Effect of 17-Hydroxy-11-Dehydrocorticosterone and Adrenocorticotropic Hormone upon Plasma Gamma Globulin, Fibrinogen, and Erythrocyte Sedimentation Rate.* (18460)

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(Introduced by G. W. Thorn)

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The use of 17-hydroxy-11-dehydrocorticosterone (Cortisone) in the management of rheumatoid arthritis brought into prominence by Hench and his collaborators(1) and extended to adrenocorticotropic hormone (ACTH) by Thorn and others(2) has stimulated great interest in the mechanism of action of these drugs on various metabolic processes. Studies reported in this communication describe the relationship between 3 of the blood elements which are altered by Cortisone and ACTH treatment, namely, plasma fibrinogen, plasma gamma globulin, and erythrocyte sedimentation rate.

Materials and methods. Clinical material. Observations were made on 12 patients hospitalized for typical severe active rheumatoid arthritis and 3 hospital patients (A.G., A.V., and R.S.) with actively progressing generalized scleroderma. Blood samples were obtained before, during, and after one or more courses of treatment with Cortisone or ACTH. A dose of 100 to 200 mg a day of Cortisone acetate in aqueous suspension‡ administered intramuscularly in divided doses every 6 hours was used. ACTH§ 80 to 100 mg a day was likewise given in divided doses intramuscularly each 6 hours.

Sedimentation rates. 5 ml of blood is collected in a tube containing the dried crystals from 0.2 ml of balanced oxalate(3). The sedimentation rate was determined by the Rourke-Ernstene method(4). The upper limit of normal for this procedure in this laboratory is 0.35 mm fall per minute.

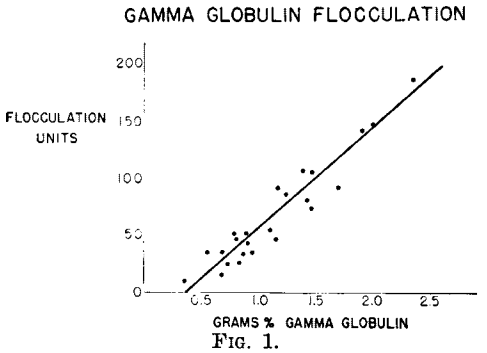

FIG. 1.

Relation between flocculation and electrophoresis methods for the determination of gamma globulin levels in various normal and pathological sera.

Gamma globulin. Kunkel's flocculation method(5) for determining gamma globulins was modified to the extent of doubling the volumes of reagents used and reading the degree of turbidity with the Klett-Summerson colorimeter. Flocculation of 1/10 ml of serum was produced with 6 ml of the zinc sulphate solution described by Kunkel(5). Fig. 1 records 30 random normal and pathological sera on which the gamma globulin content was known from previous electrophoretic analyses. There is a linear relation between the two methods. For the purpose of this study the value of 70 flocculation units has been considered the dividing line between a normal and an abnormal level.

Fibrinogen. Plasma fibrinogen was estimated

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† Stanley Tausend Fellow in Medicine.

‡ Merck and Co.

§ Armour Standard LA1A.

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2. Thorn, G. W., Bayles, T. B., Massell, B. F., Forsham, P. H., Hill, S. R., Smith, S., and Warren, J. E., *N.E.J.M.*, 1949, v241, 529.

3. Ham, T. H., and Curtis, F. C., *Medicine*, 1938, v17, 447.

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TABLE I. Effect of ACTH and Cortisone on Gamma Globulin, Fibrinogen, and Sedimentation Index.

	Gamma globulin, flocculation units			Fibrinogen, mg/ml			Sedimentation index mm/ml			
Patient	Start	End	Change	Start	End	Change	Start	End	Change	R
H.	80	56	—24	7.6	2.3	—5.3	1.35	.15	—1.20	ACTH
E.	242	183	—59							"
H.A.-1	200	100	—100							"
-2	195	112	—83	4.7	5.5	+0.8	1.90	.50	— .40	"
-3	120	49	—71							"
E.B.-1	135	75	—60	6.0	2.5	—3.5	1.00	.25	— .75	Cortisone
-2	100	60	—40	10.0	4.0	—6.0	1.20	.25	— .95	"
-3	119	65	—54	11.9	4.6	—7.3	1.50	.70	— .80	"
C.-1	130	112	—18	7.2	3.8	—2.4	1.20	1.00	— .20	"
-2	133	100	—33	14.0	7.8	—6.2	1.10	.80	— .30	"
-3	85	98	+13	6.6	6.0	—0.6	1.10	1.00	— .10	"
N.-1	54	40	—14	6.0	10.0	+4.0	0.65	.20	— .45	"
-2	48	42	— 6	8.0	5.0	—3.0	0.85	.30	— .55	"
B.	70	82	+12	8.0	2.6	—6.4	0.65	.35	— .30	ACTH
A.-1	120	66	—54							"
-2	136	53	—83	9.2	5.4	—3.8	1.85	.35	— .50	"
H.B.	65	40	—25	9.0	6.5	—2.5	1.25	.55	— .70	Cortisone
M.	120	50	—70							ACTH
B.	70	30	—41	8.0	3.5	—4.5	0.75	.25	— .50	Cortisone
C.P.	103	67	—36	10.0	7.0	—3.0	0.90	.40	— .50	"
A.G.	36	26	—10							ACTH
A.Z.	135	56	—79	6.6	2.2	—4.4	1.35	.40	— .95	"
R.	117	90	—27							"
Avg	105	67	—38	8.3	4.9	—3.4	1.16	.59	— .57	

