administered in different ways, but these changes are generally considered to be nonspecific (*2, 4*) and do not adequately account either for the symptoms or for the death of the animals (*2, 4, 5*).

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A Rapid Screening Test for the Determination of the Approximate Cholinesterase Activity of Human Blood

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Organic phosphate insecticides are coming into extensive use. The chief physiological action of these highly toxic compounds is their inhibition of the cholinesterase (ChE) activity in man, as well as in insects. Compounds having a similar action are considered as possible war gases. Among the first symptoms to appear as a result of overexposure to these compounds are nausea, vomiting, diarrhea, and headache. In very severe poisoning weakness and generalized muscular fasciculations also appear. Since these symptoms are nonspecific, it is important to know whether or not they are caused by ChE-inhibiting agents. The most practical method of detecting the true cause of the symptoms is the determination of the ChE level of the blood, which reflects the activity of the enzyme in all parts of the body.

The methods commonly used to determine blood ChE activity require apparatus found only in clinical or chemical laboratories. For screening purposes a simpler and more generally applicable test is desired, by which overexposure may be detected so that appropriate treatment could be administered with minimum delay.

Although plasma ChE is inhibited more readily than red cell ChE (*1–3*), in cases of actual human poisoning by organic phosphate insecticides both plasma and red cell ChE levels were significantly reduced (*2–4*). It seemed, therefore, that the whole-blood ChE level would serve equally well as an index of overexposure. Based on this premise, a rapid screening test that can be carried out in the field without the use of specialized equipment has been developed and is the subject of this report.

This method is essentially a visual colorimetric method in which the change in pH (ΔpH) resulting from the liberation of acetic acid from a ChE substrate (acetylcholine iodide) is estimated by the change in color of an indicator, brom thymol blue (BTB). The color of the solution at the end of 20 min will determine the approximate ChE activity. Only one drop of fingertip blood is required.

The only apparatus and materials required are a blood-diluting pipette (WBC dilution, 1:20), a dropping pipette with bulb attached, a 2- and a 1-ml rubber-stoppered serum bottle containing acetylcholine iodide[1] (6 mg) and brom thymol blue[2] (0.5 mg), respectively, and two 1-ml bottles each containing 1 ml of sterile distilled water (pH 6.8–7.0). The reagents can be added to the bottles by dissolving them separately in alcohol (300 mg substrate/25 ml and 25 mg indicator/10 ml) and adding 0.5 ml of the substrate solution to the 2-ml bottle and 0.2 ml of the indicator solution to the 1-ml bottle. After stoppering the bottles, the alcohol is removed under reduced pressure at room temperature by inserting a hypodermic needle through the rubber stopper. Before using, 1 ml of distilled water is added to each of the reagent bottles by means of the dropping pipette, and the bottles are shaken. One of the bottles that had contained distilled water can then be used as the test bottle. Acetylcholine iodide was used as the substrate because it is nonhygroscopic and stable (*5*). All these materials are easily incorporated into a small kit that can be carried in the pocket.

Because this method is based on a change in pH, the finger from which the blood sample is to be taken, as well as the lancet, must be uncontaminated with acid or alkali. After being washed with water and sterilized with alcohol, both the fingertip and the lancet must be wiped dry. After pricking the finger, blood is drawn up to the 0.5 mark on the pipette, and then the outside of the pipette is wiped with a clean piece of gauze. The blood sample is diluted with BTB solution up to the 11 mark. The blood-BTB solution is then expelled into the empty bottle, and any remaining blood in the pipette is washed into the bottle by drawing the blood-BTB solution up and down several times. A second volume of BTB is drawn up to the 11 mark and added to the blood-BTB in the test bottle. Two volumes of the substrate solution, each measured to the 11 mark, are then added to the blood-BTB solution. The test bottle is stoppered and shaken. After noting the color of the solution (it should be green) and the time, the test bottle is immediately placed in the axilla next to the skin so that the test will be carried out at constant (body) temperature. At the end of exactly 20 min the color of the solution is again observed, preferably in daylight or fluorescent light, by holding a piece of white paper about 1 in. below the bottle held in a horizontal position and looking *down* through the side of the bottle.

[1] Hoffmann-LaRoche.
[2] Harleco, water-soluble.

