permitting rapid analysis. Fig. 1 also shows the cell steady state response *versus* blood alcohol content. These data were obtained using a simulated "breath" sample prepared by passing air through standardized alcohol solutions. This plot is linear up to a blood alcohol content of 400 mg of alcohol/100 ml. of blood which is the highest value investigated. The signals at any given alcohol concentration were reproducible in consecutive duplicate tests within ±2 mg of alcohol at blood alcohol levels of 200 mg/100 ml., and within ±10 mg of alcohol at blood alcohol levels of 400 mg/100 ml. This duplication of results is well within the limits recently suggested for a breath testing device[2].

Over 2,500 tests have been run on one of the detector cells using simulated breath samples. The standard deviation of the measured alcohol concentration on the breath from the solution alcohol concentration was 3 mg of alcohol over a range of blood alcohol levels of 50 mg/100 ml. to 200 mg/100 ml., values significantly more accurate than the colorimetric alcohol detectors and well within the evidential requirements for breath alcohol measurements[2,5]. It is, in fact, better than the standard deviation observed with blood analysis[5].

Calibration checks of individual sensor cells over a period of 4 months' operation and 2,500 tests show only minor changes in signal (3% reduction per month). Thus a monthly calibration check would be adequate for a roadside screening device. The formation and build-up of acetic acid in the strong acid electrolyte have no influence on the cell response (and hence on the kinetics of alcohol electro-oxidation) over 2,500 tests.

Our studies with this instrument indicate that the electrochemical oxidation of alcohol is a more accurate technique for the measurement of alcohol in breath samples than the instruments based on colorimetric methods of detection. The electrochemical detector cell may be built into a rugged, portable unit which may be used by a police officer at the scene of a traffic offence either as a screening instrument or as an evidential device.

H. W. Bay
K. F. Blurton
H. C. Lieb
H. G. Oswin

Energetics Science Inc.,
4461 *Bronx Blvd*,
New York, NY 10470

Received December 16, 1971; revised August 4, 1972.

1 Prouty, R. W., and O'Neill, B., *Report to Insurance Institute for Highway Safety* (Washington, May 1971).
2 Smith, W. C., Harding, D. M., Biasotti, A. A., Finkle, B. S., and Bradford, L. W., *J. Foren. Sci. Soc.*, **9**, 58 (1969).
3 Day, M., Muir, G. G., and Watling, J., *Nature*, **219**, 1051 (1968).
4 Wright, B. M., *Nature*, **220**, 206 (1968).
5 Bradford, L. W., *Symposium on Alcohol and Road Traffic* (Indiana University Center for Police Training, December 1958).
6 Borkenstein, R., and Smith, H., *Medicine, Sci., Law*, **2**, 13 (1961).
7 Rightmire, R. A., Rowland, R. L., Boos, D. L., and Beals, D. L., *J. Electrochem. Soc.*, **111**, 242 (1964).
8 Hoare, J. P., *Electrochemistry of Oxygen* (Interscience, New York, 1968).

Nature (Lond.) 240, 53-54 (1972). LSD 2344

Active Transport of Lysergic Acid Diethylamide

Although it is recognized that there is little hindrance to the passage of lysergic acid diethylamide (LSD) across the blood brain barrier[1], an autoradiographic study of LSD distribution in the rat brain led to speculation that the choroid plexuses are involved in the active transport of this compound between blood and cerebrospinal fluid[2]. It was also suggested that this tissue acts as a storage site for LSD in the brain. The purpose of this communication is to elucidate the mechanism

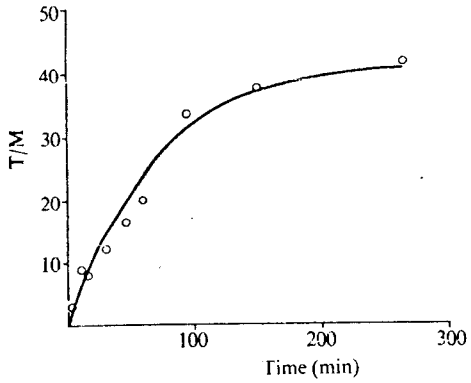

Fig. 1 Accumulation of ^{3}H-LSD by the frog choroid plexus with time. Uptake is expressed as the T/M ratio.

of LSD accumulation within the choroid plexus, and clarify the role of the choroidal epithelium in the distribution of LSD between blood and brain.

