12. Landauer, W., Baumann, L., *J. Exp. Zool.*, 1943, v93, 51.
13. Landauer, W., *J. Cell. and Comp. Physiol.*, 1954, v43, 261, Suppl. 1.

Received September 24, 1957. P.S.E.B.M., 1958, v97.

An Effect of Pyridine-2-Aldoxime Methiodide (2-PAM) on Cholinesterase at Motor End-Plates. (23653)

A. DOROTHY BERGNER AND PHILIP F. WAGLEY (Introduced by W. H. Chambers)
Clinical Research Division, U. S. Army Chemical Warfare Laboratories, Md.

Certain oximes have an effect on the end-plate potential of isolated nerve-muscle preparations(1,2). Therefore, the following experiments were done to study the action of the oxime, pyridine-2-aldoxime methiodide (2-PAM), on cholinesterase at motor end-plates in striated muscle.

Materials and methods. The oxime 2-PAM was dissolved in concentrations of 7.6 x 10^{-3} *M* and 1 x 10^{-3} *M* either in frog Ringer solution or in incubation medium for the Koelle technic(3) immediately before use. Tetraethylpyrophosphate (TEPP) was used in frog Ringer solution in approximately 1.5 x 10^{-5} *M* concentration for inactivation of cholinesterase (ChE). The *iliofibularis* muscle of *R. pipiens* was freed of surrounding connective tissue and separated into small portions lengthwise. Thus many of the muscle fibers and their motor end-plates were in intimate contact with the solutions. During each of the two 30-minute exposure periods the fibers were immersed in Ringer solution or in a solution of 2-PAM or TEPP in the pH range indicated in Table I and at temperatures of 24°-28°C. These preparations were rinsed in Ringer solution after each exposure period. Each was then transferred to the incubation medium containing acetylthiocholine as substrate either with or without 2-PAM and incubated for 30 minutes at 24°-28°C. The tissues were then mounted in glycerine on slides and examined microscopically. ChE activity was assessed by the relative amounts of copper sulfide (CuS) precipitated on the subneural apparatus and recorded quasi-quantitatively; ++++ indicating maximum activity and 0, too little activity to be detectable by this method.

Results. Photographs of representative end-plate areas showing various degrees of ChE activity are illustrated in Fig. 1. The

TABLE I. Histochemical Demonstration of Interactions between 2-PAM, TEPP and ChE in Frog Muscle.

	Exposure periods,* pH 7.75-7.10		Incubation period pH 5.35-5.30	ChE activity
	30 min.	30 min.	30 min.	
2-PAM = 7.6 × 10^{-3} *M*				
(1)	Ringer sol	Ringer sol	Medium + 2-PAM	+ ‡
(2)	2-PAM in Ringer sol †	2-PAM in Ringer sol †	Medium only	+++
(3)	TEPP *Idem*	Ringer sol	Medium "	0
(4)	TEPP "	2-PAM in Ringer sol	Medium "	+++
(5)	TEPP "	2-PAM *Idem*	Medium + 2-PAM	+
(6)	2-PAM "	TEPP "	Medium only	0
2-PAM = 1 × 10^{-3} *M*				
(7)	Ringer sol	Ringer sol	Medium + 2-PAM	+++
(8)	TEPP in Ringer sol	2-PAM in Ringer sol	Medium *Idem*	++
(9)	2-PAM *Idem*	TEPP *Idem*	Medium only	0

* After each exposure period the preparation was rinsed in Ringer solution.
† These exposures were made at pH 5.35-5.30 also with identical results.
‡ Control preparation, incubated without 2-PAM, showed maximum (++++) deposition of CuS.

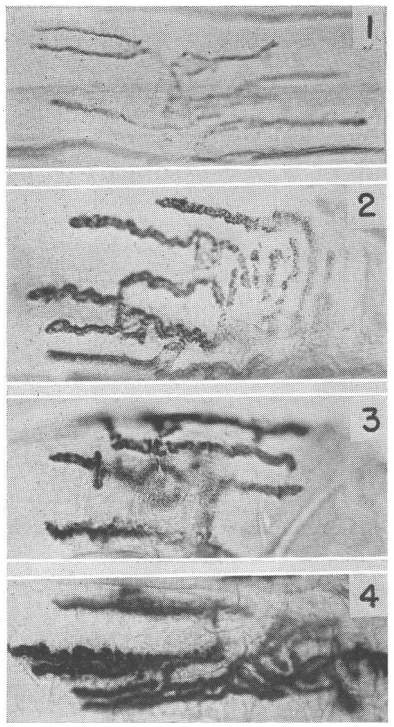

FIG. 1. Subneural apparatus of motor end-plate in *iliofibularis* muscle of frogs, stained for cholinesterase by a modified Koelle technique. ChE activity, as assessed by CuS deposit is illustrated as follows: 1, +; 2, ++; 3, +++; 4, ++++. Muscles were incubated for 30 min; magnification 275 ×.

enzyme is localized in the sub-neural apparatus.

As shown in line 1 of Table I, if 2-PAM was present during incubation of tissue at pH 5.35 to 5.30 in a concentration of 7.6 x 10^{-3} *M*, there was decided inhibition of ChE activity. This effect was readily reversible (line 2) where exposure to 2-PAM in Ringer solution prior to but not during the incubation period diminished ChE activity only slightly. Furthermore, if the ChE was inactivated by TEPP, as shown in line 3, subsequent exposure to 2-PAM restored much of the activity (line 4). However, incubation with 2-PAM of a preparation previously exposed to both TEPP and 2-PAM showed less total ChE activity, as shown in line 5. Exposure of tissue to 2-PAM prior to but not during exposure to TEPP did not protect the ChE from the latter, as shown in line 6. When these experiments were repeated using 2-PAM in a concentration of 1 x 10^{-3} *M*, the ChE depressing effect of 2-PAM during the incubation period was less striking; compare line 7 with line 1. Concomitantly, the reactivating effect of 2-PAM was greater in the lower of the 2 concentrations even though it was added to the incubation medium containing tissue previously exposed to both TEPP and 2-PAM; compare line 8 with line 5. Presumably this was the result of less of the direct inhibiting effect of 2-PAM on ChE since the oxime was present in lower concentration. Again, at this concentration, 2-PAM gave no protection to ChE against later exposure to TEPP, line 9.

