Identical subcellular distribution of palmitoyl-CoA and arachidonoyl-CoA synthetase activities in human blood platelets

Anne M. BAKKÉN and Mikael FARSTAD

Laboratory of Clinical Biochemistry, University of Bergen, University Hospital Haukeland, N-5021 Bergen, Norway

Fractionation of human blood platelets showed that palmitoyl-CoA synthetase and arachidonoyl-CoA synthetase activities had an identical distribution among subcellular fractions. The activity was highest with arachidonic acid as substrate in all fractions, with an enzyme activity of 50 nmol/min per mg of protein, in a 'dense-tubular-system'-enriched fraction. The ratio activities with arachidonate and palmitate as substrates was about 1.5 in all fractions. Heat inactivation did not distinguish between arachidonoyl-CoA synthetase and a palmitoyl-CoA synthetase. On the other hand, heat inactivation indicated two pools of long-chain acyl-CoA synthetases: one in a mitochondria- and one in the dense-tubular-system-enriched fraction.

INTRODUCTION

Formation of CoA esters is the initial step in all known metabolic pathways of fatty acids, with the exception of prostanoid synthesis from arachidonic acid and other prostanoid precursors. Incorporation of palmitate into platelet lipids was described by Deykin & Dessler (1968) and by Andreoli (1968), and later other metabolic pathways were described (Cohen & Wittels, 1970; Cohen *et al.*, 1970; Doery *et al.*, 1970; Spector *et al.*, 1970).

Palmitoyl-CoA synthetase activity in platelets has been studied in several publications from this laboratory (Farstad & Sander, 1971; Farstad *et al.*, 1973; Vollset & Farstad, 1979; Berge *et al.*, 1980), and a bimodal localization has been demonstrated. Wilson *et al.* (1982) also described an arachidonoyl-CoA synthetase activity in platelets. Laposata *et al.* (1985) suggested that this was an enzyme specific for arachidonic acid and other prostanoid precursors. They found that arachidonoyl-CoA synthetase activity was more susceptible to heat inactivation than was oleoyl-CoA synthetase activity, that eicosatrienoic acid, but not oleic acid, competitively inhibited the formation of arachidonoyl-CoA, and that proteins with arachidonoyl-CoA synthetase activity and oleoyl-CoA synthetase activity could be separated by chromatography on hydroxyapatite.

We now report studies of the total activity and subcellular distribution of acyl-CoA synthetase activity in human blood platelets using fatty acids from dodecanoate (laurate) to docosahexaenoate as substrates. The results indicated identical subcellular distribution of both palmitoyl-CoA and arachidonoyl-CoA synthetase activities, and a constant ratio of the palmitate/arachidonate activation in the various subcellular fractions. Inactivation studies also indicated different stability of enzyme pools in the mitochondria and in the dense-tubular-system-enriched fraction. The activity was very high in a dense-tubular-system-enriched fraction, and the specific activities with palmitate and arachidonate as substrates were 35 and 50 nmol/min per mg of protein respectively.

MATERIALS AND METHODS

Reagents

[1-¹⁴C]Dodecanoic acid (sp. radioactivity 36.3 mCi/mmol), [1-¹⁴C]palmitic acid (sp. radioactivity 58 mCi/mmol), [1-¹⁴C]stearic acid (sp. radioactivity 57 mCi/mmol), [1-¹⁴C]oleic acid (sp. radioactivity 56.7 mCi/mmol), [1-¹⁴C]linoleic acid, (sp. radioactivity 58 mCi/mmol), [1-¹⁴C]linolenic acid (octadeca-9,12,15-trienoic acid; sp. radioactivity 56.2 mCi/mmol), [1-¹⁴C]arachidonic acid (sp. radioactivity 60.1 mCi/mmol), [1-¹⁴C]eicosa-5,8,11,14,17-pentaenoic acid (sp. radioactivity 55 mCi/mmol) were purchased from Amersham International (Amersham, Bucks., U.K.). [1-¹⁴C]Docosa-4,7,10,13,16,19-hexaenoic acid (sp. radioactivity 55 mCi/mmol) and [1-¹⁴C]tyramine (*p*-hydroxyphenethylamine) hydrochloride (sp. radioactivity 44 mCi/mmol) were purchased from New England Nuclear (Boston, MA, U.S.A.). [1-¹⁴C]Myristic acid (sp. radioactivity 55 mCi/mmol) was purchased from CEA (Commissariat à l'Energie Atomique) (Saclay, France). Scint-Hei 3 from Koch-Light Laboratories (Colnbrook, Slough, Berks., U.K.) was used as the scintillator. ATP, CoA, fatty acids (of the same length and saturation as their radioactive counterparts), dithiothreitol, Triton X-100, 5-hydroxytryptamine ('serotonin'), EDTA and Hepes were purchased from Sigma (St. Louis, MO, U.S.A.). Unlabelled docosa-4,7,10,13,16,19-hexaenoic acid was from Aldrich, Steinheim, Germany. All other chemicals were of the highest purity commercially available.

