Changes in the Arginase and Alkaline Phosphatase Contents of the Mammary Gland and Liver of the Rat during Pregnancy, Lactation and Mammary Involution

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In a previous paper (Folley & Greenbaum, 1946) we have shown that the lactating mammary gland of the rat exhibits appreciable arginase activity and that in fact it is probably the second richest source of arginase in the body. Since it seemed likely that arginase is concerned in the metabolism of the mammary gland, probably with the synthesis of milk constituents, it was decided to study changes in the arginase content of mammary tissue of the rat during the different phases of growth and function characteristic of pregnancy, lactation and involution. The liver arginase content was also studied at the same time because pregnancy and lactation impose a considerable strain on the metabolism of the body which might be reflected in changes in the liver arginase levels.

The only other enzyme known at present to occur in striking concentration in mammary tissue is the alkaline phosphatase (Folley & Kay, 1935), the role of which in mammary metabolism is not definitely known. This work also provided an opportunity of studying changes in the phosphatase activity of mammary tissue in different states of growth and activity. To furnish an indication of possible changes in the general phosphatase levels of the body, and for comparison with the mammary phosphatase, the kidney phosphatase was also determined.

The evidence presented in this paper indicates that arginase plays a more important role in lactation than has hitherto been suspected.

EXPERIMENTAL

Rats. Hooded Norway rats were used, mated for the first time for this experiment at an age of 3 months. The groups were randomly selected and killed at 5, 10, 15 and 20 days after mating and at 5, 10, 15 and 20 days after parturition (pregnancy in this colony usually lasts 22 days). The pups of two further groups were weaned at 21 days and the mothers killed in the period of mammary involution 3 and 6 days after weaning. In addition, enzyme determinations on liver and kidney tissue from a group of unmated rats were made. The number of rats in each group is given in Table 1. Diet was as described previously (Folley & Cowie, 1944).

Table 1. *Distribution of rats among experimental groups*

	Pregnancy				Lactation				Involution	
Day	5	10	15	20	5	10	15	20	3	6
No. of rats	10	10	10	10	11	11	10	10	7	6

Preparation of tissue homogenates. The general procedure regarding dissection of the tissues was as described previously (Folley & Greenbaum, 1946). An improved tissue homogenizer was used in which the blades revolved at 15,000 r.p.m., and all metallic parts in contact with the fluid were made of stainless steel. Weighed amounts of liver, mammary gland and kidney tissue were dispersed (5 min.) in 9, 19, and 39 times their weight of normal saline respectively, one drop of toluene being added as preservative.

Arginase determination. Pure *l*(+)-arginine monohydrochloride was prepared from gelatine by method 1 of Brand & Sandberg (1932). Arginase activity was determined by a method generally similar to that used previously (Folley & Greenbaum, 1946) but differing in that determinations of the urea produced were replaced by colorimetric (Sakaguchi) estimations of the residual arginine by the method of Albanese & Frankston (1945). This change enabled a reduction to be made in the scale of the estimation, thus effecting a big saving in arginine, and also enabled more estimations to be run. There is the further advantage that one can carry out estimations of fully activated arginase in the presence of added Co^{++} without any danger of error due to decomposition of arginine by urease preparations (Hellerman & Perkins, 1935–6). The enzyme determinations were set up in the same proportions as before but scaled down from 25 ml. to 10 ml. flasks with a corresponding reduction of the other volumes. The procedure was the same up to the point of filtering after inactivation of the enzyme. The filtrate was then diluted 1 in 100 and 5 ml. of this taken for estimation by the method of Albanese & Frankston, with the difference that exactly 2 min. instead of 1 min. were allowed for the action of the hypochlorite. This slight modification was found to make very little difference to the colour produced or to the shape of the standardization curve; it was done for convenience in working through large numbers of samples. The colour was immediately measured in a Miller photoelectric absorptiometer using an Ilford Spectrum green filter (604), corrected for the colour developed by the reagents alone, and

the arginine calculated from a standard curve obtained by the same method. The solutions were kept at 21° throughout. Not all samples of hypochlorite were satisfactory; red label Deosan (Deosan Ltd., London, N. 7) proved suitable and was adopted. Ornithine, in concentration equivalent to 50% hydrolysis of the substrate, was shown not to interfere with colour development. Macpherson (1946), in discussing a similar method of arginine estimation, states, but without details of the concentrations involved, that 'NH_3 gives rise to erratic results and should be removed'. From our previous results (Folley & Greenbaum, 1946) with a method which necessarily involved determinations of preformed NH_3 we might expect that the 5 ml. of diluted filtrate taken for the colorimetric determination would contain not more than 4μg. of free NH_3. In the present experiments our results have never been erratic and moreover we have found that addition of a further 4μg. NH_3 had no effect on the final colour.

