

TABLE I. Comparison of Creatinine Values Obtained by the Enzymatic and Adsorption Methods.

Apparent	Serum creatinine, mg %	
	Enzymatic	Adsorption
.67	.50	.51
.68	.49	.46
.74	.58	.49
.78	.56	.63
.85	.66	.67
.88	.71	.66
.90	.77	.68
.90	.78	.80
.94	.76	.72
.98	.76	.71
.99	.80	.76
.99	.84	.82
1.01	.75	.82
1.17	.95	.93
1.17	1.04	.95
1.19	.99	.99
1.21	1.05	.94
1.68	1.45	1.49
2.90	2.57	2.78
3.02	2.59	2.80
3.45	3.16	3.22
3.80	3.41	3.78
4.10	3.84	3.86
5.83	5.18	5.45
7.16	6.70	6.40
10	9.48	9.70
11.4	10.8	11
12.6	11.5	11.7
13.6	12.6	13.2
18.7	17.9	18

values.

Results. The results obtained with 30 serum samples are shown in Table I. There is good agreement between the true creatinine values obtained by the enzymatic and the adsorption methods. To demonstrate further that Lloyd's reagent adsorbs creatinine chromogen specifically, a sample of blood fairly high in residual chromogen was incubated with the bacterial enzyme until all the creatinine had been decomposed. The bac-

teria were centrifuged off and the supernatant fluid was treated with Lloyd's reagent in the usual manner. No non-creatinine chromogen was adsorbed.

A report has appeared from Newburgh's laboratory which indicates that the use of 1:4 tungstate filtrates of plasma or serum give considerably lower creatinine values than 1:10 filtrates(9). Even though our experiments were performed with 1:5 tungstate filtrates, it seemed desirable to check this finding. We determined the creatinine content of 6 normal human sera in 1:5 and 1:10 tungstate filtrates. The values for the 1:10 filtrate averaged 7% higher than the 1:5 filtrate (range, 3 to 13%). This is considerably less than the 21% average difference observed by Camara *et al.*(9), but still represents a significant discrepancy. We, therefore, recommend the use of 8 ml of 1:10 tungstate filtrate instead of 4 ml of 1:5 filtrate in the adsorption procedure. The volume of the blank tubes and standards should be adjusted accordingly. Otherwise, the details of the method are unchanged.

Conclusions. Serum creatinine can be determined specifically on tungstate filtrates by using either the Miller-Dubos creatinine-splitting enzyme, or by adsorption on Lloyd's reagent and subsequent elution by alkaline picrate.

The authors were greatly helped by Dr. Allan M. Butler in carrying through this study, and we express our thanks to him.

9. Camara, A. A., Arn, K. D., Reimer, A., and Newburgh, L. H., *J. Lab. Clin. Med.*, 1951, v37, 743.

Received September 6, 1951. P.S.E.B.M., 1951, v78.

Comparative Effects of Cortisone and 11-Deoxycorticosterone on Tissue Arginase Levels of Adrenalectomized Lactating Rats. (19108)

S. J. FOLLEY AND S. C. WATSON. (Introduced by Robert Gaunt.)

From the National Institute for Research in Dairying, University of Reading, England.

Arginase levels of rat liver(1-3), kidney

(2,3) and mammary gland(2) are decreased

1. Fraenkel-Conrat, H., Simpson, M. E., and Evans, H. M., *J. Biol. Chem.*, 1943, v147, 99.

2. Folley, S. J., and Greenbaum, A. L., *Biochem J.*, 1946, v60, 40, 46.

after adrenalectomy. Pair-feeding experiments(4) have shown that this decrease is not due to post-operative anorexia and is therefore a more direct, perhaps a primary response to adrenal removal. Replacement experiments on adrenalectomized rats have given somewhat conflicting results. Fraenkel-Conrat *et al.*(1) found cortisone more active than 11-deoxycorticosterone acetate (DCA) in restoring the liver arginase levels. Folley and Greenbaum (2) obtained results suggesting that cortisone and 11-dehydrocorticosterone might be more effective than DCA in restoring both liver and mammary gland arginase levels, but later results from the same laboratory, published only in preliminary form(5), suggested that 11-dehydrocorticosterone was no more effective than DCA. In short-term experiments Kochakian and Vail(3) observed little or no restorative effect on liver arginase levels with cortical extract or DCA though both treatments partially restored the kidney arginase levels. The subsequent development of an improved technic for tissue arginase determination in our laboratory(6), coupled with the increased availability of cortisone have prompted a further study of the effect of corticoids on the arginase levels of liver, mammary gland, intestine and kidney in the lactating rat.