Preparation of platelet lysates and subfractions

A 450 ml sample of human blood was obtained in plastic bags containing 63 ml of ACD solution (100 ml of ACD solution contains 327 mg of citric acid monohydrate, 2.63 g of sodium citrate dihydrate, 222 mg of monobasic sodium phosphate monohydrate, 2.90 g of anhydrous dextrose and 27.5 mg of adenine) from the Blood Bank at this Hospital. The blood donors were healthy volunteers claiming to have omitted all medications 8 days before the blood donation. A 3 ml

portion of 77 mM-EDTA, pH 7.4, was added to 37 ml of ACD-treated blood before centrifugation to make the resuspension of the platelets easier (Holmsen *et al.*, 1977). The blood was centrifuged at 2000 $g_{\max}$ at 18 °C for 6 min in a HB-4 rotor of a Sorvall type RC 5B. centrifuge. Platelet-rich plasma (PRP) was pipetted off and centrifuged at 4250 $g_{\max}$ at 4 °C for 15 min. The platelet-free plasma was discarded, and the isolated platelets were washed once in 130 mM-NaCl/12 mM-Hepes/2 mM-EDTA, pH 7.4.

The washed platelets were contaminated with a few erythrocytes, which were removed by specific haemolysis as described by Holmsen *et al.* (1977): 1.5 vol. of ice-cold water were added to 1 vol. of platelet suspension; 2 min thereafter the suspension was made iso-osmotic by adding 0.41 vol. of 0.7 M-NaCl. The platelets were isolated by centrifugation and resuspended in a buffer containing 25 mM-Tris/HCl/112 mM-KCl/3 mM-ATP/3 mM-MgCl₂, pH 7.4 (osmolality 275 mosmol/kg). The final suspension contained about 4×10^9 platelets/ml (120×10^9 platelets in 30 ml) from two donors. The suspension was homogenized by equilibration with nitrogen at 4.14 MPa (600 lbf/in²) for 15–20 min at 0–4 °C before explosive decompression. The whole homogenate was centrifuged at 1000 $g_{\max}$ at 4 °C for 22 min to remove unbroken cells. The supernatant, i.e. the cytoplasmic extract or 'E' fraction, was centrifuged at 12000 $g_{\max}$ at 4 °C for 20 min. The pellet was resuspended in the Tris/HCl buffer and was used as the mitochondrial or 'M' fraction. The pellet was washed once, and the combined supernatants were centrifuged at 24000 $g_{\max}$ for 20 min at 4 °C in a SW 41 rotor in a Beckman preparative ultracentrifuge (model L8-70). The pellet was used as the light mitochondrial fraction or 'L' fraction and resuspended in the same buffer. During this centrifugation procedure, dense granules and α -granules mostly sediment with the 'M' fraction, and the lysosomes sediment with the 'L' fraction (Gogstad, 1980). The supernatant was centri-

fuged in the same rotor at 100000 $g_{\max}$ for 1 h at 4 °C. The pellet, which contained the 'dense tubular system' and other membrane fractions, was used as the 'P' fraction. The final supernatant was the particle-free fraction or the 'S' fraction.

Protein determination

Protein concentration was determined in all fractions by a Bradford (1976) method, with bovine serum albumin as standard.

