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Nonoxidative ethanol metabolism: Formation of fatty acid ethyl esters by cholesterol esterase

(myocardial alcohol metabolism/cardiomyopathy/atherosclerosis)

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ABSTRACT **The recent identification of myocardial metabolites of ethanol—fatty acid ethyl esters—suggests that some of the pathophysiological derangements associated with alcohol-induced heart muscle disease may be a consequence of products of myocardial ethanol metabolism. The donor of the fatty acid moiety in the formation of fatty acid ethyl esters has been identified as nonesterified fatty acid. Fatty acid esterification with ethanol is shown to be mediated by cholesterol esterase (sterol-ester acylhydrolase, EC 3.1.1.13), a finding that identifies a singular nonoxidative ethanol metabolism by an enzyme. A potential basis for the protective effect of ethanol ingestion on atherogenesis is also suggested because fatty acid ethyl esters inhibit cholesterol esterification catalyzed by pancreatic cholesterol esterase and hepatic and aortic microsomal fatty acyl-CoA:cholesterol *O*-acyltransferase (EC 2.3.1.26).**

Although an association between alcohol and heart disease was first described by Walshe in 1873 (1), the recognition of alcoholism as an important component in the history of patients with noncoronary myocardial disease was not made frequently until 1964 (2). Approximately 1–2% of chronic abusers develop overt signs of cardiac dysfunction (3); among the cardiomyopathic disorders, alcohol-induced heart muscle disease (AIHMD) is the most common, accounting for 3% of cardiac admissions to Veterans Administration or city hospitals (4).

AIHMD is associated with chronic alcohol abuse in the absence of vitamin or caloric deprivation (5, 6). However, the bases for the depression of myocardial contractility (7, 8), development of cardiac arrhythmias (9, 10), accumulation of triacylglycerides (11–13), and decreased β-oxidation of fatty acids (11–14) have not been elucidated. Because $^{14}CO_2$ has not been detected in hearts perfused with [^{14}C]ethanol (11, 15) and because intermediary myocardial metabolism of ethanol has not yet been characterized thoroughly, the molecular events underlying the pathophysiology of AIHMD have remained undefined.

In recent studies [^{14}C]ethanol incorporation into a family of fatty acid ethyl esters (FAEE) was documented in heart homogenates and isolated, perfused hearts at rates of $\approx$60 and $\approx$20 nmol/g per hr, respectively (16). These observations provided identification of a cardiac product of ethanol metabolism. Heat-inactivation studies suggested that FAEE synthesis was enzymatically mediated. The present work identifies cholesterol esterase as the enzyme catalyzing the formation of FAEE from ethanol and nonesterified fatty acid. As part of an effort to evaluate the biological significance of FAEE, the inhibitory capacity of these products towards cholesterol esterification was demonstrated. The results bear not only on myocardial ethanol metabolism but also more generally on ethanol and cholesterol metabolism and their interrelationships.

METHODS AND MATERIALS

Materials. All reagents used were of the highest grade commercially available. Radioactive tracers were purchased from New England Nuclear or Amersham. Ethyl [^{3}H]oleate was prepared from [^{3}H]oleate by hydrogen chloride gas-catalyzed esterification in ethanol. 1-Palmitoyl-2-[*carboxyl*-^{14}C]linoleoyl phosphatidylcholine was prepared by acylation of the lyso-derivative with [*carboxyl*-^{14}C]linoleic acid (17). [^{14}C]Ethanol was purified by distillation from KOH as described (16).

Heart Homogenates. A 10% suspension (wt/vol) of rabbit ventricle in Krebs–Henseleit buffer was homogenized for 15 sec with a Polytron (Brinkman), and the resulting homogenate was incubated at 37°C and extracted with acetone at selected intervals (16).

Preparation of Labeled Lipids. Isolated rabbit hearts were perfused (18) with Krebs–Henseleit buffer saturated with 95% O_2/5% CO_2 at 37°C. After 15 min of equilibration, [^{14}C]linoleic acid (10 μCi; 1 Ci = 3.7×10^{10} becquerels) was injected, and perfusion was continued for 10 min, at which time lipids were isolated by extraction (19) and by thin-layer chromatography on silica gel (20). Specific radioactivities determined by liquid scintillation spectrometry and assay of ester concentration (21) were: polar lipids, 143 dpm/nmol; diacylglycerides, 137 dpm/nmol; fatty acids, 325 dpm/nmol; triacylglycerides, 15 dpm/nmol; and cholesterol ester (after addition of [^{14}C]cholesterol oleate), 2,100 dpm/nmol.

