Amyloidosis and Hypertension in Cholesterol-Fed Rabbits.* (18965)

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Amyloidosis was observed at necropsy of rabbits fed cholesterol for long periods. Deposits of similar structure and distribution, but which did not take specific stains (paramyloid), were found in other cholesterol-fed rabbits. In this communication the histological findings, relevant biochemical observations and changes in blood pressure are reported.

Technic. Eighteen rabbits 6 to 8 weeks old were fed a basic diet of Rockland Farm pellets for periods varying between 12 and 104 weeks. The cholesterol ration for each week was dissolved in ether and poured over the pellets. Following evaporation of the ether, the rabbits readily ate the cholesterol-coated pellets. Chemical and electrophoretic determinations were made by technics outlined previously(1). Mean arterial pressure was determined at weekly intervals by direct puncture of the central ear artery using a 20-gauge needle attached to a mercury manometer. In 138 determinations on normal control rabbits the mean value was 88.1 mm Hg, with a standard deviation of 11.7 mm Hg. No control animal had a mean arterial pressure of more than 120 mm Hg.

Incidence of amyloidosis and paramyloidosis. Of 18 rabbits fed cholesterol for 3 months or longer, 7 or 39%, developed amyloidosis or paramyloidosis. The weekly cholesterol ration in 6 of the 7 animals was 3 g and in the other 20 g. The shortest period in which deposits developed was 12 weeks. However, 2 rabbits, fed cholesterol for 100 and 104 weeks, respectively, did not exhibit amyloidosis or paramyloidosis at autopsy. The weekly cholesterol dosage, duration of feedings and occurrence of amyloidosis or paramyloidosis are shown in Table I.

TABLE I. Occurrence of Amyloidosis in Cholesterol-Fed Rabbits.

Rabbit No.	Cholesterol, g per wk	Duration of feeding (wk)	Amyloidosis or paramyloidosis
1	20	12	+
2	20	16	—
3	20	20	—
4	20	28	—
5	3	29	+
6	20	32	—
7	3	36	+
8	3	36	—
9	3	44	—
10	3	44	—
11	3	48	—
12	3	48	+
13	3	53	+
14	3	56	—
15	3	73	+
16	3	82	+
17	3 / 20	60 / 40 (100)	—
18	3	104	—

Histologic findings. Amyloid and paramyloid deposits were found only in the kidney, liver and spleen. The earliest glomerular lesions consisted of small, ovoid, homogeneous anuclear deposits which stained pink in the hematoxylin-eosin preparations. These were found in many glomeruli, but only portions of glomeruli were affected. In the more advanced cases, all the glomeruli were involved. The glomeruli were markedly enlarged and their capillaries often almost completely obliterated by the eosinophilic, homogeneous substance. Bowman's space was obliterated in some of the glomeruli. Generally, the homogeneous substance stained blue with azocarmine, light tan in the van Gieson and red in the Gram preparations. Appreciable amounts of lipid within the glomeruli were demonstrated with Sudan IV in 2 of the 7 rabbits. Paraffin and frozen sections of the kidneys were stained with Congo red and methyl violet in 6 of the 7 animals. Results indicative of amyloid (bright red with Congo red, metachromasia with methyl violet) were obtained in 2 instances with both stains and

* Aided by grants from Mr. Percy Uris and Mr. Louis Adler.

1. Fishberg, A. M., Friedfeld, L., Hoffman, I., Stoller, E. R., and Fishberg, E. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 301.

TABLE II. Distribution and Staining Reactions of Amyloid and Paramyloid Deposits.

Rabbit No.	Glomerular deposits		Splenic deposits		Hepatic deposits	
	Congo red	Methyl violet	Congo red	Methyl violet	Congo red	Methyl violet
1	—	—	+	—	0	0
5	+	+	—	+	—	+
7	—	—	+	—	—	—
12	—	—	0	0	0	0
15	+	—	—	—	0	0
16	+	+	—	+	+	—

+ Amyloid stain positive. — Amyloid stain negative. 0 No deposit observed.

in another with Congo red alone. More brilliant staining of the amyloid deposits was achieved in the frozen sections. The collecting tubules were widely distended with casts, which compressed and flattened the tubular epithelium. The casts were pink in hematoxylin-eosin preparations, green with van Gieson and blue with Gram's stain. Generally the casts were scarlet with azocarmine but some, especially in kidneys with older lesions, were blue. In no instance did the casts react positively to either Congo red or methyl violet. The epithelial cells of the convoluted tubules contained large hyaline droplets, best defined when the autopsy was performed very promptly after death. These appeared deep red in the azocarmine, blue in the Gram, and dark brown in the van Gieson preparations. The droplets did not give a positive reaction with either amyloid stain. The renal arteries were normal. In the spleen the amyloid was deposited in the pulp without involving the center of the follicles or the arteries. The hepatic amyloid was not extensive and was largely confined to the midzone of the lobules. The results of the Congo red and methyl violet stains in the 6 amyloid or paramyloid rabbits in which they were carried out are shown in Table II.

