Effect of Growth Hormone on Acetic Acid-Extractable Collagen of Hamster Skin. (23724)

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Unpublished work of Dr. John Berg* indicated that growth hormone given to old hamsters increased the acetic acid-soluble collagen in the skin. The present experiments were designed to test the validity of this observation.

Preliminary data. At the outset it was necessary to determine changes with age in the concentration of acetic acid-soluble collagen in skin of untreated hamsters. A simple procedure for doing this has been reported (1). In the present experiments the hair was removed with clippers and the skin was scraped free of subcutaneous fat. One tenth g of skin, retained in a single piece as far as possible, was placed in 5 cc of dilute acetic acid in a covered stender dish at refrigerator temperature for 24 hours. About 1.5 cc of the extract was then placed in a 75x10 mm test tube and approximately 0.3 g of salt added. The amount of collagen precipitate which resulted was graded 0 to 4 plus as a rough quantitative measure of the collagen extracted. A few strands of precipitate floating to the top was 1 plus; a veil forming over the salt and floating to the top, a 2 plus and a dense precipitate sticking to the salt, a 3 plus. A 4 plus indicated the collagen solution was so concentrated that the salt was immediately surrounded by a jel which caused the salt granules to stick together forming a mass at the bottom of the test tube. In many instances the precipitate was also measured after diluting the original extract 1:8. This dilution was made by adding to 0.25 cc of the extract 1.75 cc of dilute acetic acid. Extracts were made using both 1:1000 and 1:3000 acetic acid in distilled water. The difference in acid concentration used for extraction did not seem to affect the type but only the quantity of the precipitate obtained. Since the abdominal skin contains a higher concentration of acetic acid-soluble collagen than the skin of the back, both were tested. The results of these tests on untreated male golden hamsters from the National Institutes of Health Colony showed the concentration of acid-soluble collagen in the skin to be less in the older animals than in the younger. Using 33-day-old animals the extracts of back skin gave 4 plus precipitates even when extracted with 1:3000 acetic acid. By 64 weeks the collagen extract of the back skin using 1:1000 acetic acid was 3 plus and using a 1:3000 acetic acid dilution for extraction it was 2 plus. By 30 months the corresponding values had fallen to 2 plus or less using the 1:1000 extract and were no more than 1 plus using the 1:3000 extract. The abdominal skin seemed to have a slightly lower concentration of acetic acid-soluble collagen at 64 weeks than at younger ages but 4 plus precipitates were still present. By 29 months 1 and 2 plus precipitates were obtained from 1:1000 acetic acid extracts of abdominal skin. From these results it was felt that if a difference existed in the concentration of acid-soluble collagen in the skin of treated animals from that in control animals it could be detected starting with hamsters about 60 weeks of age.

Growth hormone. Armour lyophilized veterinary growth hormone, lot #R-377-237 was used for experiments I and II and lot #R50109† for Exp. III. Data from the Armour laboratories indicated the potency of lot R-377-237 was 137% of the Armour Standard, the thyroid stimulating hormone 0.11 ± 0.02 U.S.P. unit/mg, oxytocin 0.6 U.S.P. unit/50 mg, pressor effect less than 1 unit/50 mg and depressor effect 5 γ estimated per 50 mg. Lot R50109 was 106.2% of Armour Standard, thyroid stimulating hormone 0.008 U.S.P. unit/mg, vasopressin 0.01 U.S.P. unit/mg, oxytocin 0.008 U.S.P. unit/mg, prolactin 0.1 International unit/mg and almost no adreno-corticotropic hormone, fol-

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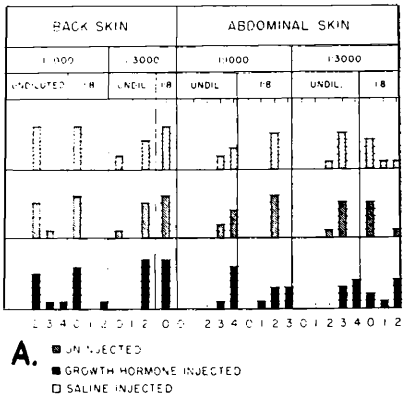
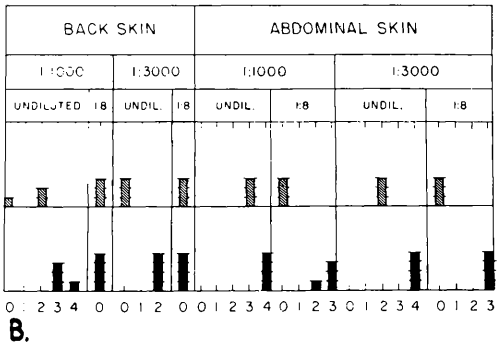
licle stimulating hormone or luteinizing hormone. For Exp. I and II the growth hormone solution for injection was prepared by suspending it in 0.9% NaCl made alkaline by adding one drop of N/10 NaOH to 20 cc. The solution was kept refrigerated and only 5 cc of the hormone was prepared at one time so that a fresh solution was available approximately every 6 days. For Exp. III the NaOH was omitted.

