Biochemical Pharmacology, Vol. 36, No. 13, pp. 2055–2059, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

COMMENTARY

ETHANOL AS A NEUROTOXIN

BRIAN E. LEONARD
Pharmacology Department, University College, Galway, Republic of Ireland

It is universally accepted that ethanol consumption can have a pronounced effect on behaviour, the brain being affected more obviously than any other system. While the initial effects of the drug appear to be stimulant, ethanol resembles the general anaesthetics in its action, as this apparent stimulation results from the unrestrained activity of various brain regions that have been freed from inhibition as a consequence of the depression of inhibitory control mechanisms. Thus, ethanol depresses both excitatory and inhibitory polysynaptic potentials and is more effective in inhibiting synaptic than impulse propagation and in potentiating pre-synaptic inhibition. In general, the effects of ethanol on the CNS are proportional to its blood concentration.

Whereas the effects of occasional ethanol consumption on CNS activity are readily reversible, chronic and excessive ingestion of ethanol is directly associated with serious neurological and mental disorders associated with memory loss, sleep disturbances, psychotic episodes and brain damage. In addition, nutritional and vitamin deficiencies which arise as a consequence of an inadequate diet, combined with faulty gastro-intestinal absorption of substances such as thiamine and carnitine, are closely associated with such neuropsychiatric syndromes as Wernicke's encephalopathy, Korsakoff's psychosis, polyneuritis and nicotinic acid deficiency encephalopathy [1, 2]. Partly as a consequence of the dramatic increase in first admissions to psychiatric hospitals in Europe and North America in recent years, attention has been directed towards the effects of chronic ethanol consumption on brain function and morphology. The studies of Courville [3] on post-mortem brains of alcoholics show that atrophy of the frontal lobes is extensive, and he suggested that such changes arose from chronic anoxia. Later studies, using air encephalography or computer-assisted tomographic (CAT) scanning devices, have shown distinct dilation of the lateral ventricles and cortical sulci [4–6]. Epstein *et al.* [5] further demonstrated that patients with enlarged sulci show impaired psychological performance, while other investigators found that cognitive dysfunction is correlated with the width of the anterior horn of the ventricles [7]. The results of these, and other [8], studies clearly suggest that the psychological malfunction seen in chronic alcoholism is associated with brain damage. The purpose of this review is to consider the various proposals which may explain how such damage occurs.

Three major mechanisms have been proposed in an attempt to explain the neurotoxicity of ethanol. First, many of the toxic effects have been ascribed to the accumulation of acetaldehyde together with an increase in the ratio of NADH to NAD^+ [9]. Many of the metabolic changes that occur following ethanol consumption, as exemplified by an increase in the lactate and fatty acid concentrations and decreased tricarboxylic acid cycle activity, appear to be a direct reflection of the increased NADH:NAD^+ ratio [10]. The effect of the accumulation of acetaldehyde in inhibiting protein synthesis [11] undoubtedly contributes to these metabolic effects. Second, ethanol has membrane fluidizing effects [12] that correlate well with the pharmacological actions of the drug on the brain. It has been speculated that the disordering effects of ethanol on nerve membranes are responsible for many of the abnormalities in function that are mediated by such membrane-bound proteins as Na^+, K^+-ATPase and adenylate cyclase [13, 14]. As such processes are linked to central neurotransmitter transport and function, it has been hypothesized that the changes in neurotransmission associated with chronic consumption are secondary to those in neuronal membrane activity. The third hypothesis which has been proposed to explain the central effects of the drug relates to the production of such specific psychosis-producing agents as salsinol and tetrahydropapaveroline [15]. Of these three possibilities, only the second will be considered in detail in the present review as this has the advantage of more completely unifying both the morphological and the functional changes seen following chronic ethanol consumption.