Pool Size of Myocardial Lipids. Pool sizes for lipid classes were determined by assay of the ester content of each lipid purified from myocardial extracts, with the recovery calculated on the basis of the amount of ^{14}C-labeled lipid tracer added before extraction, and were expressed per gram of rabbit myocardium: phospholipids, 15 μmol; diacylglycerides, 0.1 μmol; nonesterified fatty acid, 0.8 μmol; triacylglycerides, 5.0 μmol; and cholesterol esters, 0.1 μmol. Fatty acid compositions of individual lipids were determined by gas chromatographic quantitation of the fatty acid methyl esters derived from the isolated lipids by treatment with 14% BF_3 in methanol.

Cholesterol Esterase. Purified bovine pancreatic cholesterol esterase (sterol-ester acylhydrolase, EC 3.1.1.13) (Sigma) produced a single major protein band on $NaDodSO_4$ gel electrophoresis with a minor contaminant of low molecular weight. Protein concentration was determined by the method of Lowry *et al.* (22). For assaying cholesterol ester synthesis, vesicles (23) were prepared containing egg phosphatidylcholine (5.0 μmol/ml), cholesterol (2.0 μmol/ml), and [^{14}C]oleic acid (from 0.1

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Abbreviations: AIHMD, alcohol-induced heart muscle disease; FAEE, fatty acid ethyl esters.

to 6.0 μmol/ml; 100 dpm/nmol) in 50 mM phosphate (pH 6.0). In some cases, ethyl linoleate (0.05–0.5 μmol/ml) was also included in the vesicles. After addition of 40 μl of enzyme (168 μg) to 1.0 ml of vesicles and 200 μl of 24 mM sodium taurocholate in 50 mM phosphate (pH 6.0) and incubation at 37°C for 2 hr, extraction with acetone was carried out. The amount of synthesized cholesterol ester, which was isolated by thin-layer chromatography on silica gel developed with petroleum ether/diethyl ether/acetic acid, 75:5:1 (vol/vol), was calculated based on the specific radioactivity of [^{14}C]oleic acid and was corrected for chromatographic losses by using tritiated standards.

For FAEE synthesis, 40 μl of enzyme (168 μg) was added to 1.0 ml of 50 mM phosphate (pH 7.35) containing from 0.1 to 6.0 μmol of [^{14}C]linoleic acid and from 21 to 2,560 μmol of ethanol; controls omitted enzyme. Incubation was at 37°C for selected intervals. FAEE formation was verified by gas chromatography and gas chromatography/mass spectroscopy (16) and was quantitated by liquid scintillation spectrometry after extraction and thin-layer chromatography with ethyl [^{3}H]oleate (5 pmol) as an internal standard.

Microsomal Cholesterol Esterification. Microsomes from rabbit aorta and liver were isolated by differential centrifugation (24, 25) and suspended in 100 mM phosphate (pH 7.4) to give ≈7.5 mg of protein per ml. Assay of fatty acyl-CoA:cholesterol *O*-acyltransferase (EC 2.3.1.26) activity was carried out by using 5 mM ATP and 0.5 mM coenzyme A (25), except that [^{14}C]oleic acid (4 dpm/pmol) or ethyl linoleate or both were added to the reaction mixture in vesicles of phosphatidylcholine to avoid addition of acetone. Incubation was for 30 min at 37°C, and the cholesterol [^{14}C]oleate formed was assayed. Results were expressed as the percentage of activity in the presence of ethyl linoleate compared to that in its absence.

