240 min of restoration of blood flow reaches the preischaemic values in control fractions, while it is significantly lower than the preischaemic in phenobarbitone fractions.

The data presented in table 1c and in table 2 indicate that, though the reduction of protein synthesis in ischaemic liver is mainly the consequence of a decreased ribosomal activity, the capacity of recovery after restoration of blood flow depends on the build-up in the cytosol of a critical amount of low mol. wt soluble inhibitors. The capacity of recovery is completely abolished after 120 min of ischaemia, when a ribosomal inhibition similar to that found after 60 min of ischaemia is coupled with a maximal amount of cytosolic inhibitors. Moreover, in phenobarbitone fractions, the capacity of recovery is significantly impaired (−25% in comparison with the control) already after 60 min of ischaemia, when a ribosomal inhibition similar to that found in control fraction is coupled to a higher cytosolic inhibition (+82% in comparison with the control). Though the existence of soluble inhibitors of protein synthesis has been postulated by many authors[12-14], their nature has never been exactly defined. On the basis of our data, 2 possibilities may be considered to interprete the nature of the cytosolic inhibitors: a) an increased amount of cold amino acids, deriving from the catabolism of proteins, which accumulate in the hepatocyte owing to the interruption of the blood flow, with a consequent dilution of the radioactive label added, and b) the build-up in the cytosol of true inhibitors of low mol. wt, such as NADH, ADP, Pi, which have been demonstrated to accumulate in the ischaemic hepatocyte[15] and to interfere at various levels with the extrinsecation of the protein synthetic process[16-18].

From our data it appears that, at variance with the results obtained in other pathological conditions such as starvation[7] and diabetes[19], phenobarbitone does not exert any positive effect on the rate of protein synthesis of the ischaemic and post-ischaemic hepatocyte. From the data shown in table 2, it appears that in phenobarbitone fraction a greater amount of cytosolic inhibitors accumulates earlier than in control fraction. Their identification with NADH and/or ADP and Pi previously proposed is further substantiated since phenobarbitone administration leads to an increased rate of turnover of ATP[20], and hence, at the onset of ischaemia, to an increased rate of formation of ADP, Pi and, for the known relationship[21], of NADH. The earlier accumulation of these inhibitors in phenobarbitone-treated ischaemic livers in comparison with the controls may explain the ineffectiveness of phenobarbitone to improve the capacity of the hepatocyte to recover from the ischaemic insult.

1 Acknowledgment. The authors thank Prof. A. Bernelli-Zazzera for helpful discussion and criticism on the manuscript.
2 F. Cajone, G. Ragnotti, A. Bernelli-Zazzera and M. Bassi, Exp. molec. Path. *14*, 392 (1971).
3 F. Cajone and L. Schiaffonati, Br. J. exp. Path. *55*, 575 (1974).
4 F. Cajone, L. Schiaffonati and A. Bernelli-Zazzera, Lab. Invest. *34*, 387 (1976).
5 R. Kato, L.A. Loeb and H.V. Gelboin, Biochem. Pharmac. *14*, 1164 (1965).
6 R. Kato, W.R. Jondorf, L.A. Loeb, T. Ben and H.V. Gelboin, Molec. Pharmac. *2*, 171 (1966).
7 G. Ragnotti and M.G. Aletti, Biochem. J. *146*, 1 (1975).
8 A. Bernelli-Zazzera and G. Gaja, Exp. molec. Path. *3*, 351 (1964).
9 J.S. Porterfield, Bull. W.H.O. *22*, 373 (1960).
10 W. Mejbaum, Hoppe-Seylers Z. Physiol. Chem. *258*, 117 (1939).
11 A. Korner, Biochem. J. *101*, 627 (1966).
12 A.J. Munro, R.J. Jackson and A. Korner, Biochem. J. *92*, 289 (1964).
13 A. Von der Decken, Eur. J. Biochem. *4*, 87 (1968).
14 W.J.P. Mainwaring, Biochem. J. *113*, 869 (1969).
15 G. Gaja, M.E. Ferrero, R. Piccoletti, A. Bernelli-Zazzera, Exp. molec. Path. *19*, 248 (1973).
16 H. Freudenberg and J. Mager, Biochim. biophys. Acta *232*, 537 (1971).
17 M.R.V. Murthy, Biochim. biophys. Acta *119*, 586 (1966).
18 S. Pestka, Am. Rev. Biochem. *40*, 697 (1971).
19 G. Ragnotti and M.G. Aletti, Biochim. biophys. Acta, in press.
20 R. Mc Cauley, J. O'Neill and D. Couri, Biochem. Pharmac. *21*, 1929 (1972).
21 R.L. Veech, L. Rajman and H.A. Krebs, Biochem. J. *117*, 499 (1970).

