ADRENALECTOMY AND REPLACEMENT THERAPY IN LACTATING RATS

2. EFFECTS OF DEOXYCORTICOSTERONE ACETATE ON LACTATION IN ADRENALECTOMIZED RATS

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It is now well established that the numerous physiological disturbances resulting from adrenalectomy include a marked inhibition of lactation (see reviews by Turner [1939], Folley [1940] and Petersen [1944]). In a previous study of replacement therapy in adrenalectomized lactating rats [Folley & Cowie, 1944] we have shown that in our rats and under the conditions of our experiments, deoxycorticosterone acetate was more effective in maintaining lactation than either 11-dehydrocorticosterone or 17-hydroxy-11-dehydrocorticosterone. Neither with the steroid nor an extract of adrenal cortex, however, did we find it possible, with the doses given, to achieve complete restoration of lactation as judged by the only available criterion, the growth rates of litters of standard size measured under strictly controlled conditions. Our results differed from those of Gaunt, Eversole & Kendall [1942] who obtained normal lactation in adrenalectomized rats treated with 17-hydroxy-11-dehydrocorticosterone or with an extract of adrenal cortex, but only partial and inconsistent maintenance with deoxycorticosterone acetate.

In our experiments two doses of deoxycorticosterone acetate were used and a graded dose-response relation was obtained. It thus seemed likely that still greater doses would give better, perhaps complete, maintenance of lactation. We have therefore investigated the dose-response relations over a wider range of dosage. It seemed important to confirm if possible the existence of a regular relation between dose and response as Gaunt et al. [1942] reported erratic results with deoxycorticosterone acetate and Gaunt [1941] had earlier found that this steroid brought about no improvement of lactation in adrenalectomized rats. The existence of such a regular dose-response relation would provide additional evidence that the favourable effect of deoxycorticosterone on lactation in our adrenalectomized rats is a real and reproducible phenomenon.

We have also examined the degree of inhibition of lactation produced by adrenalectomy and its relation to the lactational performance of simultaneously studied control rats.

EXPERIMENTAL

Methods

In all save one experiment (see p. 22), the rats used were uniparous females 4½–5 months old; their diet and the general experimental procedure were as described previously [Folley & Cowie, 1944]. The stock diet, which was fed ad lib., has been found to contain 0-40 % Na₂O and 0-95 % K₂O calculated on a moisture-free basis. In addition the
rats received cows' milk *ad lib.*, raw liver twice weekly (approx. 5 g./rat) and raw carrot daily except Sundays; the effect of these supplements on the intake of Na and K is not likely to be great [see Cowie & Folley, 1946a].

As before, adrenalectomy was performed on the 4th day of lactation and all control rats were subjected to a sham operation. Deoxycorticosterone acetate was subcutaneously injected once daily, in most experiments from the day of operation until the day before weaning (see Table 3). The hormone was dissolved in sesame oil in such concentration that the daily dose was usually contained in 0.5 ml. In all save a few instances untreated rats received similar injections of sesame oil alone. No differences in lactational performance have been found between untreated and oil-injected controls [Folley & Cowie, 1944].

**Effect of adrenalectomy on lactation**

We had previously found [Folley & Cowie, 1944] that in our rats the effects of adrenalectomy on lactation were less severe than those observed by Gaunt *et al.* [1942]. We have now acquired sufficient data to present a more generalized picture of effects of adrenalectomy on lactation in rats of this colony. The mean growth curves of the litters of composite groups of adrenalectomized and sham-operated rats made up of groups used in several separate experiments are shown in Fig. 1. These curves, which may be taken as generally representative for our rats, show, in confirmation of our previous findings, that under the conditions of our experiments, adrenalectomy does not in general cause complete inhibition of lactation. In this colony the 16th day of lactation marks the end of the period over which the growth curve of the litter may

