spected directly and were sometimes photographed for a permanent record.

Results and Discussion. The action of low concentrations of α-lobeline in producing axone reflex sweating was usually facilitated by paraoxon, while higher concentrations (≧ 1:1,750000) of α-lobeline were inhibited by paraoxon in more than one-half the trials (Table). The Table gives combined results on 11 subjects, so that dose-response characteristics for individual subjects tend to be obscured. As a rule, the results on each subjects followed the trend that low concentrations of α-lobeline were facilitated by paraoxon and high concentrations inhibited (Figure). Moreover, α-lobeline itself shows the characteristic, previously reported for the nicotinic agents[5], of producing a progressively diminishing axone reflex response at concentrations higher than an optimum. These results suggest that endogenous acetylcholine, here acting additively with α-lobeline, can participate in the initiation of axone reflex sweating. Whether in fact this occurs under physiological conditions remains unknown.

Zusammenfassung. Paraoxon beeinflusst die Wirkung von α-Lobelin über den Axonreflex, der Schwitzen menschlicher Haut auslöst. Geringe Konzentrationen von α-Lobelin werden durch Paraoxon verstärkt, während höhere gehemmt werden. Daraus wird gefolgert, dass endogenes Acetylcholin an der Auslösung des Axonreflexes beteiligt sein kann.

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Department of Physiology, University of California Medical Center, Los Angeles (Calif.), June 6, 1961.

[5] S. Rothman and J. M. Coon, J. Pharmacol. exp. Therap. *73*, 1 (1941).

[6] *Acknowledgements.* This work was supported by a contract between the Office of Naval Research, Department of the Navy, and the University of California, NR 101-385. Paraoxon was kindly supplied by Dr. R. B. March, University of California, Riverside, and spectrophotometric determination of its concentration by Dr. G. Stevenson, University of California, Los Angeles.

Qualitative Effect of Strychnine and Brucine on Spontaneous Potentials from Explants of Telencephalon

The cells of origin of the spontaneous potentials from explants[1] of brain tissue in culture have not yet been determined. Some evidence as to their identity can be obtained from their reactions to pharmacological agents with a known effect on brain tissue. The study reported below concerns the reaction of spontaneous potentials from explants of 15 day chick embryo telencephalon to the analeptic drugs strychnine and brucine.

Each explant was taken in the usual way from the telencephalon of 15 day chick embryos and placed onto the upper aspect of a piece of cellulose sponge on a coverglass in such way as to lie between the sponge and a 36 gauge platinum electrode. The reference electrode (also of 36 gauge platinum wire) was cemented onto the side of a Kahn tube into which the coverglass with the sponge, explant and electrode were placed. A supernatant made from balanced salt solution TDL1[1] and 0.25% human serum protein at 37°C was added to the Kahn tube in such a way as to come half way up the cellulose sponge and immerse the lower end of the reference electrode without touching the explant itself (except insofar as the supernatant permeated the cellulose sponge). The Kahn tube was sealed with a serum stopper containing an air filter to prevent any change in the pressure inside the

[1] A. W. B. Cunningham, M. Dougherty, and B. J. Rylander, Nature *186*, 477 (1960).

