

The degree of ossification was in these cases insufficient to produce obvious density. Histologic changes, however, were apparent in all animals receiving phosphorus for at least one week.

Summary and Conclusions. Twenty-two albino rats were given small amounts of elementary phosphorus supplementary to an adequate diet. Roentgenograms taken before and during the experimental period demonstrated the development of an area of increased density in the subepiphyseal region.

Histologic studies revealed a failure of resorption of the usual number of primary epiphyseal trabeculae. Since bone was deposited upon these persistent spicules in an otherwise normal fashion an apparent sclerosis was produced. Although the degree of ossification was distinctly greater than normal there was no evidence of direct stimulation of osteoblastic activity. The lesion was dependent entirely upon the unusual preservation of mineralized cartilage. It was thought that the persistence of these primary trabeculae was related to the influence of elementary phosphorus deposited in this region.

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Experimental Atherosclerosis and High Protein Diets.*

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The relationship of protein and experimental atherosclerosis has been summarized by Duff.¹ No clear cut experimental evidence was available that the feeding of proteins in conjunction with cholesterol promotes experimental atherosclerosis. Nor was there any indication that a sterol-free protein diet can produce atherosclerotic lesions.

In the course of a study on the possible effects of a high protein diet on cholesterol-induced arteriosclerosis, young adult chinchilla rabbits were fed one of 3 diets:

(A) A normal stock diet (15% digestible protein, dry weight) composed of white flour, alfalfa leaf meal, linseed oil meal, salt mixture, ground carrots, and water.

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¹ Duff, G. L., *Arch. Path.*, 1935, **20**, 81, 259.

(B) A high protein diet (approximately 38% protein) composed of essentially cholesterol-free defatted casein,[†] white flour, alfalfa leaf meal, salt mixture, ground carrots, and water.

(C) A high protein diet (approximately 39% protein) composed of white soy bean flour (Nusoy), linseed oil meal, alfalfa leaf meal, salt mixture, ground carrots, and water.

These diets were fed for 6 months ($\frac{1}{3}$ of the animals receiving the casein and soy bean diets were fed for 3 months only). Part of the rabbits on each diet received in addition a daily dose (60 or 250 mg) of cholesterol, dissolved in vegetable oil, mixed with the diet. Administration of cholesterol took place during the first 3 months of each period of feeding. Absorption was demonstrated by rise in blood cholesterol values. All animals gained weight and when sacrificed at the end of the experiment were sleek and well nourished. The aorta and viscera were studied histologically, using fat and elastic tissue stains on the rolled aorta and on the heart.

The experimental procedure and the results are summarized in Table I.

Three observations may be made from the above data. One is the occurrence of lesions indistinguishable from those of cholesterol sclerosis in rabbits fed a practically cholesterol-free (high protein-casein) diet.

The occurrence of atherosclerosis on an essentially cholesterol-free diet in two separate groups of rabbits used in consecutive years is of considerable interest, especially in view of the extreme rarity of this type of spontaneous lesion (Duff, *Arch. Path.*, 1935, 20, 81).

TABLE I.
Summary of Feeding Experiments.

Diet	Chol. fed	Rabbits			Degree of sclerosis of aorta		
		No. used	No. sclerotic	% sclerotic	+	++	+++
Basic	None	8	0	0			
Casein	"	12	6	50	4	1	1
Soybean	"	8	0	0			
Basic	+	21	15	70	6	7	2
Casein	+	13	10	75		3	7
Soy bean	+	16	6	35	5	1	

+ = Lesion doubtful grossly, but present microscopically.

++ = One to several small atheromatous plaques visible grossly.

+++ = Numerous plaques present.

[†] The casein was defatted by repeated extraction with hot alcohol according to the method of Sperry.⁶ After extraction it sometimes still contained traces of cholesterol (0.024-0.107 mg in 100 g).

⁶ Sperry, W. M., *J. B. C.*, 1926, 68, 357.

The total amount of contaminating cholesterol ingested during 6 months in the casein-containing diet was approximately 2.7 mg. However, the plasma cholesterol of several of these rabbits became abnormally high, rising from less than 100 mg per 100 cc at the beginning of an experiment to 150 mg, and even 280 mg. Such a rise cannot be accounted for quantitatively by the small amount of cholesterol ingested. Since the rabbit normally does not ingest cholesterol, and does not absorb plant sterols,² the obvious conclusion is that synthesis of cholesterol was abnormally accelerated by the casein in the diet.

A second result is the increase in cholesterol sclerosis when cholesterol and casein are fed, compared to the incidence on a normal diet plus cholesterol. This increase is more apparent if the degree and extent of sclerosis are taken into consideration, rather than merely the number of animals affected. The differences shown in the table are rendered even more significant by the fact that over half of the rabbits fed the soy bean diet with cholesterol (10 of 16) and over half of those fed the basic diet with cholesterol (12 of 21) were given 250 mg of cholesterol daily, while all the animals on the casein diet with cholesterol received only 60 mg of the latter daily. Nevertheless the incidence and degree of sclerosis were highest in the last group.

The third significant observation is the apparent inhibition of lipid deposition in the aortas of rabbits fed soy bean and cholesterol. The mechanism of this is not clear. The thyroid glands of these rabbits did not differ grossly or histologically from those on the other diets. Furthermore, soy bean contains only traces of iodine.³ The choline content of the soy bean diet is small (10 mg daily), and moreover is the same as that of the basic diet. Another lipotropic factor, methionin,⁴ is present in greater amount in casein than in glycinin, the principal protein of soy beans.⁵ There remains the possibility that some other factor is present in soy bean that restricts the deposition of cholesterol.

Summary. A diet high in soy bean flour diminishes the incidence and degree of experimental sclerosis in rabbits fed cholesterol, while a diet rich in defatted casein augments the degree of sclerosis. Rabbits, fed the high protein, defatted casein diet alone may develop atheromatous lesions indistinguishable from those produced by cholesterol.

² Schönheimer, R., *Z. f. Physiol. Chem.*, 1929, **180**, 32.

³ Adolph, W. H., and Ch'en, S. C., *Chinese J. Physiol.*, 1930, **4**, 437.

⁴ Channon *et al.*, *Biochem. J.*, 1938, **32**, 977.

⁵ Baernstein, H. D., *J. Biol. Chem.*, 1932, **97**, 663.