Brit. J. Pharmacol. (1955), **10**, 220.

PHASE-BOUNDARY POTENTIALS AND GANGLION-STIMULANT ACTIVITY

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(RECEIVED FEBRUARY 2, 1955)

The theory that nerve action potentials are phase-boundary potentials evoked by acetylcholine in contact with nerve lipoid has been advocated in recent years by Barnes and Beutner (1946, 1947, 1949a, 1949b, 1949c). It is based on the demonstration (Beutner and Barnes, 1941) that weak solutions of acetylcholine develop negative potentials in contact with certain oily substances under the artificial conditions of an "oil cell," such as is shown diagrammatically in Fig. 1. Feldberg (1945) considered the possibility that such boundary potentials might be associated with the depolarizing property of acetylcholine at autonomic ganglia and at motor endplates.

Furthermore, after measuring phase-boundary potentials of several other substances, Barnes and Beutner (1949c) concluded that the different actions produced by altering the chemical structure of acetylcholine could be explained in terms of phase-boundary potentials.

It seemed to us that the choline esters and ethers previously examined by Hey (1952) would be very suitable drugs with which to test the validity of the Barnes-Beutner theory; for, if it is tenable, then the phase-boundary potentials developed by drugs highly active as nerve-cell stimulants should be high, whereas those developed by drugs relatively weak in this respect should be low.

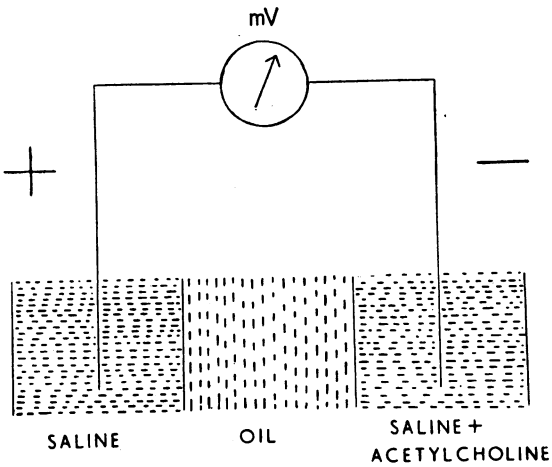

FIG. 1.—Schematic diagram of an "oil cell" for demonstrating phase-boundary potential developed at the interface between solution of acetylcholine and an oil phase.

METHODS

We used oil cells similar to those described by Beutner and Barnes (1941) and Barnes and Beutner (1942). Re-distilled guaiacol was used for the oil phase, since these authors claimed (1947) that guaiacol has certain physical properties in common with protoplasm—for example, its electrical conductivity, and its ability to absorb lipoid-soluble substances.

Solutions of 0.003 M of each of the esters and ethers were made up in 0.7% saline and tested in the modified oil cell shown in Fig. 2. This consisted of a glass U-tube provided with a drain tap to allow thorough cleaning after each separate estimation. 2 ml.

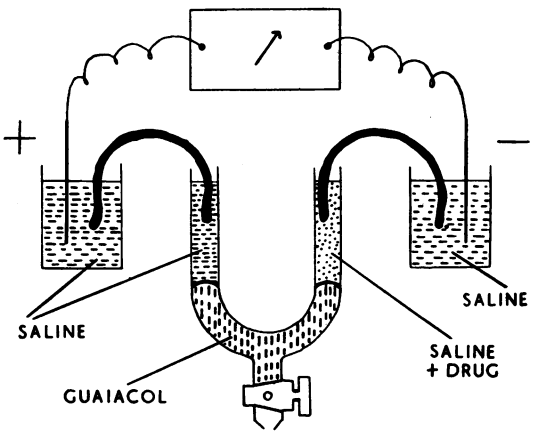

FIG. 2.—Diagram of apparatus used in this investigation of phase-boundary potentials developed by solutions of esters and ethers of choline in contact with guaiacol. Electrical continuity between the limbs of the U-tube and the outer saline-containing vessels is achieved by saline wicks; the measuring instrument is connected to chlorided silver wire electrodes dipping into the two outer vessels.

guaiacol was first run into the tube, followed by 1 ml. saline into the left-hand limb and 1 ml. drug solution into the right-hand limb. Each limb was connected, by a saline wick, to an outer vessel containing 0.7% saline. Chlorided silver wire electrodes dipped into the outer vessels, and the potential between these was measured on a Cambridge *p*H meter set to read millivolts. Three estimations were made for each drug solution and the mean potential calculated.

A few experiments were performed using lecithin instead of guaiacol; membranes consisting of frog's skin (Barnes, 1940) were also tried.

Results

Choline Esters and Nicotine

The phase-boundary potentials developed between 0.003 M solutions of the esters and guaiacol, and between nicotine acid tartrate and guaiacol, are shown in Table I; in the second column are the means of several determinations for each drug

TABLE I

PHASE-BOUNDARY POTENTIALS AND GANGLION-STIMULANT ACTIVITIES OF CHOLINE ESTERS AND NICOTINE

Except for methacholine, relative stimulant activities are from data of Hey (1952)

Compound	Mean Potential (mV) Developed by 0·003 M Solutions Against Guaiacol	Molar Ganglion-stimulant Activity, Relative to Choline Phenyl Ether = 100·0
Acetylcholine	35	2·2
Methacholine	45	0
Carbachol	18	7·0
Propionylcholine ..	47	3·4
*iso*Butyrylcholine ..	50	8·4
Trimethylacetylcholine ..	51	21·4
Benzoylcholine	68	2·5
Phenacetylcholine ..	70	7·4
Nicotine acid tartrate ..	41	47·5

—seven for acetylcholine, and three for each of the other drugs—expressed to the nearest mV. The value for acetylcholine, incidentally, agrees closely with that quoted by Barnes and Beutner (1949c). Data in the third column indicate the corresponding molar ganglion-stimulant activities relative to choline phenyl ether = 100.0, as previously determined by Hey (1952) upon the vasomotor ganglia of cats.

