Apparent Free Histidine Plasma and Urine Values in Rheumatoid Arthritis Treated with Cortisone and ACTH.* (17876)

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Preliminary observations(1,2,3,4) on the urinary excretion and plasma levels of amino acids in rheumatoid arthritis made it seem logical to determine these values before, during and after treatment with the new antirheumatic agents described by Hench *et al.* (5).

This paper summarizes a study of 15 patients with active rheumatoid arthritis treated with 17 - hydroxy - 11 - dehydrocorticosterone (Cortisone)† and adrenocorticotrophic hormone (ACTH)‡ and reports urinary excretions and plasma levels of histidine during active disease and through remission. This group was uncomplicated by any other medication.§

Experimental. Five patients (2 females and 3 males) were treated with Cortisone. Ten patients (8 females and 2 males) were treated with ACTH. The average age was 50.8 years with a range of 27 to 70 years. The average duration of the disease was 5.1 years with a range of 1.5 to 16 years. All patients suffered from active rheumatoid arthritis, had been under observation by the investigators for 6 months or longer, and were hospitalized during the entire period of investigation. They were observed during an initial pre-treatment period and were under metabolic control during the entire experimental period. The evaluation of therapy response was both subjective and objective. Stiffness, rest pain, and motion pain were determined by the patients' answers. Heat, swelling, redness, tenderness, strength of grip and degrees of function were determined daily by inspection and measurement. Blood pressure and weight recordings were made daily. Complete blood counts and sedimentation rates were performed at selected intervals. Cortisone was given in an initial dose of 300 mg intramuscularly and on each successive day 100 mg of the preparation was used. ACTH was given intramuscularly in variable dosage for 8 to 17 days. Daily dosage varied from 40 to 160 mg per day and was given in divided doses every 6 hours. Subsequent cases have been found to experience satis-

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1. Wallraff, E. B., Stephens, C. A. L., Jr., Borden, A., Holbrook, W. P., Hill, D. F., Kent, L. J., and Kemmerer, A. R., *Fed. Proc.*, 1949, v8, 399.

2. Stephens, C. A. L., Jr., Borden, A., Holbrook, W. P., Hill, D. F., Kent, L. J., Wallraff, E. B., and Kemmerer, A. R., *Proc. Seventh Internat. Congress*, May 30, 1949, in press.

3. Holbrook, W. P., Hill, D. F., Stephens, C. A. L., Jr., and Kent, L. J., unpublished data.

4. *Proceedings of the First Clinical ACTH Conference*, John R. Mote, M.D., Editor, The Blakiston Company, Philadelphia, 1950, p. 386.

5. Hench, P. S., Kendall, E. C., Slocumb, C. H., and Polley, H. F., *Proc. Staff Meet., Mayo Clinic*, 1949, v24, 181.

† All Cortisone was provided by Merck and Co.

‡ All ACTH was provided by Armour and Co. (Dr. John Mote, Medical Director).

§ During the past year a total of 51 patients with rheumatoid arthritis have been studied, 14 receiving Cortisone and 37 ACTH. In attempting to accelerate and prolong the favorable effects of treatment, a number of this group received during the control period and/or through the treatment period, various agents such as ascorbic acid, testosterone, benedryl, and adenosine triphosphate (ATP), thyroid, adrenalin, gold, X-ray therapy, amino acid feeding and aspirin. Amino acid studies were done on this group of patients but are not being reported at this time. However, we should like to state that the urinary histidine excretion response to be described has not occurred in this group except in association with clinical remission.

TABLE I.
Excretion of Apparent Free Histidine During Cortisone Administration.
(5 patients with rheumatoid arthritis).

Patient	Total dosage, mg	Control values, mg per 24 hr	Treatment values, mg per 24 hr	Diff. between max. (control and treatment) mg per 24 hr
B	1600 (14)*	53 (41– 73)	126 (117–136)	63
Y	1800 (14)*	57 (47– 62)	144 (101–186)	124
S	1000 (8)*	63 (53– 73)	131 (104–148)	75
N	1000 (8)*	98 (95–100)	149 (110–167)	67
R	1000 (8)*	84 (54–114)	141 (64–180)	66
Avg	1280 (10.4)*	71 (58– 86)	138 (99–163)	77

* Days on treatment.

TABLE II.
Excretion of Apparent Free Histidine During ACTH Administration.
(10 patients with rheumatoid arthritis).

Patient	Total dosage, mg	Control values, mg per 24 hr	Treatment values, mg per 24 hr	Diff. between max. (control and treatment) mg per 24 hr
K	310 (15)*	23 (20– 26)	81 (62–117)	91
D	675 (17)*	104 (104–104)	224 (143–334)	230
G	640 (11)*	63 (60– 66)	133 (81–174)	108
L	680 (11)*	70 (64– 76)	201 (124–285)	209
P	600 (11)*	62 (61– 62)	166 (118–231)	169
K	620 (11)*	119 (97–140)	176 (134–260)	120
B	370 (12)*	23 (18– 28)	157 (109–211)	183
W	380 (12)*	53 (44– 61)	144 (96–175)	114
F	370 (12)*	30 (23– 37)	106 (54–146)	109
M	960 (11)*	83 (79– 87)	374 (148–493)	406
Avg	560.5 (12.3)*	63 (57– 69)	176 (107–243)	174

* Days on treatment.

factory remissions on 40 mg (or less) ACTH per day.

