equal amounts of oxytocin. The slight increase in cutaneous water uptake produced by 6 mμg vasotocin was reduced by simultaneous injection of the same amount of oxytocin. As the amphibian neurohypophysis apparently contains both vasotocin and oxytocin, attempts to elucidate amphibian neurohypophysial function by studying the effects of injections of non-fractionated neurohypophysial extracts may provide misleading results as regards presence and potency of neurohypophysial hormones.

1. Jørgensen, C. B., *Acta Physiol. Scand.,* 1950, v22, Suppl. 78.

2. Jard, S., Morel, F., *Compt. rend. Acad. Sci.,* 1961, v252, 339.

3. Acher, R., Chauvet, J., Lenci, M. T., Morel, F., Maetz, J., *Biochim. Biophys. Acta,* 1960, v42, 379.

4. Heller, H., Pickering, B. T., *J. Physiol.,* 1961, v155, 98.

5. Sawyer, W. H., Munsick, R. A., van Dyke, H. B., *Gen. Comp. Endocrinol.,* 1961, v1, 30.

6. Jørgensen, C. B., Wingstrand, K. G., Rosenkilde, P., *Endocrinology,* 1956, v59, 601.

7. Boissonnas, R. A., Guttmann, St., Berde, B., Konzett, H., *Experientia,* 1961, v17, 377.

Received March 12, 1962. P.S.E.B.M., 1962, v110.

Effects of Hormone Administration on Collagen Biosynthesis in the Rat.* (27496)

L. A. BAVETTA, I. BEKHOR AND M. E. NIMNI
Department of Biochemistry and Nutrition, School of Dentistry, University of Southern California, Los Angeles

We recently reported on the ineffectiveness of methyl testosterone as well as growth hormone in counteracting the catabolic effects of methyl prednisolone on nitrogen metabolism and body weight(1). Nevertheless, growth hormone in presence of methyl prednisolone (Medrol)† partially prevented the inhibitory effect of the former on collagen biosynthesis around the subcutaneously implanted polyvinyl sponges. To understand further the mechanisms involved in the interaction of different hormones on these processes, we investigated the combined effects of methyl prednisolone and stilbestrol on rate of collagen biosynthesis. Simultaneous addition of androgens (methyl testosterone) was also investigated for this purpose.

In connection with our previous findings that growth‡ hormone partially prevented the inhibitory effect of methyl prednisolone on collagen formation we decided to further increase the level of this hormone to try to enhance this protective effect. Simultaneously with the specific studies on collagen, rate of synthesis of other tissue proteins was also investigated. Weight changes and rate of uptake of radioglycine by the perineal muscles were studied. The rate of development of these muscles, particularly the levator ani, has been very frequently interpreted as a measurement of the anabolic activity throughout the organism(2,3), particularly in reference to anabolic activity of androgenic steroids.

Methods. The experimental procedure was similar to that previously described(1). Young growing male Holtzman rats of average initial weight 160 to 170 g were used. The animals were implanted subcutaneously with sterile polyvinyl sponges (Ivalon) and fed the following diets: A basal diet (control) containing 24% vitamin-free casein, 61% corn starch, 10% corn oil, 5% of Wesson salt mixture, .2% choline HCl and an adequate supply of vitamins. 6-Methyl prednisolone (20 mg/kg diet), methyl testosterone (20 mg/kg diet) and stilbestrol (3 mg/

* This investigation was supported by U.S.P.H.S. grants.

† Medrol kindly supplied by the Upjohn Co., Kalamazoo, Mich.

‡ Growth Hormone kindly supplied by Armour Co., Kankakee, Ill.

kg diet) were added to the basal diets when indicated. Growth hormone (1 mg/rat/day) was administered intraperitoneally in alkaline saline solution. At the end of the 14th day all animals were sacrificed 5 hr after intraperitoneal injection of a tracer dose of 1 μC of 1-C^{14}-glycine (45 mC/mM) (7.3 $\times$ 10^5 counts per min.) per 100 g of body weight.

Skin, sponge and tissues under study were removed and weighed, and protein fractions and collagen isolated and counted as previously described(4).

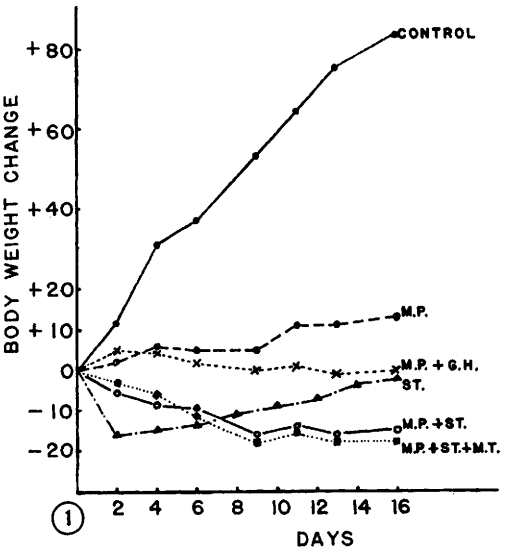
Results. Supplementation of the basal diet with methyl prednisolone (0.20 mg/rat/day) almost completely stopped growth. The daily injection of growth hormone (1.0 mg/rat/day) increased growth retardation. Further addition of stilbestrol to the diets (0.026 mg/rat/day) with or without methyl testosterone (0.17 mg/rat/day) produced an even greater growth retardation, animals actually losing body weight (Fig. 1).

