Interaction of Methandrostenolone and Adrenocortical Hormones. (27484)

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Methandrostenolone (17α-methyl-17β-hydroxyandrosta-1,4-dien-3-one; Dianabol®) is a steroid with weak androgenic and strong anabolic activity. It is essentially without progestational effects and is only a weak gonadotrophin inhibitor. Its pharmacological properties were first described in detail by Desaulles *et al.*(1,2) and more recently its clinical use has been reported by numerous investigators(3,4,5). Clark and Mills have reported that in patients with rheumatoid arthritis methandrostenolone augmented the action of corticoids, thus bringing about a reduction in the amount of corticosteroid needed for treatment(6).

In view of these findings, we report here some observations concerning the interaction between methandrostenolone and corticosteroids in rats.

Corticosteroid inhibition of growth. The effect of methandrostenolone on growth inhibiting action of glucocorticoids was studied in intact and castrate male rats. In the intact rats given a single intramuscular injection of dexamethasone trimethylacetate (0.5 mg/rat), administration of an aqueous suspension of methandrostenolone at 1.0 and 5.0 mg/rat subcutaneously (s.c.) for 10 days antagonized partially the growth inhibiting effect of this potent long acting corticosteroid (Fig. 1). As noted in the graph, the addition of methandrostenolone enabled the animals to return to a positive weight balance much sooner than the group on dexamethasone trimethylacetate alone.

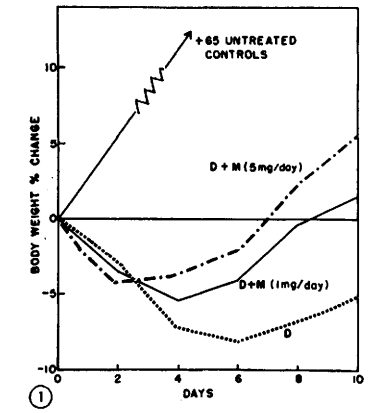
The doses of methandrostenolone used in the experiments in Fig. 1 were large and clearly in the androgenic range. Smaller doses were tried in young (125 g) castrate male rats whose growth rate was inhibited by daily s.c. administration of 1.5 mg/rat of cortisone acetate for 28 days. Results are shown in Fig. 2. Although of lesser magnitude, the same growth-promoting effects (statistical significance: $P = 0.05$) were apparent as in Fig. 1 with doses of methandrostenolone having little or no effect on the seminal vesicle. In addition there was a marked myotrophic response. While such effects on body weight have been observed on numerous occasions, consistent dose-response curves have never been obtained; hence the method is not suitable for bioassay work.

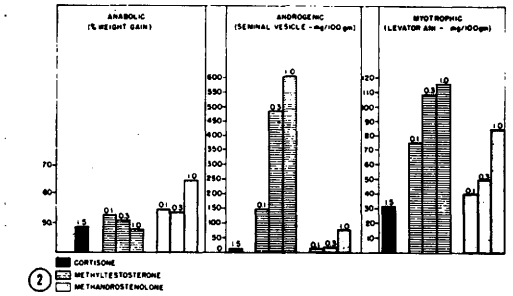
In the low dose experiments methyltestosterone was without effect. The apparent inverse dose-response curve to methyltestosterone is, judging by other similar experiments, only fortuitous.

Similar effects with methandrostenolone on body growth have been reported in non-cortisone treated, intact, and castrate rats(1).

Corticosteroid effects on inflammation. Studies on experimental inflammation were performed using methandrostenolone alone and methandrostenolone in combination with the glucocorticoids. The corticoids were given in doses which caused a minimal response to see if an additive effect was produced by other steroids given at the same time. Inflammation was produced in rats by using the granuloma pouch technic of Selye (7) as modified by Robert and Nezamis(8) and the cotton pellet granuloma method as described by Meier *et al.*(9).

In the granuloma pouch experiments administration of methandrostenolone alone at doses as high as 8 mg/rat/day s.c. did not appreciably affect the volume of exudate (Table I). A decrease occurred in thymus weight, a result generally seen with related steroids at high doses. Treatment with hydrocortisone (0.25 and 0.5 mg/rat/day s.c.) or with dexamethasone (0.0025 mg/rat/day s.c.) produced minimal anti-inflammatory responses. The administration of methandrostenolone (8 mg/rat/day s.c.) together with hydrocortisone or methandrostenolone (2 to 8 mg/rat/day s.c.) with dexamethasone to these granuloma pouch animals resulted in a significant decrease in volume of exudate as compared to that obtained with the glucocorticoids alone. In the series in which hydro-





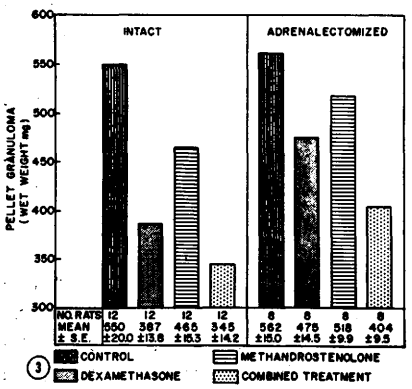


FIG. 1. Per cent body wt change over a 10-day period in rats receiving a single intramusc. inj. (0.5 mg/rat) of dexamethasone trimethylacetate (D) and in rats given dexamethasone trimethylacetate plus daily subcut. injections (1 and 5 mg/rat) methandrostenolone (D + M). Each group represents the avg of 6 animals.

