Increased Permeability of the Hemoencephalic Barrier Produced by Physostigmine and Acetylcholine[1]

Margaret E. Greig and William C. Holland[2]
Department of Pharmacology,
Vanderbilt University School of Medicine,
Nashville, Tennessee

Barbour and Abel (*1*) observed that acid fuchsin, when injected into the lymph sac of frogs, caused tetanic convulsions, but that there was generally a marked delay (often 24 hr) before the onset of the convulsions. These observers believe that the delay was caused by the slow rate of absorption of the dye by nervous tissue. They then removed the cord, treated it with acid, and observed the degree of staining of the cord. The time of onset and the degree of convulsions paralleled the amount of dye in the cord.

TABLE 1

EFFECT OF PHYSOSTIGMINE AND ACETYLCHOLINE ON THE RATE OF ONSET OF CONVULSIONS IN FROGS PRODUCED BY ACID FUCHSIN

Group	I Acid fuchsin	II Physostigmine + acetylcholine	III Acid fuchsin + physostigmine + acetylcholine
Number of frogs	19	23	26
Number that did not have convulsions ...	5	23*	4
Number that had convulsions in less than 1 hr	4	0	22
Number that had convulsions in a period longer than 1 hr ...	10	0	0
Average time for convulsions to occur ...	12.6 hr		34 min
Range of times for convulsions to occur ...	41 min–26 hr		18 min–49 min

* Four frogs in this group died without evidence of convulsions.

In our work on factors affecting the permeability of dog erythrocytes it was found that a disturbance of the acetylcholine-cholinesterase system affected the permeability (*2*), and it was felt that a simple method of determining whether other cells were also affected might be found by studying the effect of physostigmine, a specific inhibitor of cholinesterase, and acetylcholine on the rate of passage of dye through the hemoencephalic barrier as indicated by the degree of staining of the cords of frogs treated with physostigmine, acetylcholine, and acid fuchsin and the time of onset of convulsions.

The drugs were injected into the dorsal lymph sacs of the frogs in the following quantities: acid fuchsin 5 mg, acetylcholine bromide 1 mg, and physostigmine 0.1 mg for each 5 g of body weight. The time when convulsions occurred was then determined.

Experimental results are summarized in Table 1. It may be seen that of the 19 frogs receiving acid fuchsin alone, 14 went into convulsions and the average time for the onset of convulsions in these frogs was 12.6 hr. Of the 26 frogs receiving acid fuchsin, physostigmine, and acetylcholine, 22 went into convulsions, and the average time for the onset of convulsions in this group was 34 min. In this group if the frogs did not convulse in an hour they did not convulse at all. Of the 23 frogs receiving physostigmine and acetylcholine, four died without showing signs of convulsions. Tests for acid fuchsin in nervous tissue by Abel's method were positive when the frogs were in convulsions caused by physostigmine and the dye, and negative if convulsions had not begun. We also observed, as did Abel, that the frog's eye became deeply pigmented at the time of onset of convulsions.

On applying the chi-square test of significance to these results it was found that the probability that this was a chance distribution was less than 0.001.

The permeability of the hemoencephalic barrier of frogs to acid fuchsin appears to be increased by the inhibition of cholinesterase by physostigmine. This change in permeability is similar to that found in dog erythrocytes treated with physostigmine. The acetylcholine-cholinesterase system may have a widespread function in maintaining the normal permeability of the living cell.

[1] Funds for carrying on this work were kindly supplied by the Mallinckrodt Chemical Works.

[2] U. S. Public Health Fellow of the National Institutes of Health.

References

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Dispersion Staining with Phase Contrast Microscope Accessories. The Microscopic Identification of Quartz

Germain C. Crossmon

Bausch & Lomb Optical Company, Rochester, New York

Results similar to the transmitted light bright field Christiansen effect (*1*) and the dark field dispersion staining method (*2–6*) can be obtained with chemicals and minerals by means of phase contrast microscope accessories. The colors obtained with phase objectives as compared to non-phase are much more vivid—brilliant enough to produce good color transparencies. A further advantage is that they can be observed best at focus rather than above or below a good focus, a necessary condition with non-phase objectives. As compared to the