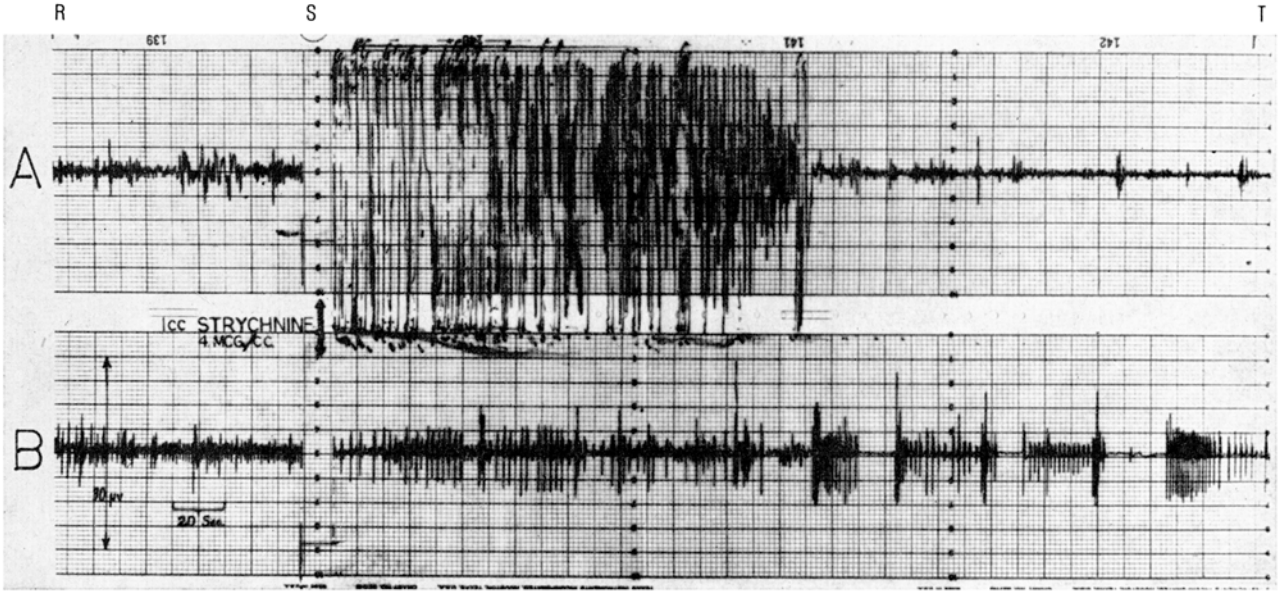

Fig. 1. Simultaneous effect of mcg/cm³ strychnine sulphate on the spontaneous potentials from two explants of 15 day chick embryo telencephalon. In both explants the section R to S represents the normal potential production from the explants. The strychnine is added at the point S and the section S to T represents the effect of the strychnine. The increase in magnitude of the potentials in both explants is obvious as is the subsequent inhibition of the potentials in Explant A. X-axis each major division represents 10 sec. Y-axis eight major division represents 30 μV.

Kahn tube and a length of Teflon tubing to allow the introduction of the supernatant containing the drug. The stopper was inserted so as to allow the egress of the two platinum electrodes. The Kahn tube was placed in a shielded incubator at 37°C and connected by shielded cables to amplifiers and a paper strip recorder.

The explants used had been in culture for 48 h. The slow and careful injection of an additional volume of warm supernatant to cover the explant followed by immediate withdrawal of an amount equal to that just added, leaving the original volume (2 cm^3) of supernatant, usually caused a reduction in the activity of the explant. The strychnine and brucine in solution were applied to the explants in this way. Since it was possible that an explant might have been displaced by this maneuver and to ensure that the results to be reported were due to the drug, many more explants were exposed to the drug than are to be reported and all explants had the same type of response. Also the same response was obtained when the drug was applied repeatedly to the same explant or to other explants.

Results. 4 mcg/cm^3 strychnine sulphate dissolved in warm supernatant was applied simultaneously to explants in the manner described above and the potentials produced by the explants increased in magnitude without apparent increase in frequency. Explant A (Figure 1, upper trace) showed the greatest increase in magnitude of potential but the effect was relatively short-lived because inhibition of the cell or cells which were initially stimulated. The cells were not dead because a lesser potential

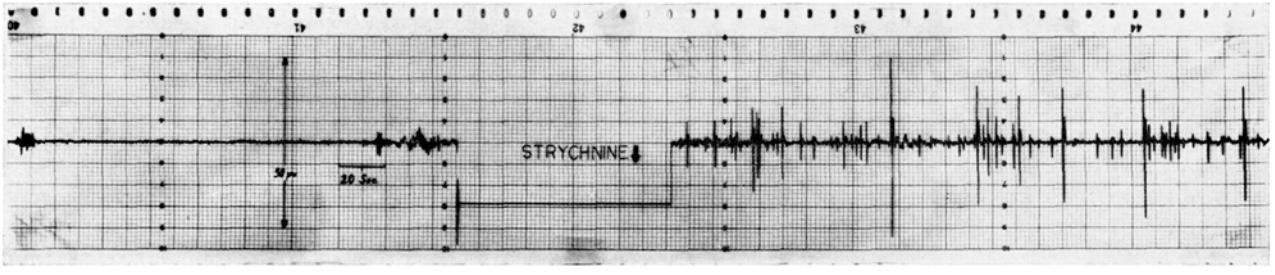

Fig. 2. The effect of 4 mcg/cm^3 of strychnine sulphate on the spontaneous potentials from an explant of 15 day chick embryo telencephalon. The increase in magnitude of the potentials after addition of strychnine is obvious. X-axis each major division represents 10 sec. Y-axis eight major divisions represents 30 μV.