Methods. Uniparous Norway rats were adrenalectomized or sham-operated on the 4th day of lactation and killed as convenient on days 14-16. A group of adrenalectomized rats received 3.0 mg DCA in 0.5 ml arachis oil in 2 divided doses daily from the day of operation until the day before autopsy; this dose was chosen as being that which has so far been found to give the best maintenance of lactation in our rats after adrenalectomy (7). Cortisone (free alcohol) was adminis-

tered to another group by subcutaneous implantation of 3 x 15 mg pellets on day 4; the mean total absorption (measured on 7 rats) was 1.22 ± 0.04 mg/day, thus providing a daily dose much larger than we have previously been able to administer to rats in experiments on tissue arginase levels(7). Arginase (fully activated with Mn^{++}) was determined on homogenates of liver, abdominal mammary gland, kidney and intestine as described by Folley and Greenbaum(4) for the first 3 tissues. In the case of intestine a 6-inch length measured from the pylorus was opened up, the contents carefully washed out, and the washed tissue homogenized into 29 volumes of Mn-saline, the incubation time in the enzyme determination being 10 min. As before, the milk retained in the mammary tissue was determined and the mammary arginase activities expressed on a "milk-free" basis.

Results. Data for body weight changes, organ weights and milk content of mammary tissue are given in Table I. The marked increase in weight during the lactation period of lactating rats receiving 3 mg DCA daily, previously observed(7), is confirmed. Adrenalectomy caused a decrease in weight of the liver and kidneys(4) which was fully reversed by DCA but hardly affected by cortisone.

The tissue arginase levels are given in Table II and the significance of differences between pairs of mean values are given in Table III. In connection with the probabilities given in Table III the relatively small numbers of rats in each group should be borne in mind. It is evident that adrenalectomy caused a significant decrease in the arginase activities of all 4 tissues and that cortisone was far more effective than DCA in restoring these levels towards the control values. In fact cortisone restored the arginase activities of liver, mammary gland and kidney to values which were not significantly different from those of the sham-operated rats, though we believe it possible that significance might have been attained had larger groups been available. On the other hand, while DCA exerted slight but statistically significant restorative effects on the mammary gland and kidney arginase it had no significant effect on the liver arginase. This last result contrasts

3. Kochakian, C. D., and Vail, V. N., *J. Biol. Chem.*, 1947, v169, 1.

4. Folley, S. J. and Greenbaum, A. L., *Biochem. J.*, 1948, v43, 581.

5. Cowie, A. T., Folley, S. J., French, T. H., and Greenbaum, A. L., *J. Endocrinol.*, 1947, v5, xxxiii.

6. Folley, S. J. and Greenbaum, A. L., *Biochem. J.*, 1948, v43, 537.

7. Cowie, A. T. and Folley, S. J., *J. Endocrinol.*, 1947, v5, 24.

TABLE I. Effect of Corticoids on Body Wt Changes, Wt of Liver and Kidneys, and Milk Content of Mammary Tissue of Rats Adrenalectomized on 4th Day of Lactation. Mean values with stand. errors are given.

Treatment	No. of rats	% change in body wt of mothers between 4th to 14th days of lactation	Mean body wt of mothers at autopsy, g	Mean wt of liver, g	Mean wt of kidneys, g	Mean % retained milk in abdominal mammary tissue
Sham operation	6	+ 5.3 ± .9*	252	12 ± .44	1.70 ± .04	62.5 ± 4.3
Adrenalectomy	6	— 3.1 ± 1.9†	216	8 ± .22	1.38 ± .03	41.4 ± 7.6
Adrenalectomy + cortisone	5	— 3.9 ± 1.9‡	221	9.3 ± .87	1.54 ± .07	59.4 ± 3.4
Adrenalectomy + DCA	5	+12.8 ± 2.6‡	271	12.1 ± .73	1.63 ± .08	57.4 ± 3.2

* Determined on 9 rats. † Determined on 11 rats. ‡ Determined on 7 rats.

TABLE II. Effect of Corticoids on Tissue Arginase Levels of Lactating Rats Adrenalectomized on 4th Day of Lactation. Mean values with standard errors are given.

Treatment	Tissue arginase [Folley & Greenbaum (6) units]					
	Liver		Mammary gland	Intestine	Kidney	
	Units/g moist tissue	Units/100 g rat	Units/g moist tissue	Units/g moist tissue	Units/g moist tissue	Units/100 g rat
Sham operation	1596 ± 167	7618 ± 753.5	219.8 ± 11.61	60 ± 3.96	57.1 ± 5.26	38.7 ± 3.77
Adrenalectomy	512 ± 42.6	1913 ± 157.1	19.8 ± 3.89	37 ± 3.65	24.6 ± 1.96	15.6 ± .92
Adrenalectomy + cortisone	1277 ± 144.6	5466 ± 920.3	189.6 ± 8.20	82.8 ± 7.15	50.2 ± 3.19	35.2 ± 3.08
Adrenalectomy + DCA	541 ± 80.7	2408 ± 372.8	94.5 ± 9.81	55 ± 3.02	36.5 ± 2.56	21.9 ± 1.55

TABLE III. Significance of Difference Between Pairs of Means.

