Effects of Methylandrostenediol on Weight and Cholesterol Content of Adrenals of Cortisone or Thiouracil-Treated Rats.* (26862)

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Methylandrostenediol (MAD) and other androgens will prevent adrenal atrophy in rats treated with cortisone acetate (1, for review). The adrenal-sparing action of androgens has been termed "ACTH-like" because: (a) the effect is exerted directly on adrenal cortex; (b) the hypophysis is not required (1). The term, "ACTH-like," may be inappropriate inasmuch as androgens do not influence adrenal weight in normal rats, and little is known of the functional capacity of androgen-maintained adrenals. In this regard, Saffran and Vogt(2) found that MAD treatment depressed adrenal corticosteroid production by 50% in adult male rats under stressful conditions. A similar depression of adrenal function was observed in rats treated with cortisone or cortisone plus MAD. To the contrary, Dean *et al.*(3) reported a 5-fold decrease in corticoid production of male rats following castration and implied that gonadal steroids are necessary for maximal adrenal secretory capacity. These apparently divergent observations could be partially reconciled if a substrate requisite for corticoidogenesis were androgen-dependent. Cholesterol is a normal precursor of corticoids and changes in adrenal cholesterol have served as useful criteria of ACTH-like activity(4). Thus, cholesterol content may reflect functional capacity of adrenals subjected to other stimuli. Adrenal cholesterol content was, therefore, determined in male and female rats treated with cortisone, MAD and their combination.

Adrenal atrophy, similar to that induced by cortisone, has been observed in rats fed a goiterogen(5, for review). This effect provided a corollary experiment evaluating the "ACTH-like" action of androgens. Accordingly, the effect of MAD on weight and cholesterol content of adrenals were observed in thiouracil-fed rats.

Materials and methods. Male and female rats (Long-Evans strain) of 50 to 70 g body weight were used. Cortisone acetate and methylandrostenediol (aq. susp.) were injected subcutaneously in 1 and 2 mg daily dosages respectively for 7 days. Thiouracil was fed for 20 days at a level of 0.5% in ground fox chow diet.

Adrenals were weighed prior to maceration and extraction with three 2 ml portions of boiling alcohol-acetone (1:1). Cholesterol content of adrenal extracts was determined by the method of Shoenheimer and Sperry as described by Hawk, Oser and Summerson(6). Body weight retarding effects of cortisone acetate dictated that adrenal weights be calculated relative to final body weight.

Results. Cortisone acetate induced adrenal atrophy in male and female rats whereas MAD had no effect alone (Tables I and II). Normal adrenal body weight ratios were, however, observed in rats of both sexes treated concomitantly with cortisone and MAD.

Adrenal cholesterol concentration was significantly depressed to ⅛ the normal value in males and to ⅓ the normal value in female rats treated with cortisone acetate. MAD alone depleted adrenal cholesterol to ¼ the normal level in males. Cholesterol levels in MAD-treated females were even lower than those observed in cortisone-treated females (12% *vs* 33% of normal).

Combined treatment with cortisone acetate and MAD maintained adrenal cholesterol levels at 75% of the normal value in male rats, but the combination of steroids did not prevent adrenal cholesterol decreases in female rats.

When adrenal atrophy was induced by feeding thiouracil to female rats, adrenal cholesterol concentration was not significantly altered. MAD did not prevent thiouracil-

* Supported by a grant from E. R. Squibb and Sons.

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TABLE I. Influence of Cortisone Acetate and MAD on Adrenal Weight and Cholesterol Concentration in Male Rats.

Treatment	No. rats	Body wt, g	Adrenal wt, mg/100 g	Adrenal cholesterol, % Total	Free
None	8	78 ± 2*	19.3 ± .8	1.66 ± .12	.16 ± .01
Cortisone acetate, 1 mg daily × 7	19	62 ± 3	10.6 ± .5	.25 ± .05	<.01
MAD,† 2 mg daily × 7	15	89 ± 4	18.2 ± .9	.44 ± .04	.10 ± .002
Cortisone acetate, 1 mg + MAD, 2 mg daily × 7	21	67 ± 5	17.6 ± .5	1.09 ± .11	.05 ± .002

* S.E. of mean. † MAD = methylandrostenediol.

TABLE II. Influence of Cortisone Acetate, MAD and Thiouracil on Adrenal Weight and Cholesterol Concentration in Female Rats.

Treatment	No. rats	Body wt, g	Adrenal wt, mg/100 g	Adrenal cholesterol, % Total	Free
None	14	104 ± 3*	19.5 ± .9	3.49 ± .32	.26 ± .02
Cortisone acetate, 1 mg daily × 7	13	68 ± 5	10.9 ± .7	1.20 ± .37	.07 ± .03
MAD, 2 mg daily × 7	8	105 ± 4	17.4 ± .8	.40 ± .08	.06 ± .01
Cortisone acetate, 1 mg + MAD, 2 mg daily × 7	13	64 ± 4	17.1 ±1.0	1.34 ± .22	.05 ± .01
0.5% thiouracil in diet × 20	5	82 ± 5	12.4 ± .2	3.18 ± .30	.21 ± .06
Idem + 2 mg MAD × 20	5	93 ± 4	13.6 ± .8	1.01 ± .22	.17 ± .02

* S.E. of mean.

induced adrenal atrophy, but did deplete adrenal cholesterol by 67%.

