Subcellular localization of hexadecanedioic acid activation in human liver

Jon Elling Pettersen and Magne Aas

Institute of Clinical Biochemistry, University of Oslo, Rikshospitalet, Oslo, Norway

Abstract The activation of hexadecanedioic acid has been studied in subcellular fractions of human liver. The activation capacity in a total homogenate of human liver was found to be 0.5 μmole/min/g wet wt of tissue, about 10% of that for palmitic acid. Hexadecanedioic acid was activated by the mitochondrial and microsomal fractions. The mitochondrial enzyme is probably localized outside the inner mitochondrial compartment. The subcellular distribution of the hexadecanedioic acid activation was almost identical with the distribution of palmitic acid activation. Hexadecanedioic and palmitic acids seemed to compete for the same enzyme.

Supplementary key words acyl CoA synthetase · hexadecanoyl CoA:carnitine *O*-hexadecanoyltransferase · tissue fractionation

We have previously reported that patients with ketosis excrete considerable amounts of *n*-hexanedioic and *n*-octanedioic acids in the urine (1, 2). In vivo experiments with ketotic rats showed that these short-chain dicarboxylic acids may be formed from long-chain monocarboxylic acids by an initial ω-oxidation followed by degradation of the long-chain dicarboxylic acids thus formed (3). In humans, short-chain dicarboxylic acids may also be formed from long-chain monocarboxylic acids by an initial ω-oxidation (4, 5). In vivo experiments in which long-chain dicarboxylic acids were given to animals (6–8) or humans (6, 8) indicate that these acids are degraded by the β-oxidation mechanism.

Degradation by β-oxidation presupposes an activation by CoA. By in vitro studies with rat liver mitochondria we (9, 10) have recently demonstrated that hexadecanedioic acid may be activated by ATP-Mg^{2+} and CoA and transported into the inner mitochondrial compartment as the mono-L-carnitine ester (hexadecanedioylcarnitine). The present study concerns the activation of hexadecanedioic acid in human liver.

MATERIALS AND METHODS

Chemicals and instrumentation were as described earlier (10).

Liver fractionation

Liver biopsy specimens weighing 1.40 and 1.45 g, respectively, were taken from two patients. Patient 1 was a 42-yr-old man undergoing colectomy for ulcerative colitis, and patient 2 was a 72-yr-old woman undergoing cholecystotomy for cholelithiasis. The liver biopsies were macroscopically normal. In both cases, however, microscopic examination showed some fibrosis and slight chronic inflammation. The biopsies were immediately cooled on ice, weighed, cut into small pieces, and homogenized with a Potter-Elvehjem homogenizer in 0.25 M sucrose containing 5×10^{-4} M ATP to counteract enzyme inactivation (11). Subcellular fractions were prepared according to de Duve et al. (12), with minor modifications as described by Norum and Bremer (13). The fractions obtained were the nuclear fraction and the cytoplasmic extract. The latter was further separated into the heavy mitochondrial (mitochondrial) fraction, the light mitochondrial (lysosomal) fraction, the microsomal fraction, and the particle-free supernate. The subfractions were then frozen (−20°C) until the enzyme assays were performed (within 1 month).

Assay procedures

The protein content of the fractions was determined by the method of Lowry et al. (14).

Marker enzymes. Monoamine oxidase (EC 1.4.3.4) was measured by a slight modification (15) of the method described by McCaman et al. (16). The assay conditions for acid phosphatase (EC 3.1.3.2) were as described by Wattiaux and de Duve (17), and glucose-6-phosphatase (EC 3.1.3.9) was assayed according to de Duve et al. (12). The CoA-dependent incorporation of ^3H-labeled free carnitine into acylcarnitine catalyzed by carnitine acyltransferase (18) was assayed by an isotope-exchange assay as described by Norum (19). Because of the complex kinetics of the exchange reaction (20), the activity was expressed in arbitrary units (cpm). Long-chain acyl CoA synthetase (EC 6.2.1.3) was assayed by the method of Farstad, Bremer, and Norum (21).

