Role of *S*-adenosylhomocysteine hydrolase in adenosine metabolism in mammalian heart

Jürgen SCHRADER, Wolfgang SCHÜTZ* and Hubert BARDENHEUER

Department of Physiology, University of Munich, Pettenkoferstrasse 12, D-8000 Munich 2, Germany

(Received 11 September 1980/Accepted 5 December 1980)

S-Adenosylhomocysteine hydrolase of mammalian hearts from different species is exclusively a cytosolic enzyme. The apparent K_m for the guinea-pig enzyme was $2.9\ \mu\text{M}$ (synthesis) and $0.39\ \mu\text{M}$ (hydrolysis). Perfusion of isolated guinea-pig hearts for 120 min with L-homocysteine thiolactone ($0.23\ \text{mM}$) and adenosine ($0.1\ \text{mM}$), in the presence of erythro-9-(2-hydroxynon-3-yl)adenine to inhibit adenosine deaminase, caused tissue contents of *S*-adenosylhomocysteine to increase from 3.5 to $3600\ \text{nmol/g}$. When endogenous adenosine production was accelerated by perfusion of hearts with hypoxic medium (30% O_2), L-homocysteine thiolactone ($0.23\ \text{mM}$) increased *S*-adenosylhomocysteine 17-fold to $64.3\ \text{nmol/g}$ within 15 min. In the presence of 4-nitrobenzylthioinosine ($5\ \mu\text{M}$), an inhibitor of adenosine transport, *S*-adenosylhomocysteine further increased to $150\ \text{nmol/g}$. L-Homocysteine thiolactone decreased the hypoxia-induced augmentation of adenosine, inosine and hypoxanthine in the tissue and the release of these purines into the coronary system by more than 50%. Our findings indicate that L-homocysteine can profoundly alter adenosine metabolism in the intact heart by conversion of adenosine into *S*-adenosylhomocysteine. Adenosine formed during hypoxia was most probably generated within the myocardial cell.

Adenosine has received much attention in recent years because of its possible involvement in regulating heart, smooth-muscle, neural and immune function (Fox & Kelley, 1978; Baer & Drummond, 1979). Specific adenosine receptors located in the membrane have been identified in various tissues (Olsson *et al.*, 1976; Schrader *et al.*, 1977a), and are thought to modulate the activity of adenylate cyclase directly (Londos *et al.*, 1978).

In the heart, as well as in most other organs, formation and release of adenosine appear to be critically dependent on the equilibrium between supply and demand for oxygen (Berne *et al.*, 1979). Factors leading to tissue hypoxia by either diminished oxygen supply or enhanced oxygen demand are known to be potent stimuli for adenosine production.

The site of adenosine formation is generally assumed to be the plasma membrane, mainly because of the location of 5'-nucleotidase (Rubio *et al.*, 1973). Functional studies, however, suggest that adenosine may also be formed and accumulate

intracellularly (Schrader & Schütz, 1979). Another possible source for myocardial adenosine formation, besides dephosphorylation of 5'-AMP, is the pathway leading from *S*-adenosylmethionine via *S*-adenosylhomocysteine to adenosine. Adenosine may thus be formed as an obligatory product of *S*-adenosylmethionine-dependent methylation reactions. A key enzyme in this pathway directly leading to the formation of adenosine is *S*-adenosylhomocysteine hydrolase (EC 3.3.1.1), which was first described by de la Haba & Cantoni (1959) in rat liver and which was more recently purified to homogeneity (Richards *et al.*, 1978). The reaction catalysed by this enzyme is reversible, with equilibrium far in the direction of *S*-adenosylhomocysteine synthesis (de la Haba & Cantoni, 1959).

