TABLE III.
Effect of Incubation with Citrated, Oxalated, and Amberlite Plasmas of Man, Dog, and Rabbit on Activity of Full Strength Human Thrombin.
Each figure in the table represents the average clotting time in seconds obtained in several experiments.*

Length of incubation (sec.)		60	120	180	240	300
Human plasma	Amberlite	8	11	13.5	29.5	34
	Citrated	12	14	16	21	29
	Oxalated	12	23	64	450	†
Dog "	Amberlite	8.5	12	18.5	27	52
	Citrated	11	13	17.5	25	34
	Oxalated	13	26.5	49	90	225
Rabbit "	Amberlite	9	11.5	14	22	40
	Citrated	11	12.5	15	20.5	34
	Oxalated	11	35	49	58.5	87

* Equal volumes of full strength human thrombin and of the plasma to be tested were incubated in a water bath at 37°C; the clot was wrapped about a glass rod coated with collodion and removed. After the incubation, 0.1 cc of the mixture were added to 0.2 cc of oxalated homologous plasma as a source of fibrinogen and the clotting time was determined and recorded.
† No clotting in 1 hr.

citrated plasma responded similarly, the behaviour may be linked with the mechanism of decalcification of the 3 agents.

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Effect of Polyoxyalkylene Sorbitan Monooleate on Blood Cholesterol and Atherosclerosis in Cholesterol-Fed Rabbits.*

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(Introduced by John G. Kidd.)
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In an attempt to alter the solubility or colloidal stability of cholesterol *in vivo* and thus modify the development of experimental cholesterol atherosclerosis, Hueper[1] administered several commercial detergents to cholesterol-fed rabbits and observed no striking alteration in blood cholesterol levels or degree of atherosclerosis.

This report deals with the effect of oral polyoxyalkylene sorbitan monooleate, Tween 80,‡, a surface-active agent of low toxicity, on the level of blood cholesterol and on the development of atherosclerosis in cholesterol-fed rabbits.

Experimental. Adult male and female rabbits averaging 3.2 kg in weight were fed a stock diet of Rockland rabbit pellets to which were added either:

A. Cholesterol—1 g; peanut oil—3 cc; Tween 80—10 cc.
B. Cholesterol—1 g; peanut oil—3 cc.
C. Tween 80—10 cc.

The ingredients were added separately to the stock diet in individual containers, and thoroughly mixed.

In Experiment 1, 6 rabbits were fed Diet A, and 5 Diet B daily. In Experiment 2, 12

* Aided by a grant from the United States Public Health Service.

† This work was done during the tenure of a Life Insurance Medical Research Fellowship.

1 Hueper, W. C., *Arch. Path.*, 1944, **38**, 381.

‡ Manufactured by the Atlas Powder Co., Wilmington, Del.

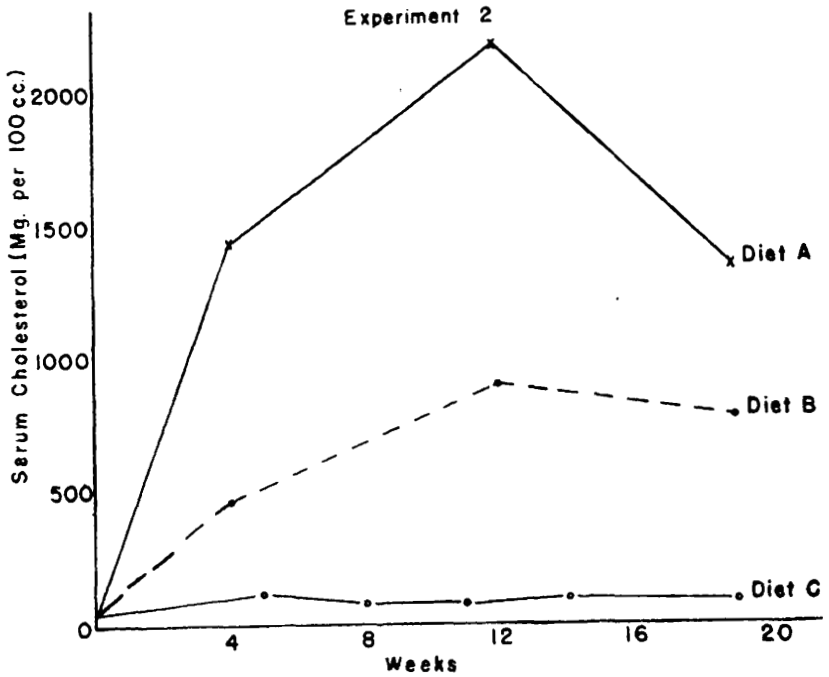

FIG. 1.
Mean Serum Cholesterol Levels.
Diet A—Cholesterol, peanut oil, and Tween 80 (12 rabbits).
Diet B—Cholesterol and peanut oil (11 rabbits).
Diet C—Tween 80 (3 rabbits).

rabbits were fed Diet A, and 11 Diet B 3 times a week, and 3 additional rabbits were fed Diet C daily. Blood was drawn from the marginal ear vein, and serum total cholesterol determined by a modification of the method of Bloor, Pelkan, and Allen.[2] Tween 80 *per se* does not influence the colorimetric determination of cholesterol, as it does not give the Leibermann-Burchard reaction itself or when added to serum *in vitro*. The degree of atherosclerosis of the aorta was determined by gross examination of the fresh specimen, and after fixation and staining of the entire aorta in Sudan IV.

