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Some Properties of the Encephalomyocarditis, Columbia SK and Mengo Viruses.* (30196)

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Five strains of virus variously known as encephalomyocarditis (EMC), Columbia SK (COL SK) and Mengo have been studied in this laboratory to compare their characteristics *in vivo* and *in vitro*. These viruses were isolated in widely separated parts of the world from a variety of animal species and have been passaged extensively in rodents and cultured cells. The immunologic relationship of the 5 strains as well as the morphology of plaques which they form in L cell monolayers, sensitivity to inhibitors in agar (presumably sulfated polysaccharides)(1), and hemaggluination properties are considered here. In addition, the results of studies on the pathogenicity of these viruses for laboratory animals are briefly summarized.

Materials and methods. Virus strains. M. Eaton supplied the EMC-M strain which was originally isolated in Florida(2). It had received approximately 80 passages in mice. EMC-L was derived from the Florida isolate and had been transmitted repeatedly in L cells in the laboratories of K. Habel and I. Lowe. A mouse adapted strain of Mengo virus which was isolated in Uganda was made available by L. Kilham(3). The T-61 variant of COL SK virus(4) was obtained from E. Furusawa who had grown it in Ehrlich ascites cells. A strain designated 1138 was isolated in Panama by Murnane *et al* from a naturally infected pig(5). It has been transmitted in mice by the intracerebral (i.c.) and intraperitoneal (i.p.) routes. The L cell adapted r and r^+ variants of EMC virus were supplied by K. Takemoto. The 2 differ in sensitivity to sulfated polysaccharides found in agar(1). Each of the virus strains was passaged in this laboratory using L-939† or primary and secondary mouse embryo (ME) monolayer cell cultures or mice or all 3 systems.

Animal studies. Groups of 12-week-old male Swiss mice were inoculated i.p. with decimal dilutions of virus. The infectivity endpoint was included in all titrations. Signs of illness were recorded as they appeared. The hearts of all animals were removed for histological examination as soon as possible after death or when the experiment was terminated on day 14.

Antiserum. Young rats or guinea pigs were inoculated i.p. on a single occasion with cell culture fluids containing high titers of infectious virus and were bled 2 to 3 weeks later. Sera were routinely adsorbed with a 20% suspension of kaolin.

Hemagglutination (HA) and hemagglutination-inhibition (HI). Tests were carried out by previously described methods(6) using antigens prepared in cultures of ME or L cells and a 0.05 M H_2BO_3 - 0.12 M KCl diluent adjusted to pH 8 with 1 N KOH. Sedimentation conditions are given in Tables I and IV.

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† Microbiological Associates, Bethesda, Md.

TABLE I. Antigenic Relationship of 5 Strains of Virus by Hemagglutination-Inhibition.

Antiserum	Antigen 1138	Mengo	COL SK	EMC-M	EMC-L
1138	*640**	160	320	640	320
Mengo	160	*320*	160	160	80
COL SK	10	10	*40*	20	10
EMC-M	40	10	<10	*80*	40
EMC-L	10	<10	<10	80	*80*

* Reciprocal of serum dilution completely inhibiting hemagglutination of guinea pig erythrocytes at 25°C.

Agar extract. A 2% suspension of Difco agar in Hanks' solution was autoclaved for 15 minutes at 120°C. Solidified agar was alternately frozen (—65°C) and thawed twice and the liquid phase which formed was separated after light centrifugation. Serial 2-fold dilutions of the extract were incubated for 60 minutes with 10^2 or 10^3 tissue culture infectious doses (ID_{50}) of virus and the mixture inoculated into tube cultures of ME cells. Thereafter the extent of the cytopathogenic effect (CPE) was evaluated at 24-hour intervals and compared with appropriate controls. A 1+ to 4+ grading system was used to quantitate the extent of CPE. Each of the virus strains received several passages in ME cells before being used in the test.

Virus assays. L cell monolayers in 35 mm plastic plates were washed with Hanks' balanced salt solution and inoculated with 0.2 ml of virus. The inoculum was withdrawn after 60 minutes and the monolayer overlaid with 2 ml of a medium containing 0.9% Noble agar, 0.25% gelatin, 5% heated (56°C, 30 minutes) chicken serum, 2× concentrated Eagle's basal medium and antibiotics. Plates were incubated at 36°C in a 5% CO_2 atmosphere. A second agar overlay containing neutral red was added after 48 hours and the plates read on the fourth day. In some experiments diethylaminoethyl dextran (DEAE-dextran)‡ was added to the overlay at a concentration of 500 μg/ml.

