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BAUMGARTNER, A. M., F. M. BAUMGARTNER. 1944. Hawks and owls in Oklahoma 1939-1942: Food habits and population changes. *Wilson Bull.* 56:209-215.

BENT, A. C. 1938. Life histories of North American birds of prey. Part II. U.S. Natl. Museum Bull. 170:295-357.

ERRINGTON, P. L. 1930. The pellet analysis method of raptor food habits study. *Condor* 32:292-296.

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ERRINGTON, P. L. 1932. Studies on the behavior of the Great Horned Owl. *Wilson Bull.* 44:212-220.

MILLER, L. 1930. The territorial concept in the Great Horned Owl. *Condor* 32:290-291.

ISOLATION OF PHOSPHOLIPASE A₂ FROM *AGKISTRODON BILINEATUS* VENOM

Venom produced by the Mexican moccasin, *A. bilineatus*, contains phosphomonoesterase, phosphodiesterase, 5'-nucleotidase, esterases, thrombin-like, L-amino acid oxidase, protease, phospholipase A₂, and NAD nucleosidase activities (Tu et al., 1967; Denson et al., 1972; Sifford and Johnson, 1978; Brunson et al., 1978). Of these enzymes, phospholipase A₂ (PhL-A₂) was chosen in this work for possible isolation. This choice of PhL-A₂ for isolation was due primarily to its heat stability and to its distribution in the eluates obtained by ion exchange chromatography of the crude venom as evidenced previously (Sifford and Johnson, 1978).

Assay procedures with minor modifications (Sifford and Johnson, 1978) included phospholipase A₂ using the clearing of an egg yolk suspension (Marinetti, 1965), phosphomonoesterase and phosphodiesterase (Richards et al., 1965), esterase (Tu et al., 1965), 5'-nucleosidase (Lo et al., 1966; Ging, 1956), and L-amino acid oxidase (Paik and Kim, 1965). Hyaluronidase was assayed according to the turbidimetric procedures of Kass and Seastone (1944).

A 450 mg sample of crude venom (Sigma) was separated on Concanavalin A covalently bound to Sepharose 4B gel (Con A) into glycoproteins (anthrone reagent positive) and nonglycoproteins (anthrone reagent negative) by employing the methods of Iscove et al. (1974) and Asperg and Porath (1970). In fractionations by ion exchange chromatography employing DEAE Sephadex A-50, the methods of Cheng and Ouyang (1967), Ouyang et al. (1971), and Johnson and Sifford (1978) were used. Proteins were desalted by using Sephadex G-10 columns at 4°C. Sephadex G-75 and G-50 columns were used to separate PhL-A₂ from higher molecular weight molecules.

An immunizing schedule was prepared according to Ownby et al. (1979). Preimmune serum was obtained from approximately 12 month old New Zealand white rabbits. An immunizing dose was prepared by dissolving 17 mg of lyophilized *A. bilineatus* crude venom in 20 ml of sterile physiological saline. A 0.5 ml aliquot of this solution was then mixed with 0.5 ml of Freund's complete adjuvant. Injections of 0.5 ml then were made subcutaneously into each thigh. Booster injections were prepared by mixing 0.5 ml of *A. bilineatus* venom (0.8 mg/ml) and 0.5 ml of Freund's complete adjuvant. One week later, subcutaneous injections of 0.5 ml of the solution were made in each shoulder. Four weeks after the booster injections, antiserum via heart puncture was collected and stored at -20°C.

Rabbit antiserum for the purified phospholipase A₂ fraction was prepared by injecting an immunizing dose containing 0.3 ml of purified enzyme (0.1 mg/ml) and 1.0 ml Freund's complete adjuvant. Subcutaneous injections of 0.65 ml of this solution were made into each thigh. One week later, booster injections of the same dose were administered into each shoulder. Four weeks later, antiserum was collected and stored at -20°C.