I used an *in vitro* preparation of a choroid plexus to measure (i) unidirectional fluxes across the epithelium, and (ii) accumulation within the epithelium from the solutions bathing the ventricular and/or vascular surface of the tissue[3,4]. The posterior choroid plexus was removed from bullfrogs (*Rana catesbiana*) and LSD accumulation within the plexus was measured during incubation in 3 ml. of physiological saline containing ^{3}H-LSD. The tissue was then rinsed in saline, drained of surface fluid, weighed and dissolved in tissue solubilizer. The total radioactivity in the tissue was determined by liquid scintillation counting and uptake was expressed as the T/M ratio:

$$\frac{T}{M} = \frac{\text{concentration of LSD per ml. of tissue water}}{\text{concentration of LSD per ml. of incubation media}}$$

To measure the unidirectional fluxes of ^{3}H-LSD across the choroid plexus the tissue was mounted in between two lucite half chambers. A tracer quantity of isotope was added to the solution on one side of the plexus and the rate of appearance of the isotope in the "cold" solution on the opposite side was measured. At the conclusion of the experiment the amount of isotope in the plexus was determined as outlined above. In a few experiments ^{3}H-LSD was added to both the ventricular and the serosal solutions, and after equilibration with the tissue the external solutions were changed rapidly, and the efflux of isotope from the plexus was measured by sampling the ventricular and serosal solutions at regular intervals. Unidirectional fluxes are expressed in mol/cm^2/h and the uptake from the ventricular or serosal solutions is expressed in mol/cm^2. All errors are quoted as the standard error of the mean with the number of estimates in parentheses.

Experiments were carried out mostly at room temperature (22–24° C) in a physiological saline containing 85 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, and 25 mM $NaHCO_3$ gassed with 95% O_2–5% CO_2. ^{3}H-LSD (New England Nuclear Corp., lot number 281–190) had a specific activity of 1.9 Ci/mmol and its radiochemical purity was greater than 93%. The purity was checked by thin-layer chromatography on silica gel plates using ethanol : acetic acid : chloroform (10 : 2 : 18). In all experiments the final concentration of LSD in the physiological saline was 3 μM, which is in the concentration range used to produce psychological effects. In general experimental procedures were as described before[5].

Fig. 1 shows the time course of ^{3}H-LSD accumulation. The T/M ratio increased rapidly during the first few minutes and thereafter more slowly, reaching a steady state after about 3 h. There was no indication of instantaneous binding of LSD to the tissue. The steady state T/M ratio was 45 ± 3 (12). As Fig. 2 shows, the accumulation of LSD within the plexus was reduced by 65% in the presence of ouabain (1.5×10^{-5} M), iodoacetate/cyanide (3 mM) and by low temperature (0° C).

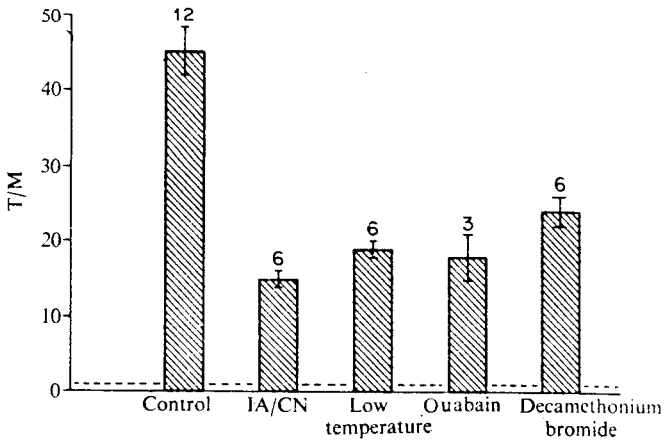

Fig. 2 Effect of inhibitors on ^{3}H-LSD accumulation by the choroid plexus. The T/M ratios were obtained after 2.5 h incubation in the physiological saline, (i) at room temperature (control), (ii) at 0° C, (iii) with 3 mM iodoacetate/cyanide (IA/CN), (iv) with 1.5×10^{-5} M ouabain, and (v) with 1 mM decamethonium bromide. The T/M ratio corresponding to a simple passive distribution of the LSD between the medium and tissue water is indicated.

Furthermore, the quaternary ammonium compound, decamethonium bromide (1 mM), reduced the uptake of LSD by 50%.

The ^{3}H-LSD was accumulated equally well across either the ventricular or the serosal surface of the plexus; in the steady state the uptake from the serosal solution corresponded to 13 ± 1 (6) $\times 10^{-10}$ mol/cm^2 (T/M ~ 3), whereas the uptake from the ventricular solution corresponded to 22 ± 1 (5) $\times 10^{-10}$ mol/cm^2 (T/M ~ 5). This was in sharp contrast to the amino-acids which were actively accumulated only across the ventricular surface of the tissue[5].

Two procedures were used to establish the state of the ^{3}H in the plexus. (1) After equilibration of the tissue with ^{3}H-LSD in the external solutions, the efflux of isotope into the ventricular and serosal solutions was measured. More than 75% of the accumulated isotope was free to wash out into the ventricular and serosal solutions. This again contrasts with the amino-acids which washed out into the ventricular solution[5]. The fact that the serosal unstirred layer is effectively much thicker than the ventricular unstirred layer[4] probably accounts for the difference in the amount of isotope washed out into the two solutions. (2) In two experiments the radioactivity in the choroid plexus was extracted by sonication in methanol. Thin-layer chromatography revealed that more than 80% of the tritium migrated as a single spot corresponding to ^{3}H-LSD, and that the remainder stayed at the origin. This suggests that 80% of the ^{3}H accumulated within the choroid plexus was free ^{3}H-LSD and that the remainder was bound to tissue proteins.

