THE EFFECT OF CORTISONE AND ADRENOCORTICOTROPHIC HORMONE ON THE CONCENTRATION OF CIRCULATING ANTIBODY*

By MOGENS BJØRNEBOE, M.D., EDWARD E. FISCHEL, M.D., AND HERBERT C. STOERK, M.D.

(From the Kommunehospitalet, Third (Medical) Department, Copenhagen, Denmark; the Department of Medicine, Columbia University College of Physicians and Surgeons, and The Edward Daniels Faulkner Arthritis Clinic of The Presbyterian Hospital, New York; and the Merck Institute for Therapeutic Research, Rahway)

PLATE 1

(Received for publication, July 29, 1950)

INTRODUCTION

The elevated serum globulin occasionally associated with some diseases, for example lupus erythematosus disseminatus and rheumatoid arthritis usually falls toward normal during the administration of cortisone or adrenocorticotrophic hormone (ACTH) (1, 2). It is believed that in certain of these diseases, the increase in serum globulin may, at least in part, result from antibody production to unknown antigens (3).

Previous studies of the effect of adrenal cortical hormones or of ACTH on antibody production have yielded conflicting results. An increase in serum hemagglutinin levels in animals immunized with sheep erythrocytes and treated with Upjohn's lipo-extract (ACE) was reported by Chase, White, and Dougherty (4). The animals used were not adrenalectomized. Eisen *et al.* (5) found that the antibody production and gamma globulin of adrenalectomized rats treated with ACE were no different from those of untreated adrenalectomized animals. They found no change in titer of sheep cell agglutinins or hemolysins, and, furthermore, no change in amount of precipitin to pneumococcus polysaccharide measured quantitatively. These investigators concluded that adrenal cortical activity is not essential for the elaboration of antibodies.

An experimental procedure for the rapid production of marked hyperglobulinemia due to specific antibodies was developed by Bjørneboe using polyvalent pneumococci as antigen (6). The procedure permits quantitative immunochemical analysis of large amounts of antibody. The present report is concerned with the use of this standardized system to determine whether ACTH and cortisone exert an effect on circulating antibody globulin, as they appear to do

* Aided by grants from the Helen Hay Whitney Foundation, the Masonic Foundation for Medical Research and Human Welfare, and the King Christian X Fund.

in diseases associated with hyperglobulinemia. In addition, histological studies have been made to investigate the suggested role of plasma cells (7, 8) and constituents of lymphoid tissue in antibody production.

EXPERIMENTAL

The Effect of ACTH on Antibody Production.—

Thirteen New Zealand red rabbits weighing about 2 kg. each were immunized with polyvalent pneumococcal vaccine.[1] The vaccine was a suspension of formalin-killed pneumococci, 10^9 per cc., of types 6, 9, 11, 13, 15, 17, 22, 23 and was washed twice by centrifugation before use. The animals were given an initial intravenous injection of 2 cc. and thereafter, 4 cc. of vaccine intravenously approximately every other day.

Five of the animals (Group P) were injected intramuscularly every 8 hours with 2 mg. of an ACTH preparation[2] equivalent in activity to between 0.5 mg. to 1.0 mg. of the Armour standard. The total daily dose was therefore equivalent to about 1.5 to 3.0 mg. of the Armour standard per rabbit per day. ACTH treatment was begun on the day before immunization was started and was continued for 4 weeks. During the 3rd week of immunization, eosinophile counts made by the Dunger method as described by Thorn *et al.* (9) showed that the ACTH-treated Group P had about one-third of the number of eosinophiles[3] present in the control rabbits of group K.

The animals were bled the day before immunization was started and again after 14 and 28 days, allowing 3 or 4 days to elapse after the last dose of vaccine. One of the control animals died with weight loss and diarrhea on the 12th day and another died of a traumatic hemorrhage on the 14th day. Antibody was measured as specific antibody nitrogen by the quantitative agglutination method of Heidelberger and Kabat (10, 11). Each serum sample was analyzed in duplicate.

The concentration of antibody nitrogen present in the serum after 14 and 28 days of immunization is listed in Table I. It is apparent that there is generally less antibody in the animals treated with ACTH than in the control group. After 14 days, only one of the ACTH-treated rabbits of Group P produced amounts of antibody comparable to that of the control animals. By the 28th day, more overlapping of treated and control values occurred. The mean amount of antibody in the ACTH Group P was approximately half of that of the control Group K at both the 14th and 28th days. Although the number of animals was small, the differences were sufficiently marked to warrant further investigation.

The succeeding experiments were performed to determine the validity of the above results and to study the effect of cortisone on the amount of circulating antibody. The cortisone was given concurrently with immunization in one experiment, as in the above studies with ACTH. In addition, as a sequel to this procedure, a number of the untreated control animals were given cortisone after

[1] The vaccine was kindly made available by Dr. F. Kauffman, The State Serum Institute, Copenhagen.