RESULTS AND DISCUSSION

Identification of the Fatty Acid Source for FAEE Formation. Because not all fractions of myocardial lipids are available commercially in suitable radioactive form, we prepared major potential fatty acid donors present in heart that might contribute their fatty acid moiety to form FAEE. These were then incubated with rabbit heart homogenates (1 g/10 ml) containing 42 mM [^{12}C]ethanol. In addition, commerically available ^{14}C-labeled lipids added in tracer amounts such as [^{14}C]triolein, [^{14}C]tripalmitin, cholesterol [^{14}C]oleate, [^{14}C]palmitoyl CoA, and [^{14}C]oleoyl CoA were also incubated separately under these conditions for 60 min at 37°C. From the determined amount of FAEE synthesis, the data indicate that the only quantitatively significant precursor for ethyl ester synthesis was nonesterified fatty acid (Table 1), with 17.2 nmol of ethyl [^{14}C]linoleate formed from [^{14}C]linoleic acid. Because 41% of the 52 nmol of total FAEE formed is ethyl linoleate, the 17.2 nmol of product accounts for nearly all product formation. In contrast, none of the other potential donors, including fatty acid CoA, contributed more than a trivial amount to FAEE biosynthesis.

These results were confirmed by separately incubating for 60 min ^{14}C-labeled palmitic, oleic, linoleic, and arachidonic acids with rabbit heart homogenates and [^{12}C]ethanol. In each case, fatty acid served as a significant source for FAEE synthesis (Table 2); importantly, no significant product formation occurred when extraction was performed at zero time of incubation. Thus, the lipid precursor in FAEE biosynthesis is nonesterified fatty acid.

Mechanism of FAEE Synthesis. The fatty acid composition of FAEE was more unsaturated than that in phospholipids (not shown), nonesterified fatty acids, and triacylglycerides (Table 3) but similar to that of cholesterol esters, suggesting that enzymatic selection of fatty acids for cholesterol esterification may be operative in FAEE formation. Of the mammalian enzymes that esterify cholesterol, lecithin:cholesterol acyltransferase (EC 2.3.1.43) and fatty acyl-CoA:cholesterol *O*-acyltransferase were excluded from catalyzing FAEE synthesis because phosphatidylcholine and fatty acid CoA do not serve as a fatty acid source (Table 1). Cholesterol esterase, on the other hand, seemed likely to catalyze FAEE synthesis because it esterifies cholesterol with fatty acid.

Because this enzyme has not been isolated yet from heart, subsequent studies used purified pancreatic enzyme. Cholesterol esterases from liver, intestine, pancreas, and aorta appear to be similar if not virtually identical (27); hence, results obtained with the pancreatic enzyme may reasonably reflect eventual observations in the heart. When cholesterol esterase (168 μg) was added to 42 mM ethanol/1 mM [^{14}C]linoleic acid/phosphate buffer, pH 7.35, the formation of ethyl linoleate (verified by gas chromatography/mass spectroscopy; diagnostic ion fragments with mass-to-charge ratio m/e = 308, 265, and 88) was rapidly catalyzed, with 480 nmol/hr synthesized per mg of enzyme. Product formation was linear with time up to 30 min,

Table 1. Formation of FAEE from potential myocardial lipid precursors

Precursor	^{14}C incorporated, nmol/g/hr
Polar lipids	<0.1
Diacylglycerides	<0.1
Fatty acids	17.2
Triacylglycerides	<0.1
Cholesterol esters	0.2
Fatty acid CoA	<0.1

^{14}C-Labeled precursors were incubated with rabbit heart homogenate (1 g/10 ml) in Krebs–Henseleit buffer (pH 7.4) with 42 mM [^{12}C]ethanol for 60 min at 37°C. Extraction with acetone, followed by thin-layer chromatography on silica gel, afforded fatty acid ethyl esters (R_F = 0.50), which were assayed for ^{14}C incorporation. Labeled precursors included those from two sources—those prepared from isolated perfused hearts labeled for 10 min with [^{14}C]linoleic acid and then reincubated with heart homogenate (lines 1 through 5) after specific radioactivity determination and those prepared or purchased commercially, including 1-palmitoyl-2-[^{14}C]linoleoyl phosphatidylcholine (line 1), [^{14}C]tripalmitin or [^{14}C]triolein (line 4), cholesterol [^{14}C]oleate (line 5), and [^{14}C]palmitoyl CoA or [^{14}C]oleoyl CoA (line 6)—all with a specific radioactivity of 100 dpm/nmol. Based on specific radioactivity and determined pool size for each myocardial lipid, the nanomoles of FAEE formed per gram of heart per hour were calculated. Under these conditions, 52 nmol of total FAEE were formed per g per hr (predominantly including ethyl palmitate, oleate, and arachidonate)—a value obtained based on parallel incubations with 42 mM [^{14}C]ethanol as the sole radioactive source.