Little attention has been paid in the past to the role of *S*-adenosylhomocysteine hydrolase in the metabolism of adenosine in whole tissue. In view of the regulatory function ascribed to myocardial adenosine [dilation of coronary vessels (Olsson, 1970; Schrader *et al.*, 1977a; Berne *et al.*, 1979) and attenuation of the action of β -adrenergic stimulation (Schrader *et al.*, 1977b; Dobson, 1978)], we report on the possible significance of *S*-adenosylhomocysteine hydrolase and L-homocysteine for

* Present address: Department of Pharmacology, University of Vienna, Währinger Strasse 13a, A-1090 Vienna, Austria.

the formation and release of this nucleoside in isolated beating hearts.

Materials and methods

[8-¹⁴C]Adenosine (sp. radioactivity 58 mCi/mmol) was obtained from Amersham-Buchler, Braunschweig, Germany. *erythro*-9-(2-Hydroxynon-3-yl)adenine was purchased from Burroughs Wellcome, Research Triangle Park, NC, U.S.A. L-Homocysteine thiolactone was from Sigma, and silica-gel thin-layer plates (Polygram SIL G/UV₂₅₄) were from Macherey-Nagel, Düren, Germany. All nucleosides, *S*-adenosylhomocysteine, adenosine deaminase (EC 3.5.4.4), nucleoside phosphorylase (EC 2.4.2.1) and xanthine oxidase (EC 1.2.3.2) were obtained from Boehringer, Mannheim, Germany. 4-Nitrobenzylthioinosine was a gift from Dr. Alan R. P. Paterson, Edmonton, Canada.

S-Adenosylhomocysteine hydrolase

Ventricular myocardium was initially homogenized (Waring Blendor) for 1 min in 10 vol. (v/w) of 10 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], containing 0.25 M-sucrose, 1 mM-mercaptoethanol and 1 mM-MgCl₂, strained through a double-layered nylon sieve and again homogenized for 1 min (Potter/Elvehjem homogenizer, Teflon pestle). A crude membrane preparation was obtained by centrifugation of a sample of the homogenate at 1000 g for 10 min, and washing the resulting pellet three times with the same buffer. For preparation of the cytosolic fraction, the homogenate was first centrifuged at 3000 g for 10 min and the resulting supernatant was again centrifuged at 220 000 g for 60 min. The cytosolic fraction was then dialysed for 4 h against an excess of 10 mM-Hepes buffer, pH 7.4, containing 1 mM-mercaptoethanol, 1 mM-MgCl₂ and 0.01% activated charcoal to adsorb all purine compounds.

The rate of hydrolysis of *S*-adenosylhomocysteine was determined by measuring the formation of uric acid by using a newly developed spectrophotometric assay. The reaction mixture (2 ml) contained 25 mM-Hepes, pH 7.4, 2 mM-MgCl₂, 0.1 mM-NaH₂PO₄, 0.5–20 μM-*S*-adenosylhomocysteine, 8 units of adenosine deaminase, 2 units of purine nucleoside phosphorylase and 2 units of xanthine oxidase at 37°C. Reaction was started by addition of the cytosolic fraction containing *S*-adenosylhomocysteine hydrolase (160–380 μg of protein). Since *S*-adenosylhomocysteine hydrolase activity was adjusted to be the rate-limiting enzyme, changes in absorbance at the wavelength of uric acid (295 nm, reference beam at 325 nm) directly reflected the activity of the enzyme. Protein was determined as described by Bradford (1976).

Synthesis of *S*-adenosylhomocysteine from

adenosine and L-homocysteine was assayed in a 0.2 ml reaction mixture containing 25 mM-Hepes, pH 7.4, 2 mM-MgCl₂, 20 μM-*erythro*-9-(2-hydroxynon-3-yl)adenine to inhibit adenosine deaminase and 0.2–50 μM-[8-¹⁴C]adenosine (2 × 10⁴ c.p.m./assay tube). Activity of *S*-adenosylhomocysteine hydrolase was determined at either constant adenosine (50 μM) or constant L-homocysteine (2 mM) concentrations while L-homocysteine or adenosine was varied respectively. L-Homocysteine was prepared from its thiolactone by alkaline hydrolysis (Hershfield & Kredich, 1978). Reaction was started by addition of cytosolic protein (90 μg). Incubation was for 10 min at 37°C, followed by heat-inactivation at 95°C for 2 min. A 200 μl portion was spotted with carrier amounts of adenosine and *S*-adenosylhomocysteine on silica-gel UV₂₅₄ thin-layer plates, which were developed for 3 h in butan-1-ol/ethyl acetate/methanol/25% (v/v) NH₃ (7:4:3:3, by vol.). *R_F* values for adenosine and *S*-adenosylhomocysteine were identified under u.v. light, and the spots were scraped off and counted for radioactivity (Schrader & Gerlach, 1977).