Results. The viruses listed in Table I caused lethal infections in 12-week-old mice. Signs of central nervous system involvement were observed in animals infected with each strain. EMC-M produced a rapidly fatal encephalomyelitis; animals receiving EMC-L, COL SK, Mengo and 1138 developed a more protracted illness manifested first by signs of myelitis and later encephalitis. Myocardial necrosis and focal collections of mononuclear cells were found consistently in the hearts of infected mice. In no case was the myocardial damage extensive nor could its severity be related to survival time or dose of virus administered. Lesions were not present in the hearts of control animals. Details of these studies will be reported elsewhere.

Each of the viruses replicated in L and ME cells producing characteristic CPE and large amounts of infectious virus. However, 2 of the mouse adapted strains (EMC-M and Mengo) required several serial passages in cell cultures before they caused reproducible CPE.

HI tests were carried out to establish the immunological relationship of the 5 virus strains. The results (Table I) established their antigenic similarity. Certain of the hemagglutinins employed in these tests exhibited a low degree of reactivity with antiserums prepared against the EMC-M and EMC-L strains. Comparable results were obtained when sera from other rats immunized with these same 2 viruses were used. However, tests carried out in a reciprocal fashion showed that the EMC-M and EMC-L hemagglutinins were reactive with all heterologous antisera.

Three of the 5 strains (EMC-M, EMC-L, COL SK) were not highly antigenic in rats; animals receiving a single inoculation of virus either developed low levels of HI antibody or had no detectable response at the lowest serum dilution tested (1 in 10). Sera used for the studies shown in Table I were among those showing the highest homologous titers.

Plaques formed under agar in L cell monolayers by Mengo, EMC-M and 1138 strains can be seen in Fig. 1. These viruses had been passaged repeatedly in mice but had not been grown *in vitro*. Mengo produced round, pinpoint sized plaques (diameter 0.3 mm); those formed by the 1138 strain were slightly larger (diameter 0.5 mm) and had somewhat irregular margins. In contrast, EMC-M plaques

‡ Pharmacia, Uppsala, Sweden.

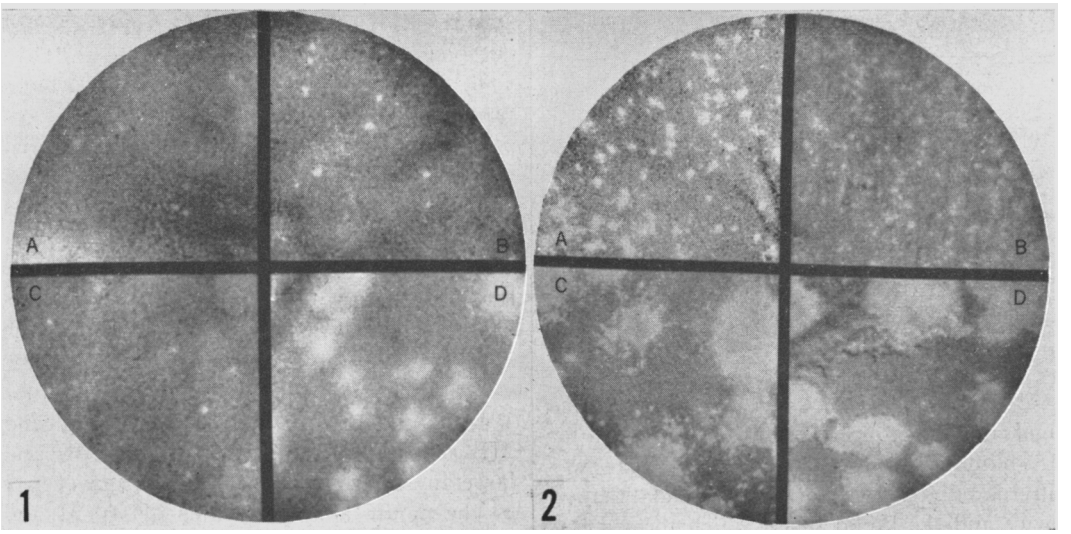

FIG. 1. Virus plaques in L cell cultures. A = Mengo; B = 1138 brain suspension after 11 i.c. passages in mice; C = 1138 heart suspension after 20 i.p. passages in mice; d = EMC-M magnification ×3.