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**Fig. 1.** Mean growth curves of litters of composite groups of adrenalectomized and sham-operated rats. ○—○, litters of 27 sham-operated rats with a total of 212 pups on the 4th day of lactation. ●—●, litters of 27 adrenalectomized rats with a total of 204 pups on the 4th day of lactation. The figures near the curves give the percentages of pups surviving.
be taken as a criterion of the lactational performance of the mother, since the eyes of the young open at about this time and thereafter they have access to the food provided for the mother. From Fig. 1 it will be seen that at the 16th day 88% of the young of the adrenalectomized rats were still alive as compared with a 97% survival of the control young which, however, were almost twice as heavy. At weaning the respective survival rates for the young of adrenalectomized and sham-operated rats were respectively 44.5% and 95.5% and the mean weight per pup of the former was approximately 50% of that of the latter. These results denote a marked, but far from complete, inhibition of lactation. That this is not invariably the case will, however, be seen later.

The possibility arises that the partial nature of this inhibitory effect is due to the presence in our adrenalectomized rats of sufficient accessory cortical tissue to allow lactation at a reduced level. If so it might be possible to improve the lactational performance of such rats by administration of anterior-pituitary adrenotrophic hormone which should produce functional hypertrophy of any accessory cortical tissue. The results of an experiment in which 1 unit daily of a purified preparation of adrenotrophin containing at least 32 sudanophobic units [Reiss, Bálint, Oestreich & Aronson, 1936] per mg. was administered to adrenalectomized rats are given in Table 1. It will be seen that lactation was not improved by the administration of this preparation, indeed the lactational performance of both intact and adrenalectomized rats receiving the hormone was slightly inferior to that of the relevant controls.

Table 1. Effect of treatment with adrenotrophin and deoxycorticosterone acetate (doca) on lactation in adrenalectomized rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total no. of young on 4th day</th>
<th>Mean wt. (g.) of young on day</th>
<th>Per cent of young alive on day</th>
<th>No. of mothers dead by 21st day</th>
<th>No. of mothers dead by 21st day</th>
<th>Per cent change in wt. of mothers between 4th and 21st days</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>6</td>
<td>25-20</td>
<td>10-4</td>
<td>4</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>Adrenotrophin*</td>
<td>6</td>
<td>30-18</td>
<td>10-4</td>
<td>4</td>
<td>16</td>
<td>21</td>
</tr>
</tbody>
</table>

Sham-operated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total no. of young on 4th day</th>
<th>Mean wt. (g.) of young on day</th>
<th>Per cent of young alive on day</th>
<th>No. of mothers dead by 21st day</th>
<th>No. of mothers dead by 21st day</th>
<th>Per cent change in wt. of mothers between 4th and 21st days</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>6</td>
<td>24-20</td>
<td>10-4</td>
<td>4</td>
<td>21</td>
<td>100</td>
</tr>
<tr>
<td>Adrenotrophin*</td>
<td>7</td>
<td>36-18</td>
<td>10-4</td>
<td>4</td>
<td>21</td>
<td>100</td>
</tr>
<tr>
<td>doca: 0-1 mg.‡</td>
<td>6</td>
<td>23-21</td>
<td>10-1</td>
<td>9</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>doca: 1-0 mg.‡</td>
<td>5</td>
<td>17-19</td>
<td>9-9</td>
<td>2</td>
<td>23-2</td>
<td>33-2</td>
</tr>
<tr>
<td>doca: 3-0 mg.‡</td>
<td>6</td>
<td>24-21</td>
<td>10-7</td>
<td>2</td>
<td>25-9</td>
<td>34-8</td>
</tr>
</tbody>
</table>

* Each rat received 17 daily subcutaneous injections of 1 Sudanophobic unit [Reiss et al. 1936] of a preparation of purified adrenotrophin containing at least 32 units per mg. beginning on the day of operation (4th day).
† Since two rats died on the 20th day the figure for the change in weight between the 4th and 19th days is given.
‡ Each rat received 17 daily injections of the stated dose beginning on the day of operation (4th day).

Relation between the intensity of lactation in intact rats and the degree of inhibition due to adrenalectomy

The results of a number of separate experiments indicated that the degree of lactational inhibition due to adrenalectomy varied somewhat from one experiment to another. Similar variations, usually relatively slight, in the lactational performance of the
control groups were also apparent and suggested a possible relation between the initial level of lactation and the severity of inhibition due to adrenalectomy.

