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# EFFECT OF DIET ON THE GROWTH AND SURVIVAL OF ADRENALECTOMIZED RATS TREATED WITH CORTICOSTERONE[1]

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*Abstract.* Dietary protein content did not influence the life sustaining capacity of a daily 300 μg dose of corticosterone in adrenalectomized rats. A lower daily dosage of 150 μg was less effective when animals were fed a 5% casein diet. Depletion of body protein stores prior to adrenalectomy did not significantly alter the response pattern of animals maintained on a daily 300 μg dose of corticosterone. Reducing the dietary protein content from 20% to 10% interfered with the capacity of the daily 150 μg dosage of corticosterone to support body weight gain in adrenalectomized rats. No significant changes in body weight were noted in adrenalectomized animals fed a 5% casein diet. Protein depletion had a favorable effect on this aspect of the hormone's activity when adrenalectomized rats were treated with the daily 300 μg dosage and refed 10% or 20% casein diets. Adrenalectomized animals maintained under these experimental conditions gained more weight than nondepleted animals fed the same diets.

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The influence of dietary protein content on the life sustaining and growth promoting effects of adrenal steroids appears to be related to the chemical structure of the steroid. The level of casein as protein in a diet influenced the action of desoxycorticosterone acetate. The critical level was 5%, below which the steroid was sharply reduced in effectivness (Mauer 1952). Cortisone acetate (150 μg/day), on the other hand, while permitting 100% or 74% survival was not effective in supporting a normal body weight gain regardless of the dietary protein content (Lenz and Leathem 1965). Reducing the dietary level of casein to 5% had a modest influence on cortisone activity with respect to survival, but deletion of casein from the diet negated this aspect of the hormone's action (Wolf 1955).

At present, little is known about the relationship between body protein stores and the physiological activity of glucocorticoids. The concept of protein stores was first proposed by Voit (1866) who observed that cats excreted large amounts of nitrogen during the initial stages of starvation. This loss of nitrogen was taken to represent the dissipation of the labile body proteins. Subsequently, Allison (1951) defined protein reserves as "those tissue proteins that may be needed for the maintenance of cellular integrity during periods of nutritional stress" and that protein reserves can include 40% to 50% of the total body protein (Allison and Wannemacher 1965).

The following experiments were undertaken to evaluate the effect of dietary protein quantity and the state of body protein stores on the physiological activity of corticosterone in the adrenalectomized rat by measuring survival and body weight gain.

## MATERIALS AND METHODS

Weanling male rats of the Long-Evans strain were maintained on a diet of Purina Laboratory Chow until a body weight of 60 g to 65 g was attained. At this time the rats were adrenalectomized and placed on experimental diets. Bilateral adrenalectomy was performed under

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ether anesthesia through incisions made in the skin and muscles in the lumbar region. Care was taken to remove the encapsulated glands with as much adherent fat as possible.

Experimental diets containing either 5%, 10%, or 20% casein were fed to groups of 16 to 20 rats for a 20 day experimental period commencing on the day after adrenalectomy (table 1). Intact animals subjected to the same dietary regimes served as controls. All animals were fed food and water *ad libitum*.

The influence of body protein stores on corticosterone activity was investigated in rats fed a protein free diet for ten days prior to adrenalectomy. Following the operation, groups of animals were fed either a 5%, 10% or 20% experimental diet *ad libitum* for 20 days. Starting on the day following adrenalectomy, body weights were recorded daily and the animals were given daily subcutaneous injections of 300 μg or 150 μg of corticosterone (Nutritional Biochemicals). At the end of the 20-day experimental period and 24 hours after the last injection, all animals were sacrificed with sodium pentabarbital (Nembutal) and body weights and organ weights were determined *post mortum*. Values from each group were pooled and tested for statistical significance with a two way analysis of variance.

