

THE EFFECT OF CORTISONE TREATMENT ON THE IN VITRO RELEASE OF HISTAMINE FROM SERUM AND BLOOD OF SENSITIZED RABBITS

W. C. SPAIN, M.D., VINCENT J. FONTANA, M.D., AND MARGARET B. STRAUSS, M.S.,
NEW YORK, N. Y.

THERE have been many reports upon the usefulness of cortisone and ACTH in various allergic disorders. Bordley et al.,¹ Randolph and Rollins,² Rose,^{3, 4} Samter,⁵ Carryer,⁶ and Carey⁷ have demonstrated their effectiveness in asthma, hay fever, and urticaria. The mechanism whereby these hormones exert their influence is unknown. Since the allergic manifestation involves antigen-antibody reaction, many workers have attempted to study this response by observing the effect of cortisone and ACTH upon anaphylactic shock in the guinea pig. Leger, Leith, and Rose,⁸ Friedlaender and Friedlaender,⁹ Harris and Harris,¹⁰ Malkiel,¹¹ and Landau,¹² all failed to observe from cortisone or ACTH any alleviating effect upon anaphylactic shock. Loveless¹³ found no associated change in the concentration of sensitizing or of protecting antibodies after 14 days of ACTH therapy in one allergic individual.

The allergic reaction is now generally considered to be due to histamine or a histamine-like substance along with other metabolites. A study, therefore, has been attempted of the in vitro histamine release from (1) the blood of cortisone-treated sensitized rabbits exposed to the specific antigen and (2) from the blood of cortisone-treated normal rabbits exposed to a mixture of rabbit antiserum and specific antigen. Spain, Strauss, and Neumann¹⁴ have previously shown that untreated allergic serum and antigen mixtures released histamine when added to normal rabbit blood whereas treated allergic serum and antigen mixtures did not release histamine. Carryer and Code¹⁵ studied histamine release into rabbit plasma as a gauge of antigen-antibody combination and detected no difference when cortisone was present. In their procedure they added cortisone only to the test tube mixture. In the following experiments cortisone was administered in large dosages to the rabbits before their blood was used.

PROCEDURE

Rabbits were sensitized with five intracutaneous injections of 10 ml. each of undiluted egg white every second day. One week after the last injection precipitins to egg white were observed by the micro-ring technique, usually with an antigen dilution of 1:20,000 or 1:50,000. Cortisone* was administered intramuscularly in total dosages ranging from 75 to 150 mg. over 2 to 4 day periods

From the Department of Medicine, New York University Post-Graduate Medical School and the Medical Service University Hospital, New York.

Received for publication, Jan. 26, 1952.

*A saline suspension of Cortone Acetate supplied by Merck & Co., Inc.

as shown in Tables I and II. Blood was withdrawn under nembutal anesthesia by cardiac puncture. Heparin, 0.09 mg. per ml. blood, was used as an anti-coagulant. All glassware in which blood was placed was silicone coated.

Histamine was purified by the method of McIntire, Roth and Shaw¹⁶ and determined by the method of McIntire, White, and Sproull¹⁷ by reaction with 2,4-dinitrofluorobenzene. The only modification employed was that a Lumetron (Model 402E) Photoelectric Colorimeter with a mercury vapor lamp of high ultra violet transmission and a special auxiliary transformer was used with a M365 filter and 1 ml. micro cells. A Beckman spectrophotometer was not available. The adaption of the Lumetron proved completely satisfactory as gauged by recovery experiments of standard histamine added to normal blood.

In the first type of experiment, 5 ml. of antiegg white rabbit blood (pre- or postcortisone) were added to 2.3 ml. of egg white diluted 1:10 with saline and also to 2.3 ml. of saline (control). Control samples were run with each sample of blood analyzed since an appreciable amount of histamine, present normally in rabbit serum and increased under certain conditions, must be subtracted as a base from the amount found in mixtures of antiserum and antigen. Each of these mixtures was run in duplicate. After incubation at 37° C. for 30 minutes they were centrifuged. From the total supernatant of 4.8 ml., 2 ml. aliquots of each mixture were taken for the histamine determination.

In the second type of experiment, the serum employed was obtained from the sensitized rabbit not treated with cortisone. As the source of bound histamine, 5 ml. of normal rabbit blood or blood from an otherwise normal rabbit pretreated with cortisone was added to a previously incubated mixture of 0.4 ml. of antiegg serum and 0.4 ml. of egg white, 1:10. Saline was added to maintain the same volume as that in the first type of experiment and the rest of the procedure was as described above.

Seven rabbits were sensitized to egg white and their heparinized blood was used for study of in vitro histamine release after mixture with antigen. Both pre- and postcortisone blood samples were obtained in five rabbits. One rabbit died before treated and untreated blood samples could be taken so that two different sensitized animals were used in one pre- and postcortisone series. The sera of all these rabbits gave positive precipitin tests to egg white in a 1:20,000 dilution or more.

In an old egg white sensitized rabbit whose serum no longer contained precipitins to high dilutions of antigen there was no release of histamine.