Effects of chronic ethanol administration on the lipid composition of neuronal membranes

The molecular action of ethanol on cell membranes has been the subject of intense investigation in recent years. Although the exact nature of these interactions and the subsequent changes in the biochemical processes have not been delineated clearly, some aspects of the subject have been reviewed recently [16, 17]. Studies using electron spin resonance and fluorescence polarization show that ethanol and other aliphatic alcohols perturb the fine structure of the cell membrane. In addition, [^{14}C]ethanol has been shown to bind reversibly to synaptic membranes [18] and to interact directly with membrane proteins by either altering the protein conformation or disrupting the relationship between the membrane proteins and lipids [19, 20]. As a consequence of these actions, a perturbation in membrane function occurs [21].

The specificity of action of ethanol on neuronal membranes may be at least partly related to the lipid composition of the membranes. Goldstein *et al.* [22]

examined the effects of ethanol on brain tissue of mice, using an electron paramagnetic resonance method to explore the changes in membrane fluidity following *in vitro* and *in vivo* administration of the drug. They showed that there is a concentration-dependent increase in membrane fluidity when ethanol is administered *in vitro*. However, following chronic administration *in vivo*, the neuronal membranes are resistant to the fluidizing effect of the drug when added to a membrane fraction *in vitro*, which the investigators suggest is a reflection of the development of tolerance. The development of tolerance appears to be associated with an increase in the cholesterol content of the membranes [22]; a replacement of unsaturated by saturated fatty acids in the membrane may also play an essential role in the resistance of the membranes to the fluidizing effects of ethanol [23, 24]. Rubin and Rottenberg [25] have used the term homeoviscous adaptation to describe this process and suggested that the cross-tolerance between ethanol, sedative-hypnotics, and general anaesthetics may arise because all three classes of drugs have a similar action on biological membranes.

Not all reported changes in membrane lipid composition following chronic ethanol administration, however, are consistent with this hypothesis. Thus, Sun and Sun [26] reported a proportionate increase in polyunsaturated fatty acids in synaptosomal membranes from guinea pigs, whereas Wing and coworkers [27] found that changes in membrane fatty acyl composition after ethanol vary with the membrane type. Mice selectively bred for their differences in sensitivities to the pharmacological effects of ethanol show no differences in the synaptic membrane phospholipid, fatty acid, or cholesterol composition [28]. In a detailed study of the effects of chronic ethanol on the fatty acyl composition of erythrocyte membranes, Wing *et al.* [28] demonstrated that the monounsaturated fatty acyl groups of phosphatidylethanolamine are particularly sensitive to ethanol and suggested that this may have a pivotal role in such processes as membrane transport and fusion. These authors hypothesized that, as in ethanol tolerant rats isolated synaptosomal membranes show altered Ca^{2+} sensitivities to neurotransmitter release, changes in the phosphatidylethanolamine fatty acyl composition may play a key role.

These changes in membrane lipid composition have also been detected in clinical studies. Thus, Lesch and coworkers [29] reported that demyelination and lipid loss occur in the brains of chronic alcoholics, while Alling and Bostrom [30] showed that the mammillary bodies are particularly sensitive to the effects of ethanol as shown by a loss of myelinated fibres. The proportion of polyunsaturated fatty acids in the phospholipid fraction of brain myelin has also been shown to decrease following chronic ethanol treatment [31]. One of the primary metabolic effects would appear to be an induction of essential fatty acid deficiency [32], and there is circumstantial experimental evidence to support the view that such a deficiency may be instrumental in causing learning deficits, particularly if the deficiency occurs during the critical periods of brain development [33, 34].

