Failure of Cortisone to Affect Rate of Disappearance of Antibody Protein.* (18695)

EDWARD E. FISCHEL, HERBERT C. STOERK, AND MOGENS BJØRNEBOE.

From the Department of Medicine, Columbia University College of Physicians and Surgeons and the Edward Daniels Faulkner Arthritis Clinic of Presbyterian Hospital, N. Y.; Merck Institute for Therapeutic Research, Rahway, N. J.; and the Kommunehospital, Copenhagen, Denmark.

The administration of cortisone or adrenocorticotrophic hormone (ACTH) during immunization results in low levels of circulating antibody(1-5). After immunization has been established, injection of these hormones may be followed within 6 hours(1,4) to 3 days(3) by a diminution of circulating antibody. The low serum antibody levels might have resulted from an increased rate of degradation of antibody protein, from inhibition of synthesis of antibody or from both of these factors. It was the purpose of the present study to determine whether or not there is an increased rate of disappearance of passively administered antibody from the circulation of cortisone-treated animals.

Methods. Antibody globulin. A pool of 650 cc of rabbit serum contained 1,069 mg of antipneumococcus antibody nitrogen to pneumococcus types 6, 9, 11, 13, 15, 17, 22, and 23 as measured by quantitative agglutination method of Heidelberger and Kabat(8). Antibody globulin was concentrated by precipitation with half saturated sodium sulfate (6,7). The precipitate was separated, washed once with one-half saturated sodium sulfate, recentrifuged and then dissolved in distilled water to make a total volume of 160 cc. The resulting solution had a total protein concentration of 9.0% of which 8.9% was globulin (by the Howe method). Four New Zealand rabbits, 2 males and 2 females, weighing about 3,100 g each, were used. Two rabbits, A and E, a male and a female, were given cortisone†, 10 mg intramuscularly daily for 3 days preceding injection of globulin solution, and for subsequent 11 days. The other two rabbits, B and F, were maintained as untreated control animals under otherwise identical conditions. On the fourth day of cortisone administration to rabbits A and E, each of the 4 rabbits received 38 cc of concentrated antibody globulin solution intravenously. The solution was injected in 2 divided portions 10 minutes apart. Twenty minutes after the second injection, 15 cc of blood was collected from the opposite marginal ear vein for determination of antibody concentration in the serum. Subsequent bleedings of 10 cc each were made after 24 hours, 48 hours and 4 days. On the seventh day, 20 cc of blood was removed and, on the tenth day, the animals were exsanguinated. Antibody determinations were performed in duplicate by the quantitative agglutination technic of Heidelberger and Kabat(7,8).

Results. The amounts of antipneumococcal antibody nitrogen remaining at various intervals following injection of antibody solution are presented in Table I and Fig. 1. It is readily apparent that no difference exists in rate of disappearance of antibody protein in the cortisone-treated rabbits. Indeed, the

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† Cortone acetate was kindly provided for this study by Merck & Company, Rahway, N. J.

TABLE I. Concentration of Antipneumococcal Antibody after Passive Immunization in Normal and Cortisone-Treated Rabbits.

Rabbit	Treatment	Antipneumococcal Antibody Nitrogen (mg AbN/ml serum) Days 0	1	2	4	7	10	Wt change* in 2-wk period (g)
A	Cortisone—10 mg daily from	1.04	.69	.52	.44	.36	.18	−180
E	3 days before antibody admin.	.90	.65	.46	.39	.28	.15	−135
B	None	.97	.64	.46	.37	.32	.20	±0
F		1.27	.78	.57	.46	.32	.20	+165

* Rabbits weighed approximately 3100 g 3 days before antibody was injected.

striking similarity of the curves is evident.

Twenty minutes after the administration of the second half of the 38 cc of antibody solution, the rabbits had between 0.90 and 1.27 mg antibody nitrogen (AbN) per ml of serum. During the first 2 days, the rate of disappearance of antibody protein was rapid. This was thought to be due largely to the establishment of a plasma-tissue equilibrium (9). Subsequently the rate of disappearance was slower and more constant, and when the data were plotted (Fig. 1) an approximate straight line relationship was obtained. The amounts of AbN/ml of serum remaining on the fourth, seventh and tenth days are very similar in all animals.

During the 2-week period of cortisone administration, rabbits A and E, with initial weights of 3,050 and 3,125 g, lost 180 and 135 g, respectively. In the same period of time, untreated control animal B, with an initial weight of 3075 g had a transient weight gain and then returned to the initial weight. Control rabbit F, initially 3125 g, steadily gained 165 g. These changes in weight occurred apparently independently of the rate of elimination of antibody.

Discussion. The unaltered rate of disappearance of antibody globulin from circulation of cortisone-treated rabbits is evident from the similarity of the curves in Fig. 1. In addition, there is a parallel between these curves and those constructed from the data of Heidelberger *et al.*(10) and of Bjørneboe(11).

In the 8 animals studied independently in 1942(10), 1945(11) and 1950, the rate of disappearance of passively administered antibody is almost identical. From the "straight line" part of the curve in Fig. 1, after the second day, which is admittedly arbitrarily taken, it is possible to calculate the maximum half life of the passively administered antibody protein to be about 6 days for each animal of the current study. A similar rate of degradation may be calculated for antibody globulin from data of the previous studies(10,11).

Unlike passively administered antibody which only "enters into the metabolic reactions which lead to its disappearance"(10) actively formed antibody globulin was found to partake of synthetic and degradation mechanisms in a manner similar to that of other serum proteins(12). Using isotope methods, a half life of about two weeks was found for such *actively* metabolized protein. In humans, a similar half life period for gamma globulins has been found(13). The disappearance rate of passively administered gamma globulin in humans has been measured under abnormal conditions, *i.e.*, 2 cases of hypoproteinemia, one of the "familial idiopathic" variety, and one associated with a lymphoma. A half life of only 3 to 4 days was found(14).

