

Alteration of Serum Protein Patterns Following Adrenalectomy in Rabbits.* (27279)

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During the past decade, since ACTH and adrenocorticosteroids have become available, great advances have been made toward understanding the role of the adrenal gland and its secretions in health and disease. Many observations made on adrenalectomized animals prior to this period, nevertheless, still remain without adequate explanation.

One such observation concerns the serum protein alterations which occur in the adrenally insufficient animal. In 3 publications(1, 2,3) the phenomenon of increased amounts of protein in sera of cats and dogs following adrenalectomy was pointed out, the increase being wholly accounted for by increases in the globulin fraction. Substitution therapy employing crude preparations of adrenal cortical extract or desoxycorticosterone resulted in restoration of total protein values to normal with the globulin values remaining elevated.

McCullagh and Lewis(4) in studies done with the Tiselius free electrophoresis technic on plasma protein from 19 patients with Addison's disease, demonstrated that during periods of adrenal insufficiency both total protein and globulin values were elevated. The increase in globulin was not found consistently in any certain fraction or fractions. Their experience with substitution therapy with desoxycorticosterone and large amounts of adrenal cortical extract paralleled that described above in experimental animals.

The relationship of the adrenal gland to this distortion of the serum protein pattern thus is well established. Further investiga-

tion seems appropriate in that many diseases in man for which the adrenal steroids are beneficial, such as rheumatoid arthritis or disseminated lupus erythematosus, are characterized by a similar alteration of the serum protein pattern.

Materials and methods. Animals. Young white New Zealand male rabbits weighing 2.3 to 4.0 kg were used. All were obtained from a single breeding source (Totem Farms, Edmonds, Wash.) and, to ensure their freedom from infection, were kept routinely for 2 weeks in the animal quarters before being studied. They were fed Purina rabbit pellet ration and greens, and following adrenalectomy, were maintained additionally on 1% salt water solution.

Surgical procedure. The rabbits were anesthetized with a nembutal-alcohol mixture injected intraperitoneally. Preoperatively, the day of surgery, each animal was injected intramuscularly with 300,000 U Benzathine Penicillin G and 25 mg of cortisone acetate. No other medication was given to these animals subsequently.

The initial 9 rabbits were operated in 2 stages, but with increasing experience, the bilateral adrenalectomy was done in one stage. The adrenal glands were approached through bilateral flank incisions. As mentioned by others(5), removal of the left adrenal gland posed no problem. The gland on the right side, sharing a common fascial capsule with the inferior vena cava, was teased off as much as possible with blunt dissection. The gland then was partially excised and the remaining portion wiped off with a dry gauze sponge until no adrenal tissue was evident grossly. Bleeding from the adrenal bed was minimal and usually subsided spontaneously. Finally, the adrenal bed was wiped off carefully with another gauze sponge soaked in absolute ethanol, this procedure sufficing to destroy any adrenal cortical tissue remnants.

Experimental procedure. Blood was taken

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from the marginal ear vein of each rabbit at regular intervals, usually on alternate days in the immediate postoperative period, and once or twice a week thereafter. Approximately 2 to 4 ml of blood was collected in a plain tube and allowed to clot, and serum was removed from the sample. A small quantity also was collected in an oxalated tube for microhematocrit determinations at regular intervals. Various studies usually were carried out immediately on these sera; if not, the samples were frozen and kept at -20°C until analyzed.

A detailed postmortem examination of the animal was performed routinely on 23 of the 26 animals involved in the experiments, particular attention being paid to a search for accessory adrenal tissue.

Protein methods. Total serum protein was determined by the Biuret method of Weichselbaum(6) standardized by a microKjeldahl analysis for nitrogen. The serum protein was fractionated by paper electrophoresis with the Spinco Model R apparatus. The strips were stained with Bromophenol blue and analyzed with the model RB Analytrol densitometer.