The effects of ethanol on lipid metabolism can be ascribed to a deficiency in the absorption of thiamine and carnitine, combined with a reduction in the activities of the *delta-6* and *delta-5* desaturases (which are responsible for the conversion of dietary linolenic acid to the higher polyunsaturated fatty acids, gamma-linolenic and arachidonic acids). The thiamine-deficient diet, combined with impaired absorption of the vitamin [35, 36], is associated with a diminished pyruvate dehydrogenase activity, which therefore reduces the essential precursors for lipogenesis. The inhibition of the desaturases by ethanol results in the synthesis of a higher proportion of saturated and monounsaturated fatty acids [37], while the reduction in the tissue concentrations of carnitine [38] reduces fatty acid oxidation as this dietary cofactor plays an essential role in the transport of fatty acids from the cytoplasm into the mitochondria where oxidation occurs [39]. The final effect of chronic ethanol administration, therefore, is associated with a shift in the membrane composition from unsaturated to saturated fatty acids, with cholesterol and glycerophospholipids containing a higher proportion of saturated fatty acids than would occur in the absence of ethanol [40]. Such changes are aided by an increase in phosphohydrolase activity which results in an enhanced conversion of phosphatidic acid to 1,2-diacylglycerol [41, 42].

In addition to their role in membrane structure, the polyunsaturated fatty acids also act as precursors of prostaglandins [43]. Änggard [44] has shown that chronic ethanol treatment increases the urinary excretion of immuno-reactive prostaglandin PGF_2. Thus, a combination of a decrease in the synthesis of the precursor unsaturated fatty acid, arachidonic acid, and an increase in the release and metabolism of the prostaglandins following chronic ethanol administration can explain the subsequent decrease in the products of the cyclooxygenase pathway. There is also some evidence that some of the changes associated with chronic ethanol abuse may be associated with the altered availability of prostaglandins [45–47]. It may be concluded that the reduction in the unsaturated fatty acid composition of neuronal membranes may underlie the effects of ethanol on membrane fluidity and changes in permeability, thereby predisposing the animal to brain damage. In experimental studies, Walker *et al.* [48] have demonstrated that the number of hippocampal and cerebellar neurons, and the number of dendritic processes, decrease following chronic ethanol administration. Evidently, good nutritional status does not protect the animals from these detrimental effects of ethanol [49]. Such changes in cell structure, combined with cell loss, could account for the gross histopathological changes seen in chronic alcoholics.

Possible reversal of the effects of ethanol on membrane lipids

If the inhibition of the *delta-5* and *-6* desaturases is an important factor that underlies the toxicity of ethanol, then it may be postulated that the administration of polyunsaturated fatty acids whose synthesis is reduced by the drug should alleviate some of its

neurotoxic effects. In support of this hypothesis, Goheen *et al.* [50] have shown that a mixture of polyunsaturated fatty acids rich in arachidonic acid prevents liver damage which would normally result from the chronic administration of ethanol. Studies by Corbett and Leonard [51], on the incidence of sound-induced seizures that occur following the abrupt withdrawal of chronically administered ethanol to rats, have also shown that the incidence and severity of the seizures are reduced following the concurrent administration of gamma-linolenic acid. Recently, these studies have been extended to show that the reduction in the turnover of γ-aminobutyric acid (GABA) in the brainstem and hippocampus following chronic ethanol administration is antagonized by the concurrent administration of gamma-linolenic acid (given as a component of Evening Primrose Oil) or carnitine [52]. As there is evidence from the studies of others that the intracisternal administration of GABA or GABA agonist drugs attenuates ethanol withdrawal seizures [52], it may be concluded that the lowering of the seizure threshold and the reduction in the availability of GABA following chronic ethanol administration are correlated. There is no evidence from our experimental studies [51] that either gamma-linolenic acid or carnitine has a direct effect on GABA turnover, so it would appear that these substances antagonize the action of ethanol by facilitating the incorporation of polyunsaturated fatty acids into neuronal membranes.

So far it is unclear whether chronic gamma-linolenic acid treatment has any beneficial effects in the treatment of alcoholism. A preliminary study on severely alcoholic patients by Glen *et al.* [53] suggests that prolonged treatment with this fatty acid (as a component of Evening Primrose Oil) following a standard detoxification programme results in an improvement in some aspects of cognitive function and a reduction in the dose of diazepam that needs to be administered to prevent alcohol withdrawal convulsions. Analysis of the lipid composition of the erythrocyte membranes of these patients clearly showed that such treatments increased the higher polyunsaturated fatty acid (e.g. arachidonic acid) component of these membranes. Qualitatively similar changes have also been found in the nerve terminals and platelets of rats to whom gamma-linolenic acid, alone or together with ethanol, had been administered [54].