Methods. Male golden hamsters from the Nat. Inst. of Health Colony were used. The animals were housed in individual cages, given water and fed on Purina chow and carrots. The experiment was performed 3 times. In Exp. I 7 hamsters, 62 weeks of age, were injected intraperitoneally with 0.1 mg of growth hormone in 0.2 cc of alkaline saline 6 days a week for 46 days. Six uninjected controls and 6 controls injected intraperitoneally with alkaline saline were used. Exp. II consisted of 4 hamsters 83 weeks of age, injected intraperitoneally with 0.2 mg of growth hormone in 0.2 cc of alkaline saline 6 days a week for 54 days, and 3 uninjected controls of the same age maintained concurrently. Exp. III consisted of 2 hamsters 29 months and one 31 months of age given growth hormone intraperitoneally equivalent to that used in Exp. II. This treatment was continued for 51 days. Two controls 29 months of age were maintained concurrently. In all instances a complete autopsy was performed on each animal and all organs were weighed. Acetic acid-extractable collagen was determined on both back and abdominal skin as previously described; the back skin being taken at the level of but not including the sex spots and below.

Results. Exp. I. The values for the acetic acid-extractable collagen in the skin are given in Fig. 1A. There is essentially no difference between the uninjected and alkaline saline injected controls. On the average the growth hormone injected animals had more acid-soluble collagen in the skin of the abdomen than the control animals. There was very little difference between the control and experimental groups in the skin of the back. The average weight gain of the growth hormone treated animals during the experiment was 12 g whereas the control animals injected with alkaline saline lost 1 g. Sterile precautions were not taken with these animals and many of the injected animals had an infectious process such as peritonitis, epididymitis





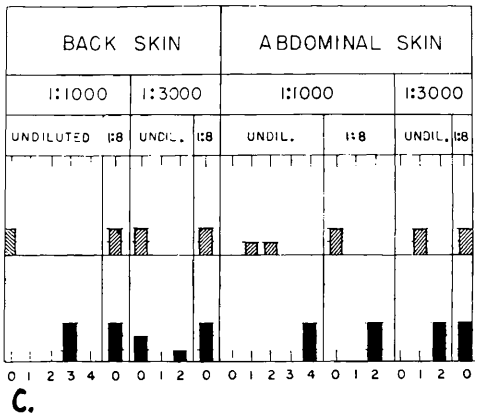


FIG. 1. Effect of intraper. injections of growth hormone on concentration of acetic acid-soluble collagen in skin of hamsters. Each line in a column represents one animal. A. Hamsters 62 wk old. 0.1 mg growth hormone 6 days a week for 46 days. B. Hamsters 83 wk old. 0.2 mg growth hormone 6 days a week for 54 days. C. Hamsters 29 and 31 mo old. 0.2 mg growth hormone 6 days a week for 51 days.

or orchitis.

Exp. II. The values for the acetic acid-extractable collagen in the skin are given in Fig. 1B. In all instances the growth hormone treated animals had a higher concentration of acetic acid-extractable collagen in the skin of both the back and abdomen than did the controls. Experimental animal No. A4 was small with severely scarred kidneys but the amount of extractable collagen in the skin was still higher than in the controls. Using 1:1000 acetic acid, the back skin of the experimental animals gave 3 and 4 plus precipitates and the controls 0 and 2 plus. Using abdominal skin, the treated animals gave a 4 plus precipitate and the controls 3 plus. When diluted 1:8 all precipitates for back skin were 0 and those of the abdominal skin were 2 plus and 3 plus for the experimental animals and 0 for the controls. Using 1:3000 acetic acid for extraction, the differences were even more pronounced. The skin of the back from the experimental animals gave a 2 plus reaction and the controls 0. The abdominal skin of the experimental animals was 4 plus and the controls 2 plus. When the 1:3000 acetic acid extract was diluted 1:8, the back skin of both experimental and control animals was 0, while the abdominal skin of the experimental animals was 3 plus and the controls again were 0. The growth hormone treated animals gained an average of 22 g and the controls 9 g. None of the animals had peritonitis, epididymitis or orchitis.

Exp. III. The values for the acetic acid-soluble collagen in the skin are given in Fig. 1C. For both the abdominal and the back skin there was an increase in the concentration of acetic acid-soluble collagen of the growth hormone injected animals as compared to the controls. There were no infections as the result of the injections in these animals.