Table 2. Incorporation of individual fatty acids into myocardial FAEE

Fatty acid	^{14}C incorporated, nmol/g/hr
Palmitate	6.0
Oleate	10.0
Linoleate	17.2
Arachidonate	7.2

^{14}C-Labeled nonesterified fatty acids, including palmitate, oleate, linoleate, and arachidonate, were incubated with rabbit heart homogenate (1 g/10 ml) in Krebs–Henseleit buffer (pH 7.4) containing 42 mM [^{12}C]ethanol. Each was added in tracer quantities to provide specific radioactivities of 325 dpm/nmol, respectively. After 60 min of incubation at 37°C, the amount of the individual FAEE formed was quantitated as described in the legend to Table 1. Under these conditions, a total of 52 nmol of FAEE were formed per g per hr but the quantitatively most significant FAEE are shown here. Palmitoleic, stearic, linolenic, and long-chain fatty acids each accounted for no more than 3 nmol/g per hr.

Table 3. Percentage fatty acid composition of myocardial lipids

	Nonesterified fatty acid	Triacyl glycerides	Cholesterol esters	FAEE
Palmitate	29.8	28.2	9.8	6.6
Stearate	28.2	4.9	3.2	3.9
Oleate	14.2	23.1	21.2	23.0
Linoleate	8.8	28.7	41.3	42.8
Arachidonate	3.0	5.2	14.4	10.0

Rabbit myocardial lipids were extracted immediately from fresh whole-heart homogenates at 4°C (25), separated by thin-layer chromatography on silica gel developed with petroleum ether/diethyl ether/acetic acid, 97:52:3 (vol/vol) (26), and converted to their respective methyl esters with 14% BF_3 in methanol at 65°C for 10 min while protected from light and maintained under nitrogen gas. Fatty acid compositions were determined by gas chromatography and are expressed as percentages with standard deviation of ±5%. The fatty acid composition of FAEE was determined similarly from product isolated after incubation of rabbit heart homogenate in Krebs–Henseleit buffer, pH 7.4/42 mM ethanol for 60 min at 37°C.

with a plateau occurring by 60 min. It was linear also as a function of added enzyme from 25 to 250 μg.

The kinetics of ethanol and linoleic acid incorporation into ethyl linoleate catalyzed by cholesterol esterase were determined (Fig. 1). With linoleic acid concentration constant at 6 mM and ethanol concentration varied from 10 mM to 2.56 M, progressively more ethyl linoleate was formed, increasing linearly with concentrations of ethanol to ≈750 mM and then reaching a plateau; 2.56 M ethanol did not irreversibly denature the enzyme. A Lineweaver–Burk plot was linear with a calculated K'_m of 670 mM and V'_{max} of 150 nmol/min per mg with respect to ethanol. In the absence of enzyme, even at 2.56 M ethanol, <0.1 nmol of product was formed. When ethanol concentration was maintained constant at 600 mM (a less-than-saturating concentration to avoid changes in the physical state of substrate and enzyme) and linoleic acid concentration was varied from 0.1 to 6 mM, a saturation effect for product formation was observed at ≈4 mM (data not shown). Lineweaver–Burk