Results. Normal rabbits. The average total serum protein value for 20 animals was 5.80 g/100 ml (Table I). Values for the individual protein fractions obtained in this group of rabbits are quite comparable to those obtained in 1945 by Deutsch and Goodloe(7) with the Tiselius free electrophoresis apparatus. Characteristically, the gamma globulins are rather low in rabbits, the average in this group of animals being 6.5% as compared to 4.3% obtained by the above investigators.

Adrenalectomized animals. Table I records the average values for total serum protein and the different electrophoretic fractions and shows the serial alterations which occurred with time following adrenalectomy. Shortly following operation, the alpha-2 and beta globulins showed significant elevations, despite the fact that total protein and gamma globulin values remained relatively constant. By the fifth day, although one animal already had manifested a distinct elevation of its gamma globulin level from 8 to 16% the ma-

jority showed only a continued elevation of the alpha-2 and beta globulins.

By the 10th day, the mean serum gamma globulin value had achieved a significant increase, the beta globulin value had begun to decrease, and the alpha-2 globulin had returned to a normal value. In all of the animals the alpha-2 and beta globulin fractions had returned to normal levels by the 15th day, but the gamma globulin values remained elevated indefinitely. Two of these animals still are living after more than 150 days following adrenalectomy and still manifest gamma globulin levels of approximately 20% at this time.

During the period of these alterations in the globulin pattern, serum albumin concentration decreased slightly and persisted at this lower level, reciprocating for the elevation in the globulin fractions. Total serum protein values, however, showed an opposite effect, increasing by approximately a gram by the 15th day and gradually returning to more normal values.

Initial hematocrit determinations on this group of rabbits prior to the operation showed a mean value of $44 \pm 2.1\%$. Following the blood loss from surgery, there was an initial decrease in the hematocrit, but these values restored themselves to preoperative levels by 2 to 4 weeks after surgery, increasing to slightly higher levels of approximately 46% to 48% shortly thereafter, and persisting at these increased levels thenceforth.

The alterations of serum protein patterns which occurred following adrenalectomy are shown in graphic form in Fig. 1, where mean concentration of each protein fraction (expressed as percent of total serum protein) is plotted as a function of time after adrenalectomy. Fig. 2 depicts serum protein changes which occurred in one illustrative animal following adrenalectomy. The day following bilateral adrenalectomy, total protein and albumin values decreased and alpha-2 and beta globulin fractions increased. On the 4th postoperative day, total protein value had returned to normal, but the albumin persisted at a decreased level and alpha-2 and beta globulins remained elevated. On the 10th day, total protein concentration had increased

to a value greater than preoperative level; the albumin, alpha-2 and beta globulins had returned toward normal; and gamma globulin level had increased from 6% to 11%.

The adrenalectomized rabbits in this ex-

periment did surprisingly well. Eight of the 26 animals died during or shortly following the operation, either from the effects of anesthesia, or most commonly from uncontrollable hemorrhage resulting from accidental

TABLE I. Influence of Adrenalectomy on Serum Protein Concentration in the Rabbit.

