Effect of Adrenalectomy on the Arginase Levels of Liver, Mammary Gland and Kidney in Lactating Rats Studied by the Paired Feeding Technique

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(Received 29 April 1948)

The liver arginase in the rat decreases following adrenalectomy (Fraenkel-Conrat, Simpson & Evans, 1943; Folley & Greenbaum, 1946; Kochakian & Vail, 1947), and in lactating rats the mammary-gland arginase behaves similarly (Folley & Greenbaum, 1946). It is possible that the decline in lactation caused by adrenalectomy (see, e.g., Cowie & Folley, 1947*a*) is connected with the decrease in mammary-gland arginase, for there is evidence (Folley & Greenbaum, 1947*a*) that this enzyme system plays an important role in the metabolism of the lactating mammary gland.

Since adrenalectomy is often followed by a marked reduction in food intake (see Ingle, 1944), it is important to find out whether the depressed tissue-arginase levels characteristic of adrenalectomized animals are a primary consequence of the loss of the adrenals or merely a secondary consequence of the post-operative anorexia. Particularly is this so for lactating rats, in which the food intake rises rapidly and steadily over the first 17 days of the lactation period to a value some three times that of pregnancy, so that even though the effect of adrenalectomy during lactation is to prevent this rise rather than to cause an actual fall, the disparity in food intake between adrenalectomized and intact rats soon becomes very marked (Cowie, Folley, French & Greenbaum, 1948).

An investigation of this question by the paired feeding method is described below. The arginase levels of the kidney were studied in addition to those of mammary gland and liver, since Kochakian & Vail (1947) have reported that the kidney arginase, like that of liver and mammary gland, is decreased after adrenalectomy.

EXPERIMENTAL

Animals. Hooded Norway rats were used, approx. 5-6 months old and undergoing their first lactation. The diet was the latest version of the stock diet used in this colony in which 10 of the parts of whole wheat have been replaced by wheat germ (see Cowie & Folley, 1948). The technique of paired feeding, including certain small obligatory dietary modifications, was as used in a previous study by Cowie & Folley (1948). Litters were reduced to 8 at birth and the mothers adrenalectomized or sham-operated on the 4th day, with autopsy on the 17th day.

Tissue-arginase determination. Enzyme assays were made on tissue homogenates made as described previously (Folley

& Greenbaum, 1947*a*), liver, 'abdominal'* mammary glands and kidney being dispersed for 5 min. into 9, 19 and 29 parts respectively of normal saline. The homogenates were fully activated with Mn^{++} after preparation (see Folley & Greenbaum, 1948*a*) so that the results give the presumed levels of enzyme protein in the tissues. It was shown previously (Folley & Greenbaum, 1948*a*) that, in view of difficulties at present unsurmountable in the way of determining the degree of activation of the 'native' arginase system, the 'potential' arginase, as given by the activity of Mn^{++} -activated homogenates, is probably the most biologically significant determination that can be made, particularly since such evidence as we have indicates that the enzyme is almost fully activated in the tissues of our rats.

The determinations were made by the method of Folley & Greenbaum (1948*a*), the reaction being carried out at 37° and pH 9.45 in presence of 0.227 M-substrate. The reaction times were as follows: liver, 5 min.; mammary gland, 5 or 10 min.; kidney, 10 min. The results are expressed in arbitrary arginase units as defined by Folley & Greenbaum (1948*a*). As in a previous study (Folley & Greenbaum, 1947*a*) the mammary-gland results have been expressed on a 'milk-free' tissue basis, the lactose content of milk, secreted by adrenalectomized and pair-fed sham-operated rats, being assumed to be the same as for normal lactating rats. Since this assumption may not be quite true (see discussion by Folley & Greenbaum, 1948*b*) the results for the mammae of the two former groups may be subject to a slight error.

RESULTS

Table 1 gives data relating to the body weights of the rats, organ weights, and milk content of the mammary tissues, etc. The tissue-arginase levels are given in Table 2. Of the mammary glands only the 'abdominal' ones were considered suitable for this work, and thus it was impossible to calculate the total mammary-gland arginase activity per 100 g. rat; instead, values for the total 'abdominal' mammary-gland arginase per 100 g. rat are given.