Animal experiments

Isolated hearts from guinea pigs, prepared as described by Schrader *et al.* (1978b), were perfused via the aorta with a modified Krebs-Henseleit solution (Schrader & Gerlach, 1977) equilibrated with either O₂/CO₂ (19:1) (normoxia) or O₂/CO₂/N₂ (6:1:13) (hypoxia). Hearts were electrically paced at 270 beats/min, and intraventricular pressure development and coronary perfusion pressure were continuously monitored in each heart. After an equilibration period of 20 min under normoxic conditions (perfusion pressure 60 cmH₂O), perfusion was changed to constant flow in order to assure constant O₂ supply under all experimental conditions. Flow was initially adjusted to yield a perfusion pressure of 80 cmH₂O. When intraventricular pressure development and perfusion pressure had attained constant values, perfusion was continued for an additional 15 min with normoxic or hypoxic medium, with or without L-homocysteine thiolactone and/or 4-nitrobenzylthioinosine.

Determination of *S*-adenosylhomocysteine, adenosine, inosine and hypoxanthine

Hearts, still attached to the perfusion cannula, were frozen between two aluminium blocks precooled to the temperature of liquid N₂ and freeze-dried. After removal of connective and atrial tissue, ventricular myocardium was extracted with 10 ml of 0.5 M-HClO₄ and the extract was carefully neutralized with KOH. The neutralized extract was then treated with activated charcoal as described by Schrader & Gerlach (1977), and adenosine and

S-adenosylhomocysteine were separated on silica-gel UV₂₅₄ thin-layer plates in the solvent system mentioned above. The u.v.-absorbing areas representing adenosine and S-adenosylhomocysteine were scraped off, eluted and quantified spectrophotometrically by successive enzymic degradation to uric acid (Schrader & Gerlach, 1977). Degradation of S-adenosylhomocysteine to adenosine was accomplished by addition of 2 units of S-adenosylhomocysteine hydrolase (sp. activity 40 μ mol/min per mg of protein), prepared from rat liver as described by de la Haba & Cantoni (1959). With a double-beam dual-wavelength spectrophotometer (Perkin-Elmer model 356) as little as 100 pmol of adenosine and S-adenosylhomocysteine could be detected.

Recovery of S-adenosylhomocysteine and hydrolytic cleavage of S-adenosylhomocysteine to adenosine during the extraction procedure was determined in each experiment by addition of 10 nCi of S-[8-¹⁴C]adenosylhomocysteine to the acid extract. Recovery of S-adenosylhomocysteine was $44.5 \pm 1.6\%$ ($\pm$ S.E.M., $n = 18$). Hydrolysis of S-adenosylhomocysteine was $2.15 \pm 0.35\%$ ($\pm$ S.E.M., $n = 11$). Values given for S-adenosylhomocysteine and adenosine are corrected for individual recoveries

and amount of adenosine formed from S-adenosylhomocysteine respectively. S-[8-¹⁴C]adenosylhomocysteine was prepared from [8-¹⁴C]adenosine (sp. radioactivity 51 mCi/mmol), 20 μ M-erythro-9-(2-hydroxynon-3-yl)adenine, 1 mM-EDTA and 2 units of S-adenosylhomocysteine hydrolase. Incubation for 10 min at 37°C was terminated by heat-inactivation at 95°C for 2 min. Purification of labelled S-adenosylhomocysteine was by t.l.c. on silica-gel plates as described above.