Van Slyke & Archibald (1946) are of the opinion that arginase activity should be measured by urea production instead of by residual arginine, because of the possibility of decomposition of arginine, without the production of urea, by enzymes other than arginase. This objection might conceivably apply particularly to our 20 hr. method for mammary gland homogenates. In 16 experiments, however, in which arginase determinations by the 20 hr. method were done on amounts of rat milk equal to the amounts of mammary tissue normally present in our reaction flasks, the decomposition of arginine was negligible, indicating not only the absence from rat milk of arginase but also of other enzymes capable of decomposing arginine without the formation of urea. It seems likely that the same would apply to mammary gland homogenates.

For both liver and mammary gland estimations the same units, '30 min.' and '20 hr.' respectively, and the same calibration curves were used as before. All determinations were begun within 2 or 3 hr. of the preparation of the homogenates.

Alkaline phosphatase. Alkaline phosphatase was determined as before (Folley & Greenbaum, 1946) using a Miller photoelectric absorptiometer with an Ilford Spectrum red filter (608).

Estimation of lactose. Lactose was determined in rat milk and in mammary gland homogenates by an adaptation of the method of Hinton & Macara (1927). In the case of milk a protein-free filtrate was prepared by diluting 0·5 ml. of milk to 25 ml., and to 10 ml. of this adding 0·5 ml. of dialyzed iron, standing for 10 min., diluting to 50 ml. and filtering. Mammary gland homogenates were deproteinized by adding 0·3–0·5 ml. dialyzed iron to 5·0 ml. of homogenate, standing 10 min., diluting to 50 ml., and filtering. This was done immediately after homogenization, as we have evidence which suggests that these homogenates exhibit an increase in iodine uptake on standing at room temperature. To 10 ml. of either filtrate were added 2·5 ml. of 10% KI and 10 ml. of 0·005 N-Chloramine T. The flasks were then stoppered with bungs, which had been steeped in KI, and placed in a constant temperature room at 20° for 90 min. At the end of this time they were removed, 2 ml. of 2 N-HCl were added and the liberated iodine titrated with 0·005 N-thiosulphate. Hinton & Macara give as a factor, 1 ml. 0·04 N-thiosulphate ≡ 6·81 mg. lactose. In this adapted method this figure corresponds to 0·851 mg. lactose/ml. 0·005 N-thiosulphate. In practice this was found to give low results, and as a result of many estimations of pure lactose and of recovery of added lactose from mammary gland homogenates the empirically determined factor of 0·870 was adopted.

Milking of rats. Groups of five rats similar to, but not identical with, those in the main experiment were set aside for milking at 10, 15 and 20 days of lactation. These rats could not be used in the experiment because the handling of the glands in milking interfered with subsequent lactation.

A machine similar to that described by Cox & Mueller (1937) was tried but the yields were insufficient for analysis. The procedure finally adopted was hand milking following an intraperitoneal injection of 5 i.u. oxytocin ('Pituitrin', Parke, Davis and Co.). With suitable manipulation of the glands and teats, sufficient milk for analysis could easily be obtained. In the case of the rats at the 10th day of lactation the milks were collected and analyzed as two composite samples representing two and three rats respectively. The samples from the five rats at the 15th and at the 20th days of lactation respectively were collected and analyzed individually.

RESULTS

Milk content of mammary tissue. In a previous paper (Folley & Greenbaum, 1946) attention was drawn to errors in enzyme determinations on mammary gland tissue due to milk retained in the alveoli and the finest ducts, for the complete removal of which no method is known. Unless the proportion of retained milk can be determined, the true weight of tissue taken for homogenization cannot be ascertained. Since it is likely that at full lactation the proportion of such milk is considerable, enzyme activities expressed per unit weight of moist tissue will be in serious error except in the extremely unlikely event of the enzyme concentration in milk and tissue being equal.

Arginase determinations on milk from rats at the 10th, 15th and 20th days of lactation, both in the presence and absence of Co^{++}, were found to be uniformly negative, and the error would, therefore, be maximal. It would also be serious in the case of phosphatase, which determinations on the rat showed to be in much lower concentration in the milk than in the mammary tissue (Table 2).

It is therefore clear that enzyme determinations on mammary tissue at different stages of pregnancy and lactation, during which the residual milk is certain to vary enormously, would have no meaning unless the milk content of the tissue could be determined and the appropriate corrections applied.

Milk contains two major constituents which are peculiar to it and which are normally found nowhere else in the body, namely casein and lactose, both of which offer possibilities in connexion with the development of a method for determining milk in mammary gland homogenates.