In the current study, the lack of an ap-

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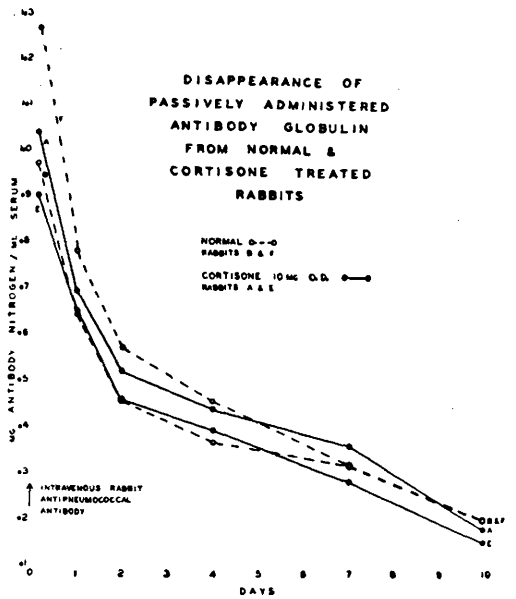

FIG. 1

preciable difference in the disappearance rates of antibody globulin in normal and cortisone-treated animals may be related to several factors. That a distinct loss of nitrogen occurs following the administration of 11-oxygenated adrenal steroids has been well documented(15). However, the mechanism which leads to the nitrogen loss is not well defined. The increased amount of urinary nitrogen may derive from dietary and other mobile sources of nitrogen, *i.e.*, due to an inhibition of the normal rate of synthesis of protein; or it may result from the accelerated breakdown of protein(16-18). The current study was designed to test catabolic rates in the absence of an anabolic effect on the antibody proteins. The results are compatible with the hypothesis that cortisone inhibits synthesis of antibody globulin, since no evidence for accelerated catabolism was found. An objection to this view may be presented. Accelerated protein catabolism may have occurred in the cortisone-treated animals without affecting the passively administered antibody. The globulin, although homologous, may have been handled as a foreign protein and not subjected to the usual catabolic mechanisms since it was not synthesized by the passively immunized rabbit. As another alternative, an inhibition of globulin synthesis may explain the finding of low antibody levels in actively immunized, cortisone-treated rabbits. In this instance, no change would be expected in the rate of disappearance of passively administered protein "tagged" by virtue of its antibody activity. Although it appears somewhat difficult to ascribe the rapid decrease of antibody in actively immunized animals treated with cortisone(1,3,4) to this mechanism, the sustained low antibody levels observed over longer periods of active immunization are probably due in large part to inhibition of globulin synthesis by cortisone (3-5). Morphological studies indicate a disturbance of tissues thought to be associated with globulin formation(3).

In addition to ACTH or cortisone, diminished amounts of circulating antibody in actively immunized animals have been observed following x-ray irradiation(19) and pyridoxine deficiency(20), conditions in which lymphoid atrophy is as pronounced as in hyperadrenalism. In experiments similar to the one reported here, but employing hemagglutinin titers, the rate of disappearance of passively administered antibody was found unaltered in pyridoxin deficient rats(21) or in rabbits treated with x-ray(22). In both studies neither homologous nor heterologous proteins were affected by the procedure employed.

Summary. 1. Four rabbits were given equal amounts of concentrated rabbit antipneumococcal globulin solution. Two of the rabbits were pretreated with cortisone and continued to receive the hormone. During the subsequent ten days, no difference was observed

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from the controls in the rate of disappearance of the "labelled" protein from the circulation. 2. Factors concerned with the interpretation of the data are discussed with reference to the effect of cortisone on protein metabolism.

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Composition of Meconium. Serological Study of Blood-Group-Specific Substances Found in Individual Meconiums.* (18696)

DOROTHY J. BUCHANAN† AND S. RAPOPORT.

From the Children's Hospital Research Foundation; Department of Biological Chemistry, College of Medicine, University of Cincinnati; and the University of Cincinnati Research Foundation, Cincinnati.

Blood group substances with the same serological activity as the red blood corpuscles, while demonstrable in various secretions of the body, can not usually be found in feces. However, Witebsky and Satoh(1) mentioned that Yosida(2) had found blood group activity in meconium. Sekimoto(3,4) showed that some samples of human meconium exhibited high activity while others showed little. He separated the infants into secretors and non-secretors and believed that transition types might also be present. Okajima(5) found that the amount of blood group substance diminished rapidly after the first few days of life. Witebsky *et al.*(6) found that a blood-group-destroying-power first appeared in the lower ileum or caecum and that its effectiveness increased in the lower part of the large intestine. Thus in adults, the colon and rectum are free of the blood group activity. It was not determined whether this destroying principle, which was assumed to be an enzyme, is formed in the body or by bacteria growing in the intestinal tract. Blood group substances have also been demonstrated in saliva. However Schiff and Sasaki(7) found that some individuals do not possess the ability to secrete blood group substances. The ability to secrete these substances is hereditary and transmitted as a simple Mendelian dominant. The finding of large amounts of blood group substances in human meconium made it of great interest to determine whether their presence in meconium corresponded with the occurrence of blood group activity in saliva. Also, in view of the conflicting evidence(8,9) concerning the correspondence of the blood group activity of amniotic fluid and the blood type of the infant, comparative studies in this direction, with simultaneous determination of the activity of meconium, were undertaken.

The present report elaborates on the preliminary serological investigations of the blood-group-specific substances found in human meconium(10), while the chemical

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† Present address, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tenn.

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