TABLE I. Percentage of eggs of 4 geographic strains of *S. japonicum* showing positive circumoval precipitation in homologous, heterologous, and normal sera.

Series	Eggs vs serum	No. of eggs	Eggs with positive reactions No.	%
CE	CE vs CAS	215	129	60.0
	CE vs FAS	175	59	33.7
	CE vs JAS	282	127	45.0
	CE vs PAS	168	42	25.0
	CE vs NS	100	0	.0
FE	FE vs CAS	777	248	31.9
	FE vs FAS	606	461	76.0
	FE vs JAS	781	263	33.7
	FE vs PAS	447	57	12.8
	FE vs NS	100	0	.0
JE	JE vs CAS	274	109	39.8
	JE vs FAS	288	93	32.3
	JE vs JAS	310	235	75.8
	JE vs PAS	177	68	38.4
	JE vs NS	100	0	.0
PE	PE vs CAS	109	39	35.8
	PE vs FAS	100	32	32.0
	PE vs JAS	150	85	56.7
	PE vs PAS	120	82	68.3
	PE vs NS	100	0	.0

were shown in Table 1. In all of the 4 series, eggs with normal serum showed no reaction. The percentages of eggs showing positive reactions were much higher with the homologous antiserum than with the heterologous antisera. The difference between the percentage of the eggs showing positive reactions with the homologous antiserum and the percentages with the heterologous antisera was found to be significant at the 5 % level in each series by the chi square test.

Discussion and conclusion. Although the circumoval reaction is species specific for *S. japonicum,* the results of the present studies show that with this test, there were cross reactions among the 4 geographic strains. A significantly larger proportion of eggs showing positive circumoval precipitation, however, was seen when the eggs were incubated with the antisera of the heterologous strains. This indicates that the geographic strains of *S. japonicum* have already become partly distinct in their serological characteristics.

1. Oliver-Gonzalez, J., Bauman, P. M., Benenson, A. S., *J. Infec. Dis.,* 1955, v96, 95.

2. Hsü, H. F., Hsü, S. Y. Li, *Proc. 6th Internat. Congr. Trop. Med. & Malaria,* 1958, v2, 58.

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Isomerized Fat and Serum Cholesterol in Swine.* (26968)

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Although the effect of saturation of fatty acids on serum cholesterol levels in a variety of experimental animals and in man has been studied extensively, no investigation dealing with fatty acid isomerization in this relationship has been noted. The present study was designed to measure the relative effects of natural (cis) fats and hydrogenated fat which contains isofatty acids including transisomers on serum cholesterol level. In addition relatively pure triolein (cis) was compared with trielaidin (trans).

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Experimental. Adult sows, ranging in age from approximately 3 to 7 years and in weight from 300 to 500 lbs, were used as the experimental animal. A purified diet (composition shown in Table I) was fed at a constant level for each animal in an amount that just maintained body weight. The diet was mixed with water to form a slurry and was fed in 2 equal portions during each day. Extra water was provided only at feeding times. The pigs were housed individually in a closed barn, but temperature was not constant. Body weight was measured weekly.

The pattern of feeding was to give a low fat diet for 3 weeks followed by 3 weeks on a diet containing the test fat at a level of

TABLE I. Diet Composition.

	Basal low fat %	Experimental test fat %
Soy protein (Promine)	14.9	18.8
Glucose (Cerelose)	76.8	49.2
Corn oil (Mazola)	2.0	2.5
Test fat	—	21.5
Cellulose (Solka-Floc)	2.0	2.5
Minerals*	4.0	5.0
Fat sol. vitamins†	+	+
Water sol. vitamins‡	+	+
Choline	.3	.3
	100.0	99.8
Total Calories (calculated)	395	500
Ratio of amount of diet fed	1.27 g	1.00 g

* Minerals in g/100 g diet: $CaHPO_4 \cdot 2H_2O$, 0.77; $CaCO_3$, 1.05; KH_2PO_4, 1.15; NaCl, 0.50; $CuSO_4 \cdot 5H_2O$, 0.002; $FeSO_4 \cdot 7H_2O$, 0.165; KI, trace, $MnSO_4 \cdot H_2O$, 0.123; $CoCl_2 \cdot 6H_2O$, 0.00008; $MgCO_3$, 0.38; $ZnSO_4 \cdot 7H_2O$, 0.48.

† Fat sol. vit. mg/100 g diet dissolved in 2 g corn oil: vit. A acetate, 4.472; calciferol, 0.0135; α tocopherol, 18.0

‡ Water sol. vit. mg/100 g diet: thiamine HCl, 0.14; riboflavin, 0.27; niacin, 1.12; Ca pantothenate, 1.10; pyridoxine, 0.16; vit. B_{12}, 0.013; folic acid, 0.067; menadione, 0.112.

40% of total calories. Extra carbohydrate was supplied during the low fat period so that total calorie intake was constant. The groups of pigs were thus fed in 3-week periods so that a low fat period preceded and followed a given test fat period. Blood was obtained at weekly intervals by clipping of the end of the tail. Serum total cholesterol was determined on duplicate 0.3 ml samples by the method of Abell *et al.*(1). Cholesterol values for the second and third week of each period were averaged for the results given in Table II. An attempt was made to use the same pigs for all of test fats. However, the experiment ran for many months and some pigs had to be discarded because of degenerative disease or lack of appetite. When new pigs were introduced they were kept on the low fat diet for approximately 2 months and were then introduced into the study only if serum cholesterol values were reasonably constant and fell into the range exhibited by the experimental animals. The number of pigs used varied from 10 to 7 as shown in Table II.

