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Effects of Dietary Factors on Production of Adrenal Steroid Hormones

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ALTHOUGH many dietary factors have been considered to be related to the production of adrenocortical hormones, the data are in most instances insufficient to provide conclusive evidence of such relationships. Among the nutrients thought to be concerned with function of the adrenal cortex, those that have been most thoroughly investigated are ascorbic acid, pantothenic acid, and sodium. It is the effect of these nutritional factors on the production of adrenal steroids that will be discussed here.

ASCORBIC ACID

One of the first observations which related a dietary factor to adrenocortical hormone secretion was the demonstration that vitamin C is present in the adrenal cortex in high concentration.[1] Not only does this gland contain a large amount of ascorbic acid but one of the cardinal manifestations of scurvy in the experimental animal is the occurrence of adrenal hypertrophy.[2] Further investigation demonstrated that as the adrenal cortex responds to corticotropin (ACTH) by secreting its characteristic hormones, there is a concomitant fall in the adrenal ascorbic acid concentration.[3] These facts stimulated interest in the relationship of this water-soluble vitamin to adrenocortical function and suggested that ascorbic acid is implicated in the synthesis of adrenal steroid hormones.

In an effort to determine the role of ascorbic acid in adrenocortical function, an investigation of the secretion of adrenal steroid hormones by scorbutic guinea pigs was undertaken in our laboratory.[4] Guinea pigs were fed a vitamin C-free diet and maintained on this regimen until severe signs of scurvy, such as loss of weight, joint swelling, and bloody diarrhea, were evident. ACTH was then administered to these animals and changes in the number of eosinophils in the peripheral blood were followed. It was found that ACTH administration resulted in a marked decline in circulating eosinophils, thus indicating a release of cortical hormones at a time when the adrenal gland on direct chemical analysis contained only traces of ascorbic acid.

This observation was corroborated by Nadel and Schneider[5] who measured the urinary excretion of hydrocortisone by scorbutic guinea pigs. These investigators found that increased quantities of this steroid hormone were present in the urine of animals that had severe manifestations of vitamin C deficiency. Further confirmation of these findings was ob-

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tained by Done *et al.*[6] who measured the concentration of adrenocortical hormones in the peripheral blood of guinea pigs with severe scurvy. It was demonstrated that a ten-fold increase in blood 17-hydroxycorticoids occurred in vitamin C-deficient guinea pigs when compared to control animals.

As a result of these and similar studies, it is now generally felt that ascorbic acid is not directly concerned with the synthesis and release of adrenocortical hormones but that vitamin C deficiency functions as a nonspecific stress and, as such, results in increased steroid secretion by the adrenal cortex.

PANTOTHENIC ACID

Attention was first drawn to the relationship between pantothenic acid and the adrenal cortex by the experiments of Morgan[7] and Daft and associates[8] in which it was demonstrated that adrenal hypertrophy, hemorrhage, and necrosis were prominent manifestations of pantothenic acid deficiency. These observations were confirmed and extended by Deane and McKibbin[9] who found, using histochemical technics, that the adrenal cortex of pantothenate-deficient animals has a reduced content of lipid, sudanophilic material, and ketosteroids. The occurrence of these structural alterations stimulated investigation of the functional status of the adrenal cortex in animals deficient in this vitamin.

Winters, Schultz, and Krehl[10] studied the effects of administration of ACTH and epinephrine to pantothenic acid-deficient rats and found that these agents failed to produce a significant decrease in the number of circulating eosinophils, whereas cortisone injection was followed by a marked decline in the eosinophil count. These investigators also demonstrated reduced fasting blood sugar levels and increased sensitivity to insulin in deficient animals.[11] Dumm and co-workers[12] made the important observation that cholesterol, a key substance in the synthesis of adrenal steroids, is present in reduced amount in the adrenal cortex of pantothenate-deficient rats following acute stress. Although these investigations, as well as others, suggested that there is diminished secretion of steroid hormones by the adrenals of pantothenate-deficient animals, cortical hormone production was measured by use of indirect methods. For this reason, studies designed to assess the secretory capacity of the adrenal cortex of pantothenic acid-deficient rats have been conducted in our laboratory. In these experiments, steroid secretion of the isolated adrenal glands of pantothenate-deficient and control animals in response to stimulation by ACTH has been studied.[13] The advantage of this approach is that it permits direct chemical determination of hormones secreted by the adrenal cortex.