As will be seen from Table I, there seems to be little correlation between ganglion-stimulant activity and phase-boundary potentials within this series. Methacholine, though not specifically tested by Hey, is well known to be devoid of nicotinic activity: nevertheless it develops a potential of 45 mV. Carbachol, on the other hand, gives a relatively low potential of 18 mV. Larger ester groupings, as in benzoylcholine, tend to develop high potentials without, necessarily, having enhanced nicotinic activity. Conversely, the high stimulant activity of nicotine acid tartrate is not associated with a high boundary potential.

Choline Ethers

In Table II the choline ethers tested are arranged in descending order of ganglion-stimulant activity. It will be noted that members of this series exhibit a wide range of stimulant activity (370 to 0.4) accompanied by a minimum of structural alteration. This wide variation in pharmacological activity is not, however, reflected in the phase-boundary potentials; these are consistently high throughout the series and are probably dominated by the affinity of the aryl moieties for the oil phase.

TABLE II

PHASE-BOUNDARY POTENTIALS AND GANGLION-STIMULANT ACTIVITIES OF CHOLINE ETHERS

Compound $R\text{-}O.CH_2.CH_2.N(CH_3)_3Br$ R =	Mean Potential (mV) Developed by 0·003 M Solutions Against Guaiacol	Molar Ganglion-stimulant Activity, Relative to Choline Phenyl Ether = 100·0
m-Bromophenyl	75	370
3: 5-Dibromophenyl ..	69	337
m-Chlorophenyl	76	220
Phenyl	76	100
m-Tolyl	78	13·1
p-Chlorophenyl	75	10·4
3: 5-Xylyl	75	5·2
p-Tolyl	79	0·4

The phase-boundary potentials developed by the choline ethers (Table II) were measured immediately after the drug solutions reached the guaiacol. If the drug solutions were allowed to remain in contact with the guaiacol phase for 10 min. before the potential was measured, slightly lower readings were obtained. However, the proportionate reduction in voltage was the same for each drug.

We have also investigated the effect of altering the molar concentration of the ether solutions and have been able to confirm the observation of Beutner and Barnes (1941) that the size of the phase-boundary potential developed by a solution of a choline derivative is proportionate to the logarithm of its concentration.

Tests on Other Boundary Systems

In an attempt to imitate physiological conditions more closely, certain of the ethers were tested on cell models in which lecithin replaced guaiacol, or in which a partition of frog's skin was used. The

potentials developed, though always less than one tenth of those obtained with guaiacol, showed a similar consistency throughout the ether series. Thus, a closer approach to "physiological" conditions afforded no closer correlation between phase-boundary potentials and stimulant activity.

Discussion

On the basis of their theory of phase-boundary potential, Barnes and Beutner (1949b) have boldly rejected some of those present-day views of drug action which embody steric considerations; they have challenged Ing's concept of precise receptor fit, and have accused both Ing and Pfeiffer of showing "preoccupation with skeletal rather than dynamic aspects of autonomic drug action." We believe, however, that Barnes and Beutner are themselves preoccupied with phase-boundary potentials to the exclusion of many other important factors.

Not only do many of the observations given here fail to accord with the theory, but certain of those reported by Barnes and Beutner themselves also, in our opinion, fail to do so. For example, the values which they give (Barnes and Beutner, 1949b) for the phase-boundary potentials of equimolar solutions of tetramethylammonium and tetraethylammonium bromides (20 and 74 mV, respectively) do not seem to predict the likely stimulant or blocking actions of these drugs on ganglia or on motor endplates; tetraethylammonium, which blocks sympathetic ganglia without depolarization (Paton and Perry, 1953), would be expected by the theory to evoke a *lower* boundary potential than its stimulant tetramethyl analogue.

Our results with the esters and ethers of choline make it clear that, in measuring phase-boundary potential, we are dealing with an isolated physicochemical property, dependent upon solubility in oil, and that this property has little bearing on the stimulant behaviour of the drugs *in vivo*.

Barnes and Beutner (1949a) claim that the oil-cell method provides a basis for distinguishing between "sympathetic" and "parasympathetic" drugs. (They presumably mean drugs acting upon structures with postganglionic sympathetic and parasympathetic innervations, respectively.) They showed that the "sympathetic" drugs dissolve in triglyceride oils, on which they form negative phase-boundary potentials. In contrast, "parasympathetic" drugs, like acetylcholine, develop boundary potentials with guaiacol but not with triglyceride oils. On the grounds that they have demonstrated a boundary-potential effect between nicotine and tributyrin, they go so far as to imply that the pressor effect of nicotine is a direct adrenaline-like action. Few would accept this suggestion. In any event, we find that nicotine does, in fact, develop a boundary potential of 41 mV in contact with guaiacol.

In conclusion, we agree with Perry (1953) that Barnes and Beutner's models can only be regarded as a vast over-simplification of a most complex situation. The behaviour of drugs on nervous structures must be intimately bound up with other factors besides phase-boundary potential—with molecular size and shape, electron density, lipoid solubility, and probably many other properties.

Summary

1. Simple apparatus is described for the measurement of phase-boundary potentials developed between solutions of drugs and guaiacol.

2. Examination of the phase-boundary potentials of esters and ethers of choline, and of nicotine, has failed to reveal any obvious correlation with ganglion-stimulant activity.

3. The Barnes-Beutner "theory of phase-boundary potential" is inadequate to account for the pharmacological behaviour of choline compounds on autonomic ganglia.

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