RESULTS

From Table I it will be seen that an average of 1.8 gamma of histamine was released from mixtures of rabbit antiegg blood and egg white and an average of 1.4 gamma of histamine was released from mixtures similar except that the blood was taken after treatment of the animal with cortisone. Since there was no significant difference between these two sets of results under these experimental conditions, there was no evidence of inhibition of the histamine release as a result of cortisone therapy.

TABLE I. THE EFFECT OF TREATMENT WITH CORTISONE ON THE RELEASE OF HISTAMINE FROM MIXTURES OF SENSITIZED RABBIT BLOOD AND ANTIGEN

MIXTURES			GAMMA HISTAMINE		
NO. ANTIEGG RABBIT BLOOD (5 ML.)	ML. EGG WHITE 1:10	ML. 0.85 PER CENT SALINE SOLUTION	IN TOTAL SUPER- NATANT	RELEASED BY ANTIGEN-ANTIBODY REACTION	
				IN TOTAL SUPERNATANT	PER ML. BLOOD PLASMA
71	2.3	0.0	4.6	1.3	0.5
71	0.0	2.3	3.3		
71 PC-1	2.3	0.0	5.9	3.3	1.3 PC*
71 PC-1	0.0	2.3	2.6		
58	2.3	0.0	8.4	4.9	2.0
58	0.0	2.3	3.5		
58 PC-1	2.3	0.0	6.4	5.4	2.2 PC*
58 PC-1	0.0	2.3	1.0		
61	2.3	0.0	5.4	4.4	1.8
61	0.0	2.3	1.0		
61 PC-2	2.3	0.0	5.8	2.9	1.2 PC*
61 PC-2	0.0	2.3	2.9		
84	2.3	0.0	7.9	4.1	1.6
84	0.0	2.3	3.8		
53 PC-2	2.3	0.0	4.7	2.8	1.2 PC*
53 PC-2	0.0	2.3	1.9		
55	2.3	0.0	9.3	6.2	2.5
55	0.0	2.3	3.1		
55 PC-2	2.3	0.0	3.8	2.7	1.1 PC*
55 PC-2	0.0	2.3	1.1		
91	2.3	0.0	8.7	5.8	2.3
91	0.0	2.3	2.9		
91 PC-2	2.3	0.0	5.7	3.2	1.3 PC*
91 PC-2	0.0	2.3	2.4		

*PC—postcortisone

PC-1—total dosage of 75 mg. cortisone; 12.5 mg. every 8 hours for 2 days.

PC-2—total dosage of 150 mg. cortisone; 12.5 mg. per dosage for 4 days.

Table II shows the results obtained when normal heparinized rabbit blood or blood from an otherwise normal rabbit pretreated with cortisone was used as the source of bound histamine and only the serum from the sensitized animal and antigen were added to the blood. There was no inhibition of the histamine release by the use of blood from normal rabbits which were pretreated with cortisone.

DISCUSSION

There have been numerous reports on the efficacious results of cortisone therapy in hay fever, asthma, and other clinical allergic manifestations, with

TABLE II. THE EFFECT OF CORTISONE-TREATED NORMAL RABBIT BLOOD ON THE RELEASE OF HISTAMINE FROM ANTISERA AND ANTIGEN MIXTURES

MIXTURE				GAMMA HISTAMINE	
RABBIT ANTI- EGG SERUM 0.4 ML.	EGG WHITE 1:10 ML.	0.85 PER CENT SALINE SOL. ML.	RABBIT BLOOD 5 ML.	IN TOTAL SUPERNATANT	RELEASED IN TOTAL SUPERNATANT
#65	0.4	1.5	normal	4.1	1.2
#65	0.0	1.9	normal	2.9	
#65	0.4	1.5	No. 64 postcortisone	5.2	1.9
#65	0.0	1.9	No. 64 postcortisone	3.3	
#65	0.4	1.5	No. 59 postcortisone	5.1	2.0
#65	0.0	1.9	No. 59 postcortisone	3.1	
#64 and #59 rabbits received 100 mg. cortisone, 12.5 mg. every 6 hours for 2 days.					

many attempts to explain it. In studying the influence of cortisone upon the antigen-antibody reaction, the majority of investigators have stated that cortisone has no apparent effect on anaphylactic shock or on the response of the sensitized smooth muscle to its antigen in *in vitro* preparations. Thus the evidence seems to indicate that there is no interference with the end result of the antigen-antibody reaction in the guinea pig. Since allergic and anaphylactic reactions seem to be produced by free histamine or histamine-like substances and other metabolites, the effect of cortisone upon histamine release in hypersensitive rabbits was studied. Some of us have previously shown that serum of a hay fever patient treated with ragweed injections when added to ragweed extract, did not release histamine under the same experimental conditions as the serum of an untreated hay fever patient and ragweed extract, which did release histamine from its bound form in normal rabbit blood. Our present results indicate that there is no demonstrable inhibition of the histamine release in mixtures of anti-egg blood from rabbits treated with cortisone, and egg solution. Therefore, if there be an effect of cortisone on such hypersensitive conditions, it would seem to act beyond the stage at which histamine is released but before the onset of such pharmacological effects of histamine on the tissues as capillary dilatation, smooth muscle contraction or stimulation of the secretory glands. Therefore the relief from clinical allergic symptoms effected by cortisone in spite of no apparent inhibition of the antigen-antibody reaction nor inhibition of the histamine release, could conceivably be due to some type of alteration in the cement substance of the cell.

