compound. It has now been found that compared with free astaxanthin the esterified pigment occurring in lobster hypodermis shows an absorption band of exactly similar shape, but with its wavelength maximum shifted 1–2 mμ. to shorter wavelengths (Table 1). All the data were obtained using the Beckman photoelectric spectrophotometer.

SUMMARY

1. It has been confirmed that esterified astaxanthin occurs in the hypodermis of the lobster, *Homarus vulgaris* Edw. and the prawn, *Nephrops norvegicus* L., and that the unesterified pigment occurs in the eggs of the lobster.

2. It has not been possible to confirm the presence of esterified astaxanthin in the carapaces of these species; from the evidence presented it is considered that the pigment is in fact free astaxanthin.

3. The lobster hepatopancreas contains only traces of β-carotene.

4. Free and esterified astaxanthin, the former predominating, have been identified in the sea flea, *Tigriopus fulvus* Fisch. In gravid females about 50 % of the pigment is in their eggs.

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REFERENCES

Fraser, J. H. (1936). *J. Anim. Ecol.* **5**, 23.
Goodwin, T. W. & Srisukh, S. (1949). *Biochem. J.* **45**, 263.
Karrer, P. & Würgler, E. (1943). *Helv. chim. Acta*, **26**, 116.
Kuhn, R. & Lederer, E. (1933). *Ber. dtsch. chem. Ges.* **66**, 488.
Kuhn, R., Lederer, E. & Deutsch, A. (1933). *Hoppe-Seyl. Z.* **220**, 229.
Kuhn, R. & Sörensen, N. A. (1938). *Z. angew. Chem.* **51**, 465.
Stern, K. G. & Salomon, K. (1938). *J. biol. Chem.* **122**, 461.
Wald, G. (1943). *Vitamins and Hormones*, **1**, 195.

The Intermediary Metabolism of the Mammary Gland

2. RESPIRATION AND ACID PRODUCTION OF MAMMARY TISSUE DURING PREGNANCY, LACTATION AND INVOLUTION IN THE RAT

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In a previous paper (Folley & French, 1949*c*) we have shown that slices of lactating mammary tissue respire fairly actively in a medium containing glucose. In the rat the activity is less than that of nervous tissues and kidney, but is of the same order as that of liver; unlike that of liver, however, the respiration is markedly increased over endogenous values in the presence of glucose. The respiratory quotient (R.Q.) of lactating mammary tissue (in glucose) is well above unity in the mouse, rat, rabbit, and, to a lesser degree, the guinea pig, but below unity in ruminants (goat, cow).

Our results on the rat indicated that Q_{O_2}, and perhaps R.Q., is lower in early than in full lactation. This preliminary finding was in line with that of Kleiber, Smith & Levy (1943), who reported a higher respiration for lactating tissue than for tissue taken from pregnant rats, provided the results were calculated to a dry tissue basis. On a moist tissue basis, however, there was no difference in metabolic rate at the end of pregnancy and at the twenty-first day of lactation, because the dry-matter content of the gland was much higher in pregnancy than in lactation.

The relation between the functional activity of a tissue and its respiratory metabolism is of considerable interest, and the mammary gland readily lends itself to a study of this question. We have previously shown (Folley & French, 1949*c*) that, in the rat, experimental depression of lactation due to restriction of the food intake or to adrenalectomy, lowers Q_{O_2}, decreases the R.Q. to values near unity, and increases the aerobic glycolysis. Another approach to this question is to study the metabolism of mammary tissue at various stages of the lactational cycle, using the term in its widest sense to include late pregnancy and post-lactational involution. The present paper reports results of such a study.

METHODS

Animals. Hooded Norway rats undergoing their first lactations were used. The stock diet, fed *ad lib.*, was as described previously (Cowie & Folley, 1948) save that 10 of the parts of whole wheat were replaced by wheat germ. All

litters were reduced to 8 (if possible 4 of each sex) at parturition. Groups were killed (by dislocation of the spine) on the twentieth day of pregnancy (i.e. 1–2 days before parturition), and on days 1, 8, 15 and 22 of lactation, litters being allowed access to the mothers up to the time of autopsy. Obviously the group killed on day 1 may have included some rats which would have failed to lactate successfully had they been allowed to survive. This could hardly have affected the mean results for this group of 10, however, since, of the rats set aside for this work, only about 10% failed to lactate. In order to allow the effects of weaning to be studied in comparison with those of continued suckling, a group was weaned on the twentieth day and killed 2 days later.