In Table 2 are given the litter-growth indices [see Cowie & Folley, 1946b] for control rats together with the corresponding values for adrenalectomized rats expressed as percentages of the control values obtained in five experiments, in each of which, as is our usual practice, rats were chosen at random for adrenalectomy or sham-operation on the 4th day of lactation from a batch of normally lactating females.

Table 2. Relation between the intensity of lactation in the rat and the degree of inhibition due to adrenalectomy

<table>
<thead>
<tr>
<th>No. of rats in group</th>
<th>Mean no. of pups per litter over days 6-11</th>
<th>Litter-growth index g./day*</th>
<th>No. of rats in group</th>
<th>Mean no. of pups per litter over days 6-11</th>
<th>Litter-growth index g./day</th>
<th>% of control</th>
<th>Per cent of original pups alive on day 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>8-0</td>
<td>16-7</td>
<td>9</td>
<td>7-9</td>
<td>6-7</td>
<td>40-1</td>
<td>99-0</td>
</tr>
<tr>
<td>6</td>
<td>7-5</td>
<td>15-3</td>
<td>6</td>
<td>7-3</td>
<td>5-9</td>
<td>38-6</td>
<td>98-0</td>
</tr>
<tr>
<td>5</td>
<td>7-8</td>
<td>14-6</td>
<td>6</td>
<td>7-3</td>
<td>5-0</td>
<td>34-3</td>
<td>84-5</td>
</tr>
<tr>
<td>6</td>
<td>7-8</td>
<td>13-7</td>
<td>6</td>
<td>7-5</td>
<td>3-7</td>
<td>27-0</td>
<td>61-0</td>
</tr>
<tr>
<td>4</td>
<td>8-0</td>
<td>12-2</td>
<td>6</td>
<td>7-5†</td>
<td>Very small</td>
<td>Very small</td>
<td>0</td>
</tr>
</tbody>
</table>

* The litter-growth index of a group of rats is defined as the mean daily gain in weight per litter over the 5-day period from the 6th to the 11th days [Cowie & Folley, 1946b].
† Calculated over days 6-8 since numerous deaths among the litters occurred from day 10 onwards.

It is apparent that there is a negative correlation between the lactational performance of the controls and the degree of lactational failure due to adrenalectomy. This conclusion is in general confirmed by the figures for the survival rates of the litters of the adrenalectomized groups at the 16th day, given in the last column of Table 2. Survival rates at the 16th day, and to a lesser extent at weaning (21st day), are useful as a secondary criterion of the degree of lactational failure in cases where the latter is of such a degree that the young begin to die before the 16th day.

The litter-growth indices of the various groups of control rats, given in Table 2, illustrate the variation in the intensity of lactation which occurs in our rats from time to time. In the experiment in which the lactational performance of the control rats was the lowest observed in the present series of experiments, the effects of adrenalectomy were so severe (see Table 2 and Fig. 4) as to amount to almost complete inhibition, all the young of the adrenalectomized rats being dead by the 14th day, i.e. 10 days after the operation. The shape of the litter-growth curve for this group (Fig. 4) was such that it was impossible to determine the litter-growth index and for comparative purposes it was assumed to be about zero. This is the only experiment so far in which we have observed such severe effects of adrenalectomy upon lactation—effects much more severe than those observed by Gaunt et al. [1942].

Effects of deoxycorticosterone acetate on lactation in adrenalectomized rats

An experiment was carried out in which in addition to the usual groups of sham-operated and adrenalectomized animals, groups of adrenalectomized rats received 0-1, 1·0 or 3·0 mg. of deoxycorticosterone acetate daily from the 4th to the 20th days
Fig. 2. Effect of various doses of deoxycorticosterone acetate on lactation in adrenalectomized rats. The continuous lines are mean growth curves of litters of groups of adrenalectomized rats receiving the indicated daily doses of deoxycorticosterone acetate. The upper and lower broken lines are mean growth curves of litters of groups of sham-operated and untreated adrenalectomized rats respectively.

