Biosynthesis of Acetyl-Coenzyme A in the Electric Organ of *Torpedo marmorata* in Relation to Acetylcholine Metabolism

By MARIE-FRANCOISE DIEBLER* and YVETTE MOROT-GAUDRY†
Département de Neurochimie, Laboratoire de Neurobiologie Cellulaire, C.N.R.S.,
91190 Gif sur Yvette, France

(Received 24 January 1977)

Formation of acetyl-CoA through acetyl-CoA synthetase (forward reaction) and through choline acyltransferase (backward reaction) was investigated in tissue extract from the electric organ of *Torpedo marmorata*. When the tissue extract was submitted to gel filtration on Sephadex G-25, the formation of acetyl-CoA by acetyl-CoA synthetase appeared fully dependent on ATP and CoA and partially dependent on acetate (an endogenous supply of acetate is discussed). Choline acetyltransferase was a potent source of acetyl-CoA, only requiring acetylcholine and CoA, and was much more efficient than acetyl-CoA synthetase for concentrations of acetylcholine likely to be present in nerve endings.

The origin of acetyl-CoA used for the biosynthesis of acetylcholine has been investigated in animal tissues by several groups of workers. Although important data have been obtained from mammalian brain on the metabolites of the tricarboxylic acid cycle involved in the production of acetyl-CoA, its transport across the inner mitochondrial membrane to the cytoplasmic sites of acetylcholine synthesis have not been elucidated (Hebb, 1957; Browning & Schulman, 1968; Nakamura *et al.*, 1970; Söllenberg & Sorbö, 1970; Tuček, 1970; Israël & Tuček, 1974; Lefresne, 1974; Tuček & Cheng, 1974). Information on acetylcholine metabolism at the neuromuscular junction has been provided by studies on purely cholinergic materials. In contrast with brain, where pyruvate but not acetate is the precursor of acetylcholine (Israël & Tuček, 1974; Dreyfus, 1975), in the corneal epithelium, lobster nerve, the electric organ and the neuromuscular junction (Williams & Cooper, 1965; Browning & Schulman, 1968; Cheng & Nakamura, 1970; Israël & Tuček, 1974; Lefresne, 1974; Dreyfus, 1975) both these acetate and pyruvate appear to be incorporated.

It is known that the activation of acetate to acetyl-CoA involves the activity of acetyl-CoA synthetase (EC 6.2.1.1) according to the following overall reaction (Webster, 1967; Tuček, 1970):

* Present address: Groupe de Recherche sur le Développement et la Pathologie du Système Nerveux Central et du Muscle de l'Enfant, U 154, Hôpital St Vincent de Paul, 74 Avenue Denfert-Rochereau, 75014 Paris, France.

† To whom reprint requests should be addressed.

Because acetate serves as a precursor of acetylcholine and, like choline, it is recycled at the synaptic level (Israël & Dunant, 1975; see also MacIntosh & Collier, 1976), we have investigated the metabolic pathways involving the two enzymes, i.e. acetyl-CoA synthetase and choline acetyltransferase (EC 2.3.1.6). The formation of acetyl-CoA was therefore investigated in extracts of the electric organ of *Torpedo marmorata*.

Experimental

Preparation of tissue extracts

Torpedo marmorata were obtained from the Marine Station of Arcachon, France. Dorso-ventral slices of the electric organ (about 1 cm thick) were removed, cut into pieces and instantly frozen by immersion in isopentane cooled to -170°C . Each sample was ground in a mortar chilled with liquid N_2 and the powder was stored at -80°C until needed. Tissue extracts were made by suspending the powder in 10mM-potassium phosphate buffer, pH 7.4, at a 1:5 ratio (w/v) and the resulting suspension was centrifuged at $12000g$ (r_{av} , 10.8 cm) for 20 min. The supernatant fluid of the tissue extract was either used directly for assays or submitted to gel filtration. For this purpose, 0.5 ml portions were applied at 4°C on columns (1 cm $\times$ 6 cm) of Sephadex G-25 equilibrated and eluted with 10mM-potassium phosphate buffer, pH 7.4. Samples (0.5 ml) were collected and fraction 4 contained enzymic proteins but no ATP or other small molecules. Fractions 4 from several columns were pooled for enzymic determinations.