General. At autopsy the 3 abdominal* mammary glands from one side were carefully dissected off and weighed, after which samples were taken for determination of retained (extracellular) milk, by determination of the lactose content on a homogenate (Folley & Greenbaum, 1947), and of total dry-matter content (Folley & Greenbaum, 1948). Tissue slices for the manometric experiments were cut from the other 3 glands as described previously (Folley & French, 1949*c*).

R.Q., Q_{O_2} (μl. O_2/mg. final dry wt./hr.) and $Q^{O_2}_{acid}$ were determined on duplicate slices from each rat by the method of Dickens & Šimer (1931) using Dickens & Greville (1933) flasks. The gas phase was 5% CO_2 and 95% O_2, and the medium the Ringer-bicarbonate of Krebs & Henseleit (1932). In all experiments with substrate, 0·3% glucose was used. Determinations in duplicate without substrate were also performed on slices from about half the rats in each group.

RESULTS

Oxygen uptake. Group mean values for $-Q_{O_2}$ are given in Table 1. At the end of pregnancy $-Q_{O_2}$ (glucose) is low, but has increased considerably by the first day of lactation. Thereafter the values rise steadily to a peak level, reached somewhere between the eighth and fifteenth days, which appears to be maintained sensibly constant until the twenty-second day, the slight drop at this time being not statistically significant. By contrast, glands from rats weaned on the twentieth day and killed on the twenty-second day show a marked fall in respiration rate (see also Fig. 1).

* 'Abdominal' refers to the 2 abdominal and 4 inguinal glands.

The increase in $-Q_{O_2}$ between the twentieth day of pregnancy and the first day of lactation is hardly affected by absence of substrate, but thereafter as lactation progresses $-Q_{O_2}$ increases but slightly, if at all, and it is doubtful whether there is a decrease at weaning. The values for the eighth, fifteenth and twenty-second days of lactation confirm our previous finding (Folley & French, 1949*c*) that glucose markedly increases the respiration of mammary gland slices. Values for the ratio $\frac{Q_{O_2}\text{ (glucose)}}{Q_{O_2}\text{ (no substrate)}}$, given in Table 1, show that this is a property only of the fully lactating gland.

The interpretation of these results is complicated by the changes in the dry-matter content of the mammary tissue (Table 2). Table 2 shows that the fully lactating gland contains less than half the dry matter of the gland at the end of pregnancy, the values agreeing very well with those of Kleiber *et al.* (1943). The question thus arises whether the increase in $-Q_{O_2}$ represents a real increase in respiration rate or is an artifact due to the disappearance of metabolically inert dry matter, such as fat or protein, contained in colostrum stored in the alveoli. On the other hand, there seems no doubt about the reality of the decrease in respiration following weaning, since the increase in the tissue dry matter at this time is relatively slight.

We have attempted to elucidate this problem by calculating for various stages the total respiration of the six abdominal glands, the weight of which, corrected for retained extracellular milk, should remain more or less constant over late pregnancy and lactation, since glandular growth largely ceases by mid-pregnancy (see Folley & Greenbaum, 1947). This approach seems preferable to an attempt simply to express the respiration rate on a moist tissue basis, since the nature of mammary gland slices (see Folley & French, 1949*c*) precludes accurate determination of their moist weights. The total respiration of the abdominal glands is easily calculated for 8, 15 and 22 days of lactation if Q_{O_2}, the total moist weight of the abdominal glands and their retained extracellular milk content, are known. The calculation involves

Table 1. *Respiratory metabolism of slices of rat mammary gland during pregnancy, lactation and involution*

(Errors are indicated in this and succeeding tables by giving mean ± S.E.M.)

