COMPARISON OF GLUCOCORTICOID ACTIVITY OF SOME SYNTHETIC ADRENOCORTICOIDS BY A NEW METHOD BASED UPON INTRAVENOUS GLUCOSE TOLERANCE CURVE IN RAT*

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Previously the author carried out the intravenous glucose tolerance test with rats, and reported that the glucose tolerance curve for the adrenalectomized animal returned to the pre-operative level on the administration of hydrocortisone acetate (Tobe, 1957). Recently new compounds representing modifications of cortisone and hydrocortisone have appeared. Fried and Sabo (1953, 1954) showed that halogenation at the 9 alpha position of hydrocortisone markedly enhances the glucocorticoid activity, while other workers found that it causes relatively greater increase in mineralocorticoid potency (Borman et al., 1954; Liddle et al., 1954; Swingle et al., 1955). Dehydrogenation of hydrocortisone at positions C-1 and C-2 definitely exaggerates the glucocorticoid function, but the sodium retaining activity appears to be considerably diminished (Perlman and Tolksdorf, 1955; Herzog et al., 1955; Swingle et al., 1956). When, however, two such differing modifications of hydrocortisone are both present in the same molecule, the biological activity of the steroid is greatly altered with respect to both gluco- and mineralocorticoid activity (Stafford et al., 1955; Hirschmann et al., 1955; Swingle et al., 1956). In the present experiments the effects of these modified corticoids and the parent ones upon intravenous glucose tolerance curve in adrenalectomized rats were compared.

MATERIAL AND METHODS

The steroids tested were J-dehydro-9α-fluorohydrocortisone (J9α-FHC), 9α-fluorohydrocortisone (9α-FHC), J-dehydrohydrocortisone (J-HC), J-dehydrocortisone (J-C), hydrocortisone (HC) and cortisone (C) which were prepared in the Merck Institute, Rahway, N. J., U. S. A. They were used as the acetate suspending in Aq. Vehicle No. 1 (Merck).

Forty two male rats of the Wistar strain, weighing approximately 200 g and fed on a commercial laboratory chow (Oriental & Co., Mc 5), were used. Under ether anesthesia all the animals were bilaterally adrenalectomized according to the technique described by Ingle and Griffith (1949). They were divided into 6 groups. Each group was divided into 3 subgroups of generally 3 rats according to the different doses of the steroids. The steroids were subcuta-

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neously injected in a daily dose of 0.25 mg, 0.5 mg and 2.5 mg for two consecutive days beginning from the fourth post-operative day. But 9α-FHC was given in 0.25 mg and 0.5 mg and C in 2.5 mg and 5.0 mg.

Intravenous glucose tolerance test was performed according to the method by Samuels et al. (1937). After 4 hours fast, the animals were given into the saphenous vein 1.25 g/kg of a 50% glucose solution. Before and 10, 20, 30, 60, 90 and 120 mins. after the glucose administration, the blood samples of 0.02 cc were taken from a tail vein, and the blood sugar value was determined by King and Garner's method (1947).

RESULTS

As reported in the previous paper, there is great fluctuation between individuals in the intravenous glucose tolerance curve for rat. In view of this, each comparison was made with one same animal. And for analysis's sake, observation was made in the following three points and numerically evaluated:

I. The curve from 10 to 30 mins. after the glucose administration In all cases 10 min. blood sugar level after adrenalectomy was lower than before it, and the curve between 10 and 30 mins. was steeper in fall after the operation than before it. The administration of some steroids returned the 10 min. blood sugar level to the pre-operative one, and turned the slope of the curve similar to the pre-operative one. When a steroid returned the curve nearly to the pre-operative one in both level and form, it was graded as 2 points (Fig. 1). When it scarcely changed the

![Graph 1](image1)

![Graph 2](image2)

↓: Intravenous injection of glucose, •••• Before adrenalectomy, ×---× After adrenalectomy, o---o After adrenalectomy and administration of corticoids (Fig. 1~3)

Fig. 1. Glucose tolerance curve after the administration of 9α-FHC

Fig. 2. Glucose tolerance curve after the administration of cortisone
curve from the post-operative one, it was graded as 0 (Fig. 2); and the intermediate one was graded as 1 (Fig. 3).

![Graph](image)

**Fig. 3. Glucose tolerance curve after the administration of \( \Delta^1 \)-C**

II. *The curve from 30 to 120 mins. after the glucose administration*  The blood sugar level at 30 mins. after the glucose administration in the adrenalectomized animal was about 110 mg/dl, the difference from pre-operative value (220 mg/dl) being exhibited most remarkably at this time. The following part of the curve was in perfect agreement with the exponential curve. The administration of some steroids returned it to pre-operative state in both level and form. Then they were graded as 2 points (Fig. 1). In regard to point of 1 (Fig. 3) and 0 (Fig. 2) it was the same as in the above.