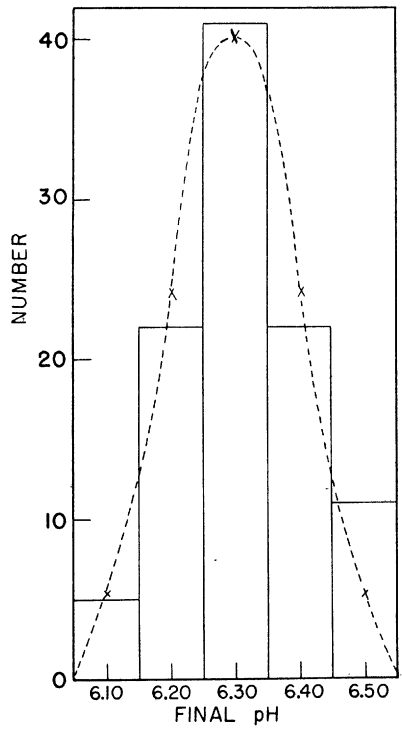

FIG. 1. Normal human whole blood: Distribution of final pH values in cholinesterase activity determinations on 101 subjects (52 white males and 49 white females, ages 19–62). ———— = histogram of determinations; – – – – – = calculated normal curve.

With this method, approximate whole-blood ChE activities of 52 white males and 49 white females between the ages of 19 and 62 were determined. The results were checked by the glass electrode. In the case of 92% of the people tested by the color indicator method, the test solution had an orange color at the end of 20 min. The remainder of the test solutions had an orange-brown color.

The initial pH of the whole-blood test solutions was found to average 7.22, with a range of 7.20–7.35 when determined by the glass electrode within a few minutes after the blood samples were taken. However, because of the loss of carbon dioxide from the blood, higher pH readings are obtained when the solutions are exposed to the atmosphere for any length of time. For this reason the final pH of the test solutions was considered to be the only criterion of ChE activity. In Fig. 1 it can be seen that the final pH range of the samples varied from 6.05 to 6.55, with a mean of 6.30 and standard deviation of 0.10. A final pH of 6.30 or less was considered to represent 100% activity, and pH 7.22 represented zero activity. On this basis a final pH of 6.50 (two standard deviations from the mean) is equal to an activity of 78%. Thus 96% of the individuals tested had a whole-blood ChE activity of 78–100%. The individuals whose color test solutions were orange-brown were also found to have low whole-blood ChE activities, based on the final pH of their test solutions.

The color of the test solutions at the end of 20 min was correlated with the final pH by using the glass electrode. A range of ChE activities (0–100%) was achieved by inhibiting the ChE to various degrees by the addition of varying amounts of sodium fluoride. The following color scale for ChE activity was thus devised: orange, 100%; brown, 75%; olive-brown, 50%; olive-green, 25%; and green 0%.

In addition to determining the whole-blood ChE activities of the individuals tested, ChE activities of plasma and red cells were also determined, using Michel's method (*6*). Individuals found to have orange-brown test solutions and low whole-blood ChE activities were also found to have either low plasma or both low plasma and low red cell activities.

No statistically significant differences were found between the whole-blood, plasma, and red cell ChE activities of the four age groups tested, or between men and women. However, it can be seen from Table 1 that in each age group the men had a slightly higher plasma activity than the women. The mean ΔpH value of 0.76 obtained for the activity of red cells from both males and females agree very closely

TABLE 1

	Men					Women				
Age Group	19–29	30–39	40–49	50–60	Total	19–29	30–39	40–49	50–62	Total
Number	16	11	10	15	52	15	9	10	15	49
Whole Blood										
Mean final pH*	6.25	6.39	6.31	6.28	6.29	6.31	6.28	6.31	6.37	6.32
SD	0.12	0.08	0.12	0.09	0.107	0.084	0.075	0.098	0.102	0.093
Mean percentage Activity*	98	93	96	99	96.8	98	99	96	94	96.6
SD	6.0	7.0	6.4	2.2	5.7	4.3	2.3	5.7	8.3	6.0
Plasma										
Mean ΔpH*	0.92	0.99	0.86	0.88	0.91	0.75	0.86	0.66	0.79	0.76
SD	0.17	0.15	0.20	0.17	0.16	0.16	0.27	0.17	0.16	0.18
Red Cells										
Mean ΔpH*	0.70	0.77	0.78	0.81	0.76	0.74	0.74	0.78	0.74	0.76
SD	0.09	0.08	0.08	0.09	0.03	0.10	0.09	0.14	0.09	0.03

* Differences between age groups and between men and women are not statistically significant.

with the value of 0.753 obtained by Michel for males (*6*) and the values obtained by other investigators for both males and females, but is lower than the value of 0.861 obtained by Wolfsie and Winter (*7*). The value obtained for plasma activity of males (0.91) was higher than the value found by Michel (0.703) but agrees with the value obtained by Wolfsie and Winter (0.912).

