intimal lipid in the aorta might not be expected to produce the same result, because medial nutrition is not dependent on fluid permeation alone since *vasa vasorum* penetrate the media. It is hoped that future investigation may elucidate the roles of these factors.

Although there may be some justification for considering that the lesions which have been observed in choline-deficient rats may be related to human atheroma, further discussion is not warranted until the results of more extensive investigations are at hand. It should be of interest however to those concerned with the problem of cardiovascular disease that lesions closely related morphologically to human atheroma can be produced by a dietary deficiency in an animal, the rat, which has previously been considered refractory to aortic disease. It is possible that by following this type of investigation, knowledge of atheromatous conditions in man can be extended.

Summary. Lesions have been observed in the aortas, carotid and coronary arteries in 26 of 116 rats fed a low choline diet for periods up to 216 days. None of these animals exceeded 300 days of age at the time of sacrifice. The initial lesion consists microscopically of the deposition of stainable lipid in the endothelial cells of the intima. In later stages, a proliferation of intimal cells had taken place so that small plaques resulted. In the large vessels (aortas and carotids) the subjacent media had undergone necrosis and eventual calcification. The possible relation of these lesions to atheroma in man is discussed.

The authors are indebted to Mr. William D. Wilson and to Miss E. Berman for their assistance in preparing microsections.

1. Griffith, W. H., and Wade, N. J., *J. Biol. Chem.*, 1939, v131, 567.

2. Hartroft, W. S., and Ridout, J. H., *Am. J. Path.*, 1951, v27, 951.

3. Wilson, W., *Bull. Int. Assn. Med. Mus.*, 1950, v31, 216.

4. Sobin, S., and Landis, E. M., *Am. J. Physiol.*, 1947, v148, 557.

5. Hartroft, W. S., *Trans. Roy. Soc. Can.*, 1941, Section V, 67.

6. Hummel, K. P., and Barnes, L. L., *Am. J. Path.*, 1938, v14, 121.

7. Wilens, S. L., and Sproul, E. E., *Am. J. Path.*, 1938, v14, 177, 201.

8. Ham, A., *Histology*, 1st Ed., Montreal, J. B. Lippincott Co., 1950, p339.

9. Wilsler, R. W., Eilbert, M. L., Schroeder, M. A., and Cohen, L., *Fed. Proc.*, 1952, VII, 434 (Abstr.).

10. Bragden, J. H., and Boyle, E., *Am. J. Path.*, 1952, v28, 527 (Abstr.).

11. Wilens, S. L., *Science*, 1951, v114, 389.

12. Hartroft, W. S., *Liver Injury*, Hoffbauer, F. W., Editor, Trans. Ninth Conf., New York; Josiah Macy, Jr. Foundation, 1950, p109.

Received September 24, 1952. P.S.E.B.M., 1952, v81.

Use of Normal Rabbit Serum in Production of Hypercholesteremia in Cholesterol Fed Rats.* (19886)

MEYER FRIEDMAN, RAY H. ROSENMAN, AND SANFORD O. BYERS.

From the Harold Brunn Institute, Mount Zion Hospital, San Francisco, Calif.

Recently we have found(1) that the hypercholesteremia following the injection of Triton WR 1339, is due to an alteration in the plasma protein complex induced by the injection of this surface active substance. The hypercholesteremia in the rat following the administration of sodium cholate also has been found (2) by us to be due to an *initial* alteration in the plasma protein complex brought about in some way by cholate. In other words, the injection of either of these substances apparently allows the blood of the treated animal to retain a greater fraction of that cholesterol *normally entering and eventually leaving the blood stream.*

These findings suggested the possibility that other forms of hypercholesteremia also might

* Aided by grants from the American Heart Association, the Monterey Heart Asso. and the U. S. Public Health Service.

TABLE I. Response of Rats Plasmapheresed with Normal Rabbit Serum to High Cholesterol Feeding.

No. of rats	Avg wt (g)	Estimated rabbit serum given (cc)*	Plasma cholesterol (mg/100 cc)				
			Before plasmapheresis	After plasmapheresis: Immed.	24 hr	48 hr	144 hr
		A. Rats given rabbit serum—high cholesterol diet					
9	261 (215-300)†	5.5 (4.1-6.5)	45 (31-53)	50 (43-58)	77 (61-93)	115 (92-136)	122 (112-131)
		B. Rats given rabbit serum—cholesterol free diet					
5	258 (214-289)	5.3 (5 -5.5)	44 (40-48)	51 (41-57)	63 (50-69)	68 (60-79)	52 (46-60)
		C. Rats—high cholesterol diet					
5	266 (254-280)	0	49 (43-54)	—	59 (48-82)	64 (43-77)	61 (43-72)

* Calculated as ½ the total vol of rabbit serum given in first plasmapheresis plus total vol given in second plasmapheresis.

† Range.

be due to this mechanism rather than to some intrinsic derangement in the handling of cholesterol by any particular organ or tissue of the body. It therefore was decided to investigate the plasma mechanism underlying the hypercholesteremia occurring in the rabbit placed upon a high cholesterol diet. If the hypercholesteremia of the cholesterol fed rabbit is due *primarily* to some property of its plasma, then transfer of this plasma to a species that ordinarily is refractory to diet induced hypercholesteremia should produce hypercholesteremia in this species when combined with a high cholesterol intake. The following preliminary report indicates that when the rat is so treated, it becomes hypercholesteremic.