For analysis of adenosine, inosine and hypoxanthine, released by the heart, coronary-effluent perfusate was collected in 10 ml of 0.6 M-HClO₄. The different purine compounds were determined as described previously (Schrader & Gerlach, 1977).

Results

S-Adenosylhomocysteine in hearts of different mammalian species

In all species investigated, S-adenosylhomocysteine hydrolase was found to be present solely in the cytosolic fraction (220 000g supernatant) prepared from ventricular myocardium; no activity could be detected in a washed particulate membrane preparation. There were, however, considerable species differences (Table 1). Although the highest activity values were found in hearts of guinea pigs, lowest values were determined for the dog heart, differing by a factor of about 20.

The apparent K_m value for guinea-pig heart S-adenosylhomocysteine hydrolase was 2.9 μ M when assayed in the hydrolytic direction (Table 2). Similar values have been reported for other tissues (Richards *et al.*, 1978; Hershfield & Kredich, 1978; Kajander *et al.*, 1976). When measured in the direction of S-adenosylhomocysteine synthesis, the apparent K_m value for homocysteine was in the millimolar range, whereas the K_m for adenosine was as low as 0.39 μ M.

Influence of L-homocysteine on adenosine metabolism during normoxic and hypoxic perfusion

Perfusion of well-oxygenated (95% O₂) isolated guinea-pig hearts for 15 min with 0.23 mM-L-homo-

Table 1. *S-Adenosylhomocysteine hydrolase in mammalian hearts of different species*

Enzyme activity was determined in the direction of S-adenosylhomocysteine (0.5 mM) hydrolysis in the 220 000g supernatant of ventricular myocardium. The assay for S-adenosylhomocysteine hydrolase was carried out as described in the Materials and methods section. Values are from n different heart preparations and are means $\pm$ S.E.M.

	<i>n</i>	S-Adenosylhomocysteine hydrolase activity (nmol/min per mg of protein)
Guinea pig	5	1.29 ± 0.17
Cat	5	0.27 ± 0.05
Rat	7	0.21 ± 0.04
Rabbit	2	0.092
Dog	3	0.079 ± 0.011

Table 2. *Kinetic properties of S-adenosylhomocysteine hydrolase determined in the 220 000g supernatant of guinea-pig ventricular myocardium*

The assay of S-adenosylhomocysteine hydrolase activity was carried out as described in the Materials and methods section. Synthesis of S-adenosylhomocysteine was measured at constant adenosine (50 μ M) or constant L-homocysteine (2 mM) concentrations while L-homocysteine or adenosine was varied respectively. Apparently V_{max} and K_m values were determined from Lineweaver-Burk plots. Values represent means $\pm$ S.E.M. for n hearts.

	<i>n</i>	V_{max} (nmol/min per mg)	K_m (μ M)
Hydrolysis	4	1.29 ± 0.17	2.9 ± 0.8
Synthesis (50 μ M-adenosine)	3	2.03 ± 0.10	257.0 ± 57.0
Synthesis (2 mM-L-homocysteine)	3	1.96 ± 0.03	0.39 ± 0.03

Table 3. *Influence of L-homocysteine thiolactone on tissue content of S-adenosylhomocysteine and adenosine in isolated perfused guinea-pig hearts*

S-Adenosylhomocysteine and adenosine were determined by spectrophotometric techniques as described in the Materials and methods section. L-Homocysteine thiolactone (0.23 mM) and 4-nitrobenzylthioinosine (5 μ M) were applied for 15 min. Hearts were perfused at constant flow (8–10 ml/min) with a saline medium, equilibrated with either O₂/CO₂ (19:1) (normoxia) or O₂/CO₂/N₂ (6:1:13) (hypoxia). Values are the means $\pm$ s.e.m. for *n* experiments. Asterisks indicate values differing significantly from respective control values by unpaired *t*-test analysis (**P* < 0.02; ***P* < 0.005). Abbreviation: n.d., not determined.