		Glucose (0·3%)			No substrate			
Stage	Days	No. of rats	$-Q_{O_2}$	R.Q.	No. of rats	$-Q_{O_2}$	R.Q.	$\frac{Q_{O_2}\text{ (glucose)}}{Q_{O_2}\text{ (no substrate)}}$
Pregnancy	20	10	1·3±0·1	0·83±0·01	5	1·5±0·05	0·62±0·03	0·87
Lactation	1	10	4·4±0·3	1·00±0·05	5	4·0±0·3	0·73±0·01	1·07
	8	8	7·1±0·6	1·62±0·03	4	4·5±0·5	0·76±0·01	1·58
	15	8	10·3±0·4	1·60±0·06	4	5·2±0·4	0·78±0·02	2·03
	22	8	9·6±0·3	1·53±0·03	4	6·3±0·2	0·74±0·02	1·49
Weaning	2	5	5·5±0·9	0·76±0·03	3	5·0	0·64	1·14

Table 2. *Total respiration of abdominal mammary glands of rats during pregnancy and lactation*

Stage	Days	No. of rats	Mean body wt. (g.)	Moist wt. of 3 abdominal mammary glands (including milk) (g.)	Milk content of mammary tissue* (%)	Total dry-matter content of mammary tissue (including milk) (%)	Dry-matter content of 'milk-free' mammary tissue (calc.)† (%)	Total respiration of 6 abdominal mammae‡ (μl. O_2/hr.)
Pregnancy	20	10	290	2·56	12·8±0·7	58·1±1·7	—	3,870§
Lactation	1	10	224	3·35	40·4±3·1	37·2±1·8	—	—
	8	8	248	3·12	37·8±3·0	28·6±0·6	30·4	8,370
	15	8	250	3·75	42·2±2·5	25·7±0·3	25·8	11,500
	22	8	250	3·49	57·6±2·1	26·0±0·3	26·6	7,540
Weaning	2	5	251	5·58	41·5±3·6	31·2±0·6	—	—

* Lactose content of rat milk taken as 3·23% (Folley & Greenbaum, 1947).
† Dry-matter content of rat milk taken as 25·60% (Folley & Greenbaum, 1948).
‡ Calculated on the assumption that all the milk in a slice is leached out during the manometric determination.
§ Calculated on the assumption that no colostrum is leached out from a slice during the manometric determination.

a number of reasonable assumptions, the most important of which are the following: (*a*) that the method of Folley & Greenbaum (1947) for calculating the extracellular milk content of mammary tissue from the lactose content of tissue homogenates is reasonably valid; (*b*) that the variations in the total solids and lactose contents of the milk over the period of lactation in question are negligible; and (*c*) that all extracellular milk is leached out from the slice during the respiration experiment. In calculating the milk content of the tissue at these stages the lactose content of rat milk was taken as 3·23% (Folley & Greenbaum, 1947) and the total solids content as 25·60% (Folley & Greenbaum, 1948).

These assumptions are, however, not valid for the gland in late pregnancy because (*a*) the gland then contains colostrum, the composition of which is unknown for the rat, but which by analogy with what is known for the heifer (Engel & Schlag, 1925) is almost certain to contain a very large amount of solids and little lactose, and (*b*) it seems probable that much of the colostrum will remain in the slice throughout the manometric determination. The total respiration of the abdominal glands for the twentieth day of pregnancy was therefore calculated on the assumption that no colostrum is leached out. This has the advantage of giving a maximum value for the total respiration since if, as is probable, some colostrum is lost during the measurements, the figure given is an overestimate. No such calculation was attempted for the first day of lactation since this is probably a period of transition from colostrum to milk, involving unknown and rapidly changing conditions to which no set of assumptions seemed to apply.

The values for the total respiration given in Table 2 indicate an increase of the order of threefold between the end of pregnancy and the fifteenth day of lactation, the increase being somewhat less at the eighth and twenty-second days.