Methods. Normal male, Long Evans rats (approximately 12 weeks old) weighing 214-300 g were used in this study. Fourteen of these rats were anesthetized, their blood heparinized and then by means of cannulation of the inferior vena cava, they were bled as completely as possible. Usually 8.0 cc of blood could be obtained from each rat. The blood was centrifuged, the plasma discarded and replaced by an equal volume (usually 3-4 cc) of previously pooled, *normal* rabbit serum (cholesterol content: 62 mg per 100 cc). The reconstituted blood was returned to the rat and after 3 minutes, the entire procedure was repeated. Since the total plasma volume of rats of this weight was approximately 7.5 cc(3), the plasmapheresis probably replaced 66-80% of the rat's own plasma with rabbit serum. No ill effects were observed in the rats following this procedure nor 48 hours later when each rat was injected again with 3 cc of the same pooled rabbit serum. No studies were done to determine how rapidly this serum was destroyed or utilized in the rat but no more than 6% of the initial amount of serum protein was excreted in the urine during the first 24 hours. Following the plasmapheresis, 9 of the 14 rats were given 100 mg of cholesterol in 3 cc of olive oil by stomach intubation and placed thereafter upon a high cholesterol diet. This diet was a mixture of pulverized Purina laboratory chow containing an added 2% cholesterol and 5% corn oil. The remaining 5 plasmapheresed rats were placed upon a cholesterol free diet(4). Five normal, untreated rats also were placed for control purposes upon the high cholesterol diet. Blood samples were obtained before and immediately after plasmapheresis and also, in most of the rats, at the end of 24, 48 and 144 hours at which time the experiment was discontinued. These samples were analyzed for cholesterol according to a previously described method(4).

Results. As Table I indicates, rats whose own plasma had been replaced for a short time at least by *normal* rabbit serum, became hypercholesteremic when placed upon a high cholesterol diet. Their average plasma cholesterol rose from 50 to 115 mg per 100 cc (an increase of 130%) in 48 hours. The

plasma cholesterol rose even higher (122 mg per 100 cc) in those rats tested at the end of 144 hours. This increase did not appear to be due to some *non-specific* reaction of the rat to the introduction of rabbit serum because the five rats given the same serum and in equal amount but placed upon a cholesterol free diet did not have a marked rise in their plasma cholesterol either at 24, 48 or 144 hours. Likewise, the normal, untreated rats placed upon the high cholesterol diet showed only a slight rise in plasma cholesterol.

Discussion. The induction of hypercholesteremia in the rat by substituting rabbit serum for its own plasma and then feeding a high cholesterol diet of course suggests that the hypercholesteremia induced by dietary means in the rabbit itself is also mediated in part at least by a peculiarity of this animal's plasma—a peculiarity which can be transferred to another species. In other words, the plasma phenomenon in respect to cholesterol induced in other animals by Triton or cholate administration may already be an intrinsic property of rabbit plasma. If this is so, the dietary induced hypercholesteremia occurring in the rabbit is of similar mechanism to that type of hypercholesteremia set in motion by the administration of these detergents. Studies concerning this point are now in progress.

Summary. Rats subjected to a procedure which substituted normal rabbit serum for their own plasma became rapidly hypercholesteremic when placed upon a high cholesterol diet.

1. Friedman, M., and Byers, S. O., *J. Exp. Med.*, in publication.

2. Byers, S. O., Friedman, M., and Biggs, M., to be published.

3. Lippman, R. W., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 188.

4. Byers, S. O., Friedman, M., and Michaelis, F., *J. Biol. Chem.*, 1950, v184, 71.

Received September 24, 1952. P.S.E.B.M., 1952, v81.

Complement-Fixing Murine Typhus Antibodies in Vitamin Deficiency States* IV. B_{12} Deficiency. (19887)

KENNETH WERTMAN AND JOSEPH L. SARANDRIA.

From the Division of Bacteriology, Department of Biological Sciences, University of Pittsburgh.

The authors have presented data(1-3) concerning the effects of thiamine, pantothenic acid, pyridoxine, nicotinic acid, riboflavin and folic acid deficiencies upon complement-fixing antibody production by rats in response to a suspension of washed murine rickettsiae (*Rickettsia typhi*) as the antigenic stimulus. The present paper is concerned with the effects of vit. B_{12} deficiency upon the production of complement-fixing antibody under similar experimental conditions. The purpose of this investigation, and others in progress, is an attempt to explain the increased susceptibility and mortality that occurs in malnourished populations exposed to typhus fever(4) and other infections. Wertman and Sarandria(1) reported that pantothenic acid and thiamine deficiencies influenced the antibody response when a relatively small amount of immunizing material was injected into the rat. However, the influence was not as apparent when a relatively large amount of the same antigenic material was employed. In addition, they also reported(2) a severe impairment of antibody response in pyridoxine deficient rats when a rickettsial antigen (*Rickettsia typhi*) containing 0.0073 mg N was injected. Rats deficient in nicotinic acid did respond and produce complement-fixing antibodies when stimulated with the same amount of antigen. These same investigators also reported(3) that the riboflavin requirement for antibody production was not as critical as the folic acid requirement. The inanition control (paired-weighed) and the normal control animals (fed

* This investigation was supported by a research grant from the Upjohn Co., Kalamazoo, Mich.