Collection of urine and blood specimens. Twenty-four hour urine specimens were collected under toluene, diluted to appropriate volume and aliquots stored at -15°C. Fasting blood was obtained by venipuncture and prevented from clotting with heparin. Tungstic acid filtrates were prepared according to the method of Hier and Bergeim(6,7) and stored at -15°C. A modification of the microbiological technic of Henderson and Snell (8) was used for the assay of free histidine. Hydrous histidine hydrochloride ($C_6H_9N_3O_2$·

6. Hier, S. W., and Bergeim, O., *J. Biol. Chem.*, 1945, v161, 717.

7. Hier, S. W., and Bergeim, O., *J. Biol. Chem.*, 1946, v163, 129.

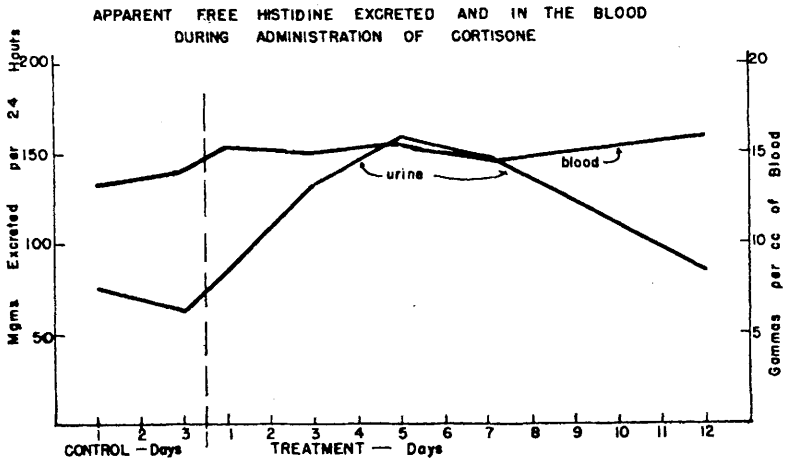

FIG. 1.
Average of 2 ♀ and 3 ♂ rheumatoid arthritics.

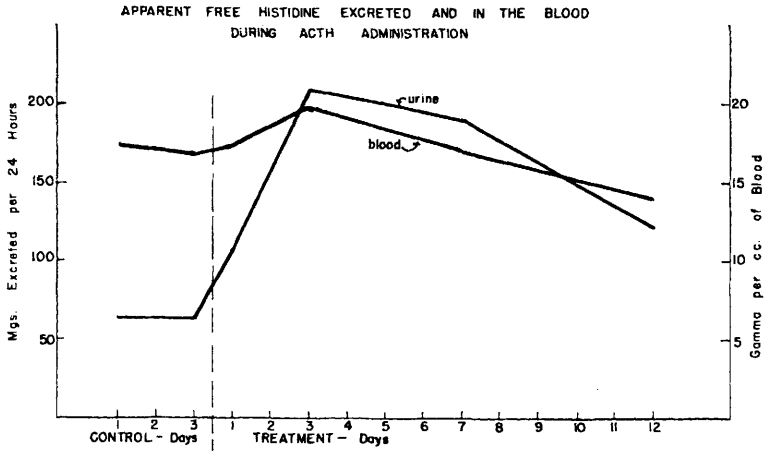

FIG. 2.
Average 8 ♀ and 2 ♂ with rheumatoid arthritis.

$HCL{\cdot}H_2O$) ("Merck") was used as a standard. All values of apparent free histidine are expressed in terms of this compound. The test organism was *Leuconostoc mesenteroides P-60*. All specimens from each patient were assayed in one series to eliminate errors due to variations in organism response. Cortisone and ACTH were found to have no stimulatory or inhibitory effect on the microorganisms.

8. Henderson, L. M., and Snell, E. E., *J. Biol. Chem.*, 1948, v172, 15.

Clinical results. All of the patients experienced improvement within 24-48 hours following the initial injection of either hormone. The patients described a sense of well-being, decrease in joint stiffness, rest pain, motion pain and joint tenderness. Swelling disappeared slowly over the period of treatment. Strength improved rapidly and there was a marked increase in appetite and energy. Following the initial 48 hour period, improvement was slower but continued steadily as long as the medication was given. The

TABLE III.
Apparent Free Histidine Plasma Values During Cortisone Administration.
(5 patients with rheumatoid arthritis).

