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

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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.

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

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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

dark field method, the colors are not quite as brilliant and are observed best at the lower magnifications, such as with the 10× (16-mm) 0.25 N.A. and 21× (8-mm) 0.50 N.A. objectives. Weak coloration can very often be obtained with the 43× (4-mm) 0.65 N.A. objective. This is especially true when using the higher index liquids which, containing a greater proportion of cinnamaldehyde, have a higher dispersion. The use of high dispersion liquids as mounting media with the phase microscope offers the advantage that the end point in the determination of refractive index by color is probably easier to obtain than by dark field illumination. Critical numerical aperture relationships between condensers and objectives necessary in the dark field method are not required. When the method is used in conjunction with polarized light, more than one identifying color can be obtained, dependent on the orientation of the specimen. Particles not readily visible at one optical orientation can often be reorientated so they stand out in sharp contrast to the mounting medium. The method can be illustrated best by the directions given below for the identification of quartz, N_w 1.544 and N_e 1.553.

Particles, crushed to a smaller size if necessary, should be mounted under a cover glass in a liquid of high dispersion and of a refractive index of n_D 1.544 at 25° C prepared from a mixture of diethylene glycol monobutyl ether and cinnamaldehyde (Eastman Kodak Company). The quantity of the lower index constituent, to be mixed with the higher to give an index of 1.544 at 25° C, can be determined by the formula

$$V_1 n_1 + V_2 n_2 = V_x n_x$$

where V represents volume, n the index, and $V_x n_x$ the volume and index desired. Our samples of diethylene glycol monobutyl ether had an index of 1.429 and cinnamaldehyde 1.619, both readings being taken at 25° C. A 1.544 index liquid would require a mixture of 3.95 cc of diethylene glycol monobutyl ether with 6.05 of cinnamaldehyde, as shown in the computation given below:

$$1.429V_1 + 1.619V_2 = 10 \quad (1.544)$$
$$1.429V_1 + 1.429V_2 = 10 \quad (1.429)$$
$$.190V_2 = 1.15$$
$$V_2 = 6.05 \text{ cc}$$
$$V_1 = 3.95 \text{ cc}.$$

For more accurate results, the mixture prepared according to the above formula should be checked on a refractometer at 25° C. It is also advisable to recheck its index occasionally for any change, since cinnamaldehyde tends to oxidize to cinnamic acid. This shift in index can be retarded by keeping the index liquid in a small bottle and maintaining it nearly full of the mixture. Other index liquid combinations can be used, but the colors obtained will tend to be less vivid.

The results given here were obtained with dark contrast phase objectives having phase accelerating annuli of one-quarter wavelength. Critical illumination should be employed and the condenser diaphragm image and phase accelerating annulus accurately centered. A microscope illuminator having a 6-v 108-w ribbon filament lamp and daylight filter was used as a light source. Preparations mounted in the 1.544 index liquid were examined at a temperature of 25° C by means of the 10× (16-mm) 0.25 N.A. and 21× (8-mm) 0.50 N.A. objectives. Observations were made with nonpolarized light and with polarized light obtained by placing a cap analyzer over the microscope eyepiece. Grains of quartz with nonpolarized light at 25° C appear colored blue with orange. With polarized light (cap analyzer over eyepiece) grains oriented for the 1.544 index are colored blue with red, and those oriented for the 1.553 index are blue with yellow. Any inclusions or particles on the surface appear white or in colors other than those characteristic for quartz. As an additional means of identification, grains can be examined in a 1.553 (epsilon index for quartz) index liquid prepared in a similar manner at 25° C from diethylene glycol monobutyl ether and cinnamaldehyde. Quartz grains in this liquid with nonpolarized light at 25° C appear light blue, usually with a very dark red border. With polarized light (cap analyzer over the eyepiece) grains oriented for the 1.553 index are colored blue with red and those for the 1.544 index a light blue to white. The red, orange, and yellow colors are usually in the form of colored borders to the grain but occasionally, depending on its shape, can be found at any point in the particle. The blue colors always cover the major portion of the grain and vary in hue from light to dark blue, depending on whether the grain is below, equal to, or above the index liquid. This criterion in conjunction with the red, orange, and yellow coloration is of additional value in determining the grain-index relationship.

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