A method based on casein determinations was considered and discarded because of the obvious difficulties of estimating very small quantities of

casein in the presence of other proteins. Lactose has been used for estimating the amount of milk in mammary tissue (Gaines & Sanmann, 1927; Gowen & Tobey, 1927, 1928). To apply this to homogenates of rat mammary tissue it is necessary to show that the reduction measured is due solely to lactose. This was shown by estimating the reducing power of mammary gland homogenates before and after hydrolysis with 2N-HCl, when it was found that the reducing sugar content was doubled, while after incubation of the hydrolysate with bakers' yeast the reducing sugar content was restored to its original level. It is also necessary to know the lactose content of rats' milk. In Table 2 are given the results of determinations of lactose in rats' milk on the 10th, 15th and 20th days of lactation. These results are in essential agreement with those of Cox & Mueller (1937) who used a milking machine.

Table 2. *Lactose and alkaline phosphatase content of rat milk*

Rat no.	Day of lactation	Phosphatase units*/100 ml.	Lactose (g./100 ml.)	Group mean
22,356	10	36·0	2·88	3·00
22,365	10			
22,385	10			
22,390	10	24·0	3·12	
22,190	10			
22,802	15	28·6	3·19	3·37
22,804	15	30·0	3·55	
22,826	15	29·0	3·41	
22,849	15	26·6	3·39	
22,854	15	28·0	3·31	
22,822	20	29·6	3·43	3·18
22,860	20	30·0	3·40	
22,806	20	30·0	3·07	
22,798	20	36·0	3·00	
22,809	20		2·98	

* King & Armstrong (1934) units.

In calculating the milk content of mammary tissue the mean milk lactose content for the appropriate stage of lactation was used when available. Because no values were available for the 5th day of lactation the mean of all values was used here, since a test (variance ratio test) for the homogeneity of variance between the three groups (Snedecor, 1940) showed the values to be homogenous ($F = 2{\cdot}11$, degrees of freedom 2 and 9).* This could probably be done without much error as our results show no evidence of significant changes in the lactose content during lactation. In the case of pregnancy, and perhaps involution, this is less permissible since the composition of fluids withdrawn from the mammary gland of animals pregnant for the first time is very different from normal milk (Asdell, 1925; Woodman & Hammond, 1923). But nevertheless we consider that the application of the milk correction during pregnancy and involution gives a result much nearer the truth than one obtained without it.

Individual lactose estimations were made on all mammary gland homogenates, in each case leading to an estimate of milk content which could be used for correcting the corresponding enzyme determinations to a true moist tissue basis. The group mean percentages of retained milk in mammary tissue at various stages are given in Table 3.

Table 3. *Milk content* (%) *of mammary gland tissue*

	Pregnancy			
Day	5	10	15	20
Mean retained milk (%)	9·61	25·66	30·14	26·45
	Lactation			
Day	5	10	15	20
Mean retained milk (%)	41·01	45·15	51·04	54·20
	Involution			
Day		3	6	
Mean retained milk (%)		34·26	24·75	

Gross and corrected group mean mammary gland weights for the six abdominal glands are shown in Fig. 1. The uncorrected mammary gland weights show a continuous and rapid increase to the end of lactation followed by a precipitous drop after weaning, while the corrected values increase to about the middle of pregnancy and thereafter remain sensibly constant until the third day of involution. A test for the homogeneity of variance showed that values for the four stages of lactation together with the last stage of pregnancy were homogenous ($F = 1{\cdot}10$, degrees of freedom 4 and 47). These results provide independent biochemical evidence in support of the currently accepted view, based on histological and cytological evidence (see Turner, 1939), according to which mammary gland hyperplasia is held to be substantially complete by mid-pregnancy and any further apparent growth is hypertrophy due to distention of the alveolar cells, and the alveoli themselves, with secretion. It will be seen that at the end of lactation the correction factor is of the order of 2×, so that determinations made without such corrections are in serious error. Moreover the factor varies between 1·1× and 2·2× at different stages of pregnancy and lactation, thus emphasizing the particular necessity for applying a milk correction in an investigation of this kind. In

* Throughout this paper, where F values are given, the number of degrees of freedom corresponding to the greater mean square is given first.

the case of phosphatase, which occurs in low concentration in milk, a further correction for the amount of phosphatase in retained milk was applied although this was small and did not appreciably alter the results.

With regard to this method for correcting for the milk content of mammary tissue it must be remembered that lactose may occur in secretory droplets in the cytoplasm of the alveolar cells in a different concentration from that of the milk which can be drawn from the gland for analysis and further that the lactose content of the milk retained in the alveoli themselves may also differ slightly from the latter. However, it seems probable that these effects will be far too small to invalidate the correction.

