

linear refractile forms in macrophages in routinely stained bone marrow smears.

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Sources and manufacturers

- a. Virachek, Synbiotics Corp., San Diego, CA.
- b. Performed by E. W. Ho, Virology Diagnostic Laboratory, University of California, Davis, CA.

References

1. Buergelt CD, Fowler JL, Wright PJ: 1982, Disseminated avian tuberculosis in a cat. *Calif Vet* 10:13-15.
2. Drolet R: 1986, Disseminated tuberculosis caused by *Mycobacterium avium* in a cat. *J Am Vet Med Assoc* 189:1336-1337.
3. Dungworth DL: 1985, The respiratory system. *In: Pathology of domestic animals*, ed. Jubb KVF, Kennedy PC, Palmer N, 3rd ed., vol. 2, p. 494. Academic Press, Orlando, FL.
4. Farhi DC, Mason UG III, Horsburgh CR Jr: 1986, Pathologic findings in disseminated *Mycobacterium avium-intracellulare* infection. *Am J Clin Pathol* 85:67-72.
5. Hix JW, Jones TC, Karlson AG: 1961, Avian tubercle bacillus infection in the cat. *J Am Vet Med Assoc* 138:641-647.
6. Karlson AG: 1978, Avian tuberculosis. *In: Mycobacterial infections of zoo animals*, ed. Montali RJ, p. 22. Smithsonian Institution Press, Washington, DC.
7. Klatt EC, Jensen DF, Meyer PR: 1987, Pathology of *Mycobacterium avium-intracellulare* infection in acquired immunodeficiency syndrome. *Hum Pathol* 18:709-714.
8. Lawrence WE, Wickham N: 1963, Cat leprosy: infection by a bacillus resembling *Mycobacterium lepraemurium*. *Aust Vet J* 39:390-393.
9. Matthews JA, Liggitt HD: 1983, Disseminated mycobacteriosis in a cat. *J Am Vet Med Assoc* 183:701-702.
10. Pedersen NC, Ho EW, Brown ML, Yamamoto JK: 1987, Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science* 235:790-793.
11. Schiefer B, Gee BR, Ward GE: 1974, A disease resembling feline leprosy in western Canada. *J Am Vet Med Assoc* 165: 1085-1087.
12. Schwartz A, Kehoe JM: 1983, Fundamental principles of immunology. *In: Veterinary internal medicine*, ed. Ettinger SJ, 2nd ed., p. 2136. W. B. Saunders, Philadelphia, PA.
13. Thoen CO: 1984, Mycobacterium. *In: Diagnostic procedures in veterinary bacteriology and mycology*, ed. Carter GR, pp. 219-228. Charles C Thomas, Springfield, IL.
14. Tomasovic AA, Rac R, Purcell DA: 1976, *Mycobacterium xenopi* in a skin lesion of a cat. *Aust Vet J* 52:103.
15. Von Lichtenberg F: 1984, Infectious diseases. *In: Pathologic basis of disease*, ed. Robbins SL, Cotran RS, Kumar V, 3rd ed., p. 346. W. B. Saunders, Philadelphia, PA.
16. White SD, Ihrke PJ, Stannard AA, et al.: 1983, Cutaneous atypical mycobacteriosis in cats. *J Am Vet Med Assoc* 182: 1218-1222.
17. Wilkinson GT, Kelly WR, O'Boyle D: 1978, Cutaneous granulomas associated with *Mycobacterium fortuitum* infection in a cat. *J Small Anim Pract* 19:357-362.
18. Wilkinson GT, Kelly WR, O'Boyle D: 1982, Pyogranulomatous panniculitis in cats due to *Mycobacterium smegmatis*. *Aust Vet J* 58:77-78.
19. Willemse T, Groothuis DG, Koeman JP, Beyer EG: 1985, *Mycobacterium thermoresistibile*: extrapulmonary infection in a cat. *J Clin Microbiol* 21:854-856.

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Preliminary studies with bovine retina cholinesterase determinations in organophosphorus insecticide poisoning

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Organophosphorus insecticide (OP) use has increased rapidly in recent years as a direct consequence of the decline in use of the persistent organochlorine pesticides. The OP's are applied directly to livestock to control grubs and flies, and in some species are approved for use as animal drugs to control gastrointestinal helminths. Crops treated with these compounds can become components of animal feed. Life-threatening exposure of the animal often occurs due to in-

advertent contamination of feed with concentrated forms of the OP's or from unapproved use of the products.