Respiratory quotient. Group mean values are given in Table 1; they are of course independent of changes

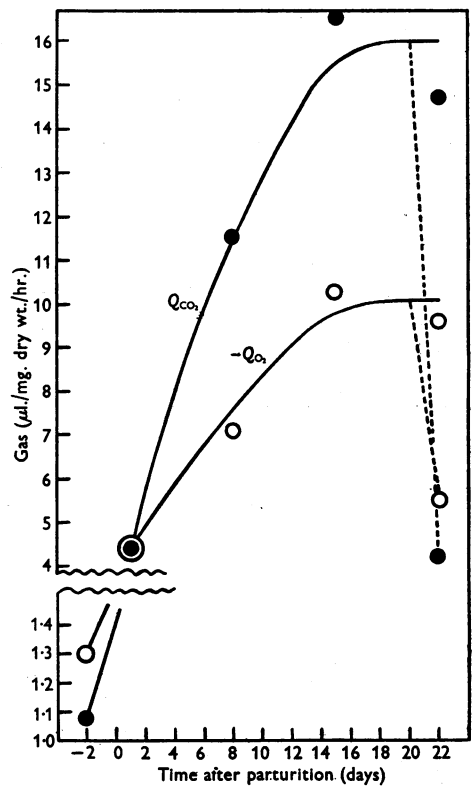

Fig. 1. Gas exchange of rat mammary gland slices during late pregnancy, lactation and after weaning. The points at the ends of the dotted lines refer to groups of rats weaned at the twentieth day of lactation.

in the dry matter or milk content of the tissue. With glucose as substrate the R.Q. is well below unity in late pregnancy, but has risen to unity on the first day

of lactation. By the eighth day the R.Q. has increased to 1·6, a value which is more or less maintained throughout lactation. This value is slightly higher than our previous value for rat mammary gland in full lactation (Folley & French, 1949*c*). After weaning for 2 days the R.Q. has dropped below unity to a value similar to that in late pregnancy. The striking increase in CO_2 output, the increase in Q_{CO_2} above $-Q_{O_2}$ near parturition and the reversal of this change following weaning, which these findings imply, are illustrated in Fig. 1. In the absence of substrate the R.Q. is below unity throughout, although the values for fully lactating tissue are a little greater than those for tissue taken in late pregnancy or after weaning.

Aerobic acid production. Changes in aerobic acid production (Table 3) are mainly considered in terms of ratios of quotients so as to eliminate complications due to changes in the dry-matter and milk content of tissue. We have previously pointed out (Folley & French, 1949*c*) that the acid produced by mammary gland slices probably includes acids other than lactic acid.

Table 3. *Aerobic acid production of rat mammary gland during pregnancy, lactation and involution*

Stage	Days	No. of rats	$Q_G^{O_2}$ (glucose)	$\frac{-Q_{O_2}\text{ (glucose)}}{Q_G^{O_2}\text{ (glucose)}}$	No. of rats	$\frac{-Q_{O_2}\text{ (no substrate)}}{Q_G^{O_2}\text{ (no substrate)}}$	$\frac{Q_G^{O_2}\text{ (glucose)}}{Q_G^{O_2}\text{ (no substrate)}}$
Pregnancy	20	10	0·9±0·1	1·45	5	4·47	2·77
Lactation	1	10	1·4±0·1	3·57	5	9·04	2·90
	8	8	1·8±0·2	4·09	4	9·28	3·76
	15	8	2·4±0·5	5·25	4	12·75	3·08
	22	8	2·0±0·2	5·16	4	6·37	2·40
Weaning	2	5	3·5±0·3	1·55	3	3·51	2·63

The ratio $-Q_{O_2}/Q_G^{O_2}$ (glucose), which gives a measure of the relative changes in respiration and aerobic glycolysis, increases from about 1·5 at the end of pregnancy to a maximum value greater than 5 at the fifteenth day of lactation. Since the total respiration of the abdominal glands increases at least threefold over this period (Table 2), it would seem that the increasing activity of the mammary gland is not accompanied by any appreciable increase in aerobic glycolysis. The extra energy requirements of lactation appear to be met solely by increased oxidation. Table 3 shows that $Q_G^{O_2}$ increases after weaning; since there is also an appreciable increase in the dry-matter content of the tissue at this time (Table 2), the actual increase in glycolysis at weaning is probably somewhat greater than indicated by the quotients.

