tant role in many biological phenomena which follow antigen-antibody combination, such as C′-fixation, skin reaction with soluble complexes, as well as anaphylactic reactions. It is of interest that aggregated gamma globulins give allergic-like responses and this suggests the possibility that some allergic dieases might be induced by molecular changes of the gamma globulin *in vivo*.

Summary. 1. Aggregated human gamma globulin, obtained by heating, gave immediate skin reactions in normal guinea pigs and inactivated complement, whereas aggregated bovine gamma globulin did not have either activity. 2. Aggregated human gamma globulin and antigen-antibody complexes are comparable on weight basis with respect to skin reactivity and complement inactivation. 3. Divalent cation(s) are required for inactivation of complement by aggregated HGG. Inactivation of C′3 by aggregated HGG is markedly dependent upon temperature. 4. A tentative hypothesis concerning the mechanism of complement fixation by antigen-antibody reaction is presented.

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Effect of Sodium Chloride Feeding on Adrenocortical Hormone Secretion of Salt Deprived Rats.* (25117)

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Sodium deprivation in rats results in increased secretion of aldosterone but total adrenocortical hormone production is decreased because of diminished synthesis of corticosterone and other steroids(1). These changes in hormone secretion take place gradually after withdrawal of sodium from the diet and require several weeks to become fully manifest. Alterations in adrenal steroid secretion induced by sodium restriction are reversible but it is not known how long it takes for cortical hormone production to become normal once adequate sodium intake is resumed. The purpose of this investigation was,

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TABLE I. Body Weights, Adrenal Weights, and Adrenocortical Hormone Secretion of Various Groups (Mean Values ± Stand. Error of Mean).

Group	Body wt (g)	Adrenal wt (mg/100 g body wt)	Adrenal steroid secretion (μg/100 mg adrenal wt)
Sodium deficient rats (16)*	120.1 ± 6.3	18.85 ± .93	55.7 ± 4.0
1 day salt replacement (8)	127.6 ± 8.0	17.37 ± .97	76.1 ± 7.4
2 " " " (8)	146.3 ± 8.7	15.55 ± .78	101.7 ± 9.6
Pair fed controls (16)	180.9 ± 2.3	13.92 ± .68	95.6 ± 6.6

* No. of animals in each group.

therefore, to determine the effect of salt replacement on secretion of cortical hormones by the adrenals of salt depleted rats.

Methods and procedure. Thirty-two male Wistar rats, weighing 50-60 g, were fed a sodium deficient, synthetic diet *ad lib.*(2). Sixteen control rats received the same diet to which was added 0.24% sodium (as sodium chloride) and were pair-fed with the deficient group. After the experiment was in progress 1 month, 16 rats were randomly selected from deficient group and fed the salt-containing (control) diet *ad libitum*. Eight of these animals consumed control diet for 1 day and the other 8 for 2 days before being sacrificed. Rats remaining in deficient group were maintained on the sodium-free regimen for 1 or 2 more days and were then sacrificed along with control animals. Animals were killed by decapitation and adrenals dissected free of surrounding tissue, weighed and bisected. Both adrenals from each rat were placed in 2 ml of Krebs-Ringer-phosphate solution, pH 7.4, which contained 200 mg % glucose. One unit of ACTH was added and the mixture incubated aerobically for 2 hours at 37°C in a Dubnoff Metabolic Incubator. On completion of incubation steroid hormones secreted into medium were recovered and determined by a method which has been previously described (3). This procedure utilizes a spectrophotometric technic which measures steroid compounds possessing an alpha, beta unsaturated ketone structure in ring A. After adrenal steroid secretion of each animal was determined, the solution containing the hormones was combined with that from other animals in the group and individual steroids separated by paper chromatography(4).

Results. (Table I). Rats fed the sodium deficient diet gained weight slowly but the final mean body weight of this group was significantly less than that of pair fed controls ($P = <.001$) or the group refed salt for 2 days, $P = <.025$). Average body weights of deficient rats and those refed salt 1 day differed only slightly.