Another method whereby the effects of ethanol on the composition and structure of nerve membranes may be attenuated involves the use of carnitine. We [51] and others [38] have shown that chronic ethanol treatment reduces tissue concentrations of carnitine in rats. In view of the importance of carnitine in the intracellular transport of unsaturated fatty acids already alluded to, it has been hypothesized that dietary supplementation with carnitine of ethanol-dependent rats may counteract some of the biochemical and behavioural effects of the drug. Preliminary studies have shown that the frequency of audiogenic seizures which occur in approximately 60% of rats following the abrupt withdrawal of ethanol is reduced to below 30% following chronic carnitine administration.* This effect of carnitine was also associated with a reduction in the ethanol-induced rise in the activity of hippocampal Ca^{2+}, Mg^{2+} ATPase activity. This is further circumstantial evidence for the view that carnitine partially reverses the ethanol-induced changes in lipid composition of the neuronal membranes and thereby prevents the changes in the activities of membrane-bound enzymes whose microenvironment in the neuronal membrane is altered by chronic ethanol treatment. The inter-relationships of thiamine, carnitine and gamma-linolenic acid deficiencies that are initiated by chronic ethanol, and the neurotoxicity of this drug, have been the subject of a short review [55].

Effect of ethanol as a behavioural teratogen

It is well established that severe structural damage occurs to the brains of infants who have been exposed to high concentrations of ethanol during the pre-natal period [56, 57]. Pre- and post-natal dystrophy, developmental retardation, and microcephaly are typical features of children with the foetal alcohol syndrome. These effects may result from the action of ethanol on all developmental stages of the CNS, especially in the periods of organogenesis associated with the first trimester of pregnancy. It is also evident that the drug can interfere with the "brain growth spurt" which occurs relatively late in pre-natal development. Mental retardation and persistent behavioural deficits (e.g. hyperexcitability) may arise as a consequence of the effects of ethanol at the later stages of brain development and can occur in the absence of obvious neurological damage [58].

Several studies in rats have shown that pre- and early post-natal exposure to ethanol produces a significant reduction in brain weight, especially in the cerebellum [59, 60], a selective loss of Purkinje cells [61], impaired maturation of the Purkinje cells and retarded synaptic development [62]. Detailed histological changes have also been reported in the dendritic spine distribution in pyrimidal cells [63], and deficits in dendritic structure and pyrimidal cell number have been observed in the hippocampus [64, 65]. These changes are qualitatively similar to those reported to occur in adult rats that have been exposed to prolonged ethanol treatment [49].

The mechanisms whereby ethanol can produce these effects are numerous, but two mechanisms seem worthy of particular attention. The first concerns the actions of ethanol on the membrane lipid composition which has already been referred to in this review. The second action, which may be particularly pertinent to our understanding of the foetal alcohol syndrome, concerns the action of ethanol on the activity of neural cell adhesion molecules (N-CAM) during early pre-natal development. These complex and highly varied oligosaccharide units, rich in sialic acid, appear to be involved in many basic membrane-related events such as cellular migration, recognition and adhestion [66]. N-CAM are also constituents of a number of key enzyme and receptor units [67]. Stibler and co-workers [68] have shown that ethanol exposure *in utero* results in a reduction

* R. Corbett and B. E. Leonard, *Proc. 14th Congress C.I.N.P.*, p. 198. Florence, Italy (1984).

in the incorporation of sialic acid into membrane-bound sialoglycoconjugates, an effect which is particularly marked in nerve terminals. It may be hypothesized therefore that a reduction in N-CAM can result in an inhibition of neuronal migration at a critical stage in development and lead to premature cellular migration and adhesion. Such changes may underlie the structural abnormalities that characterize the foetal alcohol syndrome. Such effects are undoubtedly complicated by a reduction in the placental transport of amino acids, glucose and other nutrients which could arise as a consequence of ethanol-induced hypoxia [69, 70].