Fig. 3. The influence of daily injections of deoxycorticosterone acetate from the 4th (day of operation) to the 21st day of lactation on lactation in adrenalectomized rats. \( O-\ldots-O \), relation between the lactational response and the daily dose. \( \times-\ldots-\times \), relation between the lactational response and the logarithm of the daily dose. The response is given by \( \frac{100(c-b)}{a-b} \), where \( a \), \( b \) and \( c \) are respectively the mean daily increase in weight per litter for control rats, untreated adrenalectomized rats, and treated adrenalectomized rats, in each case calculated over the 5-day period from the 6th to the 11th days of lactation.
inhibitory. The results which are shown in Fig. 2 (see also Table 1) confirm and extend our previous finding that there is a graded relation between dose and response over this dose range.

Responses calculated as described by Cowie & Folley [1946b] are plotted against dose and also against log10 dose in Fig. 3. In the first instance the points lie on a smooth curve, while in the second they give an excellent fit to a straight line, in this respect resembling many other dose-response relations. It must be remembered (a) that the response calculated in this way can only be regarded as an approximation since the litter-growth index gives only an approximate measure of the true milk yield, and (b) that no account has been taken of the possibility that, particularly in the case of the highest dosage, excess deoxycorticosterone acetate may find its way into the milk and directly affect the well-being and growth of the young. Nevertheless the regularity of the results obtained in this experiment is noteworthy and suggests that this response might be used as the basis of a method of assay of adrenal cortex extracts using deoxycorticosterone acetate as a standard.

The results in Fig. 3 suggest that the maximum response obtainable is about 60% of the theoretical. However, since the severity of the effects of adrenalectomy on lactation has been shown to vary from one experiment to another and in the same sense as the lactational efficiency of the sham-operated controls, it is of interest to see if the response to a given daily dose of deoxycorticosterone acetate shows similar variations from time to time. The results of three experiments involving the administration of 3·0 mg. of deoxycorticosterone acetate daily are given in Table 3, from which it appears that the response to this dosage varied directly with the degree of inhibition and inversely as the efficiency of lactation in the controls. The supposed

### Table 3. Effect of deoxycorticosterone acetate (doca) on lactation in adrenalectomized rats

(Mean litter size in parentheses)

<table>
<thead>
<tr>
<th>Daily dose of doca mg.</th>
<th>No. of rats in Adrenal-eectomized rats treated with doca (c)</th>
<th>Response (100 (c-b) a-b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group</td>
<td>Adrenal-ectomized group</td>
</tr>
<tr>
<td>0·1†</td>
<td>9 (8·0)</td>
<td>9 (7·9)</td>
</tr>
<tr>
<td>0·1‡</td>
<td>6 (7·5)</td>
<td>6 (7·3)</td>
</tr>
<tr>
<td>1·0†</td>
<td>9 (8·0)</td>
<td>9 (7·9)</td>
</tr>
<tr>
<td>1·0‡</td>
<td>6 (7·5)</td>
<td>6 (7·3)</td>
</tr>
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<td>3·0†</td>
<td>6 (7·5)</td>
<td>6 (7·3)</td>
</tr>
<tr>
<td>3·0‡</td>
<td>6 (7·8)</td>
<td>6 (7·5)</td>
</tr>
<tr>
<td>3·0§</td>
<td>4 (8·0)</td>
<td>6 (7·5)</td>
</tr>
</tbody>
</table>

* The litter-growth index of a group of rats is defined as the mean daily gain in weight per litter over the 5-day period from the 6th to the 11th days [Cowie & Folley, 1946b].
† Each treated rat received 10 daily doses beginning on the 4th day of lactation, i.e. the day of operation.
‡ Each treated rat received 17 daily doses beginning on the 4th day of lactation, i.e. the day of operation.
§ Calculated over days 6–8 since numerous deaths among the litters occurred from day 10 onwards.