Patient	Total dosage, mg	Control values, γ per ml	Treatment values, γ per ml	Diff. between max. (control and treatment) γ per ml
B	1600 (14)*	13.6 (13.2–14.2)	17.5 (13.7–21.3)	7.1
Y	1800 (14)*	12.1 (–12.1)	14.4 (12.1–18.7)	6.6
S	1000 (8)*	16.0 (15.8–16.4)	16.3 (15.6–17.4)	1.0
N	1000 (8)*	14.8 (14.6–14.9)	14.5 (13.0–17.3)	2.4
R	1000 (8)*	13.6 (13.4–13.7)	14.9 (13.7–16.3)	2.6
Avg	1280 (10.4)*	14.0 (13.8–14.3)	15.5 (13.6–18.2)	3.9

* Days on treatment.

sedimentation rate dropped an average of 37.1 mm per hour (Modified Westergren method). No toxic effects were noted except in the one case that received the large dose of 160 mg per day. This patient developed transient hypertension, weight gain and oliguria. In the remaining cases there was no alteration in weight on the constant diet used. Blood pressure did not alter significantly. No edema developed. The pulse rate was not significantly changed. There were no apparent differences in the clinical response to either of the hormones.

Chemical results. Urine. All patients treated with Cortisone or ACTH had a significant rise in apparent free histidine excretion. (Tables I and II) Both control excretion and increase of excretion on medication show considerable individual variation. From the composite graphs (Fig. 1 and 2) it can be seen that apparent free histidine excretion rises upon initiation of medication and continues to rise to a peak. The urinary excretion curves produced by Cortisone and ACTH are comparable except that in the case of the latter, the peak is reached sooner and the increase is of a greater magnitude. While still on either medication, the patients show a decrease from the peak. When medication is discontinued, there is a more rapid fall toward the control levels. This type of curve has occurred only in association with clinical remission. From the data at hand, and within the limits of dosage employed, there is a significant‖ correlation between ACTH dosage and the maximum increase in excretion herein reported(9).

Blood. The average values for histidine in the blood during treatment (Tables III and IV) are higher than the control in 9 patients and lower in 6. However, the maximum values reached during the treatment period are significantly higher‖ than the highest control values(10). Fig. 1 and 2 present the average plasma levels before, during and after treatment with Cortisone and ACTH. While the differences in plasma levels reported are small it seems logical to assume that even a small rise in plasma levels would be commensurate with high urinary excretion values. The plasma levels are representative of values existing during only one period of the day while the excretion values provide a measure of the total 24-hour urinary excretion. Furthermore, expressed in terms of average blood volumes a rise of 5γ per ml would represent a total increase of approximately 25 mg. The possible relationship of these findings to changes in kidney function or to other changes in metabolism produced by ACTH or Cortisone have not

‖ $P < .05$ considered significant.

9. Wallace, H. A., and Snedecor, G. W., *Iowa State College Bulletin*, 1931, v30, 8.

10. Brandt, A. E., *J. Am. Stat. Assn.*, 1933, v28, 434.

TABLE IV.
Apparent Free Histidine Plasma Values During ACTH Administration.
(10 patients with rheumatoid arthritis).

Patient	Total dosage, mg	Control values, γ per ml	Treatment values, γ per ml	Diff. between max. (control and treatment) γ per ml
H	310 (15)*	15.5 (15.3–15.8)	16.4 (11.5–18.1)	2.3
D	675 (17)*	16.4 (15.8–17.4)	16.2 (15.1–17.6)	0.2
G	640 (11)*	17.2 (15.7–18.7)	22.1 (19.4–23.9)	5.2
L	680 (11)*	19.0 (18.3–19.7)	18.5 (16.9–19.9)	0.2
P	600 (11)*	15.1 (15.0–15.2)	13.3 (12.3–14.5)	−0.7
K	620 (11)*	16.9 (16.7–17.0)	18.9 (18.3–19.9)	2.9
B	370 (12)*	19.7 (19.3–20.1)	16.3 (13.0–18.3)	−1.8
W	380 (12)*	12.1 (11.1–13.1)	13.1 (8.7–15.6)	2.5
F	370 (12)*	14.5 (14.3–14.7)	12.7 (10.3–14.9)	0.2
F	960 (11)*	24.5 (23.9–25.0)	27.4 (20.0–38.5)	13.5
Avg	560.5 (12.3)	17.1 (16.5–17.7)	17.5 (14.6–20.1)	2.5

* Days on treatment.

as yet been studied adequately.

Summary. 1. Five rheumatoid arthritics treated with Cortisone and 10 treated with ACTH show a striking increase in urinary excretion of apparent free histidine. This increase in histidine urinary excretion has to date occurred only in association with clinical remission.

2. Plasma levels were not significantly altered during the *average* treatment period, but the maximum value reached during treatment showed a significant increase when compared with the highest control values.

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Mucolytic Enzyme Systems XIII. Effect of Compounds on Hyaluronidase and its Inhibition by Human Serum.* (17877)

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During the course of the investigations on hyaluronidase and its inhibition, previously reported, further modifications of the procedures for the preparation of the substrate and the viscosimetric measurement of the hyaluronidase activity were instituted. These, as well as the effects of certain ions and or-

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