Treatment comparisons	Liver		Mammary gland	Intestine	Kidney	
	Arginase units/g moist tissue	Arginase units/100 g rat	Arginase units/g moist tissue	Arginase units/g moist tissue	Arginase units/g moist tissue	Arginase units/100 g rat
Sham operation v. adrenalectomy	†	†	†	†	†	†
v. adrenalectomy + cortisone	*	*	*	†	*	*
v. adrenalectomy + DCA	†	†	†	*	†	†
Adrenalectomy v. adrenalectomy + cortisone	†	†	†	†	†	†
v. adrenalectomy + DCA	*	*	†	†	†	†

* Not significant ($P > .05$). † Significant ($P < .02$). ‡ Significant ($P < .01$).

with previous findings from our laboratory (2,5) obtained with a different method of arginase activity assay. The results for the intestinal arginase are of interest. In this case the enzyme level was fully restored to normal by DCA while the cortisone treatment gave a significantly higher level than normal. It is, however, doubtful how much significance

should be attached to the results since, in our experience, arginase determinations on intestine are more liable to error than the assays on the other tissues studied since we have found that the enzyme is very readily removed from intestinal tissue by the washing procedure necessary to free it from its contents.

In the sham-operated and adrenalectomized

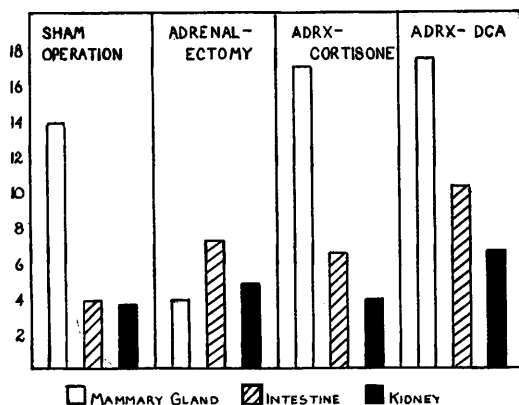


FIG. 1. Relative arginase activities (expressed per g moist tissue) of mammary gland, intestine and kidney as compared with that of liver, taken as 100.

groups the mean arginase activities of the mammary gland and kidney relative to the liver taken as 100 (Fig. 1), are very similar to the values previously found(4). As before, adrenalectomy profoundly altered the "spectrum" of relative arginase activities; neither steroid restored the distribution quite to normal but DCA gave much the more aberrant "spectrum".

Discussion. In this experiment cortisone was clearly more efficient than DCA in restoring the adrenalectomy-depleted arginase levels in all 4 tissues studied, a dose of approximately 1.22 mg/day of the former having in all cases a much greater effect than more than double this weight of deoxycorticosterone. The outcome is probably somewhat weighted in favour of cortisone by the fact that this corticoid was administered by what is probably the most efficient method, subcutaneous pellet implantation, while the deoxycorticosterone was given as acetate twice daily by subcutaneous injection in oil. Nevertheless it is extremely unlikely that the difference in

effectiveness of the two steroids can be accounted for by the difference in method of administration. It is worth noting that although cortisone effected virtually complete substitution for the adrenals as regards the arginase levels of at any rate three of the four tissues studied, in other respects (body weight changes, organ weights) the cortisone-treated animals were clearly not normal. Moreover, by permission of Dr. A. T. Cowie who will report fully on this aspect in due course, it can be noted here that, in agreement with our previous results(7) and contrary to those of Gaunt *et al.*(8), cortisone in this experiment was no more effective than DCA in restoring lactation as measured by growth rate of litters, a finding mirrored by the values for the milk content of mammary tissue given in Table I. Both corticoids effected considerable, but neither effected complete, restoration of lactation.

Summary. Cortisone was much more effective than 11-deoxycorticosterone in restoring the depleted arginase levels of the liver, mammary gland, kidney and intestine in the lactating rat adrenalectomized on the 4th day of lactation.

We are greatly indebted to Dr. S. K. Kon for placing the facilities of his rat colony at our disposal; to Dr. A. T. Cowie for access to adrenalectomized lactating rats used by him in a lactation experiment; to Mr. C. P. Cox for statistical analysis of the results; to Dr. J. M. Carlisle of Merck & Co., Inc. for cortisone; to Dr. W. J. Tindall of Organon Laboratories Ltd. for 11-deoxycorticosterone acetate; and to Ciba Pharmaceutical Products Inc. for a grant to defray publication costs.

8. Gaunt, R., Eversole, W. J., and Kendall, E. C., *Endocrinol.*, 1942, v31, 84.

Received September 10, 1951. P.S.E.B.M., 1951, v78.