STRENGTH OF CALLUS IN FRACTURED HUMERUS OF RAT TREATED WITH ANTI-ANABOLIC AND ANABOLIC COMPOUNDS

By

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A B S T R A C T

Anti-anabolic action of a lathyrus factor (BAPN) and of cortisone and anabolic action of androgenic steroids Dianabol (17α-methyl-17β-hydroxyandrosta-1,4-dien-3-one) and Ora-Testryl (9α-fluoro-11β-hydroxy-17α-methyltestosterone) on the healing of fractured humeri in rats was investigated. Tensile strength of healing callus measured at 1st, 2nd and 3rd week after fracture was determined to assess the difference in action between the compounds studied. Healing bones were also studied histologically.

Significant decrease of tensile strength of callus was observed in BAPN treated rats at 2nd and 3rd week after fracture. Less marked decrease was noted in cortisone treated animals. Certain delay of new bone formation was notable, histologically, in callus of animals treated with anti-anabolic compounds.

Both androgens studied were found to increase significantly tensile strength of callus at 1st and 2nd week after fracture. Increase of tensile strength was associated with marked stimulation of new bone formation and calcification of callus, observed on histological preparations.

Anti-anabolic and anabolic action of certain compounds on the healing of fractured humeri in rats was studied previously in our laboratory with 35S uptake method (Kowalewski & Morrison 1957; Kowalewski 1958; Kowalewski & Gort 1959; Kowalewski & Emery 1960). This method, knowingly, gives information on certain changes in ground substance occurring during chondrogenesis, osteogenesis and bone repair (Dziewiatkowski 1951). It was shown, with radiosulphate uptake procedure, that such substances as lathyrus factor, β-aminopropionitrile (BAPN) (Kowalewski & Emery 1960; Kowalewski et al. 1961).
1959) and cortisone (Kowalewski 1958; Kowalewski & Gort 1959) inhibited bone healing. Both BAPN and cortisone are known to produce osteoporosis of bone (Kowalewski & Emery 1960; Dasler 1956; Ponseti & Shepard 1954) and this may explain satisfactorily the inhibition of bone repair by these compounds, considered as anti-anabolic. It was also found that, under comparable experimental conditions, certain anabolic steroids as norethandroline (17α-ethyl-17β-hydroxy-19-norandrost-4-en-3-one) (Nilevar) promoted healing of fractures in rats (Kowalewski & Morrison 1957; Kowalewski 1958; Kowalewski & Gort 1959; Kowalewski & Gort 1959). Radiosulphate uptake method which may be specific for the determination of sulphated mucopolysaccharides of connective tissue in bone and callus, does not, however, permit the evaluation of the strength of healing bone.

Strength of callus may have clinical significance and a study of factors affecting this strength may have practical importance.

We selected for the present study two anti-anabolic factors, known to us from previous work. Both BAPN and cortisone are known inhibitors of connective tissue. We also selected two androgenic steroids, one predominantly anabolic, Dianabol, and the other, predominantly androgenic, Ora-Testryl. Dianabol (Compound 17309, Ciba) is a testosterone derivative (17α-methyl-17β-hydroxy-androsta-1,4-dien-3-one), showing only weak sex-specific effects and very strong anabolic properties.

Ora-Testryl (Fluoxymesterone, Squibb) (9α-fluoro-11β-hydroxy-17α-methyltestosterone) produces all effects of methyltestosterone (MT) but its androgenic activity is considered several times as potent as MT. Both steroids are now used clinically.

In the present study rats were treated prior to fracture of humerus and during the healing period with above mentioned drugs. Healing bones were removed from animals killed, 1, 2 and 3 weeks after fracture and the strength of callus was determined. Histological studies were also performed.

METHOD

Male Sprague-Dawley rats with average body weight of 200 g, fed a special grain diet (Kowalewski et al. 1959), were used for this study.

