Identity of Brain Alcohol Dehydrogenase with Liver Alcohol Dehydrogenase

By R. JULIAN S. DUNCAN,* JOHN E. KLINE and LOUIS SOKOLOFF

*Laboratory of Cerebral Metabolism, National Institute of Mental Health,
U.S. Department of Health, Education and Welfare, Public Health Service, Bethesda, MD 20014, U.S.A.*

(Received 8 September 1975)

A method for obtaining electrophoretically homogeneous rat liver alcohol dehydrogenase (EC 1.1.1.1) at a specific activity of 2–2.5 $\mu\text{mol/min per mg}$ of protein is presented. Antisera prepared against the purified enzyme inhibit alcohol dehydrogenase by up to 75% and cause precipitation of virtually all the enzyme. The antisera were shown by immunoelectrophoresis of a partially purified liver homogenate to be specifically directed against alcohol dehydrogenase and were used to demonstrate that the alcohol dehydrogenases of rat brain and liver share common antigens. The total activity of alcohol dehydrogenase in rat brain homogenates is normally quite low, with as much as 10% of the total activity attributable to the activity in the blood contained within the brain; in cases of severe liver damage (induced experimentally with carbon tetrachloride) this contribution may rise to as much as 60%.

Until relatively recently the question of whether brain tissue has the enzymic capacity to oxidize ethanol was unresolved (Dewan, 1943; Towne, 1964), but Raskin & Sokoloff (1968) demonstrated the ethanol- and NAD^+ -dependent reduction of lactaldehyde by the soluble fraction of rat brain. The kinetic and inhibition characteristics of this reaction were similar to but not identical with those of the same coupled reaction catalysed by liver alcohol dehydrogenase. Several other workers have confirmed the observation of alcohol dehydrogenase activity in brain homogenates (Rawat *et al.*, 1973; Taberner, 1974; Tabakoff & von Wartburg, 1975), but the question remained open as to whether the enzymes from brain and liver were the same.

The coupled oxidation of ethanol with reduction of lactaldehyde by alcohol dehydrogenase in the presence of NAD^+ is considerably faster than the oxidation of ethanol by the enzyme with NAD^+ alone (Woodley & Gupta, 1972). It is therefore possible to measure the very low enzyme activity in homogenates of brain by means of this sensitive coupled assay, but it is not possible to study other properties of the enzyme, particularly its specificity, without considerable concentration and purification of the enzyme activity. This approach is complicated, however, by the possibility that the alcohol dehydrogenase activity of the brain homogenate that is concentrated and purified may have been derived from the enzyme activity in the blood contained within the brain homogenate rather than from the cerebral

tissues themselves. The question of the existence of alcohol dehydrogenase activity within the cerebral tissues has become particularly relevant since Mukherji *et al.* (1975) reported that [^{14}C]ethanol was not incorporated into amino acids by perfused rat brain (which would presumably have been washed free of blood), although [^{14}C]acetaldehyde was readily incorporated.

The present studies demonstrate that in the normal rat there is alcohol dehydrogenase activity in brain appreciably above that contributed by the blood, and that the brain enzyme shares common antigens with the liver enzyme. This observation suggests that the large body of knowledge about liver alcohol dehydrogenase can be applied to the brain enzyme.

Methods

Enzyme assays

Liver alcohol dehydrogenase was assayed as a routine by the spectrophotometric method of Markovic *et al.* (1971) at pH 10 in 100 mM-sodium glycinate buffer containing 6 mM-ethanol and 1.5 mM- NAD^+ . The enzyme was also assayed in the direction of aldehyde reduction in a system containing 100 mM-sodium potassium phosphate, pH 7.6, 250 μM - NADH and an appropriate aldehyde concentration. The initial rate of change in E_{340} was used as the measure of enzyme activity. Brain and liver alcohol dehydrogenases were also measured by the propane-diol assay described by Raskin & Sokoloff (1970); when the purified liver enzyme was assayed in this way, its protein content was diluted 1000-fold with appropriate amounts of 1% (w/v) bovine serum

* Present address: Department of Child Health, University of Manchester, Oxford Road, Manchester M13 9PT, U.K.

albumin in 100mM-phosphate buffer, pH 7.6, before assay. All enzyme assays were carried out at 30°C. One unit of activity is the amount of enzyme that catalyses the production of 1 μ mol of NAD(H) or 1 μ mol of propanediol from the corresponding substrate in 1 min.