General conclusions

It is universally accepted that the effects of ethanol on the brain are complex and incompletely understood. Nevertheless, one of the major advances in understanding of the neurotoxicity of this drug derives from observing its effects on membrane composition and fluidity. The changes in the membrane lipid composition would appear to precede any alteration in conformational state of membrane proteins [71] and suggest that the well established changes in such membrane-bound enzymes as Na^+, K^+-ATPase [70, 72] on calcium ion fluxes [73], on central inhibitory neurotransmitter metabolism [74] and on biogenic amine metabolism [75] may be secondary to the changes in lipid composition. While the preliminary results of the experimental and clinical studies indicating that dietary supplements of polyunsaturated fatty acids can counteract some of the behavioural and neurochemical effects of chronic ethanol appear promising, clearly more extensive studies need to be undertaken before any conclusions can be made.

REFERENCES

1. R. W. Hillman, in *The Biology of Alcoholism* (Eds. B. Kissins and H. Begleiter), Vol. 3, p. 513. Plenum Press, New York (1975).
2. J. B. Turner, E. Mezey and A. W. Kimball, *Johns Hopkins med. J.* **5**, 235 (1977).
3. C. B. Courville, *Effects of Alcohol on the Nervous System of Man*. San Lucas Press, Los Angeles, CA (1955).
4. T. B. Horvath, in *Alcohol, Drugs and Brain Damage* (Ed. J. G. Rankin), p. 1. Alcoholism and Drug Addiction Research Foundation, Toronto (1975).
5. P. S. Epstein, V. D. Pisani and J. A. Fawcett, *Alcoholism: Clin. expl Res.* **1**, 83 (1978).
6. P. L. Carlen, G. Wortzman, R. C. Holgate, D. A. Wilkinson and J. G. Rankin, *Science* **200**, 1076 (1978).
7. H. Bergman, S. Borg, T. Hindmarsh, C-M. Idelstrom and S. Mutzell, in *The Biological Effects of Alcohol* (Ed. H. Begleiter), p. 771. Plenum Press, New York (1980).
8. M. Massarotti, *Pharmac. Biochem. Behav.* **18**, (Suppl. 1), 51 (1983).
9. J. J. Higgins, in *Biochemistry and Pharmacology of Ethanol* (Eds. E. Majchrowicz and E. P. Noble), p. 249. Plenum Press, New York (1979).
10. C. S. Lieber, R. Teschke, Y. Hasumura and L. M. Decarli, *Fedn Proc.* **34**, 2060 (1975).
11. T. M. Donohue, J. R. Dean, J. Tuma and M. F. Sorrel, *Archs Biochem. Biophys.* **220**, 239 (1983).
12. R. C. Lyon, J. A. McComb, J. Schreurs and D. B. Goldstein, *J. Pharmac. exp. Ther.* **218**, 669 (1981).
13. R. L. Ricci, S. S. Crawford and P. B. Miner, *Gastroenterology* **80**, 1445 (1981).
14. R. A. Rabin and P. B. Molinoff, *J. Pharmac. exp. Ther.* **216**, 129 (1981).
15. O. Beck, J. R. Bosin, A. Lundman and S. Borg, *Biochem. Pharmac.* **31**, 2517 (1982).
16. G. Y. Sun and A. Y. Sun, *Alcoholism: Clin. expl Res.* **9**, 164 (1985).