FIG. 2. Virus plaques in L cell cultures. A = COL SK; B = 1138 after 5 passages in ME cell cultures; C = EMC-L; D = EMC-M after 5 passages in L cell cultures magnification ×3.

were larger (diameter 2-3 mm) and consistently exhibited hazy, indistinct margins.

Plaque size of Mengo and 1138 strains did not substantially change with passage in mice or cell cultures. On the other hand, after several passages in L cells the EMC-M strain formed both small (diameter 0.3 mm) and large (diameter 3-5 mm) plaques (Fig. 2). Although quantitative studies were not done, by the fifth passage the number of large and small plaques produced by the EMC-M was roughly equal. However, the 2 forms appeared to be unstable since several attempts to separate them were unsuccessful.

EMC-L, a strain which had been passaged repeatedly in L cells, produced plaques which varied considerably in size. Often the larger plaques after 5 days of incubation were surrounded by small "satellites" which radiated from the margin. The proximity and orientation of these satellites to the large plaques as well as their late appearance suggests that they were derived from progeny of the larger central plaque. In contrast, COL SK formed a variety of plaques. Some were 4 to 5 mm in diameter and had ill-defined, lobulated margins whereas the majority were relatively small (diameter 0.3-0.5 mm).

Takemoto and Liebhaber(1) have isolated two variants of EMC virus (r and r^+) which differ in sensitivity to a sulfated polysaccharide inhibitor in agar. In the assay system employed here the inhibitor insensitive r variant plaques were 10-15 mm in diameter, whereas those formed by the inhibitor sensitive r^+ were 1 mm or less.

Growth of EMC-M, Mengo and 1138 in the presence of a crude extract of agar was investigated to determine if differences in the size of their plaques could be attributed to an inhibitor in agar (Table II). In tests using high concentrations of the extract appearance of CPE produced by Mengo and 1138 was delayed and usually limited to foci on the margins of the cell sheet. In contrast, widespread CPE rapidly developed in cul-

TABLE II. Effect of Extract of Agar on Appearance of CPE of 3 Strains of Virus.

Dilution of agar extract	1138 10^{3}*	Mengo 10^{3}	EMC-M 10^{2}
Undiluted	0†	0	8
1:2	1	0	8
1:4	4	0	8
1:8	5	1	8
1:16	6	2	8
Hanks' solution	8	8	8

* Amt of virus (ID_{50}) in test.

† Total score of CPE (graded 1+-4+) in 2 cultures of ME cells on third day of test.

TABLE III. Effect of DEAE-Dextran in Agar Overlay on Plaque Size.

Virus	DEAE agar*	Plaque diameter (mm) <1-1	2-3	4-5	6-7	8-9	≥10
1138	No	$5 \cdot 10^6$†					
	Yes		$5 \cdot 10^5$	$4 \cdot 10^5$			
EMC-M	No		$3 \cdot 10^6$				
	Yes		$2 \cdot 10^6$	$1 \cdot 10^6$			
Mengo	No	$5 \cdot 10^2$					
	Yes		$2 \cdot 10^2$				
EMC-r+	No	$6 \cdot 10^6$					
	Yes						$2 \cdot 10^6$

* 500 μg/ml DEAE-dextran.
† Plaque forming units/0.2 ml.

tures receiving EMC-M. Thus, the relative inhibition of the strains by the agar extract roughly correlated with the size of the plaques which they formed under agar.

Liebhaber and Takemoto(7) have reported that the effect of agar inhibitor on plaque size can be reversed by incorporating DEAE-dextran in the agar overlay. Plaques formed by the 3 viruses listed in Table II consistently were larger when DEAE-dextran containing agar was employed. However, they never were equivalent in size to the plaques of the inhibitor sensitive r^+ variant under DEAE-dextran agar (Table III). Plating efficiency of Mengo was low and was not increased by using the modified agar.

The 5 viruses agglutinated red blood cells of each of the mammalian species tested (human, guinea pig, sheep and rat). The highest HA titers were obtained with rat erythrocytes. Experiments were carried out to determine the effect of sedimentation temperature on hemagglutination. The 5 hemagglutinins exhibited differing patterns of reactivity with guinea pig erythrocytes at the 3 temperatures employed in the test (Table IV). With 2 of the 5 viruses, HA titers were substantially higher at 4°C than at 24°C and no hemagglutinin was demonstrable at 37°C. In contrast, the titers with the 3 other strains were equivalent at 4°C and 24°C and the same or only slightly lower at 37°C. Comparable results were obtained in repeat experiments using different preparations of hemagglutinin and in tests employing an unbuffered 0.14 M KCl diluent.

