Budy, Eds., Univ. of Chicago Press, Chicago, Ill., 1965, pp37-53.

2. Engfeld, B., Acta Endocrinol. Suppl. 6, 1950, v5.

3. Raisz, L. G., O'Brien, J. E., Am. J. Physiol., 1963, v205, 816.

4. Williams, G. A., Henderson, W. J., Bowser, E. M., Proc. Soc. Exp. Biol. and Med., 1964, v116, 650.

5. Au, W. Y. W., Raisz, L. G., Am. J. Physiol., 1965, v209, in press.

6. Toribara, T. Y., Terepka, A. R., Dewey, P. A., J. Clin. Invest., 1957, v36, 738.

7. Raisz, L. G., Hammack, D. F., Proc. Soc. Exp. Biol. and Med., 1959, v100, 411.

8. Kennedy, G. C., Brit. Med. Bull., 1957, v13, 67.

9. Iwanami, M., Osiba, S., Yamada, T., Yoshimura, H., J. Physiol., 1959, v149, 23.

Received May 3, 1965. P.S.E.B.M., 1965, v119.

Effects of Nicotinic Acid and Nicotinamide on Serum Cholesterol and Erythrocyte Nicotinamide Adenine Dinucleotide Levels of Rabbits.* (30374)

R. FONTENOT, H. REDETZKI AND R. DEUPREE (Introduced by T. Hernandez)
Departments of Pharmacology and Biostatistics, Louisiana State University School of Medicine, New Orleans

The effectiveness of nicotinic acid (NA) and the ineffectiveness of nicotinamide (NAM) in lowering elevated blood cholesterol levels in humans are well established. Species differences in the activity of these agents do however exist and evidence will be presented indicating that in the cholesterol fed rabbit NAM is more effective than NA when given in equal dosage. Concerning the mechanism of action, Altschul(1) recently suggested that the cholesterol lowering effects of NA and NAM might be associated with their activity to increase erythrocyte nicotinamide adenine dinucleotide (NAD) levels. The results of an analysis regarding the effects of these agents on erythrocyte NAD levels in rabbits will be discussed in support of this hypothesis.

Materials and methods. Forty-eight male albino rabbits (2-3 kg) were used. The animals received 120 g daily of a commercial rabbit chow either as stock diet or supplemented with 1 g of cholesterol. NAM and NA were prepared as sterile solutions containing 200 mg/ml. NA was solubilized by neutralization with 2 N NaOH. Both agents were administered intraperitoneally in single daily dosage of 200 mg/kg. Blood for analysis of serum cholesterol and erythrocyte NAD levels was drawn from the central ear vein.

Serum cholesterol determinations were based on a modified Liebermann-Burchardt reaction using the stabilized Hycel† reagent. NAD was analyzed enzymatically with crystallized alcohol dehydrogenase‡(2,3).

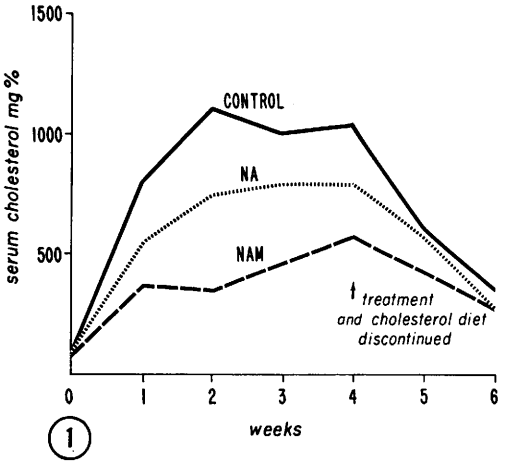
Results. Response of serum cholesterol levels to treatment with NA and NAM. Twelve rabbits were placed on the cholesterol supplemented diet. Four of these animals received intraperitoneal injections of NA administered once daily during 5 consecutive days of each week, 4 received NAM in equal dosage and injection schedule, and the remaining 4 animals served as controls. After 4 weeks, injections were discontinued and the cholesterol diet changed to normal stock diet. Blood samples for determination of serum cholesterol were taken in weekly time intervals for a period of 6 weeks. Cholesterol values of the 3 groups were compared by means of analysis of variance(4). The observed changes and the statistical evaluation of the data are presented in Fig. 1 and Table I. While both NA and NAM were found to be effective in lowering serum cholesterol levels, NAM is apparently more potent in this respect.