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maximum response of c. 60% was well exceeded in the second of these three experiments and in the third, the exceptional experiment where inhibition was complete, the response was estimated as being approximately 100%, an estimate confirmed by the growth curves shown in Fig. 4. The break in the growth curve of the control rats (Fig. 4) at the 18th day is of no practical significance; it was due to the inadvertent failure to feed three of the rats in this group during 2 days preceding weaning. In

the two experiments in which 0-1 mg. of the steroid was given daily the response, on the other hand, varied inversely as the degree of inhibition due to adrenalectomy. Clearly the results shown in Table 3 imply considerable variations from one experiment to another in the slope of the dose-response curve.

**DISCUSSION**

We have seen that while in our rats the degree of inhibition of lactation due to adrenalectomy on the 4th day may vary from time to time, the inhibition is in most cases only partial. On the average the adrenalectomized mothers seem capable of rearing almost 50% of their young to a weaning weight about 50% of the normal for this colony. In one exceptional experiment, however, the inhibition appeared to be complete or very nearly so, though it is improbable that secretion ceased entirely, since Levenstein [1936-7] found histological evidence of secretion in adrenalectomized rats which had lost their litters. In the experiments of Gaunt et al. [1942] also, the average body weight of the young of adrenalectomized rats was 50% of that of control young at the 17th day, but since the survival rate at this time was

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**Fig. 4.** Complete maintenance of lactation in a group of adrenalectomized rats receiving 3-0 mg. of deoxycorticosterone acetate daily from the 4th (day of operation) to the 21st days of lactation. The continuous line is the mean growth curve of the litters of a group of four adrenalectomized rats (28 pups at the 4th day) receiving deoxycorticosterone acetate. The upper and lower broken lines are mean growth curves of litters of groups of four sham-operated controls (32 pups at the 4th day) and six untreated adrenalectomized rats (45 pups at the 4th day) respectively. The figures near the curves give the percentages of pups surviving.
only about 45% as compared with our figure of 88% (see Fig. 1) the effects of adrenalectomy in their rats may be taken as somewhat more severe than are generally observed in ours [see also Folley & Cowie, 1944].

It is considered unlikely that the partial nature of the lactational inhibition is due to the regular presence of accessory adrenal cortex tissue in our rats for the following reasons. The amount of accessory tissue is not likely to vary from one batch of rats to another, particularly to such an extent that an exceptional batch of rats may have none as our results would make it necessary to assume. Further, survival experiments not yet published, which we have carried out on rats from the same colony, show that after adrenalectomy all save a small proportion regularly die within a predictable interval which varies slightly with the age of the rats used. The small proportion which survive apparently indefinitely, presumably possess accessory adrenal cortex tissue though it has not always been found on macroscopic inspection at autopsy. Finally, the fact that administration of adrenotrophin did not improve lactation in adrenalectomized rats, though not conclusive by itself, lends support to the belief that no accessory tissue is regularly present.

For the same reasons it is unlikely that the difference between our results and those of Gaunt et al. [1942], who observed more complete lactational inhibition than we did, could be due to differences in the amount of accessory adrenal cortex tissue possessed by our respective strains of rat. It remains possible that the differences may have been due to differences in the sodium and perhaps potassium, contents of our respective stock colony diets since lactational deficiencies in adrenalectomized rats may be slightly alleviated by administration of sodium chloride [Gaunt & Tobin, 1936; Levenstein, 1936–7; Folley & Cowie, 1944]. The variations in lactational efficiency which we have observed among groups of adrenalectomized rats in our own experiments cannot, however, be accounted for on this basis since the composition of the stock diet in this colony is kept constant. It seems more likely that these variations are connected in some way with the variations in the efficiency of lactation we have observed among our control groups. Analysis of all the data in our possession has failed to support our previous suggestion [Folley & Cowie, 1944], that such changes in lactational efficiency in the control groups may be connected with the seasonal variations described previously [Folley, Ikin, Kon & Scott Watson, 1938] in the proportion of parturient rats in this colony which totally fail to rear their young. The far slighter variations in lactational efficiency we are discussing here may possibly have as their immediate cause alterations in a delicately poised balance between factors which stimulate lactation (possibly anterior-pituitary hormones) and factors which tend to inhibit it. The latter role might well be filled by oestrogen which, as is well known, can inhibit lactation under certain conditions [see Folley, 1941] and it may be significant in the present connexion that the inhibitory action of a given dose of oestrogen is far more pronounced in adrenalectomized than in intact rats [Gaunt et al. 1942; Folley & Cowie, 1944]. Such a mechanism might thus provide an explanation of the observed inverse correlation between the lactational performance of the controls and the degree of inhibition due to adrenalectomy.

