of a small difference in the effect of cis- and trans-isomers on serum cholesterol. However, they do not indicate any major influence of isomerization such as would be obtained in commercial hydrogenation on the serum cholesterol level.

Summary. Adult sows were fed a purified diet and in alternate 3-week periods were fed the diet either with a low fat or a high fat (40% of calories) content. In the high fat periods hydrogenated fats were compared with natural plant fat mixtures designed to have the same fatty acid composition. The difference in the fats studied was in their content of isomerized fatty acids. Also triolein was compared with trielaidin. The hydrogenated fats gave the same serum cholesterol response as their corresponding natural plant fat mixtures. Trielaidin gave a lower ($P<0.05$) serum cholesterol response than triolein.

1. Abell, L. L., Levy, B. B., Brodie, B. B., Kendall, F. E., *J. Biol. Chem.*, 1952, v195, 357.

2. Roper, R., Ma, T. S., *Microchem. J.*, 1957, v1, 245.

3. Holman, R. T., Ener, S., Edmonson, P. R., *Arch. Biochem. Biophys.*, 1959, v80, 72.

4. Barnes, R. H., Kwong, E., Fiala, G., Rechcigl, M., Lutz, R. N., Loosli, J. K., *J. Nutrition*, 1959, v69, 261.

5. Keys, A., Anderson, J. T., Grande, F., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 387.

6. Hegsted, D. M., Gotsis, A., Stare, F. J., Worcester, J., *Am. J. Clin. Nutr.*, 1959, v7, 5.

7. Johnston, P. V., Johnson, O. C., Kummerow, F. A., *J. Nutrition*, 1958, v65, 13.

8. Holman, R. T., Aaes-Jorgensen, E., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 175.

Received Aug. 3, 1961. P.S.E.B.M., 1961, v108.

Effect of "Sex" of Tissue Cultures on Growth of Encephalomyocarditis Virus.* (26969)

LOUIS WEINSTEIN AND TE-WEN CHANG

Department of Medicine, Tufts University School of Medicine, and Infectious Disease Service, Pratt Clinic—New England Center Hospital, Boston, Mass.

The fact that the sex of the host may condition both susceptibility and response to viral infection has been established for naturally-occurring poliomyelitis(1,2). That this is also true in experimentally-produced disease has been noted by Weinstein and Chang (3) who found that the LD_{50} of several viruses was influenced, to a varying degree, by the sex of the animal. Male mice were observed to be much more susceptible to encephalomyocarditis virus, females were less resistant to poliovirus, while little or no sex differences were demonstrable with the agents of influenza and vaccinia.

These observations, together with the demonstration that cells derived from males and females may multiply at different rates(4,5), suggested the possibility that degree of susceptibility of isolated tissues to viral invasion might be influenced by the sex of the animal from which they were derived. This paper reports the results of studies with encephalomyocarditis virus which indicate that the "sex" of the mouse muscle culture into which it is inoculated, determines rate and extent of its growth.

Materials and Methods. Cultures of muscle of mature male and female mice were prepared by a previously described method(6) and grown in a medium consisting of 10% horse serum, 5% beef embryo extract, 45% bovine amniotic fluid, and 40% Hanks' solution.

A 10% mouse brain pool of encephalomyocarditis virus was titrated for LD_{50} by intraperitoneal injection into 6-week old animals, and stored at $-20°C$ until used. The muscle cultures were inoculated with varying doses of the brain-virus preparation. Only those tubes showing good growth of cells and

* These studies were supported by a grant from the Medical Foundation, Inc., Boston, Mass.

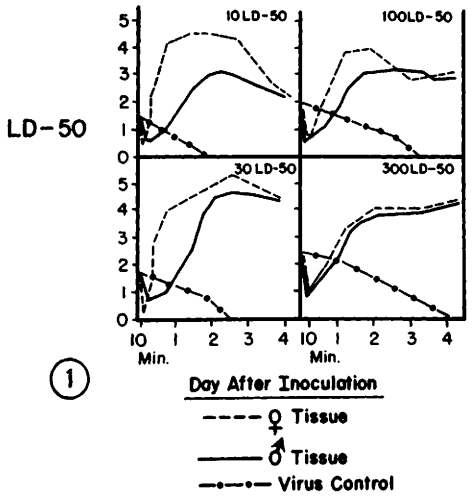

FIG. 1. Relation of size of inoculum to growth of EMC virus in male and female tissues.

