vesicle profiles. Stimulated endplates (Figures 2–3) show varying degree of reduction in vesicle number. In stimulated endplates there also appears to be a definite increase in the number of elongated vesicle profiles (Figure 2). For lack of physiological evidence, the hypothesis that elongated vesicles contain an inhibitory transmitter[10] is hardly acceptable in this case. Whether the increased proportion of elongated vesicles in stimulated endplates is caused by stimulation per se, or is due to secondary artificial factors, remains unanswered.

The synaptic vesicles are claimed to be associated with a large part of the transmitter substance[11], and a reduction in the number of synaptic vesicles seems, according to the present results, to accompany neuromuscular transmission. This apparently supports the vesicle hypothesis.

As to the mechanism of transmitter release and quantitation of transmitter, some speculative remarks can be made. Many authors, to mention only COUTEAUX and PÉCOT-DECHAVASSINE[12] and NICKEL and POTTER[13], have found evidence for fusion between synaptic vesicles and the presynaptic membrane, indicating a model for transmitter release by exocytosis. The reduced number of synaptic vesicles following stimulation in the present experiments fits this concept. However, the difference in membrane composition between synaptic vesicles and the presynaptic membrane[11] makes a definite fusion between vesicles and presynaptic membrane improbable. On the other hand, if the fusion is only temporary, the present results definitely do not favour re-usage of vesicles. Release by exocytosis of complete vesicles appears unlikely because no vesicles are present in the synaptic cleft. A last theoretical possibility concerning vesicles and transmitter release is that vesicles may release the transmitter and disappear without interaction with the presynaptic membrane. This may even gain support by the fact that only a part of the transmitter is localized in association with the synaptic vesicles[11,14]. Uncertainties thus prevail as to the actual mechanism of transmitter release, and also a possible role of elongated vesicles as a stage in the release process remains hypothetical.

Zusammenfassung. Während der Fixation wurden Nervus-phrenicus-Diaphragma-Präparate der Ratte stimuliert (50–100 Hz). Die Elektronenmikroskopie motorischer Endplatten zeigte reduzierte Gesamtzahl der synaptischen Bläschen und Zunahme der Menge der «flachen» Bläschen.

H. KORNELIUSSEN, J. A. B. BARSTAD and G. LILLEHEIL

Anatomical Institute, University of Oslo, Karl Johansgt. 47, Oslo 1, and Division for Toxicology, Norwegian Defense Research Establishment, N 2007 Kjeller (Norway), 3 February 1972.

[10] K. UCHIZONO, Nature Lond. *207*, 642 (1965).
[11] V. P. WHITTAKER, in *Excitatory Synaptic Mechanisms* (Eds. P. ANDERSEN and J. K. S. JANSEN; Universitetsforlaget, Oslo 1970), p. 67.
[12] R. COUTEAUX and M. PÉCOT-DECHAVASSINE, C. r. hebd. Séanc. Acad. Sci. Paris, Ser. D, *271*, 2346 (1970).
[13] E. NICKEL and L. T. POTTER, Brain Res. *23*, 95 (1970).
[14] M. ISRAËL and J. GAUTRON, in *Cellular Dynamics of the Neuron* (Ed. S. H. BARONDES; Academic Press, New York 1969), p. 137.

The Hyperpolarization of Neurones of the Medulla Oblongata by Glycine

Much evidence for glycine being an inhibitory transmitter substance in the spinal cord has derived from intracellular microelectrode studies demonstrating that glycine causes a hyperpolarization of spinal neurones associated with changes in membrane conductance similar to that of postsynaptic inhibition[1–3]. There is also considerable evidence that glycine is an inhibitory transmitter in the medulla oblongata. It has been shown that glycine is a potent depressant of bulbar reticular neurones and that this depression is reversibly blocked by strychnine[4].

It has recently been reported by JOHNSTON and IVERSEN[5] that there is a high affinity uptake system for glycine in the spinal cord and the medulla oblongata. Autoradiographic studies have shown that this amino acid is actively taken up by cultured medullary neurones[6].

