*Biochem. J.* (1985) **255**, 301–306
*Printed in Great Britain*



# Chain-shortening of erucic acid and microperoxisomal β-oxidation in rat small intestine

Magny S. THOMASSEN,*† Per HELGERUD‡ and Kaare R. NORUM
*Institute for Nutrition Research, University of Oslo, Blindern, Oslo, Norway*

(*Received 29 May 1984/Accepted 28 September 1984*)

1. The ability of rat small intestine to chain-shorten $C_{22:1}$ fatty acids was investigated. Radioactive chain-shortened products, mainly $C_{18:1}$, were demonstrated in intestinal-lymph lipids after intraluminal injection of [14-$^{14}$C]erucic acid. Chain-elongation to $C_{24:1}$ was also observed. 2. Adaptation to a diet containing $C_{22:1}$ fatty acids (partially hydrogenated-marine-oil diet) slightly increased the percentage of chain-shortened products. 3. Microperoxisomal β-oxidation activity, measured as $CN^-$-insensitive palmitoyl-CoA-dependent $NAD^+$ reduction, was detected in a microperoxisome-enriched fraction from mucosal scrapings. This activity was increased 1.9-fold by a soya-bean-oil diet, and 2.7-fold by a diet containing partially hydrogenated marine oil.

Rats given diets containing high-erucic-acid rapeseed oils or partially hydrogenated marine oils develop a transient lipidosis in heart and skeletal muscles (Beare-Rogers, 1977), an effect ascribed to the relatively high content of $C_{22:1}$ fatty acids in such oils. The metabolism and metabolic effects of these fatty acids have consequently been matters of great interest (for reviews, see Bremer & Norum, 1982; Christophersen *et al.*, 1982).

An increasing body of evidence suggests that the $C_{22:1}$ molecule is chain-shortened to $C_{20:1}$, and especially $C_{18:1}$, before further degradation takes place. Chain-shortening has been demonstrated in rat liver and heart (Pinson & Padieu, 1974; Christiansen, 1978; E. N. Christiansen *et al.*, 1979; Norseth, 1979), but was not detected in adipocytes from rat epididymal fat (Christophersen *et al.*, 1983). An involvement of peroxisomal/microperoxisomal β-oxidation activity in this process is highly probable, and both peroxisomal β-oxidation and chain-shortening of $C_{22:1}$ fatty acids are increased in rats fed on diets containing such fatty acids (R. Z. Christiansen *et al.*, 1979; Norseth, 1979; Neat *et al.*, 1981; Norseth & Thomassen, 1983).

The absorptive cells of mammalian small intestine contain large numbers of microperoxisomes (Novikoff & Novikoff, 1972). Moreover, palmitoyl-CoA oxidase, the enzyme catalysing the first step in peroxisomal β-oxidation, has been found in microperoxisomes from mouse small intestine (Small *et al.*, 1983). These observations suggest that absorptive cells in rat small intestine may have the capacity to chain-shorten $C_{22:1}$ fatty acids. This question has been addressed in the present study.

## Experimental

### *Materials*

Soya-bean oil and PHMO were obtained from DeNoFa and Lilleborg Fabrikker A/S, Fredrikstad, Norway. High-erucic-acid rapeseed oil was supplied by AB Karlshamns Oljefabriker, Karlshamn, Sweden. The fatty acid compositions of the dietary oils are given by Thomassen *et al.* (1982). The contents of $C_{22:1}$ fatty acids in rapeseed oil and PHMO were 43.5% and 14.5% respectively. Vitamin and salt mixtures were from ICN Pharmaceuticals, Cleveland, OH, U.S.A., [14-$^{14}$C]Erucic acid (99% radiopurity) was from CEA, Gif-sur-Yvette, France, and erucic acid (99% pure) from Sigma Chemical Co., St. Louis, MO, U.S.A. Clofibrate was obtained from Weiders Farmasøytiske A/S Oslo, Norway, and

Abbreviation used: PHMO, partially hydrogenated marine oil.

* To whom correspondence and reprint requests should be addressed.
† Present address: Norwegian Food Research Institute, P.O. Box 50, N-1432 Aas-NLH, Norway.
‡ Present address: Surgical Department B, Rikshospitalet, Oslo, Norway.

palmitoyl-CoA from P-L Biochemicals, Milwaukee, WI, U.S.A. Other chemicals were commercially available products of high purity.