Immunoelectrophoresis methods outlined by Campbell et al. (1963) and Garvey et al. (1977) were employed to determine PhL-A₂ purity. Dodecyl Sulfate-Polyacrylamide gel electrophoresis procedures of Weber and Osborn (1969) were used by Dr. Collis Geren (University of Arkansas at Fayetteville) to assay crude venom and fraction samples.

A. bilineatus crude venom contains nonglycoprotein and glycoprotein enzymes (Fig. 1). The larger nonglycoprotein fraction (Fraction I), comprising approximately 80% of the crude venom proteins, contained numerous enzyme activities. These included PhL-A₁, phosphomonoesterase, phosphodiesterase, 5'-nucleosidase, hyaluronidase, TAMEase, BAEase, and L-amino acid oxidase activities.

Fraction I, obtained by Con A chromatography, was pooled, lyophilized, and then desalted with Sephadex G-10. Fractionation of this desalted nonglycoprotein fraction by ion exchange chromatography (DEAE Sephadex A-50) yielded three large fractions and several minor fractions. PhL-A₂ activity was concentrated in the second major fraction (Fig. 2). PhL-A₂ activity (14,000 units/mg) in this fraction was much higher than that of the crude venom (234 units/mg).

The PhL-A₁-containing fraction obtained by chromatography with DEAE Sephadex A-50 was divided into two samples. Even-numbered tubes (42 through 66) were pooled to from one sample while odd-numbered tubes (41 through 67) formed the other sample. These samples, after lyophilization and desalting, were fractionated with Sephadex G-75. In both instances, PhL-A₂ activity (24,400 units/mg) was observed only in the low molecular weight fraction (Fig. 3).

The low molecular weight PhL-A₂ fraction (tubes 16-22) obtained by Sephadex G-75 chromatography was pooled, lyophilized, desalted, and applied to a Sephadex G-50 column. This fractionation yielded a fraction free of the larger molecules (Fig. 4).

Immunoelectrophoresis and disc electrophoresis of crude venom samples indicated a complex mixture of proteins although the PhL-A₂ fraction obtained by the sequence of Con A-Sepharose 4B, DEAE A-50, Sephadex G-75, and Sephadex G-50 chromatography procedures was greatly purified (Figs. 5-8). Close examinations of both types of electrophoresis patterns, however, indicated trace contaminations. These contaminations could be due, in part, to TAMEase and L-amino acid oxidase since the distributions of these enzymes in *A. bilineatus* venom overlap PhL-A₂ after DEAE Sephadex A-50 chromatography (Sifford and Johnson, 1978). At present, work is directed toward purification of large amounts of PhL-A₂ in order that more enzyme characteristics may be obtained.

We thank Dr. Collis Geren for his work with the gel electrophoresis, Dr. L. W. Hinck for his assistance with the immunological procedures, John Ruff for his assistance throughout this work, and Mrs. Alice Chandler for typing the manuscript.

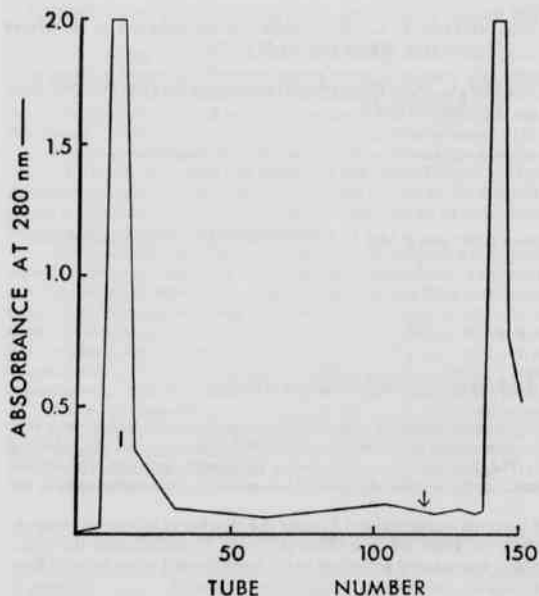


Figure 1. Chromatography of *Agkistrodon bilineatus* crude venom (450 mg) on a Concanavalin A Sepharose 4B column (2.5 × 15 cm) at 4°C by two stage elution. The arrow indicates the start of the second stage elution (using α -methyl-D-mannoside). Eluates were collected at a flow rate of 17 ml/hr.