Subcellular markers

Amine oxidase (EC 1.4.3.4) was assayed as described by Aas (1971); carnitine palmitoyltransferase (EC 2.3.1.21) as described by Bird *et al.* (1985); NADH dehydrogenase (EC 1.6.99.3) by a procedure modified from that of Wallach & Kamat (1966) and as described by Record *et al.* (1982); lactate dehydrogenase (EC 1.1.1.27) as described by the Scandinavian Committee on Enzymes (1981). Acyl-CoA synthetase (EC 6.2.1.3) was assayed by measuring the formation of [1-¹⁴C]acyl-CoA from 1-¹⁴C-labelled fatty acid; CoA and ATP as previously described by Bar-Tana *et al.* (1971) and A. M. Bakken, M. Farstad & H. Holmsen (unpublished work) respectively. Briefly, the reaction mixture contained 50 mM-Hepes, pH 7.4, 10 mM-MgCl₂, 5 mM-dithiothreitol, 70 μ M-CoA, 2 mM-ATP, 0.1 % Triton X-100 (v/v), 150 μ M-1-¹⁴C-labelled fatty acid and $(50-100) \times 10^6$ platelets in a final volume of 250 μ l. The incubation time was 3 min at 37 °C. 5-Hydroxytryptamine was assayed as described by Ingebretsen *et al.* (1985).

Optimal conditions for acyl-CoA synthetase activity with different fatty acids as substrates

Recently we (A. M. Bakken, M. Farstad & H. Holmsen, unpublished work) determined the apparent K_m values for CoA, ATP and 12 fatty acids from octanoate to docosahexaenoate. Except for octanoate

Table 1. Total activities and distribution of acyl-CoA synthetase with various fatty acids as substrate, and some marker enzymes, in fractions isolated by differential centrifugation of human platelet homogenate from two 0.5 litre portions of blood from two donors

The enzyme activities and the protein content in the fractions are expressed as a percentage of the total in the E fraction (i.e. cytoplasmic extract). M, 12000 g fraction; L, 24000 g fraction; P, 100000 g fraction; S, final particle-free supernatant.

Enzyme	Total activity (nmol/min)	Distribution (%) in fraction:				Recovery (%)
		M	L	P	S	
Dodecanoyl-CoA-synthetase	383	43	17	16	29	105
Myristoyl-CoA synthetase	598	41	17	17	30	105
Palmitoyl-CoA synthetase	656	43	16	15	31	105
Stearoyl-CoA synthetase	538	40	17	16	22	95
Oleoyl-CoA synthetase	604	40	16	15	25	96
Linoleoyl-CoA synthetase	588	44	13	12	33	102
Linolenoyl-CoA synthetase	743	38	14	12	36	100
Arachidonoyl-CoA synthetase	1190	40	15	12	35	102
Eicosapentaenoyl-CoA synthetase	1100	39	13	11	35	98
Docosahexaenoyl-CoA synthetase	675	42	18	15	41	116
Amine oxidase	35	58	31	9	0	98
Carnitine palmitoyltransferase	115	51	23	8	14	96
NADH dehydrogenase	16933	27	17	19	29	93
Lactate dehydrogenase	348 200	5	1	1	96	103
[Protein (mg)]	105	12	4	3	78	97]

Fig. 1. Relative specific activities of marker enzymes, carnitine palmitoyltransferase (CPT) and amine oxidase (MAO) for mitochondria, NADH dehydrogenase (NADH DH) for the 'dense tubular system', and lactate dehydrogenase (LDH) for the particle-free supernatant, and of acyl-CoA synthetase activity with palmitate (C_{16:0}), linoleate (C_{18:2}), arachidonate (C_{20:4}) and docosahexaenoate (C_{22:6}) as substrates

M is the mitochondrial or 12000 g fraction, L is the light mitochondrial or 24000 g fraction, P is the dense-tubular-system-enriched or 100000 g fraction, and S is the particle-free fraction. Incubation conditions were as described in the Materials and methods section with all substrates for acyl-CoA synthetase activity at 8–10 times the apparent K_m values.

and decanoate, apparent K_m values for all saturated and unsaturated fatty acids were between 11 and 20 $\mu\text{mol/l}$. For CoA, apparent K_m values were between 4 and 9 $\mu\text{mol/l}$ for all acids from myristate to docosahexaenoate. For ATP, apparent K_m values were between 130 and 200 $\mu\text{mol/l}$ for saturated fatty acids from dodecanoate to stearate, and between 55 and 96 $\mu\text{mol/l}$ for the unsaturated acids. In the present study, all assays were performed at concentrations of all three substrates at 8–10 times the apparent K_m values.

