sociation of complete, articulating limbs of young female bovines with human inhumations suggests, however, an intimate association with the cattle herd. Indeed, a combination of osteometric and cultural evidence supports the conclusion that the Non Nok Tha rice farmers possessed domestic bovines of both economic and spiritual importance.

Although the origins of a trend to bovine domestication in southeast Asia cannot be determined without further fieldwork, there can be little doubt that northeast Thailand was occupied by an innovative agricultural and herding society far earlier than previously believed. The early development of bronze technology and farming in northeast Thailand, unlike that in southwest Asia and Mesoamerica, appears to have been followed by a period of cultural conservatism lasting at least two and possibly four millennia until the adoption of iron, the domestic water buffalo, and wet rice farming probably during the first millennium A.D.

C. F. W. HIGHAM
B. F. LEACH
Anthropology Department, University of Otago, Dunedin, New Zealand

References and Notes

1. C. Sauer, *Agricultural Origins and Dispersals*, (American Geographical Society, New York, 1952).
2. C. F. Gorman, *Science* **163**, 671 (1969).
3. W. Solheim, R. H. Parker, D. Bayard, "Preliminary Reports on Excavations at Ban Nadi, Ban Sao Lao, Pimai No. 1, Honolulu" (Social Science Research Institute, University of Hawaii, Honolulu, 1966); W. Solheim, II, *Curr. Anthropol.* **9**, 59 (1968). Ten additional radiocarbon samples have been dated from the 1968 excavations [see (*4*)].
4. D. Bayard, *Asian Perspect.*, in press.
5. C. Higham and M. Jurisich, "The Faunal Remains from Non Nok Tha, Changwat Khon Kaen, Thailand," in preparation.
6. C. Higham and R. H. Parker, "Prehistoric Investigations in Northeast Thailand, 1969–70" (Anthropology Department, University of Otago, New Zealand, 1970).
7. I. Adams, *Amer. Anthropol.* **50**, 256 (1948); R. Pendleton, *Thailand: Aspects of Landscape and Life* (Duell, Sloan & Pearce, New York, 1962).
8. C. Higham, *J. Zool. Soc. London* **157**, 63 (1969).
9. C. R. Rao, *Advanced Statistical Methods in Biometric Research* (Wiley, New York, 1952).
10. ———, *Linear Statistical Inference and Its Applications* (Wiley, New York, 1965).
11. We thank W. G. Solheim, II, for permitting us to analyze the prehistoric material and for his encouragement and advice; D. Bayard and R. H. Parker (University of Otago), who criticized this report during its preparation; and Khun Noom Yoonaidham (Thai Fine Arts Department) and Khun Chin You-di (Thai National Museum), who made possible the research work in Thailand. Supported by the New Zealand University Grants Committee, the Wenner Gren Foundation, and NSF grant GS 1877.

17 August 1970; revised 27 October 1970

Conductance Changes Produced by Acetylcholine in Lipidic Membranes Containing a Proteolipid from Electrophorus

Abstract. *Ultrathin lipidic membranes containing one ten-thousandth of a special proteolipid from electric organ of* Electrophorus *reacted to the addition of acetylcholine by a rapid and transient increase in conductance. Such a change was not induced by choline and is greatly reduced by a previous application of* d-*tubocurarine. These properties, resembling those from chemically excitable membranes, were not observed with another proteolipid from the same tissue.*

The mechanism of action of synaptic receptors involves (i) a molecular interaction of the transmitter with the receptor site, which is followed by (ii) a change in the postsynaptic membrane conductance resulting in the physiological response. Studies on the isolation of receptors are beset with the difficulty that only the first step may be experimentally approached. Previous studies from this laboratory have shown that special proteolipids (that is, hydrophobic lipoproteins) extracted from the nerve-ending membranes of the cerebral cortex had the property of interacting with dimethyl-*d*-tubocurarine (*1*), serotonin (*2*), and atropine (*3*); while proteolipids from electric organs of *Torpedo* and *Electrophorus* showed high affinity binding for acetylcholine and other cholinergic agents (*4*).

