Lipogenic Enzymes in Rat Maternal Adipose Tissue in the Perinatal Period

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(Received 13 September 1979)

1. The specific activities of fatty acid synthetase, acetyl-CoA carboxylase and pyruvate dehydrogenase were measured in rat adipose-tissue extracts in pregnancy and lactation. Fatty acid synthetase specific activity correlates very closely with the rate of fatty acid synthesis, the enzyme specific activity decreasing after mid-pregnancy in a manner very similar to the rate of fatty acid synthesis. Acetyl-CoA carboxylase specific activity also decreases dramatically after mid-pregnancy. Initial pyruvate dehydrogenase specific activity shows a decrease between 2 days *pre partum* and 2 days *post partum*, but total enzyme activity shows no significant change in the same period. 2. Immunotitrations of fatty acid synthetase and pyruvate dehydrogenase activities were carried out; the titrations showed that the change in the fatty acid synthetase activity is due to a change in the enzyme amount; the amount of pyruvate dehydrogenase does not change. Therefore the decrease in fatty acid biosynthesis in subcutaneous and parametrial adipose tissue in late pregnancy and early lactation is associated with a decrease in the amount of at least one of the enzymes involved in fatty acid biosynthesis. The correlation of these events with known hormonal changes is discussed.

In pregnancy and lactation changes in maternal fat metabolism occur which are related to the substrate requirements for foetal development and for milk production.

Fat storage in adipose tissue occurs during most of pregnancy, whereas lipid mobilization predominates during lactation (Spray, 1950; Beaton *et al.*, 1954; Flint *et al.*, 1979). The change from anabolic to catabolic metabolism that occurs around parturition is associated with a sharp reduction in the rate of fatty acid synthesis (Knopp *et al.*, 1973; Smith, 1973; Flint *et al.*, 1979) and in the activity of lipoprotein lipase (Otway & Robinson, 1968; Hamosh *et al.*, 1970).

In the present study we show that the decrease in the rate of fatty acid synthesis around parturition is accompanied by a decrease in the specific activities of fatty acid synthetase and acetyl-CoA carboxylase and by a reduction of the proportion of pyruvate dehydrogenase in the active state. Furthermore the decrease in the specific activity of fatty acid synthetase is due to reduction of the amount of the enzyme. Changes in fatty acid synthesis in adipose tissue are therefore associated with profound changes in the turnover characteristics of enzymes catalysing the process.

Experimental

Animals

Experiments were carried out with Wistar rats provided by A. Tuck and Son, Rayleigh, Essex, U.K. (the time of mating being determined by the appearance of vaginal plugs) or with Wistar rats provided at the appropriate stage of pregnancy or lactation by the Joint Animal Breeding Unit, Nottingham School of Agriculture, Sutton Bonington, Leics., U.K.

Materials

Insulin was a gift from Boots Pure Drug Co., Nottingham, U.K., and $\text{NaH}^{14}\text{CO}_3$ was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. CoA, malonyl-CoA, thiamin pyrophosphate, NAD^+ , NADPH, sodium pyruvate, ATP, dithiothreitol and bovine serum albumin were obtained from Sigma (London) Chemical Co., Kingston upon Thames, Surrey KT2 7BH, U.K. Freund's complete adjuvant was obtained from Miles Laboratories, P.O. Box 37, Stoke Poges, Slough SL2 4LY, U.K. All other chemicals were of A.R. grade. Acetyl-CoA was prepared as described by Smith *et al.* (1966). Pigeon liver arylamine acetyl-

transferase was prepared as described by Tabor *et al.* (1953). Pig heart pyruvate dehydrogenase phosphate phosphatase was prepared by the method of Severson *et al.* (1974), and purified pig heart pyruvate dehydrogenase was prepared by the method of Linn *et al.* (1972) as modified by Cooper *et al.* (1974). These enzyme preparations were gifts from Dr. R. M. Denton, Dr. W. A. Hughes and Mr. J. G. McCormack, Department of Biochemistry, University of Bristol Medical School, Bristol BS8 1TD, U.K.

Methods

Rats were killed by cervical dislocation and the parametrial fat-pads and the subcutaneous adipose tissue from the inguinal region were removed and weighed.