In experiments on the effect of antisera on alcohol dehydrogenase the enzyme was incubated at 30°C for 15 min with the serum, and then the other components of the assay system were added. In experiments in which various amounts of antiserum were used, serum from an unimmunized rabbit was added to ensure that the total serum concentration remained constant.

Protein was measured by the method of Lowry *et al.* (1951) with bovine serum albumin as the standard.

Purification of liver alcohol dehydrogenase

Alcohol dehydrogenase was purified from rat liver by a method based on those of Markovic *et al.* (1971) and Andersson *et al.* (1974). All fractionations on columns were performed at room temperature (22–25°C) except where otherwise indicated. Other steps were carried out at 0–4°C. Unless noted to the contrary, all buffers contained 2.5mM-dithiothreitol.

For a typical preparation 75g of frozen rat liver (type I rat liver; Pel-Freez, Rogers, AR, U.S.A.) was fragmented into small pieces, thawed, washed thoroughly in cold water, and then homogenized by means of a Polytron homogenizer (Kinematica G.m.b.H., Lucerne, Switzerland) in 225 ml of buffer (50mM-Tris/HCl, pH 8.4). The homogenate was centrifuged at 35 000g for 45 min, and the supernatant was fractionated with solid $(\text{NH}_4)_2\text{SO}_4$. During the addition of the salt the pH was maintained between 8.0 and 8.4 with 2M-Tris base solution. Material precipitating between 0.21 and 0.485 g of $(\text{NH}_4)_2\text{SO}_4$ /ml of solution was resuspended in 37 ml of 15mM-Tris hydrochloride/NaOH, pH 8.5 (buffer 1), and after centrifugation at 39 000g for 10 min to remove insoluble matter was passed through a column (50cm $\times$ 4.5cm; flow rate 20ml/cm² per h) of Sephadex G-25 equilibrated in buffer 1. Fractions from the Sephadex G-25 column which contained alcohol dehydrogenase were pooled and passed through a column (20cm $\times$ 4.5cm; flow rate 16ml/cm² per h) of DEAE-cellulose (DE52; Whatman, Maidstone, Kent, U.K.) equilibrated in buffer 1. The enzyme, which was retarded slightly on this column, was eluted with the same buffer; the active fractions were cooled in ice, pooled, titrated to pH 7.0 with 1M- KH_2PO_4 , and then immediately passed through a column (5cm $\times$ 1.4cm; flow rate 50ml/cm² per h) of CM-cellulose (CM52) at 4°C. This column had been equilibrated with buffer 1, which had been cooled and then titrated to pH 7.0 with 1M- KH_2PO_4 . Alcohol dehydrogenase is not retarded under these conditions.

Active fractions were pooled, titrated to pH 8.0 with 2M-Tris base, then passed through an affinity column (2.5cm $\times$ 1.4cm; flow rate 40ml/cm² per h) of AMP-agarose (AG-AMPTM type 2; P-L Biochemicals, Milwaukee, WI, U.S.A.) at 4°C. Loosely bound protein was washed off the column with buffer 1 at 4°C, containing 100 μ M-NAD⁺. Alcohol dehydrogenase was then specifically eluted by the addition of 1mM-pyrazole to this buffer system (Andersson *et al.*, 1974). Those fractions containing protein (it was not possible to test for activity in the presence of this concentration of pyrazole) were pooled, warmed to room temperature, and passed through a column (1.5cm $\times$ 1.4cm; flow rate 48ml/cm² per h) of hydroxyapatite (Bio-Gel HT; Bio-Rad Labs, Richmond, CA, U.S.A.). Protein was adsorbed to the column, but the NAD⁺ and pyrazole were not adsorbed and were removed by washing the column with buffer 1 containing 5% ethanol but no dithiothreitol. Alcohol dehydrogenase was eluted with a solution containing 15mM-Tris hydrochloride, 50mM-potassium phosphate, pH 8.0, 5% (v/v) ethanol, but no dithiothreitol.

For some purposes a less highly purified enzyme was required. In these cases the liver extract was treated as described up to but not including the DEAE-column step and was then dialysed against the same buffer solution as was used to elute the enzyme from hydroxyapatite.