Enzyme assays

Acetyl-CoA synthetase (forward reaction). Activity was measured as the rate of acetyl-CoA formation from CoA, ATP and acetate as described by Wieland & Weiss (1963). Assays were buffered at pH 7.4 with 10 mM-potassium phosphate buffer and included (final vol. 1.5 ml): 117 mM-KCl; 13 mM-ATP (dipotassium salt); 13 mM-MgCl₂; 0.6 mM-CoA (trilithium salt); 1.75 mM-potassium acetate. Reaction was begun by the addition of tissue extract (0.1–0.3 ml) to preincubated substrates and stopped by heat inactivation (100°C, 5 min). Experiments were carried out at 38°C for 0 min (blank controls) and for 45 min (samples). To avoid errors in calculation (Pearson, 1965; Bergmeyer & Möllering, 1966) an internal standard of acetyl-CoA was then added to each sample. For this purpose, the incubated mixture, cooled for 2 min on ice, was subdivided into three fractions (each 0.5 ml), and acetyl-CoA (6 or 12 nmol) was introduced into two of them. The amount of acetyl-CoA in each test tube was determined at 25°C in the presence of 400 mM-Tris/HCl, pH 8.4, 0.32 mM-NAD⁺, 0.75 mM-malate and 22 units (μ mol/min) of malate dehydrogenase. After equilibrium, citrate synthase (11 units; μ mol/min) was added. The concentration of acetyl-CoA was linearly related to NADH fluorescence (340–460 nm) measured before and after the addition of citrate synthase.

Choline acetyltransferase (forward reaction). Enzymic activity was measured at 25°C as the rate of acetylation of choline with labelled acetyl-CoA as described by Fonnum (1975).

Choline acetyltransferase (backward reaction). This was measured under the same experimental conditions as acetyl-CoA synthetase. Acetylcholine chloride was added at various concentrations (0–200 mM) in either the presence or the absence of ATP and acetate. Eserine salicylate (0.1 mM final concn.) was mixed with the tissue extract. In some experiments this assay was combined with a radio-metric method. The tissue extract was filtered on a column of Sephadex G-25 and the protein fraction no. 4 was used as the enzyme source. [³H]Acetylcholine (300 μ Ci/ml, specific radioactivity 15 mCi/mmol) and 20 mM-acetylcholine were added to the incubation medium. Blanks contained boiled enzyme preparations. The rate of synthesis of unlabelled acetyl-CoA was determined as described above and simultaneously [³H]acetyl-CoA was measured in portions of each reaction mixture. For that purpose, after stopping the reaction as described above, 10 μ l of medium was spotted on a cellulose F plate (20 cm $\times$ 20 cm) (Merck, Darmstadt, Germany). The plate was run in the upper phase of the solvent system butan-1-ol/acetic acid/water (4:1:5, by vol.) for 4 h. The absorption band of the acetyl-CoA was detected with an u.v. lamp and was compared with an external

standard (R_f = 0.15). The area of cellulose gel corresponding to this band was removed and transferred to 0.2 ml of ethanol. Before counting for radioactivity, extracts were mixed with 10 ml of a scintillation cocktail prepared as described by Dunant *et al.* (1972).

ATP assay. The ATP assay was carried out immediately after the preparation of the supernatant fluid or the collection of the fractions from the Sephadex G-25 column. The method used was that with firefly luciferin-luciferase as previously described (Strehler, 1974), with minor modifications (Meunier, 1976).

Efficiency of Sephadex G-25 column. The efficiency of the Sephadex G-25 column was tested by filtering 0.5 ml of the crude extract preincubated for 0, 15, 30 and 45 min with [¹⁻¹⁴C]acetate (0.05 μ Ci); radioactivity in the effluents was determined in 0.1 ml portions mixed with liquid-scintillation-counting medium.

Protein assay

Protein was determined as described by Lowry *et al.* (1951), with bovine serum albumin as standard.

Materials

These were purchased as follows: [¹⁻¹⁴C]acetate (50 mCi/mmol), [³H]acetylcholine (250 mCi/mmol), [¹⁻¹⁴C]acetyl-CoA (58 mCi/mmol), The Radiochemical Centre, Amersham, Bucks., U.K.; CoA, acetyl-CoA, malate dehydrogenase, citrate synthase, Boehringer, Mannheim, Germany; acetylcholine (chloride), NAD⁺, ATP (dipotassium salt), firefly luciferin-luciferase, Sigma Chemical Co., St Louis, MO, U.S.A. Other materials were purchased from Merck, Darmstadt, Germany.