III. *The blood sugar level at 120 mins. after the glucose administration*  When the steroid raised the blood sugar level at 120 mins. after the glucose administration higher than that before the glucose administration, it was graded as 2 points (Fig. 1), when it kept the level lower, it was graded as 0 (Fig. 2) and when it brought the level nearly to the pre-injective one, it was graded as 1 (Fig. 3).

The results of the assessment of the steroids by above described scoring method are listed in Table 1. When the mean is 6 points, the glucose tolerance curve can be considered to have perfectly been restored by the steroid replacement, and when it is 0, the steroid can be assumed to be perfectly ineffective. Points 1~5 will indicate the intermediate potencies.
Table 1. Scoring points for the adrenocorticoids by means of a new method based upon the change in glucose tolerance curve in rat

<table>
<thead>
<tr>
<th>Steroids</th>
<th>Dose (mg)</th>
<th>Scoring Point</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Δ^1-dehydro-9α-fluorohydrocortisone</td>
<td>0.25</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>9α-fluorohydrocortisone</td>
<td>0.25</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Δ-dehydro-hydrocortisone</td>
<td>0.25</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Δ^-1-dehydro-cortisone</td>
<td>0.25</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>hydrocortisone</td>
<td>0.25</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>cortisone</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
</tr>
</tbody>
</table>

The points for the steroids in a daily dose of 0.25 mg were as follows: 9α-FHC, 4.6; Δ^-9α-FHC, 4.3; Δ^-HC, 1.6; Δ^-C, 1.3; and HC, 1.0. When the daily dose was raised they increased showing nearly same tendency as above. Only for C it was 0 even when the daily dose was 2.5 mg, and attained 1.0 when it was raised to 5 mg.
DISCUSSION

Fried and Sabo (1953, 1954) synthesized 9α-halogen-substituted products from cortisone and hydrocortisone, and reported that they are more potent in glucocorticoid activity than the original steroids by the liver glycogen deposition test on adrenalectomized rats. Borman and Singer (1954) also obtained similar results. Leathem and Wolf (1954) and Swingle et al. (1955), testing the maintenance potency and mineralocorticoid activity on adrenalectomized animals, confirmed that they have 10~20 times the potency of the original ones.

It has been found that 1α-non-saturated derivatives of cortisone and hydrocortisone have enhanced glucocorticoid activity in the liver glycogen deposition in adrenalectomized rats (Herzog et al., 1955; Stafford et al., 1955; Perlman and Tolksdorf, 1955).

There are several reports on 1α-non-saturated-9α-halogen-substituted products. In regard to the glucocorticoid activity of 1α-9α-FHC it is reported that the steroid possesses about 25 times (Hirschmann et al., 1955) or 50 times (Stafford et al., 1955) the potency of hydrocortisone, and that it is the most potent glucocorticoid known. Also there are many reports on mineralocorticoid activity of the potent synthetic adrenocorticoids shown by experiments on laboratory animals and clinical studies on human subjects.

The views of many workers are in agreement in that the potent synthetic adrenocorticoids are superior to any of the hitherto known corticoids in the glucocorticoid activity in the liver glycogen deposition. In the present experiments, the effects of these synthetic adrenocorticoids and original ones upon the blood sugar curve after the glucose administration were investigated by a new scoring method with respect to blood sugar level and type of the curve. The results showed that 9α-FHC had the highest effect, followed by 1α-9α-FHC approximately equal to the former, and then 1α-C, 1α-HC, H and C in the descending order. This is approximately in conformity with the experimental results of other workers obtained by the liver glycogen deposition test. In some cases, however, the change in the glucose tolerance curve after steroid injection occurred relatively early after the glucose administration, in some delayed, and in others the blood sugar level did not return to the preinjective one throughout 120 mins. after the glucose administration. Subsequently, even when scoring points of two steroids are same in mean, they can not always be said to have same potency in quality. It is difficult to compare the potency of steroids by the points, but the analytical observation in three points as mentioned above makes it available. It was found that the order of the steroids in potency by this method is varied by the difference in dose. For example, the points for 1α-C and 1α-HC in a dose of 0.25 mg were respectively 1.3 and 1.7, the latter being superior to the former, while in a dose of 0.5 mg they were respectively 4.3 and 2.0. The point for HC was 1.0 in a dose of either 0.5 mg or 2.5 mg. Therefore it is necessary to make observation 2 or 3 doses to compare the potency of steroids exactly by means of this method.

From these results it is concluded that the method is more simple than the liver glycogen deposition test and expected to be useful in practice, by which one can compare the potency of glucocorticoids not only quantitatively but also
SUMMARY

Activities of some potent synthetic adrenocorticoids and the original ones were compared by a new scoring method based upon the change in intravenous glucose tolerance curve in rat. $9\alpha$-FHC was the most potent, followed by $J^1-9\alpha$-FHC, $J^1$-C, $J^1$-HC, HC and C, but without significant difference between the first and the second, and the fourth and the fifth of them. The method is expected to be useful for the estimation of glucocorticoid activity.

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REFERENCES