penia in Dogs 2 and 5 nor did it prevent the appearance of nor modify the severity of purpuric manifestations in Dog 5. In Dog 1 in which there was a fatal outcome, little if any beneficial effect upon the marrow was noted from the use of alpha-tocopherol; while the favorable course of Dog 2 may well have been due to the early discontinuance of diethylstilbestrol. In no instance did alpha-tocopherol tend to delay the appearance of the hemorrhagic tendency nor accelerate the return to normal above that noted in Dogs 3 and 4.

Dogs receiving alpha-tocopherol and diethylstilbestrol showed changes in the erythrocyte count, leukocyte count, hemoglobin values, and hematocrit determinations similar to those receiving diethylstilbestrol alone. Leukocytoses similar to that observed in Dog 4 have been described previously by Castrodale.[1] In the 2 dogs in which death occurred (Dogs 1 and 6) postmortem examinations were done within 12-18 hours and both showed evidence of severe anemia, numerous hemorrhages throughout the body, and liver necrosis which was extensive in Dog 6. There was a marked hypoplasia of all elements of the bone marrow in Dog 1. Rib biopsies performed at the start of the investigations on Dogs 4, 5, and 6 showed normal marrow. Marrow studies postmortem or by biopsy at the conclusion of the studies showed: a markedly hypoplastic marrow in one (Dog 1), hyperplastic marrow in 2 (Dogs 3 and 4), and normal marrow in 3 (Dogs 2, 5, and 6). The type of marrow found appeared to have no significant relationship to the dosage of diethylstilbestrol nor to the administration or nonadministration of alpha-tocopherol.

Summary. Diethylstilbestrol caused a prolongation of the bleeding and coagulation time in dogs, thrombopenia, an increase in the capillary fragility, leukocytosis and a gradual anemia. In 2 dogs death resulted.

The changes in the circulating blood and bone marrow produced by diethylstilbestrol appear to be temporary in some instances. The return to normal, despite the continued administration of the drug, might indicate that a tolerance to diethylstilbestrol had been acquired. This return to normal was as prompt and as great without as with the administration of alpha-tocopherol.

The present studies failed to confirm the report of Skelton, Shute, *et al.* that alpha-tocopherol exerts an antipurpuric action on the hemorrhagic diathesis induced in dogs by diethylstilbestrol.

We are indebted to Hoffmann-LaRoche, Inc., for supplying the ephynal acetate and to Eli Lilly & Co. for supplying the diethylstilbestrol.

15942

Failure of Adrenal Cortical Activity to Influence Circulating Antibodies and Gamma Globulin.*

HERMAN N. EISEN,† MANFRED M. MAYER,‡ DAN H. MOORE, ROSE-RUTH TARR, AND HERBERT C. STOERK.§

From the Departments of Pathology and Medicine and the Electrophoresis Laboratory, College of Physicians and Surgeons, Columbia University, New York City.

Studies attempting to demonstrate a relationship between adrenal cortical activity and circulating antibodies have yielded con-

* The expense of this study was in part defrayed by the Bertrand Fund.

† Now at New York University College of Medicine.

‡ Now at School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.

§ Now at Harvard Medical School, Boston, Mass.

tradictory results.[1] Serum antibody levels in adrenalectomized animals have been reported to be unchanged,[2] elevated[3,4] and diminished.[5] Recently, however, Chase, White and Dougherty[6] have suggested that gamma globulin and antibodies are released from lymphocytes under the influence of the adrenal cortical hormone. This hypothesis is based upon the observation that the injection of adrenal cortical extract (ACE) brings about the "dissolution" of fixed and circulating lymphocytes[7,8] and, concomitantly, causes increased amounts of gamma globulin[9] and antibody[10,11] to appear in the blood. It was also reported that repeated simultaneous administration of ACE and antigen to intact animals produces higher antibody titers than does injection of antigen alone.[6]

The differences in antibody titers in the latter experiments,[6] though persistently obtained in several species, were rather small, since the values reported were agglutination endpoint titers, which are generally considered subject to error by a factor of 2. Furthermore, the changes produced by injection of ACE into an intact animal are probably the result of several variables and should not be attributed exclusively to direct action by the extract. It is well known that the administration of ACE produces adrenal atrophy. Since the extract does not completely replace all adrenal cortical functions there may result an indeterminate deficit of some functions and perhaps an excess of others. The findings in such an experiment,[6] therefore, reflect not only the effect of ACE, but possibly also a complex dysfunction of the animals' own adrenals.

