# THE IMPORTANCE OF THE 19-METHYL AND THE C(20) KETONE GROUPS FOR THE THYMOLYTIC ACTIVITY OF THE ADRENOCORTICAL STEROIDS

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Evidence is presented to show that reduction of the ketone grouping at carbon 20 in the α-ketol side chain of the adrenocortical steroid to form either the α- or β-hydroxy derivative, produced a profound, if not complete, loss in the ability of the analogue to involute the thymus gland of the immature albino rat. The 19-nor-derivative of hydrocortisone (cortisol) was unable to produce a significant decrease in the thymus weight at the doses employed, suggesting that the 19-methyl is one of the groups which are essential for the thymolytic activity of the corticosteroids. A 19-norhydrocortisone derivative in which the 4-dehydro-3-keto grouping in ring A was replaced by a phenolic type of structure similar to that found in the oestrogenic steroids had no effect on the thymus gland of the rat.

EARLIER work[1] has shown that adrenal corticosteroids which are capable of producing thymic involution in the rat possess the following molecular configuration: a double bond between carbons 4 and 5, a ketone group at the 3-position, an oxygen function at carbon 11, and an α-ketol side chain at carbon 17. This conclusion was based on the observation that steroid compounds which lacked one or more of these groupings did not induce a significant decrease in thymus weight of the test animals even when administered at relatively high doses.

In the present study, particular attention has been directed to the effect of the ketone group at carbon 20 in the α-ketol side chain and of the 19-methyl group on the thymolytic activity of hydrocortisone (cortisol) and some of its analogues.

## EXPERIMENTAL

Intact 25 to 30-day old rats, derived from an inbred Wistar strain and raised in the animal colony of the Food and Drug Directorate, were used. The steroids were dissolved in maize oil and administered subcutaneously in the back three times daily for 2 days. On the third day the thymus glands were removed and weighed, and the thymus weight in mg./100 g. of final weight was recorded for each rat.

By using this procedure it was possible to obtain a significant involution of the thymus gland with a total dose of hydrocortisone ranging from 0·3 to 0·4 mg./100 g. weight.

## RESULTS

The data in Table I show that replacement of the ketone at carbon-20 of hydrocortisone by either an α-hydroxyl or a β-hydroxyl produced a marked decrease in the ability of the corticosteroid analogue to induce

thymic involution. In fact none of the 20-hydroxycorticosteroids produced a significant decrease in the relative thymus weight at dose levels 20 to 30 times greater than the minimum effective dose of hydrocortisone. Likewise the 19-nor compounds had no effect on the thymus glands of the

TABLE I

EFFECT OF CORTICOSTEROID ANALOGUES ON THE RELATIVE THYMUS WEIGHT

| Corticosteroid | No. of rats | Total dose *per rat* mg./100 g. | Mg. thymus/100 g. ± S.E. |
|---|---|---|---|
| Control (maize oil alone) | 5 | 0·0 | 369·6 ± 18·2 |
| Hydrocortisone | 8 | 0·60 | 238·4 ± 6·3 |
| | 8 | 1·20 | 154·5 ± 8·4 |
| 20β-Hydroxyhydrocortisone (Reichstein's E) | 8 | 4·79 | 377·8 ± 12·5 |
| | 8 | 9·58 | 378·6 ± 10·4 |
| 20α-Hydroxyhydrocortisone | 6 | 6·23 | 370·2 ± 11·4 |
| 20α-Hydroxyprednisolone | 6 | 6·27 | 346·7 ± 14·2 |
| 20β-Hydroxyprednisone-21-acetate | 8 | 2·42 | 382·3 ± 21·8 |
| | 7 | 4·84 | 376·4 ± 13·8 |
| | 7 | 9·67 | 367·7 ± 16·6 |
| 11β,20α-Dihydroxy-4-pregnene-3-one | 6 | 6·03 | 402·3 ± 21·7 |
| 11β,20β-Dihydroxy-2-methyl-4-pregnene-3-one | 6 | 6·20 | 361·5 ± 20·0 |
| 19–Norhydrocortisone | 6 | 2·34 | 369·2 ± 15·4 |
| 3,11β,17α,21-Tetrahydroxy-19-nor-1,3,5(10)-pregnatriene-20-one | 6 | 2·30 | 385·7 ± 10·5 |

test animals at the doses used. These findings suggested therefore that both the C-(20) ketone and the 19-methyl group had a profound effect on the thymolytic potency of hydrocortisone and its analogues.

## DISCUSSION

According to Abelson, Ulrich and Long[2], 20β-hydroxyhydrocortisone (Reichstein's Compound E) was the most abundant metabolite found after the administration of hydrocortisone-4-$^{14}$C to the rat. The monohydrate of 20β-hydroxyhydrocortisone showed activity in the liver glycogen assay in the adrenalectomised mouse, but was less active than hydrocortisone. Szpilfogel, van Hemert, and de Winter[3] reported that the 20β-hydroxy derivatives of both prednisone and prednisolone were active in the liver glycogen deposition test for glucocorticoid activity. However Beyler, Hoffman and Sarett[4] found that 20α-hydroxyprednisone was inactive in the liver glycogen assay. Apparently the 20β-ols had glucocorticoid activity while the 20α-ols did not.

The results obtained in this investigation indicate that neither the 20β-ol nor the 20α-ol derivatives of prednisone or prednisolone were able to produce thymic involution in the intact immature rat at the relatively high doses used.

According to Ehrenstein, 19-nordesoxycorticosterone showed little or no physiological activity when administered at a dose level of 0·3 mg./day to adrenalectomised rats. Removal of the 19-methyl group from hydrocortisone decreased the thymolytic activity to the point where it could not be detected by the assay procedure employed. Likewise substitution of a phenolic type of ring structure for ring A of 19-norhydrocortisone reduced if not abolished the ability of the corticosteroid to involute the thymus gland. Since both testosterone and progesterone retain at least qualitatively their respective biological activities after removal of the 19-methyl

group[6,7], it is concluded that the structure specicificity of the adrenal corticosteroids differs remarkedly from that of the androgens and the progestins.

The results of this study indicate that both the C(20) ketone and the 19-methyl group can be considered to be necessary for thymolytic activity in corticosteroids.

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