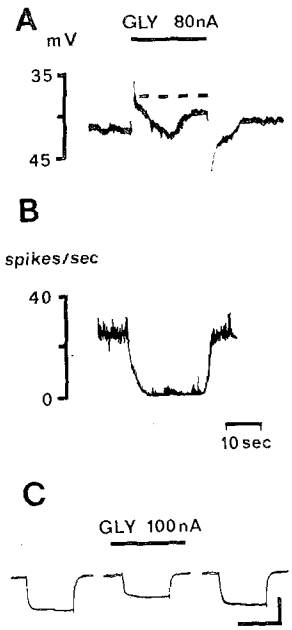
In the present study the action of microelectrophoretically administered glycine on the membrane potential and membrane conductance of neurones of the medulla oblongata of the cat has been investigated.

The experiments have been carried out on unanaesthetized, decerebrate cats. Decerebration was performed during halothane-nitrous oxide anaesthesia by coagulation at the midcollicular level[7]. Most animals were respiring spontaneously, but a few cats were paralyzed with intravenous gallamine triethiodide (Flaxedil) and artificially ventilated. The methods have been described in detail in a previous paper[4]. For the intracellular studies a combined microelectrode was used, consisting of a single recording micropipette (tip diameter less than 1 μm) filled with 3 *M* KCl or 2 *M* K-citrate and glued to a 4-barrel micropipette from which glycine (0.5 *M*, pH 3–3.5) was ejected microelectrophoretically. The recording electrode was fixed with Epoxylite and Deiberit 502 (dental wax) parallel to the multibarrel micropipette projecting 10–60 μm beyond its orifice[2,8]. The recording electrode was connected through an Ag-AgCl wire to a cathode follower. Potentials were displayed on an oscilloscope from which they were photographed by a Grass camera. In a few cells the membrane potential has also been recorded on a rectilinear ink recorder. The resistance of the cell membrane was measured by passing hyperpolarizing current pulses of 30–40 msec duration through the recording electrode by means of a device similar to that described by FEIN[9].

Considerable difficulties were experienced recording intracellularly from brain stem neurones. The membrane potential of the majority of cells decayed rapidly after im-

[1] R. WERMAN, R. A. DAVIDOFF and M. H. APRISON, J. Neurophysiol. *31*, 81 (1968).
[2] D. R. CURTIS, L. HÖSLI, G. A. R. JOHNSTON and I. H. JOHNSTON, Expl Brain Res. *5*, 235 (1968).
[3] G. TEN BRUGGENCATE and I. ENGBERG, Brain Res. *11*, 446 (1968).
[4] L. HÖSLI and A. K. TEBÈCIS, Expl Brain Res. *11*, 111 (1970).
[5] G. A. R. JOHNSTON and L. L. IVERSEN, J. Neurochem. *18*, 1951 (1971).
[6] L. HÖSLI and E. HÖSLI, Brain Res., in press.
[7] E. A. KOLLER and M. JENNY, Brain Res. *14*, 549 (1969).
[8] G. TEN BRUGGENCATE and I. ENGBERG, Brain Res. *25*, 431 (1971).
[9] H. FEIN, Ieee Trans. Bio-Med. Engin. *BME-13*, 211 (1966).

palement with the microelectrode. However, in a small number of cells it was possible to record reasonably stable membrane potentials (–40 to –65 mV) for several min. One neurone with a spike of 65 mV was held in good conditions for approximately 45 min. In the present study only cells with membrane potentials over –40 mV have been included (13 cells). Glycine ejected with currents of 60–100 nA hyperpolarized the membrane (2–8 mV) with a time course similar to that observed in spinal and in Deiters' neurones[2, 3, 8, 10]. Figure A illustrates a hyperpolarization by glycine (80 nA) of a medullary neurone which was firing at a rate of approximately 25 spikes/sec. The hyperpolarization was accompanied by complete depression of firing of this neurone (Figure B).



Action of glycine on membrane potential (A), firing rate (B) and membrane conductance (C) of two different neurones of the medulla oblongata. A) Membrane potential (–41 mV, initially –50 mV) recorded with a K-citrate electrode on a rectilinear ink recorder. Glycine (GLY) was ejected with a current of 80 nA from one barrel of an extracellular 4 barrel micropipette. Duration of glycine ejection is indicated by bar above trace. The horizontal broken line indicates the coupling artifact (4 mV) that was estimated after withdrawal of the intracellular microelectrode from the cell. B) Ratemeter record of the firing rate (spikes/sec) of the same neurone as A). Time 10 sec for A) and B). C) Measurements of membrane conductance of another neurone by the injection of hyperpolarizing current pulses (2 nA) before, during and after administration of glycine (100 nA) for approximately 10 sec. Calibrations: 10 mV, time 20 msec.