METHODS

An outline of the procedure used to measure the secretion of steroid hormones by the isolated rat adrenal gland[14] is presented in Table I. The adrenals are removed from the animal, dissected free of surrounding tissue, weighed, and bisected. Each adrenal is placed in a beaker containing 2 ml of Krebs-Ringer-phosphate-glucose solution, pH 7.4. One unit of ACTH is added and the mixture incubated aerobically for two hours at 37° C in Dubnoff metabolic incubator. On completion of incubation, the steroids are extracted from the

TABLE I

Flow Sheet Describing Recovery and Quantitative Determination of Steroids Secreted by the Isolated Rat Adrenal

One rat adrenal (bisected) placed in 2 ml Krebs-Ringer-phosphate solution, pH 7.4, with 1 unit ACTH

O_2 | 37° C for 2 hours

Extracted with 5 ml Ethylene Dichloride ($EtCl_2$)

centrifuged

Aqueous layer aspirated and 4 ml of $EtCl_2$ removed

$EtCl_2$ taken to dryness under N_2 at 50° C

Residue dissolved in 3 ml CH_3OH

Spectrophotometric determination | Porter-Silber determination

aqueous medium with ethylene dichloride and the latter solution taken to dryness. The residue containing the steroids is dissolved in methyl alcohol and aliquots of this solution are utilized for determination of steroid concentration by a spectrophotometric technic and by the Porter-Sibler method. The spectrophotometric procedure detects steroids which possess an alpha-, beta-unsaturated, ketone structure and, thus, measures total production of biologically active steroid hormones by the adrenal gland. The Porter-Silber method detects steroids such as cortisone and hydrocortisone which contain a 17,21 dihydroxy-20-ketone side chain.

Recovery of steroids utilizing this method was determined by adding a known amount of cortisone to the incubation mixture. It was demonstrated that recovery of this steroid was practically complete as measured by both the spectrophotometric and Porter-Silber methods (Table II).

Table III compares the steroid secretion by isolated adrenals of normal rats as measured by the two procedures. It is apparent that the spectrophotometric assay detects more than twice the quantity of steroid material determined by the Porter-Silber reaction. This observation is explained by the fact that the hormone secreted in largest quantity by the rat adrenal is corticosterone, a steroid which is measured only by the spectrophotometric method.

TABLE II

Recovery of Cortisone From Incubation Medium[14]

	Spectrophotometric method	Porter-Silber method
10.3 mcg cortisone added	10.78 ± 0.19 mcg* (104.7%) 11**	9.6 ± 0.11 mcg* (93.2%) 12**

* Mean ± Standard Error of Mean.
** No. of Determinations.

TABLE III

Steroid Secretion by the Rat Adrenal Gland *in vitro*; 19 Determinations in Each Series[14]

	Spectrophotometric method	Porter-Silber method
Actual steroid secretion/adrenal	11.4 ± 0.77 mcg	4.86 ± 0.24 mcg
Steroid secretion per 100 mg adrenal wt	80.1 ± 5.28 mcg	34.1 ± 4.58 mcg

* Mean ± Standard Error of Mean.

RESULTS

The results of our studies in pantothenic acid-deficient rats demonstrate that cortical hormone secretion per adrenal was significantly reduced when compared to that of pair-fed and ad libitum-fed controls (Table IV). Because there was a marked increase in the ratio

TABLE IV[13]

Steroid Secretion by the Isolated Rat Adrenal Gland

	Spectrophotometric determination (micrograms of steroid secreted per adrenal)	Porter-Silber determination (micrograms of steroid secreted per adrenal)	Spectrophotometric determination (micrograms of steroid secreted/100 *mg* adrenal tissue)	Porter-Silber determination (micrograms of steroid secreted/100 *mg* adrenal tissue)
Group I: Pantothenic acid-deficient animals (25)*	4.68 ± 0.38	2.57 ± 0.18	40.1 ± 3.05	22.8 ± 1.79
Group II: Pair-fed animals (18)*	7.67 ± 0.57[a]	3.57 ± 0.32[a]	62.7 ± 4.0[a]	29.2 ± 1.97[b]
Group III: Ad lib-fed animals (10)*	9.66 ± 0.93[a]	3.50 ± 0.34[b]	67.3 ± 3.9[a]	24.6 ± 2.3[c]

Mean Values ± Standard Error of the Mean:
[a] Difference from Group I statistically significant, P = <0.01.
[b] Difference from Group I statistically significant, P = <0.05.
[c] Difference from Group I not statistically significant.