Urinary changes. Tests for urinary protein were done in 12 of the 18 rabbits fed cholesterol. Of these, 8 were without amyloid or paramyloid. None of the 8 had proteinuria. Of the 4 rabbits with amyloid or paramyloid, 2 had proteinuria. Electrophoretic study of the urine of these 2 rabbits revealed that globulins constituted a high proportion of the urinary protein (Fig. 3).

Changes in blood chemistry. The hyperglobulinemia produced in rabbits by cholesterol feeding has been reported previously (1). Identical changes were observed in the sera of hypercholesteremic rabbits which developed amyloidosis (Fig. 3). All the rabbits had great hypercholesteremia. Monthly determinations of the nonprotein nitrogen of the serum revealed significant nitrogen retention in only one of the animals with renal lesions and that only terminally.

Changes in blood pressure. Blood pressure was measured in 12 of the 18 rabbits. In the amyloid or paramyloid group of 7 rabbits, blood pressure was determined in 5. All rose above the control levels. Hypertension is regarded as present in those animals in which

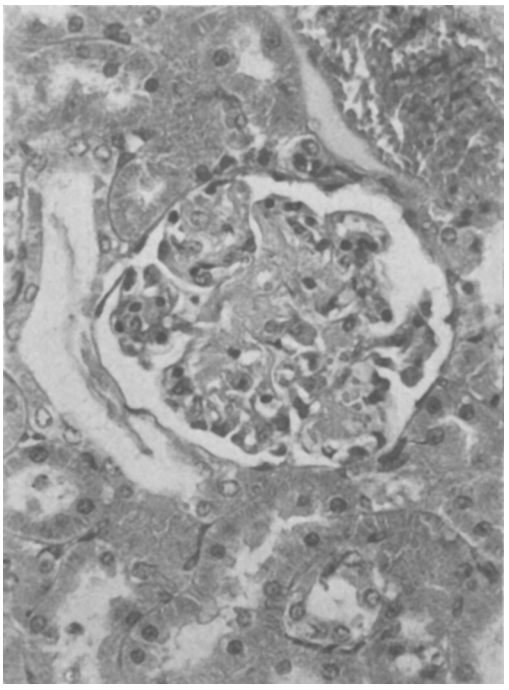

FIG. 1. Rabbit 1. Amyloid in glomerulus of rabbit fed 20 g of cholesterol per week for 12 weeks (hematoxylin and eosin).

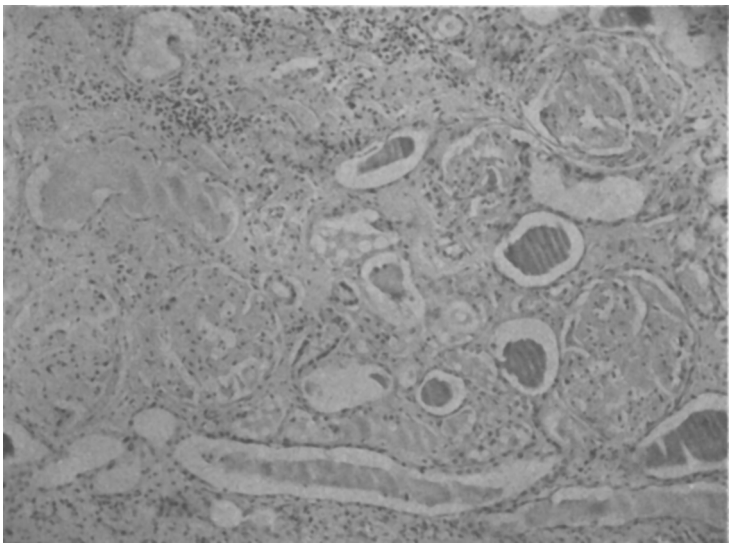

FIG. 2. Rabbit 15. Far advanced amyloidosis of the kidney in a rabbit fed 3 g of cholesterol per week for 73 weeks (hematoxylin and eosin).

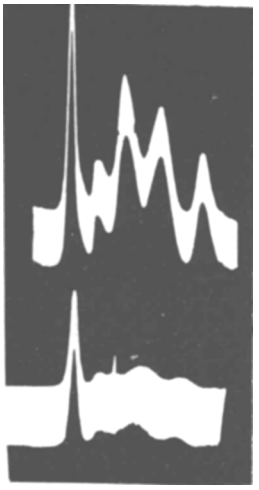

FIG. 3. Rabbit 5. Electrophoretic patterns of serum (above) and urine (below) after ingestion of 3 g of cholesterol weekly for 29 weeks. Necropsy revealed renal amyloidosis. Relative percentages of protein components:

	Serum	Urine
Albumin	24	48.7
α_1 globulin	7.2	16.5
α_2 "	29.4	24.8
β "	27.5	10.5
γ "	12	—

the arterial pressure rose above the mean of the 138 normal control measurements plus 3 times the standard deviation, *i.e.*, 123 mm Hg. This condition was fulfilled in 4 of the 5 animals with amyloidosis.