## RESULTS

**Survival.** Adrenalectomized rats fed a 20% casein diet and treated with 300 μg or 150 μg of corticosterone per day exhibited a 100% survival rate over the 20 day experimental period (table 2). Reducing the dietary level of casein to 10% or 5% did not interfere with the favorable action of the hormone on survival when animals were maintained with a threshold dose (300 μg/day) of steroid. An effect on survival was noted, however, when rats maintained on the 150 μg regime were fed a 5% casein diet. Adrenalectomized rats maintained under these conditions exhibited a survival rate of 80%.

In order to assess the influence of body protein reserves on the animal's responsivity to corticosterone, a protein-free diet was fed for 10 days prior to adrenalectomy causing body weight loss of appx. 10 g (table 3). A survival rate of 100% resulted when these rats were refed a 20% casein diet for 20 days and treated with a daily corticosterone dose of 300 μg or 150 μg. The 150 μg dose proved less effective, however, when 10% casein diet was fed during the period of protein repletion (tables 2 and 3).

**Body Weight.** Normal rats fed a 20% casein diet for 20 days gained a mean of 87.3 grams whereas the adrenalectomized rats treated with 300 mg of corticosterone

TABLE 1

*Composition of experimental diets.*

| Component | 0% Casein (PFD)† | 5% Casein* | 10% Casein* | 20% Casein* |
|---|---|---|---|---|
| Casein | 0.0 | 5.0 | 10.0 | 20.0 |
| Dextrose | 30.0 | 27.5 | 25.0 | 20.0 |
| Dextrin | 35.7 | 33.2 | 30.7 | 25.7 |
| Wesson Salt Mixture** | 4.0 | 4.0 | 4.0 | 4.0 |
| Cellu Flour | 2.0 | 2.0 | 2.0 | 2.0 |
| Crisco | 25.0 | 25.0 | 25.0 | 25.0 |
| Corn Oil | 2.0 | 2.0 | 2.0 | 2.0 |
| Choline chloride | 0.2 | 0.2 | 0.2 | 0.2 |

**Vitamin Supplements per 100 g of Diet**

| | |
|---|---|
| Vitamin A | 900 USP Units |
| Vitamin D | 180 USP Units |
| Alpha-tocopherol | 4.0 mg |
| 2-Methyl-1,4 napthoquinone diacetate | 1.0 mg |
| Thiamine Hydrochloride | 0.8 mg |
| Riboflavin | 1.6 mg |
| Pyridoxine Hydrochloride | 0.8 mg |
| Niacin | 4.0 mg |
| Calcium Pantothenate | 4.4 mg |
| Para-aminobenzoic Acid | 4.0 mg |
| Inositol | 21.6 mg |

*Grams/100 g diet.

**Modification of Osborne-Mendel salt mixture (Science 75: 339, 1932).

†PFD = Protein free diet.

TABLE 2

*Influence of dietary protein and corticosterone maintenance therapy on the 20-day survival of adrenalectomized immature male rats.*

| Group | No. | Mean Body Weight (g) | | | % Survival |
|---|---|---|---|---|---|
| | | Initial | Final | Change | |
| **20% Casein** | | | | | |
| Control* | 20 | 62.2+ ±0.3 | 149.5 ±7.3 | +87.3 ± 7.1 | 100 |
| ADX+300 μg C** | 18 | 61.8 ±0.6 | 106.8 ±4.0 | +45.0++ ± 4.7 | 100 |
| ADX+150 μg C | 19 | 62.5 ±1.1 | 102.0 ±5.8 | +39.5++ ±5.5 | 100 |
| **10% Casein** | | | | | |
| Control | 18 | 62.5 ±0.6 | 96.6 ±3.2 | +34.1 ± 2.6 | 100 |
| ADX+300 μg C | 20 | 61.8 ±0.5 | 84.5 ±2.1 | +22.7 ± 2.1 | 100 |
| ADX+150 μg C | 20 | 62.0 ±0.7 | 67.8 ±1.8 | + 5.8++ ± 1.5 | 100 |
| **5% Casein** | | | | | |
| Control | 16 | 61.3 ±0.4 | 60.5 ±1.5 | − 0.8 ± 1.2 | 100 |
| ADX+300 μg C | 20 | 62.1 ±0.4 | 59.2 ±1.8 | − 2.9 ± 1.7 | 100 |
| ADX+150 μg C | 19 | 60.9 ±0.4 | 55.5 ±1.9 | − 5.4 ± 1.9 | 80++ |

