Summary. When unenriched corn grits are incorporated in a synthetic ration to the extent of 60% of the sucrose, the nicotinic acid requirement of the dog is markedly increased and good growth does not result until the nicotinic acid content of the corn grits is increased to about 5.0 mg % (more than 5 times the unenriched level) which furnishes the dog with about 3 times as much nicotinic acid as is required for comparable or better growth on a synthetic or whole milk ration.

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Electrical Activity of Acetylcholine at the Phase Boundary Between Cholesterol and Sodium Chloride Solution.

R. BEUTNER AND T. CUNLIFFE BARNES.

From the Departments of Pharmacology and Physiology, Hahnemann Medical College and Hospital, Philadelphia.

In previous papers[1,2] we have reported that acetylcholine chloride added to physiological salt solution produces a potential difference between that solution and various oils (nitrobenzene, cresol, guaiacol, etc.) so that the negative pole is on the side of the acetylcholine added.

In the apparatus an oil layer separates 2 layers of 0.9% NaCl solution. The experiments are attempts to duplicate in a simple model the potential differences found in the nervous system. Our object is to explain the potential that gives rise to the nerve impulse.

In the models previously described[1,2] the oils used were chemically different from the lipids of the plasma membrane of the living nerve. The electrical measurements with our oil cell technic cannot be done with pure cholesterol directly since this is a solid. The cholesterol must be dissolved in a water-immiscible yet conducting solvent which must not itself establish an electric negativity with acetylcholine. Such solvents are hard to find. It was found that benzyl alcohol gave no potential with acetylcholine in concentrations as high as 2%. In other words, no potential difference was measured between the two interfaces of benzyl alcohol and sodium chloride solution when acetylcholine was added to the latter on one side.

TABLE I.
Phase Boundary Potential Difference Between Acetylcholine in Saline Solution in Contact with Benzyl Alcohol Containing 0.6% Cholesterol and Pure Saline.

Conc. of acetylcholine %	Potential difference between interfaces. Oil in contact with saline containing acetylcholine is negative.
0.05	6.0 millivolts
0.10	10.0 "
0.15	14.0 "

All experiments were performed at room temperature (25°C).

The low solubility of cholesterol in benzyl alcohol (precipitation occurs at concentrations above 0.6%) may explain the small magnitude of the acetylcholine potentials in these mixtures (Table I). It is a remarkable fact that 0.05% acetylcholine can produce a negativity of 6 millivolts at a phase boundary between a saline and an oil layer which is 99% inert (benzyl alcohol).

The nervous system contains many other lipids besides cholesterol—especially lecithin. It was found that 4% lecithin dissolved in the inactive solvent benzyl alcohol produced an electric negativity of 13 millivolts when 0.5% acetylcholine was added to the saline at one side of the oil layer. Unfortunately lecithin emulsifies in the aqueous layers, which makes the potential difference unstable.

The experiments described above demon-

[1] Beutner, R., and Barnes, T. C., *Science*, 1941, **94**, 211.

[2] Beutner, R., and Barnes, T. C., *Biodynamica*, 1942, **4**, 47.

strate the electrical activity of acetylcholine at a phase boundary, which may explain the electrical phenomena in cholinergic nerves. These findings have theoretical interest and suggest that acetylcholine is more than a "chemical mediator" or "synaptic transmittor." Acetylcholine is capable of producing variations of electrical potential differences in the nervous system of a size and sign (invariably negative) similar to the electrical currents associated with the "nerve impulses."

Conclusion The addition of acetylcholine to saline solution in contact with cholesterol dissolved in an inert solvent (benzyl alcohol) gives rise to a negative electrical potential difference. It is suggested that this electrical negativity may play an important role in the origin of the negative variation associated with the nerve impulse.

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Locomotor Reactions of Fundulus Embryos with Abnormal Mauthner's Neurones.

JANE M. OPPENHEIMER.* (Introduced by J. S. Nicholas.)

From the Department of Biology, Bryn Mawr College, and Osborn Zoological Laboratory, Yale University.

Some *Fundulus heteroclitus* embryos have been prepared in which the central nervous system is characterized by various deviations from normal in both the number and arrangement of Mauthner's neurones. In view of the divergent opinions expressed in the literature[1-3] concerning these cells, it has seemed desirable to investigate the behavior of these embryos for information concerning the function of the giant neurones.

The operations, which involved the implantation of grafts into the prospective brain region of gastrula stages, were performed by methods already described.[4] The operated embryos were allowed to develop through to swimming stages, and their reactions were observed at least twice daily during the progress of development. The embryos were fixed in Bouin's solution, sectioned at 10 micra, and impregnated with silver by a modification of Bodian's method.

In 7 embryos Mauthner's axones were lacking in the posterior medulla and cord. Four of these embryos, although capable of swimming both spontaneously and in response to artificial stimulation, were abnormally passive and tended to remain immobile, if left undisturbed, for long periods of time. The 3 remaining embryos exhibited vigorous movements. One of these was kyphotic because of disturbance to portions of the brain other than Mauthner's neurones, to such a degree that it was mechanically unable to maintain its normal postural relationships. Yet even in spite of the great mechanical obstacle to normal locomotion, it made continual efforts to produce the swimming reaction.

Four embryos showed one Mauthner's axone coursing posteriorly through the cord, instead of 2. In one of these the fiber extended down the cord only as far as the posterior trunk region, and was located in the dorso-lateral white rather than in its usual ventral position; this embryo, like some of those described above, was abnormally passive and at times immobile. In the 3 remaining embryos the axone ran in its usual position in the cord: one of these

* All operations were performed at the Osborn Zoological Laboratory, Yale University. Part of the histological work was completed in that laboratory during the tenure of a John Simon Guggenheim Memorial Foundation Fellowship.

1 Bartelmez, G. W., *J. Comp. Neur.*, 1915, **25**, 87.

2 Detwiler, S. R., *J. Exp. Zool.*, 1933, **64**, 415.

3 Coghill, G. E., *Psych. en Neur. Bladen*, 1934, **38**, 386.

4 Oppenheimer, J. M., *J. Comp. Neur.*, 1941, **74**, 131.