

intake, introduced 9.1, 13.65, and 18.2% fibrin, fed at 10, 15, and 20% planes of protein in the rations, respectively.

It will be noted from Table II that, on the 10% blood fibrin ration, during an experimental period of 76 days, on the same 456 g food intake, a change from VBC 1 to VBC 2 produced an increase of 21.2% in the protein efficiency ratio (PER), as indicated by gains in body weight per gram of protein intake; and a change of VBC 1 to VBC 3

produced an increase of 41.4% in the PER. On the rations containing 15 and 20% blood fibrin, on the same food intake, there were pronounced increases in the PER, *i.e.*, an increase of 45.1 to 71.4%, when the VBC 1 was changed to VBC 2, but no further increases in PER when VBC 1 was further increased to VBC 3.

*Summary.* When blood fibrin (Armour) was the protein in the ration, on controlled food intake, increasing the concentration of mixtures of various components of the vitamin B complex, resulted in marked increases in the protein efficiency ratio, expressed as gains in body weight per gram of protein intake.

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### Beta-Hyperglobulinemia Produced by Cholesterol Feeding in the Rabbit.\* (18178)

A. M. FISHBERG, L. FRIEDFELD, I. HOFFMAN, E. R. STOLLER AND E. H. FISHBERG.

*From the Joseph and Helen Yeamans Levy Foundation, Beth Israel Hospital, New York City.*

Recent work(1) has focused attention on the possibility that deposition of cholesterol in atherosclerosis is correlated, not with analytically demonstrable hypercholesterolemia, but rather with the circulation of relatively large aggregates of cholesterol or cholesterol combined with protein as a lipoprotein. That clinical hypercholesterolemia is often accompanied by hyperglobulinemia, including rise in beta-globulin, has been found in diabetes, obstructive jaundice and myxedema. Dubach and Hill(2) have shown analytically that the hypercholesterolemia produced in rabbits by cholesterol feeding is accompanied by increase in serum globulin. The experiments herein reported show that the hyperglobulinemia resulting from chol-

esterol feeding in rabbits is due almost entirely to increase in beta-globulin.

*Procedure.* 28 rabbits 6 to 8 weeks old were fed a basic diet of Rockland Farms pellets for periods varying between 5 weeks and 27 months. 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. The rabbits consumed between 3 and 20 g of cholesterol per week. Total protein, albumin and globulin were determined by precipitating with 23% Na<sub>2</sub>SO<sub>4</sub> followed by micro-Kjeldahl as in(3). Electrophoretic separations were made at pH 8.56 in barbiturate buffer. Cholesterol was determined by a modification of the method of Bloor, Pelkan and Allen(4). Readings were made at wave length 420 mu. Phospholipid

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3. Hawk, P. B., Oser, B. L., Summerson, W. H., *Practical Physiological Chemistry*, Ed. XII, Philadelphia, 1947, 546.

4. Bloor, W. R., Pelkan, K. F., and Allen, D. M., *J. Biol. Chem.*, 1922, v52, 191.

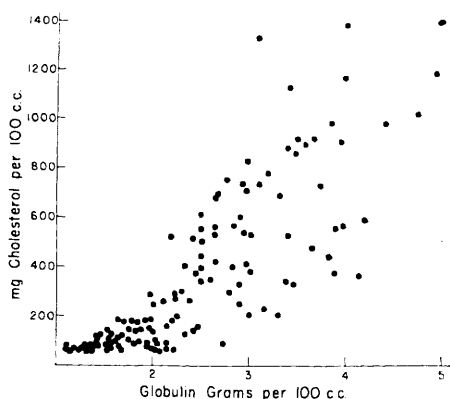


FIG. 1a

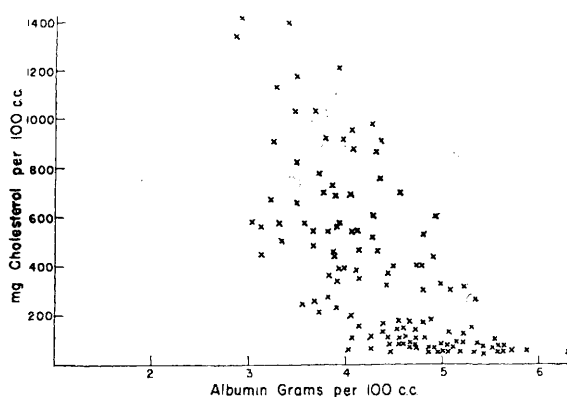


FIG. 1b

Relation of serum cholesterol level to concentration of albumin (b) and globulin (a) in 28 rabbits.

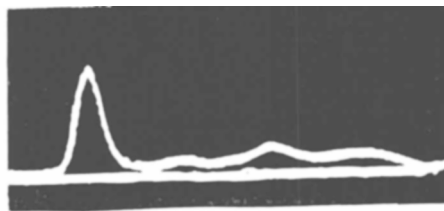
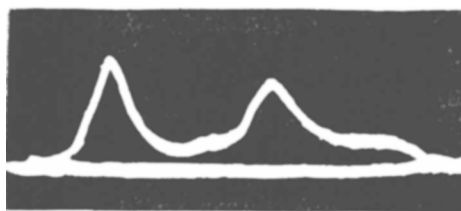


FIG. 2

Electrophoretic patterns of sera of Rabbit 573.