Assay of hexadecanedioic acid activation. Our method is based on the same principle as that described by Far-

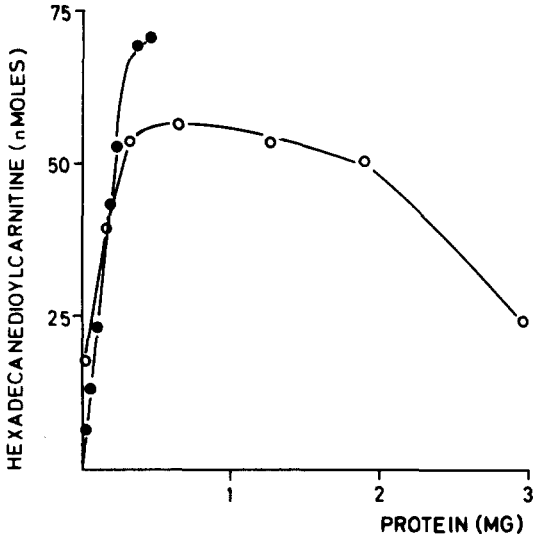

Fig. 1. Formation of hexadecanedioylcarnitine as a function of the amounts of carnitine palmityltransferase and microsomal protein from human liver (patient 1). When the amounts of carnitine palmityltransferase were varied (○), 0.24 mg of protein of microsomal fraction was used. When the amounts of microsomal fractions were varied (●), 1.26 mg of protein of carnitine palmityltransferase was present. Other conditions were as stated in Materials and Methods.

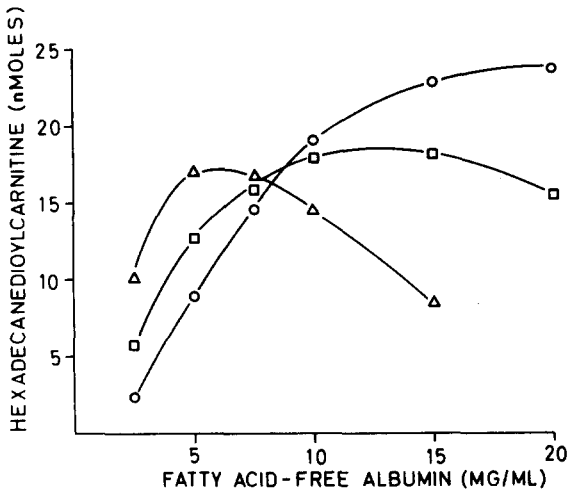

Fig. 2. Hexadecanedioylcarnitine formation as a function of the concentration of fatty acid-free bovine serum albumin. Three different concentrations of hexadecanedioate were used: 1 mM (△), 2 mM (□), and 3 mM (○). Microsomal fraction from patient 1 (0.09 mg of protein) was used as enzyme source. Other conditions were as described in Materials and Methods.

stad et al. (21) for the assay of long-chain monocarboxylic acid activation. The standard incubation mixture contained in 2 ml: 0.1 M Tris–HCl buffer (pH 7.4), 0.1 mM CoA, 5 mM ATP, 5 mM $MgCl_2$, 4 mM L-[*Me*-^{3}H]carnitine, 1.5 mM potassium hexadecanedioate, 5 mM GSH, 5 mM KCN, carnitine palmityltransferase (1.26 mg of protein), 0.75% (w/v) fatty acid-free albumin, 0.13 M KCl (to obtain maximum activity of the long-chain acyl CoA synthetase (22), and appropriate amounts of human liver subcellular fraction. In some experiments (Table 2) Triton X-100 was added to the incubation medium in an effort to expose any acyl CoA synthetase localized inside the mitochondria (15, 23). The reaction was started by the addition of activating enzyme and was continued for 30 min at 35°C. The incubation was stopped by the addition of 1 ml of 2.4 N HCl. Acylcarnitines were extracted with 1.5 ml of butanol. The butanol phase was washed once with 3 ml of butanol-saturated water. Further washing to remove all contaminating free carnitine would also remove part of the hexadecanedioylcarnitine. Total activity was measured by counting 0.5 ml of the butanol phase. The rest of the butanol phase was taken to dryness, redissolved in methanol, and chromatographed (9) on Kieselgel H (Merck) plates developed in chloroform–methanol–concentrated ammonia–water 50:30:8:2.5 (by vol). In this thin-layer chromatography system, a satisfactory separation is obtained for hexadecanedioylcarnitine (R_F 0.30–0.35) and acylcarnitines formed from endogenous (long-chain monocarboxylic) fatty acids (R_F 0.45–0.55).