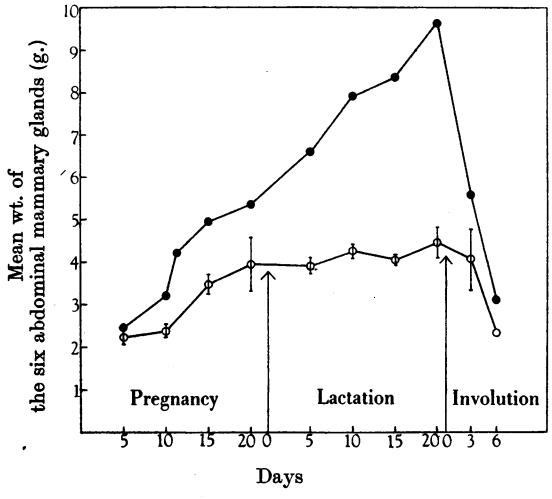

Fig. 1. Mean weights of the six abdominal mammary glands for groups of rats during pregnancy, lactation and mammary involution. The black dots represent the gross mammary gland weights; the circles represent values corrected for milk retained in the tissue. The vertical lines passing through the circles represent twice the S.E.M. When no vertical line is given on the corrected weight curve the S.E.M. is too small to show.

Variations in arginase activity of the mammary tissue. Changes in mammary arginase/g. of moist tissue are shown in Fig. 2. During the period of mammary growth, i.e. early pregnancy, there is relatively little increase in arginase content nor is there any appreciable increase up to the 5th day of lactation. But after the 5th day of lactation there begins a rapid increase in arginase activity which continues uninterruptedly to the end of lactation at which point it is some six times the value at the beginning of lactation. After weaning there is a precipitous fall back to the level characteristic of early pregnancy. A curve showing the total arginase activity of the six abdominal glands is not given

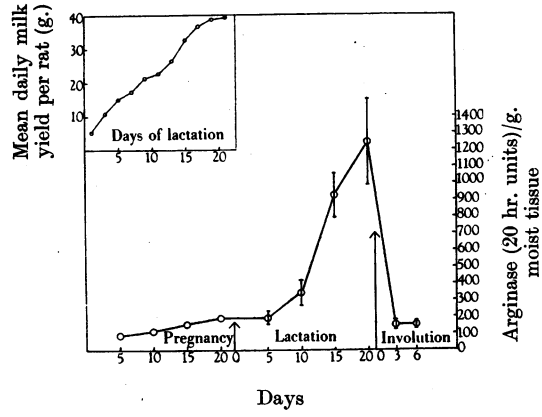

Fig. 2. Variations in mammary gland arginase. The vertical lines are equal to twice the S.E.M. When no vertical line is given, the S.E.M. is too small to show. The inset represents the lactation curve of the rat, constructed from the data of Brody & Nisbet (1938).

since the true weight of the glands remains sensibly constant throughout lactation (Fig. 1) and the curve is obviously of the same form.

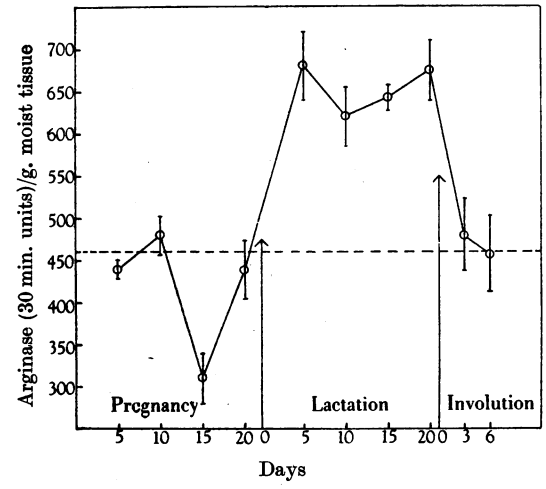

Fig. 3. Variations in the liver arginase/g. moist tissue. The horizontal broken line is the non-pregnant control level. The vertical lines are equal to twice the S.E.M.

Variations in liver arginase activity. The variations in liver arginase/g. of moist tissue are shown in Fig. 3. The liver arginase/g. in pregnancy remains constant and not significantly different from the non-

pregnant control level except the values for the 15th day which showed a marked fall. A test for the homogeneity of variance among the pregnant groups shows that the drop at the 15th day is significant ($F = 7{\cdot}99$, degrees of freedom 3 and 36). No explanation for this can be given at the present time.