Results

Production of acetyl-CoA by acetyl-CoA synthetase

In the first set of experiments, we attempted to evaluate the activity of acetyl-CoA synthetase in the electric organ of *Torpedo marmorata*. This tissue activates acetate to acetyl-CoA at the rate of 20.01 ± 1.69 nmol/min per g wet wt. (mean $\pm$ s.e.m.; n = 9). The corresponding specific activity was 1.7 ± 0.14 nmol/min per mg of protein.

Control experiments showed that, in the absence of CoA, 98% of the enzyme activity disappeared. A marked decrease of acetyl-CoA synthesis, by 87, 97 and 62%, was obtained after omission of ATP, MgCl₂ and acetate respectively (Table 1). As noted below, the residual activity was due to the presence of CoA, ATP and acetate in the crude extract. For acetate, Fig. 1 shows that there is enough acetate in the extract to support the synthesis at half its maximum rate; the endogenous concentration can therefore be estimated as approx. 1.3 mM.

Table 1. Substrate requirements of acetyl-CoA synthetase activity of a crude tissue extract

Acetyl-CoA synthetase activity was measured at 38°C as described in the Experimental section.

Incubation medium	Acetyl-CoA formation (nmol/min per g wet wt.)	Activity (%)
Complete system	26.5	100
Minus CoA	0.45	2
Minus ATP	3.5	13
Minus acetate	10.0	38
Minus MgCl ₂	0.9	3

Fig. 1. Effect of increasing concentrations of acetate on the rate of acetyl-CoA synthesis in tissue extract

The rate of acetyl-CoA synthesis (nmol/min per g wet wt.) was determined in a tissue extract at 38°C in the presence of 0.6mM-CoA, 13mM-ATP, 13mM-MgCl₂, 117mM-KCl, and at a range of acetate concentrations (mM). For details, see the Experimental section.

To remove CoA, ATP and acetate from the crude extract we submitted it to gel filtration on Sephadex G-25. Measurements of radioactive acetate or endogenous ATP in the effluent of the column showed that they were recovered after the protein peak (Fig. 2). The enzymic activity measured in fraction 4 from this column showed a complete requirement for CoA and ATP (Figs. 3b, 3c). Apparent *K_m* values for CoA and ATP were estimated as respectively 0.05 and 0.4mM (Figs. 3a, 3b, 3c).

In spite of the fact that acetate was efficiently removed by gel filtration, a source of acetate still persisted in the protein fraction no. 4 and was able to support acetyl-CoA synthesis at its maximum rate (Fig. 3a). This result suggests that the source of acetate present in this fraction has a relatively high molecular weight and no exchange between this source and radioactive acetate added to the extract before gel filtration takes place (Fig. 2c).

Fig. 2. Chromatography on Sephadex G-25 of tissue extract. Chromatography on Sephadex G-25 of 0.5ml of a tissue extract. Effluent volume per fraction was 0.5 ml (except fraction no. 2, 1ml). Elution pattern of: (a) protein (mg/ml); (b) ATP (nmol/ml); (c) [1-¹⁴C]-acetate (c.p.m./ml). For details, see the Experimental section.

Production of acetyl-CoA by choline acetyltransferase

Choline acetyltransferase is a relatively reversible enzyme (Potter *et al.*, 1968; White & Wu, 1973), but it was surprising to find that even at its maximum rate, the production of acetyl-CoA by acetyl-CoA synthetase was much less efficient than by the backwards reaction of choline acetyltransferase at substrate concentrations likely to be found in nerve endings. Fig. 4 shows the effect of increasing concentrations of acetylcholine (0–200mM) on the synthesis of acetyl-CoA, expressed as a percentage of a control from which acetylcholine was omitted. The control therefore represents solely the production of acetyl-CoA by acetyl-CoA synthetase. An increase of about 10-fold was found for 27mM-acetylcholine, which is the concentration estimated in the nerve endings (Dunant *et al.*, 1974). For higher acetylcholine concentrations, the increase is as high as 30-fold.

