EVIDENCE AGAINST CORTICOTROPHIN-LIKE ACTION OF MELANOPHORE HORMONE ON THE ADRENAL CORTEX OF MICE

By

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Jores (1933) reported a pronounced enlargement of the adrenal cortex of immature rabbits after treatment with melanophore hormone. Similar results were obtained with guinea pigs (Holmquist, 1934) and rats (Jores & Beck, 1934). These findings are now considered with renewed interest as they subscribe to the recent claims that melanophore hormone is either identical or very similar to corticotrophin (Sulman, 1952 a, b, c, d; Johnsson & Högberg, 1952). However, as melanophore hormone preparations obtained now are more potent and are tested rigorously for any possible contaminant hormones, the problem of the action of melanophore hormone on the adrenal cortex merits a re-investigation with preparations obtained by a latest method. The data which we have presented in this report clearly indicate that contrary to the earlier findings, melanophore hormone fails to elicit any response in the adrenal cortex.

EXPERIMENTAL PROCEDURE

Twentyfour female mice of a strain imported from Switzerland, weighing 28.1 ± 3.1 gm., were used in this study. Out of this total number 8 were injected with melanophore hormone, 8 with ACTH ('Corticotropin' Wilson Lab., Chicago) and the remaining animals served as the controls.

Melanophore hormone was prepared from posterior pituitary powder (kindly supplied by Messrs. Armour Co., Chicago) by the method of Landgrebe et al. (1943) and was assayed for melanophore activity according to the procedure of Frieden et al. (1948), using the common Indian Frog Rana tigrina as the test animal. ACTH was administered in a dosage of 0.5 mg. (in 0.25 ml. of sterile distilled water) thrice daily at an interval of 8 hours. An equal amount
of melanophore hormone was injected in a similar manner. The control animals received only 0.25 ml. of sterile distilled water thrice daily. The injections were given by the intramuscular route and were continued for 7 days.

Autopsy followed 24 hours after the final treatments. The adrenals were carefully dissected out, weighed to the nearest mg., and finally fixed in 10% neutral formalin. For gross histological purposes, serial sections of the gland were prepared by the paraffin method and stained with Ehrlich’s hematoxylin followed by 0.5% acid fuchsin. Sudanophilia was studied in frozen sections of the adrenal stained with Sudan IV. Adjacent sections were examined under polarized light for birefringence. The distribution of ketosteroids was also studied in frozen sections by the 2,4-dinitrophenylhydrazine reaction (Albert & Leblond, 1946).

RESULTS

The data presented in Table 1 will clearly indicate that melanophore hormone failed to influence the adrenal weight. On the other hand, ACTH significantly increased the weight of the gland.

Histologically, the cortex showed marked hypertrophy of the inner zones after ACTH treatment. The parenchymal cells of these zones were considerably enlarged in size and were intensely fuchsinophilic. An increased vascularity was also evident in these areas. The glomerular zone, however, did not show any histological response to ACTH treatment. In contrast to this picture, the cortex of the melanophore hormone-treated mice was in no way different from that of the controls. There was no hypertrophy of the cellular parenchyma of the inner zones which was to be expected if melanophore hormone acted like ACTH on the cortex. The staining reactions and the vascularity of these areas too were similar to those of the controls.

In the control mice, the maximum concentration of the sudanophilic lipid droplets were seen in the outer fasciculata. In comparison, the glomerulosa and

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of mice</th>
<th>Mean weight of the adrenal (mg.) with S. D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (treated with distilled water)</td>
<td>8</td>
<td>4.5 ± 0.98</td>
</tr>
<tr>
<td>Corticotrophin-treated</td>
<td>8</td>
<td>11.2 ± 1.0*</td>
</tr>
<tr>
<td>Melanophore hormone-treated</td>
<td>8</td>
<td>4.2 ± 1.08**</td>
</tr>
</tbody>
</table>

* Significantly heavier than the controls (t = 11.60, P < .001).
** Insignificant (t = .61).
the rest of the cortex appeared to be somewhat less sudanophilic. There was no lipid-free sudanophilic zone between the glomerulosa and the fasciculata. Adjacent sections examined under polarized light showed a similar birefringence pattern. The maximum concentration of birefringent particles of varying size were seen in the outer fasciculata whereas in the rest of the cortical areas this phenomenon was less prominent. ACTH treatment caused almost a total loss of sudanophilic and birefringent material from the inner zones. The glomerulosa, however, continued to exhibit the presence of birefringence particles. The distribution of sudanophilic and birefringent lipids in the adrenal cortex of melanophore hormone-treated mice were similar in every way to those of the controls. In frozen sections of the adrenal cortex of control mice subjected to 2,4-dinitrophenylhydrazine test, the characteristic yellow colour of varying intensity developed throughout the cortical areas. Similar sections from the ACTH-treated animals showed only faint reactions to this test. Melanophore hormone treatment, on the other hand, caused no change in the reactivity of the cortex to the phenylhydrazine test. The distribution of yellow colour was similar to that of the control animals.

**DISCUSSION**

As a well known stressor, ACTH evokes a number of clearly defined responses in the adrenal cortex. Some of these are an enlargement in size, depletion of lipid droplets and a loss of ketosteroids (Selye, 1950; Greep & Deane, 1949). In the present study too these changes are discernible in an unmistakable manner. Now, if melanophore hormone is considered to possess a corticotrophin-like action, it is to be expected that it will elicit the same changes in the adrenal cortex in an analogous manner. Unfortunately, using a highly purified preparation of melanophore hormone and keeping the treatment schedule strictly similar to ACTH, we are unable to find these changes in the cortex. On the basis of these evidences, therefore, it is clear that melanophore hormone has no ACTH-like action on the adrenal cortex and that the stimulating effects reported by the earlier workers (Jores, 1933; Jores & Beck, 1934; Holmquist, 1934) are ascribable to the presence of a contaminating substance, probably ACTH, in their preparations. The plausibility of such an explanation is further borne out by the findings of Johnsson & Högberg (1952) who demonstrated that melanophore hormone and ACTH resemble so closely that one could be prepared by the procedure generally adopted in the case of the other.

Sulman (1952 d) considers the two hormones under report, as fractions of an ACTH-complex much in the same manner as FSH and LH constitute the gonadotrophic complex. Morris (1952), on the other hand, questions the validity of this concept and simply regards them as two distinct hormones with closely

190
similar properties. Whatever may be the case, the present findings clearly indicate that at least in their actions on the adrenal cortex, the two hormones are totally unlike.

**SUMMARY**

Injection of a highly purified preparation of melanophore hormone fails to evoke changes in the adrenal cortex of mice similar to those produced by corticotrophin. It is believed that the action of the two hormones on the adrenal cortex are totally dissimilar.

**REFERENCES**