Table 2 shows that the arginase activities of the tissues of sham-operated rats pair-fed with adrenalectomized rats were not significantly different from those of controls fed *ad lib.* (see probabilities in Table 3). The total liver and 'abdominal' mammary-gland arginase values were, however, significantly diminished in the pair-fed controls due to a marked reduction in the size of these organs (Table 1). The total arginase of the kidney, which decreased very

* I.e. the pair of abdominal and the two pairs of inguinal glands.

Table 1. *Body weights and weights of organs of lactating rats autopsied on the 17th day of lactation*

Treatment	No. of rats	Body wt. at		Mean daily food intake between days 4 and 17 (g.)	Liver wt. (g.)	Kidney wt. (g.)	Wt. of 'abdominal' mammary glands (corr. for milk content) (g.)	Milk content of mammary tissue (%)
		4th day (g.)	17th day (g.)					
Sham-operated (fed <i>ad lib.</i>)	8	251 (209-286)	260 (234-302)	36	13.7±0.6 (11.7-17.0)	1.90±0.19 (1.71-2.24)	4.4±0.1 (3.9-5.0)	44.4±3.2 (29.4-58.3)
Sham-operated (pair-fed)	9	278 (232-318)	209 (175-230)	24.5	8.1±0.2 (7.5-8.8)	1.64±0.04 (1.45-1.80)	2.7±0.3 (2.4-3.4)	28.2±1.8 (21.9-35.6)
Adrenalectomized (fed <i>ad lib.</i>)	9	281 (246-338)	266 (226-298)	24.5	11.4±0.4 (10.1-13.6)	1.76±0.13 (1.57-1.93)	3.2±0.2 (2.3-3.9)	24.4±1.4 (15.7-27.8)

For each group the range is given in parentheses.

Table 2. *Arginase content of tissues of lactating rats autopsied on the 17th day of lactation*

Treatment	No. of rats	Liver		'Abdominal' mammary glands ('milk-free')		Kidney	
		Units/g. moist tissue	Units/100 g. rat	Units/g. moist tissue	Units/100 g. rat	Units/g. moist tissue	Units/100 g. rat
Sham-operated (fed <i>ad lib.</i>)	8	1414±56 (1211-1626)	7448±402 (5850-8950)	162±9 (116-188)	277±18 (199-366)	50±1.2 (44-55)	37±2.0 (30-43)
Sham-operated (pair-fed)	9	1532±87 (1141-2045)	5957±317 (4640-7300)	147±10 (93-185)	193±11 (117-230)	50±2.4 (37-61)	39±2.2 (31-52)
Adrenalectomized (fed <i>ad lib.</i>)	9	488±30 (336-612)	2105±114 (1540-2520)	20±2.3 (13-35)	25±3.1 (16-46)	26±1.2 (22-33)	17±0.8 (14-21)

The results are expressed in arbitrary arginase units (Folley & Greenbaum, 1948a). For each group the mean is given with s.e.m. and the range in parentheses.

Table 3. *Values of 'P' for comparisons between mean values given in Table 2*

Comparison	Liver arginase		Mammary-gland arginase		Kidney arginase	
	Units/g. tissue	Units/100 g. rat	Units/g. tissue	Units/100 g. rat	Units/g. tissue	Units/100 g. rat
Control v. pair-fed control	0.28	0.01	0.28	0.001	0.86	0.49
Control v. adrenalectomy	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002
Pair-fed control v. adrenalectomy	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002

The figures given are probabilities calculated by the *t* test (see Fisher, 1938).

little in size in response to the reduction in food intake, was, however, the same in both groups.

In the adrenalectomized rats there was a considerable and unequivocal decrease in the arginase levels of all three tissues below those of the pair-fed controls, both when the results were expressed per g. moist tissue or as total enzyme per 100 g. rat (see probabilities in Table 3). The mammary-gland arginase concentration was reduced most (to approximately one seventh) and that of the kidney least (to approximately one half).

The abdominal mammary glands in both the adrenalectomized rats and the pair-fed controls were not only smaller than those of controls fed *ad lib.* (Table 1), but contained less retained milk. This was in keeping with the fact that lactational performance was subnormal in both groups. Body-weight losses during the experiment were much higher in the pair-fed controls, as previously observed by Cowie & Folley (1948), and as before were probably connected

with the abnormal spontaneous activity exhibited by these rats.