All values given are the means of duplicates, which never varied more than 10%.

RESULTS

Influence of different amounts of tissue, carnitine palmityltransferase, and albumin on the rate of hexadecanedioate activation

Fig. 1 shows that maximum stimulation of the hexadecanedioylcarnitine formation was obtained with a transferase content of 0.32–1.26 mg of protein. With amounts of transferase greater than 1.89 mg of protein, a distinct decrease in hexadecanedioylcarnitine formation was seen, probably due to protein binding of substrate (hexadecanedioic acid). Therefore, the amount of transferase used in the experiments was 1.26 mg of protein. Hexadecanedioylcarnitine formation was proportional to the amount of added microsomal protein up to at least 0.23 mg. Similar experiments were performed also with the other subcellular fractions (not shown). The tissue fractionation experiments were performed with amounts of tissue corresponding to the first rectilinear part of the curve; thus, the concentration of carnitine palmityltransferase was never rate limiting in the system.

Albumin was added to the incubation medium to minimize the activation of endogenous fatty acids (22). In experiments with a microsomal fraction as enzyme source, different amounts of hexadecanedioic acid and fatty acid-free albumin were used (**Fig. 2**). With hexadecanedioic acid concentrations of 1–3 mM, hexadecanedioylcarnitine formation was greatest when the molar ratio of hexadecanedioic acid to albumin was 9–14. The highest formation of hexadecanedioylcarnitine was found with 3 mM hexadecanedioic acid. To avoid high concentrations of albumin, however, 1.5 mM hexadecanedioic acid and 0.75% (w/v) of albumin (molar ratio 13.5) was used in the ex-

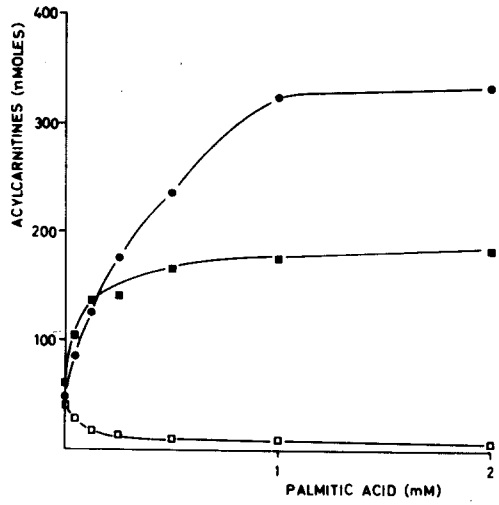

Fig. 3. Acylcarnitine formation by human liver microsomes with increasing amounts of palmitic acid in the absence or presence of hexadecanedioic acid (1 mM). Microsomal fraction from patient 2 (0.18 mg of protein) was used as enzyme source. Other conditions were as described in Materials and Methods. Long-chain monocarboxylic acylcarnitines formed in the absence of hexadecanedioic acid, ●; long-chain monocarboxylic acylcarnitines formed in the presence of hexadecanedioic acid, ■; hexadecanedioylcarnitine formed in the presence of hexadecanedioic acid, □.

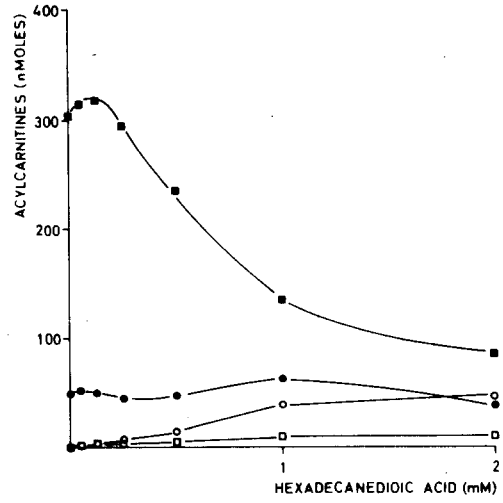

Fig. 4. Acylcarnitine formation by human liver microsomes with increasing amounts of hexadecanedioic acid in the absence or presence of palmitic acid (1 mM). Microsomal fraction from patient 2 (0.18 mg of protein) was used as enzyme source. Other conditions were as described in Materials and Methods. Long-chain monocarboxylic acylcarnitines formed in the absence of added palmitic acid, ●; long-chain monocarboxylic acylcarnitines formed in the presence of added palmitic acid, ■; hexadecanedioylcarnitine formed in the absence of added palmitic acid, ○; hexadecanedioylcarnitine formed in the presence of added palmitic acid, □.

