showed that 2 types of cell predominated. One type resembled the histiocyte and contained in its cytoplasm varying quantities of sudanophilic material. Some were engorged with lipid. The other type of cell was round and its cytoplasm swollen with unidentified, homogeneous, unstaining material. In some lesions one type of cell predominated; in other lesions, the other type. These lesions were found in 1 of the corn-oil fed group, in 3 of the butter fed group, and in 2 of the control group.

Discussion. The feeding of butter to swine resulted in an elevation of serum cholesterol. Feeding of isocaloric quantities of fat as corn oil did not produce significant elevation in serum cholesterol levels, so the rise in the butter-fed group was presumably not caused by increased ingestion of fat *per se*. The fact that elevation in serum cholesterol was confined to an elevation in high-density lipoproteins was surprising. Manipulations of diet in man and most other experimental animals, if they affect the serum lipids at all, usually alter concentration of the low-density lipoproteins. It has been shown in the rat, however, that fasting is accompanied by an increase in high-density lipoproteins and that carbohydrate feeding is accompanied by a decrease in these same classes(7).

Summary. 1) Addition of butter to the diet of swine caused a significant elevation in serum cholesterol levels. This did not occur in other animals fed isocaloric quantities of corn oil. The increase in cholesterol was limited to the high-density (alpha) lipoproteins. 2) Necropsy after 9 weeks of experimental diets revealed lesions somewhat resembling human atheromata in 50% of aortae. The same incidence was found in control animals on a conventional diet.

1. Gottlieb, H., and Lalich, J. J., *Am. J. Path.*, 1954, v30, 851.

2. Lewis, L. A., and Page, I. H., *Circulation*, 1956, v14, 55.

3. Sperry, W. M., and Webb, M., *J. Biol. Chem.*, 1950, v97, 187.

4. Stewart, C. P., and Hendry, E. B., *Biochem. J.*, 1935, v29, 1683.

5. Bragdon, J. H., *J. Biol. Chem.*, 1951, v190, 513.

6. Havel, R. J., Eder, H. A., and Bragdon, J. H., *J. Clin. Invest.*, 1955, v34, 1345.

7. Bragdon, J. H., Havel, R. J., and Gordon, R. S., Jr., *Am. J. Physiol.*, in press.

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Distribution of Coxsackie Virus in Experimentally Infected Cockroaches, *Periplaneta americana.** (23195)

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The transmission of the polio-encephalomyelitis group of viruses is not clearly understood(1,2). The most probable natural mode of transmission is fecal-oral dissemination with the alimentary tract as portal of entry. This hypothesis is supported by the finding of virus in feces and throat washings from patients and in sewage and flies(3). Persistence and possible multiplication of virus in non-biting flies(4) suggests that polio-encephalomyelitis viruses may escape the intestinal-oral cycle in the natural mammalian carrier by transmission through arthropods to new hosts. Incrimination of flies as potential vectors(3,4) suggests a similar role for cockroaches, since in many areas of the world the association of cockroaches with man's food and excreta exceeds that of flies. Supporting evidence is limited to recent experimental findings. Hurlbut(5) reported that after injection of human poliomyelitis virus (Lansing strain) into the haemocoele of the cockroach,

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Periplaneta americana, virus could be recovered 15 days later by passage of triturated whole cockroach to normal mice. He was not able to demonstrate virus in roach feces or eggs. Woke and Rosenberger(6) reported that injection of mouse strain FA encephalomyelitis virus into haemocoeles of several species of invertebrates resulted upon titration of the entire triturated invertebrate in evidence for virus survival to 28 days. The authors(7) showed that *Periplaneta americana* under experimental conditions could acquire GDVII strain mouse encephalomyelitis virus. After being fed, the virus was harbored intracorporeally for as many as 7 days and excreted daily in amounts sufficient to kill mice. Further experiments(8) with *Periplaneta americana* fed a single meal containing Coxsackie virus (Group A, Type 4, Minnesota 1 stain) proved that test roaches excreted daily for as many as 15 days sufficient virus to paralyze and kill test mice. Control experiments showed that paralysis and death of test mice resulted from infection by Coxsackie virus and not from extraneous bacterial or viral infection.

The present paper reports for the American cockroach, *Periplaneta americana,* survival of active Coxsackie virus from time of oral ingestion.