Assay of fatty acid synthetase and acetyl-CoA carboxylase specific activities. Adipose tissue was homogenized at 4°C in 0.02 M-sodium phosphate buffer, pH 7.0, containing 0.15 M-NaCl; fat was removed from the homogenate by centrifugation (7 min at 500 $g_{av.}$) at 4°C. Enzyme activities were then measured in the infranatant.

Fatty acid synthetase activity was measured by following the oxidation of NADPH in the presence of acetyl-CoA and malonyl-CoA (Speake *et al.*, 1975) by 50 and 100 μ l samples of the tissue extract. Acetyl-CoA carboxylase activity was determined by measurement of $NaH^{14}CO_3$ incorporation into malonyl-CoA (Manning *et al.*, 1976) by 50 and 100 μ l samples of the tissue extract.

Assay of pyruvate dehydrogenase. Preparation of tissue extracts and assay of initial and total pyruvate dehydrogenase activities were carried out as described by Stansbie *et al.* (1976) with 100 and 200 μ l samples of the tissue extracts.

Immunological procedures. (a) Preparation of antisera. An antiserum to rabbit mammary-gland fatty acid synthetase was raised in sheep (Paskin & Mayer, 1976). Crude antiserum was obtained by allowing the blood to clot at 4°C overnight followed by centrifugation for 20 min at 14 000 $g_{av.}$

The crude antiserum was shown to cross-react with rat adipose-tissue fatty acid synthetase by double-diffusion analysis (results not shown) carried out in 1% (w/v) agarose gels prepared in 0.02 M-sodium phosphate buffer, pH 7.0, containing 0.15 M-NaCl (Piazzi, 1969).

Purified pig heart pyruvate dehydrogenase (0.1 mg/ml) in 0.02 M-sodium phosphate buffer, pH 7.0, containing 0.15 M-NaCl was emulsified with an equal volume of Freund's complete adjuvant. Equal volumes (1 ml) of the emulsion were injected into four intramuscular and four subcutaneous sites on a sheep. The injections were repeated after 2, 4 and 6 weeks; at 6 weeks, 10 ml of blood was removed and tested for anti-(pyruvate dehydrogenase) anti-

bodies. The following week 500 ml of blood was collected by the Seldinger wire method (Mayer & Walker, 1978). Crude antiserum was prepared as described above and was shown to give multiple immunoprecipitation lines (results not shown) with purified pig heart pyruvate dehydrogenase by double-diffusion analysis (Ouchterlony, 1968; Piazzzi, 1969). In preliminary experiments the antiserum completely immunoinhibited rat adipose-tissue pyruvate dehydrogenase activity (results not shown).

(b) Immunotitration of fatty acid synthetase and pyruvate dehydrogenase activities. Fat tissue was homogenized at 4°C in 0.2 M-potassium phosphate buffer, pH 7.3, containing 0.02 M-EDTA, 0.01 M-

Fig. 1. Fatty acid synthetase specific activity in adipose tissue during pregnancy and lactation

Rats were killed 10 and 2 days *pre partum* and 2 and 7 days *post partum*. Fatty acid synthetase activity was measured as described in the Experimental section. Results are expressed as means $\pm$ S.E.M. or as mean $\pm$ half the difference between the two measurements (where errors are large enough). ●, Fatty acid synthetase activity in parametrial adipose tissue; ■, fatty acid synthetase activity in subcutaneous adipose tissue. The numbers in parentheses indicate the numbers of animals studied at each time point.

dithiothreitol and 1% (w/v) Triton X-100; the homogenate was centrifuged for 7 min at 500 g_{av} to remove the fat, and the infranatant was collected. Samples of infranatants (100 μ l) were then assayed for either fatty acid synthetase or total pyruvate dehydrogenase activity, and titrated with the appropriate antiserum. Increasing volumes of the tissue extracts were added to either 10 μ l of anti-(fatty acid synthetase) or 0.2 μ l of anti-(pyruvate dehydrogenase) sera. The incubation volume was kept constant in each titration by addition of an appropriate volume of the buffer used for homogenization.

Incubations were carried out for 15 min at 4°C and 2 h at 4°C in immunotitrations of pyruvate dehydrogenase and fatty acid synthetase respectively. Immunoprecipitates were removed by centrifugation in a Quickfit microcentrifuge for 6 min at maximum speed (14000 g_{av}) and the supernatants then assayed for either fatty acid synthetase or total pyruvate dehydrogenase activity. Crude serum from a non-immunized sheep was used as control serum in all experiments. Enzyme activities are defined so that 1 munit is 1 nmol of substrate converted/min.