Electrophoresis

Polyacrylamide-gel electrophoresis was done on continuous gels containing 5% (w/v) acrylamide (Deibler *et al.*, 1972). The pH 2.4 system, for which the gel is cast in the presence of 8M-urea and for which the electrode and gel buffer is 1M-acetic acid, was generally used, but the pH 7.0 and 8.6 systems were also used. Before being layered on the gel the protein solution was mixed with an equal volume of 8M-urea in water (for the pH 2.4 gels) or with a few crystals of sucrose for the other systems. The normal protein load was 30–50 μ g/gel. Electrophoresis was carried out at 2.5mA/gel for 90 min at pH 2.4 and for 4 h at other pH values. Gels were stained for protein with Amido Schwarz (diffusion destaining) or for alcohol dehydrogenase activity with 1.5mM-NAD⁺, 6mM-ethanol, phenazine methosulphate (0.03mg/ml) and Nitro Blue Tetrazolium (0.3mg/ml) in 100mM-Tris/HCl, pH 8.6. Stained gels were scanned at 590 or 600nm with a Gilford gel-scanning instrument.

Cellulose acetate electrophoresis was performed on Titan IIITM plates (Helena Labs, Beaumont, TX, U.S.A.) with 25mM-histidine hydrochloride/Tris base, pH 6.0, as the plate buffer and the same buffer at a concentration of 100mM in the electrode wells. Electrophoresis was continued at 2mA per plate for 40 min. For immunoelectrophoresis the plate was loaded with approx. 10 μ g of purified liver alcohol

dehydrogenase and with 75–100 μ g of a partially purified liver enzyme preparation at separate spots on opposite sides of the centre line of the plate. After electrophoresis approx. 5 μ l of serum from an immunized rabbit was loaded along the centre line of the plate and diffusion allowed to occur for 36 h at 4°C. Soluble protein was removed from the plate by gentle agitation for 1 h with 0.9% (w/v) NaCl at room temperature. Protein still adhering to the plate was stained with Ponceau S.

Preparation of antibodies

Purified liver alcohol dehydrogenase was freeze-dried and then dissolved in sufficient 0.9% (w/v) NaCl to give a protein concentration of 2 mg/ml. The enzyme solution was emulsified with an equal volume of complete Freund's adjuvant and injected subcutaneously into young adult rabbits at a dose of 1 mg of alcohol dehydrogenase per animal. The rabbits were given identical booster injections at intervals of 2 weeks. Blood samples were taken from ear vessels.

Immunoglobulins against alcohol dehydrogenase were removed from some samples of antiserum by treatment of the antiserum (2 ml, about 140 mg of protein) with purified alcohol dehydrogenase (about 500 μ g). The relative amounts of alcohol dehydrogenase and serum were determined from a titration curve of the type shown in Fig. 2. After a period of 1 h at 0°C for the antigen-antibody reaction to be completed, the mixture was centrifuged, and the supernatant stored at 4°C until used. Other samples of the antiserum were treated with an appropriate volume of the enzyme buffer before use.

Blood alcohol dehydrogenase

The concentration of alcohol dehydrogenase in blood was changed by induction of liver damage with carbon tetrachloride. Rats (200 g body wt.) were injected intraperitoneally with 0.5 ml of a mixture of corn oil and carbon tetrachloride (1:1, v/v). Of the rats 20% (two out of ten) died within 20 h of the injection; the remainder were killed at that time. Brain and whole blood (not heparinized) alcohol dehydrogenase activities in normal and carbon tetrachloride-treated rats were assayed by the procedure of Raskin & Sokoloff (1970). Aspartate aminotransferase (L-aspartate-2-oxoglutarate aminotransferase, EC 2.6.1.1) in the serum of the rats was assayed by the method of Bergmeyer & Bernt (1963).

Results

Purified alcohol dehydrogenase

Alcohol dehydrogenase in Pel-Freez frozen rat liver has an activity of about 2 units/g of tissue and is stable indefinitely at -20°C. Alcohol dehydrogenase, purified as described, was electrophoretically homo-

geneous (>98%) on polyacrylamide gels at pH 2.4, 7.0 and 8.6, and on cellulose acetate at pH 6.0 and 7.0. A densitometric scan of a representative pH 2.4 gel is shown in Fig. 1. The trace amounts of protein in other peaks probably represent polymeric forms of alcohol dehydrogenase; the peaks decreased in area if the enzyme preparation was treated with dithiothreitol just before electrophoresis and were increased severalfold in solutions of enzyme that had been freeze-dried (R. J. S. Duncan & L. Sokoloff, unpublished work; see also Markovic *et al.*, 1971). The minor peaks were not resolved in the other electrophoresis systems.