*Animals and diets*

Male rats of the Wistar strain were purchased from Møllegaard Breeding Laboratory, Ejby, Denmark. The compositions of the standard pelleted and semi-synthetic diets, as well as details about housing conditions, were as described by Nilsson *et al.* (1984).

*Collection of intestinal lymph*

The animals were given an intraperitoneal injection of barbiturate and then operated as previously described (Dueland *et al.*, 1982) with the insertion of one tube in the main intestinal lymph duct and one tube to the upper part of the duodenum for intraluminal injection of a bolus of rapeseed oil or soya-bean oil containing free erucic acid. The total volume of oil given was 0.5ml.

*Lipid extraction and analysis by g.l.c.*

Lipids were extracted from the lymph samples by the method of Folch *et al.* (1957). Total lipid fatty acids were methylated by using a mixture of benzene, methanolic HCl and dimethoxypropane at room temperature overnight (Mason & Waller, 1964), and analysed by radio-g.l.c. as described by Hagve & Christophersen (1983). The complete fatty acid composition was determined separately by g.l.c. essentially as described by Thomassen *et al.* (1982). Carrier gas flow was 0.5ml/min, splitless injection was performed at 40°C, and column temperature was programmed to rise rapidly from 40 to 200°C, and then by a slow gradient (0.5%/min) to 240°C.

*Preparation of microperoxisome-enriched fraction*

Rats were killed between 11:00 and 12:00h by a blow on the head and cutting of the carotid arteries. The proximal 30cm of jejunum was rapidly removed, flushed with ice-cold iso-osmotic saline (0.9%NaCl), opened longitudinally and washed. The mucosa was scraped off, re-washed and collected by low-speed centrifugation, followed by homogenization in a Dounce homogenizer. Whole cells and cell debris were removed by centrifugation at $3200\boldsymbol{g}_{av.}$-min. The resulting supernatant (E fraction) was then centrifuged at $100000\boldsymbol{g}_{av.}$-min to remove most of the mitochondria (M fraction). The microperoxisome-enriched fraction (P fraction) was then prepared by centrifugation of the postmitochondrial supernatant as described by Norseth *et al.* (1982), and the resulting pellet was resuspended in the homogenization medium. Subcellular fractionation of intestinal mucosa is known to be hampered by considerable cross-contamination of the fractions because of the presence of mucus (Merchant & Heller, 1977). In the present study, about 25% of the total catalase activity and also some $CN^-$-insensitive palmitoyl-CoA-dependent $NAD^+$ reduction activity was detected in the M fraction. However, more than 50% of the catalase activity, and about two-thirds of the combined activity of $CN^-$-insensitive palmitoyl-CoA-dependent $NAD^+$ reduction recovered in the M and P fractions, resided in the microperoxisome-enriched (P) fraction. The recovery of this last activity was difficult to estimate, owing to the very low activity in the E fractions.

*Enzyme and protein assays*

$CN^-$-insensitive palmitoyl-CoA-dependent $NAD^+$ reduction was measured spectrophotometrically as described by Lazarow & de Duve (1976), with the modifications introduced by Flatmark *et al.* (1981). The assay was performed at pH8.0 at room temperature (20°C).

Catalase (EC 1.11.1.6) activity was determined as described by Chance & Maehly (1955), and protein was determined by using the Folin–Ciocalteu reagent (Lowry *et al.*, 1951), with bovine serum albumin as standard.

*Statistical analysis*

Dunnett's (1955) multiple comparison test was used to evaluate the significance of differences between population means; $P > 0.05$ was taken to be not significant.

## Results and discussion

*Absorption of rapeseed oil and metabolic conversion of [14-$^{14}$C]erucic acid*