In both Exp. I and Exp. II there was no pronounced difference in the weight of organs or their histologic appearance that could be attributed to the effect of the growth hormone. The possible changes as the result of age preclude a statement as to the effect of growth hormone on the very old animals in Exp. III. The number of animals was small and discouraged statistical analysis.

Discussion. In Exp. I there was an indication that the growth hormone increased the amount of soluble collagen in the skin of the old hamsters. Exp. II was modified to amplify this effect if such existed. That is to say, older hamsters were used and a larger dosage of the growth hormone was given for a longer period of time. The results of this second experiment seem to indicate that the concentration of acid-soluble collagen in the skin of old hamsters is increased by the administration of growth hormone. In Exp. III still older hamsters were used and here the results confirmed the effect of growth hormone in increasing the acetic acid-soluble collagen component of both back and abdominal skin. The growth hormone appears to alter the collagen metabolism rather than to maintain it at the younger level. The older animals in Exp. III, Fig. 1C, which were treated with growth hormone have a higher concentration of acetic acid-soluble collagen in the skin than do the younger untreated control animals in Exp. II, Fig. 1B. If the collagen metabolism had been maintained at a younger level rather than altered, these two groups would be more nearly alike.

In the present study the animals were mature, even old, yet the alteration of collagen metabolism still occurred on the administration of growth hormone. They did increase in weight but whether this reflects an anabolism of structural proteins including collagen is not known. On gross observation of skin strips taken from over the right thigh no definite change in the thickness could be detected from that of the controls. Whether the old collagen is changed, new collagen is being laid down, or both, requires further investigation. The gross and microscopic observations of the tissues in these animals were not useful in deciding whether the growth hormone was acting through one or more intermediate endocrine glands.

Summary. Concentration of acetic acid-extractable collagen in the skin of hamsters was used as a measure of the effect of growth hormone on collagen metabolism. The intraperitoneal administration of growth hormone increased the concentration of the acetic

acid-soluble collagen in the skin of old hamsters.

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1. Banfield, W., *Anat. Rec.*, 1952, v114, 157.

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Antigenic Relationships in Insect Extracts.* (23725)

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Recent findings have indicated that dust from disintegrated insects may constitute a common type of antigen responsible for asthma and hay fever(1). One of the problems raised in this connection is whether the observed group reactivity to insects in allergic subjects is due to identical allergens in the extracts of various insects. An unequivocal answer may be obtained only by isolation and comparison of purified allergens from different insects. Useful information about the relationship of extract constituents which may also serve as a guide to fractionation of insect extracts may, however, be obtained by immunological study.

Antigens of several grass pollen extracts were studied by Gosselin *et al.*(2) using hemagglutination. They found many cross reactions among the grasses but also a high degree of specificity by inhibition technics. Gel diffusion has been applied extensively to allergen extracts by Wodehouse(3). Although our plan of study of antigenic relationships of insect extracts included such technics as anaphylactic shock, the production of asthma in the guinea pig from antigen aerosols, Schultz-Dale reaction and passive transfer reactions with reagins, the present study is confined to hemagglutination and specific precipitation in agar.

Materials and methods. The insects used for this study were chosen to satisfy the following requisites: (1) previously demonstrated wheeling skin reactions and clinical allergy in man; (2) close taxonomic relationship between some forms; (3) fairly distant biologic relationship between others; (4) availability of adequate quantities of a pure breed of insect. *Insects.* Mayfly (Ephemoptera), fly (Phormia regina), cricket (Acheta domestica), cockroach (Blatella germanicum) and silk pupa (Bombyx mori) were dried, ground, the ether soluble fraction discarded and dried again. *Extracts.* One gram of dried powder was extracted overnight in 10 ml saline with shaking. Extracts were clarified by centrifugation, merthiolate was added and the solution was frozen. This extract was designated 1:10, and subsequent dilutions were expressed on this basis. *Antisera.* Rabbits were immunized with 4 ml of 1:10 extract in Freund's adjuvant. After 4 weeks test bleedings were made and the gel precipitin pattern observed. Where necessary a second injection was made at this time. Sera were prepared from periodic bleedings and pools made in accordance with gel precipitation data. *Hemagglutination.* The tannic acid technic of Boyden(4) as modified by Stavitsky(5) was employed both for hemagglutination and hemagglutination inhibition titers. Hemagglutination inhibition was performed by preincubating 4 agglutinating doses of serum in all tubes with graded dilutions of antigen followed by addition of coated cells. Extracts were usually diluted to 1:100 for the purpose of coating sheep cells since more concentrated extracts often caused spontaneous agglutination or lysis. *Agar Diffusion.* The triangle plate method of Jennings

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