Day & No. of animals		Total protein	Albumin	α -1	α -2	β	γ	Globulin & A/G ratio
Preop. (20)	g/100 ml	5.80 $\pm .25^*$.06†	4.10 $\pm .40$.09	.35 $\pm .07$.01	.38 $\pm .11$.02	.53 $\pm .15$.04	.39 $\pm .08$.02	1.70 $\pm .32$.07
	%		71 ± 3.7 .8	6.0 ± 1.1 .2	6.5 ± 1.3 .3	10 ± 1.6 .4	6.5 ± 1.1 .3	A/G 2.41
1-2 days (18)	g/100 ml	5.94 $\pm .71$.17	3.82 $\pm .66$.16	.36 $\pm .08$.02	.51 $\pm .15$.03	.74 $\pm .25$.06	.44 $\pm .09$.02	2.04 $\pm .43$.10
	%		65.3 ± 4.4 1.0	6.1 ± 1.4 .3	8.7 ± 2.2 .5	12.5 ± 2.7 .6	7.4 $\pm .8$.2	A/G 1.87
5 days (18)	g/100 ml	6.30 $\pm .74$.17	4.05 $\pm .29$.07	.32 $\pm .10$.02	.52 $\pm .15$.04	.85 $\pm .23$.05	.51 $\pm .24$.06	2.26 $\pm .40$.09
	%		64 ± 3.8 .9	6 ± 1.9 .4	8 ± 2.2 .5	13 ± 2.7 .6	8 ± 3.02 .7	A/G 1.79
10 days (17)	g/100 ml	6.54 $\pm .73$.17	4.21 $\pm .60$.14	.39 $\pm .07$.02	.45 $\pm .21$.05	.79 $\pm .19$.05	.73 $\pm .34$.08	2.32 $\pm .49$.12
	%		64 ± 6 1.4	6 ± 1.1 .3	7 ± 2.75 .7	12 ± 2.5 .6	10.6 ± 3.3 .8	A/G 1.81
2 wk (15)	g/100 ml	6.84 $\pm .35$.09	4.54 $\pm .38$.10	.40 $\pm .11$.02	.41 $\pm .12$.03	.76 $\pm .20$.05	.73 $\pm .20$.05	2.31 $\pm .36$.09
	%		66.6 ± 4.0 1.0	5.8 ± 1.4 .4	6.0 ± 1.4 .4	11 ± 2.6 .7	10 ± 2.9 .8	A/G 1.97
3 wk (13)	g/100 ml	6.45 $\pm .66$.18	4.38 $\pm .47$.13	.38 $\pm .06$.02	.40 $\pm .14$.04	.72 $\pm .21$.06	.73 $\pm .24$.07	2.24 $\pm .44$.12
	%		66 ± 6.3 1.8	6 $\pm .9$.31	6.2 ± 2.1 .6	9.9 ± 2.4 .7	11 ± 3.3 .9	A/G 1.95
4 wk (10)	g/100 ml	6.10 $\pm .42$.13	4.05 $\pm .52$.16	.31 $\pm .10$.03	.36 $\pm .10$.03	.60 $\pm .30$.09	.72 $\pm .33$.10	2.10 $\pm .72$.23
	%		67.5 ± 10 3.1	5.2 ± 1.6 .5	5.9 ± 1.3 .4	9.7 ± 4.2 1.3	11.7 ± 4.9 1.6	A/G 2.01

* Stand. dev.

† Stand. error of mean.

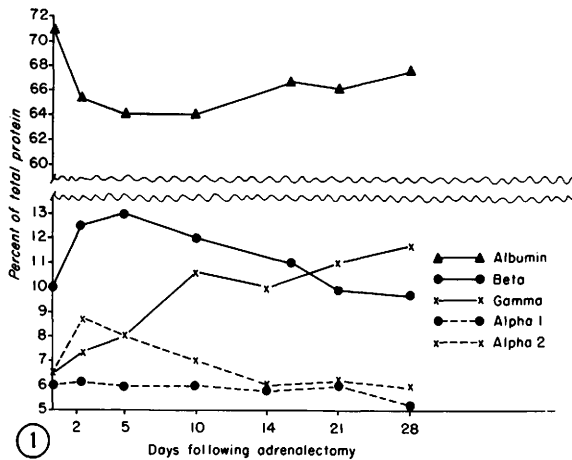
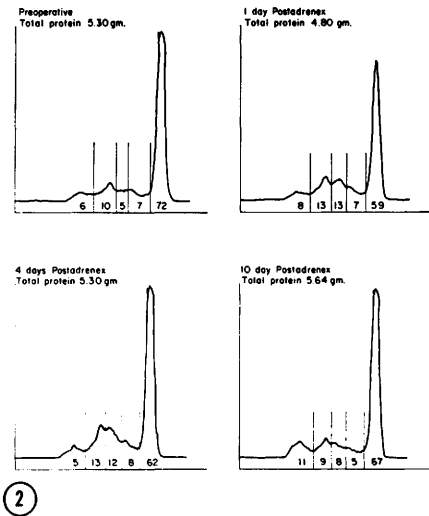


FIG. 1. Effect of adrenalectomy alone on mean % concentrations of various electrophoretic fractions of serum proteins in rabbits.