	<i>n</i>	S-Adenosylhomocysteine (nmol/g)	Adenosine (nmol/g)
Normoxia (95% O ₂)			
Control	5	3.47 $\pm$ 0.36	1.64 $\pm$ 0.11
L-Homocysteine thiolactone	4	5.89 $\pm$ 0.59*	1.52 $\pm$ 0.17
Hypoxia (30% O ₂)			
Control	5	3.66 $\pm$ 0.82	3.53 $\pm$ 0.63
L-Homocysteine thiolactone	6	64.3 $\pm$ 6.5**	2.53 $\pm$ 0.28*
4-Nitrobenzylthioinosine	12	n.d.	29.20 $\pm$ 6.50**
L-Homocysteine thiolactone + 4-nitrobenzylthioinosine	7	151.1 $\pm$ 14.9**	4.04 $\pm$ 0.60**

Table 4. *Effect of L-homocysteine thiolactone on the hypoxia-induced release of adenosine, inosine and hypoxanthine into the coronary-effluent perfusate of guinea-pig hearts*

Adenosine, inosine and hypoxanthine were determined by spectrophotometric techniques as described in the Materials and methods section. Conditions of perfusion were the same as given in the legend to Table 3. Values are means $\pm$ s.e.m., *n* = 7. Asterisks indicate significant differences (***P* < 0.005) by paired *t*-test analysis when perfusion was initiated in either the absence or presence of L-homocysteine thiolactone (0.23 mM).

	Purine release (pmol/min per g)		
	Adenosine	Inosine	Hypoxanthine
Normoxia (90% O ₂)	86 $\pm$ 12	750 $\pm$ 178	188 $\pm$ 41
Hypoxia (30% O ₂)	1209 $\pm$ 353	5295 $\pm$ 1840	2292 $\pm$ 706
Hypoxia (30% O ₂) + L-homocysteine thiolactone	439 $\pm$ 110**	2514 $\pm$ 789**	1575 $\pm$ 532**

cysteine thiolactone caused the tissue content of S-adenosylhomocysteine to increase by about 70% (Table 3). Under these conditions adenosine content did not significantly change.

Completely different results were obtained when endogenous adenosine production was accelerated by perfusion of the hearts with hypoxic medium (30% O₂). Hypoxia alone caused adenosine to increase from 1.64 to 3.53 nmol/g, but S-adenosylhomocysteine content did not change. In the presence of L-homocysteine thiolactone, however, S-adenosylhomocysteine increased by a factor of 17, whereas adenosine decreased by about 50%. Similar changes were also observed in inosine and hypoxanthine, which decreased from 8.1 $\pm$ 0.5 and 6.0 $\pm$ 0.5 nmol/g to 5.1 $\pm$ 1.3 and 3.9 $\pm$ 0.9 nmol/g respectively ($\pm$ s.e.m.; *P* < 0.002; *n* = 5).

In an attempt to increase tissue adenosine content further, we used 4-nitrobenzylthioinosine, a potent inhibitor of nucleoside transport across cellular membranes (Paterson *et al.*, 1977). 4-Nitrobenzylthioinosine (5 μ M) greatly potentiated the hypoxia-induced increase in tissue adenosine. In the presence

of such elevated cellular adenosine contents, L-homocysteine caused a further increase in myocardial S-adenosylhomocysteine, but adenosine again was decreased.

Analyses of coronary-effluent perfusates of isolated guinea-pig hearts showed that hypoxia (30%) also greatly augmented the release of adenosine and its degradation products, inosine and hypoxanthine (Table 4). L-Homocysteine thiolactone (0.23 mM) drastically diminished this effect. The total decrease in the amount of purine compounds liberated under these conditions was 64 nmol/15 min per g. This value compares well with the tissue content of S-adenosylhomocysteine accumulating over the same period of time (Table 3). There was no detectable release of S-adenosylhomocysteine from isolated hearts in experiments with L-homocysteine thiolactone.