periments. This gave a hexadecanedioylcarnitine formation that was about 25% lower than the maximum.

The optimal concentrations of ATP, Mg^{2+}, CoA, and carnitine were found to be the same as in the rat liver experiments (10).

Activation experiments with hexadecanedioic and palmitic acids added together as substrates

As shown in **Fig. 3,** increasing amounts of long-chain monocarboxylic acylcarnitines were formed when increasing amounts of palmitic acid were added as substrate, both in the absence and presence of hexadecanedioic acid (1 mM). It can be seen that with up to 0.13 mM palmitic acid the formation of long-chain monocarboxylic acylcarnitines was largest when hexadecanedioic acid was present. This may have been due to an exchange of this acid with endogenous monocarboxylic fatty acids bound to proteins. With 0.25 mM or more of palmitic acid, smaller amounts of long-chain monocarboxylic acylcarnitines were formed when hexadecanedioic acid was present. Moreover, when hexadecanedioic acid was present and increasing amounts of palmitic acid were added, decreasing amounts of hexadecanedioylcarnitine were formed. These findings may be due to a competition between the two substrates for the same enzyme(s).

As shown in **Fig. 4,** increasing amounts of hexadecanedioylcarnitine were formed when increasing amounts of hexadecanedioic acid were added (with no added palmitate). The formation of carnitine esters of endogenous fatty acids was practically unchanged. Also in the presence of 1 mM palmitate the formation of hexadecanedioylcarnitine increased with increasing concentrations of hexadecanedioic acid. The added palmitate, however, strongly reduced hexadecanedioylcarnitine formation. The formation of long-chain monocarboxylic acylcarnitine remained high and practically unchanged until the hexadecanedioic acid concentration exceeded 0.25 mM; it then decreased. These findings may support the suggestion that the two substrates compete for the same enzyme(s).

Activity and distribution of marker enzymes in subcellular fractions of human liver

Table 1 shows the activity of various enzymes in human liver. Palmitate activation was about half of that found in rat liver (15, 24, 25) and corresponded well to the values found by Farstad, Aas, and Sander for human liver (26). The activities of glucose-6-phosphatase and acid phosphatase were also about half of those in rat liver (15, 21, 23, 25), while the activity of monoamine oxidase was about two-thirds of that found in human liver by Farstad et al. (26) and in rat liver by Aas (15).

Table 1 further shows that the activities of monoamine oxidase, carnitine palmityltransferase, acid phosphatase, glucose-6-phosphatase, and long-chain acyl CoA synthetase were distributed in the same manner as in rat liver subfractions (12, 13, 15, 21, 23–25). This suggests that these enzymes probably have the same subcellular localization in human liver as in rat liver. They may therefore be used as marker enzymes for subcellular particles in fractionation studies of both human and rat liver.

TABLE 1. Hexadecanedioate activation and the activity of some marker enzymes in subcellular fractions of human liver.