17. C. D. Stubbs and A. D. Smith, *Biochim. biophys. Acta* **779**, 89 (1984).
18. A. Y. Sun, *Ann. N.Y. Acad. Sci.* **273**, 295 (1976).
19. D. C. Lin, *Biochem. Pharmac.* **29**, 771 (1980).
20. N. P. Franks and W. R. Lieb, *Nature, Lond.* **300**, 487 (1982).
21. H. Sandermann, *Biochim. biophys. Acta* **515**, 209 (1978).
22. D. B. Goldstein, J. H. Chin, J. A. McComb and L. M. Parsons, in *Biological Effects of Alcohols on Mouse Biomembranes* (Ed. H. Begleiter), p. 1. Plenum Press, New York (1980).
23. J. H. Chin, L. M. Parsons and D. B. Goldstein, *Biochim. biophys. Acta* **513**, 359 (1978).
24. J. M. Littleton, S. J. Greive, P. J. Griffiths and G. R. John, in *Biological Effects of Alcohol* (Ed. H. Begleiter), p. 7. Plenum Press, New York (1980).
25. E. Rubin and H. Rottenberg, *Pharmac. Biochem. Behav.* **18**, (Suppl. 91), 7 (1983).
26. G. Y. Sun and A. Y. Sun, *Res. Commun. Chem. Path. Pharmac.* **24**, 405 (1979).
27. D. R. Wing, D. J. Harvey, J. Hughes, P. G. Dunbar, K. A. McPherson and W. D. M. Paton, *Biochem. Pharmac.* **31**, 3431 (1982).
28. D. R. Wing, D. J. Harvey, S. J. Belcher and W. D. M. Paton, *Biochem. Pharmac.* **33**, 1625 (1984).
29. P. Lesch, E. Schmidt and F. W. Schmidt, *Z. klin. chem. biochem.* **11**, 159 (1973).
30. C. Alling and K. Bostrom, *Acta neuropath.* **50**, 77 (1980).
31. G. Y. Sun, V. Danopaulos and A. Y. Sun, *Curr. Alcohol Res.* **7**, 83 (1980).
32. C. Alling, W. Becker, A. W. James and E. Anggard, *Alcoholism: Clin. expl Res.* **8**, 238 (1984).
33. D. F. Candwell and J. A. Churchill, *Psychol. Rep.* **19**, 99 (1966).
34. R. Paoletti and C. Galli, *Ciba Fdn Symp.* **121**, 140 (1971).
35. A. D. Thompson, H. Baker and C. M. Leevy, *J. Lab. clin. Med.* **76**, 36 (1970).
36. O. Frank, A. Luisada-Opper, M. F. Sorrell, A. D. Thompson and H. Baker, *Expl molec. path.* **15**, 191 (1971).
37. A. M. Nervi, R. O. Peluffo and R. R. Brenner, *Lipids* **15**, 263 (1980).
38. D. Rudoman, C. W. Swell and J. D. Ansley, *J. clin. Invest.* **60**, 716 (1977).
39. I. B. Fritz, *Adv. Liped Res.* **1**, 285 (1963).
40. R. C. Reitz and R. J. Schilling, in *Alcohol Tolerance and Dependence* (Eds. H. Riger and J. Crabbe), p. 251. Elsevier, Amsterdam (1980).
41. R. C. Reitz, *Prog. Lipid Res.* **18**, 87 (1979).
42. R. G. Sturton, S. C. Buherwith, S. L. Burditt and D. N. Brindley, *Fedn Eur. Biochem. Soc. Lett.* **125**, 296 (1981).
43. L. S. Wolfe, *J. Neurochem.* **38**, 1 (1982).
44. E. Änggard, *Pharmac. Biochem. Behav.* **18**, (suppl. 1), 401 (1983).
45. D. J. Segarnick, H. Dryer and J. Rotrosen, *Biochem. Pharmac.* **34**, 1343 (1985).
46. M. Thomas, A. L. A. Boura and B. R. Vijayakumar, *Res. Commun. Chem. Path. Pharmac.* **24**, 405 (1979).
47. R. F. Anton, C. J. Wallis and C. L. Randall, *Prostaglandins* **26**, 421 (1983).

