ADRENALECTOMY AND REPLACEMENT THERAPY
IN LACTATING RATS

5. THE EFFECT OF ADRENALECTOMY ON LACTATION
STUDIED IN PAIR-FED RATS

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(Received 5 December 1947)

In previous studies of the effects of adrenalectomy on lactation in the rat [Folley & Cowie, 1944; Cowie & Folley, 1947a, b] we have shown, in agreement with others [Gaunt, 1941; Gaunt, Eversole & Kendall, 1942], that the operation causes a serious secretory decline but not the complete abolition of lactation. As is well known [see Ingle, 1944; Young, 1945], adrenalectomized animals, at any rate in the absence of salt therapy, may show a reduction in appetite, and since a diminution in food intake would adversely affect lactation, it is pertinent to inquire how far the lactational decline following adrenalectomy is due to anorexia, and how far to the disruption of some more direct functional relationship between the adrenals and mammary glands. We have attempted to answer this question by means of the paired-feeding technique. The experiments described in this paper give a partial answer to this question, but at the same time they serve to illustrate a defect inherent in the paired-feeding technique which in many cases may make interpretation of results obtained by this method far from easy.

This complication arises from the fact that the metabolism of an intact animal on a restricted diet may differ from that of a similar animal, the appetite of which is impaired because of the removal of an endocrine gland, not only in respect of the primary metabolic consequences of the difference between the endocrine systems of the two animals, but also in respect of the reaction of the intact animal to the changed dietary conditions. Thus the attempt to eliminate one extraneous factor, the difference in food intake, from the experimental situation may be liable to introduce further ones. This, as Ingle [1947] has pointed out, applies particularly to paired-feeding experiments on hypophysectomized or, as the results described in this paper emphasize, adrenalectomized animals, which exhibit minimal voluntary activity in contrast with pair-fed, intact controls the voluntary activity of which is often greatly enhanced in consequence of restriction of the diet.

EXPERIMENTAL

The rats (uniparous females approximately 5 months old) and the general procedure used by us in lactation studies have been described in previous papers [Cowie & Folley, 1947a, b, c]. The diet was as given to the stock rats in this colony except that liquid milk ad lib. was not given but, instead, dried whole milk was mixed with the stock diet in the proportion 375 : 1000 as in a previous experiment, involving the feeding of a high-protein diet [Cowie & Folley, 1947b]. Supplements of raw liver and
ADRENALS AND LACTATION

vegetables (carrot and lettuce) in small amounts were fed as usual since these could be neglected in considering the total caloric intake. In the first of the two experiments the composition of the stock diet was as before [see Cowie & Folley, 1947b], while in the second a slightly modified stock diet, in which ten of the aliquot parts of whole wheat were replaced by wheat germ, was used. This change was made for the whole colony for reasons unconnected with this work.

Adrenalectomy, or in the case of the controls a sham operation, was performed on the 4th day of lactation. Each rat and her litter were weighed daily from parturition until the experiment was ended on the 17th day of lactation. Feeding of the mixture of stock diet and dried milk was begun at parturition, the mixture being offered ad lib. until the day of operation, and the daily food intake measured. During this period rats which persistently scattered their food, thus preventing accurate determination of the amount eaten, were eliminated from the experiment.

Each adrenalectomized rat was paired with a sham-operated partner which had littered (and had therefore been operated upon), in most cases 1 day, or in a few cases 2 or more days, later. Each pair was made up of rats which had shown, during the pre-operative period, as nearly as possible the same appetite, and whose litters were closely alike as regards birth weight and subsequent growth rate. The adrenalectomized member of a given pair was each day given food in excess of its anticipated requirements, and the amount uneaten weighed 24 hr. later; its sham-operated partner, on the corresponding day of lactation, was given the same amount of food.

In one or two cases in which the appetite of the adrenalectomized rat declined to such an extent that its pair-fed control began to suffer a serious loss of weight, the experiment ended on the 15th day to avoid exposing the intact control to unnecessary hardship. For this reason the body-weight changes and food intake figures in Table 2 are given from the 4th to the 15th day.

This paper deals with two paired-feeding experiments, in the first of which the adrenalectomized rats were given tap water to drink ad lib., while in the second the tap water was replaced by 1% NaCl.

RESULTS

The mean growth curves for the combined litters of the adrenalectomized rats receiving no salt therapy and for their pair-fed controls are shown in Fig. 1, together with a curve for a group of sham-operated rats on unlimited food intake, which was run at the same time for comparison. Litter-growth indices [Cowie & Folley, 1947c] and data relating to the growth and survival rates of the litters are given in Table 1. Data relating to changes in body weight of the mothers during the experiment and to food intake are given in Table 2.