Effects of [N-(2-oxo-3,5,7-cycloheptatrien-1-yl)] aminooxoacetic acid ethyl ester (AY-25,674) on cyclic 3′,5′-nucleotide formation and phosphodiesterase activity

T.A. Pugsley and W. Lippmann[1]

Biochemical Pharmacology Department, Ayerst Research Laboratories, P.O. Box 6115, Montreal (Quebec, Canada H3C 3JI), 29 May 1978

Summary. The orally-effective antiallergic compound [N-(2-oxo-3,5,7-cycloheptatrien-1-yl)] aminooxoacetic acid ethyl ester (AY-25,674) exhibited a potency equivalent to or 3 times less than theophylline in inhibiting guinea-pig lung and beef heart PDE, respectively. AY-25,674 did not affect the basal activity of guinea-pig lung adenyl cyclase. Although part of the antiallergic activity of AY-25,674 may be due to the ability to elevate cyclic AMP levels by PDE inhibition, other modes of action appear to be of greater relevance.

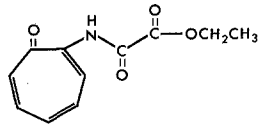
[N-(2-Oxo-3,5,7-cycloheptatrien-1-yl)] aminooxoacetic acid ethyl ester (AY-25,674, figure) is a new orally-effective antiallergic agent in the rat[2]. It inhibited passive paw-anaphylaxis in the rat and the antigen-induced release of histamine from sensitized rat mast cells[2]. In addition, the compound has been demonstrated to inhibit passive lung anaphylaxis in the rat[3].

Nucleoside-3′,5′-monophosphate phosphodiesterase (PDE) hydrolyzes cyclic AMP to AMP and is of relevance as a control mechanism for intracellular concentrations of cyclic AMP[4]. Various drugs which have been demonstrated to be PDE inhibitors have been associated with a variety of physiological responses. In this regard, the secretion of mediators of allergy in response to antigen challenge is inhibited by a number of agents which raise intracellular cyclic AMP concentration, e.g., β-adrenergic receptor agonists, PDE inhibitors (methylxanthines), prostaglandins and dibutryl cyclic AMP[5]. Moreover, the antiallergic drugs theophylline[4] and disodium cromoglycate (DSCG)[6] and also doxantrazole, a new potential antiallergic drug[7], have

been reported to inhibit PDE of beef heart, guinea-pig and human lung[8-12].

In the present study, the effects of AY-25,674 on cyclic AMP PDE activity of beef heart and guinea-pig lung were determined and compared with those of theophylline, DSCG, doxantrazole and 2'-amino-carbonyl-3'-methoxyoxanilic acid, ethyl ester (Wy-16,922), a new orally-effective antiallergic agent in the rat[13]. In addition, the effects of these compounds on basal cyclic AMP levels were measured in guinea-pig lung homogenates in vitro.