In the present study an attempt was made to avoid the above difficulties by employing quantitative methods for the measurement of antibodies, and by studying the effect of ACE in adrenalectomized animals maintained on sodium chloride and desoxycorticosterone acetate (DCA). The latter (DCA) has been found to have no demonstrable effect on serum antibody levels.[10] The objectives of the experiments described below were to determine, firstly, whether adrenal cortical activity is essential for the formation and release of antibodies, and secondly, whether ACE is effective in enhancing circulating antibody levels in the absence of the adrenal cortex.

Experimental. Twenty-seven male albino rats, ranging in age from 14 to 18 weeks, and in weight from 215 to 252 g, were subjected to bilateral adrenalectomy under ether anesthesia. Following the operation, and for the duration of the experiment (30 days), all animals were maintained on Rockland rat diet, and on drinking water containing 1% NaCl. Each animal also received a subcutaneous injection of 0.4 mg DCA‖ every second day. Thirteen of these animals (Group I) were given, in addition, a daily subcutaneous injection of 0.5 ml of adrenal cortical lipoextract (ACE).¶ The remaining 14 animals served as controls (Group II). Six unoperated male albino rats of similar age and weight were included as additional controls (Group III). The 3 groups were composed of littermates.

All animals were immunized with a formalinized pneumococcus type IS (Dawson M Strain) vaccine (20 μg N per ml). Following an initial intraperitoneal injection

[1] Perla, D., and Marmorston, J., *Natural Resistance and Clinical Medicine*, Little, Brown & Co., 1941, Boston.

[2] Khorazo, D., *J. Immunol.*, 1931, **21**, 151.

[3] Také, N. M., and Marine, D., *J. Inf. Dis.*, 1923, **33**, 217.

[4] Jaffe, H. L., and Marine, D., *J. Inf. Dis.*, 1924, **35**, 334.

[5] Perla, D., and Marmorston-Gottesman, J., *J. Exp. Med.*, 1928, **47**, 723.

[6] Chase, J. H., White, A., and Dougherty, T. F., *J. Immunol.*, 1946, **52**, 101.

[7] Dougherty, T. F., and White, A., *Am. J. Anatomy*, 1945, **77**, 81.

[8] Dougherty, T. F., and White, A., *Science*, 1943, **98**, 367.

[9] White, A., and Dougherty, T. F., *Endocrinology*, 1945, **36**, 207.

[10] Dougherty, T. F., Chase, J. H., and White, A., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 135.

[11] Fox, S. A., and Whitehead, R. W., *Am. J. Physiol.*, 1935, **113**, 44.

‖ Generously supplied by Schering Corp., Bloomfield, N.J.

¶ Upjohn Company, Kalamazoo, Michigan.

TABLE I.
Comparison of Body Weights and Weights of Thymus and Pituitary Glands.

Group	Procedure	No. animals	Initial body wt, g	Final body wt, g	Thymus wt, (±Σm) mg	Pituitary wt, (±Σm) mg
I	Adrenalectomized 0.4 mg DCA 0.5 cc ACE	13	283	310	230 ± 12.5	13.2 ± 0.5
II	Adrenalectomized 0.4 mg DCA	11	267	296	318 ± 16.7	10.8 ± 0.4
III	Non-operated controls	6	272	302	226 ± 10.7	10.1 ± 0.4

of 0.5 ml on the 8th day after operation, each animal received 3 weekly courses of 4 intravenous injections each. The daily dose in the first course was 0.5 ml, in the second course 0.8 ml (average), and in the third course 0.5 ml, a total of 160 μg of bacterial N per animal. Occasionally a subcutaneous or intraperitoneal injection was given in lieu of an intravenous one. Such substitution occurred in 1.9%, 9.6% and 2.8% of the injections for Groups I, II and III respectively.