It will be seen from the curves that though restriction of the food intake of intact rats considerably reduced their lactational performance, they still lactated definitely better than adrenalectomized rats. This is confirmed by the growth and survival data for the litters, particularly the litter-growth indices, given in Table 1. Thus, while the litter-growth index for pair-fed controls was 57.5% of that for controls receiving unlimited food, the index for adrenalectomized rats was only 41.8% of this control value.

The data for the body-weight changes of the mothers from the day of operation to the 15th day (Table 2) are of interest, since they show that the adrenalectomized rats

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more or less maintained their weight over this period, while the pair-fed controls, on the other hand, suffered a loss in weight of 19-7% during this period. In this connexion it may be noted that throughout the experiment the voluntary activity of the pair-fed controls was markedly greater than that either of the adrenalectomized rats, which exhibited very little activity at all, or of the intact controls given unlimited food. This was obvious, for example, during the daily weighing when our routine procedure is, after removing a given mother and her litter from their cage, to confine the mother in a wooden box fitted with a hinged lid, while the litter is being weighed. Usually the rats remain perfectly quiet in the box, but in the case of the pair-fed controls in these experiments, their determined efforts to escape needed in almost every case the restraint of a suitable weight placed on the lid.

The mean litter-growth curves for the second experiment, in which the adrenalectomized rats received salt therapy, are given in Fig. 2. On this occasion no rats were available to form a comparison group of sham-operated animals receiving an unlimited diet. As before, the litter-growth indices and growth and survival data for the litters are given in Table 1 and the changes in the body weight of the mothers and the food-consumption data in Table 2. It will be seen that the mean daily food consumption, calculated over the period from the 4th to the 15th day, of adrenalectomized rats receiving saline was significantly greater than that recorded in the previous experiment when no saline was given. Furthermore, these adrenalectomized animals actually gained weight during the post-operative period. It is therefore not surprising to find that the litter-growth index indicated a slightly better lactational performance

![Fig. 1. Mean growth curves of the litters of groups of fifteen rats adrenalectomized on the 4th day of lactation (curve A), fifteen sham-operated on the 4th day of lactation and pair-fed with the adrenalectomized rats (curve B), and eight sham-operated on the 4th day and thereafter continued on an unlimited diet (curve C).](image-url)
by adrenalectomized rats when salt was given than when it was not. But it was still clearly inferior to that of pair-fed controls which, moreover, had taken advantage of the larger offerings of food which enabled them to lactate better and with a smaller concomitant body-weight loss than in the previous experiment. It must be remembered, however, that it is difficult to make close quantitative comparisons between lactational performances in these two experiments, because the second one did not include a group of controls on unlimited diet which would form a common base-line of comparison between the two experiments. Lactational performance, as indicated by the litter-growth index, of groups of intact rats in this colony varies somewhat from time to time [Cowie & Folley, 1947a] so that, as we have pointed out before [Cowie & Folley, 1947c], it is desirable that a group of sham-operated controls be included in each experiment whenever possible.

Table 1. Data relating to the growth and survival of the litters of adrenalectomized and pair-fed, sham-operated rats

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Treatment</th>
<th>No. of mothers</th>
<th>Total no. of young on 4th day</th>
<th>Mean weight (g.) of young on day</th>
<th>Percentage of young alive on day</th>
<th>Mean litter-growth index* (g./day) with s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sham operation (fed ad lib.)</td>
<td>8</td>
<td>29 33</td>
<td>10-0 33-7</td>
<td>98 97</td>
<td>15-3 ± 0-7</td>
</tr>
<tr>
<td></td>
<td>Sham operation (pair-fed)</td>
<td>15</td>
<td>60 59</td>
<td>10-3 22-9†</td>
<td>99 84†</td>
<td>8-8 ± 0-6</td>
</tr>
<tr>
<td></td>
<td>Adrenalectomy</td>
<td>15</td>
<td>62 58</td>
<td>10-2 20-3†</td>
<td>100 80†</td>
<td>6-4 ± 0-6</td>
</tr>
<tr>
<td>2</td>
<td>Sham operation (pair-fed)</td>
<td>9</td>
<td>35 36</td>
<td>10-3 26-3</td>
<td>99 94</td>
<td>10-4 ± 0-8</td>
</tr>
<tr>
<td></td>
<td>Adrenalectomy + 1-0% saline</td>
<td>9</td>
<td>33 38</td>
<td>10-1 22-1</td>
<td>100 86</td>
<td>7-6 ± 0-8</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, 1947c].
† These values are for the litters of thirteen mothers only, since two pairs of rats were killed on the 15th day (see text).
‡ Calculated by the 't' test from the paired data.