* Indicates the Number of Animals in Each Group.

of adrenal weight to body weight in the deficient animals, steroid production is also presented as micrograms of steroid secreted per 100 mg of adrenal tissue. When synthesis of adrenal cortical hormones by deficient animals is expressed in this manner, it remains significantly less than that of the pair-fed group. Hormone secretion of deficient animals is also less than that of ad lib-fed controls when measured by the spectrophotometric method, but when determined by the Porter-Silber procedure, there was not a significant difference. It has been mentioned earlier that the Porter-Silber reaction measures less than one half of the total hormone secretion by the adrenal cortex. From these studies we have concluded that, in pantothenic acid-deficient animals the synthesis of adrenal steroids is diminished and that this vitamin is necessary to maintain the functional integrity of the adrenal cortex. These findings are supported by the experiments of Longwell and associates who found that in *in vivo* secretion of adrenocortical hormones by pantothenate deficient rats was diminished.[15]

Since it has been shown that pantothenic acid is essential for elaboration of adrenocortical hormones in response to ACTH, the mechanism by which this vitamin facilitates steroid hormone secretion must be sought. It is known that pantothenic acid does not exist in the free state in the animal body and that the metabolically active form of the vitamin is coenzyme A. Coenzyme A functions as an intracellular carrier of acetyl groups and is necessary for the condensation of these two carbon fragments in the formation of fats, carbohydrates, cholesterol, and steroids. The primary reaction leading to the formation of these complex substances from acetate is the condensation of two molecules of acetyl Co A to form aceto-acetyl CO A. The exact chain of reactions leading to formation of the steroid molecule from this compound is not known, but it is presumed that further condensations take place. Thus, since pantothenic acid is an integral part of coenzyme A, a deficiency leads to depletion of body stores of this coenzyme. It seems reasonable to assume that the end result will be a failure of synthesis of complex substances including the adrenal steroid hormones.

SODIUM

Restriction of sodium intake has been shown to produce alterations in the adrenal cortex of animals and man. Several investigators[16,17] have demonstrated increased size and decreased lipid content of the zona glomerulosa in sodium-deficient animals and these changes have also been observed in human adrenals.[18] It is also known that sodium deprivation alters hormone secretion of this gland. Luetscher and Axelrad[19] observed that restriction of sodium intake in normal men was followed by increased urinary excretion of aldosterone but that there was no significant change in the excretion of other adrenal hormones. These experiments were of short duration and studies of the effects of prolonged sodium restriction on adrenocortical hormone secretion have not been reported. For this reason, an investigation of the effects of severe, chronic sodium deficiency on adrenal steroid secretion was undertaken in collaboration with Dr. Phyllis M. Hartroft, Department of Pathology, Washington University School of Medicine.[20]

Young, male Wistar rats were fed a sodium-deficient, synthetic diet which was otherwise nutritionally adequate. Pair-fed controls received the same diet to which was added 0.6 per cent sodium chloride. Equal numbers of animals from each group were sacrificed 2, 4, 7, 9, 14, 30 and 60 days after beginning the experimental diets. Serum-sodium, potassium, blood hematocrit, body and adrenal weights were determined when animals were sacrificed. One adrenal from each rat was incubated in a physiologic medium with ACTH according to the method described earlier.[14] The steroids secreted into the medium were recovered and quantitatively determined by the spectrophotometric technic. In addition, paper chromatographic separation of the steroid compounds secreted by the adrenals of deficient and control rats was carried out using the toluene-propylene glycol chromatographic system.[21] The remaining adrenal from each animal was used for histologic study.

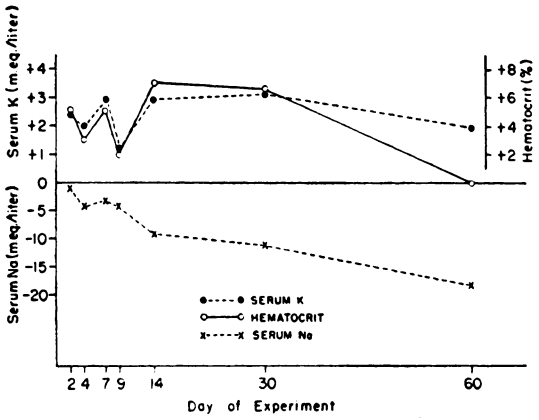

Fig. 1. Serum Sodium and Potassium and Blood Hematocrit of Sodium Deficient Animals Compared with that of Pair-Fed Controls.[20]