Similar results were obtained in the absence of substrate, although under these conditions the ratio of respiration to glycolysis is higher. The values for the ratio $\frac{Q_G^{O_2}\text{ (glucose)}}{Q_G^{O_2}\text{ (no substrate)}}$ show relatively little change, but there is an indication of a slight increase in autoglycolysis relative to the glucolysis as the secretory activity of the tissue diminishes.

DISCUSSION

The present results show that, allowing for simultaneous changes in the dry-matter content of the tissue, the onset of lactation in the rat and the subsequent progressive increase in milk yield (see data of Brody & Nisbet, 1938) are correlated with an increase in the metabolic rate of mammary tissue. Our values for Q_{O_2} are at all stages two to three times as great as those of Kleiber *et al.* (1943), possibly because those workers did not use thin slices, so that their tissue may not have been in equilibrium with oxygen. Studies of the oxygen uptake of the ruminant udder by the arteriovenous method show on the whole (e.g. the results of Reineke, Stonecipher & Turner, 1941, on the goat) very little difference between dry and lactating udders as regards arteriovenous differences in oxygen. Our findings on the rat indicate that the enhanced energy consumption in lactation is met by an increase in respiration rather than glycolysis, so in the ruminant, and probably other mammals as well, the increased energy requirements of lactation may involve an increase in blood flow. Jung (1932) reported that the blood flow through the lactating goat udder was about four times the value for the dry gland, an increase in fair agreement with our estimate of a threefold increase in the total respiration of the abdominal glands in the rat.

Even more significant, because in a sense they are qualitative rather than purely quantitative, are the changes in R.Q. (in glucose). The R.Q., well below unity at the end of pregnancy, has risen to unity shortly after parturition and thereafter rises to a value of 1·5–1·6 which is maintained throughout lactation. Despite criticisms of the validity of the R.Q. as an indicator of metabolic processes (Soskin, 1941), it seems safe to conclude that the change in R.Q. of mammary tissue at parturition implies a profound change in the intermediary metabolism of the mammary gland at the start of secretion.

A brief consideration of what this change implies may not be out of place. In previous papers (Folley & French, 1948*a*, 1949*c*) we concluded that the high

in vitro R.Q. of lactating mammary tissue indicates the synthesis of fat from oxygen-rich materials. It is probable, however, that even in late pregnancy, when the R.Q. is low, some fat synthesis is proceeding; and indeed Popják & Beeckmans (1949) has demonstrated the incorporation of ^{14}C into the fatty acids of neutral fat isolated from the mammae of pregnant rabbits given labelled acetate. There thus appears to be a turnover of fat in the gland even during pregnancy, but the high R.Q. following parturition given by slices of ruminant udder in the presence of acetate and of non-ruminant gland in the presence of glucose (Folley & French, 1948*b*, 1949*a*) undoubtedly indicates net synthesis of fat by lactating tissue. We have suggested that part at least of the milk fat, perhaps particularly the shorter-chain fatty acids which distinguish it from body fat, is synthesized in the mammary gland itself from acetate (Folley & French, 1948*b*, 1949*a*) which the ruminant absorbs in large quantities from the rumen. Since Bloch & Rittenberg (1945) have shown in the rat that considerable amounts of acetate are continually produced, it seems likely that acetate is a substrate for synthesis of milk fat by the mammary gland in all mammals. The above-mentioned experiments of Popják & Beeckmans (1949) confirm this for the rabbit. The apparent ability, as indicated by the high R.Q., of mammary slices from non-ruminants, to effect net synthesis of fat from carbohydrate *in vitro* (Folley & French, 1948*a*, 1949*c*) would be explicable if the pathway from carbohydrate to fat passes through acetate. Bloch (1947) does not favour this possibility, but the position is admittedly rather obscure, and further *in vitro* work on the mammary gland might well help in clarification. The main difficulty hitherto has been the inability of lactating tissue from non-ruminants to utilize acetate *in vitro* in contrast to slices from ruminant udders (Folley & French, 1948*b*, 1949*a*); but in recent experiments (Folley & French, 1949*b*) we have shown that lactating mammary slices from rabbit and rat will utilize acetate in presence of small concentrations of glucose, the R.Q. being above unity, and in the rabbit often greater than in the presence of glucose alone. It seems that mammary slices from various species are subject to different limiting conditions, and it may be that non-ruminant mammary tissue, which contains very little glycogen (Folley & French, unpublished), requires glucose to provide the glycerol necessary for glyceride synthesis, a process which may well favour the synthesis of fatty acids. Other possibilities are that glucose is necessary to provide additional carbon for fatty acid synthesis, as suggested by Bloch (1947), or to provide energy for the activation of acetate.