The demonstration that the reverse activity of choline acetyltransferase was indeed responsible for the acetyl-CoA synthesis described was supported by the following facts. (1) Choline acetyltransferase was present in the protein fraction. After gel filtration, its activity was tested in the presence of [¹⁴C]acetyl-CoA and choline, by the method of Fonnum (1975) (Fig. 5). (2) The high increase in the synthesis of

Fig. 3. Kinetics of acetyl-CoA synthetase activity (forward reaction)

Rate of acetyl-CoA synthesis (nmol/min per mg of protein) was determined in fraction 4 (Fig. 2) as a function of: (a) CoA concentration (mM) with 13 mM-ATP as the fixed substrate and at the following acetate concentrations of 0 (●), 0.5 (▲), 1.0 (△) and 1.75 (○) mM; (b) CoA concentration (mM) with 1.75 mM-acetate as the fixed substrate and at ATP concentrations of 0 (●), 0.13 (▲), 0.25 (△), 0.65 (○), 1.3 (■) and 13 (□) mM; (c) ATP concentration (mM) with 1.75 mM-acetate as the fixed substrate and at CoA concentrations of 0 (●), 0.03 (▲), 0.21 (△) and 0.6 (○) mM.

Fig. 4. Effect of increasing concentrations of acetylcholine on acetyl-CoA formation in the tissue extract

Formation of acetyl-CoA was measured in a tissue extract in the presence of 0.6 mM-CoA, 13 mM-ATP, 13 mM-MgCl₂, 1.75 mM-acetate, 117 mM-KCl and at a range of acetylcholine concentrations (mM). Results are expressed as percentage of the activity of controls where acetylcholine was omitted from the incubation medium.

Fig. 5. Elution pattern of choline acetyltransferase activity after gel filtration of the tissue extract

Chromatography on Sephadex G-25 of a 0.5 ml sample of tissue extract. Effluent volume per fraction was 0.5 ml (except no. 2, 1 ml). Elution profile of: (a) proteins (mg/ml); (b) forward reaction of choline acetyltransferase activity expressed as acetylcholine formation (nmol/min per ml of fraction) and determined by the method of Fonnum (1975).

acetyl-CoA did not require the presence of ATP and acetate but was dependent on the amount of CoA (apparent K_m for CoA, approx. 0.3 mM) (Fig. 6). (3) As expected if the observed increase in acetyl-CoA was due to backward reaction of choline acetyl-

Fig. 6. Kinetics of choline acetyltransferase (backward reaction)

Rate of acetyl-CoA synthesis (nmol/min per mg of protein) was determined in a protein fraction (fraction no. 4). Results are shown as double-reciprocal plots with: (a) $[S] = \text{CoA}$ (mM) as the variable substrate, at acetylcholine concentrations of 5 ($\blacktriangle$), 10 ($\triangle$) and 20 ($\circ$) mM; apparent K_m for CoA, approx. 0.3 mM; (b) $[S] = [\text{acetylcholine}]$ (mM) as the variable substrate, at CoA concentrations of 0.09 ($\blacktriangle$), 0.3 ($\triangle$) and 0.6 ($\circ$) mM; apparent K_m for acetylcholine was 50 mM. Incubation mixture contained also 13 mM-MgCl₂, 117 mM-KCl, no ATP and no acetate.

Fig. 7. Effect of increasing concentrations of choline on the acetyl-CoA formation by choline acetyltransferase working backwards

Assays were performed on protein fraction 4. The rate is expressed as a percentage of a control where choline was omitted from the incubation medium; experiments were made with 10 ($\blacktriangle$) and 20 ($\bullet$) mM-acetylcholine. Incubation mixture contained also 13 mM-MgCl₂, 117 mM-KCl, no ATP and no acetate. For details, see the Experimental section.

Table 2. Choline acetyltransferase activity (backward reaction) in crude tissue extract or in protein fraction. The acetyl-CoA formation through choline acetyltransferase activity (backward reaction) was measured in the tissue extract or in protein fraction 4 (Fig. 5), (a) as incorporation of the [³H]acetyl moiety of labelled [³H]acetylcholine into [³H]acetyl-CoA (expressed as c.p.m./min per mg of protein) and (b) as rate of acetyl-CoA synthesis (expressed as nmol/min per mg of protein). Incubation mixture contained: 0.6 mM-CoA, 0.150 mCi of [³H]acetylcholine, 20 mM-acetylcholine, 13 mM-MgCl₂, 117 mM-KCl.