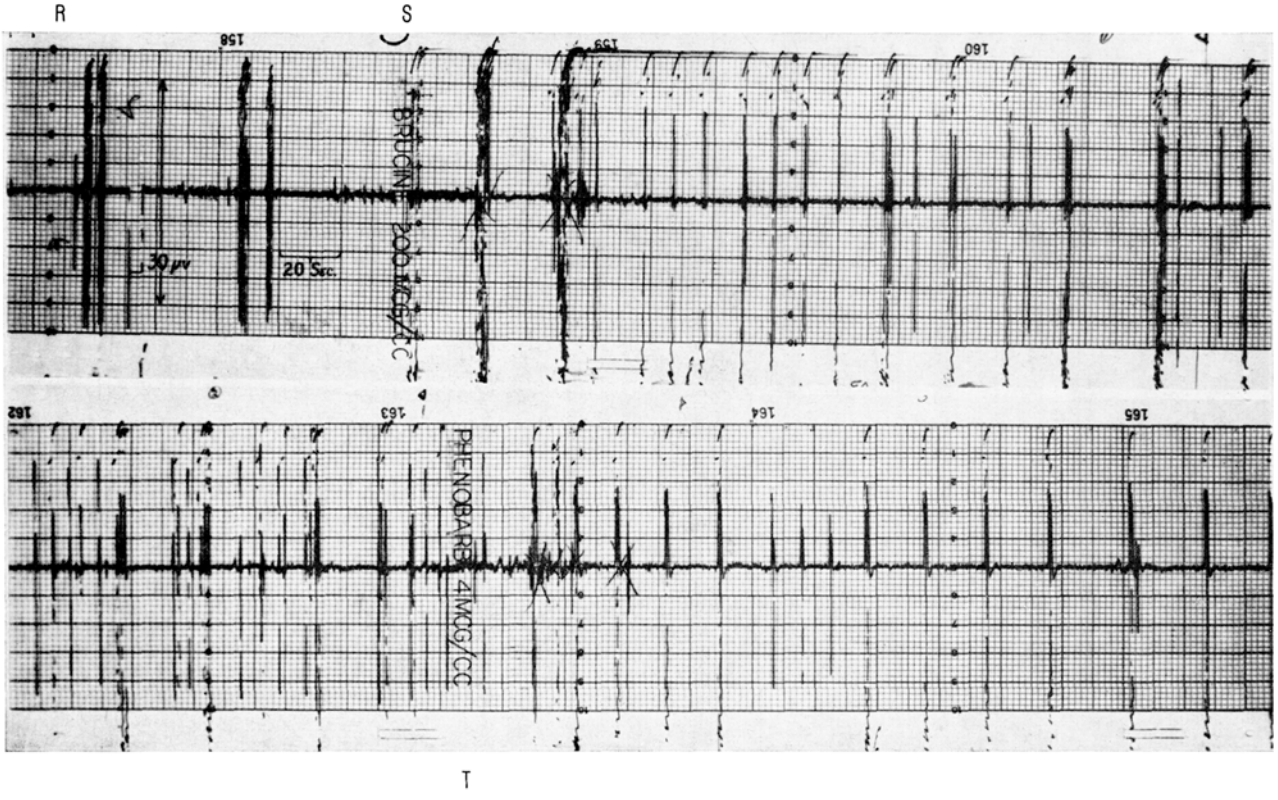

Fig. 3. The effect of 200 mcg/cm^3 brucine sulphate on the spontaneous potentials from an explant of 15 day chick embryo telencephalon. The section R to S is the normal activity of the explant. The brucine is added at the point S and the section from S to T represents the effect of the brucine. At the point T sodium phenobarbital is added to the final concentration of 4 mcg/cm^3 and its effect in diminishing the effect of the brucine is obvious. X-axis each major division represents 10 sec. Y-axis eight major divisions represents 30 μV.