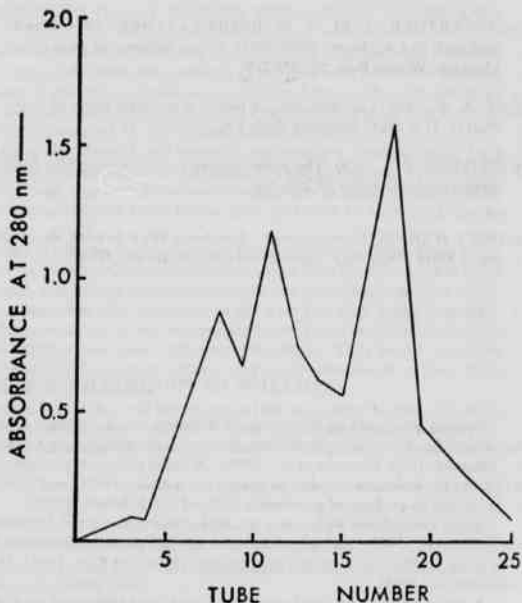


Figure 3. Chromatography of the phospholipase A₂ containing fraction, collected from DEAE A-50 ion exchange chromatography, on a Sephadex G-75 column (1 × 100 cm) at 4°C. Eluates of 4.0 ml/tube were collected.

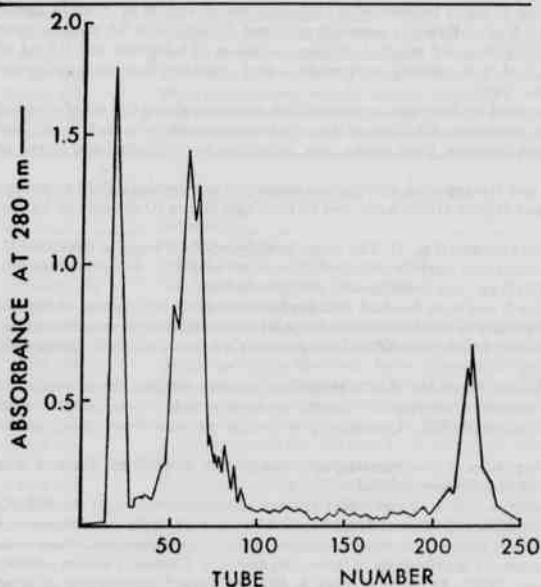


Figure 2. Chromatography of *Agkistrodon bilineatus* venom nonglycoproteins on a DEAE Sephadex A-50 column (2.5 × 56 cm) at 4°C by two stage elution with ammonium acetate buffer. Eluates of 5.0 ml each were collected at a flow rate of 17 ml/hr.

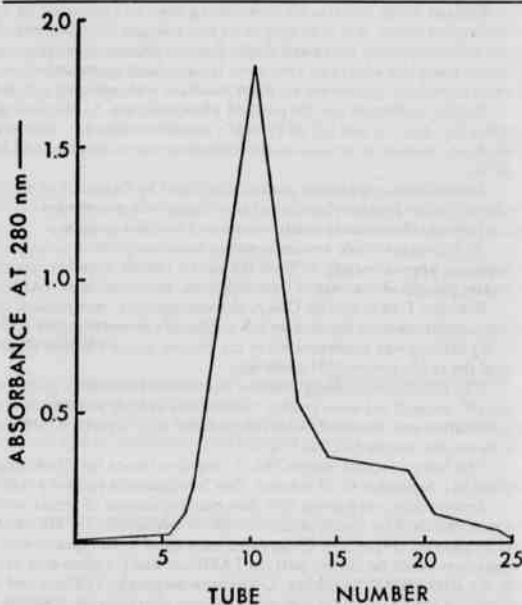


Figure 4. Chromatography of phospholipase A₂ containing fraction, from Sephadex G-75 chromatography, on a Sephadex G-50 column (1 × 100 cm) at 4°C. Eluates of 4.0 ml/tube were collected.