	$10^{-2} \times [\text{H}] \text{Acetyl-CoA synthesis}$ (c.p.m./min per mg of protein)	Rate of acetyl-CoA synthesis (nmol/min per mg of protein)
Tissue extract	52	1.1
Protein extract (fraction no. 4)	60	1.3

Discussion

It has been shown that synapses of neuromuscular type (electric organ of *Torpedo marmorata*, or neuromuscular junction of the rat) utilize acetate as a preferential precursor for acetylcholine synthesis (Israel & Tuček, 1974; Dreyfus, 1975). It was useful to study the activation of acetate to acetyl-CoA in electric tissue. No data on the acetyl-CoA synthetase was previously available for the electric organ. Moreover, information on the brain acetyl-CoA synthetase comes from a tissue which does not accept acetate as an acetylcholine precursor (Schuberth, 1965; Tuček, 1967; Neidle *et al.*, 1969; Aas, 1971; Tencati & Rosenberg, 1973; Reijnierse *et al.*, 1975, 1976).

transferase, we were able to reverse it partially by adding choline to the incubation medium. Fig. 7 shows that choline acetyltransferase working backwards in the presence of 5 mM-choline and 20 mM-acetylcholine, is still as efficient as acetyl-CoA synthetase. (4) Finally, when [³H]acetylcholine was added to the incubation medium, it was shown that the label was recovered in the acetyl moiety of the acetyl-CoA newly synthesized by the tissue extract or the protein fraction no. 4 (Table 2).

In optimum conditions, acetyl-CoA synthetase activity was 20 nmol/min per g wet wt. of tissue. The kinetic parameters of the enzyme were determined; apparent K_m values for CoA, ATP and acetate were respectively 0.05, 0.4 and 0.035 mM. When soluble acetate was removed from the tissue extract by gel filtration, the protein fraction (no. 4) was still able to support the acetate requirement of acetyl-CoA synthetase activity at its maximum rate. This result was surprising, and several hypotheses have been considered. If the acetyl-CoA synthetase was present in fraction 4 already as an acyl-enzyme, it would no longer require ATP (Webster, 1967), and this was not the case. It was also possible to rule out specific or non-specific adsorption of acetate on proteins, since no exchange took place with radioactive acetate even for a long incubation time (45 min). Presumably a high-molecular-weight acetate donor is present in fraction 4, and further work will be necessary to identify it.

More surprising was the finding that acetyl-CoA synthetase was 30 times less efficient in producing acetyl-CoA than choline acetyltransferase (reverse reaction) when these were optimum conditions for their respective substrates. This result led us to consider the origin of acetyl-CoA in the nerve endings, where high acetylcholine and CoA concentrations are expected to be found. It is known that acetylcholine concentration in nerve endings is about 30 mM (Dunant *et al.*, 1974). The residual acetyl-CoA synthetase activity in a tissue extract results from an endogenous CoA concentration of 0.8 mM (see Table 1). As the extracellular space is 70% of the total volume of the tissue, we can assume a cellular CoA concentration of at least 2.7 mM. It seems reasonable to suppose that at these concentrations choline acetyltransferase working backwards is efficiently producing acetyl-CoA. Moreover, it is well known that CoA, and to a lesser extent acetylcholine, inhibit the forward reaction at these concentrations (Kaita & Goldberg, 1969; White & Cavallito, 1970; Glover & Potter, 1971; White & Wu, 1973). We have verified that this was also true for *Torpedo* choline acetyltransferase and we have obtained 70–90% inhibition of acetylcholine synthesis at these concentrations. The only limitation to the backward reaction of choline acetyltransferase activity would be the presence of large amounts of choline in the nerve endings. With an acetylcholine/choline molar ratio of 4:1 the backward choline acetyltransferase reaction still produces as much acetyl-CoA as does acetyl-CoA synthetase. It would be necessary to suppose that all the choline measured in the tissue (200–1000 μ M) (Israël *et al.*, 1973) is present in the nerve endings (4% of the tissue volume), to give a concentration of 20 mM which is able to block the backward choline acetyltransferase reaction. This is extremely unlikely. If choline was uniformly distributed

throughout the tissue, it would not exceed 3 mM in the nerve ending, a concentration not able to reverse appreciably the backward reaction of choline acetyltransferase.

Our results suggest that fluctuations of both enzyme activities either in the forward or in the backward direction will occur in conditions where transmitter concentration will decrease, triggering uptake of choline and acetate in the nerve endings.

This work was supported by the Centre National de la Recherche Scientifique, France. We are grateful to Mrs. Paule Mastour-Franchon for technical assistance, to Dr. M. Israël, Dr. N. Morel and Dr. S. Tuček for helping to start this work and to Dr. P. Lefresne for critical discussion.