When rats, operated as described in the Experimental section, were given an intraluminal injection of high-erucic-acid rapeseed oil we found that the percentage of erucic acid [$C_{22:1\ (13)}$] in lymph lipids was significantly decreased relative to the oil (results not shown), in good agreement with previous studies on rats given rapeseed oil or mustard-seed oil in the diet (Caselli *et al.*, 1979; Vadsev & Kako, 1978). These results have been ascribed to a lower digestibility of erucic acid (Caselli *et al.*, 1979), but may also be due to the incorporation of fatty acids of endogenous origin into the lymph lipids (Gangl & Ockner, 1975). However, it is also a possibility that a metabolic conversion of the $C_{22:1}$ fatty acids into shorter monoenes takes place in the intestinal wall, as first suggested by Vadsev & Kako (1978). This may also be indicated by our finding that, although the percentage of $C_{22:1}$ acid in lymph lipids at the peak

of absorption was decreased to about 75%, the percentage of $C_{20:1}$ acid was unchanged and that of $C_{18:1}$ acid increased to about 125% of that of the rapeseed oil injected. No direct test has, however, to our knowledge been performed as to the ability of the intestine to shorten $C_{22:1}$ fatty acids. We therefore injected [14-$^{14}$C]erucic acid, dissolved in soya-bean oil, directly into the intestinal lumen of rats, operated as described in the Experimental section, and studied the composition of the lymph lipids by radio-g.l.c. The rats were previously fed on a standard pelleted diet, or adapted to a diet containing $C_{22:1}$ fatty acids (PHMO diet). As shown in Fig. 1, no significant difference was observed in the rate of transfer of radioactivity from lumen to lymph in the two groups of rats. Between 2 and 8h after the injection of [14-$^{14}$C]-erucic acid, radioactive material accumulated at a fairly constant rate, and at the end of this period about 80% of the total radioactivity recovered in each experiment (40–70% of the amount injected) had appeared in the lymph.

The main part of the radioactivity was recovered as erucic acid, but a significant amount was also found in $C_{18:1}$, $C_{20:1}$ and $C_{24:1}$ fatty acids. Fig. 2 shows the percentages of radioactivity recovered as $C_{18:1}$ and $C_{24:1}$ acids during the course of the absorption, whereas the percentage of $C_{18:1}$ acid was high and fairly constant from the first 2h onwards, the percentage of $C_{24:1}$ acid seemed to increase gradually with time. About 2% of the radioactivity appeared in a peak that was eluted as $C_{20:1}$ acid. This percentage did not change significantly, as a function of either absorption time or the feeding regimen (results not shown).

The percentage recovered as 18:1 acid was, on the other hand, slightly increased in the rats given the PHMO diet. Thus, feeding PHMO may evoke an increase in the chain-shortening capacity also in rat small intestine, in line with previous findings in liver and heart (E. N. Christiansen *et al.*, 1979; Norseth, 1979). Our results suggest, however, that the extent of increase is less in the intestine than in liver and heart.

The percentage recovered in $C_{24:1}$ acid seemed to be somewhat decreased in the rats given

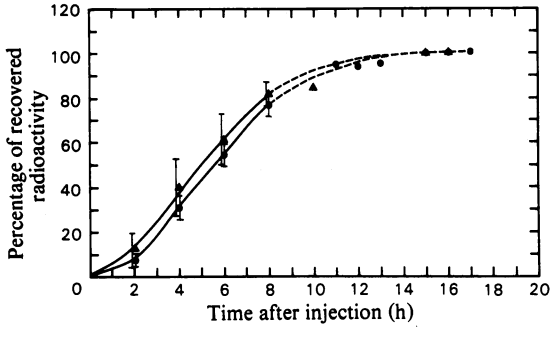


Fig. 1. *Recovery of radioactivity in intestinal lymph after intraduodenal injection of [14-$^{14}$C]erucic acid*
Rats operated as described in the Experimental section were given an intraduodenal injection of 0.5ml of soyabean oil containing about 5% free [14-$^{14}$C]erucic acid. Intestinal lymph was collected for the next 16–20h, and the radioactivity recovered was measured by liquid-scintillation counting. The Figure shows the cumulative appearance of recovered radioactivity (means ± S.D.) for three animals fed on a standard pelleted diet (▲) or on a PHMO diet (●).