Methods and materials. The measurement of PDE activity of beef heart and guinea-pig lung were carried out according to previous methods[11,14-16]. The protein concentration was determined by the method of Lowry et al.[17]. The beef heart and guinea-pig lung PDE assays were carried out exactly as previously described by Lippmann[16] and Tateson and Trist[11], respectively. The IC_{50} was determined by plotting % inhibition versus the logarithm of the inhibitor concentration and is the inhibitor concentration producing a 50% reduction in enzyme reaction velocity compared with the non-inhibited controls. The measurement of adenyl cyclase activity of guinea-pig lung was carried out exactly as described by Weinryb et al.[18]. The materials used were beef heart PDE (Boehringer Mannheim, 15153 EPAY, control No. 7205306), Russel's viper venom (Sigma Chemical Co.), [8-^{3}H] cyclic AMP (sp.act. 21 Ci/mmole, Schwarz-Mann), [α-^{32}P]-ATP [adenosine-5'-triphosphate,tetra-(triethylammonium)salt (sp.act. 33.54 Ci/mmole, New England Nuclear)], Dowex I-X-2 (minus 400 mesh, chloride form) and Dowex 50 W-X-8 (100-200 mesh, hydrogen form, BioRad Laboratories), theophylline monoethanolamine (K&K Laboratories), doxantrazole (Wellcome Research Laboratories), Wy-16,922 (Wyeth Laboratories), and disodium cromoglycate (Fisons Ltd); AY-25,674 was synthesized by Dr J.F. Bagli, Chemistry Department, Ayerst Research Laboratories.



[N-(2-Oxo-3,5,7-cycloheptatrien-1-yl)]aminooxoacetic acid, ethyl ester: AY-25,674.

Table 1. Effect on cyclic AMP phosphodiesterase activities

Agent	Beef heart PDE inhibition: IC_{50} (μM)	Guinea-pig lung
AY-25,674	1515	1940
Theophylline	515	1475
DSCG	2750	$\simeq$ 10500* (10% at 2500 μM)
Doxantrazole	20	64
Wy-16,922	1340	$\simeq$ 7400*

Final cyclic AMP concentration: beef heart 0.117 μM; guinea-pig 10.0 μM. * Extrapolated value.

Table 2. Effect on the activity of guinea-pig lung adenyl cyclase

Agent	Concentration (M)	Stimulation of basal activity (%)
Isoproterenol	1×10^{-5}	76
Norepinephrine	1×10^{-4}	78
Epinephrine	1×10^{-4}	84
PGE_1	5×10^{-5}	25
AY-25,674	1×10^{-4}	7
	1×10^{-5}	0
Wy-16,922	2.0×10^{-3}	0
DSCG	2.5×10^{-3}	0
Theophylline	1×10^{-3}	5
Doxantrazole	1×10^{-5}	0

Activities of agents examined are expressed as the percent stimulation of basal activity which was 60.88 ± 2.78 pmoles/100 μg protein/15 min incubation.

Results and discussion. AY-25,674 inhibited guinea-pig lung and beef heart PDE in vitro displaying a potency similar to (guinea-pig lung PDE) or 3 times less (beef heart PDE) than theophylline (table 1). However, both AY-25,674 and theophylline are relatively weak as PDE inhibitors, e.g. as compared to doxantrazole; DSCG is even weaker in activity. Wy-16,922 exhibited a weaker inhibition of guinea-pig lung PDE than AY-25,674 and was equivalent to AY-25,674 with regard to inhibition of beef heart PDE.

None of the known or new antiallergic agents examined (table 2) stimulated the guinea-pig lung adenyl cyclase preparation, which lacks PDE activity, indicating a lack of a direct stimulation of cyclic AMP formation. Isoproterenol, norepinephrine, epinephrine and PGE_1 stimulated cyclic AMP formation (table 2) and this is presumably due to a direct effect on their respective receptors[5,19]. The lack of effect of Wy-16,922 on cyclic AMP formation is consistent with a reported lack of effect of this agent on cyclic AMP[13].

