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.

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

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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.

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The bactericidal activity of the serum of these rabbits was also found to be defective, when tested *in vitro* against *Salmonella typhi* 0 901. As in immune hemolysis, the full bactericidal activity could be restored by addition of the missing C′ activity(11). In the present investigation, the defective rabbits were tested for their capacity to clear *S. typhi* of the same strain from the circulation.

Materials and methods. S. typhi bacteria of the same strain (0 901)† as used in previous experiments(11) on the bactericidal activity of the complement defective sera were used. Heat-killed bacteria were labeled with I^{131} according to Biozzi *et al*(1), using 1.0 mc I^{131} for labeling 2.7×10^{10} bacteria. After 7 washings in 30 ml saline each, 4.1% = 0.04 mc were found to be fixed to the bacteria. In the injected suspension fluids, 2.7% of this latter radioactivity was attributable to unfixed I^{131}.

For sensitization of *Salmonella typhi*, 2-fold serial dilutions of rabbit anti-*S. typhi* 0 901 serum in veronal buffered (pH 7.2; 7×10^{-3} *M*) saline were set up. To 0.1 ml of the various dilutions, 5×10^7 labeled bacteria in 0.1 ml buffered saline was added and the mixture was incubated for 60 minutes at 37°C. Agglutination occurred up to a 1:640 dilution. For sensitization, *S. typhi* were then incubated under the same conditions in the relation of 5×10^7 labeled bacteria to 0.1 ml antiserum 1:1200, which is one-half the concentration required for detectable agglutination. Rabbit sera were titrated for agglutinating antibodies against *S. typhi* (Table I) as in (2).

The clearance of *S. typhi* was followed in 3 randomly chosen normal rabbits of normal C′ activity and in 3 rabbits of the defective strain (Table I). Blood samples of all animals were collected shortly before the clearance and the serum used for estimation of complement activity and for titration of agglutinating antibodies against *S. typhi* 0 901.

A suspension of 3×10^9 labeled bacteria in 2.0 ml saline was prepared and injected into a marginal ear vein. The blood dripping continuously from the marginal vein of the other ear was collected in 1.0 ml portions and the time of the last drip of each portion noted as sample time. The blood clearance was then measured in a well-type scintillation counter by the technique as given in(2). The phagocytic index $k = \frac{\log C_1 - \log C_2}{t_2 - t_1}$ was calculated from the slope of the semilogarithmic plot in Fig. 1, as were the times needed by each individual animal to clear 50% of the bacteria.

The radioactivity localized in liver, spleen and kidney of rabbit #55 after the blood was cleared of 95% of the radioactivity injected was measured by putting specimens of the various organs into receptor tubes and counting these in a scintillation counter. The total radioactivity localized in one organ then was calculated by comparing the fresh specimen weight with the fresh total organ weight.

Results. The clearance rates of unsensitized *S. typhi* in the normal rabbits were $k = 0.2690$, 0.3370 and 0.3780. The C′-defective rabbits cleared the bacteria with the same efficiency ($k = 0.2944$ and 0.2905). This rate was not influenced when sensitized *S. typhi* were injected ($k = 0.2970$) instead of unsensitized. The data on these experiments are summarized in Table I. Semilogarithmic plots of radioactivity circulating *vs* time are given in Fig. 1, each curve representing an individual rabbit.

Rabbit #55 was sacrificed 10.0 minutes after injection of *S. typhi* and the liver, spleen and kidney immediately recovered. Of the total radioactivity of 2.85×10^5 counts/min injected, 2.4×10^5 c/min = 84% were found in the liver, 1.6×10^4 c/min = 5.6% in the spleen, 1.8×10^3 c/min = 0.63% in the kidneys, 4.6% still circulating at the time the last blood sample was collected, as calculated on the basis of total blood weight equaling 1/13 of the body weight. This would leave 5.2% of the total radioactivity injected unaccounted for.

Discussion. The requirement of specific antibodies for rapid clearance of foreign cells from the blood stream by fixed macrophages of the RES has been demonstrated by various investigators (reviewed in 3), in experiments performed on rats, mice or rabbits using het-

† Courtesy of Behring-Werke, Marburg/Lahn, Germany.

TABLE I. Summary of Experimental Data and Results.

	Normal rabbits, No.			C′ defective rabbits, No.		
	55	68	102	12	81	83
Wt, g	4200	3400	3400	3900	3450	3250
C′ titer at beginning of exp (50% units)	81	48	81	<5	<5	<5
Aggl titer against *S. typhi*	<1:4	1:8	<1:4	1:32	<1:4	<1:4
No. of bacteria injected	3×10^9	3×10^9	3×10^9	3×10^9	3×10^9	3×10^9
Bacteria sensitized before injection	No	No	No	No	No	Yes
Total radioactivity at time of inj., counts/min	2.85×10^5	3.3×10^5	3.3×10^5	2.85×10^5	3.0×10^5	3.0×10^5
50% of this cleared after min	1.15	.92	.78	1.20	1.05	1.00
Phagocytic index, k =	.2690	.3370	.3780	.2944	.2905	.2970

erologous erythrocytes, *E. coli* or *S. typhi*, as the foreign cells. As shown by Benacerraf *et al*(2), there is a direct linear correlation between the amount of antibodies reacting with the bacteria and the speed of phagocytosis by the RES.