The hydrogenated fats were specially prepared from soybean oil so as to have iodine numbers in the range of 105 or 80. Two separate lots of each hydrogenated fat (described as hyd. fat No. 1 or No. 2) were supplied through the courtesy of Dr. Fred Mattson, Proctor and Gamble Co.

The various lots of fatty acids were methylated by the technique described by Roper and Ma(2). The different batches of hydrogenated fat were analyzed for fatty acid composition by gas chromatography using a Barber and Coleman Model 10 gas chromatographic instrument. A 6 ft. column packed with diethylene glycol succinate (DEGS) in a ratio of 1:5 w/w on Celite 545, 80-100 mesh, was maintained at 180°C with Argon used as the Carrier gas at a flow rate of 59

TABLE II. Serum Cholesterol Values in Swine Fed Experimental Fat Diets.

Fat component in diet	No. animals	Serum Cholesterol: Low fat control periods	Fat periods	Increase
		mg/100 ml	mg/100 ml	mg/100 ml
#1 Hyd. Fat I.V. 105–110	10	67.2 ± 1.4*	86.6 ± 2.1	19.4 ± 2.1
Fat Mixture A	10	67.0 ± 1.5	85.6 ± 2.5	18.6 ± 2.5
#1 Hyd. Fat I.V. 70-80	10	68.8 ± 1.6	93.4 ± 2.6	24.2 ± 2.6
Fat Mixture B	10	69.8 ± 1.0	90.4 ± 1.7	20.6 ± 1.7
#2 Hyd. Fat I.V.-105	8	66.2 ± 3.2	88.8 ± 2.2	22.6 ± 2.2
Fat Mixture C	7	68.9 ± 3.3	85.3 ± 2.1	16.4 ± 2.1
#2 Hyd. Fat I.V.-80	8	67.2 ± 3.1	91.0 ± 3.5	23.8 ± 3.5
Fat Mixture D	7	67.3 ± 3.0	98.0 ± 3.6	30.7 ± 3.6
Triolein	8	67.0 ± 1.2	93.9 ± 3.1	26.9 ± 3.1+
Trielaidin	8	69.4 ± 1.2	87.4 ± 2.0	18.0 ± 2.0

* Mean ± standard error.
+ Significant at 0.05 probability.

TABLE III. Saturated and Unsaturated Fatty Acid Content of Dietary Fats.

Fats	Sat. fatty acid (palmitic & stearic)	Monoene (palmitoleic & oleic)	Diene (linoleic)	Triene (linolenic)
#1 Hydrogenated Fat (I.V. 108-110)	15.1	48.8	35.5	.6
Fat mixture A	14.6	55.3	29.7	—
" " C	13.1	50.2	36.7	—
#1 Hydrogenated Fat (I.V. 70-80)	22.2	63.0	14.8	—
Fat mixture B	18.1	62.4	19.6	—
" " D	15.2	72.7	11.7	.3
#2 Hydrogenated Fat (I.V. 105)	17.9	48.0	32.8	1.2
Fat mixture CN	11.2	50.7	37.7	.4
#2 Hydrogenated Fat (I.V. 80)	21.9	67.0	11.1	—
Fat mixture DN	21.6	68.3	7.3	—
				Ratio cis/trans
Triolein	3.3	87.7	2.7	2.84/ .70
Trielaidin	3.1	89.2	2.0	.70/2.76

ml per min. An applied potential of 500 volts was maintained on the ionization detector cell containing 56 μc of Radium-226. The cell bath was heated to 240°C, while the flash heater was maintained at 287°C. This technique was found to have a standard error of estimate of $\pm$ 0.25%.

The combined values for palmitic and stearic and for palmitoleic and oleic together with values for dienes and trienes are given in Table III. Natural oils and fats were similarly analyzed and mixtures made so as to simulate the fatty acid composition of the hydrogenated fats. Fat mixtures A and B were made from cottonseed oil (Wesson oil) and olive oil. These were intended to correspond to batch No. 1 hydrogenated fat, iodine No. 105-110 and 70-80 respectively. Fat mixture C was composed of safflower oil and olive oil and D was safflower oil and beef tallow. These were intended to correspond to batch No. 2 hydrogenated fat, iodine No. 105 and 80 respectively. The fatty acid analyses of these mixtures are shown in Table III.

A purified commercial "triolein" was elaidinized and re-esterified with glycerol to give a "trielaidin" (prepared by the Aero Chemical Corp., Newark, N. J.). Fatty acid analyses for these 2 preparations are given in Table III. The 2 monoenes were analyzed for relative cis- and trans-isomerization by infrared spectroscopy(3). The "triolein" and the "trielaidin" gave a ratio of cis to trans of 2.84:0.70 moles per kg and 0.70:2.76 moles per kg respectively.

Results and discussion. There were no statistically significant differences between any pair of fats except the triolein and trielaidin. The natural form (triolein) gave slightly higher cholesterol values than the unnatural form (trielaidin). The more saturated hydrogenated fat of batch 2 had a natural fat mixture for comparison that was a closer approximation of fatty acid composition than was the case with batch 1. In this pair the natural mixture also gave a slightly higher but not significantly different serum cholesterol. The one clear-cut effect that was noted was that triolein had a definite serum cholesterol increasing effect. This might have been surmised from the olive oil feeding experiments in swine reported by Barnes *et al.*(4), but differs from results obtained in man(4,5). In this respect cholesterol responses of the pig more closely resemble observations made in the rat(6).

Trans-fatty acids are absorbed and deposited in the tissues; furthermore, they appear to be metabolized(7). Perhaps the most striking difference is the complete lack of activity of trans-linoleic acid as an essential fatty acid and even the possibility of certain trans acids being essential fatty acid antagonists(8). The results obtained in the present study do not rule out the possibility

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.

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

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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

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