Our previous finding that deoxycorticosterone acetate will restore lactation to a considerable degree after adrenalectomy has been confirmed and we have now obtained further evidence that in any one experiment the response is regularly
related to the dose. In different experiments the response to a daily dose of 3-0 mg., calculated by a method which takes account of the lactational performance of intact and adrenalectomized controls, has amounted to a restoration of the lactational deficit due to loss of the adrenals varying from 60 to 100 %.

It may be worth noting here that the rats used in the experiment in which deoxycorticosterone acetate gave a 100 % response differed from those used in all other experiments reported here and previously [Folley & Cowie, 1944] in that they were appreciably older (approx. 10 months old) and were undergoing their second lactation when used for experiment.

Claims to have demonstrated the existence of a specific lactation hormone (corti-lactin) in the adrenal cortex [Brownell, Lockwood & Hartman, 1933; Spoor, Hartman & Brownell, 1941] are difficult to reconcile (a) with the fact that in one experiment deoxycorticosterone acetate gave complete restoration of lactation, and (b) with the complete restoration with 17-hydroxy-11-dehydrocorticosterone reported by Gaunt et al. [1942].

We do not propose to speculate at present on the possible reasons for variations in the lactational response of adrenalectomized rats to deoxycorticosterone acetate observed in these experiments, nor to comment further on the fact that while at the 3-0 mg. level the response varied directly with the degree of inhibition, the opposite was the case in two experiments in which 0-1 mg. was administered. It may be pointed out, however, that if the absolute level of lactation attained by the rats receiving 3-0 mg. had been taken as a measure of the response, the latter would have shown little variation in all three experiments. Nevertheless it would seem that our method of estimating the response [Cowie & Folley, 1946b], taking into account the lactational performance of intact and adrenalectomized controls run at the same time as the treated group, has the sounder theoretical basis.

SUMMARY

1. Adrenalectomy on the 4th day of lactation causes a marked, but usually only partial, inhibition of lactation in our rats. Variations in the degree of inhibition from one experiment to another have been observed and in one experiment inhibition was complete or nearly so.

2. There was a marked negative correlation between the degree of inhibition due to adrenalectomy and the lactational performance of sham-operated controls, which itself varied somewhat from one experiment to another.

3. The fact that in most cases adrenalectomy does not entirely abolish lactation is not due to the presence in our rats of accessory adrenal cortex tissue.

4. Lactation was maintained, in most cases partially, in adrenalectomized rats by daily administration of deoxycorticosterone acetate. Over the dose range studied there was a graded relation between dose and response. The relation between log dose and response over this range appeared to be linear.

5. The lactational response to a given dose of deoxycorticosterone acetate has been found to vary widely from one experiment to another; the response to 3-0 mg. daily varied directly with the degree of lactational inhibition due to adrenalectomy. In one experiment in which inhibition in untreated adrenalectomized rats was, to all intents and purposes, complete, lactation was fully restored to the control level by 3-0 mg. of deoxycorticosterone acetate daily.
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We are greatly indebted to Dr S. K. Kon for generously placing at our disposal the facilities of the rat colony maintained by him at this Institute for nutritional investigations, and to Dr M. Reiss for a supply of standardized adrenotrophin. Our best thanks are also due to Dr A. N. Macbeth of Organon Laboratories, Ltd., for generous supplies of deoxycorticosterone acetate and to Mr W. S. Ferguson of Messrs I.C.I. Ltd., Jealott’s Hill Research Station, for determining the Na₂O and K₂O contents of our stock colony diet. We are happy to acknowledge the help of the Agricultural Research Council who provided one of us (A.T.C.) with a special research grant.

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