as clottable protein after the method of Morrison(6). 4.0 ml of venous blood was discharged into a tube containing 0.75 ml of acid citrate dextrose solution‖ and gently mixed. Plasma was obtained by centrifugation. One or 2 ml of this plasma, depending on the expected level, were made up to 19 ml in 0.85% sodium chloride solution. To this was added 1 ml of a solution containing 50 units of human thrombin.¶ The clot was allowed to retract overnight, separated, washed, dried, and weighed. The normal fibrinogen content of plasma is 2.5-3.0 mg per ml.

Results. Table I presents the values obtained on the 16 patients studied. The average fall during hormone treatment in the gamma globulin flocculation units was from 105 to 67. The average fall in mg per ml of fibrinogen was from 8.3 to 4.9, and finally, the average fall in the sedimentation index was from 1.16 to 0.59 mm per second. These changes are significant. Fig. 2 indicates the scatter of results, each dot representing the change in a single treatment period.

In patient A.G. the figures indicate that treatment suppressed a normal gamma globulin to a subnormal value. Fig. 3 is the plasma electrophoretic pattern on this same patient before and at the end of treatment, showing these same changes pictorially.

Sedimentation indices are affected by many factors, including the plasma concentration of both fibrinogen and gamma globulin. Grey and Mitchell(7) demonstrated that an increase of 0.2 g% of this protein is sufficient to raise the one-hour rate of fall of erythrocytes 300%, while an increase of the gamma globulins of 0.4 g% changes the sedimentation rate 100%. Both of these quantities are well within the range of change in protein concentration that we have described here. In

6. Morrison, P., *J. Am. Chem. Soc.*, 1947, v69, 2723.

‖ 2.20 g % trisodium citrate $2H_2O$
2.20 g % dextrose
0.80 g % citric acid

¶ Kindly supplied by the Department of Physical Chemistry, Harvard Medical School.

7. Grey, S., and Mitchell, E., PROC. SOC. EXP. BIOL. AND MED., 1942, v51, 403.

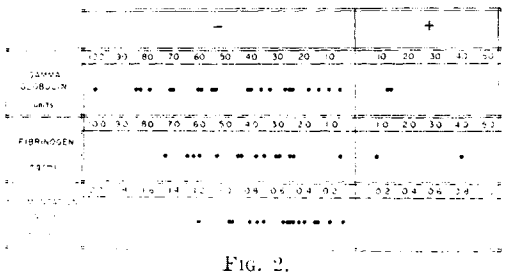
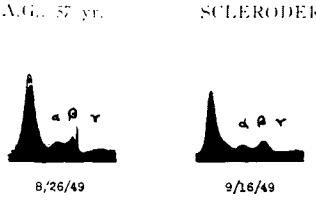


FIG. 2.

The change in gamma globulin, fibrinogen, and sedimentation rate during ACTH or cortisone therapy.



Electrophoretic patterns before and after a single 21-day period of ACTH therapy.

FIG. 3.

The suppression of a normal gamma globulin level to a subnormal value by ACTH.

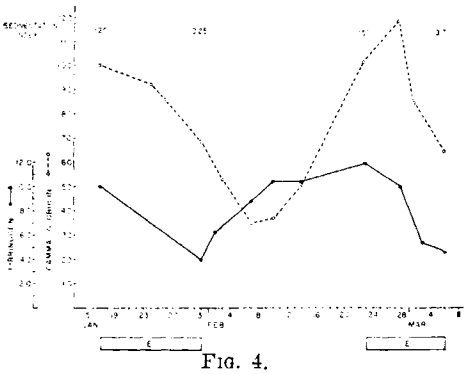


FIG. 4.

The interrelationship between the gamma globulin and the fibrinogen during cortisone therapy in rheumatoid arthritis.

general, fibrinogen concentrations most closely paralleled sedimentation rates, as has been reported by Ham and Curtis(8) for the Rourke-Ernstene method. In patient H.A., however, the fall in sedimentation rate correlated with the fall in gamma globulins, as treatment in this case was accompanied by no significant change in the fibrinogen level. Patient E. V. (Fig. 4) presents the usual pattern of the inter-relationship between these proteins during and after hormone treatment. Fibrinogen levels were more rapidly affected by treatment than were gamma globulin levels. This is easily seen in the period immediately following her first course of treatment, when the gamma globulins continued to fall, while fibrinogen levels promptly rose again. Reciprocal divergences of these values were present during the first week of the second course of treatment, when the gamma globulins continued to rise, while the fibrinogen was already on its way down.