We now describe some experiments in which the second step—that of eliciting a response—has been explored by the use of ultrathin black artificial lipidic membranes separating two aqueous phases (*5*). Control membranes were made with a solution of chloroform, methanol, and tetradecane (1.0 : 0.8 : 0.4) containing, per milliliter, 10 mg of synthetic cholesterol (Sigma, 99 percent) and 10 mg of total phospholipds from bovine cerebral cortex. After extraction (*6*) the phospholipids were evaporated several times, to precipitate the contaminating proteolipids, and then they were purified through a column of silicic acid. Experimental membranes were made as indicated, but with addition of proteolipids from *Electrophorus* in a proportion of protein to phospholipids of 1 : 10,000. The proteolipids were extracted and purified as described (*4*). Two proteolipid peaks separated by column chromatography on Sephadex LH-20 from a total lipid extract of the electric organ were used. Peak 1 has no binding capacity for acetylcholine and peak 3, the so-called "receptor" peak, is the one that binds the cholinergic agents (*4*). The membranes were made across a hole 1 mm in diameter in a Teflon septum separating two chambers containing 100 m*M* NaCl and 50 m*M* tris(hydroxymethyl)aminomethane (*p*H 7).

The instrumental arrangement was similar to that of Ehrenstein *et al.* (*7*). A potential difference across the membrane was maintained constant by a voltage source, and it was measured via calomel electrodes with a Keithley d-c voltmeter 200B. The current was determined with a Keithley 150A microammeter and recorded with a Heat EUW servo-recorder. In most cases the drugs were added in 50-μl portions, by means of a fine polyethylene tube ending at 2 mm from the positive side of the membrane.

In the control membranes (without proteolipid) the current voltage (I/V) curves showed an ohmic relation between 0 ± 100 mv, and the resistance thus calculated was $4.2 \pm 0.6 \times 10^5$ ohm cm^2 (mean $\pm$ S.E.; $n = 10$); this value is similar to that reported (*8*). When the membranes were made by adding to the original mixtures proteolipids from peak 1 or 3, the resistance became nonlinear in the voltage range mentioned, and their values were about ten times smaller. At 100 mv the mean value was $5.0 \pm 0.9 \times 10^4$ ohm cm^2 ($n = 12$).

The injection of acetylcholine ($10^{-2}M$ in the pipette) upon the membrane containing the proteolipid of peak 3 produces a rapid five- to tenfold increase in d-c current intensity, which is of a transient nature (Fig. 1A). This result was obtained on 20 different membranes and in some cases it was elicited with only 5 μl of the acetylcholine solution. Since the applied voltage is maintained constant the d-c effect reflects an increase in the conductance of the membrane. Choline applied in a similar way and concen-

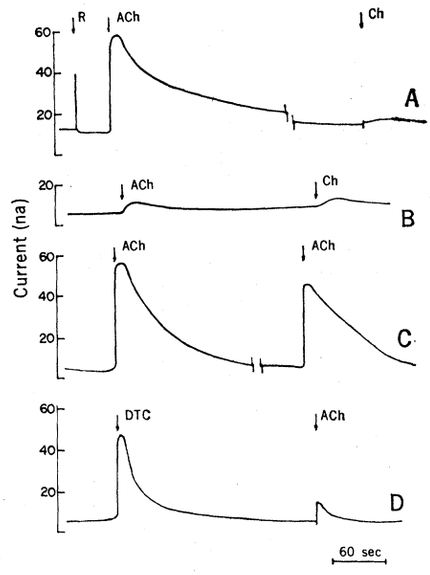

Fig. 1. Records of d-c currents across artificial lipidic membranes containing proteolipids from *Electrophorus*. A, C, and D correspond to peak 3, and B to peak 1. *ACh*, acetylcholine; *Ch*, choline; *DTC*, *d*-tubocurarine; *R*, control; the artifact is due to the injection of the bath solution.

tration failed to elicit such a response (Fig. 1A). When acetylcholine was applied to control membranes or to those containing the "nonreceptor" proteolipid (peak 1) a minimal conductance increase was detected (Fig. 1B). The transient conductance increase induced by acetylcholine may be repeated by successive injections on the same membrane (Fig. 1C). The addition of *d*-tubocurarine ($10^{-3}M$ in the pipette) also elicits a conductance response; however, after this application the action of acetylcholine is much reduced (Fig. 1D). To discard the influence of changes in tonic concentration control experiments were made by injecting different solutions. Thus, with distilled water, 300 m*M* NaCl, or 300 m*M* KCl there were no significant changes in conductance.

Some material of bacterial origin may induce electrical excitability in artificial membranes (*9*). Since the extraction of the proteolipid is done with organic solvents from lyophilized, freshly dissected electric organ this type of contamination can be discarded.

The experimental conditions so far used do not permit us to establish with certainty the initial concentration of the drugs reached at the membrane interface since the 50-μl portions of the drugs applied diffuse and are diluted into the 10-ml content of the chamber and they cannot be washed out. However, those shortcomings do not alter the main facts here reported, and new chambers of better design should decrease these difficulties and permit more elaborate pharmacological experiments.