Effect of L-homocysteine and infused adenosine on S-adenosylhomocysteine contents

When isolated guinea-pig hearts were perfused simultaneously with adenosine (0.1 mM) and L-

Fig. 1. Increase in tissue *S*-adenosylhomocysteine in the presence of *L*-homocysteine and adenosine

Isolated guinea-pig hearts, electrically paced at 270 beats/min, were perfused at constant flow (10 ml/min) with a modified Krebs–Henseleit solution containing *L*-homocysteine thiolactone (0.23 mM) and adenosine (0.1 mM). *erythro*-9-(2-Hydroxynon-3-yl) adenine (5 μ M) was either absent from (○) or present in (●) the perfusion medium.

homocysteine thiolactone (0.23 mM), tissue *S*-adenosylhomocysteine content considerably increased with time (Fig. 1). In the presence of *erythro*-9-(2-hydroxynon-3-yl)adenine, which inhibits adenosine deaminase, the increase in *S*-adenosylhomocysteine was even greater. After 120 min of perfusion under these conditions, *S*-adenosylhomocysteine increased from 3.47 nmol/g to a value as high as 3600 nmol/g. Despite the greatly enlarged *S*-adenosylhomocysteine pool, contractile-force development of the isolated hearts was not significantly altered.

Discussion

The present paper demonstrates that perfusion of intact hearts with *L*-homocysteine greatly diminishes tissue content and release of vasoactive adenosine during hypoxia. This effect does not result from changes in oxygen supply to the heart, but is due to the conversion of adenosine into *S*-adenosylhomocysteine by action of *S*-adenosylhomocysteine hydrolase. *L*-Homocysteine therefore can uncouple production of adenosine from the accumulation of

this nucleoside in the tissue and its release into the vascular system. This action of *L*-homocysteine may provide a suitable experimental tool with which to evaluate in further studies the possible role of adenosine in the heart and other tissues (Fox & Kelley, 1978; Baer & Drummond, 1979).

S-Adenosylhomocysteine hydrolase was shown to bind adenosine tightly (Hershfield & Kredich, 1978). From the kinetic parameters of a highly purified *S*-adenosylhomocysteine hydrolase [$V_{\max}$, 0.5 μ mol/min per mg of protein; mol.wt. 237 500 (Richards *et al.*, 1978)], the adenosine-binding properties of the enzyme (2 mol of adenosine/mol of enzyme; M. Hershfield, personal communication) and the specific activity of the cardiac enzyme (38.1 nmol/min per g of ventricle in the guinea pig), it can be calculated that about 0.6 nmol of adenosine/g of ventricle is bound to *S*-adenosylhomocysteine hydrolase. Such a value would decrease the measured tissue content of adenosine in the guinea-pig heart (1.64 nmol/g) by about 37%. It is noteworthy that the dog heart, which exhibits a very low *S*-adenosylhomocysteine hydrolase activity, is also characterized by a low tissue content of adenosine [0.3 nmol/g (Olsson, 1970)]. Thus the amount of *S*-adenosylhomocysteine hydrolase present in each tissue may be a major factor determining the total tissue content of adenosine.

S-Adenosylhomocysteine hydrolase proved to be exclusively a cytosolic enzyme in the heart. It is therefore reasonable to assume that adenosine, the substrate for this enzyme, must have accumulated intracellularly in order to explain its conversion into *S*-adenosylhomocysteine. The possibility that adenosine was first released by the heart and then trapped by *L*-homocysteine after its reuptake appears unlikely, since inhibition of adenosine transport by 4-nitrobenzylthioinosine (Paterson *et al.*, 1977) not only greatly augmented tissue content of adenosine, but also potentiated the effect of *L*-homocysteine on *S*-adenosylhomocysteine (Table 3).

No values have been reported so far for the normal tissue content and plasma concentration of *L*-homocysteine (Mudd & Levy, 1978). Only in plasma of patients with homocysteinuria was *L*-homocysteine measured to be in the millimolar range (Mudd & Levy, 1978). On the basis of our results, and owing to the ubiquitous distribution of *S*-adenosylhomocysteine hydrolase (Eloranta, 1977), homocysteinuria is likely also to be associated with severe alterations in the metabolism of adenosine.