"Enzyme"	Absolute Values for Total Homogenate	Percentage Values						
		N + E	N	M	L	P	S	Recovery
Patient 1. Hexadecanedioate activation	0.41	100	2.9	18.4	9.6	36.2	14.0	81.1
Palmitate activation	5.8	100	2.4	17.9	12.1	39.7	11.8	83.9
Carnitine palmityltransferase		100	3.0	49.8	13.2	10.7	11.0	87.7
Monoamine oxidase	0.54	100	2.4	40.2	12.2	25.3	7.4	87.5
Acid phosphatase	1.6	100	0.0	26.4	25.8	27.5	7.0	86.7
Glucose-6-phosphatase	6.2	100	0.6	12.6	15.7	34.4	0.0	63.3
Protein	102.5	100	4.6	17.3	7.1	19.3	38.2	86.5
Patient 2. Hexadecanedioate activation	0.56	100	3.9	21.5	9.8	49.2	12.6	97.0
Palmitate activation	6.3	100	3.0	14.6	9.0	44.6	10.5	81.7
Carnitine palmityltransferase		100	7.7	44.3	13.8	13.3	17.3	96.4
Monoamine oxidase	0.59	100	6.3	39.6	12.1	34.4	2.9	95.3
Acid phosphatase	2.4	100	3.6	20.1	27.2	27.6	8.2	86.7
Glucose-6-phosphatase	6.0	100	0.6	1.2	6.9	55.0	0.0	63.7
Protein	156.0	100	8.1	12.7	4.9	22.6	42.9	91.2

Enzyme assays were performed as described in Materials and Methods. Absolute values for total homogenate (N + E) are expressed in μmoles/min/g wet wt of tissue except for protein, which is given in mg/g wet wt of tissue. E, cytoplasmic extract; N, nuclear fraction; M, mitochondrial fraction; L, lysosomal fraction; P, microsomal fraction; S, particle-free supernate.

Subcellular distribution of hexadecanedioic acid activation in human liver

Table 1 also shows the subcellular distribution of the activation of hexadecanedioic acid in human liver in relation to marker enzymes for subcellular particles. The same distribution of enzyme activities was found in both patients. The distribution of hexadecanedioic acid activation is almost identical with that of palmitic acid activation, i.e., it is localized in both the mitochondrial and microsomal fractions.

The activation capacity for hexadecanedioic acid in the total homogenate of human liver was found to be 0.5 μmole/min/g wet wt of tissue, i.e., about 10% of that found by Farstad et al. for palmitic acid (26).

Table 2 shows that addition of Triton X-100 to the incubation medium did not increase the activation capacity of the mitochondrial fraction for either palmitate or hexadecanedioate. Thus, the enzyme(s) activating hexadecanedioic and palmitic acids appears to be localized outside the inner mitochondrial compartment.

DISCUSSION

In contrast to the current procedure, methods based on CoA disappearance or hydroxamate formation do not differentiate between the activation of mono- and dicarboxylic acids. This is of importance because there is an increased activation of endogenous fatty acids when hexadecanedioic acid is added to the incubation mixture. With the current procedure we have recently (10) demonstrated the formation of carnitine esters of long-chain dicarboxylic acids in rat liver subcellular fractions. In those studies the absolute requirement for both CoA and ATP-Mg^{2+} showed that the CoA ester of hexadecanedioic acid was an intermediate in the formation of hexadecanedioylcarnitine (10). For reasons given previously (10), the formation of hexadecanedioylcarnitine in our assay system can be used to determine the rate of activation of hexadecanedioic acid.

The acyl CoA synthetase(s) that activates long-chain monocarboxylic acids is localized in the mitochondrial and microsomal fractions in both rats (15, 21, 24, 25) and humans (26), and the mitochondrial enzyme(s) is localized in the outer membrane (15, 27). We have previously shown that the activation of hexadecanedioic acid occurred in the mitochondrial and microsomal fractions in rat liver, with a distribution identical with that of palmitic acid activation (10). The mitochondrial activating enzyme(s) seemed to be localized outside the inner mitochondrial compartment (9). Other results (10) indicated that hexadecanedioic and palmitic acids appeared to compete for the same enzyme(s).

The results of the present study with human liver are similar to those obtained previously with rat liver (9, 10). The activation capacity in the total homogenate of human liver for hexadecanedioic acid (0.5 μmole/min/g wet wt of tissue) was less than in rat liver (1.0 μmole/min/g wet wt of tissue) (10). In livers from both rats (10) and humans, the activation capacity for hexadecanedioate was about 10% of that for palmitate. The hexadecanedioic acid activation in human liver was localized in the mitochondrial and microsomal fractions. A relatively large amount of the activation took place in the microsomal fraction. This may be partly artificial because the fibrosis of the livers required a rather severe homogenization procedure. Thus, mitochondrial outer membrane may have been stripped off during the homogenization procedure and sedimented with the microsomal fraction. Monoamine oxidase, which in rat liver (28) and bovine heart (29) is a marker enzyme for mitochondrial outer membrane, was in both the mito-

