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Further study is planned using polyethylene film as one glazing and a rigid material such as filon as the second glazing to cut costs and increase the effectiveness, therefore promoting the utilization of solar energy.

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NEUTRAL SUGARS IN SELECTED PIT VIPER, ELAPID, LIZARD AND SCORPION VENOMS

Carbohydrates exist in venoms in the form of glycoproteins and as free sugars. Aragon et al. (1977) reported that venom from the Central American *Bothrops asper* is very rich in both glycoproteins and free sugars. Glycoproteins are reported in a wide variety of snake venoms (Oshima and Iwanaga, 1969; Basu et al., 1970; Hatton, 1973; Ruff et al., 1980; Marlas, 1982). Viperid and crotalid venoms often contain relatively large amounts of bound carbohydrates when compared with venoms of elapids. These carbohydrates include neutral sugars, amino sugars, and sialic acid (Oshima and Iwanaga, 1967). In this paper we quantitatively compare L-fucose, D-galactose, D-glucose, and D-mannose neutral sugars of whole venoms from snakes, lizards, and scorpions. The venoms were also analyzed for the presence of D-arabinose and D-xylose.

L-fucose and lyophilized venoms of *Agkistrodon bilineatus*, *Heloderma horridum*, *H. suspectum*, *Androctonus australis*, and *Naja naja atra* were purchased from Sigma Chemical Company. The other venoms, also lyophilized, were a gift from Dr. H. L. Stahnke of the Poisonous Animals Research Laboratory at Arizona State University. The other carbohydrate standards were purchased from Chem Service, Inc.; 1-dimethylamino-2-propanol from Aldrich Chemical Company; methanol from MCB Manufacturing Chemists, Inc.; and pyridine from Fisher Scientific Company. All liquid reagents were redistilled prior to use.

Gas chromatography was performed using a Perkin-Elmer Model 3920B instrument equipped with dual flame ionization detectors and 6-ft., 1/8-in.-o.d. nickel columns packed with 1% stabilized diethylene glycol adipate on 100-200 mesh Chromosorb W (HP) by the procedure described by Mawhinney et al. (1980). Data were collected, stored, and analyzed by a Varian Vista 401 Chromatography Data System.

Neutral sugars were obtained by heating 2 to 4 mg samples of venom with 1.0 ml of 0.6 N HCl per mg of venom at 100° for 4 h and eluting in sequence through 0.8 x 8-cm columns of Dowex 1-4X (CO₃⁻ form, 50-100 mesh) and Dowex 50-8X (H⁺ form, 200-400 mesh) with distilled H₂O. One ml of internal standard solution containing 0.0186 mg of phenyl β-D-glucopyranoside was added to the effluent before the sample was concentrated by lyophilization. To convert neutral sugars to oximes, the effluent was mixed with 0.2 ml of a solution containing 0.6 g of hydroxylamine hydrochloride, 2.0 ml of methanol, 5.47 ml of pyridine, and 0.53 ml of 1-dimethylamino-2-propanol and heated at 70° for 5 min in a Teflon-capped Reacti-vial. After cooling to room temperature, a stream of dry air was directed into the open vial to remove excess reagent. Acetate derivatives were prepared by adding 1.0 ml of pyridine-acetic anhydride (1:3 v/v), mixing, and heating the vial at 70° for 25 min. The vial was cooled to room temperature, after which the solution was reduced to a syrup using a stream of dry air. To remove salts, the contents were dissolved in 1.0 ml of chloroform and washed once with 1.0 ml of 1.0 N HCl and three times with 1.0 ml each of distilled water. The chloroform was evaporated with a stream of dry air (Mawhinney et al., 1980). For conversion to aldononitrile acetate (Varmer, et al., 1973), 0.6 ml of pyridine and 1.8 ml of acetic anhydride were added and the mixture was heated at 90° for 30 min. The solution was evaporated to dryness at 40° under diminished pressure with a stream of nitrogen directed into the vessel.

Neutral sugars are present in pit viper, elapid, lizard, and scorpion venoms (Table). D-arabinose and D-xylose were not detected in venoms of *Crotalus molossus*, *C. scutulatus*, and *N. naja*. Only trace amounts, less than 1 μg per mg of venom, of these sugars were indicated in the other venom analyses. Venom of *A. piscivorus piscivorus* was relatively low in D-mannose. Otherwise, pit viper venoms contained abundant D-mannose, comparable amounts of L-fucose and D-galactose, and relatively small amounts of D-glucose. *A. bilineatus* venom was highest in all the sugars assayed, except D-glucose. D-mannose was not the major sugar in the elapid venom tested; however, D-mannose was dominant in the lizard venom. *Centruroides sculpturatus* venom was higher in total neutral sugar than the other scorpion venoms.