The most important point brought out by the figure is the sharp rise following parturition. The values obtained throughout lactation do not differ significantly among themselves ($F = 0{\cdot}73$, degrees of freedom 37 and 3) and must be regarded as constant, but the general lactation level is very significantly higher than the level at the 5th and 10th days of gestation ($F = 12{\cdot}1$, degrees of freedom 5 and 56). Following weaning there was a sharp drop back to the level characteristic of early pregnancy.

Our data provide some suggestion of a regular increase in liver weight in late pregnancy and lactation, over and above that due to general body growth (Fig. 4). This tends to enhance the increase in

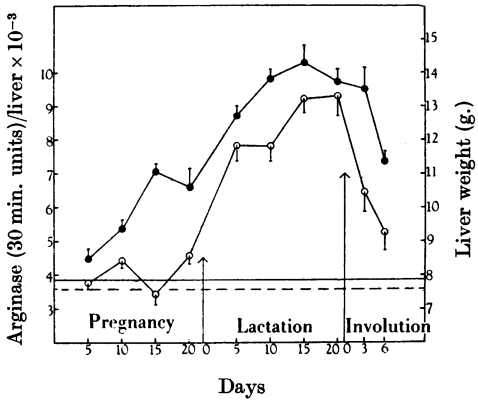

Fig. 4. Variations in the total liver arginase and the liver weight. Circles represent total liver arginase; black dots represent liver weight. The intact horizontal line is the non-pregnant control level for liver weight. The broken horizontal line is the non-pregnant control lever for total liver arginase. In order to avoid confusion each vertical line represents the S.E.M. in one direction only.

liver arginase following parturition when calculated on the basis of the whole liver, and there is some indication that the total liver arginase rises slowly over the whole lactation period.

The enhanced liver arginase activity (per g. moist tissue) characteristic of lactation stands in contrast to the state of affairs during pregnancy where the values were not significantly different from the non-pregnant rats, except in the case of the 15th day of pregnancy where the value was smaller. It is interesting that the increased strain on the maternal metabolism due to the needs of the growing foetuses is not of a nature which requires an increase in the liver arginase activity, whereas lactation evidently necessitates such an increase.

Variations in the mammary gland phosphatase. In contradistinction to the mammary gland arginase, the mammary gland phosphatase (Fig. 5) increases steadily throughout pregnancy and reaches a maximal value soon after parturition, which is maintained constant throughout lactation (confirmed by a test for homogeneity of variance for the four lactation values giving a value of $F = 1{\cdot}27$, degrees of freedom 3 and 38). Again there is a sharp decrease after weaning but it is not so precipitous as in the case of arginase, i.e. the involution level is still

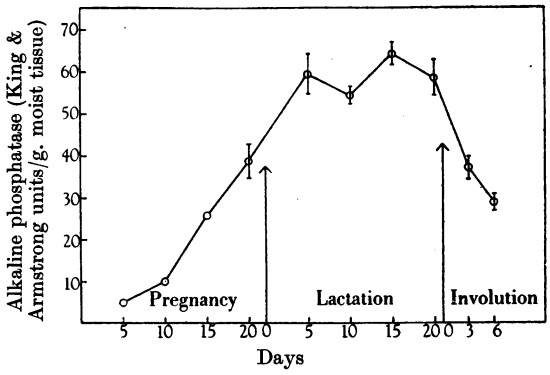

Fig. 5. Variations in mammary gland alkaline phosphatase. The vertical lines are equal to twice the S.E.M. Where no vertical line is given the S.E.M. is too small to show.

some three times that of early pregnancy. Kidney phosphatase/g. of moist tissue (not shown in the figure), used as a reference standard, remained substantially constant throughout.

DISCUSSION

Increase in mammary gland arginase content at mid-lactation. It is not clear whether the dramatic rise in mammary arginase at mid-lactation is a process initiated between the 5th and 10th days of lactation or is of the nature of an autocatalytic increase in response to a stimulus operating at some time during pregnancy. Inspection of Fig. 2, indeed, suggests that most of the curve up to the end of lactation could be fitted by a logistic equation corresponding to a unimolecular autocatalytic chemical reaction. If so, this would agree with the second alternative. It seems desirable, however, in the present state of knowledge, to discuss our results on the basis of the former, less complicated, assumption, as it seems possible that while the relatively

small but nevertheless statistically significant increase between the 5th and 20th days of pregnancy may be connected with the phase of mammary growth (and Edlbacher & Merz (1927) have suggested a correlation between cellular multiplication and tissue arginase), the increase occurring during lactation must certainly have no connexion with mammary growth, which has long since ceased.