TABLE 2. Effect of Triton X-100 on the activation of hexadecanedioic acid in human liver mitochondria

Substrate	Addition	Reaction Product: Hexadecanedioyl-carnitine	Reaction Product: Long-chain Monocarboxylic Acyl-carnitine
		nmoles	
Hexadecanedioic acid (1.5 mM)	None	16.6	23.8
Hexadecanedioic acid (1.5 mM)	Triton X-100 (0.5%, w/v)	16.1	22.9
Palmitic acid (0.5 mM)	None		50.5
Palmitic acid (0.5 mM)	Triton X-100 (0.5%, w/v)		47.5

Incubation conditions were as described in Materials and Methods. Freshly prepared mitochondrial fraction (patient 2) from 5 mg wet wt of tissue (0.1 mg of protein) was used as enzyme source.

chondrial and microsomal fractions, while carnitine palmityltransferase, which is a marker for the inner mitochondrial membrane (13, 27), was limited almost exclusively to the mitochondrial fraction.

The subcellular distribution of hexadecanedioate activation was almost identical with that of palmitate activation. Furthermore, the results shown in Figs. 3 and 4 suggest that palmitic acid and hexadecanedioic acid compete for the same enzyme(s). However, the endogenous long-chain monocarboxylic acids would be expected to compete with added palmitic or hexadecanedioic acid for binding sites on albumin and on the acyl CoA synthetase(s). Therefore, it is very difficult by kinetic studies to conclusively establish whether or not hexadecanedioic acid is activated by long-chain acyl CoA synthetase (EC 6.2.1.3). In conclusion, we suggest that palmitic and hexadecanedioic acids may be activated by the same enzyme(s). Purified enzyme preparations would be needed to prove this.

The authors are indebted to Professor J. Bremer for helpful discussions. The biopsy material was kindly supplied by Surgical Dept. B, Rikshospitalet, Oslo. The microscopic examinations were done at the Institute of General and Experimental Pathology, Rikshospitalet, Oslo. The technical assistance of Mrs. Kari Frogner and Miss Guri Bjerkholt is gratefully acknowledged.

Manuscript received 19 October 1973 and in revised form 29 March 1974; accepted 30 May 1974.