RESULTS

Activity and subcellular distribution of acyl-CoA synthetase(s) in human blood platelets

Table 1 and Fig. 1 show the results of a typical experiment. The total activity and the subcellular distribution of acyl-CoA synthetase are shown compared with marker enzymes in fractions obtained by differential centrifugation of a platelet homogenate. The recovery of protein and enzymes was 93–116% (Table 1).

Fig. 1 shows the distribution of marker enzymes, amine oxidase and carnitine palmitoyltransferase for the mitochondria (in the M and L fractions), NADH dehydrogenase for the dense tubular system (in the P fraction), lactate dehydrogenase for the particle-free supernatant (the S fraction) and the subcellular distribution of acyl-CoA synthetase activity with palmitate, linoleate, arachidonate and docosahexaenoate as substrates. The distribution of NADH dehydrogenase and lactate dehydrogenase indicate that the M fraction to some extent was contaminated with whole platelets. The L fraction therefore seemed to be a more pure mitochondrial fraction than the M fraction. Also the P fraction and the L fraction were to some degree mutually contaminated. These results are, however, in agreement with most attempts to isolate subcellular fractions of human blood platelets (Gerrard *et al.*, 1976; Fukami *et al.*, 1978; Berge *et al.*, 1980; Gogstad, 1980; Mauco *et al.*, 1984).

Dense granules, α -granules and lysosomes co-precipitated with the M and L fractions, since the latter were precipitated at a centrifugal force of 24000 g_{max} ; 18% of the 5-hydroxytryptamine was found in the M fraction, 2% in the L fraction, 1% in the P fraction and

Fig. 2. Mean specific activities of acyl-CoA synthetases in isolated fractions of human blood platelets from four healthy blood donors

The various fractions were as described in the legend to Fig. 1. All substrates were used at 8–10 times the apparent K_m values. For details, see the Materials and methods section.

84% in the S fraction. Although this indicates a considerable disruption of dense granules, the intact ones sedimented with the M fraction. However, as the vast majority of acyl-CoA synthetase activity is found in the mitochondria and in the endoplasmic reticulum in all cells investigated (Farstad *et al.*, 1967, 1973; Aas 1971; Groot *et al.*, 1976), the presence of other granules in the M and L fractions was considered of less importance.

The distribution of acyl-CoA synthetase activity (Table 1 and Fig. 1) was very much the same with either of the substrates tested. This indicates that enzyme activity with near identical specificity is distributed both in the mitochondria and the dense tubular system. Similar results were also found with dodecanoate, myristate, stearate, oleate, linolenate and eicosapentaenoate (Table 1).

Fig. 2 shows that the mean acyl-CoA synthetase specific activity in all subcellular fractions was very much higher than reported for a partially purified arachidonoyl-CoA synthetase (Laposata *et al.*, 1985). Whereas they obtained a maximal specific activity of about 7 nmol/min per mg of protein after hydroxyapatite chromatography of repeatedly sonicated platelet membrane fractions, a peak activity of about 50 nmol/min per mg of protein was observed in the P fraction in the present study. The values are mean values from two experiments, each consisting of pooled platelets from two donors. The differences between means were less than $\pm 10\%$ for all acids in all fractions. Fig. 2 also shows that the pattern of activity within the different fatty acids was almost identical in the various fractions. The relative activity of arachidonate to palmitate was about 1.5 in all fractions.

