used in Neutralization Tests with Toluca-3 Virus.

Virus type	Source	Homologous titer *vs* ≧100 ID_{50}	Dilution used
Poliovirus 1-3	NIH*	≧1:1000	1:50
Coxsackie A 1-8, 10, 16, 17, 19, 22, 24	Wennert	≧500	1:20
" " 9	NIH	≧1:1000	1:50
" " 21	Wenner	≧1:1000	1:50
" " 11-15, 18, 20B	NIH	≧1:200	1:10
" B 1-5	"	≧1:1000	1:50
" " 6	Wenner	≧1:1000	1:50
ECHO 1-3, 5, 6, 7-9, 11, 12, 14	"	≧1:2000	1:100
" 6', 6", 13, 15-17, 19, 20, 22-26	"	≧1:1000	1:50
" 18	"	≧1:500	1:25
" 21, 27	"	≧1:200	1:10
" 28	"	≧1:100	1:5
" 4	"	1:90	1:5
" 29, 30, 31, 32	NIH	≧1:1000	1:50
Unclassified picornavirus 353	"	≧1:100	1:5
" " 1059	"	≧1:2000	1:100
" " 1734	"	≧1:320	1:16
" " 11757	"	≧1:1280	1:64
" " 33342	"	≧1:2000	1:100

* NIH sera for the polioviruses, Coxsackie viruses, and ECHO viruses were produced in the authors' laboratory and those for the unclassified picornaviruses were produced by Dr. Karl M. Johnson.

† Sera produced under the auspices of National Foundation, Nat. Cancer Inst., and Nat. Inst. of Allergy & Infect. Dis.

types 1 through 6, and Coxsackie A types 1 through 20, 20A, 20B, 21, 22, and 24. In addition the same dosage of Toluca-3 virus was not inhibited by antisera to ECHO types 29, 31, and 32 prepared in the authors' laboratory when they were used at dilutions of 1:10 or 1:20. These latter sera are those referred to in Table I.

No sera were available from the child from whom Toluca-3 was isolated. However, some, but not all, individual human sera selected at random had neutralizing antibody against 300 $TCID_{50}$ of Toluca-3 virus when tested at a dilution of 1:8.

The titer of Toluca-3 virus was not affected by exposure to ethyl ether for approximately 18 hours at 4°C, nor was it affected by exposure to a pH of 4.0 for one hour at 37°C. A pool of Toluca-3 virus had a titer of $10^{4.0}$ $TCID_{50}$ per ml after exposure at 50°C for one hour in Hanks' BSS but had a titer of $10^{6.0}$ $TCID_{50}$ per ml when exposed to the same conditions in the presence of 1 M Mg Cl_2.

The size of Toluca-3 virus was estimated by filtration through gradocol membranes. As seen from Table II it has a size approximating that of the recognized enteroviruses.

Some of the above experiments were carried out with virus material prepared before terminal dilution purification had been carried out. The purity of this material was established by showing that an antiserum prepared with virus purified by 3 terminal dilutions had as high a titer against the early virus pool as it did against the purified pool.

Toluca-3 virus with a titer of at least $10^{6.5}$ $TCID_{50}$ per 1.0 ml was inoculated into 6 litters of suckling mice less than 16 hours old. Each of the litters contained 8 mice. Two of the litters were inoculated intracerebrally with 0.02 ml amounts, 2 were inocu-

TABLE II. Results of Gradocol Membrane Filtrations with Toluca-3 Virus.

Avg pore diameter of membrane ($m\mu$)	Titer of filtrate ($TCID_{50}$ per 1.0 ml)
Before filtration	$10^{7.0}$
350	$10^{7.0}$
55	$10^{5.5}$
37	$10^{2.0}$
22	0/10*

* Numerator denotes number of tubes positive; denominator denotes number of tubes inoculated with 1.0 ml of filtrate.

lated intraperitoneally with 0.05 ml amounts, and 2 were inoculated subcutaneously with 0.5 ml amounts. None of the mice appeared ill or died during a 14-day observation period except for 2 mice which were found missing within 24 hours of the time they were inoculated.

Discussion and summary. The data presented indicate that Toluca-3 virus has properties characteristic of the enteroviruses but that it is antigenically distinct from all previously described serotypes. Despite the absence of generally accepted definitions of the polioviruses, Coxsackie viruses, and ECHO viruses(4) this agent has been designated tentatively as the prototype strain of ECHO type 33 by the Panel for Picornaviruses of the National Institute of Allergy and Infectious Diseases.

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Effect of o,p'-DDD on Hepatic Metabolism of Pentobarbital in Rats.* (29853)

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The experiments described here began with the initial observation that both the depth and duration of pentobarbital anesthesia were reduced in rats treated with the compound 2,2 bis (2-chlorophenyl, 4-chlorophenyl) 1,1-dichloroethane (o,p'-DDD).† This observation suggested that o,p'-DDD might stimulate hepatic metabolism of pentobarbital since Hart and Fouts(1) reported that administration to rats of DDT (a structural analog of DDD) is capable of inducing the hepatic microsomal enzymes which metabolize hexobarbital.

Experiments were performed to test the effects of administration of o,p'-DDD on pentobarbital metabolism in the rat. Sleeping time following injection of pentobarbital was used as an indirect measure of *in vivo* metabolism while *in vitro* metabolism was determined chemically in liver slices. In an attempt to demonstrate enzyme induction by o,p'-DDD, ethionine, an inhibitor of protein synthesis(2), was given with o,p'-DDD. Inhibition of the effect of an enzyme inducer by ethionine has been put forward as evidence for an induction of *de novo* enzyme synthesis by the inducer rather than activation of existing enzymes(3).

Methods. Male rats of the Holtzman strain were maintained on a diet of Purina Laboratory Chow and water *ad libitum*. A preliminary experiment utilized 10 rats weighing 225 to 250 g, 5 of which served as controls while 5 received 300 mg/kg o,p'-DDD by the oral route. In subsequent studies 34 rats weighing 100 to 150 g were divided into the following treatment groups: (a) control, (b) o,p'-DDD 100 mg/kg in peanut oil subcutaneously, (c) o,p'-DDD 300 mg/kg orally, (d) d,l-ethionine 150 mg/kg intraperitoneally (i.p.), and (e) d,l-ethionine with o,p'-DDD.‡ The number of rats per treatment group is given in Table I. All doses quoted in both

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† Aldrich Chemical Co., Milwaukee, Wisconsin.

‡ Results were identical with a combination of ethionine and either 100 or 300 mg/kg o,p'-DDD; therefore, the data were pooled and are presented as one group.