AN ANABOLIC ANDROGEN AS A STIMULANT OF BONE HEALING IN RATS TREATED WITH CORTISONE

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It was shown in our laboratory that radiosulphate uptake by the healing fractured bone in rats may be inhibited by cortisone and promoted by certain anabolic steroids (Kowalewski & Gouws, 1957; Kowalewski & Morrison, 1957; Kowalewski, 1958). This was considered to indicate that cortisone inhibited and anabolic steroids stimulated the synthesis of certain mucopolysaccharides which participate in collagen fibrillogenesis and which specifically incorporate S^{35} (Asboe-Hansen, 1954; Layton, 1951). Special attention was given to the synthetic androgen 17-ethyl-19-nortestosterone (Nilevar) which demonstrated significantly higher anabolic effect on bone healing than other androgens did. This steroid counteracted also the catabolic effect of cortisone, as studied by S^{35} uptake method, when both hormones were administered simultaneously (Kowalewski, 1958).

The possibility of clinical use of 17-ethyl-19-nortestosterone as a hormone offsetting post-cortisone osteoporosis was therefore suggested. It was, however, evident that the simultaneous use of both steroids, as described in animal experiments, does not cover the more frequent clinical situation when treatment of an already existent post-cortisone lesion is considered. To study this problem an experiment was designed in which 17-ethyl-19-nortestosterone was administered to animals pre-treated with cortisone.

METHODS

Male rats of the Wistar strain, fed with stock grain diet (Kowalewski, Gouws & Lang, 1959) and having a weight range from 148 to 180 gm., were used for this study. A closed complete fracture of right humerus was produced following the method
previously described (Osborne & Kowalewski, 1956). The animals were kept in separate
cages and the fractures were left to heal unsupported, during a three week period.
They were then sacrificed 24 hours after the intraperitoneal injection of isotope.
Radio-sulphur ($S^{35}$ in $H_{2}SO_{4}$) was given in doses of 200 microcuries per rat, the dose being
dissolved in 2 ml. of distilled water together with 8 mg. of sodium sulphate.

Treatment. The hormones were given in ten doses during the three weeks preceding
the fracture, or during the three week period of healing of the fracture; in all cases
four doses the first week and three doses weekly for the next two weeks.

Cortisone (Merck) was given in doses of 10 mg. in 0.5 ml. of saline per rat sub-
cutaneously.

17-ethyl-19-nortestosterone (Nilevar, Searle Co.) was administered per os, the dose
being a 5 mg. crushed tablet, mixed with food. Animals were divided into the fol-
lowing five groups:
(1) No treatment.
(2) Given cortisone prior to fracture only.
(3) Treated with cortisone prior to fracture and given 17-ethyl-19-nortestosterone
during the healing period.
(4) Given cortisone during the healing period only.
(5) Given both cortisone and 17-ethyl-19-nortestosterone during the healing period.

Measurement of radiosulphate. After the rats were killed, their fractured and normal
humeri were removed, cleaned and dried at room temperature for 24 hours, following
which the bone weight was recorded. The humeri were then processed by the nitric-
perchloric acid wet-digestion method described previously (Osborne & Kowalewski,
1956).

The precipitate of radioactive barium sulphate obtained at the final stage of pro-
cessing, was transferred to planchettes and the counting carried out using a thin mica
window Tracerlab 8-M tube. A Tracerlab Superscaler S. C. 18A was used for recording.
The results were calculated in counts per minute (c. p. m.) per 100 mg. of tissue and
F/I ratio of radioactivity was computed. This is the ratio of the uptake (c. p. m./100 mg.
of bone) of $S^{35}$ by the fractured humerus (F) to the uptake of $S^{35}$ by the corresponding
intact bone (I).

RESULTS AND COMMENT

The results are summarized in Table 1. There is a significant reduction of
F/I ratio in rats given cortisone as compared with normal controls. This re-
duction is very marked in animals receiving cortisone during the period of
fracture healing. When cortisone is given prior to operation and there is no
treatment following it, the F/I ratio is higher, but still significantly under the
normal range. Administration of 17-ethyl-19-nortestosterone to rats pre-treated
with cortisone has a comparable effect to the combined use of both 17-ethyl-19-
nortestosterone and cortisone given simultaneously. In both cases the F/I ratio
is in the range of normal values.

The results of our previous work on the cortisone and 17-ethyl-19-nor-
testosterone effects on bone tissue repair, as measured by $S^{35}$ uptake technique,
are confirmed in this short experiment. It is apparent that this anabolic steroid
not only offsets the effect of cortisone when administered simultaneously with

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Table 1.
Ratio F/I of the radioactivity in fractured (F) and intact (I) humeri, three weeks after fracture and 24 hours after the injection of S\textsuperscript{35}.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of rats</th>
<th>Values</th>
<th>F/I</th>
</tr>
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</table>
| 1     | None      | 12          | Mean   | 2.02
|       |           |             | S. D.  | ± 0.23
|       |           |             | Range  | 1.59–2.63 |
| 2     | Cortisone prior to fracture | 12 | Mean | 1.31 |
|       |           |             | S. D.  | ± 0.21 |
|       |           |             | Range  | 1.17–1.42 |
| 3     | Cortisone prior to and Nilevar\textsuperscript{*} following fracture | 12 | Mean | 2.21 |
|       |           |             | S. D.  | ± 0.16 |
|       |           |             | Range  | 2.01–2.44 |
| 4     | Cortisone following fracture | 12 | Mean | 0.81 |
|       |           |             | S. D.  | ± 0.23 |
|       |           |             | Range  | 0.45–0.96 |
| 5     | Cortisone and Nilevar\textsuperscript{*} following fracture | 12 | Mean | 2.05 |
|       |           |             | S. D.  | ± 0.21 |
|       |           |             | Range  | 1.63–2.94 |

\*Nilevar = 17-ethyl-19-nortestosterone.

the catabolic hormone, but also is able to improve the pre-existing bone condition provoked by pre-treatment with cortisone. It is well known that in clinical practice one may be faced with cases presenting post-cortisone bone lesions. One possibility is to interrupt the catabolic hormone and permit the organism to recover without further hormone therapy. Another way is to begin vigorous therapy with an anabolic hormone after discontinuing cortisone. The present and previous experimental results indicate that such therapy of post-cortisone bone lesions is worthy of clinical assay.

SUMMARY

Cortisone significantly impaired the repair of a fractured humerus in rats, both when given prior to fracture and when administered during the three week healing period.

Anabolic steroid Nilevar (17-ethyl-19-nortestosterone) offset this effect of cortisone when given simultaneously with cortisone or alone to rats pre-treated with cortisone.

The action of steroids on bone healing was measured by S\textsuperscript{35} uptake procedure.
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REFERENCES