Tissue extracts from three rats were used for three separate immunotitrations at each time point (i.e. at 10 and 2 days *pre partum* and 2 days *post partum*). The results of each immunotitration at a single time point were combined and the mean values calculated for both antiserum and control serum preparations. Graphs of activity measured versus activity added were constructed with these data from each time point. The straight lines obtained were compared by a two-way analysis of variance to test if the sets of data differed significantly from each other (Snedecor & Cochran, 1967).

Protein determinations. Determinations of protein concentration were carried out by the method of Lowry *et al.* (1951), as modified by Wang & Smith (1975).

Results

Changes in fatty acid synthesis during pregnancy and lactation

Changes in the rate of fatty acid synthesis from glucose in parametrial and subcutaneous adipose tissues between days 12 of pregnancy and 15 of lactation have been reported elsewhere (Flint *et al.*, 1979).

Changes in enzyme specific activities

Fatty acid synthetase specific activity of both parametrial and subcutaneous adipose tissues (Fig. 1) falls sharply from high specific activities at 10 days *pre partum* to very low activities by day 2 of lactation; a significant increase ($P < 0.02$) in fatty acid synthetase activity is seen between days 2 and 7

of lactation in both tissues. These results confirm those obtained in preliminary experiments (Sinnott-Smith *et al.*, 1979). The changes in fatty acid synthetase activities closely parallel changes in the rate of fatty acid synthesis in both adipose tissues. Significant positive correlations were found between the rate of fatty acid synthesis and the activities of fatty acid synthetase in this (correlation coefficient 0.83, $P < 0.02$) and in our previous study (correlation coefficient 0.98, $P < 0.001$) (Sinnott-Smith *et al.*, 1979).

Acetyl-CoA carboxylase activity (Fig. 2) also shows a sharp decrease after mid-pregnancy, the activity being considerably decreased by 2 days *pre partum* and becoming undetectable on the second day of lactation. This pattern is similar to that of

Fig. 2. Acetyl-CoA carboxylase activity in adipose tissue during pregnancy and lactation

Rats were killed as described in Fig. 1 and acetyl-CoA carboxylase activity was measured as described in the Experimental section. Acetyl-CoA carboxylase activity in parametrial adipose tissue (●) and in subcutaneous adipose tissue (■) is shown. Results are expressed as means $\pm$ S.E.M. (where errors are large enough). Numbers in parentheses indicate the number of animals studied at each time point.

fatty acid synthesis, but does not follow it as closely as does fatty acid synthetase activity; the correlation coefficient between acetyl-CoA carboxylase activity and fatty acid synthesis was 0.82 (significant at $P < 0.02$), whereas that for fatty acid synthetase in this group of rats was 0.98 ($P < 0.001$).

Preliminary studies suggested that both the initial and total activities of pyruvate dehydrogenase of parametrial and subcutaneous adipose tissue decreased around parturition (Sinnott-Smith *et al.*, 1979). In contrast in subsequent experiments, in which the amount of pyruvate dehydrogenase was also determined, no decrease in total activity was observed in either parametrial or subcutaneous adipose tissue between days 12 of pregnancy and 7 of lactation (Expt. 1 of Table 1). To clarify this point, initial and total pyruvate dehydrogenase activities were measured in parametrial adipose tissue from twelve 20-day-pregnant and eleven 2-day-lactating rats. These results confirmed that there is no change in the total pyruvate dehydrogenase activity of the tissue around parturition, whereas the initial activity decreases significantly ($P < 0.05$) (Expt. 2 of Table 1). However, in this experimental series of animals, total parametrial pyruvate dehydrogenase activity was approximately one-third of that in the first series of animals (Table 1).

The reason for the decrease in the total activity of adipose-tissue pyruvate dehydrogenase observed in the preliminary experiment is not clear, but the subsequent experiments indicate that it is the exception rather than the norm.