A closed complete fracture of the right humerus was produced in all rats following the method previously described (Osborne & Kowalewski 1956). The animals were kept in separate cages and the fractures were left to heal unsupported.

Hormones were given in the following doses:

Cortisone acetate (Merck) was injected subcutaneously in dose of 5 mg per rat, 3 times per week. Treatment started 2 weeks before the fracture and continued until the end of the experiment.

Dianabol was administered per os, as a crushed 5 mg tablet, mixed with the rat’s daily diet. 3 times per week, for 1 week before fracture and until the end of the experiment.
Ora-Testryl was given as a crushed 1 mg tablet, mixed with rat's diet. Timing of this treatment was the same as for Dianabol.

BAPN treated rats were fed basal diet containing 0.2% of synthetic β-aminopropionitrile fumarate (Abbott). This diet was initiated 3 weeks before fracture and continued until animals were killed.

Rats were divided in the following five groups:

1. No treatment.
2. Given BAPN diet.
3. Treated with cortisone.
4. Treated with Dianabol.
5. Treated with Ora-Testryl.

At weekly intervals 12 rats from each group were sacrificed, weighed and the fractured humeri dissected and cleaned. Some of the bones were preserved intact for photography and microscopic study. Sections of all fractures were made in the longitudinal plane. The alignment of the fractures varied. All sections were stained with haematoxylin and eosin. Low power (×45) photomicrographs were taken.

The strength of the healing callus was determined using a specially constructed strain gauge (Fig. 1). Tensile strength of the callus was expressed in grams. Mean values for treated and control groups were compared by the Rank Sum Test and significance levels were expressed in per cent (Wilcoxon 1945).

![Fig. 1.](image)

**Fig. 1.** Picture of the strain gauge used for measurement of the strength of callus. The epiphyseal ends of the fractured humerus (A) are tied into wire mesh tubes (B). One mesh tube is attached to the calibrated spring (C). The spring is connected through a lever arm to a battery circuit and contact (D). When tension is exerted through the spring the contact is made and is indicated by the lighting of the bulb (E). It was found that 420 grams stress was needed to make the contact. This base-line factor, identical for all measurements, is not added to the recorded values. By tightening the screw arm (F) stress is placed on the callus and on the spring. The elongation of the spring is directly proportioned to the stress producing it (Hooke's Law) and can be measured by means of a calibrated millimeter scale (G) under the vertical face plate (H) of the screw arm. The stress is expressed in grams with the help of calibration graph and by interpolation.
RESULTS

The experimental data are summarized in Table 1. It may be noted that in normal control rats there is a progressive increase of the strength of the callus during the first 3 weeks of healing of fractured humerus.

Treatment with BAPN results in the decrease of callus strength, notable at the 2nd and 3rd week after fracture. Not so marked a decrease of callus strength was observed in cortisone treated rats and only at the 3rd week after fracture. Treatment of animals with anabolic androgens provoked a highly significant rise in the strength of the callus. This effect was observed only at the 1st and 2nd week of the healing period.

Histological sections of the fractures in the control group of untreated rats showed the classical picture of healing in callus. Microphotographs of 1 week (Fig. 2) and 2 weeks old callus (Fig. 3) demonstrate the difference, in the amount of cartilage and new bone, due to progress of normal healing.

Parallel to the decrease in the strength of callus in rats treated with anti-anabolic compounds, used in this study, certain delay of new bone formation was observed, when histological sections of fractured bones of animals treated with BAPN or cortisone were reviewed. It is probable that the difference between the above mentioned sections and those of control group will be found more pronounced if a specific staining procedure, like Van Gieson's for collagen, be used.

Histological picture of callus in rats treated with anabolic androgens was also different from that observed in normal controls.

In Dianabol treated rats the sections showed an appreciably larger amount of callus, including mesenchymal tissue and new bone, than in preceding 3 groups (Figs. 4 and 5).