Studies of the stability of acyl-CoA synthetase activity in homogenates of human platelets

Acyl-CoA synthetases are labile enzymes and very susceptible to inactivation by sonication (Norum *et al.*, 1966; Tanaka *et al.*, 1979). Arachidonoyl-CoA synthetase activity has been reported to be very sensitive to heat inactivation (Wilson *et al.*, 1982). Homogenates of human platelets were therefore sonicated for different periods and at various power settings. Even when the sample tube was soaked in an ice bath, sonication for 2×30 s at $26 \mu\text{m}$ (full-scale effect $30 \mu\text{m}$) resulted in more than 50% inactivation, and repeated sonication resulted in further inactivation. This is in agreement with the results obtained by Norum *et al.* (1966).

Incubation of platelet homogenates at 45°C resulted in rapid inactivation of the acyl-CoA synthetase activity (Fig. 3). Wilson *et al.* (1982) found an almost selective inactivation of the arachidonoyl-CoA synthetase activity. Fig. 3 shows, however, similar inactivation of the acyl-CoA synthetase activity with either palmitate or arachidonate as substrates. After 30 min, the remaining activity was the same with both substrates. When 3 mM-ATP was added to the incubation medium, inactivation was retarded. Under these conditions the activity with arachidonate was better preserved initially, but after 30 min the remaining activities were the same.

Fig. 3 indicates that platelet homogenates contain pools of acyl-CoA synthetase with different resistances towards 45°C heat inactivation. One pool (Fig. 3b) was inactivated with a half-time ($t_{1/2}$) of 1.5 min with either palmitate or arachidonate as substrate, and one pool with a $t_{1/2}$ of 8.75 min with palmitate and 10.5 min with arachidonate as substrate. In the presence of 3 mM-ATP

Fig. 3. Palmitoyl-CoA synthetase (circles) and arachidonoyl-CoA synthetase (squares) activities in whole homogenates of blood platelets after incubation at 45°C for up to 30 min in the presence or absence of 3 mM-ATP

(a) Activity after incubation in the presence of ATP; (b) activity in the absence of added ATP. After the inactivation, all assays were performed at concentrations of all substrates 8–10 times the apparent K_m values as described in the Materials and methods section. Activities of palmitoyl-CoA synthetase (○, ●) and arachidonoyl-CoA synthetase (□, ■) in the presence (○, □) or in the absence (●, ■) of ATP. An arachidonoyl-CoA synthetase activity of 100% in this experiment was 24 nmol/min per 10^9 platelets and for palmitoyl-CoA synthetase activity it was 14 nmol/min per 10^9 platelets.

(Fig. 3a), $t_{1/2}$ values were increased to respectively 3.5 and 2 min for the 'fast' pool, and 10.5 and 12 min for the 'slow' pool.

When L and P fractions were incubated under the same conditions, the acyl-CoA synthetase activity in the L fraction (mitochondria-enriched fraction) was more rapidly inactivated than the P fraction (dense-tubular-system-enriched fraction) with either palmitate or arachidonate as substrates (Fig. 4). After 10 min of inactivation, the arachidonoyl-CoA synthetase activity (Fig. 4b) was about 10% more inactivated than the palmitoyl-CoA synthetase activity (Fig. 4a), in both the L and P fractions in the presence of 0.5 mM-ATP. When 3.5 mM-ATP was present, inactivation was slightly more counteracted in the L fraction than in the P fraction. These observations are in agreement with the results of Tanaka *et al.* (1979), who found that the mitochondrial acyl-CoA synthetase was very unstable in crude preparations.

DISCUSSION

The present results confirm the bimodal localization of acyl-CoA synthetase activity in blood platelets (Farstad *et al.*, 1973; Berge *et al.*, 1980). The activity was, however, more than six times the activity of arachidonoyl-CoA synthetase reported by Wilson *et al.* (1982) with either arachidonate or eicosapentaenoate as substrates. In an isolated dense-tubular-system-enriched fraction (the P fraction) the activity with arachidonate as substrate was

Fig. 4. Palmitoyl-CoA synthetase and arachidonoyl-CoA synthetase activities in isolated L and P fractions after incubation of the fractions at 45 °C in the presence of 0.5 mM-ATP (closed symbols) and 3.5 mM-ATP (open symbols) for up to 10 min