S-Adenosylhomocysteine is known to inhibit numerous *S*-adenosylmethionine-dependent methylation reactions (Usdin *et al.*, 1979), and inhibition of membrane phospholipid methylation was reported to influence coupling of β -adrenergic receptors to adenylate cyclase (Strittmatter *et al.*, 1979b) as well

as ion fluxes (Strittmatter *et al.*, 1979a). Although S-adenosylhomocysteine increased in the present study to extraordinary high values, which are in the range of myocardial ATP concentration [3940 nmol/g (Schrader & Gerlach, 1977)] contractile-force development of the heart remained unchanged. Furthermore, preliminary observations indicate that under these conditions the contractile response of the isolated heart to β -adrenergic stimulation does not change. Thus the significance of methylation reactions for the function of the heart remains to be elucidated.

W. S. is a Research Fellow of the Alexander von Humboldt Foundation, Germany. The skilful technical assistance of Mrs. Chr. Schäfer-Grunitz and Mrs. D. Kiesel is gratefully acknowledged.

References

- Baer, H. P. & Drummond, G. I. (eds.) (1979) *Physiological and Regulatory Functions of Adenosine and Adenine Nucleotides*, Raven Press, New York
- Berne, R. M., Foley, D. H., Watkinson, W. P., Miller, W. L., Winn, H. R. & Rubio, R. (1979) in *Physiological and Regulatory Functions of Adenosine and Adenine Nucleotides* (Baer, H. P. & Drummond, G. I., eds.), pp. 117–126, Raven Press, New York
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- de la Haba, G. & Cantoni, G. L. (1959) *J. Biol. Chem.* **234**, 603–608
- Dobson, J. G. (1978) *Circ. Res.* **43**, 785–793
- Eloranta, T. O. (1977) *Biochem. J.* **66**, 521–529
- Fox, J. H. & Kelley, W. N. (1978) *Annu. Rev. Biochem.* **47**, 655–686
- Hershfield, M. S. & Kredich, N. M. (1978) *Science* **202**, 757–760
- Kajander, O., Eloranta, T. & Raina, A. (1976) *Biochim. Biophys. Acta* **438**, 522–531
- Londos, C., Cooper, D. M. F., Schlegel, W. & Rodbell, M. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5362–5366
- Mudd, S. H. & Levy, H. L. (1978) in *The Metabolic Basis of Inherited Diseases* (Stanbury, J. B., Wyngaarden, J. B. & Frederickson, D. S., eds.), pp. 458–503, McGraw-Hill, New York
- Olsson, R. A. (1970) *Circ. Res.* **26**, 301–306
- Olsson, R. A., Davis, C. J., Khouri, E. M. & Patterson, R. (1976) *Circ. Res.* **39**, 93–98
- Paterson, A. R. P., Babb, L. R., Paran, J. H. & Cass, C. E. (1977) *Mol. Pharmacol.* **13**, 1147–1158
- Richards, H. H., Chiang, P. K. & Cantoni, G. L. (1978) *J. Biol. Chem.* **253**, 4476–4480
- Rubio, R., Berne, R. M. & Dobson, D. G. (1973) *Am. J. Physiol.* **225**, 938–953
- Schrader, J. & Gerlach, E. (1977) *Pflügers Arch.* **367**, 129–135
- Schrader, J. & Schütz, W. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 4279
- Schrader, J., Nees, S. & Gerlach, E. (1977a) *Pflügers Arch.* **369**, 251–257
- Schrader, J., Baumann, G. & Gerlach, E. (1977b) *Pflügers Arch.* **372**, 29–35
- Strittmatter, W. J., Hirata, F. & Axelrod, J. (1979a) *Biochem. Biophys. Res. Commun.* **88**, 147–153
- Strittmatter, W. J., Hirata, F. & Axelrod, J. (1979b) *Science* **204**, 1205–1207
- Usdin, E., Borchardt, R. T. & Creveling, C. R. (eds.) (1979) *Transmethylation*, Elsevier/North-Holland, Amsterdam