Purified rat liver alcohol dehydrogenase had a specific activity of 2–2.5 (mean, 2.3) units/mg of protein in five preparations. The average recovery was 36% of the activity after the $(\text{NH}_4)_2\text{SO}_4$ precipitation, and the usual protein concentration from the hydroxyapatite column was 4 mg/ml. The purified enzyme is stable for several weeks when stored at 4°C at pH 8.0 in the presence of 5% ethanol.

Alcohol dehydrogenase in brain and in blood

Rat brain alcohol dehydrogenase had a mean activity of 18.2 (S.E.M. = ± 1.1) munits/g fresh wt. of whole brain in 34 brains. After centrifugation at 200 000 g for 30 min the soluble enzyme was stable when stored frozen at -20°C.

The mean alcohol dehydrogenase activity in whole

Fig. 1. Electrophoretogram of purified alcohol dehydrogenase

Enzyme (45 μ g of protein) was electrophoresed for 90 min at 2.5 mA on a 5% polyacrylamide gel containing 8 M-urea, pH 2.4. The protein was stained with Amido Schwarz and the gel scanned at 590 nm.

Table 1. *Effect of liver damage on the alcohol dehydrogenase activity measured in brain homogenates*

Rats (200g) were injected intraperitoneally with 0.5 ml of a mixture of equal volumes of corn oil and carbon tetrachloride. Then 20h later the animals were killed, and alcohol dehydrogenase activity was measured in whole blood and in brain by the method of Raskin & Sokoloff (1970). Aspartate aminotransferase was measured in serum by the method of Bergmeyer & Bernt (1963) to indicate the degree of liver damage induced by the carbon tetrachloride. The differences between the means of the CCl₄-treated and control groups are in all cases statistically significant at the $P < 0.001$ level. The numbers in parentheses represent the numbers of animals from which the mean values $\pm$ S.E.M. were derived.

		Alcohol dehydrogenase	Aspartate aminotransferase
Blood	Control	15.6 $\pm$ 2.5 (5) munits/ml of blood	0.11 $\pm$ 0.07 (3) units/ml of serum
	CCl ₄ -treated	73 $\pm$ 2.5 (4) munits/ml of blood	1.75 $\pm$ 0.6 (4) units/ml of serum
Brain	Control	18.2 $\pm$ 1.1 (34) munits/g fresh wt. of whole brain	Not determined
	CCl ₄ -treated	36.0 $\pm$ 0.6 (8) munits/g fresh wt. of whole brain	Not determined

Fig. 2. *Inhibition of alcohol dehydrogenase by antiserum*

Alcohol dehydrogenase (4.0 munits) was incubated for 15 min at 30°C with serum (68 μ g of protein/ μ l), then assayed spectrophotometrically with NADH and 600 μ M-lactaldehyde as described in the text. ○, Purified enzyme (2 μ g of protein); ●, partially purified liver homogenate (100 μ g of protein).

rat blood was 15.6 (S.E.M. = $\pm$ 2.5) munits/ml in samples from five rats. If 1 g of rat brain contains as much as 75 μ l of blood (Sippel, 1974), it is apparent that less than 10% of the alcohol dehydrogenase activity found in homogenates of brain is contributed by the blood present in the brain during homogenization. Changes in the activity of this enzyme in blood are, however, reflected by changes in the apparent enzyme activity measured in brain. Rats treated with carbon tetrachloride to induce liver damage had a greatly increased alcohol dehydrogenase activity in whole blood, and a significant increase in alcohol dehydrogenase measured in homogenates of brain (Table 1).

Characteristics of the enzyme-antibody reaction

Antibodies to alcohol dehydrogenase were detectable in the serum of rabbits 4–5 weeks after the first injection of the antigen. Results presented below were obtained from rabbits that had received four fortnightly injections of antigen and which were bled 1 week after the last injection. The extent of inhibition of enzyme activity by antiserum increased with time after the first appearance of antibody and varied from animal to animal, but was usually between 50 and 75%. At least two types of antibodies toward alcohol dehydrogenase were present in the antisera and could be distinguished on Ouchterlony diffusion plates (Plate 1) and after immunoelectrophoresis (Plate 2).