Figure 5. Immunoelectrophoretic patterns obtained by reacting anti-crude *Agkistrodon bilineatus* venom serum with electrophoretically separated crude venom.



Figure 6. Immunoelectrophoretic patterns obtained by reacting anti-crude *Agkistrodon bilineatus* venom serum with electrophoretically separated phospholipase A₂ containing fraction and crude venom.



Figure 7. Immunoelectrophoretic patterns obtained by reacting anti-crude *Agkistrodon bilineatus* venom serum with electrophoretically separated phospholipase A₂ containing fraction and *Agkistrodon bilineatus* crude venom.

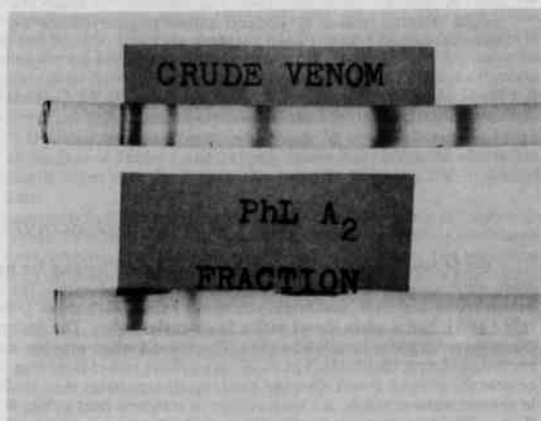


Figure 8. Patterns obtained by Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of *Agkistrodon bilineatus* crude venom and the phospholipase A₂ containing fraction.