All OP insecticides in sufficient doses share a common mechanism of acute toxic action-inhibition of the enzyme acetylcholinesterase (AChE).¹ The inhibition of this enzyme causes a buildup of the neurotransmitter acetylcholine, which is responsible for the clinical signs of toxicosis. Historically, analyses for AChE have been performed on brain tissue in postmortem cases or on whole blood or erythrocytes in antemortem cases. If whole blood is utilized for the assay, then the combined influence of AChE and pseudocholinesterase (PChE) will be monitored. The term cholinesterase (ChE) activity will be used here to denote both AChE and the combined AChE and PChE contributions of the tissue and whole blood samples, respectively.

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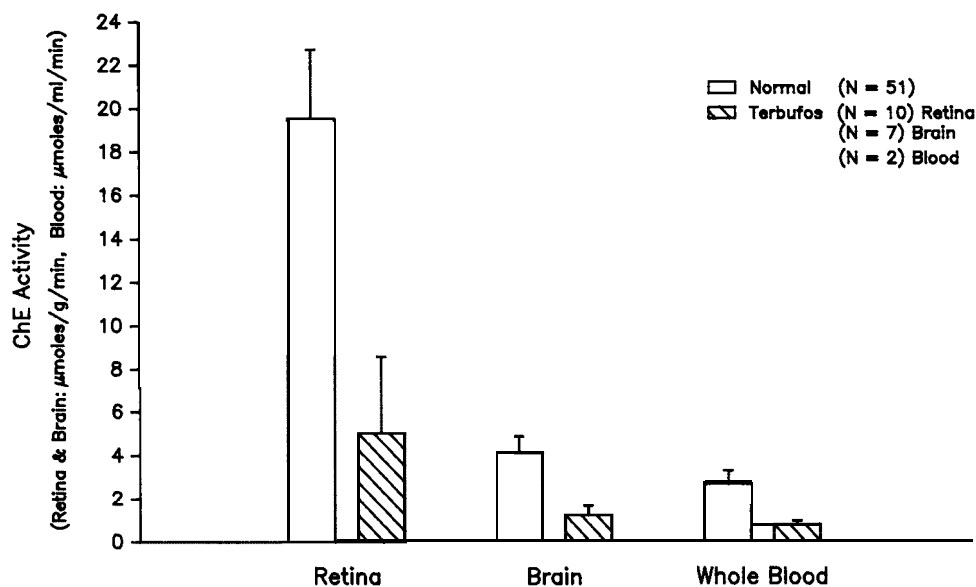


Figure 1. Retina, brain, and whole blood cholinesterase activity of normal and terbufos-exposed cattle.

The difficulties in removing the brain make it undesirable as a specimen for rapid screening tests. The brain might not be removed unless OP exposure is highly incriminated in a toxicosis. Since OP exposure is often unsuspected at the time of necropsy, some toxicosis goes unrecognized. In addition, the lack of homogeneity of brain ChE concentrations often requires (1) that the sample be completely homogenized or (2) that a specific location be assayed (i.e., caudate nucleus). Blood is an easily attainable specimen, but the variability due to plasma esterase fluctuations in addition to problems in obtaining unclotted specimens postmortem limits its value for routine screening.

The retina is a highly complex nervous tissue. Because the eye is easily removed, it is potentially the preferred tissue for use in postmortem evaluations of OP exposure in the bovine. In practice, the eye could be routinely collected, frozen, and evaluated later as part of a screening procedure. The purposes of this study were to evaluate and select assay parameters for retinal tissue, to establish baseline activity in normal animals, and to compare the retinal activity (from an OP toxicosis case) with currently accepted procedures (brain, blood). Previous work has shown that brain ChE activities paralleled those of retina in rats dosed with fenthion.⁵ Parallel responses of retina and brain ChE have been reported by other researchers.^{3,6,7}

A modification of the Ellman calorimetric assay for ChE² as described previously⁴ was utilized for all enzyme determinations, except the retina determinations were made on 100 mg of tissue/10 ml buffer rather than the 500 mg of tissue/25 ml buffer dilutions used for brain assays. A spectrophotometer^a was utilized for all assays with a wavelength setting of 412 nm and a cell temperature of 25 C.

Cholinesterase activities were determined on bovine blood and tissues collected from normal cattle at a slaughterhouse and specimens from a bovine case of spontaneous poisoning. In May 1987, a case involving terbufos^b toxicosis due to contaminated feed occurred in Rockford, Illinois, in which

90 feeder cattle died over a 3-week period in a herd in which 300-450 animals were at risk. A site investigation was made to collect tissues for evaluation of ChE activities for use in postmortem diagnosis. Eight animals were necropsied in the field and 1 eye, brain, rumen content, and liver were obtained from each. All specimens were collected within 24 hr of death. The 8 animals had been removed from the contaminated feed approximately 1 week prior to their death. Terbufos concentration ranged from <0.5 to 2.6 ppm in the rumen samples. No terbufos was detected in the liver samples. Feed collected on-site contained from < 1 to 196 ppm terbufos.