Fig. 2 shows titration curves of relative liver alcohol dehydrogenase activity remaining versus amount of antiserum added. Even at very high ratios of antibody to enzyme (such as those involved in Table 2) the relative enzyme activity remained at the plateau value reached in Fig. 2, and was the same whether the enzyme was assayed by the propanediol procedure or by direct spectrophotometry at 340 nm. Fig. 2 also shows that the enzyme-antibody titration curve was virtually identical irrespective of whether homogeneous enzyme or a fairly crude liver preparation was used; hence extraneous tissue protein does not interfere with the antibody-antigen reaction. Serum from unimmunized rabbits did not affect the activity of the enzyme. The curves in Fig. 2 were obtained with lactaldehyde as substrate, but similar curves were obtained with acetaldehyde.

Although alcohol dehydrogenase is not completely inhibited in the presence of antiserum, it was found that more than 99.5% of the enzyme activity could be removed from solution by centrifugation after incubation in the presence of a suitable concentration of antiserum. Antiserum treated in this manner had no measurable inhibitory effect on a further sample

EXPLANATION OF PLATE I

Ouchterlony diffusion of alcohol dehydrogenase and antiserum

Antiserum (350 μ g) was placed in the centre well. The peripheral wells contained: (1) purified liver alcohol dehydrogenase (20 μ g); (2) partially purified liver homogenate (100 μ g of protein); (3) high-speed centrifugal supernatant of brain homogenate (100 μ g of protein).

EXPLANATION OF PLATE 2

Immunoelectrophoresis on cellulose acetate

(a) Approx. 75 μ g of a partially purified liver homogenate; (b) approx. 10 μ g of purified liver alcohol dehydrogenase. Electrophoresis was at 2 mA for 40 min. Proteins soluble after immunodiffusion were washed out, and the plate was then stained with Ponceau S.

of enzyme, and the precipitation line on Ouchterlony plates was lost.

Plates 1 and 2 show that the antisera used were directed specifically against alcohol dehydrogenase. That there is no antibody directed against any trace impurity in the purified alcohol dehydrogenase can be seen in Plate 1; the precipitation lines produced by the crude liver preparation and the purified enzyme are identical. Plate 1 also shows that there is no visible line of precipitation produced by the supernatant fraction of brain homogenate, indicating that there is no precipitating antibody in the serum directed against any major constituent of the brain. The lack of antibody directed against material other than alcohol dehydrogenase in liver homogenate is confirmed in Plate 2. Immunoprecipitation lines obtained after electrophoresis of homogeneous liver alcohol dehydrogenase are identical with those obtained with a partially purified enzyme preparation (Plate 2), under electrophoretic conditions with polyacrylamide gels in which more than 12 bands of protein were clearly resolved. That both precipitation lines visible in Plate 1 were from antibodies directed against alcohol dehydrogenase is shown in Plate 2; the two bands of immunoprecipitation were centred on a single focus, at which alcohol dehydrogenase activity and protein could be demonstrated by staining on other plates.

Identity of brain and liver alcohol dehydrogenase

Immunological evidence that brain and liver alcohol dehydrogenases are identical is provided in

Table 2. Inhibition of alcohol dehydrogenase by specific antisera

Brain and liver alcohol dehydrogenases were preincubated at 25°C for 15 min with 25 µl of serum as indicated, then assayed by the method of Raskin & Sokoloff (1970). The values tabulated are the means of triplicates. The experiment was repeated with two different antisera and three brain preparations.

	Enzyme activity (nmol/ incubation)	
	Brain	Liver
Preincubation mixture		
Enzyme alone	106	66
Enzyme + serum from an unimmunized rabbit	106	73
Enzyme + antiserum	38 (64% inhibition)	23 (65% inhibition)
Enzyme + absorbed antiserum	112*	66*

* These values have been corrected for the propane-1,2-diol produced by alcohol dehydrogenase introduced with the absorbed antiserum (41 nmol in this experiment).

Table 2. The extent of inhibition of the brain and liver enzymes by an excess of antiserum is virtually the same. Moreover serum from which the anti-(alcohol dehydrogenase) immunoglobulins had been removed by specific precipitation with the pure liver enzyme inhibited neither the brain nor the liver enzyme. It should be noted that alcohol dehydrogenase used to precipitate the antibody was not completely removed from the serum by this procedure, and the values in Table 2 have been corrected where appropriate for this added activity.

