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 concerned with the influence of adrenalectomy

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on production of antibodies in experimental animals. Most of these experiments were conducted prior to the widespread availability of adrenocortical hormones; 2 of these studies(2,3) could not demonstrate enhancement of the antibody reaction, but all of the other investigations(4-8) showed a definite increase in antibody titers in adrenalectomized animals as compared to intact controls.

In view of present concepts relating precipitating antibodies to serum gamma globulins, it seemed evident that the observations mentioned above might be closely related to the finding of increased gamma globulins in adrenalectomized animals discussed in the preceding paper(9). To investigate this, studies correlating specific antibody responses to bovine serum albumin with serum protein alterations in adrenalectomized animals were undertaken; the results of these studies are discussed here.

Methods. The animals and serum protein determination methods employed, and the surgical technic have been described(9). Sera for antibody determinations were obtained before and on alternate days immediately after immunization, and followed in this manner until the peak titers were obtained; then the determinations were done twice weekly. Crystallized Bovine Serum Albumin‡ (BSA) was dissolved in sterile isotonic saline solution, 100 mg per 10 ml of solution. Each rabbit was injected intravenously with 50 mg of the bovine serum albumin in 1 dose, regardless of body weight. The amount of antibody present in the serum was determined by noting the highest dilution of antigen which was precipitated by the anti-serum. Serial dilutions of the original antigen solution described above, beginning with a 1:2 dilution, were overlayered with the serum in capillary tubes; after overnight incubation at 37°C, the final reading was made.

Results. With the animals employed and the dosage of antigen used in this experiment, the normal intact animals showed surprisingly uniform levels of circulating precipitating antibody in response to this primary immunization. Four of the 5 normal animals developed titers of 1:8200 and the other, a titer of 1:4100. The peak titer occurred on the 10th day in all animals.

‡ Armour Crystallized Bovine Plasma Albumin (Lot #V68802).

TABLE I. Maximum Titer of Anti-BSA Antibody.

Normal controls			Adrenalectomized rabbits		
Rabbit		Days	Rabbit		Days
3	1:8200	10	7	1: 65,000	14
4	1:8200	10	8	1:262,000	14
10	1:8200	10	9	1: 16,000	14
11	1:8200	10	18	1:131,000	14
12	1:4100	10	19	1:131,000	14
			23	1: 65,000	16
			25	1:131,000	16

It is evident from Table I that higher levels of antibodies were attained in adrenalectomized than in normal intact animals. With one exception, the increase represented 3 or 4 tube dilutions beyond the expected normal values. These maximum titers also were attained approximately 14 to 16 days after injection of the antigen, as compared to peak titers attained in normal rabbits on the 10th day. The results showed no definite relation to time of adrenalectomy, the injection of the soluble protein antigen ranging from 7 to 20 days after operation in this group of animals.

Fig. 1 illustrates the sequence of serum protein alterations in a rabbit subjected initially to adrenalectomy and then to the antigen injection. Gamma globulin had increased from 7% to 14% by 10 days after adrenalectomy, at which time the other globulin fractions showed no significant alteration. Following BSA administration in this animal, 15 days after immunization and 29 days after adrenalectomy, the gamma globulin fraction increased further to 21%, correlating well with the peak antibody titer of 1:65,000 obtained at this time. The albumin/globulin ratio had also reversed itself.

In view of the increased gamma globulin level that can be induced with adrenalectomy alone, an experiment was carried out to observe the relationship of circulating gamma globulin concentration in these adrenalectomized animals to previous exposure of the animal to an antigen. Specific antibody to bovine serum albumin first was produced by immunization with BSA; when this antibody had disappeared from the circulation, the animal was adrenalectomized to determine whether or not the specific antibody would

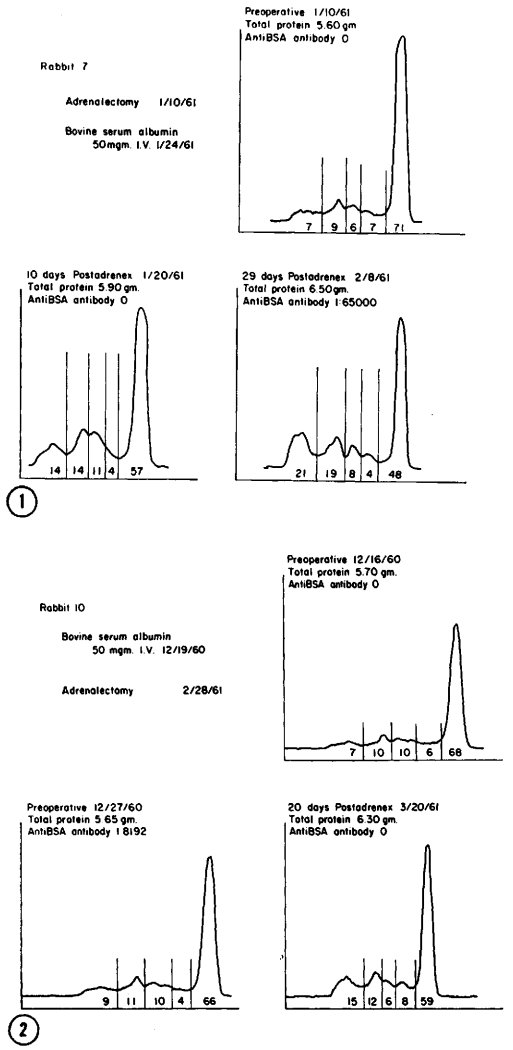

FIG. 1. Changes in electrophoretic patterns of serum protein in a rabbit, reflecting first adrenalectomy (1/20/61), then intrav. inj. of bovine serum albumin (2/8/61). Numbers below tracing represent % concentration of total proteins.