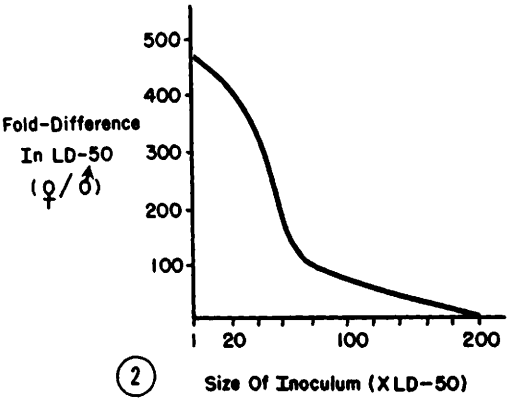

FIG. 2. Ratios of degree of growth of EMC virus in male and female tissues.

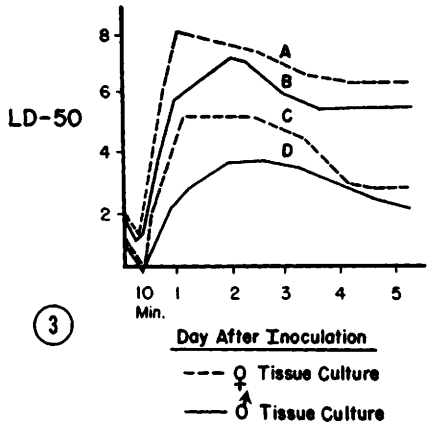

FIG. 3. Effect of age of tissue culture on growth of EMC virus in male and female cells.

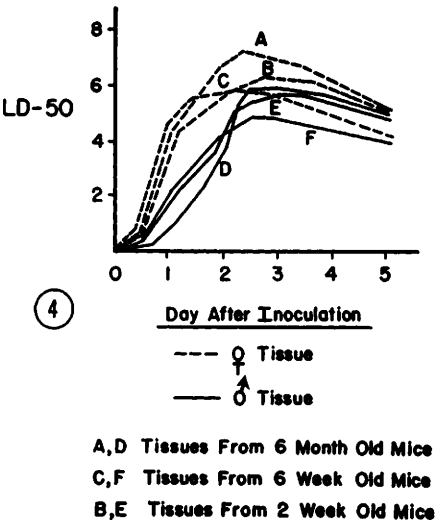

FIG. 4. Effect of age of mice from which muscle was obtained on growth of EMC virus.

in which the degree of multiplication of the muscle from both sexes was approximately the same were infected; 10 tubes of "female" and "male" tissue were used for each experiment which was repeated at least 3 to 4 times. The cultures were incubated in a stationary position at 37°C. Aliquots (0.1 ml) were removed from each tube after 24, 48, 72, 96, 120, and 144 hours and stored at −20°C until titrated.

Virus growth curves were determined by titration in animals. The fluid removed after varying periods of incubation was diluted by 10-fold steps and 0.2 ml of each dilution injected intraperitoneally into 4 to 6 week old male mice; 6 animals were used for each dilution. The LD_{50} was calculated each day until the end of experiment; deaths occurring in the first 24 hours were not included.

Results. As can be seen in Fig. 1, the rate and extent of growth of encephalomyocarditis

virus were different in cultures of "male" and "female" muscle. Degree of difference was dependent on size of the inoculum with which the tissues were infected. It was maximal when the quantity of virus used was small and decreased to the vanishing point as this was increased. Thus, with an inoculum of 10 LD_{50}, rate of multiplication and total virus yield during the first 2 days of incubation were greater in "female" cultures while with an infecting dose of 300 LD_{50} no difference between the sexes was noted. These observations were confirmed in another experiment in which inocula varying from 1 to 200 LD_{50} were employed. Data calculated on the basis of the female/male difference are presented in Fig. 2 in which it can be seen that the yield of virus was almost 500 times higher in "female" tissue when the inoculum was 1 LD_{50} and that, with 200 LD_{50}, no difference was present.

Since uninfected cultures of cells from male and female tissues may grow at different rates (4,5), a study was carried out to determine whether this was responsible for the observations described above. Cultures of muscle from male and female mice were grown for 5 and 12 days respectively; degree of cellular multiplication was greatest in the latter. Tubes from each period of growth were selected so that the number of cells in the "male" and "female" ones were approximately the same and all were infected with 10 LD_{50} of encephalomyocarditis virus. Aliquots were removed every 24 hours during 5 days of incubation at 37°C and titrated by intraperitoneal injection into mature male mice. The virus was found to grow at a more rapid rate and to a greater extent in "female" cells of both the 5- and 12-day old tissues (Fig. 3). Despite the fact that the speed and degree of viral replication were less in the younger cells, the differences between female and male cultures in relation to multiplication of virus were greater in them than in the older ones.