Results. In Experiment 1, at 4, 7, and 10 weeks the mean serum total cholesterol levels of rabbits fed Diet A (cholesterol, peanut oil, and Tween 80) were 1,490, 2,725, and 2,150 mg per 100 cc respectively, as compared with 725, 975, and 1,160 mg per 100 cc for the rabbits fed Diet B (cholesterol and peanut oil). Fig. 1 summarizes the results of Experiment 2. The serum total cholesterol levels reached a peak at 12 weeks with a mean value of 2,215 mg per 100 cc (range 900-4,350) for rabbits on Diet A, and 880 mg per 100 cc (range 285-2,205) for rabbits on Diet B. The difference between the two groups is more than 6 times the probable error, and is statistically significant. Rabbits fed Diet C (Tween 80) showed at first a very slight rise in blood cholesterol, which then returned to and remained within the normal range. The blood cholesterol levels of the rabbits fed Diet B were in approximately the same range as those previously reported in cholesterol feeding experiments (Weinhouse and Hirsch;[3] Dubach and Hill[4]).

The action of Tween 80 in augmenting the blood cholesterol level is probably due, in

[2] Bloor, W. R., Pelkan, K. F., and Allen, D. M., *J. Biol. Chem.*, 1922, **52**, 191.

[3] Weinhouse, S., and Hirsch, E. F., *Arch. Path.*, 1940, **30**, 856.

[4] Dubach, R., and Hill, R. M., *J. Biol. Chem.*, 1946, **165**, 521.

part at least, to better emulsification of cholesterol in the intestinal tract and more efficient absorption. Studies now in progress using parenteral administration of Tween 80 indicate that because of its detergent properties it may also have a more direct influence on the level of cholesterol and other lipids in the blood.

Rabbits fed cholesterol plus Tween 80 developed atherosclerosis of the aorta more uniformly, at an earlier date, and of a slightly more severe degree than the cholesterol-fed controls. Animals fed only Tween 80 had no atherosclerosis.

Studies are now in progress to determine whether Tween 80 can effect resorption of experimental atherosclerosis.

Summary. Rabbits fed Tween 80 and cholesterol developed blood cholesterol levels that were 2 to 3 times as high as those obtained by cholesterol feeding alone, and also exhibited an earlier and somewhat more severe degree of atherosclerosis.

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V. Long-term Maintenance of Two Strains on Synthetic and on Stock Diets.*

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Nielsen and Black[1] have reported that mice require a dietary source of biotin and folic acid during their early period of growth, and that this requirement is increased by incorporating sulfasuxidine in the ration. We[2] have observed that mice of 2 highly inbred strains grow well on synthetic rations containing no added biotin and folic acid. We have also shown, however, that one or both of these factors may be essential for satisfactory reproduction and lactation.[3] Unpublished work in this laboratory has revealed that mice of the C_{57} strain may be maintained on a synthetic diet with 0.75% sulfasuxidine without added biotin and folic acid but with p-aminobenzoic acid for about 6 months without showing outward changes other than slight alopecia and achromotrichia. The discrepancy between our results and those of Nielsen and Black and the fact that the literature contains few reports of long-term maintenance of mice on highly purified diets led us to analyze the records of our mouse colony in which animals have been maintained on various stock diets and synthetic rations.

Methods. Two strains of mice (the A strain, with high incidence of spontaneous mammary tumors, and the C_{57} with low tumor incidence) have been employed in these studies. At weaning the animals were placed in individual screen-bottom cages and their growth rates measured until they were 10 weeks old. Thereafter they were used for reproduction and lactation studies as previously reported.[3] During the rest intervals between matings or after the end of the reproductive studies 2 or 3 animals were housed together. The stock rations employed were Purina Dog Chow and later Purina Laboratory Chow. The synthetic diet (No. 101)[3] contained dextrose 60%, "vitamin-free" casein 23%, hydrogenated cottonseed

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1 Nielsen, E., and Black, A., *J. Nutrition*, 1944, **28**, 203.

2 Fenton, P. F., Cowgill, G. R., and Stone, M. A., *Yale J. Biol. and Med.*, 1947, in press.

3 Fenton, P. F., and Cowgill, G. R., *J. Nutrition*, 1947, **33**, 703.