Immunotitrations of fatty acid synthetase and pyruvate dehydrogenase

Titration of fatty acid synthetase and total pyruvate dehydrogenase activities at each stage of

pregnancy and lactation with antiserum or control serum were carried out. Four sets of data (one for each stage) were obtained and are plotted in Figs. 3 and 4. A straight line could be drawn through the data for each stage for each enzyme, but the four lines were found to be not significantly different from each other by two-way analysis of variance. Therefore one straight line is drawn through all the points for each enzyme titration (Figs. 3 and 4). Since the immunotitration lines are statistically identical, no immunochemically reactive but enzymically inactive fatty acid synthetase or pyruvate dehydrogenase is present in adipose-tissue extracts. This means that the activities of fatty acid synthetase and total pyruvate dehydrogenase present in the cell are proportional to the enzyme amounts; therefore the changes in fatty acid synthetase activity (Fig. 1) are due solely to changes in the enzyme amount; the amount of pyruvate dehydrogenase does not change.

Protein content and adipose-tissue weight

During pregnancy and lactation there is no change in adipose-tissue cell number, whereas the mean cell volume changes considerably; in particular, there was a marked decrease in mean cell volume around parturition (Flint *et al.*, 1979). The weight of adipose tissue (Table 2) also seems to decrease between 2 days *pre partum* and 2 days *post partum*, but this was not statistically significant.

However, there is a significant decrease in the amount of protein per fat-pad between 2 days *pre* and *post partum* in both parametrial and subcutaneous adipose tissues, but this is regained by day 7 of lactation (Table 2). Expressing the activities of fatty acid synthetase, acetyl-CoA carboxylase and the initial pyruvate dehydrogenase activity per fat-pad would then accentuate their changes in activity over the experimental period (results not

Table 1. *Pyruvate dehydrogenase specific activities*

Rats were killed at 10 and 2 days *pre partum* and at 2 and 7 days *post partum*. Pyruvate dehydrogenase activities were measured as described in the Experimental section. Results are expressed as means $\pm$ S.E.M. with the numbers of observations in parentheses.

Time	Pyruvate dehydrogenase activity (nmol/min per mg of protein)			
	Expt. 1		Expt. 2	
	Parametrial adipose tissue	Subcutaneous adipose tissue	Parametrial adipose tissue	
	Total	Total	Initial	Total
10 days <i>pre partum</i>	3.44 $\pm$ 0.77 (6)	2.15 $\pm$ 0.50 (6)	—	—
2 days <i>pre partum</i>	3.95 $\pm$ 1.12 (6)	2.06 $\pm$ 0.42 (6)	0.37 $\pm$ 0.095 (12)*	1.33 $\pm$ 0.27 (12)
2 days <i>post partum</i>	3.77 $\pm$ 1.02 (3)	2.29 $\pm$ 0.42 (3)	0.12 $\pm$ 0.03 (11)*	1.21 $\pm$ 0.14 (11)
7 days <i>post partum</i>	2.87 $\pm$ 0.36 (3)	2.52 $\pm$ 0.48 (3)	—	—

* Significantly different, $P < 0.05$.

Fig. 3. Immunotitration of fatty acid synthetase activity

Rats were killed as described in Fig. 1. Fatty acid synthetase activity was titrated against $10\mu\text{l}$ of anti-(fatty acid synthetase) serum or control serum (see the Experimental section). Each point represents the mean of measurements on two animals at 10 days *pre partum*, five animals at 2 days *pre partum* and three animals at 2 and 7 days *post partum*. S.E.M. values are not shown, but did not exceed 20% and were generally below 5%. The straight lines were obtained by linear-regression analysis with all the points for either antiserum or control serum. (a) Immunotitration of enzyme in parametrial adipose tissue; (b) immunotitration of enzyme in subcutaneous adipose tissue. Titrations with control serum (closed symbols) and antiserum (open symbols) are shown. Measurements were performed on extracts from adipose tissue of rats killed 10 days *pre partum* ($\square$, $\bullet$), 2 days *pre partum* ($\square$, $\blacksquare$), 2 days *post partum* ($\triangle$, $\blacktriangle$) or 7 days *post partum* ($\diamond$, $\blacklozenge$). The regression line for the titration of parametrial enzyme with control serum had a slope of 1.33 ± 0.113 and a correlation coefficient of 0.93, and with antiserum the regression line had a slope of 0.47 ± 0.05 and a correlation coefficient 0.92. The regression line for titration of subcutaneous enzyme with control serum had a slope of 1.44 ± 0.095 and a correlation coefficient of 0.96; with antiserum the regression line had a slope of 0.54 ± 0.07 with a correlation coefficient of 0.89.