This dramatic rise in mammary gland arginase which takes place in mid-lactation can only mean that arginase occupies a key position in the metabolism of the lactating, as contrasted with the non-lactating mammary gland. The nature of its function in lactation is a point of considerable interest and importance. The only well-established role of arginase in mammalian metabolism is as a participant in the production of urea through the 'ornithine cycle'. It may be noted, however, that this mechanism for the formation of urea has not found universal acceptance (see Bach, Crook & Williamson, 1944), and there is a possibility that urea formation may not involve arginase at all. Even if the ornithine cycle provides the true and only mechanism for urea production in the mammal, a quantitative parallelism between liver arginase content and the level of urea production does not necessarily follow. As far as we know no such parallelism has been conclusively demonstrated. However, there is a decrease in liver arginase after adrenalectomy (Fraenkel-Conrat, Simpson & Evans, 1943; Folley & Greenbaum, 1946), a procedure which presumably results in decreased deamination of amino-acids, though it is not at present known whether or not the primary cause of this effect is a reduction in food intake which results from adrenalectomy. But on the other hand liver tumours may show a high arginase content coupled with a disability to synthesize urea (Neber, quoted by Edlbacher & Koller, 1934; Greenstein, 1943). Nevertheless, in our opinion, the consensus of evidence would appear to favour the view that arginase participates in urea production in some such scheme as that postulated by Krebs & Henseleit (1932), and as a reasonable corollary, increased deamination and urea formation might well demand an increased tissue arginase.

If the mammary gland arginase is, like that of the liver, concerned with deamination and urea production, with gluconeogenesis in fact, it should be possible to demonstrate (*a*) an uptake of amino-acids by the active mammary gland and (*b*) an output of urea. Arterio-venous studies, in fact, have uniformly shown a small but definite uptake of amino-acids by the lactating gland of the cow (Blackwood, 1932; Shaw & Petersen, 1938*a*, *b*; Bottomley & Folley, unpublished results) and the goat (Lintzel, 1934; Graham, Jnr., 1937–8; Graham, Jnr., Peterson, Houchin & Turner, 1937–8; Reineke, Peterson, Houchin & Turner, 1939) but not by the non-lactating bovine udder (Blackwood, 1932). An appreciable output of urea by the udder of the lactating goat has been reported by Graham, Jnr., Houchin & Turner (1937) and Reineke *et al.* (1939).

On the basis of balance experiments on the lactating ruminant mammary gland it has been claimed (Graham, Jnr., 1937–8; Shaw & Petersen, 1938*a*, *b*; Reineke *et al.* 1939) that the uptake of amino-acids is quite insufficient to account for the observed synthesis of casein. It therefore seems probable that blood amino-acids absorbed by the mammary gland are not directly concerned in the synthesis of casein, a conclusion supported by the failure of Reineke, Williamson & Turner (1941) to detect an uptake of amino-acids by the udder of the fasting goat which nevertheless continued to secrete some milk, but may be utilized for a process analogous to gluconeogenesis. Indeed Graham, Jnr. (1937–8) speculated on the possibility that lactose precursors might arise from the deamination of amino-acids by the active mammary gland without providing any direct evidence in support of this supposition. The present results seem to strengthen the idea that the deamination of amino-acids, absorbed from the blood stream as such or perhaps in the form of protein (e.g. see Reineke *et al.* 1941), with the formation of substances concerned in carbohydrate metabolism, and also, according to modern views (see Stadie, 1945), in fat metabolism as well, is an important element in the metabolism of the lactating mammary gland.

The increase in mammary gland arginase at mid-lactation must clearly be considered in relation to our finding that the liver arginase content increases immediately after parturition and remains elevated throughout lactation.

The mammary gland is an exceptional organ, situated outside the body but receiving its metabolites from the common blood stream. At the height of its activity it shows a remarkable capacity for the synthesis of its specific protein, fat and sugar. In the rat, unlike the ruminant in which the milk yield rises to a peak relatively early in the lactation period and thereafter steadily falls off, such data as are available indicate a steady increase in milk yield over the whole lactation period of 21 days. The measurement of milk yield in the rat is an extremely difficult operation and the only data known to us are those of Brody & Nisbet (1938) obtained by a complicated, and not entirely unobjectionable, method. A mean lactation curve calculated from some of their data is shown in the inset of Fig. 2. It seems possible that if intermediates derived from the deamination of amino-acids are indeed essential substrates for milk synthesis, the liver is able during early lactation when, in the rat, milk secretion is proceeding at a relatively low level, to satisfy both