Table 1.
Effect of anti-anabolic and anabolic drugs on strength (in g) of callus of fractured humerus in rat. Each value represents mean for ten rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Week after fracture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Controls</td>
<td>640</td>
</tr>
<tr>
<td>BAPN</td>
<td>740</td>
</tr>
<tr>
<td>Cortisone</td>
<td>423</td>
</tr>
<tr>
<td>Dianabol</td>
<td>*1890</td>
</tr>
<tr>
<td>Ora-Testryl</td>
<td>*2010</td>
</tr>
</tbody>
</table>

* Significance level treated versus control, less than 1%.
** Significance level treated versus control, less than 5%.
Fig. 2.
One week old callus of fractured humerus in normal rat.

Fig. 3.
Two week old callus of fractured humerus in normal rat.
**Fig. 4.**
One week old callus of fractured humerus in rat treated with Dianabol.
Compare with Fig. 2.

**Fig. 5.**
Two week old callus of fractured humerus in rat treated with Dianabol.
Compare with Fig. 3.
Fig. 6.
One week old callus of fractured humerus in rat treated with Ora-Testryl.
Compare with Figs. 2 and 4.

Fig. 7.
Two week old callus of fractured humerus in rat treated with Ora-Testryl.
Compare with Figs. 3 and 5.
In animals treated with Ora-Testryl the sections showed a much greater amount of new bone formation than any of the other groups. The bone trabeculae were larger and showed more evidence of calcification (Figs. 6 and 7).

Limited numbers of microphotographs showing marked histological differences between the groups were included in this report.

**DISCUSSION**

In the present experiment the effect of certain anti-anabolic and anabolic compounds on the strength of healing callus of a fracture was studied. Cortisone and BAPN, a lathyrogenic sweet pea toxin, represented anti-anabolic factors. Cortisone is known to exert an inhibitory effect on the chemical and cellular processes associated with tissue repair (Asboe-Hansen 1957). BAPN is also an inhibitor of connective tissue metabolism. It provokes, knowingly, the syndrome of lathyrysm with osteoporosis, lesions of bone matrix and alteration of mineralization (Ponseti & Shepard 1954). Both cortisone (Asboe-Hansen 1957) and sweet pea toxin (Dasler 1956) are responsible for abnormal metabolism of connective tissue mucopolysaccharides.

In the present experiment an attempt was made to find whether these known anti-anabolic factors do influence the strength of the callus in healing fractured bone. The tensile strength of the callus does not necessarily represent a function of a definite histological or biochemical pattern of healing bone. We expected, however, that anti-anabolic compounds will decrease this strength. The decrease of tensile strength of callus occurred indeed in both cortisone and BAPN treated rats. This effect was significant in BAPN treated rats, studied the 2nd and 3rd week after fracture. It was, however, less marked in healing humeri of rats treated with cortisone.

Interesting results were obtained with androgenic steroids considered in this study as anabolic factors.

Protein-anabolic effect of certain androgens is well known (Saunders & Drill 1958; Kochakian & Dolphin 1955). They are used clinically as growth hormone and they stimulate both linear growth and skeletal maturation of bones (Sobel et al. 1956). They are effective in counteracting the anti-anabolic effect of various stressors (Selye & Mishra 1958) and in protecting against post-cortisone calcium loss (Fisher & Hastrup 1954). They also counteract the cortisone and BAPN effects on 35S uptake of fractured bones in rats (Kowalewski & Gort 1959; Kowalewski & Emery 1960).

In the present study both androgens were found to increase significantly the tensile strength of the callus at 1st and 2nd week after fracture. Parallel to this increase of strength were the changes in histological picture of healing fractures.
Stimulation of mesenchymal growth, increase of new bone formation and calcification of callus were observed in fractures of rats treated with androgens. Under described experimental conditions certain anti-anabolic and anabolic compounds are able to alter normal healing process of bone fracture. One may speculate on the possible use of some anabolic steroids in the treatment of certain cases in human orthopaedic clinic. Such a clinical trial is certainly indicated.

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REFERENCES