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Title: A SCREENING TEST FOR CHOLINESTERASE DEFICIENCY

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Introduction. Each year in the U.S., about 6000 patients who have atypical cholinesterase (ChE) are anesthetized. Generally, surgical patients are not screened to determine those at risk because of the lack of a reliable, easily-used screening test. Determination of ChE activity is best performed at pH7.4, since this enzyme exhibits an activity optimum at that pH[1]. This requires the use of a pH-stat with automatic titration. The procedure is time consuming and normally not available in clinical laboratories. A screening test paper ('Acholest', E. Fougera and Comp., Inc.) previously available has now been discontinued by its manufacturer because of difficulty of use. An automated assay (Automatic Chemical Analyzer, DuPont Instruments) is available but expensive. This communication describes another test paper which is much easier to use and which gives results that can be made a permanent part of the patient's record. It is based on color changes of a pH indicator by acid formed from ChE-catalyzed hydrolysis in an unbuffered system. Several technical tricks are employed to make the test cheap and easy to use.

Methods. Paper is impregnated with a solution of either (1) acetylcholine and bromthymol blue or (2) these compounds plus physostigmine sulfate. The papers are dried and cut into pieces approximately 0.5-1.0cm^2 and fastened to labels in a paired fashion. One drop of serum is added to each piece of paper thus impregnated and left to dry in air at room temperature.

Results. Acetylcholine serves as substrate for the serum enzyme in this test. Acetic acid formed by hydrolysis causes the color to change from blue to yellow. Physostigmine inhibits ChE activity, blocks acid formation and causes the paper to remain blue. This blue color, which is intensified by the high pH of dried serum, serves as a comparison control. If the serum contains active ChE, the pair of papers will show marked difference in color. The paper without physostigmine will turn yellow and the paper with physostigmine will remain blue. If the serum lacks ChE activity, then the pair of papers will be similar, i.e., both remain blue. Intermediate activity produces an intermediate color change. A gradation of color from blue to yellow (through green) can be seen in the spectrum from inactive to active patient sera. The lower limit for observing a change from blue is at a ChE activity of 0.5-6μmoles/min/ml serum. (Normal is >1.0μmole/min/ml serum). The dried papers have remained true to their colors for at least two months at this writing. And we have tested at least 50 serum samples to date without a single false-positive result.

Discussion. 'Alcholest' paper suffers from two disadvantages. It must be watched and timed to tell if the serum has normal activity. And, it must be covered and kept wet while the test is being conducted. Therefore, significant amounts of time must be devoted to each test. This new test incorporates three technical improvements: (1) The color is best read after the papers are dry and the watching of color development is not required. (2) The paper with physostigmine serves as a comparison control. Low ChE activity is indicated by shades of yellow and green in a dark blue background and is easily picked out. And (3) the label including the results can be transfered to the patient's chart. The color will stay and the results become part of patient's permanent file. The whole procedure consumes very little time and only two drops of serum, is cheap to perform and is adaptable to wide use as preoperative screening test.

References.

1. Bergmann F, Segal R, Shimoni A, Wurzel M: The pH pH-dependence of enzymatic ester hydrolysis. Biochem J 63:684-690, 1956.