48. D. Walker, D. Barnes, J. Riley, B. Hunter and S. Zornetzer, in *Psychopharmacology of Alcohol* (Ed. M. Sandler), p. 17. Raven Press, New York (1980).
49. D. Walker, B. Hunter, D. Barnes and J. Riley, in *Cerebral Deficits in Alcoholism* (Ed. D. Wilkenson), p. 123. Addiction Research Foundation, Toronto (1980).
50. S. C. Goheen, E. C. Larkin and G. A. Rao, *Lipids* **18**, 285 (1983).
51. R. Corbett and B. E. Leonard, *Neuropharmacology* **34**, 269 (1984).
52. R. Corbett and B. E. Leonard, in *Learning and Memory: Mechanisms of Information Storage in the Nervous System* (Ed. H. Matthies), p. 391. Pergamon Press, Oxford (1986).
53. A. I. M. Glen, E. M. I. Glen, F. K. MacDonald, L. E. F. MacDonald, J. R. Mackenzie, D. J. Montgomery, M. S. Manku, D. F. Horrobin and N. Morse, in *Alcoholic Beverages* (Eds. G. G. Birch and M. G. Lindley), p. 203. Elsevier, Amsterdam (1985).
54. R. Corbett, J. F. Ménez, B. E. Leonard and H. Floch, *Neurosci. Lett.* **69**, 198 (1986).
55. B. E. Leonard, *Alcohol Alcoholism* **19**, 97 (1984).
56. K. L. Jones and D. W. Smith, *Lancet* **1**, 1267 (1973).
57. S. K. Clarren and D. W. Smith, *New Engl. J. Med.* **292**, 1063 (1978).
58. A. P. Streissguth, S. Landesman-Dwyer, J. C. Martin and Din Smith, *Science* **209**, 353 (1980).
59. E. L. Abel, *Psychopharmacology* **57**, 5 (1978).
60. J. Diaz and H. H. Samson, *Science* **208**, 751 (1980).
61. S. C. Phillips and B. G. Cragg, *Expl Neurol.* **80**, 218 (1983).
62. B. Volk, M. Maletz, M. Tiedemann, G. Mall, C. Klein and H. H. Berlet, *Acta neuropath.* **54**, 19 (1981).
63. G. Stoltenburg-Didinger and H. L. Spohr, *Devl Brain Res.* **11**, 119 (1983).
64. E. L. Abel, S. Jacobsen and B. J. Sherwin, *Neurobehav. Toxic. Teratol* **5**, 139 (1983).
65. D. E. Barnes and D. W. Walker, *Devl Brain Res.* **1**, 333 (1981).
66. R. Greig and M. Jones, *Biosystems* **9**, 43 (1977).
67. R. W. McLawhon, G. S. Schoon and G. Dawson, *Eur. J. Cell. Biol.* **25**, 353 (1981).
68. H. Stibler, E. Burns, T. Kruckeberg, P. Gaetano, E. Cerven, S. Borg and B. Tabakoff, *J. neurol. Sci.* **59**, 21 (1983).
69. E. L. Abel, *Fedn Proc.* **44**, 2318 (1985).
70. C. Guerri, R. Wallace and S. Grisolia, *Eur. J. Biochem.* **86**, 581 (1978).
71. C. Guerri and S. Grisolia, *Pharmac. Biochem. Behav.* **18**, (Suppl. 1), 45 (1983).
72. Y. Israel, H. Kalant, E. Le Blanc, J. C. Bernstein and I. Solozer, *J. Pharmac. exp. Ther.* **174**, 330 (1970).
73. M. L. Michaelis, E. K. Michaelis and J. Tehan, *Pharmac. Biochem. Behav.* **18**, (Suppl. 1), 19 (1983).
74. E. Kulonen, *Med. Biol.* **61**, 147 (1983).
75. W. A. Hunt and E. Majchrowicz, *Pharmac. Biochem. Behav.* **18**, (Suppl. 1), 371 (1983).