Fig. 4. Immunotitration of pyruvate dehydrogenase activity

Rats were killed as described in Fig. 1; the total pyruvate dehydrogenase activity was titrated against $0.2\mu\text{l}$ of anti-(pyruvate dehydrogenase) serum or control serum (see the Experimental section). Each point represents the mean of measurements on six animals at 10 and 2 days *pre partum*, and three animals at 2 and 7 days *post partum*. S.E.M. values are not shown, but did not exceed 20% and were generally below 10%. The straight lines were obtained by linear-regression analysis on all the points. (a) Immunotitration of enzyme in parametrial adipose tissue; (b) immunotitration of enzyme in subcutaneous adipose tissue. Titrations with control serum (closed symbols) and antiserum (open symbols) are shown. Measurements were performed on animals killed at 10 days *pre partum* ($\square$, $\bullet$), 2 days *pre partum* ($\square$, $\blacksquare$), 2 days *post partum* ($\triangle$, $\blacktriangle$) and 7 days *post partum* ($\diamond$, $\blacklozenge$). The regression line for titration of parametrial enzyme with control serum had a slope of 0.74 ± 0.07 and a correlation coefficient of 0.92; with antiserum the regression line had a slope of 0.75 ± 0.045 and a correlation coefficient of 0.93. The regression line for titration of the subcutaneous enzyme with control serum had a slope of 0.57 ± 0.03 and correlation coefficient of 0.97; with antiserum the regression line had a slope of 0.62 ± 0.03 and a correlation coefficient of 0.98.

Table 2. *Weight and protein content*

The adipose tissues were removed from the rats, weighed and then homogenized in 2 vol. of extraction buffer (see the Experimental section). After centrifugation (7 min at 500 $g_{av.}$) the extract volume was measured and the protein content determined. From this the protein content of the fresh tissue was calculated. Results are expressed as means $\pm$ S.E.M. Numbers in parentheses indicate the numbers of animals studied. Adipose-tissue weight is expressed in g/rat and protein content is expressed as mg of protein/fat-pad.

Time	Parametrial adipose tissue		Inguinal subcutaneous adipose tissue	
	Adipose-tissue weight	Protein	Adipose-tissue weight	Protein
10 days <i>pre partum</i>	7.13 $\pm$ 0.67 (8)	18.5 $\pm$ 0.88 (8)	5.87 $\pm$ 0.32 (8)	29.5 $\pm$ 2.21 (8)
2 days <i>pre partum</i>	7.22 $\pm$ 0.53 (11)	21.0 $\pm$ 1.84 (11)	4.71 $\pm$ 0.41 (11)	16.9 $\pm$ 1.48 (11)
2 days <i>post partum</i>	5.49 $\pm$ 0.68 (6)	15.4 $\pm$ 1.33 (5)*	3.60 $\pm$ 0.38 (6)	12.9 $\pm$ 0.59 (6)*
7 days <i>post partum</i>	6.1 $\pm$ 0.97 (6)	19.6 $\pm$ 1.8 (6)	4.55 $\pm$ 0.71 (6)	17.0 $\pm$ 3.07 (6)

* Significantly different from 2 days *pre partum* ($P < 0.05$).

shown). Despite the changes in total protein content of the two fat-pads, no statistically significant change in the total pyruvate dehydrogenase activity of either parametrial or subcutaneous fat-pads was found between 10 days *pre partum* and 7 days *post partum*.

Discussion

Regulation of adipose-tissue metabolism

During pregnancy and lactation there appear to be coordinated changes in the rate of fatty acid synthesis and the lipoprotein lipase activity of adipose tissue, which reflect the accumulation and mobilization of fat in the tissue (Flint *et al.*, 1979).

The decrease in fatty acid synthetase activity (Fig. 1), which is due solely to a decrease in the amount of the enzyme and not to inactivation of a constant amount of the enzyme, parallel the decrease in the rate of fatty acid synthesis.

Changes in the specific activity of acetyl-CoA carboxylase were also very similar to those of the rate of fatty acid synthesis over the period of study. However, the better correlation of fatty acid synthetase activity (and hence amount) to the rate of fatty acid synthesis, suggest that changes in the amount of this enzyme are responsible for the changes in the rate of fatty acid synthesis. These conclusions are supported by Guynn *et al.* (1972), who suggested that inhibition of acetyl-CoA carboxylase is responsible for short-term control of fatty acid synthesis, whereas long-term changes are brought about by changes in the amount of fatty acid synthetase present in the cell.