The onset of lactation involves a change from a condition characteristic of late pregnancy in which, although cytologically the secretory phase has begun, the product bears little resemblance in composition to milk, to the post-parturient state in which, provided suckling proceeds, large quantities of normal milk are secreted. These events are accompanied by the following changes in the *in vitro* metabolic properties of the rat mammary gland: (*a*) the metabolic rate increases to an extent difficult to assess from our data, but probably at least threefold; (*b*) the slices acquire the property of responding to glucose in the medium by an increase in respiration; (*c*) the R.Q. increases above unity; and (*d*) the ratio of respiration to glycolysis increases.

The initiation of lactation is under endocrine control, the chief feature being an increased release of prolactin, and perhaps of other hormones concerned in lactation, by the anterior pituitary (see review by Folley, 1947*a*), and it would therefore seem likely that a close relationship must exist between the lactogenic hormone or hormone complex and the changes in the mammary gland metabolism occurring at parturition.

The changes which follow weaning are also of interest in this connexion. Within a short time of removing the litter the mammae exhibit changes in metabolism in the opposite direction from those which occur at parturition; the respiration decreases and, more significant, the R.Q. quickly drops below unity. Weaning also causes a definite increase in the apparent aerobic glycolysis similar to that shown previously (Folley & French, 1949*c*) to be associated with the partial inhibition of lactation resulting from inanition or adrenalectomy. Changes in mammary metabolism following weaning may be due to (*a*) the loss of the suckling stimulus, and (*b*) the effects, chemical or physical, of non-removal of milk. No attempt has been made in the present work to assess the relative role of these two primary factors, but obvious test experiments, utilizing the techniques of Selye (1934), suggest themselves. The suckling stimulus is believed to influence the function of the mammary gland through a neurohormonal arc involving as its final, centrifugal link the release of prolactin by the anterior hypophysis (see Folley, 1947*b*, for review); and the experiments of Selye (1934), and others, suggest that removal of the suckling stimulus may be the more important of the two above-mentioned factors, at any rate in the early stages of weaning. Thus the changes in the R.Q. of mammary tissue at parturition and after weaning alike raise the question how far the physiological action of prolactin on the mammary epithelium, an action which the experiments of Lyons (1942) and Meites & Turner (1948) involving intramammary duct injection of prolactin show to be direct, is bound up with the promotion of reactions leading to synthesis of fat from oxygen-rich materials. Mammary tissue may well prove particularly useful for an attack on this aspect of what is undoubtedly the

outstanding problem of endocrinology, the hormone-enzyme relationship.

The results for the unweaned group at 22 days of lactation seem to indicate that the respiration and R.Q. have begun to decline; but in order to study the normal course of lactation beyond this point it would be necessary to provide fresh litters so as to eliminate the effect of self-weaning of the young. (Incidentally, self-weaning is shown by the increased milk content of the glands at 22 days, Table 2.)

In conclusion, we may refer to the value of a more or less complete picture of changes in the respiratory metabolism of the mammary gland in relation to various phases of its physiological activity, such as is provided by this work, as forming a background for further studies of the biochemical mechanisms involved in the synthesis of milk.

SUMMARY

1. The respiratory metabolism and acid production of slices of rat mammary gland in the presence and absence of glucose have been studied during pregnancy, lactation and following weaning.