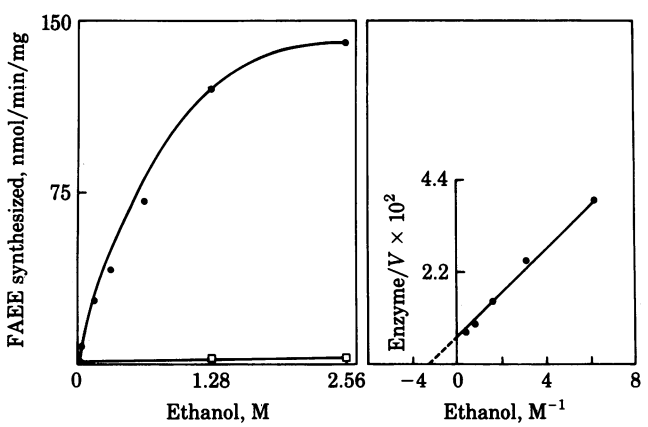

FIG. 1. Cholesterol esterase-catalyzed esterification of [^{14}C]linoleic acid with ethanol. The ethanol concentration dependence of ethyl linoleate formation catalyzed by pancreatic cholesterol esterase was determined after addition of 40 μl of cholesterol esterase (●) to 1.0 ml of reaction mixture containing 6.0 μmol of [^{14}C]linoleic acid (specific radioactivity, 100 dpm/nmol), 50 μmol of sodium phosphate (pH 7.35), and 21–2,560 μmol of ethanol and incubation at 37°C for 30 min. Control incubations (□) omitted enzyme. Acetone extraction of the mixture and thin-layer chromatography on silica gel developed with petroleum ether/diethyl ether/acetic acid, 75:5:1 (vol/vol), provided ethyl [^{14}C]linoleate, which was quantitated. Results are expressed as nmol/min of product formed per mg of enzyme versus ethanol concentration (*Left*) or are plotted in a double-reciprocal fashion [*Right*; units for ordinate, $(nmol/min/mg)^{-1}$].

graphic analysis gave a straight line and a K'_m of 1.3 mM and a V'_{max} of 130 nmol/min per mg with respect to linoleic acid.

These results indicate that cholesterol esterase will nonoxidatively metabolize ethanol at concentrations attainable *in vivo* as a consequence of social drinking. The high K_m for ethanol implies that product formation would be related in a first-order manner to ethanol concentration achieved because saturation of the enzyme binding site for alcohol would require supraphysiological concentrations. Thus, should similar kinetics pertain to human FAEE biosynthesis, the more one drinks, the more FAEE would be formed.

The identification of pancreatic cholesterol esterase as an enzyme capable of metabolizing ethanol, together with the data indicating that nonesterified fatty acid—present in heart in concentrations of ≈130 μM for linoleate, for example—participates in ethyl ester formation, suggests that cholesterol esterase in heart is the enzyme responsible for FAEE formation. Definitive conclusions await purification of this myocardial enzyme. More broadly, however, the implication is that FAEE are synthesized throughout the body because cholesterol esterase has been identified in many different organs (27). The potential importance of these products of nonoxidative ethanol metabolism may lie in their interaction with enzymes that recognize naturally occurring fatty acid esters.

Potential Biological Significance of FAEE. In this regard, product inhibition of cholesterol esterification by FAEE was examined. Intracellularly, fatty acyl-CoA:cholesterol *O*-acyltransferase, a microsomal enzyme, and cholesterol esterase, a cytosolic enzyme, catalyze cholesterol esterification (27), with the former enzyme identified in many tissues such as liver (25, 28) and aorta (24, 26, 29) and the latter enzyme identified in pancreas (30), peritoneal macrophages (31), and aorta (24). Both purified pancreatic cholesterol esterase and rabbit liver and aortic microsomes have been used as a source of fatty acyl-CoA:cholesterol *O*-acyltransferase to test the hypothesis that FAEE may inhibit intracellular cholesterol esterification because these organs synthesize FAEE at rates comparable to that of heart (unpublished data).

Incubation of pancreatic cholesterol esterase with phosphatidylcholine vesicles containing cholesterol and [^{14}C]oleic acid in the absence and presence of ethyl linoleate (from 0 to 200 μM) demonstrated a progressive diminution of cholesterol [^{14}C]oleate formation as ethyl linoleate concentration increased (Fig. 2). At 50 μM ethyl linoleate, there was 35% inhibition of esterification and at 200 μM, 47% inhibition. Liver and aortic microsome-catalyzed esterification of cholesterol was even more susceptible to ethyl linoleate inhibition (Fig. 2). Incubation of microsomes from liver and aorta, respectively, with [^{14}C]oleic acid in the presence of ATP and coenzyme A, with ethyl linoleate increasing from 0 to 200 μM, led to a progressive decrease in the rate of cholesterol [^{14}C]oleate formed to ≈10–15% of control rates at 200 μM ethyl linoleate. Fifty percent inhibition was observed at ≈25 and ≈30 μM ethyl linoleate, respectively, for aortic and hepatic microsome-catalyzed esterification of cholesterol. Virtually identical amounts of inhibition were found when 40 μM [^{14}C]oleoyl CoA (4 dpm/pmol) replaced exogenous [^{14}C]oleic acid, ATP, and coenzyme A in the assay, indicating that diminished cholesterol esterification did not reflect inhibition of fatty acid activation to its CoA ester. These results demonstrate that inhibition by FAEE of enzymatic pathways of intracellular cholesterol esterification occurs at concentrations of FAEE readily achievable after ethanol consumption.