FIG. 2. Anabolic, androgenic and myotrophic activity of methyltestosterone and methandrostenolone in cortisone-treated rats. Dosage (mg/rat/day) is shown above the column and each column represents the avg of 12 animals treated for 28 days.

FIG. 3. Response of intact and adrenalectomized rats to 7 days' treatment with methandrostenolone (4 mg/rat/day s.c.) and dexamethasone (0.005 mg/rat/day s.c.) alone and in combination as measured by wet weights of the cotton pellet granuloma.

cortisone was used as the glucocorticoid, doses of less than 8 mg of methandrostenolone were ineffective. The combined treatment had no other noticeable effect except for further decrease in thymus weight.

When two 50 mg sterile cotton pellets were implanted under the skin of intact or adrenalectomized rats, the administration of methandrostenolone (4 mg/rat/day s.c.) alone resulted in a slight decrease in the weight of the resulting granuloma (Fig. 3). Dexamethasone at a dose of 0.005 mg/rat/day s.c. inhibited granuloma growth consistently. There was an additive effect when dexamethasone and methandrostenolone were given in combination.

Anti-inflammatory activity of other steroids + dexamethasone. In view of these results other steroids were tried alone and in combination with dexamethasone for their effects on granuloma pouch inflammation. Methyltestosterone, testosterone, norethandrolone and progesterone were given s.c. at a dose of 4 mg/rat/day. Both methyltestosterone and testosterone augmented the anti-inflammatory response of dexamethasone, while norethandrolone and progesterone failed to show any activity at all in these tests. The results obtained with norethandrolone were surprising inasmuch as others have reported an additive effect for this steroid when given in combination with cortisone acetate(10).

Lack of other corticosteroid-like activity. Further studies were performed to see if methandrostenolone possessed any additional corticosteroid-like activities. Unlike the action of glucocorticoids, chronic treatment with this steroid in various doses caused no adrenal atrophy in intact rats. In like manner methandrostenolone did not prevent adrenal compensatory hypertrophy after unilateral adrenalectomy, and failed to maintain life in the immature adrenalectomized animals. It had no apparent effect on carbohydrate metabolism since it did not promote liver glycogen deposition. In acute diuresis tests employing methods previously described (11), methandrostenolone had no effect on sodium or water excretion. Like numerous other related compounds, this anabolic ster-

TABLE I. Effect of Methandrostenolone, Hydrocortisone and Dexamethasone on Granuloma Pouch Inflammation in Intact Rats.

Treatment	No. of rats	Terminal body wt (g)	Exudate (ml)	Thymus (mg)	Adrenals (mg)
Controls	28	153 ± 1.3†	6.3 ±.2	240.4 ± 11.4	43.1 ± 1.1
Hydrocortisone,					
0.25 mg/rat/day × 4	12	150 ± 1.8	4.6 ±.3	200.2 ± 16.3	41.2 ± 1.4
0.5 " " " " "	28	148 ± 1.6	4.0 ±.2	171.6 ± 8.2	38.7 ± 1.2
Methandrostenolone,					
8 mg/rat/day × 4	28	151 ± 1.6	5.4 ±.3	180.0 ± 10.6	43.0 ± .9
Methandrostenolone, 8 mg/rat/ day × 4 + hydrocortisone,*					
0.25 mg/rat/day × 4	12	146 ± 1.6	2.0 ±.3	170.0 ± 12.0	41.1 ± 1.1
0.5 " " " " "	28	144 ± 1.6	1.9 ±.3	129.0 ± 6.5	38.2 ± 1.0
Controls	42	153 ± 1.1	6.3 ±.2	250.7 ± 9.2	44.6 ± .6
Dexamethasone,					
2.5 μg/rat/day × 4	42	146 ± .9	4.8 ±.2	213.0 ± 7.6	42.0 ± 1.0
Methandrostenolone,					
2 mg/rat/day × 4	16	157 ± 1.4	6.8 ±.3	236.6 ± 11.5	43.0 ± 1.5
4 " " " " "	34	154 ± 1.1	5.9 ±.2	180.9 ± 9.1	41.0 ± .7
8 " " " " "	8	157 ± 2.9	6.2 ±.4	222.0 ± 6.3	45.6 ± 2.1
Dexamethasone,* 2.5 μg/rat/ day × 4 + methandrostenolone,					
2 mg/rat/day × 4	16	147 ± 2.5	3.5 ±.3	163.6 ± 11.9	37.4 ± 1.2
4 " " " " "	34	147 ± 1.7	3.4 ±.2	132.5 ± 5.1	40.8 ± .9
8 " " " " "	8	142 ± 1.8	2.9 ±.3	134.1 ± 14.0	40.1 ± 1.8