[2] The adrenocorticotrophic hormone was kindly furnished by Dr. Edwin E. Hays, Director of Biochemical Research, Armour and Company, Chicago.

[3] We are grateful to Dr. Charles M. Plotz for help in the eosinophile determinations.

immunization was under way to determine the effect of the hormone on preformed antibody. The course of immunization was then continued in these animals, as well as in the remainder of the untreated control group.

The Effect of Cortisone on the Amount of Antibody When the Hormone Is Administered Early in Immunization.—

Seventy New Zealand white and chinchilla rabbits weighing about 2 kg. were immunized with pneumococcal vaccine as described above. The animals were distributed into five groups proportionately according to sex, weight, and strain. Groups A, B, and C were simultaneously immunized, and at a later date Groups E and F were simultaneously immunized. Groups A and C, 28 rabbits, served as untreated controls for the 12 animals of the same strain (New Zealand white) in Group B. The Group A animals received a daily injection of 10 mg. of cortisone acetate[4] intramuscularly for 2 days before, and throughout immunization.

TABLE I

The Effect of ACTH on the Concentration of Circulating Antibody When the Hormone Is Administered from the Onset of Immunization with Pneumococci

Mg. antibody nitrogen per ml. of serum at 14 and at 28 days after beginning immunization with pneumococci.

After 14 days		After 28 days	
ACTH Group P	Control Group K	ACTH Group P	Control Group K
mg. AbN/ml.	*mg. AbN./ml.*	*mg. AbN/ml.*	*mg. AbN/ml.*
0.50	0.98	0.67	1.41
0.54	1.47	1.01	1.90
0.62	1.49	1.22	2.30
0.81	2.02	1.67	—
2.34	2.22	3.34	5.31
	2.30		4.72
	2.50		6.23
Mean....0.96	1.85	1.58	3.65

Five New Zealand red rabbits treated with ACTH, approximately 0.5 to 1.0 mg. Armour standard, every 8 hours for the entire period. 7 control rabbits of same strain.

Subsequently this procedure was repeated using two groups of chinchilla rabbits and a smaller dosage of cortisone. 15 rabbits of Group E were treated with a daily injection of 2.5 mg. of cortisone intramuscularly for 17 days. After the first 3 days, immunization was started in these animals and in the 15 animals of untreated control Group F.

As had been previously observed (6), the frequent injection of large amounts of pneumococcus vaccine is toxic and results in an appreciable mortality. 5 of 48 animals receiving the vaccine without ACTH or cortisone (Groups K, A, C,

[4] The cortisone was obtained from Merck and Company on recommendation of the Committee on Cortisone Research of the National Academy of Sciences with funds allocated by the United States Public Health Service.

and F) died in the first 2 weeks. The mortality was appreciably increased in the 34 animals which received the hormones from the beginning of immunization (Groups P, B, and E). 10 of these animals died. Half of the dead were of Group B and received the larger dosages of cortisone (10 mg. daily). However, after immunity was established, as in the animals of Groups A and F, the subsequent administration of cortisone did not appear to affect the mortality rate. Of 24 animals in these two groups, only 5 died during the 3rd and 4th weeks. There were 8 deaths among 24 animals of Groups C and E, which received no hormone during the same period of immunization.

Weight changes had no correlation with the amounts of circulating antibody. Generally the animals receiving vaccine, both untreated and hormone-treated, did not gain as much weight as did a group of unvaccinated animals of similar initial weight. Indeed, a marked loss of weight occurred in some of the immunized animals, with no apparent relationship to the subsequent levels of serum antibody nitrogen.

The results of quantitative agglutination analysis of the circulating antipneumococcal antibody in these animals is presented in Table II. The average antibody nitrogen content per milliliter of serum (AbN/ml.) of 27 rabbits of control Groups A and C on the 9th and 14th days of immunization is 0.27 and 1.47 mg., respectively. This is in excess of the average amount of antibody nitrogen per milliliter of serum of the animals of Group B given 10 mg. cortisone daily, *viz.*, 0.12 and 0.55 mg. AbN/ml. on the 9th and 14th days, respectively. Table II shows a similar, but less marked, diminution of serum antibody content in the Group E animals, given one-fourth of the amount of cortisone that the Group B animals received. On the 14th day of immunization, the 10 animals of Group E had an average of 0.81 mg. AbN/ml. serum as compared with the average of 1.17 mg. AbN/ml. serum of the 11 untreated animals of Group F immunized simultaneously. These findings are not as striking as the difference found with 10 mg. of cortisone or with 1.5 to 3.0 mg. of ACTH daily, but are consistent with the previous results.

Immunization was continued in 3 animals of Group E after cortisone was discontinued on the 14th day. 6 days later, 2 animals had appreciable increases in antibody content from 1.43 to 2.07, and 1.57 to 2.55, respectively. The 3rd animal had a slight decrease in antibody content from 2.06 to 1.88 mg. AbN/ml. In this short period of time, it appears that the depressing effect of cortisone on antibody content of serum was reversed in 2 of the 3 animals when the cortisone was discontinued.