There is considerable evidence that the immunological release of inflammatory mediators, such as histamine and SRS-A, is inhibited by raised intracellular concentrations of cyclic AMP[5]. Although the precise mechanism by which cyclic AMP inhibits mediator release is not known, it may act by reducing mast cell membrane permeability to calcium[20]. Beta-adrenergic receptor stimulants, prostaglandins, histamine$_2$[H_2]-receptor stimulants and methylxanthines, such as theophylline, are thought to act by raising intracellular cyclic AMP levels either directly by receptor stimulation or indirectly by inhibition of PDE[5]. In vitro evidence indicates that DSCG[8] and doxantrazole[11] inhibit PDE and thus probably increase intracellular cyclic AMP levels; this action may explain, at least partly, the antiallergic activity of these agents[11,21]. The inhibition of guinea-pig and human lung PDE by DSCG observed by Roy and Warren[8] was not confirmed by Tateson and Trist[11], and according to the latter investigators, these findings thus cast doubt as to whether some of the antiallergic activity of DSCG is due to PDE inhibition. The inhibition of PDE by DSCG and doxantrazole in the present study are generally in agreement with those of Tateson and Trist[11]. The clinical antiallergic activity of doxantrazole[7] has not been firmly established[22]. Thus, although it is possible that part of the antiallergic activity of the agents examined may be attributed to inhibition of PDE, it would appear that under the conditions used in this study, other modes of action are of greater relevance with respect to the mechanisms of the antiallergic activity of AY-25,674, Wy-16,922, DSCG, and also theophylline[23].

Determination of the effect of AY-25,674 on cyclic nucleotide metabolism in lung mast cells, its effect on lung cyclic GMP PDE[24,25] and/or interaction with Ca^{+2} transport[20,26-28] may contribute further information with respect to the pharmacological mode of action of AY-25,674.

1 The authors acknowledge the excellent technical assistance of Ms M.T. Silvestre.

2 R.R. Martel, J. Klicius and J. Pinski, Can. J. Physiol. Pharmac., in press.

3 R. Greenberg, R. Martel and K. Smorong, Can. J. Physiol. Pharmac., in press.

4 G.A. Robinson, R.W. Butcher and E.W. Sutherland, A. Rev. Biochem. *37*, 149 (1968).
5 H.R. Bourne, L.M. Lichtenstein, K.L. Melmon, C.S. Henney, Y. Weinstein and G.M. Shearer, Science *184*, 19 (1974).
6 J.S.G. Cox, J.E. Beach, A.M.J.N. Blair, A.J. Clark, J. King, T.B. Lee, D.E.E. Loveday, G.F. Moss, T.S.C. Orr, J.T. Ritchie and P. Sheard, Adv. Drug Res. *5*, 115 (1970).
7 J.F. Batchelor, M.J. Follenfant, L.G. Garland, J.H. Gorvin, A.F. Green, H.F. Hodson, D.T.D. Hughes and J.E. Tateson, Lancet *1*, 1169 (1975).
8 A.C. Roy and B.T. Warren, Biochem. Pharmac. *23*, 917 (1974).
9 W.A. Taylor, D.H. Francis, D. Sheldon and I.M. Roitt, Int. Archs Allergy appl. Immun. *47*, 175 (1974).
10 W.A. Taylor and D. Sheldon, Int. Archs Allergy appl. Immun. *47*, 696 (1974).
11 J.E. Tateson and D.G. Trist, Life Sci. *18*, 153 (1976).
12 N. Lavin, G.S. Rachelefsky and S.A. Kaplan, J. Allergy clin. Immun. *57*, 80 (1976).
13 M.E. Rosenthale, A.J. Begany, A. Dervinis, J. Sellstedt, C. Guinosso and M.I. Gluckman, J. Pharmac. exp. Ther. *197*, 153 (1976).
14 G. Brooker, L.J. Thomas Jr and M.M. Appleman, Biochemistry *7*, 4177 (1968).
15 A.R. Somerville, M.-L. Rabouhans and A.A. Smith, Biochem. J. *120*, 11 p (1970).
16 W. Lippmann, Experientia *30*, 237 (1974).
17 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
18 I. Weinryb, I.M. Michel and S. Hess, Archs Biochem. Biophys. *154*, 240 (1973).
19 I. Weinryb and I.M. Michel, Experientia *27*, 1386 (1971).
20 J.C. Foreman, J.L. Mongar, B.D. Gomperts and L.G. Garland, Biochem. Pharmac. *24*, 538 (1975).
21 J.C. Foreman and L.G. Garland, Br. Med. J. *1*, 820 (1976).
22 R. Pauwels, H. Lamont and M. Van Der Stracten, Acta allerg. *31*, 239 (1976).
23 S. Norn, A. Geisler, P. Stahl Skov and R. Klysner, Acta allerg. *32*, 183 (1977).
24 C.J. Coulson, R.E. Ford, S. Marshall, J.L. Walker, K.R.H. Wooldridge, K. Bowden and T.J. Coombs, Nature *265*, 545 (1977).
25 H. Bergstrand, J. Kristoffersson, B. Lundquist and A. Schurmann, Molec. Pharmac. *13*, 38 (1977).
26 L.M. Lichtenstein, Int. Archs Allergy appl. Immun. *49*, 143 (1975).
27 J.L. Mongar and P. Svec, Br. J. Pharmac. *46*, 741 (1972).
28 F. Carpenedo, R.M. Gaion and G. Fassina, Biochem. Pharmac. *24*, 2069 (1975).