Adrenal hypertrophy occurred in salt deprived rats as demonstrated by an increased adrenal weight-body weight ratio (AW/BW) when compared to that of pair-fed controls, ($P = <.001$). The AW/BW of rats given salt replacement for 1 day was less than that of the deficient group but this difference was not large enough to be statistically significant. Two days of salt refeeding resulted in a further decrease in adrenal weight, however, so that the difference in AW/BW between this group and deficient rats was significant, $P = <.05$.

Hormone secretion by adrenals of sodium depleted rats was approximately one-half that of control animals, the difference being highly significant, $P = <.001$. After 1 day of salt replacement there was an increase in steroid secretion so that difference in hormone production by this group and deficient rats was significant, $P = <.05$. A further increase in adrenal hormone secretion occurred in animals given salt refeeding for 2 days so that the value for this group was about the same as that of control rats. Difference in hormone production by the group refed salt for 2 days and deficient group was significant, $P = <.001$.

Previous investigation has shown that isolated adrenals of normal rats secrete aldosterone, corticosterone and other hormones with corticosterone being produced in largest amount(5,6,7). Adrenals of sodium depleted rats secrete relatively large amounts of aldosterone but elaboration of other hormones vir-

tually ceases if salt restriction is prolonged (1). In this study chromatographic separation of steroids secreted by adrenals of control rats showed a normal pattern. Steroid production by adrenals of rats depleted of sodium for 1 month consisted almost entirely of aldosterone and no corticosterone could be detected. Chromatographic analysis of adrenocortical hormone secretion of rats fed a salt-containing diet for 1 day after being deprived of sodium for 1 month revealed an increased amount of aldosterone, although the quantity was less than that produced by depleted animals not subjected to salt replacement. Furthermore, a small amount of corticosterone was secreted by adrenals of these rats whereas adrenals of deficient animals did not produce this hormone. The pattern of adrenal steroid secretion after 2 days of salt replacement was essentially normal, although a slight increase in aldosterone was still evident.

Discussion. Sodium deficiency induced in rats results in alterations of adrenocortical structure and function. Hypertrophy of the zona glomerulosa and increased aldosterone secretion occur and there is secondary atrophy of the zona fasciculata with reduced synthesis of corticosterone(1,2,8). These changes gradually become more marked as salt deprivation is continued and body sodium content declines. Studies of the effect of salt replacement on the adrenal cortex of sodium depleted rats have shown that structural abnormalities are partially corrected after 24 hours and are almost completely reversed within 48 hours(9). Reappearance of a normal histological pattern after salt refeeding was considered to indicate that cortical hormone secretion had returned to the original level although steroid determinations were not carried out.

In our experiment it was demonstrated that resumption of salt feeding after a period of sodium restriction resulted in a rapid increase in total cortical hormone secretion so that within 2 days normal levels were attained. Furthermore, as body sodium stores were replenished there was a decline in production of aldosterone and synthesis of corticosterone, which had virtually ceased, returned to normal. These observations show that abnormalities of adrenal steroid secretion induced by salt depletion can be quickly corrected when sodium concentration approaches physiological levels. Changes in sodium content undoubtedly affect cortical hormone production by altering activity of certain enzyme systems although the mechanism by which these reactions are influenced is not understood. Regardless of mechanism of control it is apparent that body sodium content is an important factor affecting hormone secretion by the adrenal cortex.

Summary. Effect of salt replacement on secretion of adrenocortical hormones by rats which had been deprived of sodium for 1 month was investigated. Results demonstrated that total cortical hormone production, which had been depressed, increased to equal that of control animals; that elevated aldosterone synthesis declined to previous levels; and that cortisterone secretion, which had been undetectable, increased rapidly. Thus, within 48 hours after resumption of salt feeding secretion of adrenocortical hormones returned to normal.

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