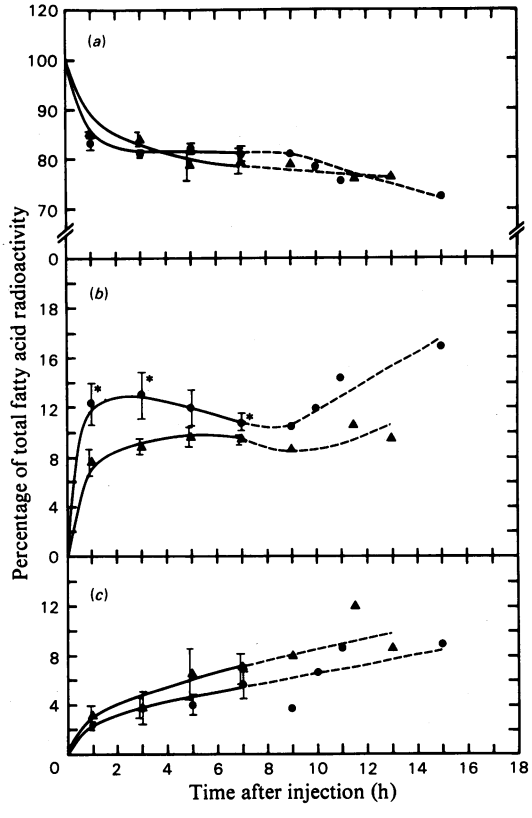


Fig. 2. *Percentage of radioactivity recovered as $C_{22:1}$, $C_{18:1}$ and $C_{24:1}$ fatty acids in lymph lipids after intraduodenal injection of [14-$^{14}$C]erucic acid*
Lipids were extracted from lymph samples collected as described in the legend to Fig. 1, and radio-g.l.c. was performed as described in the Experimental section. The Figure shows the percentage of total fatty acid radioactivity recovered as $C_{22:1}$ (*a*), $C_{18:1}$ (*b*) or $C_{24:1}$ (*c*). The values represent means ± S.D. for three animals fed on a standard pelleted diet (▲) or on a PHMO diet (●). Statistically significant differences from the standard pelleted diet group are indicated by $*0.05 > P > 0.01$.

PHMO, although the differences observed were not statistically significant. The results are, however, in good agreement with the studies on erucic acid conversion in rat lung tissue by Lecerf (1980), who observed that the conversion of erucic acid into nervonic acid ($C_{24:1}$) was less, and into oleic acid ($C_{18:1}$) more, in a group of rats given a rapeseed-oil diet as compared with other diet groups.

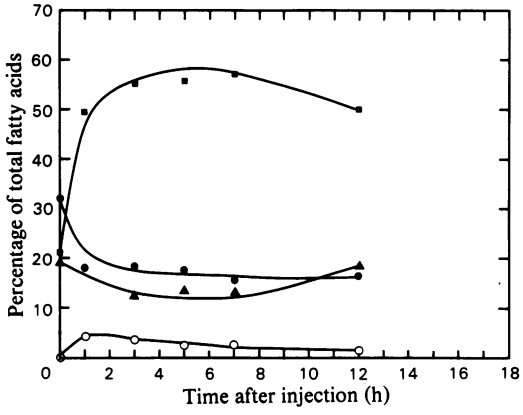


Fig. 3. *Changes in fatty acid composition of lymph lipids during the course of absorption*
Lipids were extracted from the lymph samples collected as described in the legend to Fig. 1, and g.l.c. analysis of total fatty acids was performed as described in the Experimental section. The Figure shows the percentage of some of the main fatty acids, $C_{18:2}+C_{18:3}$ (■), $C_{18:1}$ (●), $C_{16:0}$ (▲) and $C_{22:1}$ (○). The values represent means for three animals fed on a standard pelleted diet.

Because of the seemingly inverse relationship between chain-shortening and chain-elongation, the percentage of radioactivity recovered in $C_{22:1}$ acid did not differ significantly in the two groups of rats. This was also reflected in the similarity in the total fatty acid composition of the lymph lipids. Thus no great differences were observed in the time-dependent variations in fatty acid composition. The results from control animals are shown in Fig. 3. The percentage of $C_{22:1}$ fatty acid varied in both dietary groups from about 4 to 1%, and the time course of the total fatty acid pattern suggested that erucic acid, administered as the free acid, was absorbed somewhat faster than most of the fatty acids from the soya-bean oil. Table 1 gives the total fatty acid compositions of lymph lipids collected between 4 and 6 h after injection, at the time of constant rate of radioactivity transfer. A decrease was observed in the percentage of $C_{22:1}$ fatty acids as compared with the oil injected into the intestinal lumen, but no statistically significant differences were observed between the two groups of animals.