the normal demands of the body and the additional ones of the mammary gland, the added burden due to lactation being reflected in an increase in its content of arginase. But when, in mid-lactation, the rate of milk synthesis has passed a given point, the liver can no longer cope with the increased demand for deaminized residues and the mammary gland is forced to realize, to a striking degree, its potentialities, evidenced by the presence of relatively small amounts of arginase in the non-lactating gland, for gluconeogenesis. On this view the mammary gland in full lactation may be regarded as in some respects analogous to the liver of an animal in a state of 'overproduction' (e.g. pancreatic diabetes; phloridzin diabetes), in that in the effort to maintain a high output of sugar, amino-acids are increasingly used as sugar precursors. In this connexion it would be of interest to investigate whether the induction of diabetes is accompanied by a rise in liver arginase. Takehara (1938) has indeed reported increased liver arginase levels during fasting in the dog, which were restored to normal by feeding carbohydrate but not by a diet of fat and protein.

Other possible roles of mammary gland arginase may be briefly discussed. Edlbacher & Merz (1927) have postulated a correlation between tissue growth and arginase content. Though our results show some evidence of a slight increase in mammary gland arginase in pregnancy, during the earlier part of which most of the mammary growth occurs, and to this extent may be said to agree with the views of Edlbacher & Merz, the large increase characteristic of mid-lactation cannot have any connexion with mammary growth since most authorities agree (e.g. Weatherford, 1929) on the rarity of mitosis during lactation. A second possibility arises from the suggestion put forward by Edlbacher and his collaborators (see Edlbacher, 1938) that cellular arginase may be concerned with protein synthesis in the cell. During lactation the mammary gland is a site of active protein synthesis and on this view the mammary gland arginase might well be concerned with the synthesis of casein. From the present evidence as discussed above, however, it would seem more likely that the mammary arginase is primarily concerned with deamination processes.

Changes in the mammary gland alkaline phosphatase. The changes in alkaline phosphatase content of mammary tissue are strikingly different from the arginase changes in that the former are confined to the period of pregnancy. The form of the curve expressing the changes in phosphatase content of the mammary gland is generally similar to that showing the increase in weight of the mammary gland tissue. It is perhaps unwise to conclude from this that the role of the alkaline phosphatase in the mammary gland is concerned merely with the growth phase which necessarily involves synthesis of nucleoproteins, as suggested by Moog (1946), since the mammary phosphatase level remains high throughout lactation. The additional possibilities that during the secretory phase it may be concerned with the synthesis of casein or with dephosphorylation processes connected with carbohydrate metabolism, or even with the capture of blood sugar molecules by the mammary gland cells, must not be overlooked.

Phosphatase activity of mammary gland as compared with that of kidney. In our previous paper (Folley & Greenbaum, 1946) we reported an approximate value of 7 for the kidney phosphatase/mammary gland phosphatase ratio in the rat on the 21st day of lactation. This mean value referred to six animals only and no correction could then be made for the milk content of the mammary tissue. The present results which are more extensive and which have been corrected for the milk content of the mammary tissue permit a more accurate comparison of the phosphatase activity of the mammary gland with that of the kidney, as representing one of the richest sources of alkaline phosphatase in the mammalian body. Since the mammary phosphatase values for the four groups of lactating rats were found to be drawn from the same statistical population (see above) these can be considered for this purpose as one group of 42 animals. The same applies to the corresponding kidney phosphatase values since in point of fact all groups investigated including the non-pregnant non-lactating controls were also statistically homogeneous.

The mean corrected mammary gland phosphatase activity for the 42 animals was 57·8 King & Armstrong units/g. moist tissue, the corresponding mean value for the kidneys of the same animals being 163·5 King & Armstrong units/g. moist tissue. The weighted mean kidney/mammary gland ratio was $2{\cdot}88 \pm 0{\cdot}095$. It may be of interest to compare these values with those of the non-lactating mammary gland as represented by the group of ten rats at the 5th day of pregnancy. For these ten animals the mean corrected mammary gland phosphatase activity was 4·96 King & Armstrong units/g. moist tissue and the mean kidney phosphatase activity 149·7 King & Armstrong units/g. moist tissue. The weighted mean kidney/mammary gland ratio was $34{\cdot}24 \pm 4{\cdot}34$.

Unfortunately our present data do not allow us to make a re-evaluation, on the basis of values corrected for retained milk, of our previous estimate of the liver/mammary gland arginase activity ratio for the lactating rat, because experiments at present under way have revealed considerable difficulties in the determination of the ratio between our 30 min. and 20 hr. arginase units. This ratio must be known in order to compare mammary gland and liver arginase activities under the conditions of these experiments.

An accurate comparison between the arginase activities of mammary gland and liver tissue is of considerable importance and the question is being investigated further.