Beginning with the second course of pneumococcus vaccine each, but one, of the remaining 8 pneumococcus injections was accompanied by a simultaneous injection of 0.5 ml of a washed sheep erythrocyte suspension, increasing in strength from 2% to 4%. Five of these injections were given intravenously in admixture with the pneumococcus vaccine, and 2 were given separately by the peritoneal route. Five days after the last injection of antigen the animals were bled from the heart and autopsied. Hearts, kidneys, thymus and pituitary glands were weighed and prepared for histological examination.

Two-fold serial dilutions of inactivated sera from individual animals were tested for agglutination and hemolysis of washed sheep erythrocytes. Two 100% units of guinea pig complement were used in the latter determinations. The endpoints adopted were 3+ for agglutinins (large clumps) and 2+ for hemolysins (approximately 50% lysis).

Precipitins against Type I Pneumococcus capsular polysaccharide were determined on individual sera by the quantitative micromethod of Heidelberger and MacPherson.[12] Since a preliminary test showed that in the rat the complement present did not measurably contribute to specific precipitate N its removal as recommended for human serum by the above authors was omitted. The factor for conversion of the optical density of the blue-green color given by the Folin phenol reagent to micrograms of N was determined on electrophoretically separated rat serum gamma globulin and found to be 108 (Coleman Model II Spectrophotometer, 13 mm cuvettes, 650 mμ wavelength).

Sera of individual animals and pooled samples were studied electrophoretically after diluting 1:3 with, and dialyzing against, a pH 7.4 buffer 0.02 M with respect to sodium phosphate and 0.15 M with respect to sodium chloride.

Results. The animals survived the postoperative period in apparently good condition. All 3 groups gained weight at an identical rate during the 30 days of experimentation (Table I), but 3 animals in Group II died. The cause of the deaths was not apparent, but previous experience has shown that injections of pneumococcus vaccine may kill a small percentage of even normal animals. At autopsy none of the adrenalectomized animals had remnants of adrenal tissue demonstrable grossly.

The data on thymus weights, summarized in Table I, show that the thymus glands of the intact controls (Group III) and of the adrenalectomized animals receiving ACE (Group I) were identical in weight. This indicates adequate replacement of the adrenal factor responsible in normal animals for the reduction of lymphoid tissue in response to such stimuli as the injection of vaccine. The thymus weights of the adrenalectomized animals receiving DCA alone (Group II) were

[12] Heidelberger, M., and MacPherson, C. F. C., *Science*, 1943, **97**, 405.

TABLE II.
Comparison of Serum Antibody Concentrations.

Group	Procedure	No. animals	Agglutinin titers Avg	Agglutinin titers Range	Hemolysin titers Avg	Hemolysin titers Range	Precipitins (SI) $\pm\Sigma m$ μg N per cc
I	Adrenalectomized 0.4 mg DCA 0.5 cc ACE	13	1:80	1:16-1:256	1:86	0-1:160	32.1 ± 3.7
II	Adrenalectomized 0.4 mg DCA	11	1:67	1:32-1:128	1:160	1:10-1:320	35.6 ± 3.5
III	Non-operated controls	6	1:56	1:32-1:128	1:85	1:10-1:320	45.3 ± 3.7

TABLE III.
Electrophoretic Fractionation of Serum Proteins.

No. of serum samples used	Composition % Albumin	α-globulin	β-globulin	γ-globulin	Total area of pattern (arbitrary units)
	Group I. Adrenalectomized, DCA + ACE.				
1	69.4	—	13.8	16.8	1505
3	60.4	8.8	9.6	21.2	1465
4	66.0	—	13.5	20.5	1485
1	67.7	—	14.6	17.7	1300
Weighted avg	64.7	2.9	12.9	20.0	1460
	Group II. Adrenalectomized, DCA.				
1	60.6	9.5	10.2	19.7	1270
1	68.8	—	13.8	17.4	1380
3	66.0	—	13.8	20.2	1235
Weighted avg	65.5	2.0	13.0	19.5	1270
	Group III. Non-operated Controls.				
1	59.0	7.4	8.1	25.5	1490
1	63.4	—	14.7	21.9	1230
Avg	61.2	3.7	11.4	23.7	1360

significantly higher than those of the other 2 groups. This difference, however, does not indicate thymus hyperplasia in Group II, since the weights of these glands were still somewhat below the accepted norm.[13,14] Despite the difference in thymus weights between the adrenalectomized animals receiving ACE and those which did not, there was no statistically significant difference between their concentrations of circulating antibodies (Table II). This was true of hemoagglutinins and hemolysins as well as of antipneumococcus capsular polysaccharide precipitins which were measured quantitatively. The unoperated control animals had slightly greater amounts of circulating precipitin than both groups of adrenalectomized animals (Table II). Statistically, this difference is probably significant.