*Microperoxisomal β-oxidation*

By analogy with findings in other organs, it might be expected that chain-shortening of $C_{22:1}$ fatty acids is related to a microperoxisomal β-oxidation activity also in the small intestine. We therefore investigated the existence of a $CN^-$-insensitive palmitoyl-CoA-dependent $NAD^+$ reduction activity in a microperoxisome-enriched fraction from small-intestinal mucosal scrapings, and, as shown in Table 2, activities in the order of 1–4nmol of NADH produced/min per mg of protein were observed. This is in good agreement with the values obtained in a similar micro-

Table 1. *Fatty acid composition of soya-bean oil containing dissolved erucic acid, and of lymph lipids during its absorption*
Rats were fed on a standard pelleted diet, or a diet containing 20% (w/w) PHMO + 5% (w/w) soya-bean oil (PHMO diet). The intestinal lymph duct was cannulated as described in the Experimental section. The fatty acid composition of lymph lipids collected at the peak of soya-bean oil absorption, i.e. between 4 and 6 h after injection of the oil into the intestinal lumen, was determined by g.l.c. Only fatty acids >1% are included in the Table. Values represent means ± S.D. for three animals in each dietary group. No statistically significant differences ($P<0.05$) were observed between the values obtained in the two groups.

| | Fatty acid composition (%) | | |
|---|---|---|---|
| | Soya-bean oil + | Lymph lipids | |
| Fatty acid | erucic acid | Pelleted diet | PHMO diet |
| $C_{14:0}$ | | | 1.0±0.4 |
| $C_{16:0}$ | 11.6 | 13.6±1.7 | 13.4±1.3 |
| $C_{16:1}$ | | 1.1±0.2 | 1.5±0.3 |
| $C_{18:0}$ | 4.2 | 4.4±0.3 | 4.2±0.2 |
| $C_{18:1}$ | 21.3 | 17.4±1.0 | 20.5±2.9 |
| $C_{18:2}+C_{18:3}$ | 58.3 | 55.9±1.7 | 49.9±6.4 |
| $C_{20:1}$ | | | 1.0±0.4 |
| $C_{20:4}$ | | 1.2±0.2 | |
| $C_{22:1}$ | 4.8 | 2.5±0.7 | 2.8±0.5 |

Table 2. *$CN^-$-insensitive palmitoyl-CoA-dependent $NAD^+$ reduction in microperoxisome-enriched fractions from rat small-intestinal mucosa*

Rats were fed on semisynthetic diets containing 20% (w/w) of soya-bean oil (SO) or PHMO, or on a standard pelleted diet with or without clofibrate (0.3%, w/w). Microperoxisome-enriched fractions were isolated from mucosal scrapings, and assayed for $CN^-$-insensitive palmitoyl-CoA-dependent $NAD^+$ reduction as described in the Experimental section. Values represent means ± S.D. for four animals in each group. Statistically significant differences from the standard pelleted diet group are indicated by: $*0.05 > P > 0.01$; $**0.01 > P$.

| Dietary treatment | $NAD^+$ reduction (nmol/min per mg of protein) |
|---|---|
| Pellets | 1.4 ± 0.5 |
| SO, 3 days | 2.7 ± 0.4* |
| PHMO, 3 days | 4.0 ± 0.7** |
| PHMO, 7 days | 3.7 ± 1.1** |
| PHMO, 3 weeks | 3.4 ± 1.1** |
| Pellets + clofibrate, 10 days | 2.4 ± 0.8 |

peroxisome-enriched fraction from rat heart. The optimum requirements for palmitoyl-CoA and detergent (Brij 58) were very similar to those described by Flatmark *et al.* (1981) for the liver enzyme system, but no stimulation of the activity was found by the addition of FAD (50 μM).

As observed in liver and heart, high-fat feeding resulted in an increased activity of the palmitoyl-CoA-dependent $NAD^+$ reduction. Thus with a soya-bean-oil diet an activity about twice as high as that in rats fed on a standard pelleted diet was observed, whereas PHMO feeding led to a 2.7-fold increase. However, in contrast with that observed in liver and heart, no further increase was detected in the rats fed on PHMO for 1 and 3 weeks. This may possibly be related to the relatively short life-time (2–4 days) of the absorptive cells in the small intestine.