Decrease of serum alpha-tocopherol levels in rabbits after acute treatment with Δ^9-tetrahydrocannabinol[1]

M. Chiarotti and G.V. Giusti

Istituto di Medicina Legale, Università Cattolica del S. Cuore, Roma, and Cattedra di Medicina Legale dell'Università di Perugia, Terni (Italy), 2 May 1978

Summary. The i.v. administration of 10 mg of Δ^9-THC in rabbits produced, after 24 h, a 50% reduction of the initial values of serum alpha-tocopherol.

Our studies on chronic toxicity of Δ^9-THC (Δ^9-tetrahydrocannabinol) demonstrated the occurrence of a true muscular dystrophy in mice[2,3], and of a persistent alteration of bone marrow leucopoiesis in mice and rats[4,5] after 1-month treatment with Δ^9-THC at behavioral doses. Other authors reported sterility and infertility associated with testicular atrophy in experimental animals[6] and decreased serum testosterone levels in humans[7]. As these data are reconcilable with a relative alpha-tocopherol deficiency, the hypothesis of a possible interference of Δ^9-THC with serum alpha-tocopherol was taken into account.

Materials and methods. 10 male rabbits, weighing 2.5–3 kg, fed with a normal diet containing 14 mg/kg of alpha-tocopherol, were used. After 12 h fasting, 2–3 ml of blood was drawn from the auricular vein and about 1 ml of serum was obtained. Immediately after, rabbits were injected i.v. with 10 mg of Δ^9-THC suspended in the vehicle (1 ml of phosphate buffer pH 7.4 containing 2 drops of Tween 80). Samples of blood were drawn after 5 h and after 24 h. During this period, food was withheld. Control animals were injected with the vehicle alone.

Levels of alpha-tocopherol in serum were determined by gasliquid chromatography with solid injection, as described elsewhere[8].

Results and discussion. Results are summarized in the table and the figure. As can be seen, the injection of the vehicle and the food deprivation for 24 h have only a very limited influence on the alpha-tocopherol level, while the administration of Δ^9-THC produced a significant decrease of serum

Number	Δ^9-THC			Vehicle		
	t_0	t_1	t_2	t_0	t_1	t_2
1	1.33	0.48	0.63	0.96	0.93	0.96
2	1.14	1.22	0.58	1.20	1.86	1.33
3	1.14	1.00	0.85	0.53	0.43	0.56
4	1.89	0.56	0.40	3.80	3.20	3.00
5	1.68	0.53	0.45	1.60	1.20	1.00
6	1.62	1.60	1.57			
7	1.30	0.70	0.60			
8	0.45	0.10	0.13			
9	1.98	1.51	1.27			
10	1.50	0.80	0.60			
$\bar{X}$	1.40	0.86	0.71	1.61	1.52	1.37
SE	0.14	0.15	0.13	0.57	0.47	0.42

t_0, Concentrations of alpha-tocopherol in serum (mg‰) before the treatment; t_1, concentrations 5 h after treatment; t_2, concentrations 24 h after treatment.