References

- Aas, M. (1971) *Biochim. Biophys. Acta* **231**, 32–47
- Bergmeyer, H. U. & Möllering, H. (1966) *Biochem. Z.* **344**, 167–189
- Browning, E. T. & Schulman, M. P. (1968) *J. Neurochem.* **15**, 1391–1405
- Cheng, S. C. & Nakamura, R. (1970) *Biochem. J.* **118**, 451–455
- Dreyfus, P. (1975) *C. R. Hebd. Séances Acad. Sci. Ser. D* **280**, 1893
- Dunant, Y., Gautron, J., Israël, M., Lesbats, B. & Manaranche, R. (1972) *J. Neurochem.* **19**, 1987–2002
- Dunant, Y., Gautron, J., Israël, M., Lesbats, B. & Manaranche, R. (1974) *J. Neurochem.* **23**, 635–643
- Fonnum, F. (1975) *J. Neurochem.* **24**, 407–409
- Glover, V. A. S. & Potter, L. T. (1971) *J. Neurochem.* **18**, 571–580
- Hebb, C. D. (1957) *Physiol. Rev.* **37**, 196–220
- Israël, M. & Dunant, Y. (1975) in *Metabolic Compartmentation and Neurotransmission* (Berl, S., Clarke, D. D. & Schneider, D., eds.), pp. 621–639, Plenum Publishing Corporation, New York
- Israël, M. & Tuček, S. (1974) *J. Neurochem.* **22**, 487–491
- Israël, M., Hirt, L. & Mastour-Franchon, P. (1973) *C. R. Hebd. Séances Acad. Sci. Ser. D* **276**, 2725–2728
- Kaita, A. & Goldberg, M. (1969) *J. Neurochem.* **16**, 1185–1191
- Lefresne, P. (1974) Thèse de Doctorat d'Etat, Université de Paris VI
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- MacIntosh, F. C. & Collier, B. (1976) in *Handbuch der Experimentellen Pharmakologie* (Born, G. V. R., Eichler, O., Farah, A., Herken, H. & Welch, A. D., eds.), vol. 42, chapter 2, pp. 99–228, Springer-Verlag, Berlin, Heidelberg and New York
- Meunier, F. M. (1976) Thèse de Doctorat IIIe Cycle, Université de Paris VII
- Nakamura, R., Cheng, S. C. & Naruse, H. (1970) *Biochem. J.* **118**, 443–450
- Neidle, A., Van den Berg, C. J. & Grynbaum, A. (1969) *J. Neurochem.* **16**, 225–234
- Pearson, D. J. (1965) *Biochem. J.* **95**, 23 c–24 c

- Potter, L. T., Glover, A. S. & Saelens, J. K. (1968) *J. Biol. Chem.* **243**, 3864–3870
- Reijnierse, G. L. A., Veldstra, H. & Van den Berg, C. J. (1975) *Biochem. J.* **152**, 477–484
- Reijnierse, G. L. A., Veldstra, H. & Van den Berg, C. J. (1976) *Anal. Biochem.* **72**, 614–622
- Schuberth, J. (1965) *Biochim. Biophys. Acta* **98**, 1–7
- Söllenberg, J. & Sorbö, B. (1970) *J. Neurochem.* **17**, 201–207
- Strehler, B. L. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), 2nd edn., vol. 4, pp. 2112–2121, Verlag Chemie, Weinheim, and Academic Press, New York and London
- Tencati, J. R. & Rosenberg, R. N. (1973) *Biochim. Biophys. Acta* **293**, 542–551
- Tuček, S. (1967) *J. Neurochem.* **14**, 531–545
- Tuček, S. (1970) in *Drugs and Cholinergic Mechanisms in the Central Nervous System* (Heilbronn, E. & Winter, A., eds.), pp. 117–131, Försvarets Forskningsanstalt, Stockholm
- Tuček, S. & Cheng, S. C. (1974) *J. Neurochem.* **22**, 893–914
- Webster, L. T. (1967) *J. Biol. Chem.* **242**, 1232–1240
- White, H. L. & Cavallito, C. J. (1970) *Biochim. Biophys. Acta* **206**, 343–358
- White, H. L. & Wu, J. C. (1973) *J. Neurochem.* **20**, 297–307
- Wieland, O. & Weiss, L. (1963) *Biochem. Biophys. Res. Commun.* **10**, 333–338
- Williams, J. D. & Cooper, J. R. (1965) *Biochem. Pharmacol.* **14**, 1286–1289