Materials and methods. Feeding of virus to adult cockroaches (*Periplaneta americana*) strapped by cellulose tape to corks(9) was accomplished with tuberculin syringe and blunt needle: a single meal of 0.2 ml of a 10% hind limb suspension from mice moribund from Coxsackie infection was fed. The diet thereafter consisted of fox chow and water. *Harvesting of viscera* was done at 4 intervals of 5 days: 3-8 cockroaches were dissected to obtain pools of viscera representing (a) gastro-intestinal tracts, (b) salivary glands, (c) reproductive organs, and (d) fat bodies and haemocoeles. Care was taken to prevent intermixing of visceral fluids and dehydration of viscera. Tissues were stored at −20°C until tested for virus. *Virus assays* were done on each 5-day pool of viscera, triturated in a mortar with 0.85% NaCl solution and antibiotics to yield a 10% tissue suspension and a final concentration of 500 units of penicillin and 100 μg of streptomycin. After 30 minutes at room temperature, suspensions were centrifuged at 700 rpm for 10 minutes. The supernatant fluid in 0.05 ml doses was injected intraperitoneally into litters of 24 to 48-hour-old Swiss albino mice.

Results. Results of 13 experiments are summarized in Tables I and II. Table II presents the findings in graphic form and includes results of periodic tests of 24-hour fecal specimens from test cockroaches.

Pooled gastrointestinal tract samples removed from cockroaches at 5-day intervals up to 20 days following a single feeding of Coxsackie virus yielded on intraperitoneal injection sufficient virus to paralyze and kill test mice in from 2 to 4 days (Table I). Passage of 1% hind-limb suspensions from dead recipient mice to new litters demonstrated lethal content of virus. *Salivary glands* were found in about half of the experiments to contain virus; in most cases, virus recovery was limited to 5-day samples. In one experiment virus was demonstrated in the 5, 10 and 20-day samples, but not in the 15-day material. *Fat bodies and haemocoeles* yielded virus 3 times in 32 attempts. The irregularity suggests cross contamination during dissection. *Reproductive organs* similarly yielded virus only a few times. *Fecal specimens* periodically collected at 24-hour intervals and inoculated intraperitoneally contained irregularly sufficient virus to paralyze and kill test mice (Table II). *Effect of feeding Coxsackie virus-fed cockroaches to adult Swiss albino mice* was determined with adult cockroaches fed a known content of Coxsackie virus as previously described. After 2 days, 4 cockroaches were fed to 2 mice. Fecal specimens representative of each 24-hour period were collected daily from the mice and stored at −20°C until tested for virus. Supernates of fecal specimens representative of 4 successive days were prepared by the method used for roach tissues: 0.05 ml doses were inoculated intraperitoneally into 24 to 48-hour-old Swiss albino mice. Sufficient virus was present in the 1st and 2nd day fecal specimens to paralyze and kill test mice; 3 and 4-day fecal specimens were negative.

Discussion. Under the described experi-

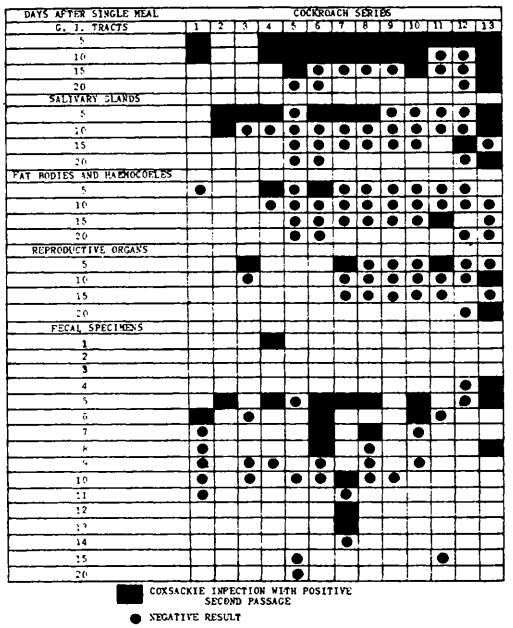
TABLE I. Recovery of Coxsackie Virus in Mice Injected with Visceral Suspensions from Virus-Fed Cockroaches.

