

Variations in the Levels of Androgens and Gonadotrophic Hormones in the Blood of Rats during Healing of Fractures

TESTOSTERONE and similar androgens promote the anabolism of proteins. Retention of nitrogen was observed after administration of androgens to gonadectomized animals^{1,2}. Male sex hormones also act directly on the skeleton. They enhance endochondrial ossification³ and increase metaphyseal growth⁴. They also increase the fusion of osteocartilagenous and bone grafts in rats and accelerate osteogenic reactions in the surrounding tissues⁵.

In the investigation reported here an attempt was made to obtain a picture regarding the changes in the levels of androgens, luteinizing and follicle-stimulating hormones in the blood of rats during the course of healing of fractures.

Mature male rats of an average weight of 200 g were used. They were divided into groups of 10 animals each. One group of normal intact rats served as control. The remaining groups were subjected to experimental fractures of the left tibiae. The operations were performed by a closed method under ether anaesthesia⁶. A time-table was arranged so that at the end of the experiment the rats were killed at intervals of 5, 10, 15, 22 and 29 days after fracture. Blood was collected separately and the sera belonging to animals of the same group were pooled.

In order to extract androgens from the sera, 4 volumes of acetone were added. The mixture was then centrifuged at 4,000 r.p.m. for 15 min. The supernatant fraction containing androgens was collected. The precipitated proteins containing gonadotrophins were washed three times with acetone-ether mixture (equal parts by volume) to complete the extraction of androgens, and the washings added to the previously obtained acetone extract. After evaporation of the solvent the extracted androgens were dissolved in cotton seed oil to bring them to the original volume of serum. The protein fraction was also dissolved in saline to restore the original volume⁷.

Androgens were assayed using the chick-comb-growth method⁸. Luteinizing hormone (LH) contents of 0.5 ml. serum of rats was determined by the method depending on the formation of corpora haemorrhagica in the ovaries of immature mice previously treated with pregnant mare serum⁹. Follicle-stimulating hormone content of 0.5 ml. of rat serum was determined by the method based on the augmentation reaction of human chorionic gonadotrophin¹⁰.

increased uptake of sulphur-35 by rat humerus¹¹ and tibia⁶ 2-3 weeks after fracture. This time corresponds to the period of maturation of the cellular elements of the fracture callus⁶. The level of androgens, in the serum, increased at the same period of cellular activity.

The principal action of luteinizing hormones on the testis is to cause the secretion of testicular hormones. Such an increase in the level of androgens is apparently a result of increased production of pituitary luteinizing and follicle-stimulating hormones. It has been reported that the effectiveness of luteinizing hormone in activating the interstitial cells of the testis is increased in the presence of follicle-stimulating hormone¹² and this may explain the increased level of follicle-stimulating hormone two weeks after the operation.

In conclusion, it is noteworthy that in rats with experimental fractures (but otherwise kept under natural conditions) the level of serum androgens rises during the second and third weeks post-fracture. The accompanying changes in the levels of luteinizing and follicle-stimulating hormones were, in our opinion, necessary for the stimulation of the testis to increase the production of androgens rather than possessing a direct effect on callus formation.

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PHARMACOLOGY

Toxic Products in Groundnuts

THE recent appearance of a report¹ of a symposium on this subject prompts us to record some of our observations on alleged uses of groundnuts by an Amerindian tribe in the interior of British Guiana. With our children we visited several tribes in this general area during 1961 for the purpose of collecting the natural products used medicinally by the piamen (medicine-men). All our information concerning groundnuts was obtained from members of the Wapisiana tribe (Arawakan linguistic family; location: Southern Rupununi savannahs, approximately 2° 30' N., 59° W.). One of them told us that specially prepared crushed 'Monart' (that is, the fruits of *Arachis hypogaea* L. (identified by kind permission of the Director, Royal Botanic Gardens, Kew)) used to be added to the drink of persons whose conduct was deleterious to the tribe and had the effect of producing insanity and, finally, death. The specific example of Fr. Cary-Elwes, S.J., was quoted.

Study of the journal kept by Fr. Cary-Elwes in his latter days is now in progress² so that following earlier reports³ on the hepatotoxicity of groundnuts we inquired whether this activity might explain his condition as deduced from the journal. We were informed⁴ that the evidence indicated that he had died from avitaminosis rather than

Table 1. ANDROGENS, LUTEINIZING (LH) AND FOLLICLE-STIMULATING (FSH) HORMONES IN RATS' SERUM DURING FRACTURE HEALING

Days after fracture	Chick comb-wt. mg/100 g body-wt. (androgen)	Corpora haemorrhagica/mouse (LH)	Ovary-wt./100 g body-wt. (FSH)
No fracture (Normal control)	19.28 ± 0.97	0.88 ± 0.22	14.45 ± 1.63
5 days	21.06 ± 0.91	1.88 ± 0.39	14.59 ± 1.95
10 days	21.86 ± 1.76	0.75 ± 0.16	16.50 ± 0.89
15 days	23.80* ± 1.62	0.71 ± 0.28	22.68 ± 1.78
22 days	23.69* ± 1.17	0.38 ± 0.18	16.72 ± 1.50
29 days	18.82 ± 1.77	0.50 ± 0.27	16.29 ± 1.63

* Significantly different from normal, $P=0.05$.

The results (Table 1) show that there was a statistically significant increase in the comb-weights of chicks treated with androgens extracted from the sera of rats 15 and 22 days after fracture over the comb-weights of chicks treated with serum of normal rats. The level of luteinizing hormone showed a significant increase 5 days after the fracture. Minimal levels of this hormone were observed during the third and fourth weeks. A statistically high level of follicle-stimulating hormone was detected in the blood of rats 15 days after the fracture, otherwise the values recorded for this hormone remained within normal levels.

Increased formation of cellular proteins is essential for the normal process of fracture healing. As previously mentioned, androgens play an important part in protein anabolism^{1,2}. It has been demonstrated that there is an