The Effect of Cortisone on the Amount of Antibody When the Hormone Is Administered 2 Weeks after the Onset of Immunization.—

Most of the untreated control animals of the previous experiment were used for this study. After 14 days, when antibody production had been well established in the animals of Groups

A and C, 11 rabbits of Group A were given a daily injection of 5 mg. of cortisone intramuscularly for 4 days and then 2.5 mg. for 3 days. Immunization of these animals was continued, simultaneously with continued immunization of 12 of the untreated animals of Group C.

TABLE II

The Effect of Cortisone on the Concentration of Circulating Antibody When the Hormone Is Administered from the Onset of Immunization with Pneumococci

Mg. antibody nitrogen per ml. of serum

10 mg. cortisone daily			2.5 mg. cortisone daily	
Rabbit	9th day	14th day	Rabbit	14th day
	mg. AbN/ml.	*mg. AbN/ml.*		*mg. AbN/ml.*
B2	0.06	0.46	E24	0.49
B6	0.06	0.48	E8	0.62
B12	0.09		E29	0.67
B9	0.10	0.48	E20	0.70
B1	0.13		E28	0.80
B7	0.18	0.77	E26	0.85
B4	0.21		E23	0.85
			E5	0.85
			E27	1.06
			E7	1.19
Mean.....	0.12	0.55		0.81
Mean ±S.E. of groups without cortisone.....	0.27* ± .02	1.47* ± .13		1.17‡ ± .06

* 0.27 and 1.47 are the average AbN/ml. on the 9th and 14th days respectively of 27 rabbits of Groups A and C immunized simultaneously with the B group animals. Individual titers of these controls on the 9th and 14th days are as follows: 0.13, 0.49; 0.28, 0.54; 0.19, 0.60; 0.21, 0.61; 0.07, 0.69; 0.31, 0.75; 0.22, 0.99; 0.32, 1.12; 0.23, 1.15; 0.19, 1.20; 0.16, 1.26; 0.36, 1.33; 0.28, 1.34; 0.27, 1.54; 0.40, 1.54; 0.22, 1.57; 0.37, 1.61; 0.12, 1.68; 0.35, 1.68; 0.22, 1.95; 0.17, 2.16; 0.16, 2.23; 0.27, 2.34; 0.52, 2.40; 0.43, 2.52; 0.31, 3.05; 0.47 (9th day only).

‡ 1.17 is the average antibody nitrogen level per ml. on the 14th day of 11 untreated control animals of Group F immunized simultaneously with the E group animals. These controls had the following titers: 0.41; 0.52; 0.71; 0.85; 1.04; 1.11; 1.19; 1.34; 1.46; 2.04; 2.18.

On the 16th or 17th day, 2 or 3 days after cortisone administration was begun, 6 of the 10 animals of the cortisone-treated Group A showed an absolute decrease in the amount of antibody, while none of the Group C animals showed a decrease at that time. Text-fig. 1 illustrates the course of the individual antibody titers in these animals. The mean value for each group is indicated by the dotted line. At the end of the 7th day of hormone administration (21st day of immunization), the animals of untreated control Group C showed an increase in the average antibody content from 1.34 mg. AbN/ml. on the 14th day to 2.17 mg.

AbN/ml. on the 21st day, *i.e.*, an increase to 162 per cent of the average level on the 14th day. In contrast, the cortisone-treated animals of Group A showed an absolute decrease in the average titer, from 1.79 mg. AbN/ml. on the 14th day to 1.58 mg. AbN/ml. on the 21st day, a decrease to 88 per cent of the 14th day level (Table III).

In a subsequent experiment, this effect was again demonstrated.

Eight animals of Group F were given daily injections of 5 mg. of cortisone intramuscularly after the 14th day of immunization, and in this instance it was continued for 14 days instead of 7. The results for individual animals are shown graphically in Text-fig. 2, and the averages for the group are tabulated in Table III.

TABLE III

Effect of Cortisone on Antibody Concentration of Serum after Antibody Formation Is Established

Averages of antipneumococcal antibody nitrogen per ml. serum.