*Intact *ad libitum*-fed rats.
**C = Corticosterone, ADX = Adrenalectomized.
+Mean ± standard error.
++Difference between experimental group and dietary control group significant ($P<0.01$).

gained 45.0 g (table 2). A lesser dose of hormone (150 μg) was nearly as effective and permitted a mean body weight gain of 39.5 g.

A 10% casein diet effectively supported a mean weight gain of 34.1 g in normal rats and 22.7 g in adrenalectomized rats given 300 μg of steroid daily. A 10% casein diet, however, in combination with a 150 μg dose of hormone resulted in only a 5.8 g increase in mean body weight revealing an influence of diet on hormone action. No significant increases in body weight were noted in animals fed a 5% casein diet.

Normal animals fed a protein free diet for 10 days and refed a 20% casein diet exhibited a body weight increase of 97.7 g compared to 40.6 g and 5.5 g in animals refed 10% and 5% casein diets (table 3). Body weight gain in adrenalectomized animals refed 20% or 10% casein diets was subnormal and directly related to hormone dosage level. Adrenalectomized animals refed a 5% casein diet exhibited no significant body weight gain.

Protein depletion modified the effect of corticosterone on the adrenalectomized rats treated with 300 μg of corticosterone daily and refed 10% or 20% casein diets. These animals gained more weight than nondepleted rats fed the same diets. This improved responsivity was not evident, however, in rats given a marginal (150 μg) daily dose of steroid.

## DISCUSSION

The life sustaining capacity of corticoids in adrenalectomized rats is influenced by the quantity of dietary protein (Aschkenasy 1954, Wolf 1955). Thus, survival rate is reduced in cortisone acetate (Aschkenasy 1954, Wolf 1955) and desoxycorticosterone acetate (Mauer

TABLE 3

*Influence of dietary protein and corticosterone maintenance therapy on the 20-day survival of adrenalectomized immature male rats fed a protein-free diet for 10 days prior to adrenalectomy.*

| Group | No. | Mean Body Weight (g) | | | % Survival |
|---|---|---|---|---|---|
| | | Initial* | Final | Change | |
| **20% Casein** | | | | | |
| Control** | 9 | 49.7++ ±1.1 | 147.4 ±10.3 | +97.7 ± 9.9 | 100 |
| ADX+300 μg C+ | 20 | 51.3 ±0.9 | 111.5 ± 3.7 | +60.2 ± 2.9 | 100 |
| ADX+150 μg C | 20 | 46.8 ±0.7 | 85.5 ± 3.4 | +38.7† ± 3.4 | 100 |
| **10% Casein** | | | | | |
| Control | 10 | 50.3 ±0.8 | 90.9 ± 3.1 | +40.6 ± 2.8 | 100 |
| ADX+300 μg C | 19 | 49.8 ±1.0 | 82.4 ± 4.2 | +32.6 ± 1.7 | 100 |
| ADX+150 μg C | 20 | 50.1 ±0.6 | 58.0 ± 3.7 | + 7.9† ± 3.1 | 71† |
| **5% Casein** | | | | | |
| Control | 10 | 47.5 ±0.4 | 53.0 ± 0.8 | + 5.5 ± 1.0 | 100 |
| ADX+300 μg C | 19 | 49.1 ±0.5 | 50.8 ± 1.7 | + 1.7 ± 1.3 | 100 |
| ADX+150 μg C | 20 | 49.3 ±1.3 | 49.9 ± 1.3 | + 0.6† ± 1.0 | 69† |

*Body weight after feeding a protein-free diet for 10 days.
**Intact *ad libitum*-fed rats.
+C = Corticosterone, ADX = Adrenalectomized.
++Mean ± standard error of the mean.
†Difference between experimental group and dietary control group significant ($P<0.01$).