REFERENCES

1. Pettersen, J. E., E. Jellum, and L. Eldjarn. 1971. Short chain dicarboxylic acids in human urine accompanying ketosis. *Scand. J. Clin. Lab. Invest.* **27(Suppl. 118):** 69. (Abstr.)
2. Pettersen, J. E., E. Jellum, and L. Eldjarn. 1972. The occurrence of adipic and suberic acid in urine from ketotic patients. *Clin. Chim. Acta.* **38:** 17–24.
3. Pettersen, J. E. 1972. Formation of *n*-hexanedioic acid from hexadecanoic acid by an initial ω oxidation in ketotic rats. *Clin. Chim. Acta.* **41:** 231–237.
4. Tryding, N. 1957. Intestinal absorption and metabolism of 2,2-dimethylstearic acid in man. *Ark. Kemi.* **11:** 307–312.
5. Tryding, N., and G. Westöö. 1957. On the metabolism of 2,2-dimethylnonadecanoic acid in man. *Ark. Kemi.* **11:** 313–316.
6. Emmrich, R., and I. Emmrich-Glaser. 1940. Stoffwechselversuche mit höheren geradzahligen gesättigten Dicarbonsäuren. *Hoppe-Seyler's Z. Physiol. Chem.* **266:** 183–192.
7. Verkade, P. E., J. van der Lee, and A. J. S. van Alphen. 1937. Untersuchungen über den Fettstoffwechsel. VIII. Fütterungsversuche an Hunde mit den Natriumsalzen normaler gesättigter Dicarbonsäuren. *Hoppe-Seyler's Z. Physiol. Chem.* **250:** 47–56.
8. Bernhard, K., and M. Andreae. 1937. Stoffwechselversuche mit Dicarbonsauren. *Hoppe-Seyler's Z. Physiol. Chem.* **245:** 103–106.
9. Pettersen, J. E. 1973. In vitro studies on the metabolism of hexadecanedioic acid and its mono-L-carnitine ester. *Biochim. Biophys. Acta.* **306:** 1–14.
10. Pettersen, J. E., and M. Aas. 1973. ATP-dependent activation of dicarboxylic acids in rat liver. *Biochim. Biophys. Acta.* **326:** 305–313.
11. Aas, M. 1972. Inactivation and protection of long-chain acyl-CoA synthetase. *Abstr. Commun. Mtg. FEBS.* **8:** Abstr. 1117.
12. de Duve, C., B. C. Pressman, R. Gianetto, R. Wattiaux, and F. Appelmans. 1955. Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue. *Biochem. J.* **60:** 604–617.
13. Norum, K. R., and J. Bremer. 1967. The localization of acyl coenzyme A–carnitine acyltransferase in rat liver cells. *J. Biol. Chem.* **242:** 407–411.
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
15. Aas, M. 1971. Organ and subcellular distribution of fatty acid activating enzymes in the rat. *Biochim. Biophys. Acta.* **231:** 32–47.
16. McCaman, R. E., M. W. McCaman, J. M. Hunt, and M. S. Smith. 1965. Microdetermination of monoamine oxidase and 5-hydroxytryptophan decarboxylase activities in nervous tissues. *J. Neurochem.* **12:** 15–23.
17. Wattiaux, R., and C. de Duve. 1956. Tissue fractionation studies. 7. Release of bound hydrolase by means of Triton X-100. *Biochem. J.* **63:** 606–608.
18. Bremer, J. 1962. Carnitine in intermediary metabolism. Reversible acetylation of carnitine by mitochondria. *J. Biol. Chem.* **237:** 2228–2231.
19. Norum, K. R. 1964. Palmityl-CoA:carnitine palmityltransferase. Purification from calf-liver mitochondria and some properties of the enzyme. *Biochim. Biophys. Acta.* **89:** 95–108.
20. Bremer, J., and K. R. Norum. 1967. The effects of detergents on palmityl coenzyme A:carnitine palmityltransferase. *J. Biol. Chem.* **242:** 1749–1755.
21. Farstad, M., J. Bremer, and K. R. Norum. 1967. Long-chain acyl-CoA synthetase in rat liver. A new assay procedure for the enzyme, and studies on its intracellular localization. *Biochim. Biophys. Acta.* **132:** 492–502.
22. Aas, M. 1970. Long-chain acyl-CoA synthetase in rat liver. Stimulation by high salt concentrations. *Biochim. Biophys. Acta.* **202:** 250–258.

23. Aas, M., and J. Bremer. 1968. Short-chain fatty acid activation in rat liver. A new assay procedure for the enzymes and studies on their intracellular localization. *Biochim. Biophys. Acta.* **164:** 157–166.
24. Lippel, K., J. Robinson, and E. G. Trams. 1970. Intracellular distribution of palmityl-CoA synthetase in rat liver. *Biochim. Biophys. Acta.* **206:** 173–177.
25. van Tol, A., and W. C. Hülsmann. 1969. The localization of palmitoyl-CoA:carnitine palmitoyltransferase in rat liver. *Biochim. Biophys. Acta.* **189:** 342–353.
26. Farstad, M., M. Aas, and J. Sander. 1973. Long-chain acyl-CoA synthetase activity in blood platelets and some human tissues. *Scand. J. Clin. Lab. Invest.* **31:** 205–211.
27. Norum, K. R., M. Farstad, and J. Bremer. 1966. The submitochondrial distribution of acid:CoA ligase (AMP) and palmityl-CoA:carnitine palmityltransferase in rat liver mitochondria. *Biochem. Biophys. Res. Commun.* **24:** 797–804.
28. Schnaitman, C., V. G. Erwin, and J. W. Greenawalt. 1967. The submitochondrial localization of monoamine oxidase. An enzymatic marker for the outer membrane of rat liver mitochondria, *J. Cell Biol.* **32:** 719–735.
29. Racker, E., and H. Proctor. 1970. Reconstitution of the outer mitochondrial membrane with monoamine oxidase. *Biochem. Biophys. Res. Commun.* **39:** 1120–1125.