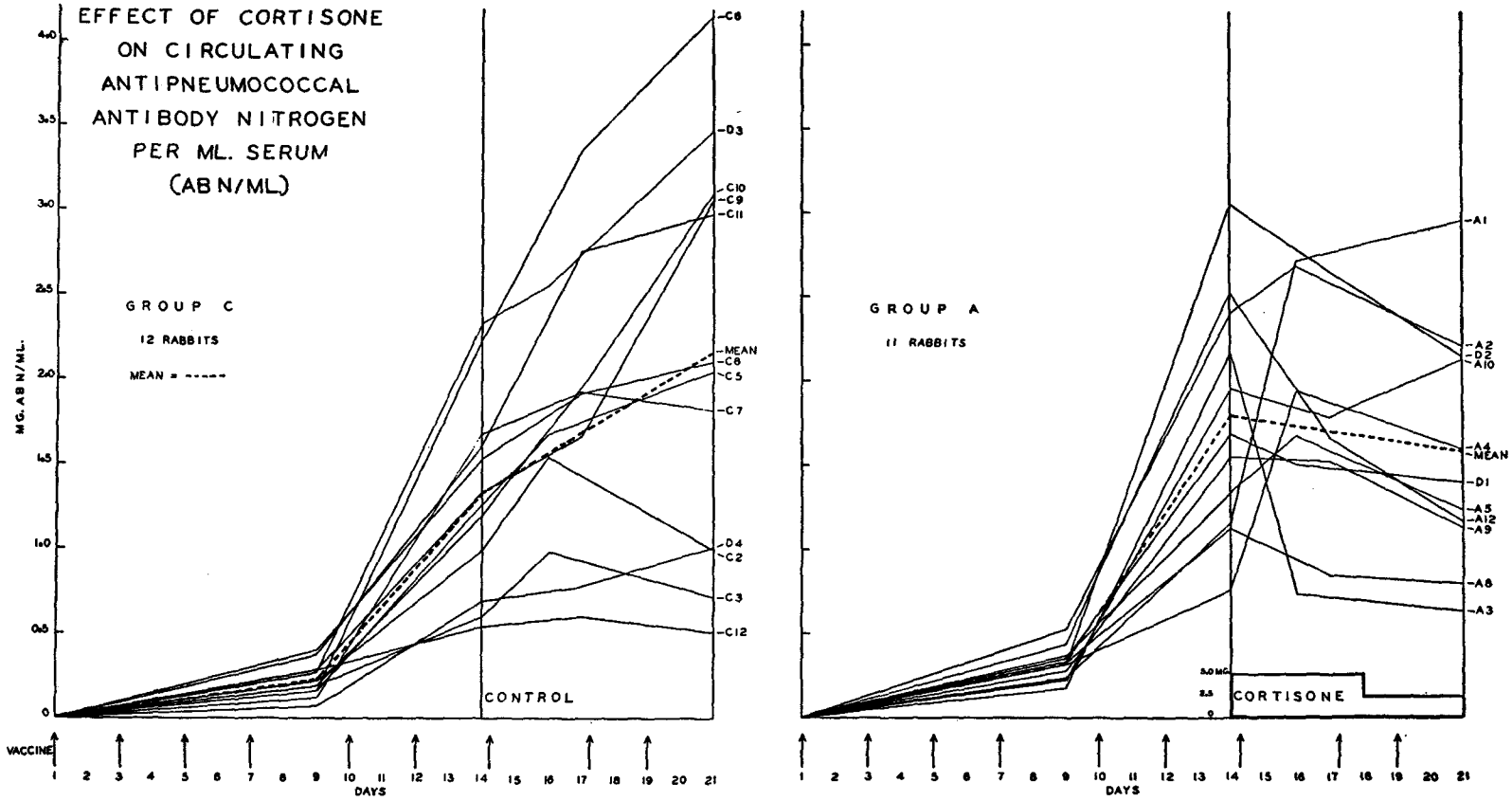
Continued immunization with polyvalent pneumococci. Beginning on the 14th day of immunization, Groups A and F received cortisone acetate intramuscularly.

	No. rabbits	Daily cortisone dosage from 14th day	14th day	Final determination			
				21st day	28th day	Δ	% of level on 14th day
			mg. AbN/ml.	*mg. AbN/ml.*	*mg. AbN/ml.*	*mg. AbN/ml.*	
Group C	12	None	1.34	2.17		+0.83	162
Group A	11	5 mg. for 4 days, then 2.5 mg. for 3 days	1.79	1.58		−0.21	88
Group K*	7	None	1.85		3.65	+1.80	197
Group F	8	5 mg. for 14 days	1.17	1.22‡	0.70	−0.47	60

* Control group of Table I, not immunized simultaneously with Group F.