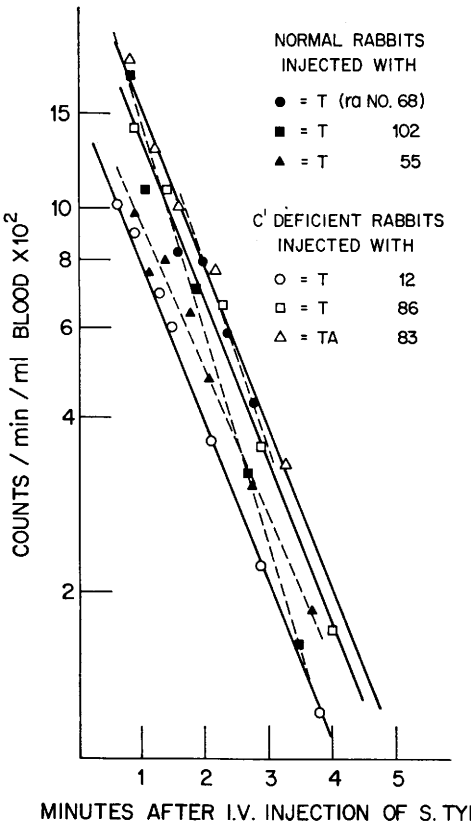

FIG. 1. Radioactivity of I^{131}-labeled *S. typhi* bacteria circulating after i.v. injection is plotted *vs* time. The speed of removal from the circulation of C'6-deficient rabbits (solid lines) equals that of normal rabbits (dotted lines). T = unsensitized *S. typhi* were injected. TA = *S. typhi* were injected after *in vitro* reaction with antibody. ra NO = rabbit no. (see Table I).

The very rapid clearance rates observed in our rabbit experiments (Fig. 1), average value for k = 0.3109, are consistent with earlier observations in this species. In experiments(2) on the clearance rate of the rabbit RES for *E. coli* under similar conditions, the average value for k was 0.300. In these experiments, the rapid disappearance of *E. coli* from the circulation was ascribed to the presence of serum antibodies at agglutination titers between 1:32 and 1:64, titrated under the same conditions as in this study. In the present investigation no attempt was made to evaluate the influence of agglutinating serum antibodies circulating in the animals (Table I), or of C′ factors other than C'6 on the clearance rate. It should also be mentioned in this respect that the agglutinin titers given in Table I do not necessarily reflect the opsonizing antibody activity of the sera(3). The one animal (#83) injected with *in vitro* pre-sensitized *S. typhi* had a clearance rate well within the range of the other rabbits.

In the presence of antibody, the clearance rate is further increased by nonspecific serum factors (opsonins), which are indistinguishable from the serum C′ system as defined in immune hemolysis(3-5). There is, however, some uncertainty as to whether all the known factors of the C′ system, especially all the subfractions of C'3, are involved(5). *In vitro*

experiments on the opsonizing effect of C′ on EA, for phagocytosis by guinea pig leukocytes, have led Nelson(6) to the conclusion that only C′1, C′4, C′2 and C′3 (the first subfactor in the sequence of the "classical" C′3 complex to react)(7), participate in opsonization. Employing a system of sheep erythrocytes, rabbit antibodies and human C′, Gerlings-Petersen and Pondman(8) similarly found a participation only of antibody, C′1, C′4, and C′2 in the *in vitro* opsonization for phagocytosis by human leukocytes, without excluding, however, the possible participation of a hydrazine sensitive subfraction of C′3, *i.e.*, the C′3a of Taylor and Leon(12).

Since there is no method for the selective *in vivo* inactivation of classical C′3 or its subcomponents, the complement defective rabbit strain is of great help in the evaluation of the role of the C′3 complex in immune clearance of *S. typhi*. The defective animals in this experiment of nature have serum which shows normal activity of C′1, C′4, C′2(9) and C′3 (= β_{1c}; unpublished). They are defective by heredity only with respect to a subcomponent of the classical C′3 complex. The missing factor is indistinguishable (10) from a component of the human C′ system tentatively designated C′6 by Nilsson and Muller-Eberhard(13). The defective sera contain only an average of 2% of the total lytic C′ activity as compared with normal rabbit serum(9). This is well below the 5% of original overall C′ activity arrived at by Spiegelberg, Miescher and Benacerraf(5) when these authors decomplemented mice by intraperitoneal injection of aggregated γ-globulin. The 95% decomplementation led to a marked and significant decrease of the normal clearance rate for *E. coli* of k = 0.046 to k = 0.013.