The symptoms caused by overexposure to anti-ChE agents usually do not appear until the ChE level of the plasma is near zero and that of the red cells is below 30% (*2, 3*). Since the color indicator method can detect 75% or less of normal activity, it should be quite adequate for relating symptoms to ChE activity. If the symptoms are found to be due to a low ChE activity, atropine should be administered immediately in recommended doses (*8, 9*).

In addition to relating symptoms to ChE activity, the color indicator test could be used as a screening test to detect individuals having a low ChE activity either as the result of overexposure to anti-ChE compounds or as the result of some pathological condition. A ChE activity below 75% should dictate the removal of such an individual from further exposure to anti-ChE compounds until his ChE activity returns to normal.

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Mutation of Mating Type in *Saccharomyces cerevisiae*[1]

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Two mating type genes (*a* and α) were first described in *Saccharomyces cerevisiae* by Lindegren and Lindegren (*1*). These authors reported that haploid cultures maintained vegetatively may ultimately lose their mating ability, but noted no interconversion between *a* and α. Leupold (*2*) has found that mating type in the yeast *Schizosaccharomyces pombe* is mutable, apparently in all directions. Such mutability has not been proved in *S. cerevisiae*, although it has been inferred by Winge (*3*), based on the observation that "a diploid area . . . is frequently encountered at the margin of an otherwise haploid colony. . . ."

TABLE 1

SEGREGATION OF ASCOSPORES FROM A CROSS BETWEEN A176.1 (*a* Tr⁻ Me⁻ Ad⁺ Ur⁺) × A191.1 (*a* Tr⁺ Me⁺ Ad⁻ Ur⁻)*

Ascospore	M.T.	Tr	Me	Ad	Ur
A667.1	α	+	+	+	−
A667.2	α	+	−	+	+
A667.3	a	−	−	−	+
A667.4	a	−	+	+	−
A668.1	a	+	−	+	−
A668.2	a	−	−	−	+
A668.3	α	−	+	+	−
A668.4	Died				
A670.1	α	+	−	+	+
A670.2	a	−	+	+	−
A670.3	a	−	−	−	−
A670.4	Lost during dissection				
A671.1	a	−	+	+	+
A671.2	α	+	−	−	−
A671.3	a	−	−	+	+
A671.4	Died				

* Tr = tryptophan-synthesizing gene; Tr⁺ = independent of tryptophan requirement; Tr⁻ = requires tryptophan for growth; Me = methionine; Ad = adenine; Ur = uracil; M.T. = mating type.

To examine this question directly the following experiment was carried out. A haploid clone of *a* mating type, which required tryptophan and methionine for growth, was mixed with another *a* haploid which required adenine and uracil. The cells were handled as described elsewhere (*4*) for prototroph recovery. Controls were run with each haploid alone. One ml of each washed cell suspension was plated on minimal agar (lacking tryptophan, methionine, adenine, and uracil). Neither control plate had any colonies; i.e., the frequency of double mutation was too low to be revealed by the plating method. The plate in which the mixture had been plated had about 10 colonies. Several of these were isolated and induced to sporulate.

The segregation data for one of these isolates are shown in Table 1. Mutation of mating type from *a* to α has probably occurred to give rise to the observed results. Ascus A667 segregates 2*a* : 2α, and the three incomplete asci show heterozygosity for mating type. It may be noted that A667 yields a 3+ : 1− ratio for the adenine gene. Population analyses run on the independent cultures revealed no heterogeneity, suggesting that mutation occurred very early in ascospore development or germination. It is unlikely that there is a significant correlation between the mutation for mating type (which must have occurred prior to conjugation) and that for adenine independence (which must have occurred after fusion).

These data, demonstrating mutation of mating type prior to a cross between two *a* haploids, do not rule out the possible occurrence of an *a* × *a* cross, giving rise to an "illegitimate" diploid (*5*). Such diploids would be homozygous for mating type, and have been reported to sporulate only poorly if at all. It has been

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