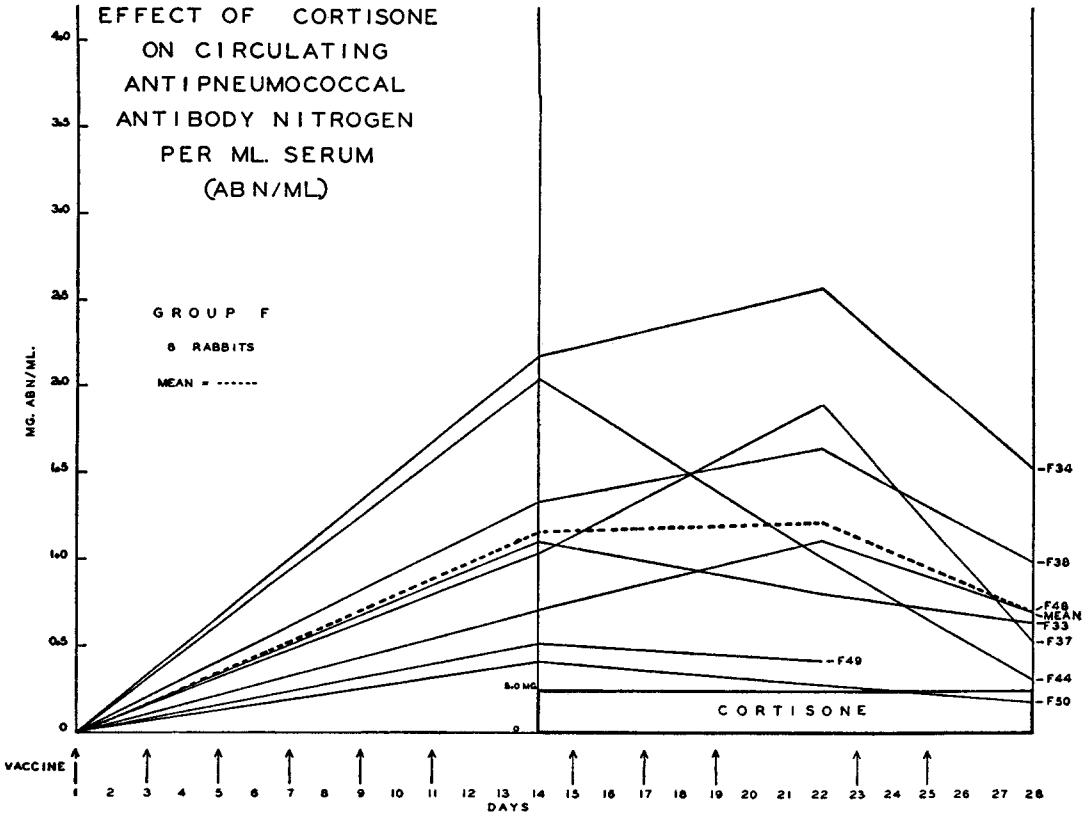
‡ Average amount on 22nd day.

In previous work (6), in control Group C (Text-fig. 1 and Table III) and in the control animals of Group K (Table III), continued immunization for a week or two after the 14th day was found to result in an increased antibody concentration during the 3rd or 4th week. A maintenance at a plateau level subsequently occurred as long as immunization with vaccine was continued (6). The animals of Group K exhibited an increase of the mean titer from 1.85 mg. AbN/ml. on the 14th day to 3.65 mg. on the 28th day, a change of 197 per cent. In the animals of Group F charted in Text-fig. 2 and summarized in Table III, cortisone administration from the 14th day resulted in a marked leveling off at the mean titer of the 14th day. Following this, an absolute decrease occurred. After 14 days of immunization, the mean AbN/ml. was 1.17 mg. On the 22nd day of immunization, after 8 days of cortisone, the average content was about the same, 1.22 mg. AbN/ml. 6 days later, however, after 14 days of cortisone


EFFECT OF CORTISONE
ON CIRCULATING
ANTIPNEUMOCOCCAL
ANTIBODY NITROGEN
PER ML. SERUM
(AB N/ML)
GROUP C
12 RABBITS
MEAN = -----
MG. AB N/ML.
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
C6
D3
C10
C9
C11
MEAN
C8
C5
C7
D4
C2
C3
C12
CONTROL
VACCINE
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
DAYS
GROUP A
11 RABBITS
A1
A2
D2
A10
A4
MEAN
D1
A5
A12
A9
A6
A3
5.0 MG.
2.5
0
CORTISONE
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
DAYS

TEXT-FIG. 1.

and with continued immunization, the average of the 7 surviving animals had dropped appreciably to 0.70, a decrease of .47 mg. AbN/ml., or 60 per cent of the amount at the beginning of cortisone administration.



TEXT-FIG. 2.

Histopathology.—Changes characteristic of prolonged immunization were present in all rabbits immunized but not treated with ACTH or cortisone. These changes were authenticated by comparison with a group of 10 non-immunized apparently normal rabbits of the same strain and weight. Splenic enlargement was evident and enlargement of the lymph nodes was suggested from the weight of these organs. Thymic weight appeared slightly reduced. In accord with previous observers, the increase in splenic weight appeared to be explained predominantly by an augmentation in the number and size of large mononuclear cells throughout the pulp. The Malpighian bodies were large and exhibited strikingly enlarged germinal centers containing numerous mitotic figures (Figs.