Table 2. Body-weight changes and food consumption of adrenalectomized and pair-fed, sham-operated rats

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Treatment</th>
<th>Mean percentage change in weight of mothers between 4th and 15th days with s.e.</th>
<th>Mean daily food consumption (g./day) between days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6 and 11*</td>
<td>4 and 15</td>
</tr>
<tr>
<td>1</td>
<td>Sham operated (fed ad lib.)</td>
<td>+ 8-1 ± 1-2</td>
<td>Not measured</td>
</tr>
<tr>
<td></td>
<td>Sham operated (pair-fed)</td>
<td>-19-7 ± 2-0</td>
<td>21-0 ± 1-1</td>
</tr>
<tr>
<td></td>
<td>Adrenalectomy</td>
<td>- 1-5 ± 1-5</td>
<td>21-1 ± 1-1</td>
</tr>
<tr>
<td>2</td>
<td>Sham operated (pair-fed)</td>
<td>-12-9 ± 2-4</td>
<td>24-0 ± 1-4</td>
</tr>
<tr>
<td></td>
<td>Adrenalectomy + 1-0% saline</td>
<td>+ 7-6 ± 2-4</td>
<td>24-7 ± 1-4</td>
</tr>
</tbody>
</table>

* i.e. the period over which the litter-growth index [Cowie & Folley, 1947c] was calculated.
DISCUSSION

The marked difference in lactational performance between adrenalectomized and sham-operated rats, previously described [Cowie & Folley, 1947a], is certainly reduced when the food intake of the two types of rat is equalized. Nevertheless, the litter-growth and survival data (Table 1 and Figs. 1, 2) show that the performance of the adrenalectomized rats was significantly inferior to that of pair-fed controls, irrespective of whether or not the former received NaCl therapy. In fact, the litter-growth and survival data (Table 1) indicate that the difference in lactational performance between the two types of rat was if anything slightly enhanced when the adrenalectomized animals received NaCl. This was probably because the NaCl therapy improved the appetites of the adrenalectomized rats, which meant that their partners received more food than the pair-fed controls in the other experiment, an increment which they seemingly proceeded to convert into milk more efficiently than their adrenalectomized pair-mates. It must be admitted that the difference between the milk yields of adrenalectomized and pair-fed rats, though significant, was relatively slight in both experiments; and at first sight it might appear that post-operative anorexia could account for most of the lactational decline following adrenalectomy and that the effect attributable to the interruption of a more direct relationship between the adrenals and the mammae is relatively insignificant.

However, the present experiments well illustrate the fact that adrenalectomized rats and pair-fed controls are not strictly comparable for studies on milk secretion, since the controls in both experiments lost considerable weight during the experiment.

**Fig. 2.** Mean growth curves of the litters of groups of nine rats adrenalectomized on the 4th day of lactation and thereafter given 1% NaCl instead of water to drink (curve A), and nine sham-operated on the 4th day of lactation and thereafter pair-fed with the adrenalectomized rats (curve B).
while the body weight of the adrenalectomized animals changed very little, or, when NaCl was administered, increased by about the same percentage as that of intact controls fed ad lib. These findings become intelligible in the light of the fact that the pair-fed controls continuously exhibited quite unusual voluntary activity, exceeding that of intact rats on a normal food intake, while the activity of the adrenalectomized rats was much reduced. Thus it seems certain that the metabolic rate of the pair-fed controls was considerably greater than that of the adrenalectomized rats and probably exceeded that of the intact rats receiving food ad lib. Such an increase in total metabolism under conditions of limited food intake would undoubtedly result in increased tissue catabolism leading to loss in body weight.

In order quantitatively to interpret the effects of restriction of the food intake on the lactational performance of the sham-operated rats in these experiments, it is necessary to know how far milk secretion tends to be maintained under these conditions at the expense of tissue catabolism, and also the relative demands resulting from the increased voluntary activity on the food and on the body tissues. The answers to these questions are of course not known, but three possibilities are considered below in a discussion in which, when we refer to the body-weight losses suffered by the pair-fed controls, we mean the net differences between the slight body-weight increase regularly observed in our intact rats during lactation [see Folley & Cowie, 1944; Cowie & Folley, 1947a, b] and the striking losses observed in the pair-fed controls.