The identical clearance rates in 3 defective and in 3 normal animals show that the capability of the rabbit RES to clear the circulation from *S. typhi* is independent of the hemolytic activity of the overall C′3 complex (more specifically C′6).

The data on the distribution of the radioactivity after the circulation was cleared of bacteria are consistent with what has already been demonstrated in mice(4,5). At the clearance rate of k = 0.2690 in rabbit #55, 10 minutes after injection of 3×10^9 bacteria, 84% of them were found fixed in the liver.

Comparison of the results presented here with previous findings on the bactericidal serum activity of the defective strain uncovers a significant difference between the clearance and the serum bactericidal mechanisms. When tested with the same strain of bacteria (*S. typhi* 0 901), the bactericidal serum action on sensitized *S. typhi* was identical with the complement reaction in immune hemolysis, especially with respect to the requirement of the unimpaired activity of the C′3 complex(11). Even undiluted defective serum was unable to kill a detectable fraction of a population of sensitized *S. typhi* in the absence of C′3 complex activity from other sources. In immune clearance, however, the absence of an average of 98% of normal overall C′ activity due to lack of C′6 remained without influence on the quantity of bacteria removed from the circulation by the RES macrophages in a given time. Therefore, the requirement for the hemolytically active C′3 complex for the killing of *S. typhi* is a feature clearly distinguishing the immune bactericidal reaction from the blood clearance of the bacteria by RES phagocytosis.

Summary. Three rabbits with an hereditary defect of the classical third component of complement (absence of C′6) were intravenously injected with 3×10^9 heat-killed *S. typhi* 0 901 labeled with I^{131}. The rates of blood clearance by the RES in these animals were found to be identical with the rates observed in normal rabbits. Ten minutes after the intravenous injection, 84% of the bacteria were found localized in the liver. It is concluded that the activity of C′6 is without influence on the removal of *S. typhi* from the blood stream by the RES. The nonparticipation of C′6 in the clearance mechanism distinguishes this reaction from the immune bactericidal action of rabbit serum against *S. typhi* 0 901.

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1. Biozzi, G., Howard, J. G., Halpern, B. N.,

Stiffel, C., Mouton, D., Immunology, 1960, v3, 74.

2. Benacerraf, B., Sebestyen, M. M., Schlossman, S., J. Exp. Med., 1959, v110, 27.

3. Biozzi, G., Stiffel, C., in Grabar-Miescher: Mechanism of cell and tissue damage produced by immune reactions, 2nd International Symposium on Immunopathology, Brooklodge, Benno Schwabe, Basel, 1961.

4. Benacerraf, B., Miescher, P., Ann. N. Y. Acad. Sci., 1960, v88, 184.

5. Spiegelberg, H. L., Miescher, P. A., Benacerraf, B., J. Immunol., 1963, v90, 751.

6. Nelson, A. R., in Grabar-Miescher: Mechanism of cell and tissue damage produced by immune reactions, 2nd International Symposium on Immunopathology, Brooklodge, Benno Schwabe, Basel, 1961, p245.

7. Linscott, W. D., Nishioka, K., J. Exp. Med., 1963, v118, 795.

8. Gerlings-Petersen, B. T., Pondman, K. W., Vox Sang., 1962, v7, 655.

9. Rother, U., Rother, K., Z. f. Immunitatsfschg., 1961, p224.

10. Muller-Eberhard, H., Rother, K., Rother, U., in preperation.

11. Rother, K., Rother, U., Petersen, K. F., Gemsa, D., Mitze, F., J. Immunol., 1964, v93, 319.

12. Taylor, A. B., Leon, M. A., Fed. Proc., 1961, v20, 19.

13. Nilsson, U., Muller-Eberhard, H., ibid., 1964, v23, 506.

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Relationship Between Spleen Weight Increase Factor (SWIF) of Mice and *Eperythrozoon coccoides.** (30376)

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A transmissible spleen weight increase factor (SWIF) encountered during the transmission of a neoplastic disease in mice was previously described(1). The possibility was considered at that time that the SWIF could be *Eperythrozoon coccoides,* since morphological elements said to be characteristic of *E. coccoides* were, under some circumstances, observed in blood smears of mice infected with the SWIF. Under other circumstances, blood of infected mice did not disclose these elements. Therefore, some reservation was expressed concerning the identity of the SWIF with *E. coccoides.* We now present data which quantitatively relate spleen weight increase to certain of these characteristic morphological elements in the blood and, hence, to *E. coccoides.*

Materials and methods. The preparation of infectious material and the mice used were previously described. When infectious plasma was desired, blood was collected by the orbital method(2) with the aid of heparinized micro blood collecting tubes.‡ After centrifugation at 800 × *g* for 15 minutes in the cold, the plasma was collected and, unless immediately used, stored in the CO_2 deep freeze.

The average response of 5 mice at 7 days determined a single experimental point. The body weight of each mouse was determined at sacrifice, its spleen excised, and the spleen weight (mg) per gram body weight determined. The mean of the 5 values and its standard error were calculated.

In many instances the determination of

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