The failure of ACE to influence the level of circulating antibodies in adrenalectomized rats was paralleled by an inability to alter the concentration of serum gamma globulin, as is indicated by the data of Table III and as is illustrated in the typical patterns of Fig. 1.

Although not bearing directly on the question of antibody formation, the following additional data were obtained. Cardiac and renal enlargement have been observed in intact animals following the repeated injection

[13] Stoerk, H. C., *Endocrinology*, 1944, **34**, 329.

[14] Stoerk, H. C., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 90.

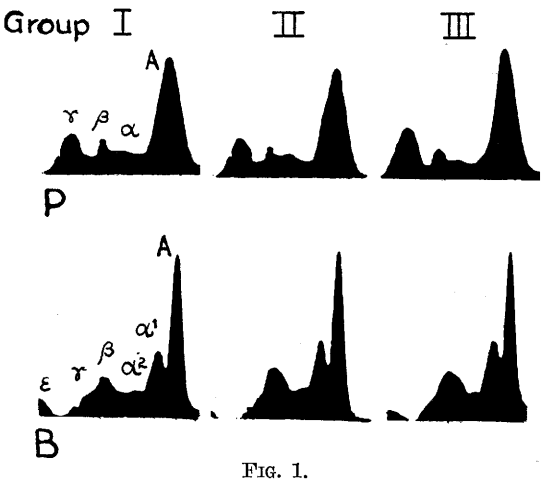

FIG. 1.
Electrophoresis patterns of sera from the 3 groups of rats. Upper row, in phosphate buffer; lower row, in barbiturate buffer.

of 10 mg,[15] and 2.5 mg[16] of DCA. The repeated injection of 0.4 mg DCA into adrenalectomized animals produced no such effect: The weights of the hearts and of the kidneys of both groups of adrenalectomized animals (I and II) were alike and were within normal limits.[17] The histological changes observed in kidneys of intact animals repeatedly injected with 2.5 mg DCA[16,18] were also present in both groups of adrenalectomized animals (receiving 0.4 mg), although to a milder degree.

The ACE-injected adrenalectomized animals (Group I) had pituitary glands which were significantly heavier (Table I) than those of the 2 control groups (II and III). This to our knowledge has not been previously observed. No apparent changes were evident, however, in the cytology of the enlarged hypophyses.

Discussion. Although the above findings indicated that adrenal cortical activity does not influence the concentration of circulating antibodies and gamma globulin, they did not exclude the possibility that considerable differences in their "turnover" had been induced: Thus ACE-injected animals may have had an augmented rate of antibody release which was obscured by an equally increased rate of antibody degradation. Conversely the adrenalectomized rats, not given ACE, may have had a diminished release of antibody which was concealed by an equally reduced rate of antibody degradation. Such changes could have occurred in the 2 groups of adrenalectomized rats, despite their having identical serum antibody and gamma globulin concentrations. This possibility had to be seriously considered in view of the widely held belief that protein catabolism is accelerated by increased adrenal cortical activity. Furthermore, it has been suggested that the metabolism of antibodies and of other serum proteins and of organ proteins are alike.[19,20] Consequently, 3 groups of rats maintained under the above experimental conditions were fed glycine tagged with heavy nitrogen and the "turnover" of their serum proteins was determined by following the rate of replacement of N^{15} by N^{14} in their total serum proteins. The results of these experiments will be reported elsewhere in greater detail.[21] It was found, however, that in adrenalectomized rats receiving DCA and salt alone the loss of N^{15} from serum protein occurred at the same rate as in similar animals receiving ACE and as in the intact controls.

It therefore appears that the integrity of the adrenal cortex is not essential for the production or the release of antibody.