The total amount of collagen synthesized in response to implantation of polyvinyl sponges is shown in Table I. Supplementation of the diets with methyl prednisolone, stilbestrol as well as testosterone decreased the amount of collagen formed in response to the polyvinyl sponge implant. The combination of methyl prenisolone and stilbestrol brought about the same response as stilbestrol alone. The greatest inhibition of sponge collagen synthesis occurred in those animals fed diets supplemented with methyl prednisolone, stilbestrol and methyl testosterone (Group V).

The hexosamine content of the sponges decreased proportionately to the collagen concentration in those animals receiving the methyl prednisolone but not in those groups receiving the sex hormones where the H/C ratio was slightly increased.

Contrary to our previous finding(1) where growth hormone injected at a level of 0.2 mg per day slightly prevented the inhibitory effect of methyl prednisolone in collagen synthesis, at the present level (1.0 mg/day) no beneficial effect was evident.

The specific activity of collagen samples isolated from the sponge of animals sacrificed 5 hours after injection of C^{14}-glycine is also shown. The composition of the skin of these



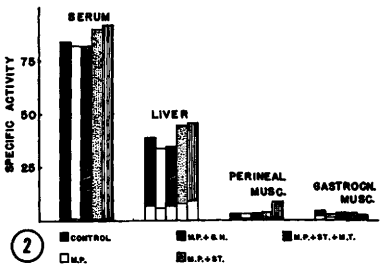


FIG. 1. Changes in body weight of rats fed the different experimental diets. The basal 24% casein diet (control) was supplemented as indicated: M.P. (0.20 mg methyl prednisolone/rat/day), G.H. (1.0 mg/rat/day growth hormone injected subcut.), S.T. (0.026 mg stilbestrol/rat/day), M.T. (0.17 mg methyl testosterone/rat/day).

FIG. 2. Specific activities of tissue proteins of rats receiving the different hormone supplements. Rats sacrificed on 13th day of experiment, 5 hr after inj. of 1-C^{14}-glycine. White area below each column represents non-protein fraction specific activity.

same animals is shown in Table II. The water, fat content, proportion of collagen present in the dry fat-free skin, and specific activity of the isolated collagen are recorded. Also included are values for the weight of the perineal muscles, expressed in absolute value and as percentage of final body weight.

The specific activity of serum, liver, perineal and gastrocnemius muscle proteins and the non-protein fractions of these tissues are schematically represented in Fig. 2.

Discussion. Observation of the growth patterns of the animals consuming the different dietary supplements shows that none of

TABLE I. Effect of Methyl Prednisolone, Growth Hormone, Stilbestrol and Methyl Testosterone on Specific Activity and Total Collagen and Hexosamine Contents of Subcutaneously Implanted Polyvinyl Sponge.*

Group	Supplement (mg/100 g diet)	Values/100 mg implanted sponge Collagen (mg)	Hexosamine (mg)	Hexo./Collagen	Specific activity sponge collagen†
I	Control	14.9 ±.2‡	1.17 ±.03	.079	90 ± 6
II	Medrol 2.0 mg	11.8 ±.3	.92 ±.10	.078	57 ± 4
III	*Idem* + GH 1.0 mg/day I.P.	12.4 ±.4	.91 ±.10	.074	58 ± 7
IV	Medrol 2.0 mg, stilbestrol 0.3 mg	10.8 ±.3	.94 ±.05	.087	59 ± 4
V	*Idem* + M. testosterone 2.0 mg	10.0 ±.2	.98 ±.10	.098	60 ± 4
VI	Stilbestrol 0.3 mg	10.5 ±.3	.96	.092	—

* 8 rats/group. † Counts per min./mg collagen. ‡ Stand. error.

these were able to counteract the growth retardation induced by addition of methyl prednisolone. Contrary to our previous finding (1) where growth hormone injected at a level of 0.2 mg/day slightly prevented the growth inhibitory effect of the corticoid, at the present level (1.0 mg/day) it increased the rate of body weight loss and it was also ineffective in significantly counteracting the antiinflammatory effect of the corticosteroid as measured by the synthesis of collagen stimulated by the implanted plastic sponges. The close dependence of the effects of growth hormone on the tensile strength of wounds with the dose at which it is administered(5) and its inefficiency at relatively high dosages(6) to counteract the inhibition of wound healing caused by corticosteroids, as judged by histological studies, has been reported.

The supplementation of diets with stilbestrol decreased the amount of collagen synthesized as a result of the implant of polyvinyl plastic sponges.

These observations relative to the inhibitory effect of dietary stilbestrol on collagen synthesis do not agree with the findings of Robertson and Sanborn(7) that, in guinea pigs, the daily injection of 0.5 mg of stilbestrol per day enhanced collagen formation in carrageenan induced granulomas.