Del Castillo *et al.* (*10*) reported electrical changes, induced by cholinergic agents, in lipidic membranes with addition of acetylcholinesterase from bovine erythrocytes. In our experiments the proteolipid is devoid of acetylcholinesterase activity and was identified as an independent macromolecular entity (*11*). Unpublished observations with electron microscopy (from this laboratory) have shown that the isolated proteolipid appears as elongated macromolecules which may undergo morphological changes under the action of cholinergic agents. Similar findings were obtained with a "receptor" proteolipid isolated from cerebral cortex (*12*).

Mueller *et al.* (*13*) have shown that some ionophoric antibiotics and the excitability inducing material (*9*), in artificial membranes, were able to induce electrical phenomena resembling those observed in excitable membranes. Our results show that artificial membranes containing the proteolipid of peak 3 may be excited by certain cholinergic agents.

The characteristics of such response provide additional support to the idea that this special proteolipid, which is present in the electroplax membranes (*11*) and which binds cholinergic agents (*4*), may represent a cholinergic receptor.

MARIO PARISI, EMILIO RIVAS
E. DE ROBERTIS
Instituto de Anatomía General y Embriología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina

References and Notes

1. E. De Robertis, S. Fiszer, E. F. Soto, *Science* **158**, 928 (1967); E. De Robertis, S. Fiszer, J. M. Pasquini, E. F. Soto, *J. Neurobiol.* **1**, 41 (1969).
2. S. Fiszer and E. De Robertis, *J. Neurochem.* **16**, 1201 (1969).
3. E. De Robertis, J. González-Rodríguez, D. Teller, *Fed. Europ. Biochem. Soc. Lett.* **4**, 4 (1969).
4. J. L. La Torre, G. S. Lunt, E. De Robertis, *Proc. Nat. Acad. Sci. U.S.* **65**, 716 (1970).
5. P. Mueller, D. O. Rudin, H. T. Tien, W. C. Wescott, *J. Phys. Chem.* **67**, 534 (1963).
6. J. Folch and M. Lees, *J. Biol. Chem.* **191**, 807 (1951).
7. G. Ehrenstein, H. Lecar, R. Nossal, *J. Gen. Physiol.* **55**, 119 (1970).
8. E. A. Henn and T. E. Thompson, *Annu. Rev. Biochem.* **38**, 241 (1969).
9. P. Mueller and D. O. Rudin, *Nature* **213**, 603 (1967).
10. J. Del Castillo, A. Rodríguez, C. A. Romero, *Ann. N.Y. Acad. Sci.* **144**, 803 (1967).
11. E. De Robertis and S. Fiszer de Plazas, *Biochem. Biophys. Acta* **219**, 388 (1970).
12. C. Vásquez, F. J. Barrantes, J. L. La Torre, E. De Robertis, *J. Mol. Biol.* **52**, 221 (1970).
13. P. Mueller and D. O. Rudin, *Nature* **217**, 713 (1968).
14. We thank Dr. Dante Chiarandini and Dr. Enrique Stefani for critically reading the manuscript and Dr. José L. La Torre for the preparation of proteolipids. Supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina) and NIH (5 R01 NS 06953-05 NEUA). M.P. and E.R. hold research career awards from CNICT.

13 October 1970; revised 6 January 1971

Alcohol Breath Tests: Gross Errors in Current Methods of Measuring Alveolar Gas Concentrations

Abstract. *Transitory contact of ethanol with the mucous membranes of the mouth or nasal passages, or both, is sufficient to drastically alter measurements of concentrations of ethanol in so-called "alveolar" gas for more than 20 minutes after such contact. Various concentrations of ethanol were taken into the mouth by human subjects and were expectorated. Readings of so-called "blood alcohol" were then taken at short intervals by means of the Breathalyzer® and were continued up to 1 hour after exposure. These readings were compared with blood-alcohol concentrations measured by quantitative chemical analysis of venous blood. When true concentrations of blood alcohol were at or close to zero (plus possible error of 0.0001 gram per 100 milliliters), readings of greater than 0.40 gram per 100 milliliters were obtained on the Breathalyzer. Repeated mouth washing and gargling with water, changes in the nature of the solvent, and stomach loading each had only a slight effect in diminishing these errors.*

It has been assumed that accurate measurements of the concentration of ethanol in blood can be determined by measurements of ethanol fractions in "alveolar" gas (*1*, p. 29). "Alveolar" gas is usually defined as the last sample