LITERATURE CITED

- ASPBERG, K. and J. PORATH. 1970. Group-specific adsorption of glycoproteins. *Acta Chem. Scand.* 24:1839-1841.
- BRUNSON, E. M., B. D. JOHNSON and D. H. SIFFORD. 1978. NAD nucleosidase of *Agkistrodon bilineatus* venom. *Amer. J. Trop. Med. and Hyg.* 27:365-368.
- CAMPBELL, D. H., J. S. GARVEY, N. E. CREMER and D. H. SUSSDORF. 1963. Immunoelectrophoresis. Pp. 149-155, *In Methods in Immunology, A Laboratory Text for Instruction and Research.* W. A. Benjamin Inc., New York.
- CHENG, H. and C. OUYANG. 1967. Isolation of coagulant and anti-coagulant principles from the venom of *Agkistrodon acutus*. *Toxicon* 4:235-243.
- DENSON, K. W. E., F. E. RUSSELL, D. ALMAGRO and R. C. BISHOP. 1972. Characterization of the coagulant activity of some snake venoms. *Toxicon* 10:557-562.
- GARVEY, J. S., N. E. CREMER and D. H. SUSSDORF. 1977. Immunoelectrophoresis. Pp. 328-336, *In Methods of Immunology, A Laboratory Text for Instruction and Research.* W. A. Benjamin, Inc., Reading, Mass.
- GING, N. S. 1956. Extraction method of colorimetric determination of phosphorus in microgram quantities. *Anal. Chem.* 28:1330-1333.
- ISCOVE, N. N., F. SIEBER and K. H. WINTERHALTER. 1974. Erythroid colony formation in cultures of mouse and human bone marrow: Analysis of the requirement for erythropoietin by gel filtration and affinity chromatography on Agarose-Concanavalin A. *J. Cell. Physiol.* 83:309-320.
- KASS, E. H. and C. V. SEASTONE. 1944. The role of the mucoid polysaccharide (Hyaluronic Acid) in the virulence of a group A hemolytic streptococci. *J. Exp. Med.* 79:319-329.
- LO, T., Y. CHEN and C. LEE. 1966. Chemical studies of Formosan cobra (*Naja naja atra*) venom. Part I. Chromatographic separation of crude venom on CM-Sephadex and preliminary characterization of its components. *J. Chinese Chem. Soc. Ser. II* 13:25-37.
- MARINETTI, G. V. 1965. The action of phospholipase A on lipoproteins. *Biochim. Biophys. Acta.* 98:554-565.
- OUYANG, C., J. HONG and C. TENG. 1971. Purification and properties of the thrombin-like principle of *Agkistrodon acutus* venom and its comparison with bovine thrombin. *Thrombos. Diathes. haemorrh.* (Stuttg.) 26:224-234.
- OWNBY, C. L., W. M. WOODS and G. V. ODELL. 1979. Antiserum to myotoxin from prairie rattlesnake (*Crotalus viridis viridis*) venom. *Toxicon* 17:373-380.
- PAIK, K. and S. KIM. 1965. pH substrate relationship of L-amino acid oxidases from snake venom and rat kidney. *Biochim. Biophys. Acta.* 96:66-74.
- RICHARDS, G. M., G. DUVAIR and M. LASKOWSKI, SR. 1965. Comparison of the levels of phosphodiesterase, endonuclease, and monophosphatases in several snake venoms. *Biochem.* 4:501-503.
- SIFFORD, D. H. and B. D. JOHNSON. 1978. Fractionation of *Agkistrodon bilineatus* venom by ion exchange chromatography. Pp. 231-242, *In Toxins: Animal, Plant and Microbial* (P. Rosenberg, ed.) Pergamon Press, Oxford and New York.
- TU, A. T., G. P. JAMES and A. CHUA. 1965. Some biochemical evidence in support of the classification of venomous snakes. *Toxicon* 3:5-8.
- TU, A. T., P. M. TOOM and D. S. MURDOCK. 1967. Chemical differences in the venoms of genetically different snakes. Pp. 351-362, *In Animal Toxins* (Russell and Saunders, eds.) Pergamon Press, Oxford and New York.

Arkansas Academy of Science

WEBER, K. and M. OSBORN, 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* 244:4406-4412.

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SAGE THRASHER (*OREOSOPTES MONTANUS*), A NEW STATE RECORD

On 24 November 1979, Cheryl Lavers and I were looking for birds in the Farville area, about 6 miles NE of Jonesboro, Craighead County, when we discovered a Sage Thrasher (*Oreoscoptes montanus*). The bird was rather Mockingbird-like (*Mimus polyglottos*) in shape, plain gray-brown crown and back, and bright yellow eye with a black pupil. Other characters differed from a Mockingbird in that it was smaller and shorter tailed and it had a white throat with a black malar stripe. The underparts, with a ground color of warm buff, were densely streaked with black chevrons arranged in length-wise rows. There was a white wingbar, and a dark tail with white outer corners. The bill was slender, very slightly decurved, and dark bluish-black in color, as were the rather short legs. The bird ran along the ground in open places, or under brush and stayed on or near the ground. It was observed catching, decapitating, then swallowing ground crickets (*Acheta* sp.). We have both observed Sage Thrashers in several western states. An examination of standard field guides (Peterson, R. T. 1961. A field guide to western birds. Houghton Mifflin Co., Boston; Robbins, C. S. et al. 1966. Birds of North America. Golden Press, N.Y.) further confirmed our identification. A description and slides of the bird have been sent to Charles Mills, Curator, the Arkansas Audubon Society, and Dr. Douglas James, Department of Zoology, University of Arkansas at Fayetteville. This is a first recorded instance of the Sage Thrasher in Arkansas.