A significant unidentified peak (Fig.), probably indicating another neutral sugar, was recorded immediately prior to the D-mannose peak in the chromatograms of *A. p. piscivorus*, *C. arrox*, *N. naja*, and *N. n. atra* venom samples. This peak was minor or absent in the remaining chromatograms. Small unidentified peaks were also recorded immediately prior to the L-fucose peak.

Sialic acid and amino sugar analyses of the above venoms are now in progress.

Table. Neutral Sugars in Various Venoms*†

Venom	L-Fuc	D-Gal	D-Glu	D-Man
Pit Vipers:				
<i>Agkistrodon bilineatus</i>	13.3	9.8	1.2	15.4
<i>Agkistrodon contortrix contortrix</i>	1.8	1.6	trace	8.8
<i>Agkistrodon piscivorus piscivorus</i>	3.5	1.2	1.7	1.0
<i>Crotalus atrox</i>	3.0	2.7	1.9	5.6
<i>Crotalus molossus</i>	2.0	2.1	trace	3.0
<i>Crotalus scutulatus</i>	2.5	8.0	trace	8.3
<i>Crotalus viridis cerberus</i>	3.1	4.8	1.5	6.0
Elapids:				
<i>Halo naja</i>	1.1	1.7	1.3	2.5
<i>Halo naja atra</i>	3.2	5.2	1.6	3.2
Lizards:				
<i>Heloderma horridum</i>	2.3	6.3	2.6	7.2
<i>Heloderma suspectum</i>	2.0	4.5	trace	3.1
Scorpions:				
<i>Androctonus australis</i>	1.3	1.0	2.4	1.4
<i>Centruroides sculpturatus</i>	5.9	6.2	4.6	4.9
<i>Uroscorpus arizonensis</i>	1.8	1.4	1.3	3.6
<i>Uroscorpus viridulus</i>	1.4	4.4	1.4	4.0

* μg of sugar/mg of venom

† trace indicates < 1.0 μg of sugar/mg of venom

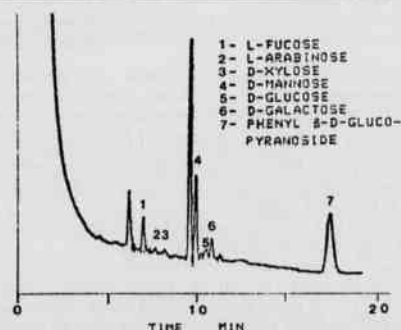


Figure. Gas chromatographic separation of neutral sugars from *C. atrox* venom as aldononitrile acetates. The initial hold was at 170° for two minutes followed by an increase of 8°/min to a final temperature of 240°. Nitrogen flow rate was 24 ml/min and sample size was 4 μl.

General Notes

LITERATURE CITED

- ARAGÓN, F., R. BOLANOS, and O. VARGAS. 1977. Carbohidrates del veneno de *Bothrops asper* de Costa Rica. Estudio cuantitativo. Rev. Biol. Trop., 25(2):171-178.
- BASU, A. S., R. PARKER and R. O'CONNOR. 1970. Disc electrophoresis of glycoproteins in snake venoms. Toxicon 8:279-284.
- HATTON, M. W. C. 1973. Studies on the coagulant enzyme from *Agkistrodon rhodostoma* venom. Isolation and some properties of the enzyme. Biochem. J. 131:799-807.
- MARLAS, C. 1982. Purification and preliminary structure of a potent platelet aggregating glycoprotein isolated from the venom of *Crotalus durissus cascavella*. Toxicon 20:289-290.
- MAWHINNEY, T. P., M. S. FEATHER, G. J. BARBERO, and J. R. MARTINEZ. 1980. The rapid quantitative determination of neutral sugars (as aldononitrile acetates) and amino sugars (as O-methylxime acetates) in glycoproteins by gas-liquid chromatography. Anal. Biochem. 101:112-117.
- OSHIMA, G., and S. IWANAGA. 1969. Occurrence of glycoproteins in various snake venoms. Toxicon 7:235-238.
- RUFF, J. D., B. D. JOHNSON, and D. H. SIFFORD. 1980. A glycoprotein proteinase in *Agkistrodon bilineatus* venom. Proc. Ark. Acad. Sci. 34:130-131.
- VARMA, R., R. S. VARMA, W. S. ALLEN, and A. H. WARDI. 1973. Gas chromatographic determination of neutral sugars from glycoproteins and acid mucopolysaccharides as aldononitrile acetates. J. Chromatogr. 86:205-210.