* Significant potentiation of dexamethasone and hydrocortisone effect at 5% level of significance (proved by 2 × 2 factorial experiments).

† Mean ± Stand. error.

oid inhibited cortisone-induced adrenal atrophy.

In rats with regenerating adrenal glands, however, methandrostenolone produced a marked inhibition in rate of regeneration of the enucleated gland(12) as well as an inhibition in development of hypertension(13).

Discussion. Results have been presented showing interactions between methandrostenolone and the glucocorticoids. In view of methandrostenolone's antagonism of the growth inhibiting effects of glucocorticoids and its inactivity in other tests for corticosteroid activity, the augmentation of corticoid anti-inflammatory activity was unexpected.

The details of the "anti-inflammatory" effects of methandrostenolone and related steroids differed with the test procedure used. Thus methandrostenolone alone was ineffective in reducing the exudate formation in the granuloma pouch but was active in inhibiting cotton pellet granulomas. Furthermore, higher doses were required to obtain any action on granuloma pouches than on pellet granulomas. That technical details may be of considerable importance is suggested by the fact that Jasmin *et al.*(10) found similar anti-inflammatory effects of norethandrolone while we did not. This discrepancy is without obvious explanation.

Nevertheless, by any criterion the dose of anabolic steroids required to demonstrate inflammation-suppressing effects is high in terms of possible human application. Hence while the data reported here help establish the principle that anabolic steroids have such action, they cannot be used to predict with any confidence that the smaller doses used in human therapy would act similarly.

The fact that a corticoid-sparing action of small doses of methandrostenolone was found in some patients with inflammatory disease has been attributed to an inhibition of the metabolic degradation of either endogenous or exogenous glucocorticoids(6). That different mechanisms may be involved in our animal work is suggested by the fact that methandrostenolone was active in adrenalectomized ani-

mals not treated with corticoids. The results were therefore not totally adrenal-dependent.

Summary. The anabolic steroid methandrostenolone partially antagonized the growth inhibiting effects of glucocorticoids in rats and also, depending on the type of experiment, augmented or added to the anti-inflammatory activity of these steroids. Similar activity was found with methyltestosterone and testosterone, whereas norethandrolone and progesterone were inactive. The action of methandrostenolone was independent of the adrenal. Methandrostenolone itself showed no other signs of glucocorticoid-like activity.

The authors wish to express their appreciation to Dr. Robert Gaunt for advice and encouragement and to Mrs. Hanna Sylwestrowicz for statistical evaluation. Thanks are due also to Nancy Howie, Margot Stein, Patricia Reilly and Viola Dube for technical assistance.

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Received March 30, 1962. P.S.E.B.M., 1962, v110.

An Orally-Active, Heat-Labile Factor in Pancreas Which Induces Salivary Gland Hypertrophy in Rats. (27485)

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During an investigation of the comparative nutritive value of raw and heated animal tissues, it was observed that rats fed a stock diet supplemented with raw pancreas exhibited retarded growth and a marked enlargement of the salivary glands. These effects did not occur when heated pancreas or other raw tissues were fed. The present study was undertaken to confirm and extend these earlier observations.

Procedure and results. Twenty-four male rats of the Holtzman strain were selected at an average body weight of 43 g (range 41 to 47 g) and were divided into 4 comparable groups of 6 each. One group was fed a basal natural food stock ration;* the remaining groups were fed the basal ration plus 2%, 3% or 4% desiccated and defatted raw pancreas† respectively, the latter being incorporated in the diet in place of an equal amount of stock ration. Animals were placed in metal cages with raised screen bottoms (3 rats per cage) and were provided the above diets and water *ad libitum.* The animals were weighed weekly; and after 26 days of feeding were killed and examined for gross pathology. Findings indicate that increment in body weight was retarded in all rats fed the desiccated and defatted raw pancreas-containing diets, the growth retardation being proportional to the level of pancreas fed. In addition to growth retardation, rats fed the desic-

* Rockland Rat Diet in meal form, A. E. Staley Mfg. Co., Decatur, Ill.

† Viokase Powder 4 N.F. Pancreatin, VioBin Corp., Monticello, Ill.