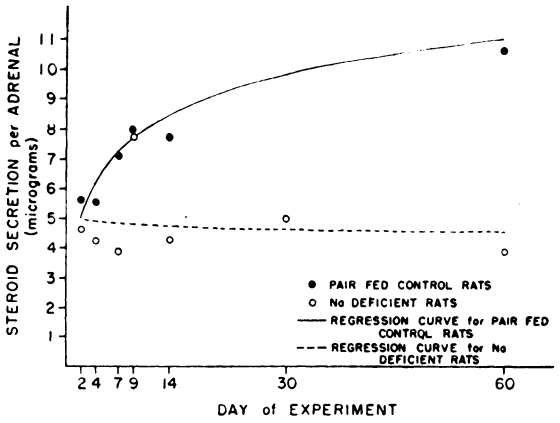

Fig. 2. Total Steroid Hormone Secretion by the Isolated Adrenals of Experimental Animals.

From Eisenstein, A. B. and Hartcroft, P. M.: Alterations in adrencortical hormone secretion induced by sodium deficiency. *Endocrinology* (in press).

The rats consuming the sodium-deficient diet gained weight throughout the period of observation but at a rate which was significantly slower than that of pair-fed controls. Although growth of the animals was decreased by sodium restriction, marked adrenal hypertrophy occurred in these rats (Table V). Serum-sodium levels declined steadily in deficient rats during the 60-day study while the potassium concentration remained elevated. The blood hematocrit was also increased in deficient animals during most of the period of observation (Fig. 1).

Steroid secretion by the isolated adrenals of deficient rats and pair-fed controls is presented in Figure 2. It is apparent that secretion of hormones by the adrenal cortex of control animals increased progressively as the animal grew. Total adrenal hormone secretion of sodium-deficient animals, however, continued at a relatively constant and significantly ($P = < 0.01$) lower rate despite hypertrophy of the gland.

It has previously been shown that isolated adrenals of normal rats secrete four steroids when stimulated by ACTH and that corticosterone is secreted in largest amount.[14] Paper chromatographic separation of hormones secreted by the adrenals of control rats in this study also revealed the presence of four compounds which were present in normal proportion (Fig. 3). Chromatographic analysis of the adrenal secretion of sodium deficient animals on the second, fourth, and seventh days also revealed normal steroid production. By the ninth day, and also on days 14, 30 and 60, increased quantities of aldosterone were secreted by the adrenals of sodium-deficient

TABLE V[20]

Mean Adrenal Weights of Sodium Deficient and Control Rats at Various Intervals After Beginning Experimental Diets

	Duration of sodium deficiency	Number of animals	Weight right adrenal (*mg*)	Weight right adrenal (*mg*) per 100 *g* body weight
Deficient	2 days	5	8.2	11.7
Control		5	7.8	10.4
Deficient	4 days	5	8.3	11.3
Control		5	8.6	10.0
Deficient	7 days	5	9.2	11.5
Control		5	10.0	10.7
Deficient	9 days	5	9.5	10.0
Control		5	9.2	8.6
Deficient	14 days	10	8.9	11.6
Control		10	8.2	7.8
Deficient	30 days	10	10.3	11.8
Control		10	11.3	7.9
Deficient	60 days	6	15.3	9.9
Control		6	17.0	6.4