The initial pyruvate dehydrogenase activity shows a decrease between 2 days *pre partum* and 2 days *post partum*, but the total activity and amount of enzyme present in the cell show no significant change. Therefore pyruvate dehydrogenase may

impose some limiting effect on the rate of fatty acid synthesis by the reduction of the proportion in the active state, but no long-term adaptations in pyruvate dehydrogenase amount seem to occur.

The specific activities of some enzymes in fatty acid biosynthesis in adipose tissue do not change during pregnancy and lactation. For example, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities do not change significantly (Smith, 1973).

The decrease in the amount of fatty acid synthetase is presumably brought about by a selective mechanism involving changes in the turnover characteristics of this enzyme (i.e. rates of synthesis and/or degradation), but the factors responsible are not yet known. The amount of fatty acid synthetase in adipose tissue is reduced by glucocorticoid administration to adult rats and increased by adrenalectomy. This occurs because the rate of synthesis of the enzyme is decreased and increased respectively (Volpe & Marasa, 1975). Also, insulin stimulates the synthesis of fatty acid synthetase in liver (Lakshmanan *et al.*, 1975).

Glucocorticoid concentrations increase sharply to a peak at the end of pregnancy (see Kuhn, 1977) while insulin concentrations decline (Sutter-Dub *et al.*, 1974; Kuhn, 1977; Flint *et al.*, 1979). Similarly the number of insulin receptors of the adipocytes rises during early pregnancy and fall around parturition (Flint *et al.*, 1979). Any, or all, of these changes could be responsible for the decrease in the amount of fatty acid synthetase of adipose tissue around parturition. However, the serum concentrations of other hormones change around parturition (Kuhn, 1977), so further factors may also be involved.

There is a decrease in the total protein concentration and a relatively greater decrease in the concentration of fatty acid synthetase of rat adipose

tissue around parturition. It would seem probable that there may be similar disproportionate decreases in the concentration of other enzymes in the tissues at this time. This possibility deserves further investigation, since it may reveal a regulated programme of turnover of specific enzymes, such as that which is well-characterized in mammary gland (Mayer & Paskin, 1978; Paskin & Mayer, 1978). However, in adipose tissue in late pregnancy and lactation, changes in enzyme-turnover characteristics result in decreased enzyme amounts, in contrast with mammary gland, where an increase in cell-specific enzyme amounts occurs. In mammary gland the amount of fatty acid synthetase increases because enzyme synthesis is increased severalfold while enzyme degradation is transiently decreased. The mechanisms responsible for these differences between the two tissues are unknown, but differential responses to hormones would seem likely to be involved. In this context it is probable that the decrease in number of insulin receptors of adipocytes around parturition may have a critical role in the regulation of enzyme synthesis and degradation in the tissue at this time.

Immunotitrations of fatty acid synthetase and pyruvate dehydrogenase activities

Immunochemical methods have been used to show whether enzyme activity changes are due to changes in enzyme amount. Two aspects of the immunotitrations are of interest.

The first is that the residual activity of both enzymes is lower than can be accounted for by immunoinhibition by the antisera. This effect is due to a loss of enzyme activities with time. This phenomenon was noted during preliminary experiments (results not shown) and in other published immunochemical studies with both long and short incubation times (Joshi & Sidbury, 1976; Volpe *et al.*, 1973). The incubation times in the titrations reported here minimized the time-dependent loss of enzyme activities.

A second anomaly was observed during titration of fatty acid synthetase activity. Activities measured with control serum indicate activation of the enzyme. This activation is probably caused by the removal of fatty acids from the incubation mixtures by binding to serum proteins. The protein concentrations of anti-(fatty acid synthetase) serum and control serum were 32 and 57 mg/ml respectively. The higher protein concentration in incubations with control serum may therefore have decreased the fatty acid concentration sufficiently to produce a de-inhibition of fatty acid synthetase activity.

We thank Miss J. Saxton for technical assistance and Dr. J. Pearson for advice on statistical methods.