2. $-Q_{O_2}$ is low at the end of pregnancy, but has increased considerably on the day following parturition; thereafter the values rise to a maximum value of approximately 10 at mid-lactation. Interpretation of these changes is complicated by changes in the dry-matter content of the tissue, which by mid-lactation has fallen to less than half of the value at the end of pregnancy. However, calculation of the total respiration of the abdominal mammary glands indicates that the initiation and subsequent increase in the intensity of lactation are accompanied by a true increase in the respiration.

3. The effect of glucose in increasing the respiration of mammary gland slices is only seen in lactating tissue.

4. The R.Q. (in glucose) is well below unity at the end of pregnancy, but has risen to unity on the day following parturition; thereafter it rises to a maximum of approximately 1·6, which is maintained throughout most of the lactation period. In absence of substrate, the R.Q. remains below unity throughout lactation.

5. Lactating gland shows a higher value for $Q_G^{O_2}$ than the gland at the end of pregnancy; the increase is, however, largely an artifact due to the changes in the dry-matter content of the tissue. The extra energy requirements of the lactating gland seem to be met solely by increase in respiration.

6. Weaning is followed by a sharp fall in respiration, the change in Q_{O_2} being too great to be attributed to the relatively slight increase in the dry-matter content of the tissue which occurs at this time. Further, the R.Q. decreases below unity and the apparent aerobic glycolysis rises.

7. The significance of the results is discussed in the light of possible mechanisms of fat synthesis in the mammary gland, and of relationships between anterior-pituitary hormones and changes in mammary gland metabolism.

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REFERENCES

Bloch, K. (1947). *Physiol. Rev.* **27**, 574.
Bloch, K. & Rittenberg, D. (1945). *J. biol. Chem.* **159**, 45.
Brody, S. & Nisbet, R. (1938). *Res. Bull. Mo. agric. Exp. Sta.* no. 285.
Cowie, A. T. & Folley, S. J. (1948). *J. Endocrinol.* **5**, 24.
Dickens, F. & Greville, G. D. (1933). *Biochem. J.* **27**, 1479.
Dickens, F. & Šimer, F. (1931). *Biochem. J.* **25**, 973.
Engel, H. & Schlag, H. (1925). *Milchw. Forsch.* **2**, 1.
Folley, S. J. (1947*a*). *Brit. med. Bull.* **5**, 135.
Folley, S. J. (1947*b*). *Brit. med. Bull.* **5**, 142.
Folley, S. J. & French, T. H. (1948*a*). *Nature, Lond.*, **161**, 933.
Folley, S. J. & French, T. H. (1948*b*). *Biochem. J.* **43**, lv.
Folley, S. J. & French, T. H. (1949*a*). *Nature, Lond.*, **163**, 174.
Folley, S. J. & French, T. H. (1949*b*). *Biochem. J.* **44**, xlv.
Folley, S. J. & French, T. H. (1949*c*). *Biochem. J.* **45**, 117.
Folley, S. J. & Greenbaum, A. L. (1947). *Biochem. J.* **41**, 261.
Folley, S. J. & Greenbaum, A. L. (1948). *J. Endocrinol.* **5**, 236.
Jung, L. (1932). *C.R. Soc. Biol., Paris*, **109**, 737.
Kleiber, M., Smith, A. & Levy, P. (1943). *Proc. Soc. exp. Biol., N.Y.*, **53**, 94.
Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
Lyons, W. R. (1942). *Proc. Soc. exp. Biol., N.Y.*, **51**, 308.
Meites, J. & Turner, C. W. (1948). *Res. Bull. Mo. agric. Exp. Sta.* no. 415.
Popják, G. & Beeckmans, M. L. (1949). *Biochem. J.* **44**, xxxvii.
Reineke, E. P., Stonecipher, W. D. & Turner, C. W. (1941). *Amer. J. Physiol.* **132**, 535.
Selye, H. (1934). *Amer. J. Physiol.* **107**, 535.
Soskin, S. (1941). *Physiol. Rev.* **21**, 140.