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also shown, however, that one or both of these factors may be essential for satisfactory reproduction and lactation.[3] Unpublished work in this laboratory has revealed that mice of the C_{57} strain may be maintained on a synthetic diet with 0.75% sulfasuxidine without added biotin and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.

TABLE I.
Summary of Observations.

Strain	C_{57}				A			
Sex	Male		Female		Male		Female	
Diet	Stock	101	Stock	101	Stock	101	Stock	101
No. of animals	14	8	30	11	9	19	21	28
Achromotrichia (slight)	2	1						
Alopecia (slight)		4	3	2	1	2		2
Skin ulceration						2		2
Eye involvement						6		1
Moist dermatitis				2		5		4
Massive fecal crusts						4		2
No. of animals with tumors							14	16
Avg time of onset—months							11½	10
Avg No. of litters born			4.3	2.7			4.0	1.9
No. of deaths								
Fecal crusts						4		2
"Tumors"							4	5
Incisor overgrowth				1				
Avg wt when sacrificed—g	28.2	30.3	29.0	26.8	27.8	25.2	—	—

oil 10%, salts 5%, roughage 2%, 8 vitamins of the B complex (thiamine, niacin, pyridoxine, riboflavin, pantothenate, choline, inositol, and p-aminobenzoic acid), cod liver oil concentrate, α-tocopherol, and linoleic acid. The animals on synthetic rations were given various supplements during the reproduction study;[3] these, however, were only administered for relatively brief time periods. Because of lack of space, it was necessary to sacrifice most of the animals when they were about a year old.

Results. The pertinent observations made on these animals in the course of their existence or at the end of the year period are summarized in Table I. Mice of the A strain maintained on synthetic diet showed numerous symptoms which they did not develop on the stock ration. Most of these changes were not observed in the C_{57} strain maintained on either diet. The eye lesions consisted of inflammation and denudation of the surrounding skin. Occasionally a dried exudate was observed in this area. Animals showing these conditions were usually hesitant to open their eyes when disturbed. The moist dermatitis which was observed almost exclusively in the A strain on synthetic diet was usually found below the chin and extended caudad for a distance of several centimeters. No hair was seen in the involved areas. Skin ulcerations, when present, were usually on the lateral body areas and on the legs. The formation of massive fecal crusts over the anus was observed in 6 animals of the A strain on synthetic diet. This condition, which has always been fatal, has also been observed in the offspring of the generation of mice reported here. We have seen this happen only in A strain mice on synthetic diets. It has even occurred in the animals subsisting on the most complete synthetic diets which we have studied.

The causes of deaths in each group of animals are arranged in the table in 4 classes. When the cause of death is described as "tumor," we mean only that the animals in question had a sizable mammary tumor which could have been the cause of death. No extensive autopsies were performed on the dead animals.

Discussion. We have not found under the conditions of our experiments that the mouse requires a dietary source of biotin or folic acid for early growth; nor have we observed any deficiency symptoms during the early period of life as did Nielsen and Black.[1] A possible explanation of this discrepancy may lie in the fact that Nielsen and Black carried out special alcohol extractions of their casein and sucrose, while we used commercial "vitamin-free" casein and dextrose C.P. without further treatment. Biotin assays on our diet 101 showed it to contain less than one μg of biotin per 100 g of ration. The fact that

Nielsen and Black did not incorporate p-aminobenzoic acid in their rations does not offer a convincing reason for the discrepancy. It seems much more likely that the difference is due to the nature of the dietary carbohydrate. We have used dextrose while Nielsen and Black used sucrose. This aspect of the problem is now being further investigated. Another possible explanation at the moment seems to be one of strain difference. Our evidence[4,5] very strongly supports the view that there is a clear strain difference in the nutritional requirements of the C_{57} and the A strain. The mice used by Nielsen and Black may have possessed such a high requirement for biotin and folic acid that they showed deficiency symptoms during a very early period of life.

The difference in the time required for A strain females to develop mammary tumors on the stock and the synthetic diets is highly suggestive and is being investigated more thoroughly.

The much greater incidence of symptoms and deaths of A strain mice on synthetic diets suggests again their greater nutritional requirements. We have shown in this laboratory[4,5] that this strain requires more riboflavin and pantothenic acid for growth than does the C_{57} strain. The findings reported here suggest also a greater need for biotin or folic acid or both in order to maintain the animal in good health during later life. The absence of possible unknown nutritional factors cannot be altogether overlooked. We have found[6] that mice of the C_{57} strain on synthetic diets had greater cecal contents and a greater bacterial population than did mice of the A strain. This strain difference was not observed in animals on stock ration.

Summary. Two strains of highly inbred mice, one tumor-susceptible and the other tumor-resistant, were maintained for a period of about one year on a stock diet or on a synthetic ration adequate for good growth performance. The tumor-susceptible A strain on synthetic diet showed numerous deficiency symptoms not seen on stock rations and not shown to any great extent by C_{57} mice on either stock or synthetic diets.

[4] Fenton, P. F., and Cowgill, G. R., *J. Nutrition*, 1947, **34**, 273.

[5] Fenton, P. F., and Cowgill, G. R., *Fed. Proc.*, 1947, **6**, 407.

[6] Gall, L. S., Fenton, P. F., and Cowgill, G. R., in press.

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Antagonism of Sulfadiazine Inhibition of Psittacosis Virus by p-Aminobenzoic and Pteroylglutamic Acids.*

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Studies on the chemotherapeutic action of the sulfonamides on certain bacteria[1] have shown that p-aminobenzoic acid (PABA) and pteroylglutamic acid (PGA) antagonize the inhibition of growth of these organisms by sulfonamide drugs. The demonstration of sulfadiazine (SD) inhibition of the growth of certain strains of psittacosis virus[2] suggests the likelihood that PABA and PGA might be active antagonists for this inhibitory effect of the sulfonamides.

A yolk sac passage strain of psittacosis

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[1] Lampen, J. O., and Jones, M. J., *J. Biol. Chem.*, 1947, **170**, 133.

[2] Early, R. L., and Morgan, H. R., *J. Immunol.*, 1946, **53**, 151.