FIG. 2. Serial changes in electrophoretic tracings of serum proteins in a rabbit following adrenalectomy. Numbers superimposed upon individual protein fractions indicate % of total protein.



puncture of the inferior vena cava.

Of the 18 animals that survived long enough to be studied, 12 showed an increase in gamma globulin of 4% or more above the original level. The majority of these animals lived beyond 30 days following adrenalectomy, and currently, there are 3 animals that have survived more than 150 days.

Death in 5 of the animals was attributable to infection as revealed by post mortem examination, and generally occurred much after the onset of "hypergammaglobulinemia" in the animals.

A group of the surviving animals died in an unusual manner. Eight of them were subjected to an ACTH test to determine whether they had residual adrenal cortical function following the surgery. Within 2 to 12 hours following ACTH administration, 6 of the 8 animals died. This high rate of mortality prohibited further use of the test and brought up a most interesting question concerning the relationship of ACTH to death in the animals. A similar experience with the use of ACTH in cases of Addison's disease in man, has been reported(8) and further studies to investigate this phenomenon will be made.

Sham operations. Two rabbits were subjected to sham operation with the adrenals left intact. Inadvertently, in one animal the

small intestine was incised, resulting in an outpouring of greenish intestinal contents into the peritoneal cavity; nevertheless, the wound was repaired and the operation carried to completion. In this, as well as the other animal, the post-operative course was uneventful. Serial electrophoretic analyses of the serum proteins revealed, as in the adrenalectomized animals, early increases in alpha-2 and beta globulins with gradual return to normal levels by 10 days. Gamma globulin concentration remained stable throughout, even in the rabbit with peritonitis.

The post-operative management of these adrenalectomized rabbits presented little difficulty; the only noticeable change in their condition throughout the post-operative period was an increased intake of salt solution beginning about 4 to 5 days after surgery and continuing for about 2 weeks. They thrived under normal care-taking conditions of the general animal quarters and appeared normal in all respects. Infections terminating in rapid death occurred sporadically.

The immediate change in serum alpha-2 and beta globulin fractions can be classified as an acute phase reaction. Chanutin and Gjessing(9) found similar changes in sera of dogs injured by various means such as scald-

ing, freezing or turpentine injection.

The rather abrupt appearance of significantly increased amounts of gamma globulin in the circulation of these adrenalectomized animals is of considerable interest. Biologically, the gamma globulins are distinguished by their antibody function, but whether or not all gamma globulin represents antibody has not been answered satisfactorily. Surgery alone could not explain this phenomenon of increasing gamma globulin levels, particularly in view of the experience with the sham operated animals. As indicated by the serum protein and hematocrit values there probably is a degree of hemoconcentration present. However, if this were responsible for the changes in the serum protein pattern alterations, all fractions should show similar changes.

The hormones of the adrenal gland seem to exert some control over the level of circulating gamma globulin. It has been well documented that large amounts of adrenal steroids can suppress the formation of antibody, and thus conceivably diminish the amount of circulating gamma globulin(10). The results of this study demonstrate, conversely, that serum gamma globulins increase in the circulation following surgical extirpation of the adrenal glands alone.

Summary. Bilateral adrenalectomy was performed in 26 rabbits. In most of the 18

survivors, significant alterations in the concentrations of the various serum protein fractions were demonstrated by paper electrophoresis. Acutely, alpha-2 and beta globulin fractions increased sharply. By the end of the second week, gamma globulin had increased significantly and alpha-2 and beta globulins had returned to normal. Associated with these serum protein pattern alterations were suggestive evidences of hemoconcentration, as revealed by increased total serum protein and hematocrit values.

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Serum Antibody and Protein Studies Following Adrenalectomy in Rabbits.* (27280)

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Cortisone is known to exert marked effects on many immunologic mechanisms, the depression of specific antibody response with large doses of this agent being one of these known actions(1). However, although the immune mechanisms in the adrenally insufficient animal have been studied to some extent, they are still largely unknown, as judged by the lack of information in the literature.

However, several studies have been con-

cerned with the influence of adrenalectomy

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