* This investigation was supported by USPHS Research Grant AM 09375 and Louisiana Heart Assn.

† Hycel, Inc., Houston, Texas.

‡ Boehringer Mannheim Corp., New York.

Response of erythrocyte NAD levels to treatment with NA and NAM. Thirty-six rabbits were divided into 2 groups of 18 each. One group received cholesterol supplemented diet; the other group regular stock diet. This was done in order to obtain information regarding the influence of high cholesterol diet on the NAM and NA induced NAD synthesis. Each of the 2 main groups was subdivided into 3 groups of 6 animals each. One group received NA, one NAM, and one served as control. The drugs were administered daily in equal dosage (200 mg/kg) for 6 consecutive days of each week. After 5 weeks, injections were discontinued and animals receiving cholesterol were placed on regular diet. Blood samples for analysis of erythrocyte NAD levels were obtained at the beginning and end of the 1st week and at the end of the 3rd, 5th, and 6th weeks.



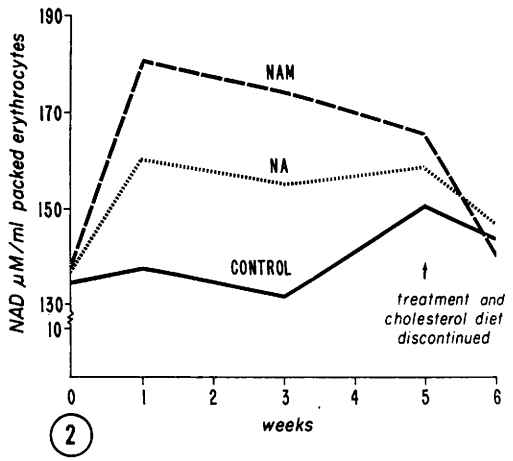


FIG. 1. Influence of NA and NAM on serum cholesterol levels of rabbits receiving a high cholesterol diet. The data points represent mean values of each group.

FIG. 2. Influence of NA and NAM on erythrocyte NAD levels. The data points represent mean values of each group.

TABLE I. Observed Values of F for Comparison Among the Means of the 3 Groups Represented in Fig. 1.*

Weeks	Control *vs* NA	Control *vs* NAM	NA *vs* NAM
1	7.40‡	21.74§	3.77
2	5.60†	37.53§	14.13§
3	2.56	17.29§	6.54†
4	1.49	4.84	.96
5	.04	1.06	.69
6	.25	.30	.00

* Since the 3 comparisons made among means observed at the same week are not all independent, control of the over-all error rate should be accomplished by making the individual comparisons at the .025 rather than the .050 level.
† $P<.050$
‡ $P<.025$
§ $P<.010$

TABLE II. Observed Values of F for Comparison Among the Means of the Three Groups represented in Fig. 2. See footnote to Table I.

Weeks	Control *vs* NA	Control *vs* NAM	NA *vs* NAM	Regular *vs* cholesterol diet
1	4.83*	22.17‡	6.30†	2.4997
3	7.01†	24.34‡	5.23*	.0001
5	.87	2.91	.60	.0082
6	.35	.54	1.84	.0005

* $P<.050$
† $P<.025$
‡ $P<.010$

The observed changes and the statistical evaluation of the data are presented in Fig. 2 and Table II. Since there was not sufficient evidence to conclude that the amount of cholesterol intake has any influence on erythrocyte NAD levels, corresponding groups were pooled for purposes of statistical analysis. While both NA and NAM were found to be effective in increasing NAD levels, NAM is apparently more potent in this respect.