1952, Leathem 1957) maintained rats following a reduction in dietary protein. Moreover, adrenalectomized rats treated with 300 μg of corticosterone and fed a 20% casein diet exhibited a 92% to 100% survival rate (Oddis and Leathem 1964, Lenz and Leathem 1965) as compared to a 31% survival rate in adrenalectomized animals fed a 10% casein diet (Gianferrari 1968). In our experiments, a daily 300 μg dose of corticosterone supported a 100% survival rate over a 20 day experimental period when animals were fed either a 20% or 10% casein diet. Survival rates of 75% have been reported for adrenlectomized rats treated with 150 μg of corticosterone and fed a 20% casein diet (Oddis 1958, Oddis and Leathem 1964). Under the conditions of our experiments, this dose permitted a 100% survival rate in adrenalectomized rats fed 10% and 20% casein diets, but was less effective with a 5% casein diet where the survival rate was only 80%. Thus, reductions of dietary casein content interfered with the life sustaining activity of corticosterone when administered at subthreshold dosages but was not evident when threshold doses (300 μg/day) were given.

Protein depletion prior to adrenalectomy reduced the life sustaining capacity of cortisone acetate (Davis 1958). In the current experiments, depletion of body protein stores did not influence the life sustaining capacity of corticosterone during refeeding with 20% casein, suggesting that the natural adrenal steroid, corticosterone, was more effective.

Although various steroid replacement

regimes are capable of supporting a body weight gain in immature adrenalectomized rats, they are not equally effective. Body weight gain is greater in adrenalectomized rats treated with a 300 μg dosage of corticosterone (Oddis and Leathem 1964) or 25 μg of desoxycorticosterone acetate (Leathem 1957, Mauer 1952) than in those treated with 150 μg of cortisone acetate (Wolf 1955). A daily dose of 94 μg to 1870/100 g (Reichlin and Brown 1960) or 150 μg (Lenz 1966) of corticosterone and desoxycorticosterone acetate in a 10:3 ratio (Lenz 1966) permitted a normal body weight gain in adrenalectomized rats. Furthermore, corticosterone (300 μg/day) permitted a 33.0 g mean body weight gain over a 20 day experimental period compared to 38.0 g in normal pair fed rats (Oddis and Leathem 1964). A daily 300 μg dose of corticosterone in the current experiment permitted a 45 g body weight gain in adrenalectomized animals fed a 20% casein diet. The body weight gains were, however, significantly less than those of the *ad libitum* fed dietary control animals.

The effectiveness of corticoid hormones on body weight gain in adrenalectomized rats is also susceptible to variations in the protein content of diet. Either a daily 300 μg or 150 μg of corticosterone was equally effective in supporting a body weight gain in animals fed a 20% casein diet; nevertheless, these body weight gains were significantly less than those recorded for the *ad libitum*-fed control animals. The effectiveness of 150 μg corticosterone in adrenalectomized rats fed a 10% casein diet was curtailed since these animals gained an average of 5.8 g. These data indicate that hormonal requirements of adrenalectomized rats can be modified by the nutritional status of the animal during the period of replacement therapy.

Animals refed protein subsequent to protein depletion exhibited an enhanced tendency for protein anabolism (Leathem 1962). The absence of corticoids during the repletion phase did not interfere with the anabolic rebound of adult animals. Adult male rats adrenalectomized after one month of protein-free feeding and maintained on 1% NaCl exhibited a 74 g weight gain and nitrogen retention comparable to pair-fed controls (Leathem 1970). The adrenalectomized rats treated in our experiments with a daily 300 μg of hormone and refed 10% or 20% casein gained more weight than their nondepleted counterparts. This finding suggests that the metabolic adjustments that occur during protein depletion alter the responsivity of the animal to the hormone. This response appears, however, to be dose-related since depleted animals maintained with a daily 150 μg dose of corticosterone were not significantly different from the nondepleted animals in terms of mean body weight gain.

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