Discussion

Several groups have been able to confirm the observation by Raskin & Sokoloff (1968) of alcohol dehydrogenase activity in homogenates of brain (Rawat *et al.*, 1973; Taberner, 1974; Tabakoff & von Wartburg, 1975). However, it had not previously been shown that the alcohol dehydrogenase found in homogenates of brain was not a consequence of the presence of enzyme in the blood in the brain. A simple reconciliation of the results of those authors demonstrating alcohol dehydrogenase in brain and those of Mukherji *et al.* (1975), who reported that perfused brain could not utilize [¹⁴C]ethanol, might have been that the enzyme was present only in the blood of the tissue and was washed out by the perfusion fluid. The activity of alcohol dehydrogenase in blood varies between species. In the serum of rabbit and sheep there is no alcohol dehydrogenase detectable, but in goat there is considerable activity (R. J. S. Duncan & L. Sokoloff, unpublished work). The present work shows that in the rat there is alcohol dehydrogenase activity in brain above that accounted for by the enzyme in blood, although the blood may contribute a portion of the activity found in brain homogenates. In pathological conditions in which alcohol dehydrogenase is released into the blood the apparent activity of the enzyme in brain can be increased as shown in Table 1; hence, the interpretation of experimentally induced increases of the activity of this enzyme in brain may require caution.

It is commonly found that alcohol dehydrogenase is only partially inhibited by its specific antibody (Fuller & Marucci, 1972). For the present work this is a fortunate occurrence, because at the very low amounts of the enzyme found in brain it is not possible to use classical tests of immunological identity such as the nature of the precipitation lines after Ouchterlony diffusion or the identity of the antigen-antibody titration curves. Since the extent of inhibition of the enzyme by an excess of antibody may be taken as a characteristic of the enzyme-antibody pair (see Fuller & Marucci, 1972), similar inhibition of the activity from different tissues is evidence that the enzymes in those tissues are identical. This evidence is given weight if it can be shown that the antiserum used

contains no antibodies directed against other proteins in the tissues concerned, and if it can be shown that removal of the specific antibody involved prevents the inhibition. Each of these criteria has been met in the present case (Plates 1 and 2 and Table 2).

The results presented here show that brain and liver alcohol dehydrogenases share common antigens and are probably immunologically identical. It is safe to ascribe the properties of the liver enzyme to that of brain, but although much is known of the specificity of alcohol dehydrogenase, it is still not possible to assign any particular substrate to the enzyme in brain, and the role of this enzyme in brain remains unclear.

References

- Andersson, L., Jornvall, H., Åkeson, Å. & Mosbach, K. (1974) *Biochim. Biophys. Acta* **364**, 1-8
- Bergmeyer, H.-U. & Bernt, E. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), 1st edn., pp. 837-842, Academic Press, New York
- Deibler, G. E., Martenson, R. E. & Kies, M. W. (1972) *Prep. Biochem.* **2**, 139-163
- Dewan, J. G. (1943) *Q. J. Stud. Alcohol* **4**, 357-361
- Fuller, T. C. & Marucci, A. A. (1972) *Enzymologia* **42**, 139-153
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Markovic, O., Theorell, H. & Rao, S. (1971) *Acta Chem. Scand.* **25**, 195-205
- Mukherji, B., Kashiki, Y., Ohyanagi, H. & Sloviter, H. A. (1975) *J. Neurochem.* **24**, 841-843
- Raskin, N. H. & Sokoloff, L. (1968) *Science* **162**, 131-132
- Raskin, N. H. & Sokoloff, L. (1970) *J. Neurochem.* **17**, 1677-1687
- Rawat, A. K., Kuriyama, K. & Mose, J. (1973) *J. Neurochem.* **20**, 23-33
- Sippel, H. W. (1974) *J. Neurochem.* **23**, 451-452
- Tabakoff, B. & von Wartburg, J. P. (1975) *Biochem. Biophys. Res. Commun.* **63**, 957-966
- Taberner, P. V. (1974) *Biochem. Pharmacol.* **23**, 1219-1220
- Towne, J. C. (1964) *Nature (London)* **201**, 709-710
- Woodley, C. L. & Gupta, N. K. (1972) *Arch. Biochem. Biophys.* **148**, 238-248