(a) Palmitoyl-CoA synthetase activity; (b) arachidonoyl-CoA synthetase activity. ○ and ● represent P fraction, and △ and ▲ represent L fraction. After inactivation, enzyme assays were performed with all substrates at 8–10 times the apparent K_m values as described in the Materials and methods section. An arachidonoyl-CoA activity of 100% was in this experiment 30 nmol/min per mg of protein in the P fraction and 24 nmol/min per mg of protein in the L fraction. A palmitoyl-CoA activity of 100% was respectively 23 and 16 nmol/min per mg of protein.

acid and eicosapentaenoic acid, but not by oleic acid; (2) selective heat inactivation of the arachidonoyl-CoA synthetase activity; and (3) separation of proteins with oleoyl-CoA or arachidonoyl-CoA synthetase activity by hydroxyapatite chromatography of twice-homogenized platelet membranes.

Some of the present results are not in agreement with the findings of Wilson *et al.* (1982) and Laposata *et al.* (1985). Whereas Wilson *et al.* (1982) found an apparent K_m for CoA of 130 $\mu\text{mol/l}$, we found an apparent K_m for CoA of about 7 $\mu\text{mol/l}$, both with palmitate and arachidonate as well as other long-chain acids as the fatty acid substrates (A. M. Bakken, M. Farstad & H. Holmsen, unpublished work). Our values are in fair agreement with actually measured CoA concentrations of about 10 $\mu\text{mol/l}$ in human platelet homogenates (Ingebretsen *et al.*, 1982).

Wilson *et al.* (1982) used 100 μmol of competing fatty acids/l in their competition experiments, with an apparent K_m of 30 $\mu\text{mol/l}$ for arachidonic acid, whereas we used concentrations of competing acids close to the apparent K_m for the actual fatty acid (A. M. Bakken, M. Farstad & H. Holmsen, unpublished work). By using palmitate instead of oleate, our competitive-inhibition experiments (A. M. Bakken, M. Farstad & H. Holmsen, unpublished work) were in fair agreement with Wilson *et al.* (1982). However, in our experiments eicosapentaenoic and docosahexaenoic acids also inhibited palmitate activation (A. M. Bakken, M. Farstad & H. Holmsen, unpublished work). Wilson *et al.* (1982) reported different sensitivity to heat inactivation at 45 °C with a $t_{1/2}$ of 8 min for arachidonoyl-CoA synthetase and 31.5 min for oleoyl-CoA synthetase. In the present investigation heat inactivation at 45 °C resulted in almost identical $t_{1/2}$ values for both synthetase activities. In the presence of 3 mM-ATP, the initial inactivation was increased slightly more for arachidonoyl-CoA than for palmitoyl-CoA synthetase activity. However, after 30 min inactivation the remaining activities with both these substrates were almost equal, in either the presence or absence of ATP.

Laposata *et al.* (1985) separated proteins with oleoyl-CoA or arachidonoyl-CoA synthetase activities by hydroxyapatite from repeatedly sonicated platelet membrane fractions. As acyl-CoA synthetase activity is sensitive to inactivation by sonication (Norum *et al.*, 1966; the present investigation), the results of Laposata *et al.* (1985) are difficult to interpret. The acyl-CoA synthetase activity in their sonicated material might have been as much as 75% inactivated. This may explain the comparably low specific activity in their partially purified enzyme. Also the arachidonate/palmitate activation ratio of about 1.5 in the various subcellular fractions differs from that observed by Laposata *et al.* (1985).

So far, the present results confirm two pools of long-chain acyl-CoA activity, both of which have near-identical specificity for saturated and unsaturated fatty acids.