In unpublished experiments, however, it was seen, in agreement with Dougherty, Chase and White,[10] that shortly after a single injection of ACE there occurred an elevation of serum antibody-N (of about 30%) in

15 Selye, H., Stone, H., Nielson, K., and Leblond, C. P., *Canad. Med. Assn. J.*, 1945, **52**, 571.

16 Knowlton, A. I., Stoerk, H. C., Seegal, B. C., and Loeb, E. N., *Endocrinology*, 1946, **38**, 315.

17 Knowlton, A. I., Loeb, E. N., Stoerk, H. C., and Seegal, B. C., *J. Exp. Med.*, 1947, **85**, 187.

18 Stoerk, H. C., and Zucker, T., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 297, and unpublished data.

19 Heidelberger, M., Treffers, H. P., Schoenheimer, R., Ratner, S., and Rittenberg, D., *J. Biol. Chem.*, 1942, **144**, 555.

20 Schoenheimer, R., Ratner, S., Rittenberg, D., and Heidelberger, M., *J. Biol. Chem.*, 1942, **144**, 545.

21 Stoerk, H. C., John, H. M., and Eisen, H. N., in preparation.

rabbits previously immunized with pneumococcus Type I. This nonspecific anamnestic rise lasted only about 12 hours and did not occur at all in animals whose previous antibody response had been relatively weak. Hence it appears that increased adrenal cortical activity may exert a transitory influence upon the distribution of preexisting antibodies. However, it is clear from the data presented above that adrenal cortical activity has no prolonged effect upon the production or release of antibodies and gamma globulin.

Summary. Identical concentrations of serum antibodies and gamma globulin were found in adrenalectomized rats repeatedly injected during immunization with ACE and in similar animals not receiving ACE. It is concluded that adrenal cortical activity is not essential for the fabrication or release of antibodies and gamma globulin.

15943 P

Evaluation of Vagotomy in Chronic, Non-Specific Ulcerative Colitis.*

CLARENCE DENNIS AND FRANK D. EDDY.

From the Department of Surgery, University of Minnesota Hospitals.

Two previous papers by one of us[1,2] bear out the conclusion of Elsom and Ferguson[3] that surgical management of chronic, nonspecific, ulcerative colitis is safer and productive of better health than prolonged conservative treatment. The surgical management is beset with many pitfalls, however, and disastrous complications still occur.

In search of a simpler method of handling the problem, consideration has been given to division of the vagus nerves, with the thought in mind that decrease in general intestinal muscular activity might result and might ease the symptoms of the disease, as Dragstedt[4] has found to be the case in duodenal and gastric ulcers.

We were plunged into the problem by a patient who had had all her colon and ¾ of her small intestine resected for colitis and ascending ulcerative ileitis. Recurrent ileitis demanded some active therapy after all known conservative measures had failed. Vagotomy was suggested by Dr. C. J. Watson of the Department of Medicine, and resulted in marked improvement, the patient having thereafter the first solid stools she had had in 4 years. Unfortunately the improvement was temporary.

A plan was formulated to employ transthoracic vagotomy in a group of cases to determine the possible value of the procedure. Moore[5] had had a very poor result from vagotomy in one fairly severe case, and we therefore confined ourselves to minimally involved cases.

In the 4 cases in which vagotomy was the only surgical therapy, studies were performed before operation, in the 2 weeks afterward, and 6 weeks to 3 months after vagotomy. Observations include number and character of stools, general health, presence of blood, pus, mucus in the stools, proctoscopic and barium enema findings, transit time of material through the intestine by X-ray and charcoal, electrocardiograms, triple histamine (Lannin) gastric analyses, 12-hour night collection of gastric juice, and insulin gastric analyses with blood glucose checks.

As indicated in Table I, lasting good re-

* Supported by a Research Grant from the Graduate School of the University of Minnesota.

1 Dennis, Clarence, *Surgery*, 1945, **18**, 435.

2 Dennis, Clarence, *Minnesota Med.*, 1945, **28**, 228.

3 Elsom, K. A., and Ferguson, L. K., *Am. J. Med. Sci.*, 1941, **202**, 59.

4 Dragstedt, L. R., *Surgery*, 1945, **17**, 742.

5 Moore, F. D., *Bull. New Eng. Med. Center*, 1945, **7**, 52.