Discussion. These data confirm and extend the histochemical observations of Gaunt *et al.* (1), who noted a loss of Schultz-positive (cholesterol) material in adrenals of male rats treated with cortisone or MAD. Present data suggest a sex difference in the effects of an androgen on adrenal function, since adrenal cholesterol was (partially) protected by MAD in cortisone-treated males, but not in cortisone-treated female rats. Also, MAD alone depleted cholesterol to a greater extent than did cortisone alone, in female rats, while inducing a lesser, but significant cholesterol loss from adrenals of male rats. Full significance of these effects awaits experimental evidence of the corticoidogenic capacity of adrenal glands of rats treated with cortisone, MAD, or their combination. Van der Vies (7) found that adrenals obtained from hydrocortisone-treated rats produced lesser amounts of corticoids when incubated *in vitro* than did adrenals of normal rats. Saffran and Vogt(2) observed *in vivo* a 50% reduction in adrenal corticoid production by male rats pre-treated with MAD, cortisone or their combination. Reduced corticoid production reported in MAD- or cortisone-treated rats correlates well with current data on cholesterol. Thus, severe depletion of cholesterol, a major corticosteroid precursor, may be responsible for reduced corticoidogenesis(2).

Less easily explained is the near-normal cholesterol level in adrenals of male rats receiving MAD and cortisone acetate concomitantly, and the observations of Saffran and Vogt(2) that animals so treated exhibit a reduced corticoid output. However, Saffran and Vogt did not obtain the expected adrenal weight maintaining effect of MAD in cortisone-treated rats, possible because they used intact adult males instead of immature rats (1).

MAD did not protect against thiouracil-induced adrenal weight loss. Since MAD is known to maintain essentially normal adrenal weights in hypophysectomized rats(1) the data suggest that thiouracil may reduce adrenal weight by some extra-pituitary

mechanism(5).

Summary. Cortisone acetate depressed adrenal weight and reduced adrenal cholesterol concentration in male and female rats. Methylandrostenediol (MAD) depleted adrenal cholesterol in both sexes while not influencing adrenal weight. When cortisone acetate and MAD were injected concomitantly, normal adrenal weights were observed in male and female rats. However, adrenal cholesterol content was protected by MAD only in cortisone-treated males. MAD did not prevent adrenal weight loss due to thiouracil, but did deplete cholesterol by 67%.

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Received July 13, 1961. P.S.E.B.M., 1961, v108.

Plasma Thromboplastin Antecedent Levels in Patients Receiving Coumarin Anticoagulants and in Patients with Laennec's Cirrhosis.* (26863)

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Naeye reported(1) that plasma thromboplastin antecedent (PTA) levels were depressed by Dicumarol (bishydroxycoumarin). However, Waaler later found(2) a normal formation of activation product (which requires PTA) in plasma from 10 patients receiving long term therapy with Dicumarol or phenylindanedione. In addition, a recent preliminary report by Rodermund, Schneider and Egli(3) describes a normal formation of activation product in subjects given Marcoumar for 10 days.

Naeye also reported(1) that PTA levels fell in liver disease. Other reports of the quantitative measurement of PTA activity in liver disease have not been found.

A specific one-stage assay for PTA has been reported from this laboratory recently(4). This test has been used to measure PTA levels in a group of patients receiving coumarin anticoagulants and in another group of patients with advanced Laennec's cirrhosis.

Methods. Blood for testing was drawn with Monocote (Armour and Co.) coated needles and silicone (Dri-film, G.E. SC-87) coated syringes. Nine parts of blood were added to 1 part of anticoagulant (made by mixing 2 parts of 0.1 M citric acid and 3 parts of 0.1 M sodium citrate) in a polyethylene tube. Platelet-poor plasma was prepared by centrifuging for 20 minutes at 14,500 g at 4°C and was tested within about 3 hours after the blood was drawn.

The technic and standardization of the quantitative assay for PTA have been reported elsewhere(4). The clotting system consists of: 0.1 ml of a "cephalin" reagent diluted in a suspension of kaolin powder, 0.1 ml of hereditary PTA deficiency substrate plasma, 0.1 ml of a 1/5 or a 1/10 dilution of the test plasma, and 0.1 ml of 35 mM calcium chloride solution. Neither the substrate plasma nor the test plasma contacts an activating surface prior to its addition to the clotting mixture.

The assay measures PTA activity accurately at levels above about 15% of a standard reference plasma. For example, the 90% confidence limits for a single observed value of 50% PTA vary between 42 and 60% of the reference plasma.

* Aided by a grant from Los Angeles County Heart Assn.