This lack of agreement may be caused by differences in species, dosage, route of administration, mechanism of induction or nature of the granulation tissue formed.

On the other hand, Taubenhaus and Amromin(8) observed histologically that granulation tissue formation in the walls of turpentine abscesses in rats was greatly depressed by administration of estrogens. Only very few thin fibroblasts and sparse collagen fibers were present in the abscesses of these animals 5 days after subcutaneous injection of turpentine. The controls at that time showed active and well-demarcated granulation tissue.

Hexosamine content of the sponges decreased proportionally to collagen concentration in those animals receiving solely the medrol supplement, but not in those groups receiving in addition sex hormones, where the hexosamine/collagen ratio increased slightly.

The specific activity of the newly-formed collagen was decreased in all the groups re-

TABLE II. Water, Fat and Collagen Content of Skin, Collagen Specific Activity, and Perineal Muscle Weight as Affected by Hormone Administration.

Group	Supplement (mg/100 g diet)	Skin % water	Skin % fat	Skin collagen in dry fat-free skin	Skin collagen spec. activity	Perineal muscle Total wt	Perineal muscle % body wt
I	Control	66.0	8.5	57.3 ± 2	25 ± 2	.568	.237
II	Medrol 2 mg	69.5	2.0	62.0 ± 2	13 ± 2	.364	.215
III	*Idem* + GH 1.0 mg/day I.P.	68.3	2.0	62.6 ± 2	6 ± 1	.327	.208
IV	Medrol 2.0 mg, stilbestrol 0.3 mg	67.9	1.7	62.8 ± 2	2 ± .3	.100	.072
V	*Idem* + M. testosterone 2.0 mg	67.4	1.9	63.8 ± 3	4 ± .3	.214	.155
VI	Stilbestrol 0.3 mg	68.9	3.1	64.0 ± 3		.093	.064

ceiving steroid supplements. Nevertheless, the rate of synthesis does not parallel the total collagen content of skin. This is relatively increased by corticoid or estrogen administration, mainly because of depletion of the metabolically more labile skin constituents, such as fat and non-collagenous proteins. The total fat-free solids have been found to be proteins in nature, on the basis of their total nitrogen content. Within this fraction these hormones appeared to increase the amount of collagenous proteins at the expense of a decrease in proportion of the non-collagenous proteins. Skin collagen synthesis was markedly decreased by addition of methyl prednisolone and even more by the supplement of diets with stilbestrol. Injection of growth hormone (Group III) also reduced further the degree of labeling of skin collagen.

The weight changes of the perineal muscles do not correlate with observed changes in body weight nor with other metabolic processes. This is clearly observed when considering the last 3 groups listed. Addition of methyl testosterone to diets containing stilbestrol protected the muscle from a more complete depletion. The only evidence of an anabolic effect of testosterone under these circumstances occurred at this level and at that of other macroscopically observed accessory sex organs. This emphasizes previous findings where the growth of the perineal muscle (which includes the levator ani) occurred under circumstances where no actual tissue synthesis occurred due to inadequacy of the dietary supply(9). We make a special point regarding the contrasting effects on this muscle because of its widespread use as an index of anabolic activity, particularly in connection with androgenic steroids.

Measurements of uptake of radioglycine by protein and non-protein fractions of different tissues did not differ significantly among groups. Skeletal muscle protein synthesis occurred to a greater extent in the controls than in the hormone treated groups. It also was increased significantly in the perineal muscles of the methyl testosterone supplemented animals but not in the gastrocnemius.

Summary. Growth hormone, methyl testosterone or stilbestrol, when given alone or in combination were not able to counteract the inhibitory effects of 6-methyl prednisolone on body weight gain and collagen synthesis at the site of subcutaneously implanted polyvinyl sponges. This experiment emphasizes the importance of dosage, since at lower concentration (0.2 mg/day) growth hormone had been found to be of benefit in this respect. The rate of uptake of radioglycine by skin collagen was depressed by the hormonal administration and the only significant increase of protein synthesis among the tissues investigated occurred at the perineal muscle of the androgen treated rats. This again points to the unique behavior of the muscles of the perineal complex and to its unsuitability for serving as an estimate of anabolic non-androgenic activity.

1. Bavetta, L. A., Bekhor, I., Shah, R., O'Day, P., Nimni, M. E., *Endocrinology,* in press.

2. Eisenber, E., Gordan, C. S., *J. Pharmac. and Exp. Therap.,* 1950, v99, 38.

3. Metcalf, W., Broich, J., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 744.

4. Nimni, M. E., Bavetta, L. A., *ibid.,* 1961, v106, 738.

5. Williamson, M. B., Neumann, G. J., *Fed. Proc.,* 1954, v13, 322.

6. Spain, D. M., Molomut, N., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 326.

7. Robertson, W. B., Sanborn, E. C., *Endocrinology,* 1958, v63, 250.

8. Taubenhaus, M., Amromin, G. D., *ibid.,* 1949, v44, 359.

9. Nimni, M. E., Geiger, E., PROC. SOC. EXP. BIOL. AND MED., 1957, v97, 606.

Received March 16, 1962. P.S.E.B.M., 1962, v110.