Discussion. Biological properties of antigenically similar strains of virus often differ. The reasons for these differences are complex and largely unknown but in part reflect the environment in which the virus is maintained after it is recovered from nature. The virus strains employed in this study were isolated in widely separated parts of the world and had been grown in a variety of animals and cell cultures. The work reported here was undertaken to compare these strains and to determine if differences between them exist. Dick(8) using mouse neutralization tests and Warren *et al*(9) employing cross protection, neutralization and complement fixation tests showed that EMC, COL SK and Mengo virus are antigenically indistinguishable. The results of the serological tests in Table I indicate that the virus strains used in this study are similar but not necessarily identical.

Despite the varied backgrounds of the 5 strains each caused myocardial lesions and signs of central nervous system disease in mice. Thus, pathogenicity for animals seems to be a stable characteristic of these viruses which is not altered by the usual methods of laboratory passage. Nevertheless, the ability of certain strains to cause lethal infections can be modified by laboratory manipulation. Furusawa and Cutting(10) have described a variant of COL SK which is lacking in "neuro-virulence" and I have selected a variant of 1138 which causes a non-fatal infection in mice manifested primarily by myocarditis.

The 3 mouse-adapted strains were made up of a homogeneous population of plaque-forming particles. It would appear that mice provide an environment conducive to the selection of strains which are comprised of viruses having similar plaque forming charac-

TABLE IV. Effect of Sedimentation Temperature on Hemagglutination by 5 Strains of Virus.

Virus strain	Sedimentation temperature 4°C	24°C	37°C
1138	640*	40	<20
EMC-M	1280	80	<20
Mengo	1280	640	160
EMC-L	640	640	160
COL SK	160	320	160

* Reciprocal of antigen dilution using guinea pig erythrocytes.

teristics. However, the data show that individual mouse strains may differ one from another in plaque size, sensitivity to agar inhibitors and hemagglutination properties.

The diameter of the plaques formed by the mouse adapted strains increased slightly when DEAE-dextran was incorporated in the agar overlay. Since the concentration of DEAE-dextrose was sufficient to allow the inhibitor sensitive r^+ variant to attain a size comparable to inhibitor insensitive r, it seems likely that the sulfated polysaccharide inhibitor in agar is only one of the factors affecting the size of plaques. The data of Ellem and Colter using their strain of Mengo and L cells overlaid with methyl cellulose support this conclusion(11).

Summary. Comparative studies were carried out using 5 strains of virus known by the designations EMC, COL SK and Mengo. Hemagglutination-inhibition tests showed that the strains were antigenically similar although not necessarily identical. Each was highly neurotropic and produced myocardial damage in mice. Plaques produced by the 5 virus strains in L cell monolayers differed morphologically. The size of the plaques formed by 3 mouse-adapted strains was roughly correlated with sensitivity to an inhibitor in extracts of agar and increased when DEAE-dextran was incorporated in the overlay. However, evidence is presented which indicates that factors other than inhibitor sensitivity also contribute to differences in plaque size. Each of the strains hemagglutinated erythrocytes of 4 mammalian species. Hemagglutination by some strains was temperature dependent, whereas with others it was not.

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Serum Alkaline Phosphatase in the Post-Natal Pig and Effect of Serum Storage on Enzyme Activity.* (30197)

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Few studies have been conducted on serum alkaline phosphatase levels in newborn pigs. According to Young and Underdahl(1), activity is highest at birth, falls considerably by the fifth day, and gradually declines to 50 days post-partum. The effect of prolonged storage on alkaline phosphatase in swine serum has apparently not been reported.

The objectives of this study were (a) to determine serum alkaline phosphatase activity in the pig at intervals from birth to 4 weeks of age, (b) to relate this enzyme activity to serum protein concentration and body weight changes, and (c) to determine whether prolonged 5° or —20°C storage affects serum alkaline phosphatase activity.

Methods. Forty-one pigs from 2 gilts and 3 sows, which were on the same ration, were included in this investigation. The pigs were caught as they were farrowed, were weighed,

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