Recent epidemiologic studies documented that deaths and coronary events due to cardiac atherosclerotic disease were reduced in individuals who consumed moderate amounts of

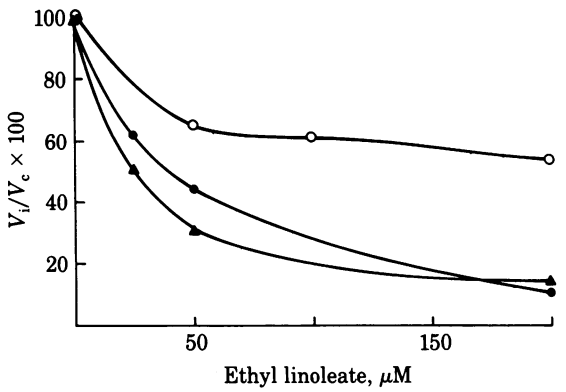

FIG. 2. Inhibition by ethyl linoleate of cholesterol esterification catalyzed by pancreatic cholesterol esterase and hepatic and aortic microsomes. The rate of cholesterol [^{14}C]oleate formation was determined in the absence or presence of ethyl linoleate (0–200 μM). Formation from exogenous cholesterol and [^{14}C]oleic acid was catalyzed by bovine pancreatic cholesterol esterase (○), or formation from endogenous cholesterol and exogenously added 5 mM ATP, 0.5 mM CoA, and [^{14}C]oleic acid was catalyzed by hepatic (●) or aortic (▲) microsomes. Results are expressed as the percentage of activity observed in the presence of ethyl linoleate to that in its absence. Each inhibition curve represents the results from at least triplicate experiments. V_i, velocity of inhibited reaction; V_c, velocity of control reaction.

ethanol on a daily basis (32). Total cholesterol concentration was increased but esterified cholesterol was reduced in such individuals (33). Although the pathophysiology of atherogenesis is complex, an early lesion in blood vessels before atheromata formation appears to be enhanced esterification of cholesterol. Marked increases in cholesterol oleate and linoleate (27, 34) and in the activity of fatty acyl-CoA:cholesterol *O*-acyltransferase and cholesterol esterase, enzymes responsible for their synthesis, are observable 1 wk after initiation of an atherogenic diet in rabbits (24, 35). The present findings suggest that inhibition of cholesterol esterification by FAEE, with retardation of an early key metabolic reaction necessary for atherogenesis, may be at least a partial mechanism for the protective effect of moderate ethanol ingestion on the development of atherosclerosis. They may also explain, in part, the decreased fraction of esterified cholesterol found in the blood of patients hospitalized for alcohol abuse (33). Interactions with other enzymes that recognize cholesterol esters are also possible with further effects on sterol metabolism. In addition to a possible pathophysiological role in AIHMD, FAEE may interact with a number of fatty acid-metabolizing and ester-recognizing enzymes to affect their activity with consequent metabolic effects.