	1/9/50		2/28/50	
	Cholesterol 1413 mg %		Cholesterol 256 mg %	
	Serum proteins (rel. %)		Serum proteins (rel. %)	
Albumin + $\alpha_1$ -globulin	39.8		47.7	
$\alpha_2$ - "	5.7		10.8	
$\beta$ - "	40.3		22.3	
$\gamma$ - "	14.2		19.2	

was determined by the method of Youngberg and Youngberg(5).

**Results.** In Fig. 1a the cholesterol content of the serum is plotted against the globulin and in Fig. 1b against the albumin level. As cholesterol rose with continued feeding, globulin increased and albumin diminished.

The electrophoretic pattern of Rabbit 573 was typical (Fig. 2). It shows that the rise in serum globulin accompanying hypercholesterolemia is due almost entirely to beta-globulin. Jan. 9, when cholesterol feeding had elevated serum cholesterol to 1413 mg/100 ml, beta-globulin constituted 40.3% of total protein; Feb. 28, after discontinuation of cholesterol feeding had been followed by fall in serum cholesterol to 216 mg/100 ml, beta-

globulin was 22.3% of total protein.

The rise in serum cholesterol in rabbits produced by cholesterol feeding is accompanied by elevation in phospholipid (Table I). However, the proportionately greater rise in cholesterol, as has been previously observed(6), results in an augmented cholesterol/phospholipid ratio.

In 2 dogs fed 10 to 20 g of cholesterol 6 days weekly for 9 and 11 months, respectively, there were relatively slight changes in serum cholesterol, phospholipid, albumin and globulin. However, each of these slight changes was in the same direction as in the rabbit.

**Discussion.** The observations throw no light on the mechanism by which rise in serum cholesterol entails rise in beta-globulin and fall in albumin. The findings harmonize with previous suggestions that beta-globulin

5. Youngberg, G. E., and Youngberg, M. V., *J. Lab. and Clin. Med.*, 1930, v16, 158.

TABLE I. Changes in Lipid and Protein Fractions During and After Cholesterol Feeding (Rabbit #42. Lipids mg, proteins g/100 cc).

	3/3*	4/14†	5/15
Total cholesterol	91	1312	450
Free "	30	352	209
Ester "	61	960	241
Phospholipids	50	325	155
Total fatty acids	922	2383	1164
Total protein	6.05	5.76	5.55
Albumin	3.47	2.33	3.05
Alpha <sub>1</sub> -globulin	.49	.39	.20
Alpha <sub>2</sub> -globulin	.56	.52	.26
Beta-globulin	.86	1.99	1.40
Gamma-globulin	.60	.51	.59
Cholesterol/phospho-lipids	1.82	4.03	2.90
Albumin/globulin	1.3	.66	.91

\* Cholesterol feeding started.

† Cholesterol feeding discontinued.

may function as a carrier for lipids in the form of a lipoglobulin. Autopsy on the rab-

bits fed cholesterol disclosed the anticipated severe atherosclerosis. In view of the large size of the beta-globulin molecule, the rise in beta-globulin which accompanies the hypercholesterolemia in these animals, with resultant cholesterol-beta-globulin complexes, accords well with those theories(1,7) which attribute atherosclerosis to the circulation and deposition in the arterial wall of giant cholesterol-containing aggregates.

**Summary.** The hyperglobulinemia accompanying hypercholesterolemia produced by cholesterol feeding in the rabbit is predominantly due to beta-globulin.

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### Effect of *p*-Aminobenzoic Acid and Vitamin C Upon Duration of Survival of Nephrectomized Rats. (18179)

M. F. MASON, GUS CASTEN AND ALLAN LINDSAY.

*From the Parkland Hospital and the Department of Medicine, Southwestern Medical College of the University of Texas, Dallas.*

In studying the rate of acetylation of *p*-aminobenzoic acid (PABA) in nephrectomized rabbits (to be published) it was observed that these animals uniformly died 3 to 4 days following removal of the second kidney. Each had received an intraperitoneal injection of 40 to 65 ml of 5% glucose solution containing 200 to 1000 mg of PABA, neutralized to pH 7.4 with NaOH, 18 to 24 hours after the second nephrectomy (ether anesthesia). After withdrawal of a series of small blood specimens from an ear vein they were returned to cages where food (Purina rabbit chow) and water were available *ad lib*. Because methemoglobin formation occurred in an occasional animal, Vit. C in amounts from 50 to 500 mg was added to the fluid injected into another group of similarly treated rabbits, and it was at once noticed that the duration of survival was strikingly increased, ranging from 5½ to 8½ days. Even more strik-

ing was the improved condition of the animals during most of the period of survival. They were alert, active, and in most respects behaved like normal rabbits until a few hours prior to death. These apparent effects of a single dose of a combination of PABA and Vitamin C have since been confirmed by an independent observer upon a limited number of nephrectomized rabbits (Dr. E. E. Muirhead; personal communication).

Inasmuch as careful records had not been kept in connection with these casual observations it was decided to study a series of rats under more controlled conditions to determine whether any increase in survival time after nephrectomy is caused by the administration of vitamin C, alone, or whether the combination of PABA and the vitamin is required.

**Experimental.** Forty-eight male albino rats weighing from 314 to 417 g, and maintained