1 and 2). The red pulp was very cellular, containing dense infiltrations of mononuclear cells. Among these cells, in most animals there were extensive accumulations of plasma cells with basophilic oval or polygonal cytoplasm, eccentric nuclei, and juxtanuclear vacuoles. Accumulations of plasma cells were seen almost exclusively outside the follicles. In sections of the mesenteric lymph nodes, the follicles also showed prominent germinal centers. Large mononuclear cells appeared increased within the medullary tissue. Cortical tissue in the thymus of immunized animals was decreased, with a relative increase of medullary tissue. No actual counts were made of Hassall bodies but their number appeared increased in many of the immunized rabbits. This change has been described as another reaction to foreign protein by Hammar (12). Other lymphoid tissues were not examined.

In the immunized animals treated with ACTH and cortisone, moderate to marked atrophy of the Malpighian bodies were frequently evident (Fig. 3). Fragmentation of lymphocytes in the follicles was seen in many instances, but was extensive in only 2 rabbits. In addition, cortisone appeared to inhibit or reverse most of the alterations associated with repeated injections of antigenic material. This was strikingly evident from the absence of hyperplasia of the germinal centers in the follicles. Furthermore, cellular infiltration of Billroth's cords and plasma cell accumulations were usually not pronounced in the cortisone-treated groups.

DISCUSSION

These studies show that a reduction in circulating antibody and in lymphoid tissue occurs with the administration of cortisone or ACTH. The literature contains many conflicting discussions about the interrelationships of antibody formation, the reticulo-endothelial system, lymphoid tissue, and the adrenal cortex (13, 14). With immunization, several morphological changes are known to occur, most of which were observed in this study.

Initially there is phagocytosis of particulate or soluble antigen by cells of the reticulo-endothelial system (15). Intense immunization is also known to be followed, at a later period, by enlargement of the spleen and lymph nodes. Antibody has been found in extracts of the lymph node draining the region injected with antigen prior to its appearance in other locations (16). At present it appears impossible to determine directly the cell type in lymphoid tissue which contains appreciable quantities of antibody. Discrepancies in the identification of various cells composing lymphoid tissue, and the difficulty of comparing antibody concentrations in extracts of different tissues or fluids contribute to this problem. Microscopically, the reaction to foreign proteins is strikingly manifested by enlargement of the germinal centers of lymph follicles (17), and the appearance of large, apparently immature, mononuclear cells outside the follicle in the medulla of lymph nodes and in the red pulp of the spleen.

Tissue lymphocytes outside of the lympho-epithelial organs thymus and tonsil,

are abundantly mixed with cells belonging to the reticulo-endothelial system. In the lympho-epithelial organs, histiocytes are extremely scarce. It appears therefore that properties suspected to be inherent in lymphocytes or in reticulo-endoethelial cells, may better be ascribed to the latter cells when they are found in lymphoid tissue but not in thymic or tonsillar tissue. Lymph nodes and spleen were found to contain measurable amounts of antibody and gamma globulins (18, 19), in contrast to the absence of these from thymus and tonsillar tissue (19–21). Harris *et al.* (33) failed to find a relative increase of gamma globulin in extracts of sensitized popliteal lymph nodes. However, the disproportionate increase in proteins with greater electrophoretic motility probably resulted from the acute inflammatory reaction, since the sensitizing antigen was injected only 5 days previously. Although the true lymphocyte does not appear to contain antibody or gamma globulin, a type of large mononuclear cell, which proliferates during immunization has been identified by Rich as belonging to the lymphocytic series (22). During immunization, the appearance of increased numbers of plasma cells is also evident, both in lymphoid tissue and at extra-lymphoid sites such as the renal peripelvic fat (23). These cells are thought to derive from the reticulo-endothelial system and were found to contain antibody (8, 23).

In the present study, a decrease of antibody nitrogen in the cortisone-treated animals was associated with a diminution of all types of mononuclear round cells, including plasma cells.

The relationship of lymphoid tissue to immunization is emphasized by the coincidence of atrophy of lymphoid tissue and depression of antibody formation. Other experimental procedures result in a destruction of atrophy of lymphoid tissue, and at the same time inhibit antibody production. Of these most prominent are the administration of x-ray (24), and nitrogen mustards (25), and pyridoxine deficiency (26). In some of these instances, other tissues and cells may also be affected, but lymphoid tissue appears to be more susceptible to early and severe damage under all of these conditions.

The atrophy of lymphoid tissue by adrenal cortical extract is well known (12, 32). It was reported that the dissolution of lymphoid tissue by a single injection of ACTH or adrenal cortical extract resulted in a marked increase in serum antibody (27). This was thought to be an explanation of the anamnestic response. When quantitative immunochemical techniques are employed, however, no appreciable increase in serum antibody is found after a single injection of ACE, ACTH, or cortisone despite a well documented diminution in lymphoid tissue and lymphocytes (5, 28, 19). Indeed, a decrease in the amount of circulating antibody has been reported (28, 19). In addition, a normal anamnestic response was stimulated by homologous antigen in adrenalectomized animals (29).