The data presented in Fig. 4 indicate that age of the animal from which the muscle used for infection was derived played some part in determining the magnitude of viral multiplication. Mice of both sexes, aged 6 months, 6 weeks, and 14 days respectively were used in these studies. The muscles of the male and females in each of these groups were cultured. Tubes exhibiting approximately the same degree of cell growth were selected, inoculated with 10 LD_{50} of EMC virus, and incubated at 37°C. Aliquots were withdrawn once daily for 5 days and LD_{50} determined by inoculating varying dilutions intraperitoneally into 6-week old male mice. Although the virus was found to multiply more rapidly and to a greater degree in "female" muscles of all age groups, the differences from the males were greatest in the 6-month old animals and least in the 2-week old ones. When titrated after 24 hours of incubation, the quantity of virus present in cultures of muscle of 6-month old mice was found to be 5700 times greater than could be demonstrated in "male" muscle of the same age; the female to male ratio of virus in tissue of 2-week old animals was only 8 to 1.

Discussion. These results indicate that susceptibility to viral invasion is conditioned by the sex of the host at all levels of complexity of cellular organization ranging from the whole animal to isolated tissue growing in artificial media. The fact that differences in growth of encephalomyocarditis virus in "female" and "male" cells were greater in muscle removed from fully mature animals raises the possibility that the determinant factor was the influence of sex hormones during life. However, a similar effect of lesser degree was also noted in cultures of the tissues of sexually-immature mice. This suggests that the basic mechanism involved in determining sex differences in susceptibility of cells to virus infection is not endocrine activity alone, although this may influence quantitative aspects of the phenomenon.

The possibility that variations in extent of cell growth present at the time of viral infection may have been responsible for the observed differences in susceptibility to virus invasion of male and female tissues merits consideration. The data, however, do not support this hypothesis for the following reasons: (a) At the time of infection with EMC virus, the number of cells in the cultures of both the male and female muscle were approximately equal. (b) Despite the fact that there were fewer cells in 5-day than in 12-

day old cultures of mouse muscle, the differences in speed and extent of growth of virus were greatest in the younger tissues. If degree of viral multiplication were dependent only on the number of muscle cells available for invasion, the female to male ratio of virus should have been highest in the 12-day old cultures because they contained a greater number of cells than the 5-day old ones at time of infection.

It is striking that differences in degree of growth of EMC virus in cultures of muscle from male and female mice could be demonstrated only with inocula of certain size. With small dose of virus, the difference between the 2 sexes was maximal; as quantity of infecting agent was increased, it became less marked until it was no longer noted when 200 to 300 LD_{50} were used. The reason for this phenomenon is not apparent.

Summary. When EMC virus was grown in cultures of mouse muscle, it multiplied more rapidly and to a greater degree in the tissue obtained from female animals. The quantity of virus used to produce infection was an important determinant in demonstration of this phenomenon. The sex differences did not appear to be related to the number of cells present in the muscle culture at time of viral inoculation.

1. Weinstein, L., Shelokov, A., Seltzer, R., Winchell, G., *New Eng. J. Med.*, 1952, v246, 296.
2. ———, *ibid.*, 1957, v257, 47.
3. ———, Chang, T. W., Unpublished data.
4. Frothingham, T. E., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 505.
5. Osterhout, S., Tamm, I., *J. Immunol.*, 1959, v83, 442.
6. Chang, T. W., Weinstein, L., PROC. SOC. EXP. BIOL. AND MED., 1959, v1C2, 181.

Received Aug. 4, 1961. P.S.E.B.M., 1961, v108.

Hemagglutination-Inhibition Antibody Responses in Human Adenovirus Infections. (26970)

LEON ROSEN
(Introduced by R. J. Huebner)
Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Nat. Inst. Health, Public Health Service, U. S. Dept. of HEW, Bethesda, Md.

Although neutralizing antibody responses in persons infected with adenoviruses tend to be type-specific, heterotypic responses occur frequently(1). Furthermore, because of certain unusual aspects of adenovirus neutralization tests it is difficult to equate the findings from different laboratories or even those obtained in the same laboratory at different times(1). Complement-fixing (CF) antibody responses generally show no type-specificity in human adenovirus infections(1).

Preliminary data indicating that adenovirus hemagglutination-inhibition (HI) antibody responses in man might be type-specific have been presented(2). This report is concerned with additional data subsequently obtained from a study of paired sera from 36 children naturally infected with adenovirus types 1, 2, 3, or 5 and from 27 adults naturally infected with adenovirus types 4 or 7.

Materials and methods. The children with adenovirus type 1, 2, 3, or 5 infections were residents of an institution (Junior Village) and were part of a population whose microbial experiences were the subject of intensive study(3). Paired sera were selected so that they spanned the first known isolate of a given adenovirus serotype in a child, yet did not include adenoviruses of other serotypes. The first serum of each pair antedated the adenovirus isolate and second was collected at varying times thereafter as indicated in the tables.

The adults with adenovirus type 4 or 7 infections were naval recruits from whom these particular serotypes were isolated during a study of respiratory illness. The first serum from these individuals was obtained at onset