Days after single meal	Mouse passage	Cockroach series* 1	2	3	4	5	6	7	8	9	10	11	12	13
(G. I. tracts)														
5	1	7/7			10 10	8 8	8 8	10/10	6/6	8/8	9/11	2/9	7/7	8/8
	2†	6/6			6/6	9 9	6 6	6/6	7/7	6/6	9/10		4/4	9/9
10	1	8/8			8/8	6 6	8 8	2/5	2/7	6/6	1/5	0/10	0/10	3/8
	2	7/7			9/9	6 6	10 10	6/6	6/6	7/7	2/6			7/7
15	1					11 11	0 7	0/12	0/7	0/7	1/5	0/7	0/7	3/8
	2					8 8					8/8			5/5
20	1					0 7	0 7					0/10	0/10	4/9
	2													7/7
(Salivary glands)														
5	1	0/5	8 9	2/5	9/9	0 5	1 9	1/9	1/8	0/6	0/7	0/8	0/7	1/5
	2		1 5		8 8		11 11	8/8	8/8					8/8
10	1		5 7	0 11	1 8	0 7	0 7	0/5	0/7	0/7	0/6	0/4	0/2	2/7
	2		9/9		0 11									7/7
15	1					0 10	0 8	0/5	0/6	0/7	0/8	0/5	1/9	0/7
	2												5/5	
20	1					0 8	0 10						0/10	2/7
	2													7/7
(Fat bodies, haemocoeles)														
5	1				5/7	1 7	8 8	0/5	0/7	0/7	0/8	0/6		
	2				9/9	0 5	9 9							
10	1				0/6	1 7	0 8	0/7	0/6	0/8	0/5	0/7	0/6	0/4
	2					0 3								
15	1					0 8	0 7	0/5	0/7	0/5	0/7	4/5	0/8	0/9
	2											9/9		
20	1					0 7	0 8						0/8	0/3
	2													
(Reproductive organs)														
5	1			6 10				2/11	0/10	0/8	0/8	1/5		0/4
	2			10 10				7/7				6/6		
10	1			0 9				0/6	0/5	0/8	0/7	0/8	0/7	3/3
	2													5/5
15	1							0/5	0/6	0/7	0/9	0/6	0/6	0/2
	2													
20	1													1/4
	2													8/8

* Mice dead of Coxsackie infection/mice inj. intrap., 0.05 ml.
† Hind limbs from mice of passage 1 employed as inoculum for passage 2.

mental conditions, samples of pooled gastrointestinal tracts removed at 5-day intervals up to 20 days from cockroaches fed a single meal containing Coxsackie virus contained sufficient virus to paralyze and kill test mice. Recovery of virus from pooled salivary glands was limited to 5-day samples with one exception when virus was recovered from 5, 10 and 20 day samples. It appears possible that a) cockroaches by feeding on mammalian excreta could acquire virus, and maintain it for a period of time, and transfer it by contamination of food, or that b) cockroaches so infected might be consumed by wild mice to transmit virus through the feces of the mice. The presence of Coxsackie virus in feces and its recovery from sewage of cities in which Coxsackie infections have occurred(3) indicates that cockroaches have opportunities for acquisition of virus. Present and previous experimental findings(7,8) suggest a possible role for cockroaches in the transmission of

TABLE II. Recovery of Coxsackie Virus from Cockroach Viscera.



sporadic Coxsackie and poliomyelitis infections in man.

Summary. Gastrointestinal tracts removed at 5-day intervals for up to 20 days from cockroaches, *Periplaneta americana,* fed a single meal of Coxsackie virus contained sufficient virus to paralyze and kill test mice. Salivary glands removed after 5 days from the test cockroaches also caused paralysis and death in test mice; in one experiment, salivary glands removed 5, 10 and 20 days after a single feeding of virus caused Coxsackie infection in test mice.

1. Sabin, A. B., *J. A. M. A.,* 1947, v134, 749.
2. ———, *Ann. Int. Med.,* 1949, v30, 40
3. Melnick, J. L., Shaw, E. W., and Curnen, E. C., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 344.
4. Melnick, J. L., and Penner, L. R., *ibid.,* 1947, v65, 342.
5. Hurlbut, H. S., *J. Inf. Dis.,* 1950, v86, 103.
6. Woke, P. A., and Rosenberger, C. R., *ibid.,* 1957, v100, 12.
7. Syverton, J. T., and Fischer, R. G., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 296.
8. Fischer, R. G., and Syverton, J. T., *Am. J. Trop. Med.,* 1951, v31, 238.
9. Wedberg, S. E., and Clarke, N. A., *J. Bact.,* 1947, v54, 447.

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Heat-Stability of *Candida albicans* and *Saccharomyces cerevisiae* Hemagglutinating Antigens. (23196)

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Erythrocyte sensitizing substances (ESS) derived from *Candida albicans* and *Saccharomyces cerevisiae* have recently been described as requiring lipid for sensitization of red cells in the hemagglutination test(1). Further studies of these antigens led to an investigation of their heat-stability. Unexpectedly it was established that these antigens prepared by similar technics react differently to heat in respect to their capacity subsequently to sensitize red cells. In the following report serologic activity of ESS from *C. albicans* and *S. cerevisiae* is compared to whole yeast-cell agglutinating antigen from these organisms before and after heat-treatment.

Materials and methods. Preparation of antigens and serologic tests employed. ESS from *C. albicans* and *S. cerevisiae* derived from liquid medium, and antisera to these organisms were obtained as described recently (1). The hemagglutination test described previously was used to test activity of these antigens(2). Whole yeast-cell antigens were prepared from cells grown at optimal temperatures on Sabouraud's dextrose agar. The cells were heat-killed at 60°C for 2 hours, washed 3 times in saline and adjusted to a No. 2 McFarland nephelometer tube. The yeast-cell agglutination test employed 0.4 ml serial dilutions of inactivated rabbit sera to