Discussion. It seems likely that the difference in the rates of response to hormone therapy of fibrinogen and gamma globulin levels reflects the rates of synthesis of these two proteins. The best estimate of the rate of gamma globulin turnover was made by Schoenheimer *et al.*(9) who found that the half life of an individual gamma globulin molecule is approximately 2 weeks. Our observation of a one-week delay of the gamma globulin response to hormone therapy would fit in well with these data. Fibrinogen, on the other hand, would seem to be a protein with a very rapid turnover, particularly when it is noted that its absolute level in the serum can change 2- or 3-fold within a few days' time. Hence, the effect of ACTH or Cortisone therapy on this protein could be expected to manifest itself rather promptly.

The role of the adrenal cortical hormones in the lysis of lymphoid tissue is readily recognized as possibly an important factor in lowering the blood gamma globulin(10). An understanding of the effect of the cortical steroids on the fibrinogen, however, must rest with a fuller knowledge of the effect of these hormones upon liver metabolism, as fibrinogen seems to be manufactured by this organ(11).

While it is possible that the changes in the blood protein levels responsible for the sedimentation rate are entirely secondary to variation in disease activity, it is also reason-

8. Ham, T. H., and Curtis, F. C., *Medicine*, 1938, v17, 413.

9. Schoenheimer, R., Ratner, S., Rittenberg, D., and Heidelberger, M., *J. Biol. Chem.*, 1942, v144, 545.

10. Selye, H., *Endocrinology*, 1937, v21, 169.

11. (a) Drury, D. R., and McMaster, P. D., *J. Exp. Med.*, 1929, v50, 569; (b) Jones, T. B., and Smith, H. P., *Am. J. Physiol.*, 1930, v94, 144.

able to assume that they are the result of a direct action of the cortical steroids upon their manufacture. Such an explanation would be consistent with the known "anti-anabolic" properties(12) of these hormones. Therefore, until more data is accumulated on this point, it will be wise to use the sedimentation rate in parallel with the eosinophil count, merely as an index of continued hormonal activity, not as a criterion of effectiveness of therapy on disease activity.

Conclusions. 1. Both ACTH and Cortisone therapy depress the gamma globulin and fibrinogen levels in the blood of patients with active rheumatoid arthritis and scleroderma.

2. Changes in these 2 blood proteins are responsible for the observed changes in the sedimentation rate.

3. At the present state of our knowledge, the sedimentation rate cannot be used as an index of the effectiveness of hormone therapy on the active disease process.

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12. Albright, F., *Harvey Lect.*, 1942-43, v38, 123.

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Fat Necrosis Produced by Exposure of Pancreatic Duct and Duodenal Mucosa.* (18461)

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In previous papers(1,2) the effects of experimental injuries to the pancreas were reported. It had been found that open transsection of the main pancreatic duct or the lengthwise incision of the main duct and its two main branches was not followed by fat necrosis unless the animal had been fed before or after the operation, or when cholinergic drugs had been given postoperatively. Extensive crushing or complete transverse tearing of the pancreas did not result in any intra-abdominal fat necrosis, regardless of whether the animals were fed or starved pre- or postoperatively. In a discussion of these results an experimental procedure was suggested of which no report was found in the pertinent literature. Nevertheless, this paper is not a claim for an original procedure and is merely a report on the use of an effective method for the experimental production of fat necrosis.

Twenty male and female mongrel dogs, weighing from 7 to 12 kg, were subjected to an aseptic operation under pentobarbital-sodium anesthesia. The main pancreatic duct was exposed and carefully excised together with a circular flap of duodenal wall approximately 2 cm in diameter, with the orifice of the duct in its center; the opening in the duodenum was closed. It was possible to perform this procedure with a minimum of contamination. The dogs were fasted pre- and postoperatively, but water was given freely after the operation. Most animals received penicillin and streptomycin postoperatively.

Of these 20 dogs, 3 showed no ill-effects. They were sacrificed on the 3rd, 4th, and 18th day respectively, and there was no evidence of intraperitoneal disease. A fourth dog, which was in good shape and would probably have survived, was sacrificed on the 8th postoperative day, and autopsy revealed a moderate amount of disseminated fat necrosis. In 2 of these 4 dogs, the duodenal flap

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1. Popper, H. L., *Surg. Gynec. Obst.*, 1949, v88, 254.

2. Popper, H. L., *Am. J. Dig. Dis.*, 1949, v16, 343.