Discussion. Although the pH of the solutions used in the exposure periods was kept between 7.75 and 7.10, the pH for the incubation period was lowered to between 5.35 and 5.30. By lowering the pH below the usual 6.0 of the Koelle formula, it was possible to hold to a minimum the reaction between the substrate, acetylthiocholine, and 2-PAM when it was added to the incubation medium(4). Both the reaction between enzyme and substrate and the competing reaction between oxime and substrate are pH dependent. The diminution in ChE activity accompanying this lowering of pH was compensated by a slightly longer period of incubation (30 instead of 25 minutes) at pH 5.3.

Previous *in vitro* studies of the action of oximes on ChE in tissues have been based on the use of homogenates of tissue, red blood cell suspensions, whole muscle and nerve-muscle preparations(5-9). In the present experiments fresh muscle fibers were employed. A concentration of 2-PAM that is only slightly inhibitory for ChE, as demonstrated with this technic, has a reactivating effect on previously inactivated enzyme. In a higher concentration, 2-PAM had a marked inhibitory effect on ChE. The oxime effect was reversible. After rinsing preparations previously exposed to 2-PAM, no protection to ChE from the effect of subsequent exposure to TEPP was demonstrated. In the presence of the higher concentration of 2-PAM, ChE previously inactivated with TEPP showed less restoration of function. This may represent a supplemen-

tary inhibiting effect of 2-PAM. If such an interpretation is correct, it seems logical to assume that 2 aspects of activity of 2-PAM were demonstrated in the preparation described by line 5 of Table I. First, TEPP-inactivated ChE was reactivated and subsequently it was inhibited by the oxime itself.

Conclusion. 2-PAM in a high concentration *in vitro* had a dual effect on motor endplate ChE previously inactivated with TEPP. The oxime reactivated the enzyme and, if allowed to remain in contact with the preparation, inhibited the reactivated enzyme. The effect of 2-PAM was reversible and after the removal of 2-PAM no protection to ChE from the later exposure to TEPP was demonstrated.

The authors are indebted to J. J. Cuculis for the photographic work.

1. Wagley, P. F., *Chem. Warfare Labs. Techn. Report* 2145, 1957, 1.
2. ———, *Bull. Johns Hopkins Hosp.*, 1957, v100, 287.
3. Koelle, G. S., *J. Pharmacol. Exp. Therap.*, 1951, v103, 153.
4. Bergner, A. D., O'Neill, J. J., *J. Histochem. and Cytochem.*, 1958, in press.
5. Childs, A. F., Davies, D. R., Green, A. L., Rutland, J. P., *Brit. J. Pharmacol.*, 1955, v10, 462.
6. Holmes, R., Robins, E. L., *ibid.*, 1955, v10, 490.
7. Kewitz, H., *Arch. Biochem. and Biophys.*, 1957, v66, 263.
8. Kewitz, H., Nachmansohn, D., *ibid.*, 1957, v66, 271.
9. Fleisher, J. H., *Fed. Proc.*, 1957, v16, 297.

Received September 27, 1957. P.S.E.B.M., 1958, v97.

Relationship of Dietary Dienoic Acid Content to That in Mouse Carcass Fat.* (23654)

S. B. TOVE AND F. H. SMITH (Introduced by G. H. Wise)
Animal Nutrition Section, Department of Animal Industry, North Carolina State College, Raleigh

The investigation here reported was suggested by results obtained in a previous study (1) that showed an increase in carcass fat dienoic acids of animals fed rigorously extracted soybean oil meal. The results of the study indicated that the increased carcass fat dienoic acids were not the result of residual dienoic acids in the meal. Hence, work attempting to isolate the causative factor was initiated. The fractionation work revealed that the ether-extracted soybean meal contained sufficient dienoic acids, which could be removed by methanol extraction, to account for the increased carcass fat levels of these acids. During these experiments it became necessary to investigate the nature of the response curve of carcass fat dienoic acids to dietary dienoic acids, since little information is available about the quantitative aspects of this relationship especially at low dietary levels.

* Approved by Director of Research for publication as Paper No. 845 of Journal Series. Supported in part by grant from Nutrition Foundation.

Methods. Weanling albino mice obtained from the North Carolina State Laboratory of Hygiene were used in these studies. They were housed individually on wire screens and received *ad libitum* an egg albumin-starch purified diet similar to diet 13 of the previous studies(1). Each animal was parenterally given 0.01 mg biotin twice weekly. The various adjuncts that were tested for their capacity to alter the carcass fat dienoic acid content were added at the expense of starch. Each experiment was of 3 weeks duration with the average of 6 replications reported. At the completion of the experiment the mice were gassed, skinned, decapitated, and the entire gastrointestinal tract removed. The mouse carcass was then placed into a 30-ml beaker, minced with scissors and dehydrated by freeze drying. The dry carcass was ground with about 1 g of Super-Cel and about 2 g of anhydrous sodium sulfate. This mixture was placed into a coarse alundum crucible and extracted with anhydrous ether for 12-18 hrs in a Pickel extractor. After evaporation of the