The action of glycine on the membrane conductance was studied in 5 cells. Glycine reversibly reduced the amplitude of the potential change produced by a hyperpolarizing current pulse passed through the recording electrode (Figure C). Glycine also blocked the action potential evoked by an intracellular depolarizing pulse in two cells. The administration of glycine depolarized 3 cells impaled with KCl electrodes probably due to the diffusion of chloride ions from the recording electrode.

Our results demonstrating that glycine causes a hyperpolarization and an increase in membrane conductance support the hypothesis that glycine is an inhibitory transmitter substance in the medulla oblongata. However, it has yet to be shown that postsynaptic inhibition of brain stem neurones is blocked by strychnine in the same manner as the hyperpolarization by glycine.

Zusammenfassung. Mikroelektrophoretisch verabreichtes Glycin erzeugt eine Hyperpolarisation und eine Zunahme der Leitfähigkeit der Zellmembran von Neuronen der Medulla oblongata der Katze. Diese Ergebnisse unterstützen die Hypothese, dass Glycin die Funktion einer hemmenden Überträgersubstanz im Hirnstamm hat.

L. HÖSLI and H. L. HAAS[11, 12]

Department of Neurophysiology, Neurological Clinic of the University of Basel, Socinstrasse 55, CH-4051 Basel (Switzerland),
29 May 1972.

[10] K. OBATA, K. TAKEDA and H. SHINOZAKI, Expl. Brain Res. *11*, 327 (1970).

[11] We are indepted to Dr. A. K. TEBÈCIS who participated in some of the experiments, to Mr. P. F. ANDRÈS who designed some of the equipment used in this investigation and to Miss V. MEIER for typing the manuscript.

[12] Some of the results have been reported at the Symposium «Physiology and Pharmacology of Synapses» Basel, 1971, Experientia *27*, 1113 (1971).

Mechanism of Allyl Alcohol-Induced Hepatic Necrosis

Although allyl formate and its metabolite, allyl alcohol, were shown to cause extensive periportal necrosis of the rat liver more than half a century ago[1], the biochemical mechanism of their hepatotoxic action remains controversial[2–5]. Recently, REES and TARLOW[6] reported that inhibition of mitochondrial respiration in vitro and blockade of protein synthesis by allyl formate depend upon the nonmicrosomal enzyme alcohol dehydrogenase and can be mimicked by the metabolite acrolein. REES and TARLOW[6] also confirmed that alcohol dehydrogenase is localized in the periportal regions of the hepatic lobule[7] and postulated that the periportal distribution of allyl formate-induced hepatic necrosis results from the alkylation of macromolecules by a metabolite, acrolein, produced within periportal hepatocytes. The present studies provide the first direct evidence in vivo that allyl alcohol causes periportal necrosis through the covalent binding of a metabolite to periportal hepatocytes.

Methods. Male Sprague-Dawley rats (200 g) were pretreated either with saline (0.5 ml i.p.) or with pyrazole (375 mg/kg i.p.) an inhibitor of hepatic alcohol dehydro-

[1] J. G. PIAZZA, Z. exp. Path. Ther. *17*, 318 (1915).

[2] H. EPPINGER, H. KAUNITZ and H. POPPER, *Die seröse Entzündung* (Springer, Vienna 1935).

[3] K. HEINEMANN, Beitr. path. Anat. *98*, 545 (1937).

[4] H. SCHÖN and E. STEIDL, Klin. Wschr. *35*, 354 (1957).

[5] W. EGER, *The Liver* (Ed. C. H. ROUILLER; Academic Press, New York 1964), chapt. 26.

[6] K. R. REES and M. J. TARLOW, Biochem. J. *104*, 757 (1967).

[7] N. J. GREENBERGER, R. B. COHEN and K. J. ISSELBACHER, Lab. Invest. *14*, 264 (1965).