In its normal range, the Sage Thrasher breeds in the sagebrush (*Artemisia*) deserts of western North America, coming as far east as western Oklahoma. It winters in the southern part of its range and Mexico, occurring as far east as central and southern Texas, with a small isolated winter colony in extreme southwestern Louisiana (A.O.U. check-list of North American birds, 1957). The species is seldom recorded as a vagrant. The following extralimital occurrences to the east of its range have been recorded: Florida, 2; Illinois, 2; Maryland, 1; New York, 2; North Carolina, 1; South Dakota, 1.

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SOME EFFECTS OF METHYL GREEN ON EXPRESSION OF DAMAGE INDUCED IN G1 *XENOPUS* CELLS BY ULTRAVIOLET LIGHT

Methyl green shows a high degree of specificity for DNA and is a component of many nuclear stains. Kurnick (1952) observed that this basic dye was readily bound by polymerized DNA; two amino groups of the dye binding to two phosphoric acid groups of DNA. Errera (1951) demonstrated that the affinity of DNA for methyl green is influenced by radiation and other agents which depolymerize DNA, or alter its internal configuration. Doudney and Haas (1958) showed that methyl green significantly influenced metabolic activities, such as DNA and RNA synthesis, in *Escherichia coli*. These results suggested that appropriate experiments, involving methyl green treatments coupled with germicidal UV exposures, might aid in describing the expression and repair of UV-induced lethal and mutational damage in prokaryotic cells. Experiments of this nature were carried out by investigators, such as Witkins (1961), which led to the notion that UV induces lesions in bacterial DNA that either are removed by repair systems or are converted to permanent structural changes during the first DNA synthetic period following the exposure. We report here an extension of such experiments to eukaryotic cells, in which some effects of methyl green on the repair of UV (254 nm)-induced damage in G1 phase *Xenopus* cells are examined.

Routine maintenance of log phase A8W243 *Xenopus* cultures, incubations, cell synchronizations, irradiations, mitotic index determinations, survival determinations (colony counts), and chromosome analysis have been described in detail (Griggs and Bender, 1973; Payne and Griggs, 1977; Griggs and Orr, 1979).

The basic dye used, methyl green, was obtained from Difco.

Figure 1 shows results of a series of mitotic index experiments performed to examine the effects of methyl green on progression of UV irradiated G1 cells through interphase and the first succeeding mitosis, (M1). These data described the appropriate time intervals for collection of the sets of mitotic cells analyzed for effects of methyl green on UV-induced aberration production (Table 1). Concentrations of the dye in the range 0.0 - 0.003 g/l appeared to induce little delay in progression of the irradiated cells above that induced by the UV alone. The similarity in average height and width of these mitotic peaks indicated that the dye did not significantly reduce the number of irradiated cells that reached G1.

The average cell cycle for non-irradiated *Xenopus* cells was approximately 26 hours (not shown); eight hours G1, 13 hours S, three hours G2, and two hours M1. Payne and Griggs (1977) carried out autoradiographic studies which indicated that early G1 phase cells, exposed to low doses of UV (0 - 150 ergs mm⁻² range), are not delayed in their progression through G1, but experience rather lengthy S phase delays. These facts, coupled with the data of Figure 1 and Table 1, indicate that chromatid aberration frequencies, resulting from UV exposure of early G1 cells, are significantly altered by methyl green only when the dye is in contact with the exposed cells as they pass through early S phase. Some relationship between the aberrant intracellular mechanism, by which methyl green augments chromatid type aberration production, and DNA synthesis is suggested by the fact that both mechanisms appear to function with peak efficiency in early S phase.

The data of Figure 2 indicate that a methyl green concentration of 0.003 g/l has virtually no effect on the expression of UV-induced lethal damage, no matter where in the cell cycle the dye is applied. These data suggest that the mechanism which expresses UV-induced aberrational damage in *Xenopus* cells differs significantly from the mechanism which expresses lethal damage, supporting results of previous studies of the overlap of UV-induced lethal and aberrational lesions in *Xenopus* cells (Griggs and Orr, 1979; Payne and Griggs, 1977).