The weighted mean relative arginase activities of the liver, mammary gland and kidney in rats of each group are given in Table 4. In the normal lactating

Table 4. *Relative arginase contents of tissues of lactating rats autopsied on the 17th day of lactation (weighted means)*

	Sham-operated (fed <i>ad lib.</i>)	Sham-operated (pair-fed)	Adrenal-ectomized (fed <i>ad lib.</i>)
Liver	100	100	100
Mammary gland	11.6	9.6	4.3
Kidney	3.6	3.2	5.6

rats the liver contained approximately nine times as much arginase as the mammary gland and about twenty-eight times as much as the kidney. In the pair-fed controls the relative potencies were not

much disturbed, but in the adrenalectomized rats the mammary gland contained only about 4% of the arginase in the liver, whilst the relative activity of the kidney had increased somewhat.

DISCUSSION

This work shows that adrenalectomy in the lactating rat is followed by a reduction in the arginase activities of the liver and mammary gland, and confirms our previous findings (Folley & Greenbaum, 1946) which were based on a method for arginase-activity assay open to certain objections on kinetic grounds (see Folley & Greenbaum, 1948*a*). It further shows, in agreement with Kochakian & Vail (1947), that adrenalectomy decreases the kidney-arginase levels. In addition, the present results demonstrate clearly that the arginase levels of adrenalectomized rats were significantly lower than those of pair-fed controls, as was indicated by a preliminary experiment under slightly different conditions (Folley & Greenbaum, 1947*b*). From this it might be argued that post-operational anorexia cannot be the primary cause of the diminution in tissue-arginase levels. There are certain points to take into consideration, however, but first it is worth noting, as additional evidence of the importance of arginase in relation to the secretory activities of the mammary gland, that the mean values for the total 'abdominal' mammary-gland arginase, which may be taken as a measure of the total mammary-gland enzyme available in the body, for the three groups of rats, varied directly as the respective lactational performances of these groups, for which the litter-growth indices (Cowie & Folley, 1947*b*) were as follows: controls (fed *ad lib.*), 13.0; pair-fed controls, 11.2; adrenalectomized rats, 6.1 (Cowie *et al.* 1948).

In the mammary gland, adrenalectomy leads not only to a decline in functional activity, but possibly also to some progressive structural involution in long-term experiments such as these (see Levenstein, 1937; Folley & Greenbaum, 1948*b*). Part of the observed decrease in mammary-gland arginase might therefore be accounted for on this basis, since it might be held that we were assaying glands which contained considerable proportions of stromal tissue, the arginase content of which, on the basis of our previous determinations on involuted glands (Folley & Greenbaum, 1947*a*), would almost certainly be low. It seems improbable, however, that much of the effect could be due to this cause because at killing the adrenalectomized rats were still lactating, even if at a considerably reduced level; and Levenstein (1937) found that in his adrenalectomized lactating rats there was not much alveolar involution, with its accompanying increase in the proportion of stroma to parenchyma, so long as the young were still alive (up to 9 days after adrenalectomy on the

day following delivery). Moreover, the pair-fed controls had, if anything, even smaller mammas than the adrenalectomized rats, but the arginase content was not significantly below that of controls fed *ad lib.* It would seem, therefore, that with the mammary gland, as probably also with the kidney, the reduced arginase content was a primary consequence of adrenalectomy, and not due to anorexia or to involutionary increase in the proportion of stroma to parenchyma. It is noteworthy that, although adrenalectomy causes a slight reduction in the Q_{O_2} (glucose) of rat-mammary-gland slices and a decrease in the high R.Q. (glucose) values (Folley & French, 1948) to values near unity, the same happens in pair-fed controls (Cowie *et al.* 1948). The behaviour of the mammary-gland arginase system in face of adrenalectomy thus stands in contrast to that of the mammary enzyme systems concerned in the metabolism of glucose, and may indicate a specially intimate, though not necessarily direct, relationship between the adrenal cortex and the arginase system.