Adrenal cortical extract when administered repeatedly during the period of active immunization has been reported to augment antibody production, as measured by hemagglutination titers (4). This finding contrasts with that reported here, based on the use of a reproducible chemical method for the determination of antibody. In addition, adrenalectomized animals produce appreci-

able amounts of antibody, comparable to those produced by animals similarly treated and given replacement therapy with adrenal cortical extract (5).

The diminution in antibody content of the serum observed in the ACTH and cortisone-treated animals may be the result of the negative nitrogen equilibrium which results from the administration of cortisone (30). The marked decrease in antibody noted (Text-fig. 1) in some animals after only 2 or 3 days of cortisone administration, and in previous experience after only several hours of ACTH or cortisone administration (28, 19), suggests an actual destruction of protein. An inhibition of synthesis of antibody would probably require more time to become as markedly manifest, since the half-life of antibody protein has been shown to be about 2 weeks (31). The reduction of circulating antibody by ACTH and cortisone may have significance clinically in bacterial and allergic diseases. However, it appears unlikely that the striking clinical effects noted with these hormones are ascribable to this action alone.

SUMMARY

The administration of adrenocorticotrophic hormone and of cortisone was found to result in a reduction in the concentration of antipneumococcal antibody in the circulation of rabbits. This reduction occurred both when the hormones were administered at the beginning of immunization and after immunization was well advanced. Marked atrophic changes in lymphoid tissue and a diminution in the number of various types of mononuclear cells followed upon the hormone administration. The possible bearing of these observations on theories concerning the sites of antibody production is discussed.

BIBLIOGRAPHY

1. Hench, P. S., Kendall, E. C., Slocumb, C. H., and Polley, H. F., *Proc. Staff Meet. Mayo Clin.*, 1949, **24,** 181.
2. Ragan, C., Grokoest, A. W., and Boots, R. H., *Am. J. Med.*, 1949, **7,** 741.
3. Fischel, E. E., *Am. J. Med.*, 1949, **7,** 772.
4. Chase, J. H., White, A., and Dougherty, T. F., *J. Immunol.*, 1946, **52,** 101.
5. Eisen, H. N., Mayer, M. M., Moore, D. H., Tarr, R., and Stoerk, H. C., *Proc. Soc. Exp. Biol. and Med.*, 1947, **65,** 301.
6. Bjørneboe, M., *Acta path. et microbiol. Scand.*, 1943, **20,** 221.
7. Bjørneboe, M., and Gormsen, H., *Acta path. et microbiol. Scand.*, 1943, **20,** 649.
8. Fagraeus, A., *J. Immunol.*, 1948, **58,** 1.
9. Thorn, G. W., Forsham, P. H., Prunty, F. T. G., and Hills, A. G., *J. Am. Med. Assn.*, 1948, **137,** 1005.
10. Heidelberger, M., and Kabat, E. A., *J. Exp. Med.*, 1934, **60,** 643.
11. Kabat, E. A., and Mayer, M. M., Experimental Immunochemistry, Springfield, Illinois, Charles C. Thomas, 1949.
12. Hammar, J. A., Die normal-morphologische Thymusforschung, Leipzig, J. A. Barth, 1936.

13. Burnet, F. M. The Production of Antibodies, Melbourne, The Macmillan Co., 1949.
14. Taliaferro, W. H., *Ann. Rev. Microbiol.*, 1949, **3**, 159.
15. Sabin, F. R., *J. Exp. Med.*, 1939, **70**, 67.
16. McMaster, P. D., and Hudack, S. S., *J. Exp. Med.*, 1935, **61**, 783.
17. Hellman, T., *in* Handbuch der mikroskopischen Anatomie des Menchen, (W. Moellendorff, editor), Berlin, Julius Springer, 1930, **6**, 233.
18. Kass, E. H., *Science*, 1945, **101**, 337.
19. Stoerk, H. C., and Solotorovsky, M., *Am. J. Path.*, in press.
20. Abrams, A., and Cohen, P. P., *J. Biol. Chem.*, 1949, **177**, 439.
21. Harris, T. N., Rhoads, J., and Stokes, J., Jr., *J. Immunol.*, 1948, **58**, 27.
22. Rich, A. R., Lewis, M. R., and Wintrobe, M. M., *Bull. Johns Hopkins Hosp.*, 1939, **65**, 311.
23. Bjørneboe, M., Gormsen, H., and Lundquist, F., *J. Immunol.*, 1947, **55**, 121.
24. Wilson, G. W., and Miles, A. A., *in* Topley and Wilson's Principles of Bacteriology and Immunity, Baltimore, The Williams and Wilkins Co., 1946, 1104.
25. Philips, F. S., Hopkins, F. H., and Freeman, M. L. H., *J. Immunol.*, 1947, **55**, 289.
26. Stoerk, H. C., Eisen, H. N., and John, H. M., *J. Exp. Med.*, 1947, **85**, 365.
27. Dougherty, T. F., Chase, J. H., and White, A., *Proc. Soc. Exp. Biol. and Med.*, 1945, **58**, 135.
28. Fischel, E. E., LeMay, M., and Kabat, E. A., *J. Immunol.*, 1949, **61**, 89.
29. Stoerk, H. C., *Fed. Proc.* 1948, **7**, 281.
30. Long, C. N. H., Katzin, B., and Fry, E. G., *Endocrinology*, 1940, **26**, 309.
31. Schoenheimer, R., Ratner, S., Rittenberg, D., and Heidelberger, M., *J. Biol. Chem.*, 1942, **144**, 545.
32. Wells, B. B., and Kendall, E. G., *Proc. Staff Meet. Mayo Clin.*, 1940, **15**, 324.
33. Harris, T. N., Moore, D. H., and Farber, M., *J. Biol. Chem.*, 1949, **179**, 369.