Unidirectional flux experiments showed that there was no significant net flux of ^{3}H-LSD across the choroid plexus in the absence of concentration gradients. The fluxes from ventricle to serosa and from serosa to ventricle were 0.18 ± 0.02 (30) and 0.17 ± 0.02 (34) $\times 10^{-9}$ mol/cm^2/h respectively. Four experiments on the effect of iodoacetate/cyanide were inconclusive. In all four experiments there was a slight increase in the apparent unidirectional fluxes when the inhibitors were added. This apparent increase in flux resembles the effect of ouabain on potassium unidirectional fluxes across this tissue[6] and in both cases it is probably a reflexion of the decrease in the intracellular concentration of the isotope. The unidirectional ^{3}H-LSD fluxes corresponded to a permeability coefficient of 1.5×10^{-5} cm/s —about a factor of five greater than the permeability of the neutral amino-acids. Similar experiments also showed that LSD was not actively transported across the arachnoid membrane.

It can be concluded from these experiments that ^{3}H-LSD is accumulated within the frog choroid plexus. Both the efflux experiments and the chromatographic analysis of the tissue radioactivity indicate that more than 75% of the tritium accumulated within the tissue occurs as LSD not irreversibly bound to the tissue. A small percentage of the isotope in the plexus, however, does seem to be bound to some cellular components. These results are consistent with findings that LSD was metabolized to a significant extent only in the liver[1] and that a fraction of ^{3}H-LSD was firmly bound to the rat choroid plexus[2].

The absence of a significant difference between the unidirectional ^{3}H-LSD fluxes across the choroid plexus makes it unlikely that this amine is actively transported across the choroidal epithelium. It is more probable that the permeability of the tissue to LSD is sufficient to account for the rapid exchanges of this drug between the blood and cerebrospinal fluid. Choroid plexuses also do not seem to be well suited as storage sites for LSD in the brain since (1) the drug is accumulated equally well from the ventricular and serosal solutions, and (2) LSD accumulated within the choroid plexus does not preferentially wash out into either the ventricular or the serosal solutions.

^{3}H-LSD is accumulated within the frog choroid plexus by a process characteristic of active transport. Accumulation occurs against considerable concentration gradients (T/M = 45) and depends on the supply of metabolic energy. The effect of ouabain (Fig. 2) suggests that an Na/K ATPase is directly or indirectly involved. There is much in common with the active accumulation of other amines in the choroid plexus. Primary amines (5-hydroxytryptamine and noradrenaline[7]), tertiary amines (narcotic analgesics[8,9] and atropine[10]) and quaternary amines (hexamethonium, decamethonium and choline[11,12]) are all actively accumulated within choroid plexuses. Kinetic studies have established that these amines share a common transport mechanism, that is, quaternary amines competitively inhibit the active accumulation of primary and tertiary amines. In the work reported here I have shown that the active accumulation of LSD was inhibited by the quaternary amine, decamethonium bromide. This suggests that the amine carrier in the choroid plexus is responsible for the active accumulation of LSD. Finally, on the basis of these results it is quite reasonable to speculate that LSD interacts with similar amine carriers and receptor sites in other cells and tissues such as those in the kidney and cerebral cortex. To assess the importance of such interactions in the hallucinogenic activity of LSD it will be necessary to measure the relative affinity of "receptor sites" in the central nervous system for LSD and the naturally occurring amines.

I thank Patricia Ilg for technical assistance, and Drs Alan Grinnell and Jared Diamond for comments on the manuscript. This study was supported by grants from the Life Insurance Medical Research Fund and the US Public Health Service.

ERNEST M. WRIGHT

Department of Physiology,
University of California Medical Center,
Los Angeles, California 90024

Received June 9, 1972.

1 Axelrod, J., Brady, R. O., Witkop, B., and Evarts, E. V., *Ann. NY Acad. Sci.*, **66**, 435 (1957).
2 Diab, I. M., Freedman, D. X., and Roth, L. J., *Science*, **173**, 1022 (1971).
3 Wright, E. M., *Brain Res.*, **23**, 302 (1970).
4 Wright, E. M., and Prather, J. W., *J. Membrane Biol.*, **2**, 127 (1970).
5 Wright, E. M., *Brain Res.* (in the press).
6 Wright, E. M., *J. Physiol.* (in the press).
7 Tochino, Y., and Schanker, L. S., *Biochem. Pharmacol.*, **14**, 1557 (1965).
8 Takemori, A. E., and Stenwick, M. W., *J. Pharmacol. Exp. Ther.*, **154**, 586 (1966).
9 Hug, C. C., *Biochem. Pharmacol.*, **16**, 345 (1967).
10 Eriksson, K. H., and Winbladh, B., *Acta Physiol. Scand.*, **83**, 300 (1971).
11 Tochino, Y., and Schanker, L. S., *Amer. J. Physiol.*, **208**, 666 (1965).
12 Tochino, Y., and Schanker, L. S., *Pharmacologist*, **6**, 177 (1964).