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1. Walshe, W. H. (1873) *Disease of the Heart and Great Vessels* (Smith, Elden, London) 4th Ed., pp. 359–363.
2. Bridgen, W. & Robinson, J. (1964) *Br. Med. J.* **2**, 1283–1289.
3. Fink, R., Marjot, D. H. & Rosalki, S. B. (1979) *Ann. Clin. Biochem.* **16**, 165–167.
4. Kramer, K., Kuller, L. & Fisher, R. (1968) *Ann. Int. Med.* **69**, 273–282.
5. Bing, R. J. & Tillmans, H. (1977) in *The Effects of Alcohol on the Heart, Metabolic Aspects of Alcoholism*, ed. Lieber, C. S. (Univ. Park Press, Baltimore, MD), pp. 117–134.
6. Regan, T. J., Ettinger, P. O., Haider, B., Ahmed, S. S., Oldewurtel, H. A. & Lyons, M. M. (1977) *Annu. Rev. Med.* **28**, 393–409.
7. Schwartz, L., Sample, K. A. & Wigle, E. D. (1975) *Am. J. Cardiol.* **36**, 963–966.
8. Weishaar, R., Sarma, J. S. M., Maruyama, Y., Fischer, R., Bertuglia, S. & Bing, R. J. (1977) *Am. J. Cardiol.* **40**, 556–562.
9. Demakis, J. G., Proskey, A., Rahimtoola, S. H., Jamil, M., Sutton, G. C., Rosen, K., Gunnar, R. M. & Tobin, J. R. (1974) *Ann. Int. Med.* **80**, 293–297.
10. Burch, G. E. & DePasquale, N. P. (1969) *Am. J. Cardiol.* **23**, 723–731.
11. Lochner, A., Cowley, R. & Brink, A. J. (1969) *Am. Heart J.* **78**, 770–780.
12. Regan, T. J., Koroxenidis, G., Moschos, C. B., Oldewurtel, H. A., Lehan, P. H. & Hellems, H. K. (1966) *J. Clin. Invest.* **45**, 270–280.
13. Kikuchi, T. & Kako, K. J. (1970) *Circ. Res.* **26**, 625–634.
14. Kako, K. J., Liu, M. S. & Thornton, M. J. (1973) *J. Mol. Cell. Cardiol.* **5**, 473–489.
15. Gailis, L. & Verdy, M. (1971) *Can. J. Biochem.* **49**, 227–233.
16. Lange, L. G., Bergmann, S. R. & Sobel, B. E. (1981) *J. Biol. Chem.* **256**, 12968–12973.
17. Lands, W. E. M. (1960) *J. Biol. Chem.* **235**, 2233–2237.
18. Bergmann, S. R., Clark, R. E. & Sobel, B. E. (1979) *Am. J. Physiol.* **37**, 911–917.
19. Bligh, E. G. & Dywer, W. T. (1959) *Can. J. Biochem. Physiol.* **37**, 911–917.
20. Calderon, P., Furnelle, J. & Christophe, J. (1979) *Biochim. Biophys. Acta* **574**, 379–390.
21. Snyder, F. & Stephens, N. (1959) *Biochim. Biophys. Acta* **34**, 244–245.
22. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
23. Brecher, P., Pynn, H. Y. & Cobeniay, A. V. (1979) *J. Lipid Res.* **8**, 154–171.
24. Brecher, P. I. & Chobanian, A. V. (1974) *Circ. Res.* **35**, 692–701.
25. Goodman, D. S., Deykin, D. & Shiratori, T. (1964) *J. Biol. Chem.* **239**, 1335–1345.
26. Hashimoto, S. & Dayton, S. (1974) *Proc. Soc. Exp. Biol. Med.* **145**, 89–92.
27. Paoletti, R. & Kritchersky, D. (1978) *Adv. Lipid Res.* **16**, 221–266.
28. Balasubramanian, S., Goldstein, J. L., Faust, J. R., Bruchede, G. Y. & Brown, M. S. (1977) *J. Biol. Chem.* **252**, 1771–1779.
29. St. Clair, R. W., Lofland, H. B. & Clarkson, T. B. (1970) *Circ. Res.* **27**, 213–225.
30. Murthy, S. K. & Ganguly, J. (1962) *Biochem. J.* **83**, 460–469.
31. Day, A. J. & Tume, T. K. (1969) *Biochim. Biophys. Acta* **176**, 367–376.
32. Klatsky, A. L., Friedman, G. D. & Siegelaub, A. B. (1981) *Circulation* **64**, (3), 32–41.
33. Sabesin, S. M. (1981) *Circulation* **64**, (3), 72–84.
34. Bottcher, C. J. F., Boelsme-Van Houte, E., ter Haar Romony-Wachter, C. C., Loodford, F. P. & Yan Gent, C. M. (1960) *Lancet* ii, 1162–1166.
35. Day, A. J. & Proudlock, J. W. (1974) *Atherosclerosis* **19**, 253–258.