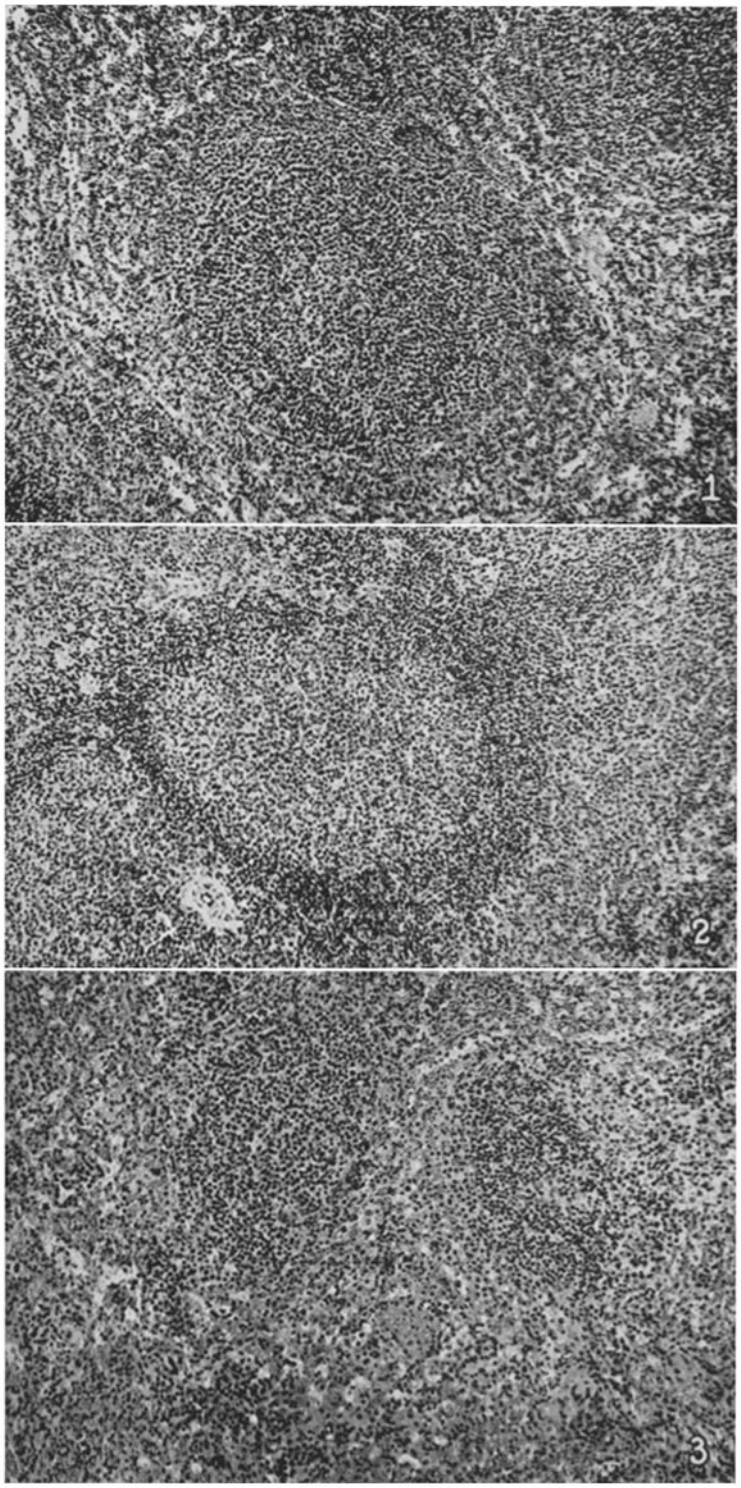
EXPLANATION OF PLATE 1

Sections of lymph follicles from the spleens of rabbits. × 150.

FIG. 1. Unimmunized control.

FIG. 2. Immunized.

FIG. 3. Immunized and treated with cortisone.



(Bjørneboe *et al.*: Cortisone and adrenocorticotrophic hormone)