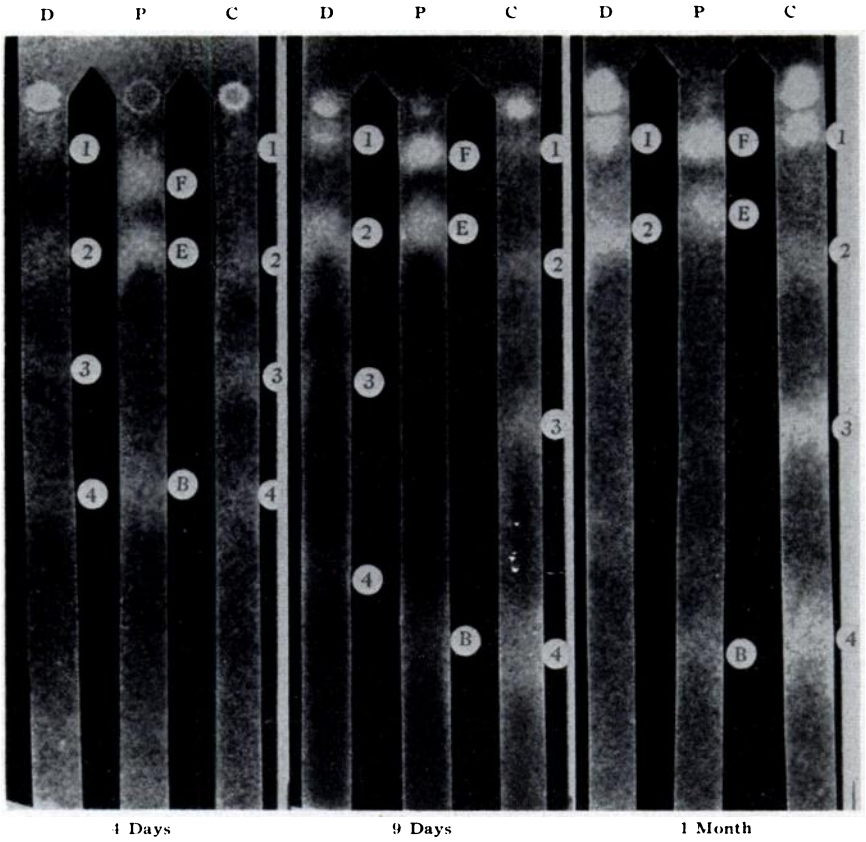

Fig. 3. Chromatograms of the Adrenal Steroids Secreted by Sodium Deficient Rats and Pair Fed Controls.[20]

Each chromatogram consists of three strips labelled D, P, and C. Strip D contains the entire steroid secretion of deficient animals sacrificed on the day indicated and the individual steroids present on the chromatogram are numbered. Strip P is a pilot limb on which hydrocortisone (F), cortisone (E), and corticosterone (B) were placed. Steroid hormones secreted by the adrenals of control animals were placed on Strip C and are also indicated by number.

Previous investigation has shown that steroid 1 is hydrocortisone, steroid 2 is aldosterone, and steroid 4 is corticosterone. The identity of steroid 3 has not been elucidated.[20] (From Eisenstein, A.B. and Harteroft, P. M.: *Endrocrinology* in press).

animals. The chromatograms also demonstrate that while aldosterone secretion became greater with increasing duration of sodium depletion, the secretion of certain other steroids including corticosterone was greatly diminished (Fig. 3).

Since it was demonstrated in these studies that sodium restriction results in diminished total adrenal steroid hormone synthesis, consideration must be given to the effect of this hormonal change on the ability of the animal to withstand other types of stress. It seems likely that a reduction in adrenocortical hormone secretion as a result of sodium depletion would make the organism less capable of withstanding other stresses such as cold, heat, infection, and injury. Another fact which must be considered is that, in addition to reduction in total hormone synthesis, aldosterone forms a large proportion of the steroids which are secreted by sodium-deficient rats, thus further diminishing the available glucocorticoids. The data obtained in this investigation are not sufficient to substantiate the idea that chronic sodium deficiency may interfere with adaptation of the organism to

stress, but do suggest that further study of this problem should be carried out.

These experiments have also shown that the adrenal cortex in response to sodium depletion is capable of selectively increasing the secretion of aldosterone while decreasing the synthesis of certain other steroid hormones. This has been demonstrated previously by the studies of Singer and Stack-Dunne.[22] It is, therefore, suggested that a *specific* stress may influence synthetic mechanisms concerned with the elaboration of adrenocortical hormones in such a way that the gland secretes increased quantities of an individual hormone. The mechanism by which a specific stress may alter steroid synthesis is not apparent, but it seems likely that factors other than ACTH are involved.

CONCLUSIONS

The effects of three dietary factors, ascorbic acid, pantothenic acid, and sodium, on production of adrenocortical hormones have been reviewed. Evidence which has been presented indicates that ascorbic acid, although it is present in high concentration in adrenal tissue, is not directly concerned with secretion of adrenocortical steroids. Pantothenic acid, however, has been shown to be intimately related to synthesis of adrenal steroid hormones. It has been suggested that pantothenic acid is essential for elaboration of adrenocortical hormones since this vitamin is an integral part of coenzyme A. It has also been shown that sodium is an important factor in determining hormone secretion by the adrenal cortex since a deficiency of this mineral results not only in increased secretion of aldosterone but leads to diminished production of certain glucocorticoids.

There are many reports which suggest that other nutrients may be concerned with adrenocortical hormone secretion. Although these data do not provide conclusive evidence of such relationships, the studies are stimulating and arouse the interest of investigators in the fields of nutrition and endocrinology. The effect of dietary factors on the production of adrenal steroid hormones remains a fertile area for future investigation.

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