Discussion. Theories about the mechanism of action of the cholesterol lowering effect of NA should include an explanation of the ineffectiveness of NAM which has been verified especially in humans by many investigators(5,6,7). The known influence of these

agents on cellular NAD levels in the liver has found consideration; but the fact that in most studied species NAM is more effective than NA in increasing NAD concentrations of this tissue speaks strongly against such an association. However, one effect of NA in humans which is not shared by NAM is the stimulation of NAD synthesis in erythrocytes (8,9). These findings and the available information of the effects of NA and NAM on erythrocyte NAD levels in different animal species(1) led Altschul to consider a regulatory influence of the erythrocytes on cholesterol metabolism. The assumption that agents which lower serum cholesterol by this mechanism effectively increase erythrocyte NAD levels finds additional support from the data presented in this study. The greater potency of NAM to lower elevated cholesterol levels in rabbits corresponds well with the observed greater effectiveness of NAM to increase erythrocyte NAD levels in this species.

Summary. The influence of NA and NAM administration on erythrocyte NAD and serum cholesterol of cholesterol fed rabbits was investigated. NAM was more effective than NA in both elevation of erythrocyte NAD concentrations and lowering of serum cholesterol. The results were discussed in support of the hypothesis of Altschul that a significant relationship exists between the effects of NA and NAM on serum cholesterol and erythrocyte NAD levels.

1. Altschul, R., Niacin in Vascular Disorders and Hyperlipemia, Charles C Thomas, Springfield, Ill., 1964, p113.

2. Holzer, H., Busch, D., Kroger, H., Hoppe Seyler Z., 1958, v313, 184.

3. Redetzki, H., Alvarez - O'Bourke, F., J. Pharmacol. and Exp. Therap., 1962 v137, 173.

4. Snedecor, G., Statistical Methods, Iowa State Press, Ames, 1956.

5. Altschul, R., Hoffer, A., Stephen, J., Arch. Biochem. Biophys., 1955, v54, 558.

6. Parsons, W., Jr., Achor, R., Berge, K., McKenzie, B., Barker, N., Proc. Staff Meetings Mayo Clinic, 1956, v31, 377.

7. Miller, O., Hamilton, J., Goldsmith, G., Circulation, 1958, v18, 489.

8. Hoagland, Ch., Ward, S., Shank, R., J. Biol. Chem., 1943, v151, 369.

9. Redetzki, H., O'Bourke, F., Ruskin, A., Fed. Proc., 1961, v20, 448.

Received May 4, 1965. P.S.E.B.M., 1965, v119.

Studies on Complement Defective Rabbits. IV. Blood Clearance of Intravenously Injected *S. typhi* by the Reticuloendothelial System.* (30375)

KLAUS ROTHER AND URSULA ROTHER (Introduced by C. A. Stetson, Jr.)
Department of Pathology, New York University School of Medicine, New York City

The clearance rate from the circulation of intravenously injected bacteria or foreign erythrocytes is greatly enhanced by specific antibodies and serum complement (C′)(1-5). There is, however, some uncertainty with respect to the participation of the individual components of C′. *In vitro* studies on the phagocytosis of erythrocytes by polymorphonuclear leukocytes suggest that C′ participates only to the intermediate reaction step of C′3(6-8). The same might be true also for the participation of C′ in immune clearance by reticuloendothelial macrophages *in vivo*.

The role of one of the subcomponents of the classical C′3 complex can conveniently be investigated in rabbits with an hereditary defect of serum complement activity. The sera of these rabbits are deficient in the activity of the classical third component of C′(9) and therefore do not lyse sensitized sheep erythrocytes (EA). It has been found recently (10) that full lytic activity can be restored to these sera by addition of a purified component of human C′, tentatively called C′6.

* This investigation was supported by Grant No. U-1412 from Health Research Council of City of New York.