Of the 11 animals in the non-amyloid group, blood pressure was measured in 7. One of these, a rabbit fed 3 g of cholesterol weekly for 104 weeks, maintained a mean pressure between 140 and 205 mm Hg for over 30 weeks, with an average reading of 175 mm Hg. This was the most severe hypertension observed in the entire series. Of the 6 remaining rabbits in the non-amyloid group in which the blood pressure was measured, 3 showed a moderate rise above the control values. In none, however, was a level of 123 mm Hg reached.

Discussion. Of 18 rabbits fed 3 to 20 g of cholesterol weekly for 12 to 104 weeks, 7 developed amyloidosis or paramyloidosis. In necropsies of 38 rabbits kept under the same conditions and with identical diets, but which were not given cholesterol, these lesions were not observed. Of the 7 rabbits with amyloidosis or paramyloidosis, 3 manifested infections at necropsy (1 pneumonitis, 1 subcutaneous abscess, 1 cellulitis of a paw). Two other rabbits had superficial ulcerations of the soles, attributed to the wire floor of the cages, and which are not uncommon in rabbits kept in such cages in this laboratory. Of the 11 cholesterol-fed rabbits without amyloidosis, 3 had infections (2 ear infections, 1 purulent mediastinal mass) and 3 ulcerations of the soles. Marked atheroma of the aorta and

TABLE III. Mean Arterial Pressure in Rabbits with Renal Amyloidosis and Paramyloidosis.

Rabbit No.	Control B.P. (mm Hg)	Maximum mean experimental B.P. (mm Hg)
1	85	125
5	90	135
7	80	115
15	100	150
16	85	140

other large arteries was present in all the rabbits.

The available evidence does not definitely indicate the mechanism through which cholesterol feeding in rabbits sometimes results in amyloidosis. However, a significant role is perhaps to be attributed to the hyperglobulinemia which develops. Hyperglobulinemia has repeatedly been demonstrated in various clinical and experimental circumstances in which amyloidosis occurs. The lesions in the kidneys of our animals closely resemble those produced by Sussman and Freed(2) by injection of a 10% globulin solution (rabbit antipneumococcic serum). Cholesterol feeding would appear to be another method by which amyloidosis can be produced through the intermediacy of hyperglobulinemia.

Since amyloidosis does not constantly result from hyperglobulinemia in any of the conditions studied (human and experimental tuberculosis, multiple myeloma, casein feeding, injection of bacteria or their products, etc.), other pathogenetic factors must also be involved. These are as obscure in the amyloidosis that develops in some rabbits fed cholesterol as in other forms of amyloidosis.

Of the 5 animals with amyloidosis in which blood pressure was measured, 4 had hypertension. Of 7 rabbits fed cholesterol which did not develop amyloidosis, only one had unequivocal hypertension. However, this animal had the highest mean pressure (175 mm Hg average for over 30 weeks) observed. That feeding cholesterol to rabbits results in hypertension has been both affirmed(3) and denied(4). Our data show that cholesterol feeding does result in hypertension in some rabbits. Though the findings indicate that hypertension is more apt to result from cholesterol feeding in rabbits if amyloidosis is produced, the blood presure may also become elevated in the absence of amyloid. Renal amyloidosis is thus not a necessary intermediary in the pathogenesis of hypertension produced in rabbits by feeding cholesterol. Whether the extensive atherosclerosis of the aorta and some of the other arteries present in all the animals affects the blood pressure remains to be established.

The finding of a high percentage of globulins in the proteinuria of rabbits in which amyloidosis was produced by cholesterol feeding harmonizes with the long known fact that the proportion of globulin in the urinary protein of human renal amyloidosis tends to be high.

Summary. Of 18 rabbits fed cholesterol for 12 to 104 weeks, 7 developed amyloidosis or paramyloidosis. These rabbits had hyperglobulinemia and their urinary protein contained a high percentage of globulins. Hypertension developed in 4 of 5 rabbits with amyloidosis and in 1 of 7 without such deposits.

2. Sussman, R. M., and Freed, S. Z., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 359.

3. Schmidtmann, M., *Verhandl. deutsch. path. Gesellsch.*, 1925, v20, 118.

4. Thoelldte, M., *Beitr. z. path. Anat.*, 1927, v77, 61.

Received July 5, 1951. P.S.E.B.M., 1951, v78.