P. A. S.-S. was supported by a CASE Studentship from the Science Research Council.

References

- Beaton, G. H., Beare, J., Ryce, M. H. & McHenry, E. W. (1954) *J. Nutr.* **54**, 291–304
- Cooper, R. H., Randle, P. J. & Denton, R. M. (1974) *Biochem. J.* **143**, 625–651
- Flint, D. J., Sinnett-Smith, P. A., Clegg, R. A. & Vernon, R. G. (1979) *Biochem. J.* **182**, 421–427
- Guynn, R. W., Veloso, D. & Veech, R. L. (1972) *J. Biol. Chem.* **247**, 7325–7331
- Hamosh, M., Clary, T. R., Chernick, S. S. & Scow, R. O. (1970) *Biochim. Biophys. Acta* **210**, 473–482
- Joshi, V. C. & Sidbury, J. B. (1976) *Arch. Biochem. Biophys.* **173**, 403–414
- Knopp, R. H., Saudek, C. D., Arky, R. A. & O'Sullivan, J. B. (1973) *Endocrinology* **92**, 984–988
- Kuhn, N. J. (1977) in *Comparative Aspects of Lactation* (Peaker, M., ed.), pp. 165–192, Academic Press, London
- Lakshmanan, M. R., Nepokroeff, C. M., Kim, M. & Porter, J. W. (1975) *Arch. Biochem. Biophys.* **169**, 737–745
- Linn, T. C., Pelley, J. W., Pettit, F. H., Hucho, F., Randell, D. D. & Reed, L. J. (1972) *Arch. Biochem. Biophys.* **148**, 327–342
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Manning, R., Dils, R. & Mayer, R. J. (1976) *Biochem. J.* **153**, 463–468
- Mayer, R. J. & Paskin, N. (1978) in *Regulation in Fatty Acid and Glycerolipid Metabolism* (Dils, R. & Knudsen, J., eds.), pp. 53–62, Pergamon Press, Oxford
- Mayer, R. J. & Walker, J. H. (1978) in *Techniques in the Life Sciences*, vol. B1/11, B119 (1–32), North-Holland/Elsevier, Amsterdam
- Otway, S. & Robinson, D. S. (1968) *Biochem. J.* **106**, 677–682
- Ouchterlony, Ö. (1968) *Handbook of Immunodiffusion and Immunoelectrophoresis*, Ann Arbor Science Publishers, Ann Arbor and London
- Paskin, N. & Mayer, R. J. (1976) *Biochem. J.* **159**, 181–184
- Paskin, N. & Mayer, R. J. (1978) *Biochem. J.* **174**, 143–161
- Piazzi, S. E. (1969) *Anal. Biochem.* **27**, 281–284
- Severson, D. L., Denton, R. M., Pask, H. T. & Randle, P. J. (1974) *Biochem. J.* **140**, 225–237
- Sinnett-Smith, P. A., Vernon, R. G. & Mayer, R. J. (1979) *Biochem. Soc. Trans.* **7**, 388–389
- Smith, R. W. (1973) *J. Dairy Res.* **40**, 353–360
- Smith, S., Easter, D. J. & Dils, R. (1966) *Biochim. Biophys. Acta* **125**, 445–455
- Snedecor, G. W. & Cochran, W. G. (1967) in *Statistical Methods*, 6th edn., pp. 299–301, Iowa State University Press, Ames
- Speake, B. K., Dils, R. & Mayer, R. J. (1975) *Biochem. J.* **148**, 309–320
- Spray, C. M. (1950) *Br. J. Nutr.* **4**, 354–360

- Stansbie, D., Denton, R. M., Bridges, B. J., Pask, H. T. & Randle, P. J. (1976) *Biochem. J.* **154**, 225–236
- Sutter-Dub, M. T., Leclercq, R., Sutter, B. J. C. & Jacquot, R. (1974) *Horm. Metab. Res.* **6**, 297–300
- Tabor, H., Mehler, A. H. & Stedman, G. R. (1953) *J. Biol. Chem.* **204**, 127–138
- Volpe, J. J. & Marasa, J. C. (1975) *Biochim. Biophys. Acta* **380**, 454–472
- Volpe, J. J., Lyles, T. O., Roncari, D. A. K. & Vagelos, P. R. (1973) *J. Biol. Chem.* **248**, 2502–2513
- Wang, C. S. & Smith, R. L. (1975) *Anal. Biochem.* **63**, 414–417