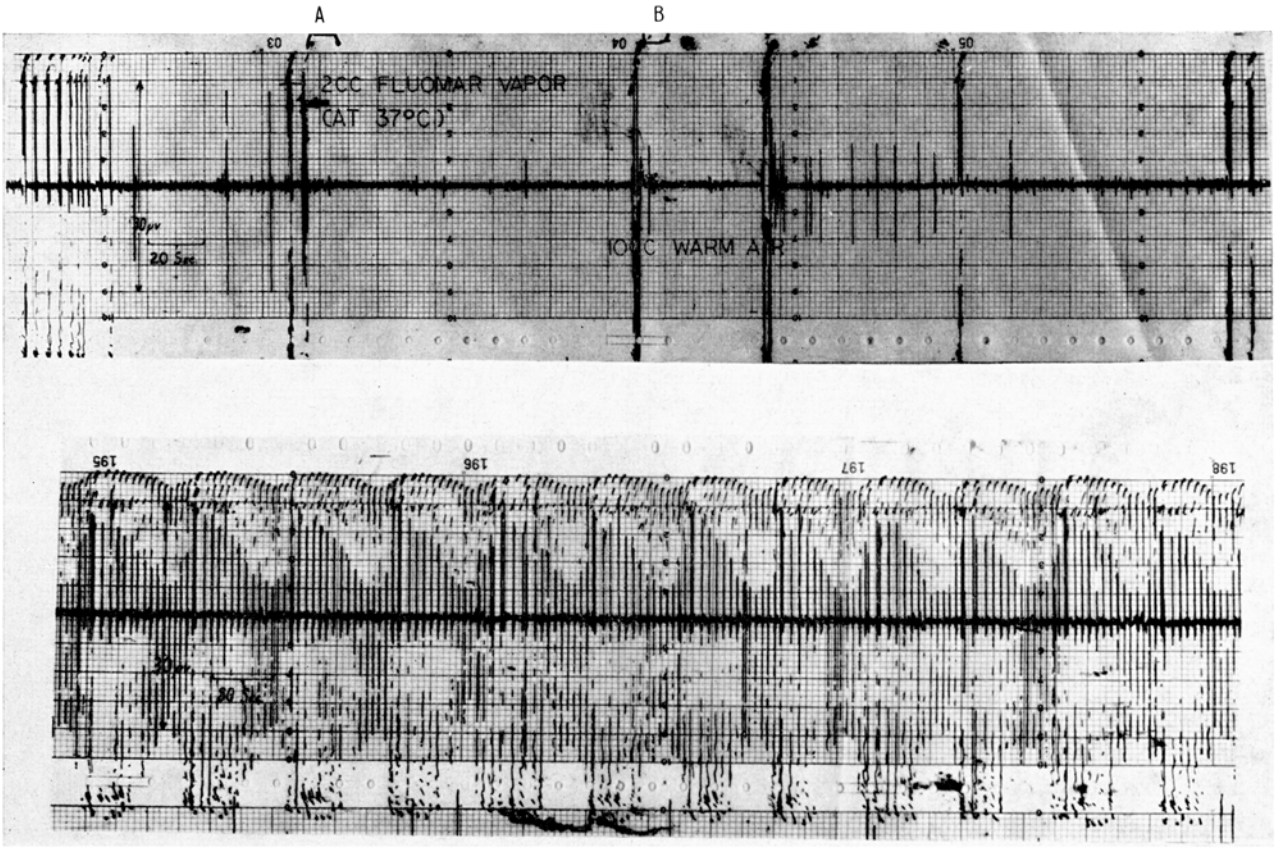

Fig. 4. The lower part of this Figure shows a continuation of the recording from Figure 3 at one quarter the paper speed of the previous Figure to show the cyclic variation in the potentials. The upper recording (made at four times the paper speed of the lower one) shows the effect on an anesthetic gas, Fluomar (using air saturated with Fluomar vapor at 37°C), on the potentials amplified by strychnine and then lessened by sodium phenobarbital. The anesthetic vapor is applied at A and washed out by two injections of air at 37°C at B. X-axis, lower recording, each major division represents 40 sec, upper recording, each major division represents 10 sec. Y-axis eight major divisions represents 30 μV.

continued to arise. Explant B (Figure 1, lower trace) showed a lesser increase in magnitude. This increased activity continued until the culture was abandoned, about 48 h later. A further example of the stimulatory effect of strychnine on a telencephalic explant can be seen in Figure 2.

The effect of brucine sulphate on telencephalic explants is seen in Figures 3 and 4. It is similar to that of strychnine but fifty times the concentration of brucine was needed for a similar activity. The effect of brucine was reduced by sodium phenobarbital and abolished by anesthetic vapor (air saturated with Fluomar Vapor at 37°C) whose effect was partially reversed when the vapor was washed out with warm air at 37°C.

Discussion. From the above results it can be seen that the explants react to strychnine and brucine in the same manner as has been reported for the nervous systems of intact animals and for cells of the central nervous system (in particular the nerve cell bodies and axons[2]). The proportionate strengths of strychnine and brucine necessary for equivalent action on the explants is the same as is needed in the living animal[3]. The mutual antagonistic effect of brucine and barbiturate is the same as that encountered in the whole animal and attributed to actions on the active elements of the central nervous system. The suppressive effect of an anesthetic gas on the potentials which have been exaggerated by the action of brucine is the same as encountered in the living animal and also can be regarded as evidence that the potentials were arising from the explant and were not artefacts. In brief the responses of the explants to these pharmacological agents are the same as those on the intact central nervous system and on nerve cells and their axons[4].

Zusammenfassung. Brucin and Strychnin ergeben Vergrösserung der spontanen Potentiale aus Telencephalus-Gewebe (*in vitro*). Die Potentialzunahme nimmt bei Zusatz von Natriumphenobarbital ab und wird durch Betäubungsmittel aufgehoben. Die Reaktion des Explants auf Pharmaka stimmt mit derjenigen des intakten Zentralnervensystems, der Nervenzellen und ihrer Axone überein.

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Tissue Dynamics Laboratory, Pathology Department, University of Texas, Medical Branch, Galveston (Texas), June 12, 1961.

[2] P. Heinbecker and S. H. Bartley, Amer. J. Physiol. *125*, 172 (1939).

[3] R. W. Morrison and A. R. Bliss, J. Amer. pharm. Assoc. *21*, 753 (1932).

[4] This work was done under a U.S. Navy Contract, NONR 1598(04).