SUMMARY

1. Changes in the mammary gland and liver arginase, and the mammary gland and kidney alkaline phosphatase, have been studied in pregnancy, lactation and mammary involution in the rat.

2. A method is described for the estimation of retained milk in mammary gland tissue enabling mammary gland enzyme determinations to be corrected to a true moist tissue basis.

3. The liver arginase content (per g. moist tissue) during pregnancy and after weaning is indistinguishable from that of unmated controls except at the 15th day of pregnancy when significantly lower values are observed. Over the whole lactation period the liver arginase is significantly increased above these values.

4. The mammary gland arginase increases slowly during pregnancy and early lactation but undergoes a dramatic increase between the 5th and 10th days of lactation. It reaches a maximum at the 20th day of lactation and after weaning falls precipitously to the level of early pregnancy.

5. The alkaline phosphatase content of the mammary gland increases rapidly during pregnancy, reaching a maximum at parturition, and remaining at the same level throughout lactation; it decreases after weaning to a level intermediate between those of lactation and early pregnancy. The alkaline phosphatase content of kidney tissue remains constant throughout pregnancy, lactation and mammary involution.

6. The results are believed to indicate that the mammary gland arginase is concerned with the deamination of amino-acids by the mammary gland, and that the increased arginase content during late lactation in the rat is due to an increased requirement of deaminized residues for the synthesis of milk constituents.

7. The results are consistent with the view that the alkaline phosphatase of the mammary gland may be concerned with the synthesis of nucleoproteins during the growth phase and possibly with the synthesis of casein and the capture of blood sugar molecules during lactation.

8. During lactation in the rat, kidney tissue contains on the average 2·9 times as much alkaline phosphatase as mammary gland tissue. During the mammary growth phase, i.e. in early pregnancy, the kidney/mammary gland ratio is approximately 34.

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Absorption of Carotene from Carrots in Man and the Use of the Quantitative Chromic Oxide Indicator Method in the Absorption Experiments

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The findings of various investigators concerning the absorption of carotene from vegetables in man differ greatly. Wilson, Das Gupta & Ahmad (1937) state that only 10–20 % of the carotene from carrots or spinach is excreted in the faeces. Van Eekelen & Pannevis (1938) found 99 % of the carotene from carrots and 95 % of the carotene from spinach in the faeces. Kreula & Virtanen (1939) and Virtanen & Kreula (1941) observed a carotene excretion varying between 64 and 99 %, and generally amounting to about 90 %. The absorption from very finely grated carrots seemed to be more favourable than from carrots merely masticated. With (1940) observed with purees of tomatoes, carrots, spinach, and carrot meal, excretion values of from 40 to 70 %. Extensive experiments carried out by the Vitamin A Sub-Committee of the Accessory Food Factors Committee (Lister Institute and Medical Research Council, 1945) showed a carotene excretion of about 75 % from carrots and of about 60 % from spinach. If the carotene is dissolved in fat, a noticeably greater part of it is absorbed. Thus With (1940) observed in man excretion values of 25–70 % while the above mentioned Committee found 28 %.

METHODS

Quantitative chromic oxide method

If carotene is determined in the usual way in the faeces, the excreta must be collected quantitatively during several days and stored, frequently for a considerable time, before analysis.

In this respect the quantitative chromic oxide indicator method adopted by Edin (1926) offers noticeable advantages. This method is based on the fact that chromic oxide is not absorbed from the alimentary canal of animals and man, and is entirely excreted in the faeces. The excretion of carotene can be calculated from the proportions of carotene and chromic oxide in the food and in the faeces, and the quantitative collection of faeces is not necessary. In order to find out the excretion of carotene contained in a food, the excretion of carotene derived from the basal diet must also be taken into consideration. This can be determined in a preliminary test. If the basal diet remains unchanged in separate experiments, the basal excretion value determined for each subject may be used in a number of absorption experiments. The net excretion is determined by subtracting from the total carotene excreted during the experimental period (calculated for instance/g. of Cr_2O_3) the carotene correspondingly excreted during the basal period.

Two experiments were made to find out the suitability of the chromic oxide indicator method for the investigation of carotene absorption in man. In one a mixture of carrot and olive oil was used as a source of carotene, in the other, grated carrot alone. In the first experiment the excretion was determined simultaneously by the method involving the collection of faeces and by the quantitative chromic oxide indicator method; in the second only the latter method was employed.

Carotene determinations

In both experiments carotene was determined by the phase separation method (Virtanen & Kreula, 1941), and in the first one also by the chromatographic method according to With (1940). For phase separation, saponification was carried out under nitrogen. The procedure now used in the phase separation method differed from that in the method