We are grateful to Professor Holm Holmsen, Department of Biochemistry, University of Bergen, for valuable discussions, and to Dr. Knut Halvorsen, the Blood Bank, Haukeland Hospital at the University of Bergen, for providing us with blood. The study was supported by grants from the Norwegian Research Council for Science and the Humanities, Council for Medical Research.

seven times the activity of a partially purified arachidonoyl-CoA synthetase (Laposata *et al.*, 1985). The evidence for an acyl-CoA synthetase specific for arachidonic acid and other prostanoid precursors (Wilson *et al.*, 1982; Laposata *et al.*, 1985) was based on the following: (1) competitive inhibition by eicosatrienoic

REFERENCES

- Aas, M. (1971) *Biochim. Biophys. Acta* **231**, 32–47
- Andreoli, V. M. (1968) *Eur. J. Pharmacol.* **4**, 404–410
- Bar-Tana, J., Rose, G. & Shapiro, B. (1971) *Biochem. J.* **122**, 353–362
- Berge, R. K., Vollset, S. E. & Farstad, M. (1980) *Scand. J. Clin. Lab. Invest.* **40**, 271–278
- Bird, M. I., Munday, L. A., Saggerson, E. D. & Clark, J. B. (1985) *Biochem. J.* **226**, 323–330
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Cohen, P. & Wittels, B. (1970) *J. Clin. Invest.* **49**, 119–127
- Cohen, P., Derksen, A. & van den Bosch, H. (1970) *J. Clin. Invest.* **49**, 128–139
- Deykin, D. & Desser, R. K. (1968) *J. Clin. Invest.* **47**, 1590–1602
- Doery, J. C. G., Hirsh, J. & Cooper, J. (1970) *Blood* **36**, 159–168
- Farstad, M. & Sander, J. (1971) *Scand. J. Clin. Lab. Invest.* **28**, 261–265
- Farstad, M., Bremer, J. & Norum, K. R. (1967) *Biochim. Biophys. Acta* **132**, 492–502
- Farstad, M., Aas, M. & Sander, J. (1973) *Scand. J. Clin. Lab. Invest.* **31**, 205–211
- Fukami, M. H., Bauer, J. S., Stewart, G. J. & Salganicoff, L. (1978) *J. Cell. Biol.* **77**, 389–399
- Gerrard, J. M., White, J. G., Rao, G. H. R. & Townsend, D. (1976) *Am. J. Pathol.* **83**, 283–293
- Gogstad, G. (1980) *Thrombosis Res.* **20**, 669–681
- Groot, P. H. E., Scholte, H. R. & Hülsman, W. C. (1976) *Adv. Lipid Res.* **14**, 75–126
- Holmsen, H., Østvold, A. C. & Pimentel, M. A. (1977) *Throm. Haemostasis* **37**, 380–395
- Ingebretsen, O. C., Bakken, A. M. & Farstad, M. (1982) *Clin. Chim. Acta* **126**, 307–313
- Ingebretsen, O. C., Bakken, A. M. & Farstad, M. (1985) *Clin. Chem.* **31**, 695–698
- Laposata, M., Reich, E. L. & Majerus, P. W. (1985) *J. Biol. Chem.* **260**, 11016–11020
- Mauco, G., Fauvel, J., Chap, H. & Douste-Blazy, L. (1984) *Biochim. Biophys. Acta* **796**, 169–177
- Norum, K. R., Farstad, M. & Bremer, J. (1966) *Biochem. Biophys. Res. Commun.* **24**, 797–804
- Record, M., Bes, J. C., Chap, H. & Douste-Blazy, L. (1982) *Biochim. Biophys. Acta* **688**, 57–65
- Scandinavian Committee on Enzymes (1981) *Scand. J. Clin. Lab. Invest.* **41**, 107–116
- Spector, A. A., Hoak, J. C., Warner, E. D. & Fry, G. L. (1970) *J. Clin. Invest.* **49**, 1489–1496
- Tanaka, T., Hosaka, K., Hoshimaru, M. & Numa, S. (1979) *Eur. J. Biochem.* **98**, 165–172
- Vollset, S. E. & Farstad, M. (1979) *Scand. J. Clin. Lab. Invest.* **39**, 15–21
- Wallach, D. F. H. & Kamat, V. B. (1966) *Methods Enzymol.* **8**, 164–172
- Wilson, D. B., Prescott, S. M. & Majerus, P. W. (1982) *J. Biol. Chem.* **257**, 3510–3515

Received 11 October 1988/16 January 1989; accepted 25 January 1989