The intact bovine eyes were frozen at -15 C if not assayed the same day of collection. The eyes were defrosted at room temperature, and the cornea and iris were removed for the retina ChE determinations. The ocular fluids and the lens were removed and incisions were made in the sclera to open the tissue flat, exposing the retina. The retina was lifted gently with tweezers and cut from the optic nerve. It was placed into a conical micro-centrifuge tube and capped until assayed. Freezing the intact eye had no effect on the subsequent removal of the retina.

The whole brain (including brain stem) was collected for brain ChE determinations. Tissues were analyzed the same day or frozen at -15 C until analysis could be performed. Prior to testing, the whole brain (including brain stem) was chopped into approximately 2- x 2-cm pieces and frozen in liquid nitrogen. The frozen pieces were ground in a Waring blender into a fine frozen powder. Aliquots of 0.5 g of the whole brain homogenates were added to 25 ml of a 0.1 M phosphate buffer, pH 8.0, and mixed with a homogenizer.^{c,4}

The ChE values in normal tissues were 2.68 ± 0.61 $\mu\text{mol/ml/min}$, 4.07 ± 0.75 $\mu\text{mol/g/min}$, and 19.57 ± 3.13 $\mu\text{mol/g/min}$ for whole blood, brain, and retinal tissues, respectively, from 51 cattle (Fig. 1). The ChE values in the terbufos-poisoned animals were 0.73 ± 0.17 $\mu\text{mol/g/min}$ for 2 unclotted blood samples, 1.14 ± 0.44 $\mu\text{mol/g/min}$ for 7 brains,

and 5.01 ± 3.51 $\mu\text{mol/g/min}$ for 10 retinas. The retina assays from poisoned animals resulted in a mean ChE activity inhibition of 76%, with a range of 49-96%. The brain ChE activity yielded a mean of 74% inhibition, with a range of 56-90%. The mean for 2 unclotted whole blood samples resulted in 73% inhibition, with a range of 67-80% (Fig. 1).

The use of OP insecticides in agriculture is very common. The routine procedures of assaying ChE in brain or rumen contents for the insecticide frequently are not used due to the difficulty of acquiring field samples or because inappropriate samples were collected. In some situations because of topical exposures or excretion, the insecticide will not be found in the rumen.

This study provided baseline ChE activities for normal and OP-exposed cattle needed to evaluate the use of the retina as a diagnostic tissue for postmortem evaluation of OP insecticide toxicosis. The eye may be easily and routinely collected and frozen for immediate testing or storage. These data show that retinal ChE activity parallels the brain activity in cattle that died from ingestion of terbufos. This research has shown that bovine retinal tissue possessed approximately 5 times the ChE activity of whole brain homogenates, retained 77% of its original activity 7 days after removal from the eye when stored at 22 C, and showed no loss of activity after 7 days at 0 to -2 C.

The significance to the animal industry is the development of a diagnostic procedure using a readily obtainable and homogeneous tissue for improved recognition of OP toxicoses. The test can be used to obtain a rapid diagnosis of an OP insecticide toxicosis, thereby preventing additional exposure of the herd and allowing for the proper treatment to minimize additional death losses.

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Sources and manufacturers

- a. Shimadzu UV/160, Shimadzu Corp., Kyoto, Japan.
- b. Counter@, American Cyanamid, Wayne, NJ.
- c. Biohomogenizer®, Biospec Products, Inc., Bartlesville, OK.

References

1. Doull J, Klassen C, Andur M, ed: 1980, Caserett and Doull's toxicology, the basic science of poisons, 2nd ed., p. 365. Macmillan Publishing Co., Inc. New York, N.Y.
2. Ellman GL, Courtney KD, Andres V Jr, Featherstone RM: 1961, A new and rapid calorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 7:88-92.
3. Frances CM: 1953, Cholinesterase in the retina. *J Physiol* 120: 435-439.
4. Harlin KS, Ness D: 1986, Brain cholinesterase-normal enzyme activity levels in several large and small animal species. *Proc Annu Meet Am Assoc Vet Lab Diagn* 29:457-459.
5. Honda Y: 1971, Studies on the electrical activity of the mammalian retina in vitro; the effect of acetylcholine upon the ERG of rabbit retinas in vitro. *Acta Soc Ophthalmol Jpn* 75:1164-1171.
6. Moon BY, Pak SY: 1969, Cholinesterase and acid phosphatase in the rabbits' retinae following severance of the optic nerve. *Yonsei Med J* 10:18-54.
7. Nicholas CW, Koell GB: 1968, Comparison of the localization of acetylcholinesterase and non-specific cholinesterase activities in mammalian and avian retinas. *J Comp Neurol* 135:1-15.