arrested by placing the suspension back at +2°C (compare curves 2, 3, and 4; Fig. 3). Furthermore, relatively little death results upon exposure to −22°C in distilled water. In one experiment the initial viable cell count was 1.4 x 10^8 per ml; after 1 day the viable count was 1.2 x 10^8 per ml.

Since the NaCl in the suspensions prevents freezing, we are not concerned with extracellular ice. However, the possible formation and role of intracellular ice must be considered. Although our experiments do not permit a definite conclusion as to the presence or absence of intracellular ice, they do demonstrate that, if this ice is formed, it is not an important lethal factor. Firstly, periodic fluctuations from −22° to +2°C and back to −22°C bring about no greater death than uninterrupted storage at −22°C (curves 4 and 5; Fig. 3). Secondly, we have never detected between +2° and −22°C a crucial temperature on each side of which survival differs sharply.

Summary. Suspensions of *E. coli* in 4.6 M NaCl stored under conditions which preclude cell division undergo at first a rapid decline in viable cells, but as the storage interval is extended the death rate continuously decreases until eventually it becomes negligible. Cells that endure the period of rapid decline are able to maintain themselves in the saline for long periods. This resistance is not heritable. In fact, once the cells are removed from the saline their resistance to it is lost. Generally, the higher the storage temperature the better is survival. Also, holding suspensions at a relatively high temperature (+2° or −9°C) prior to subsequent storage at a low temperature (−22°C) improves survival at the low temperature. These data suggest that the tolerance to 4.6 M NaCl is acquired while the cells reside in the saline and does not exist preformed in a segment of the original population. Two hypotheses have been offered to account for the adjustment to NaCl tolerance.

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Swine and Experimental Atherosclerosis. (23194)

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Gottlieb and Lalich(1) have reported that the aortas of swine obtained from slaughterhouses occasionally show atheroma-like lesions, and that incidence of these lesions increases with age. Lewis and Page(2) have reported rather high serum cholesterol levels and rather high concentrations of low-density lipoproteins in at least one strain of pig. It was therefore hoped that this species might prove a suitable one for studies on effects of diet on serum lipoproteins and on the atherosclerotic process.

Materials and methods. The experimental animals were 8 2-year-old boars of a cross-bred strain, bred and maintained at Beltsville. They had previously been used in breeding experiments. Their basal diet consisted of a mash made of 60 parts yellow corn meal, 20 parts soybean oil meal, and 2 parts of mineral mixture. This diet contains approximately 2.9% crude fiber, 58.3% nitrogen-free extract, 17.6% protein, 3.2% fat, 4.8% ash and 13.4% moisture. The experimental diets were made by adding 2.2 parts of corn oil or 2.6 parts of butter to 10 parts of the basal diet. Approximately 40% of the caloric value of these diets was thereby derived from fat. The diets were made up weekly and

TABLE I. Serum Cholesterol Levels of Swine on Control and Experimental Diets.

	Total cholesterol, mg/100 ml	t	p
Control	52 ± 21	1.47	.1–.2
Corn oil diet	65 ± 15		
Control	61 ± 23	2.38	.025
Butter diet	84 ± 18		

stored in a refrigerator. During the control period the animals were free to graze. During the experimental period the animals were housed in pairs in pens that had both inside and outside runs. They were fed once daily. At the start of the experimental period the animals weighed about 475 lb each. An effort was made to keep the body weight as constant as possible. The animals were bled from the jugular vein twice while on the basal diet. The interval between bleedings was one week. They were bled again after 3, 6, and 9 weeks on the experimental diets. They were killed and necropsied after 9 weeks on the diets. At this time 4 additional boars of the same strain and age, maintained on a conventional diet, were killed as anatomic controls. The serum was analyzed for total and free cholesterol(3), for lipid phosphorus (4), and for total lipid(5). The triglyceride was calculated by difference(5). The 2 serum samples collected during the control period and the first and third samples during the experimental period were subjected to lipoprotein fractionation by repeated ultracentrifugation at increasing solvent densities(6). The fractions were then analyzed chemically for lipid and protein content. At necropsy the heart and aorta only were examined. Tissues were fixed in formalin, sectioned with the freezing microtome, and stained with oil red O and hematoxylin.

Results. One corn-oil fed animal lost 27 lb during the experimental period. All others gained weight. The average gain for corn-oil fed animals was 31 lb and for butter fed animals was 29 lb. This gain represented approximately 5% of their original weight.

The mean serum cholesterol levels together with standard deviations for control and dietary periods are shown in Table I. Animals fed corn oil showed a slight increase over their control levels, but the difference is not significant. Animals fed butter showed a significant increase with the probability that it was due to chance 1 in 40. When the levels in the butter fed group are compared with the levels in the corn-oil fed group the p value is 0.02. The maximum rise in serum cholesterol levels occurred within the first 3 weeks of butter feeding. Thereafter the levels remained relatively constant. The cholesterol-phospholipid ratio showed no significant changes, averaging 0.84 under all conditions. The serum triglyceride levels averaged 40 mg per 100 ml. There were no significant dietary differences.

Fractionation of serum lipoproteins during the control period showed that the very low-density classes (Sf>10) carried practically all the serum triglyceride and therefore varied in concentration with the latter. As is true of all animal species so far studied, with the single exception of man, concentration of low-density lipoproteins (Sf 0-10) was less than concentration of the high-density lipoproteins. During the control period the former averaged about 84 mg per 100 ml, the latter 207. The cholesterol:phospholipid ratio in the low-density fraction (Sf 0-10) exceeded unity. In this respect swine resemble man rather than other mammals studied.

Animals on the corn oil diet showed no significant changes in lipoprotein patterns over the control period. Animals on the butter diet showed an increase in the high-density classes only. The mean increase in cholesterol content of this class was 30%, in phospholipid content 29%, and in protein content 19%.

Examination of the hearts of the 12 animals revealed no abnormalities of valves or coronary arteries. The aortas of 6 animals, however, revealed lesions. These were sharply delineated thickenings of the intima. They were longitudinal, paralleling the long axis of the aorta and varied in size up to 5 x 2 mm of the same pale color as the surrounding normal intima. They were confined to the descending thoracic aorta and appeared identical with those illustrated by Gottlieb and Lalich(1).

Microscopically these intimal thickenings

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.

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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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