As also shown in Table 2, clofibrate treatment, if anything, seemed to give only a marginal increase in this activity. It has been reported that clofibrate increases the activity of acyl-CoA oxidase in mouse small-intestinal microperoxisomes by 50% (Small *et al.*, 1983). This is in contrast with the findings in liver, where a 10-fold stimulation is observed (Lazarow & de Duve, 1976; Thomassen *et al.*, 1982). However, in rat heart clofibrate treatment gave only a 2.5-fold increase (Norseth & Thomassen, 1983). Thus the effects observed in rat small intestine seem to be more in line with those observed in rat heart microperoxisomes than in liver peroxisomes. The reason for these differences is still unknown.

In conclusion, the results obtained in this study suggest that the absorptive cells in rat small intestine possess the capacity to chain-shorten $C_{22:1}$ fatty acids. Furthermore, this capacity may, by analogy with observations in other tissues, be related to a β-oxidation activity in the microperoxisomes encountered in great amounts in these cells.

This work was supported by the Royal Norwegian Council for Scientific and Industrial Research. We thank Ms. T. Rørtveit for excellent technical assistance.

## References

Beare-Rogers, J. L. (1977) *Progr. Chem. Fats Other Lipids* **15**, 29–56
Bremer, J. & Norum, K. R. (1982) *J. Lipid Res.* **23**, 243–256
Caselli, C., Carlier, H. & Bezard, J. (1979) *Nutr. Metab.* **23**, 73–87
Chance, B. & Maehly, A. C. (1955) *Methods Enzymol.* **2**, 764–775
Christiansen, E. N., Thomassen, M. S., Christiansen, R. Z., Osmundsen, H. & Norum, K. R. (1979) *Lipids* **14**, 829–835
Christiansen, R. Z. (1978) *Biochim. Biophys. Acta* **530**, 314–324
Christiansen, R. Z., Christiansen, E. N. & Bremer, J. (1979) *Biochim. Biophys. Acta* **573**, 417–429
Christophersen, B., Norseth, J., Thomassen, M. S., Christiansen, E. N., Norum, K. R., Osmundsen, H. & Bremer, J. (1982) in *Nutritional Evaluation of Long-Chain Fatty Acids in Fish Oil* (Barlow, S.M. & Stansby, M.E., eds.), pp. 89–139, Academic Press, London
Christophersen, B. O., Krogstad, T. & Norseth, J. (1983) *Lipids* **18**, 137–141
Dueland, S., Pedersen, J. I., Helgerud, P. & Drevon, C. A. (1982) *J. Biol. Chem.* **257**, 146–150
Dunnett, C. W. (1955) *J. Am. Stat. Assoc.* **50**, 1096–1121
Flatmark, T., Christiansen, E. N. & Kryvi, H. (1981) *Eur. J. Cell Biol.* **24**, 62–69
Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497–509
Gangl, A. & Ockner, R. K. (1975) *J. Clin. Invest.* **55**, 803–813
Hagve, T. A. & Christophersen, B. O. (1983) *Biochim. Biophys. Acta* **753**, 339–349
Lazarow, P. B. & de Duve, C. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2043–2046
Lecerf, J. (1980) *Biochim. Biophys. Acta* **617**, 398–409
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
Mason, M. E. & Waller, G. R. (1964) *Anal. Chem.* **36**, 583–586
Merchant, J. L. & Heller, R. A. (1977) *J. Lipid Res.* **18**, 722–733
Neat, C. E., Thomassen, M. S. & Osmundsen, H. (1981) *Biochem. J.* **196**, 149–159
Nilsson, A., Thomassen, M. S. & Christiansen, E. N. (1984) *Lipids* **19**, 187–194

Norseth, J. (1979) *Biochim. Biophys. Acta* **575**, 1–9
Norseth, J. & Thomassen, M. S. (1983) *Biochim. Biophys. Acta* **751**, 312–320
Norseth, J., Normann, P. T. & Flatmark, T. (1982) *Biochim. Biophys. Acta* **719**, 569–579
Novikoff, P. M. & Novikoff, A. B. (1972) *J. Cell Biol.* **53**, 532–560
Pinson, A. & Padieu, P. (1974) *FEBS Lett.* **39**, 88–90
Small, G. M., Burdett, K. & Connock, M. S. (1983) *Biochem. Int.* **7**, 263–272
Thomassen, M. S., Christiansen, E. N. & Norum, K. R. (1982) *Biochem. J.* **206**, 195–202
Vadsev, S. C. & Kako, K. S. (1978) *Am. J. Physiol.* **235**, E509–E516