The case of the liver is somewhat more complex. In these experiments, as in previous pair-feeding experiments on lactating rats (Cowie & Folley, 1948), the pair-fed controls showed marked hyperactivity and lost considerable weight during the experiment. The kind of body tissue catabolized was not determined, but if, as seems certain, it included protein, one might expect that the liver-arginase levels would tend to be maintained, particularly since the liver itself decreased in size. Takehara (1938) has indeed reported increased liver-arginase concentrations in fasted rats. On the same diminished food intake the adrenalectomized rats, however, lost relatively little weight but lactated less intensely than the pair-fed controls. Lactation seems to require an enhanced liver-arginase content (Folley & Greenbaum, 1947*a*) so that, on both counts, in these rats the demands on the urea-forming machine of the liver would be less than in the pair-fed controls; consequently a decrease in the liver arginase might be anticipated, apart from a reduction due to disturbance of any direct relationship between adrenal-cortical hormones and liver arginase. Anorexia would possibly have a different influence on the liver arginase in the presence and absence of the adrenals respectively, at any rate during lactation, and the interpretation of the results of paired feeding experiments is accordingly complicated. Whether adrenalectomy lowers the liver-arginase content because of the reduction in protein catabolism due to the decreased food intake, or whether anorexia itself represents an adjustment to a decrease in protein catabolism due to loss of adrenal cortical intervention at some point in the mechanism responsible for deamination and excretion of waste nitrogen (e.g. the liver-arginase system), it is thus impossible at present to say. By analogy with the

post-adrenalectomy changes noted in the arginase levels of the mammary gland and kidney, however, it would seem reasonable to conclude that anorexia is not the primary cause of the decrease in the liver arginase following adrenalectomy.

The use of an arginase-assay method kinetically more sound (see discussion by Folley & Greenbaum, 1948a) than that used previously for the purpose (Folley & Greenbaum, 1946), together with the introduction of a means of making at least an approximate correction of the results for the milk retained in the tissue, has enabled us to make a re-evaluation of the arginase activity of the mammary gland in relation to other arginase-containing tissues in the lactating rat, which is considerably more favourable to the mammary gland than our previous estimate. Since we have now found that, in the intact lactating rat, the mammary gland exhibits about three times the arginase concentration of the kidney, our previous conclusion (Folley & Greenbaum, 1946) that the mammary gland is the second most abundant source of mammalian arginase is amply confirmed. These calculations of course refer to the total 'activatable' enzyme (presumably the total enzyme protein), but it is not anticipated that the results would be much upset if it were possible to determine the concentration of the active Mn^{++} protein (see Folley & Greenbaum, 1948a) in the tissues. Our liver/kidney ratio is lower than the ratio reported by Kochakian (1944) for the mouse. Assuming, by analogy with the constancy of the concentration of alkaline phosphatase in the rat kidney during pregnancy, lactation and involution (Folley & Greenbaum, 1947a), that the kidney arginase does not increase in lactation, this discrepancy could be accounted for, at least partly, by the increased liver-arginase content during lactation (Folley & Greenbaum, 1947a).

SUMMARY

1. Liver, kidney and mammary-gland arginase levels of rats, adrenalectomized on the 4th day of lactation and autopsied on the 17th day, were significantly lower than those of pair-fed sham-operated lactating rats. Arginase per g. moist tissue was reduced most in the mammary gland (to one seventh) and least in the kidney (to one half).

2. The tissue-arginase levels of sham-operated lactating rats pair-fed with adrenalectomized rats were not significantly lower than those of sham-operated rats fed *ad lib.*, save in the case of total arginase in the liver and in the 'abdominal' mammary glands, the reduction in which was in each case due to a decrease in the size of the gland or glands concerned.

3. It is concluded that the results indicate a closer relationship between adrenal-cortical hormones and the arginase of the mammary gland and kidney than would be the case if the decrease in the enzyme concentration were due primarily to post-operative anorexia. Reasons are advanced for the belief that the diminution of liver arginase following adrenalectomy is also not primarily due to anorexia.

4. A re-evaluation of the relative arginase activity of the mammary gland, in relation to that of other tissues in the intact lactating rat, indicates that liver contains nine times the amount in mammary tissue and twenty-eight times the amount in kidney. These ratios are much the same in intact lactating rats on a reduced food intake, but in adrenalectomized rats the activity of the mammary gland relative to the liver falls slightly below that of the kidney.

We are indebted to Dr S. K. Kon for placing the facilities of his rat colony at our disposal; to Dr A. T. Cowie for performing the adrenalectomies and to Mr S. C. Watson for technical assistance.

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