level in the bodies of workers exposed to tetryl and if it appears to be lowered start daily administration of 100 or 200 mg. No harm will be done.

Lead and Zinc Poisoning

In 1939 Holmes and Campbell[6] reported that dust of lead or its compounds destroyed some vitamin C in the bodies of workers exposed to the toxic dust. Daily doses of 200 mg of this vitamin in most cases resulted in great improvement in health. These findings have been confirmed by Marchmont-Robinson.[7]

The zinc oxide fume given off when brass is melted is causing symptoms that are somewhat reminiscent of lead poisoning. A possible relation to vitamin C destruction is suggested.

To complete this list of items of military value, it might be mentioned that Dr. Louis J. Karnosh, of Western Reserve Medical School, had 100 cases of insomia treated with vitamin C and observed excellent improvement. It would seem that both C and B_1 could be useful in many cases of nervous disturbance.

Harry N. Holmes

Oberlin College

NATURAL AND SYNTHEIC INHIBITORS OF CHOLINE ESTERASE[1]

Acetylcholine and adrenaline, which are considered to be the chemical mediators of nervous impulses, or closely related to these mediators, are substituted methyl ethanol amines. It will be shown in this note that the fact that both compounds are N-methylated may have a physiological significance, since it links both of them to interaction with the same enzyme, choline esterase. This was indicated to us by consideration of the structure of physostigmine, the most powerful inhibitor of the esterase. A large part of its inhibiting action undoubtedly is due to the methyl urethane side chain as shown by the effect of drugs of the same structural type.[2] The structure of these compounds and of other strong inhibitors of the esterase, *e.g.*, methylene blue, suggests that the N-methyl group is part of the configuration essential for their inhibiting properties.

In addition to the N-methyl group in the methyl-urethane side chain, physostigmine contains two other N-methyl groups in its indole and pyrrolidine rings. The question arises whether these N-methyl groups contribute to the action of the drug. The N-methyl indole part is of particular interest since it relates the physostigmine structure to that of adrenochrome, a substituted N-methyl indole, which has been thought to be an oxidation product of adrenaline.[3]

We have studied the action of indole, N-methyl indole and oxidation products of adrenaline on choline esterase in human serum. The enzymatic hydrolysis of acetyl choline (0.016 molar) was followed by electrometric titration; 0.1 or 0.25 ml of serum were used in a total volume of 25 ml. The solution was 0.7 per cent. in regard to sodium chloride to exclude interference from varying sodium concentration.[4]

Physostigmine in H 10^{-6} molar concentrations produces a 50 per cent. inhibition of esterase under the conditions of our experiment. Indole gives a 50 per cent. inhibition in a molar concentration of about 10^{-2}. N-methyl indole is at least twice as active as indole. The exact values are difficult to determine, since the compound is only slightly soluble in water.

The oxidation products of adrenaline differed in their action on the esterase depending on the type of oxidation and on the p_H at which the oxidation was carried out. Products obtained by oxidation with iodate or catechol oxidase[5] at acid p_H do not inhibit the esterase. Solutions which contain the enzymatic oxidation product of adrenaline show a strong inhibiting action after being alkalinized. Oxidation with bromine leads to active compounds which inhibit the esterase to 50 per cent. in molar concentration of 10^{-4}. On the basis of these experiments it can be understood that samples of adrenaline show different degrees of inhibition depending on the extent to which they have been exposed to air. The sample of adrenaline least active inhibited the esterase to 50 per cent. in molar concentration of 2.5×10^{-3}. Another one which inhibited the esterase to 15 per cent. in molar concentration of 10^{-3} doubled its inhibiting effect after exposure to air in a thin layer for several days. A large part of the colored inhibitor can be removed by charcoal or by recrystallization.

These experiments suggest the possibility that under physiological conditions, metabolic products of adrenaline may be formed which have a strong inhibiting effect on choline esterase. Formation of active oxidation products of adrenaline would not only result in a disappearance of adrenaline *per se* but also, by inhibition of the esterase, in a slower removal of acetyl choline.

Heinrich Waelsch
Herbert Rackow

College of Physicians and Surgeons,
Columbia University

[6] Harry N. Holmes and Kathryn Campbell, *Jour. Lab. Clin. Med.*, 24: 1119, 1939.

[7] S. W. Marchmont-Robinson, *Jour. Lab. Clin. Med.*, 26: 1478, 1941.

[1] This work was supported by a grant from the Joshua Rosett Research Fund.

[2] E. Stedman, *Biochem. Jour.*, 20: 719, 1926; 23: 17, 1929; E. Stedman and E. Stedman, *Biochem. Jour.*, 25: 1147, 1931.

[3] D. E. Green and D. Richter, *Biochem. Jour.*, 31: 596, 1937.

[4] G. A. Alles and R. C. Hawes, *Jour. Biol. Chem.*, 133: 375, 1940.

[5] We are indebted to Dr. C. R. Dawson for the preparation of catechol oxidase.