FIG. 2. Serial alterations of the electrophoretic patterns of serum protein in a rabbit, following intrav. inj. of bovine serum albumin (12/27/60), then adrenalectomy (3/20/61). Numbers below figures represent % concentration of total protein.

reappear with the appearance of increased gamma globulin. Four animals were studied; 3 of the 4 demonstrated a significant "hypergammaglobulinemia" following the adrenalectomy. None of these animals showed reappearance of specific anti-BSA antibody concurrent with development of increased gamma globulin levels following adrenalectomy.

Fig. 2 illustrates results of this experiment in one animal. The first tracing shows the normal pattern obtained in the control period. Following the antigenic stimulation, gamma globulin increased slightly to 9%. Two months after this pattern had been obtained, when the specific antibody had disappeared from circulation, and 20 days after bilateral adrenalectomy, gamma globulin had increased to 15%, but the anti-BSA antibody failed to reappear in the circulation.

Discussion. These observations confirm previous experiences of serum antibody levels in adrenalectomized animals. The antibody titers to bovine serum albumin were definitely increased in comparison to titers obtained in normal intact control animals. Previous studies demonstrating greater antibody increases in adrenalectomized than in control animals following antigenic stimulation all have employed multiple injections of the antigen, thus involving secondary anamnestic responses. The present experiments demonstrate that significantly higher levels of circulating precipitating antibodies also can be produced by a single injection of antigen in adrenalectomized as compared with control animals.

There is also a delay in occurrence of maximum antibody levels in the adrenalectomized group of animals. All of the normal animals showed maximum response by the 10th day, whereas maximum titers were not seen in the adrenalectomized animals until the 14th or 16th day.

The observation that the secondary hypergammaglobulinemia produced by adrenalectomy does not recall the specific anti-BSA antibody into circulation, once it has disappeared, would seem to indicate that the resultant gamma globulin increases following adrenalectomy are not related to a previous exposure of the animal to antigen, and suggest the possibility that all circulating gamma globulin may not have a specific antibody function. Obviously, such a conclusion requires further study. Nevertheless, these observations demonstrate that the adrenal gland secretions exert a profound effect upon concentration of gamma globulin in circulation. This fraction is related to specific antibody function as demonstrated by the correlation of higher antibody levels to induced hyper-

gammaglobulinemia in the adrenalectomized state.

The critical question as to the mechanism of the increased sensitivity of these animals to antigenic stimulation remains unanswered. Whether their increased antibody production is a primary effect of absence of adrenal hormones or is a secondary phenomenon reflecting decreased plasma volume, hemoconcentration, or other direct effects of adrenal depletion is not known. Further studies employing more exacting quantitating technics would seem to be required.

Summary. Increased circulating levels of antibody following a single intravenous injection of bovine serum albumin were demonstrated in adrenalectomized rabbits, as compared with normal intact controls. Additionally, there was a delay in appearance of maximum titers of antibody in the test group of animals. In animals previously immunized with bovine serum albumin, adrenalectomy produced increased gamma globulin concentrations but failed to recall specific anti-BSA antibody into circulation.

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Metabolic Rate of Tree-Shrews (*Urogale everetti*).* (27281)

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The tree-shrews (Tupaiidae) comprise a family concerning which there has been some taxonomic disagreement, some authorities considering them as belonging to the insectivores, while others believe they are the closest living representative of the primitive, generalized primates. The evidence supporting the latter view makes these animals of special interest, for the availability of a small (rat-sized) primate for laboratory investigations in endocrinology, nutrition, metabolism, and radiobiology would be desirable.

During general observations on a small colony of Philippine tree-shrews data were obtained on their metabolic rates. It was of interest to compare these rates with the high rates reported for insectivore shrews(1).

Sex and age. Of the 32 animals tested, 20 were females and 12 males. No precise ages are known. They had been captured as immature animals at least 9 months prior to the metabolic rate determinations. Roentgenograms made 1 month before these determinations showed complete epiphyseal union in all long bones in 28 of the 32 animals, although in many the persistence of the "scar" of epiphyseal union suggested its recent completion. In the remaining 4 animals (2 male, 2 female) the majority of epiphyses showed completed union, but the persistence of notches at the tibial tuberosity and of partial epiphyseal plates (chiefly at the distal ends of radius and ulna) suggested that these animals were slightly younger, perhaps in late adolescence.

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