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REPAIR OF ULTRAVIOLET AND GAMMA-RAY INDUCED LETHAL DAMAGE IN AN INSECT TISSUE CULTURE CELL LINE*

We have recently performed a series of preliminary radiation experiments which indicate that the IPL-22 insect tissue culture cell line constitutes another fruitful system for study of the roles played by intracellular repair mechanisms in the radiation resistance of eukaryotic cells. The effects of repair processes on the kinetics of ultraviolet (UV) and gamma-ray induced cell killing (loss of colony forming ability) are briefly described here.

The IPL-22 line was cloned from the IPL-21 insect line (*Spodoptera frugiperda*), which was obtained from Dr. Troy Orr of the Southwest Foundation for Research and Education in San Antonio, Texas. The line was routinely maintained in IPL-41 medium (Kansas City Biological) in plastic tissue culture flasks (Falcon). Log phase monolayer cultures with plating efficiencies near 0.70 were selected for each experiment. All experimentation was carried out at 26 degrees Celsius. Gamma-ray was administered with a custom designed Mark IV Cesium 137 irradiator at a dose rate of 40 rads/minute. Techniques employed for UV irradiations, photoreactivation (PR), cell fusions, caffeine treatments, single cell plating of treated cells, incubations, and survival determination (assays for colony forming ability) were essentially the same as those described previously for *Xenopus* cells (Griggs and Bender, 1973; Griggs and Orr, 1979; Haetten, McGuinness and Griggs, 1982).

The UV LD₅₀ (lethal dose to 50 percent of the cells) for IPL-22 cells can be estimated from the UV-alone data of Figure 1 to be near 200 ergs/mm², indicating a significantly higher resistance to the lethal effects of UV than that observed for established vertebrate tissue culture lines such as the A8 *Xenopus* line (LD₅₀ near 60 ergs/mm²) and the V79 hamster line (LD₅₀ near 75 ergs/mm²) (Griggs and Bender, 1972). The UV-alone data (Figure 1) constitutes a sigmoid or threshold curve, suggesting a multihit single target, multitarget single hit, or multitarget multihit relation (Elkind and Whitmore, 1967). However, as indicated by the UV + caffeine data of Figure 1, caffeine significantly alters the UV curve by reducing the shoulder or threshold segment. These data suggest that the threshold results, at least in part, from the operation of a caffeine sensitive intracellular repair mechanism, perhaps similar to the caffeine sensitive recombination-like repair mechanism observed in V79 hamster cells (Cleaver, 1974; Haetten et al. 1982).

IPL-22 cells photoreactivate a small fraction of the lethal damage induced by UV doses in the range 0-400 ergs/mm² (Figure 2). Direct enzymatic repair is indicated, since the reactivation light effectively diminishes the UV dose, (Rupert and Harm, 1966). It is interesting that IPL-22 cells do not appear to possess an efficient PR mechanism, as do many microorganisms (Rupert and Harm, 1966) and some vertebrate cells (Griggs and Bender, 1972).

The gamma-ray survival curve for IPL-22 cells also indicates a threshold response with an LD₅₀ near 1000 rads (gamma-ray alone points, Figure 3). This is a rather marked resistance to the lethal effects of gamma-ray as compared to the resistance shown by established mammalian cell lines (Elkind and Whitmore, 1967). The observed increase in resistance to a given dose when the dose is fractionated (Table 1) suggests that gamma-ray resistance is due in part to the operation of a dark repair mechanism, perhaps similar (or identical) to "Elkind recovery" (Elkind and Sutton, 1960).

Two experiments were carried out to explore overlap of UV and gamma-ray induced lethal lesions. As indicated by the UV + gamma data of Figure 3, UV exposures in the 0-40 ergs/mm² range actually reactivate some of the lethal damage induced by 500 rads of gamma-ray. This UV reactivation (UVR) appears to be similar to that observed in *Xenopus* cells (Cross and Griggs, 1978). Higher doses of UV have an additive, or perhaps synergistic, effect with gamma-ray. The data of Table 2 are results of an attempt at what could be termed "fusion reactivation" (FR). The synkaryons, produced by fusion of UV-irradiated parental cultures with gamma-irradiated parental cultures, exhibited a higher level of survival than either of the parental cultures (Experiment 3, Table 2). This "Reactivation" may result from a type of genomic complementation in which each viable synkaryon contains at least one undamaged copy of the essential genetic units. However, further investigation of the growth characteristics of viable synkaryons may indicate a more complex mechanism, perhaps involving some type of enzymatic repair.

The data described here indicate that a significant part of the radiation resistance exhibited by IPL-22 insect cells is due to the functioning of dark (non PR) radiation repair mechanisms. These dark mechanisms appear to function more efficiently than similar repair mechanisms

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