(a) The increased voluntary activity of the pair-fed controls may have occurred not only at the expense of the body tissues, the catabolism of which was reflected in the body-weight losses, but also at the expense of a proportion of the food which would otherwise have been available for milk secretion. This would imply that the mean daily amount of food used for maintenance as distinct from milk production, the ‘maintenance ration’ of the dairy husbandman, was somewhat greater in the pair-fed controls than in the adrenalectomized rats, and probably also than in the intact rats receiving food ad lib. The superiority in lactational performance of the pair-fed controls over their adrenalectomized counterparts would thus have been greater had there been no increase in voluntary activity. In other words, if the alternative we are considering were true, it could be said that the pair-fed controls lactated better than the adrenalectomized rats on a smaller ‘production ration’ and would have excelled them still more had their ‘production ration’ been equal to that of their pair-mates.

(b) The increased tissue catabolism represented by the body-weight losses of the pair-fed rats may have represented the exact cost of the enhanced metabolism resulting from their hyper-activity, in which case it may be concluded that their milk yield was very little different from what it would have been had they undergone no increase in spontaneous activity. If this were the case, it would follow that our results give a substantially true quantitative picture of the effect of adrenalectomy on lactation.

(c) The enhanced tissue catabolism and consequent body-weight losses exhibited by the pair-fed controls may have been only partly due to their increased voluntary activity, the rest being ascribable to an effort to maintain milk secretion. In this event it would follow that the pair-fed controls would have lactated hardly more, perhaps even a little less, efficiently than the adrenalectomized rats, had not the possession of adrenal glands in some way conferred upon them the power of secreting milk at the expense of tissue catabolism, an ability which according to our results
seems to be absent from adrenalectomized rats. Incidentally, in this latter connexion it may be remembered that Long, Katzin & Fry [1940] have suggested that the adrenal cortex may promote the mobilization of endogenous protein by exerting a direct effect on the conversion of tissue protein into amino acids. If this alternative were true, it seems possible that had these rats not increased their voluntary activity they might have secreted more milk than they actually did, but this would depend on how far under such conditions body tissues, which otherwise would have undergone catabolism to support increased voluntary activity, could have been diverted to the support of milk secretion.

The paired-feeding technique, as used in these experiments, does not permit of differentiating between the above possibilities (and others differing from one or the other of them only in degree), though it may be said at once that the third alternative seems a priori rather unlikely. As between the first and second alternatives we favour the first as being the more likely, since it seems probable that under conditions of insufficient food intake an increased metabolism due to enhanced voluntary activity would be preferentially compensated for by an increased use for maintenance of food which would otherwise be available for the support of milk secretion. This interpretation is supported by the indication from our results (Table 1) that the disparity in lactational performance between the adrenalectomized rats and their intact pair-mates was enhanced when NaCl was administered to the former with the result that not only they, but also the pair-fed controls, were able to utilize more food so that the body-weight losses of the latter were reduced.

In order to study this question adequately it would be necessary to ‘pair-feed’ not on a basis of total food intake, but on a basis of ‘production’ ration for milk secretion, a procedure involving the theoretical division of the daily ration into two moieties which is not possible on present knowledge. It is hoped, however, that further information may accrue from experiments involving the ‘forced-feeding’ of adrenalectomized rats which are contemplated.

The present results allow only of the conclusion that adrenalectomy does result in a significant decline in lactation, quite apart from secondary effects due to anorexia, to a degree which on the face of it is not very large but may well in actual fact be larger than the paired-feeding technique is capable of revealing.

**SUMMARY**

1. Since studies of the effect of adrenalectomy on lactation may be complicated by secondary consequences of post-operational anorexia, the question has been investigated in rats by the paired-feeding technique.

2. The difference in lactational performance between adrenalectomized and intact rats was considerably reduced when the food intake of the two types of rat was equalized, but the superiority of the pair-fed controls was still significant even if relatively slight. This conclusion holds irrespective of whether or not the adrenalectomized rats were given NaCl therapy, though in the former case there was a slight indication that the superiority of the controls is more marked.

3. Intact rats on a reduced food intake showed a marked increase in voluntary activity, while the activity of their adrenalectomized pair-mates was much reduced. The former lost considerable body weight during the experiment, while the body
ADRENALS AND LACTATION

weight of the adrenalectomized rats was nearly or fully maintained, or, when NaCl was given, increased.

4. It is impossible to say what effect the enhanced tissue catabolism resulting from hyper-activity had on the milk yield of the controls, but certain possibilities are discussed. It is concluded that the paired-feeding method is inadequate for quantitative studies of the effect on lactation of procedures which incidentally reduce the appetite.

We are indebted to Dr S. K. Kon for placing the facilities of his rat colony at our disposal, and to Mr S. C. Watson for technical assistance.

REFERENCES

Ingle, D. J. [1944]. The Chemistry and Physiology of Hormones, p. 83.