SUMMARY

1. When the blood of sensitized rabbits treated with large doses of cortisone was exposed to antigen, histamine was released *in vitro* in amounts comparable to that released under similar conditions from the blood of sensitized rabbits not treated with cortisone.

2. From the blood of rabbits, normal except for treatment with cortisone, histamine was released in the presence of rabbit antiserum and antigen to the same degree as from the blood of normal rabbits not treated with cortisone.

REFERENCES

1. Bordley, J. E., Carey, R. A., Harvey, A. M., Howard, J. E., Kattus, A. A., Newman, E. V., and Winkenwerder, W. L.: Preliminary Observations on the Effect of Adrenocorticotrophic Hormone (ACTH) in Allergic Diseases, *Bull. Johns Hopkins Hosp.* **85**: 396, 1949.
2. Randolph, T. G., and Rollins, J. P.: Relief of Allergic Diseases by ACTH Therapy, *Proc. Clin. ACTH Conf.* **1**: 479, 1950.
3. Rose, B.: Studies on the Effect of ACTH on Eosinophilia and Bronchial Asthma, *Proc. Clin. ACTH Conf.* **1**: 491, 1950.
4. Rose, B., Pare, J. A. P., Pump, K., and Stanford, R. L.: Preliminary Report on Adrenocorticotrophic Hormone (ACTH) in Asthma, *Canadian M. J.* **62**: 6, 1950.
5. Samter, M.: Effect of Adrenocorticotrophic Hormone (ACTH) on Patients with Allergic Diseases—Facts and Speculations, *J. ALLERGY* **21**: 296, 1950.
6. Carryer, H. M., Koelsche, G. A., Prickman, L. E., Maytum, C. K., Lake, C. F., and Williams, H. L.: Effect of Cortisone on Bronchial Asthma and Hay Fever Occurring in Subjects Sensitive to Ragweed Pollen, *J. ALLERGY* **21**: 282, 1950.
7. Carey, R. A., Harvey, A. McG., Howard, J. E., and Winkenwerder, W. L.: The Effect of ACTH and Cortisone on the Course of Chronic Bronchial Asthma, *Bull. Johns Hopkins Hosp.* **87**: 387, 1950.

8. Leger, J., Leith, W., and Rose, B.: Effect of Adrenocorticotrophic Hormone on Anaphylaxis in Guinea Pigs, *Proc. Soc. Exper. Biol. & Med.* **69**: 465, 1948.
9. Friedlaender, S., and Friedlaender, A. S.: The Effect of Pituitary Adrenocorticotrophic Hormone (ACTH) on Histamine Intoxication and Anaphylaxis in the Guinea Pig, *J. ALLERGY* **21**: 303, 1950.
10. Harris, S., and Harris, T. N.: Effect of Cortisone on Some Reactions of Hypersensitivity in Laboratory Animals, *Proc. Soc. Exper. Biol. & Med.* **74**: 186, 1950.
11. Malkiel, S.: The Influence of ACTH and Cortisone on Histamine and Anaphylactic Shock in the Guinea Pig, *J. Immunol.* **66**: 379, 1951.
12. Landau, S. W., Nelson, W. A., and Gay, L. N.: The Effect of Adrenocorticotrophic Hormone (ACTH) and Cortisone on Histamine Reactions and Anaphylactic Reactions in Guinea Pigs, *Bull. Johns Hopkins Hosp.* **88**: 395, 1951.
13. Loveless, M. H.: The Influence of ACTH on the Sensitizing and Immunizing Antibodies of Inhalant Allergy, *Bull. N. Y. Acad. Med.* **27**: 477, 1951.
14. Spain, W. C., Strauss, M. B., and Neumann, E.: In Vitro Release of Histamine by Hypersensitive (Allergic) Serum in Contrast to Immune (Treated) Allergic Serum in Antigen and Normal Rabbit Blood Mixtures, *J. ALLERGY* **21**: 318, 1950.
15. Carryer, H. N., and Code, C. F.: Effect of Cortisone Upon the Release of Histamine During In Vitro Hemolytic Reactions in Rabbit Blood, *J. ALLERGY* **21**: 310, 1950.
16. McIntire, F. C., Roth, L. W., and Shaw, J. L.: The Purification of Histamine for Bioassay, *J. Biol. Chem.* **170**: 537, 1947.
17. McIntire, F. C., White, F. B., and Sproull, M.: The Determination of Histamine with 2,4-Dinitrofluorobenzene, *Arch. Biochem.* **29**: 376, 1950.