decreased (135–120%) (Table II). It may be concluded that under aerobic conditions the inhibiting effect of D-L-glyceraldehyde, both on the respiration and the leucine-C^{14} incorporation, is very weak. Under anaerobic conditions, however, the picture is quite different (Table III). The addition of D-L-glyceraldehyde only results in absence of fermentation, with no CO_2 being produced.

The addition of D-L-glyceraldehyde in weak concentrations (0.33 mM) does not result in a reduction of glycolysis or of leucine-C^{14} incorporation, but may even produce a slight increase.

Slight inhibition of glycolysis and aminoacid incorporation begins at concentrations of 0.66 mM. At higher concentrations (1.32 mM) of D-L-glyceraldehyde glycolysis is reduced to about a half, and the leucine-C^{14} incorporation to a third. The results obtained in developing cells and in resting cells are in agreement.

Investigations are currently in progress on the influence of D-L-glyceraldehyde on RNA cellular synthesis to establish at what level the inhibition of protein synthesis begins[5].

Riassunto. In aerobiosi la D-L-gliceraldeide a notevoli concentrazioni ha una lievissima azione inibente sulla ossidazione del glucosio e sulla incorporazione di leucina-C^{14}. In condizioni di anaerobiosi invece la D-L-gliceraldeide in concentrazioni medie inibisce notevolmente sia la glicolisi che la incorporazione di leucina-C^{14}.

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The Presence of a L-Leucyl-β-Napthylamide Hydrolyzing Enzyme in Snake Venoms

Although the hydrolysis of proteins by snake venoms is well recognized, no extensive study has been carried out on venom exopeptidases. The inability of *Crotalus atrox* (Western Diamondback rattlesnake) venom to hydrolyze *N*-carbobenzoxyglycyl-L-phenylalanine, hippuryl-L-arginine, and hippuryl-L-lysine, lead BROWN[1] to the conclusion that carboxypeptidases A and B were absent in this venom. Similar conclusions were drawn by WAGNER and PRESCOTT[2] when *N*-carbobenzoxyglycyl-L-phenylalanine and hippuryl-β-DL-phenyllactate were not hydrolyzed by 4 rattlesnake venoms. In this same investigation, WAGNER and PRESCOTT found no evidence for a true amino peptidase.

Recently, TU et al.[3] studied the di- and tripeptide hydrolyzing ability of 12 species of rattlesnake and 8 cobra species. From this study, it was concluded that many of the peptides hydrolyzed by these venoms were those which would also be hydrolyzed by leucine amino peptidase. Therefore, the synthetic substrate L-leucyl-β-napthylamide, commonly used as a test for leucine amino peptidase, was employed in determining the hydrolyzing ability of a number of species from each family of poisonous snakes.

Enzymatic activity was determined by a modified method of GOLDBARG and RUTENBURG[4]. A solution consisting of 1 ml venom (1 mg/ml) was incubated at 37 °C with 1 ml of 0.02% substrate in 0.01 M phosphate buffer pH 7.1 for 1 h. The reaction was stopped by the addition of 1 ml of 25% trichloroacetic acid. Following

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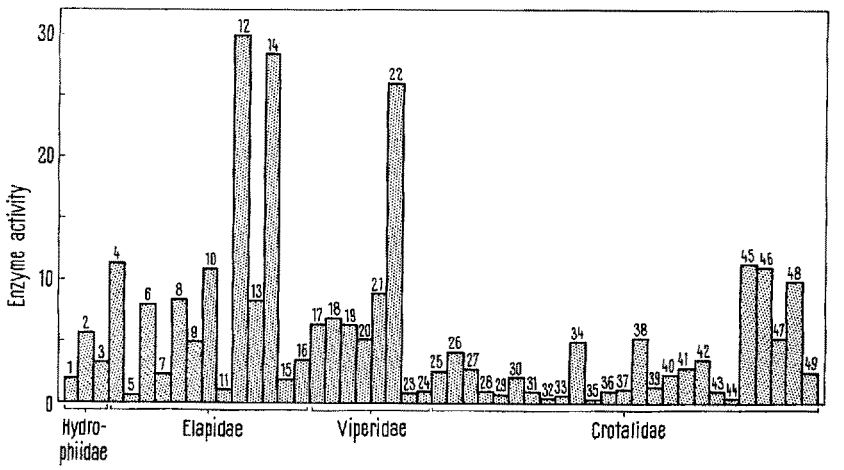

Enzymatic activity of snake venoms hydrolyzing L-leucyl-β-naphthylamide. Venoms corresponding to the numbers are:

Name	Common name	Origin
Family Hydrophiidae		
(1) *Laticauda laticaudata*	Black-banded sea snake	Japan
(2) *Enhydrina schistosa*	Common sea snake	Malay to Australia
(3) *Hydrophis cyanocinctus*	Chittal	Malay to Persian Gulf
Family Elapidae		
(4) *Naja naja*	Common cobra	India
(5) *N. naja atra*	Taiwan cobra	Formosa
(6) *N. naja siamensis*	Thailand cobra	Thailand
(7) *N. hannah (Ophiophagus hannah)*	King cobra	India
(8) *N. melanoleuca*	Black cobra	Eastern Africa
(9) *N. flava*	Yellow cobra	Africa
(10) *N. haje*	Egyptian cobra	Northern Africa
(11) *N. nigricollis*	Black-necked cobra	Africa
(12) *Dendroaspis angusticeps*	Green mamba	Africa
(13) *Bungarus multicinctus*	Banded krait	Formosa
(14) *Oxyuranus scutellatus scutellatus*	Australian taipan	Australia and New Guinea
(15) *Notechis ater humphreys*	King Island tiger	Australia
(16) *Pseudechis australis*	Common Malga snake	Australia and New Guinea
Family Viperidae		
(17) *Bitis arietans*	Puff adder	Africa
(18) *B. gabonica*	Gaboon viper	Africa
(19) *B. gabonica rhinoceros*		Africa
(20) *B. nasicornis*	Rhinoceros viper	Africa
(21) *Causus rhombeatus*	Night adder	S. Africa
(22) *Echis carinatus*	Saw-scaled viper	C. Asia, N. Africa and Middle East
(23) *Vipera ammodytes*	Sand viper	Europe
(24) *V. russellii*	Russell's viper	India
Family Crotalidae		
(25) *Crotalus basiliscus*	Mexican West Coast rattlesnake	Mexico
(26) *C. durissus totonacus*	Mexican Totonacan rattlesnake	Mexico
(27) *C. terrificus terrificus*	S. American rattlesnake	S. America
(28) *C. viridis viridis*	Prairie rattlesnake	USA
(29) *C. atrox*	Western diamondback	USA
(30) *C. horridus horridus*	Timber rattlesnake	USA
(31) *C. horridus atricaudatus*	Canebrake rattlesnake	USA
(32) *Sistrurus miliarius barbouri*	Dusky Pygmy rattlesnake	USA
(33) *Bothrops jararaca*	S. American pit viper	S. America
(34) *B. schlegeli*	Horned Palm viper	Costa Rica
(35) *B. nummifera*	Jumping viper	Costa Rica
(36) *B. picadoi*		Costa Rica
(37) *B. atrox*	Fer de lance	Mexico
(38) *B. nasuta*	Hog nosed viper	Costa Rica
(39) *Trimeresurus okinavensis*	Okinawan habu	Okinawa
(40) *T. mucrosquamatus*	Taiwan habu	Formosa
(41) *T. gramineus*	Green habu	Formosa
(42) *T. flavoviridis*	Yellow-green pit viper	Japan
(43) *Agkistrodon acutus*	Hundred pace snake	Formosa
(44) *A. halys*	Mamushi	Japan
(45) *A. contortrix laticinctus*	Broadbanded copperhead	USA
(46) *A. contortrix mokesen*	Northern copperhead	USA
(47) *A. piscivorus leukostoma*	Western cottonmouth	USA
(48) *A. piscivorus piscivorus*	Eastern cottonmouth	USA
(49) *A. bilneatus*	Tropical moccasin	C. America

centrifugation, 1 ml of the supernatant liquid was added to 1 ml of 0.1% sodium nitrite. After standing 3 min, 1 ml of 0.5% ammonium sulfamate was added and the reaction mixture allowed to stand for 2 min. To this solution, 2 ml of 0.5% *N*-(1-naphthyl)-ethylenediamine alcoholic solution was added. After 30 min, the absorbance was read at 580 nm and corrected with appropriate blanks. Enzyme activity was defined as:

$$\text{activity} = \frac{\text{absorbance change/min}}{\text{mg venom}} \cdot 1000 .$$

Although there were considerable species dependent variations in the ability to hydrolyze L-leucyl-β-naphthylamide, almost all the venoms investigated hydrolyzed this substrate. In general, Elapidae and Viperidae venoms exhibited relatively high activity, while those of Hydrophiidae (sea snakes) gave comparatively low activity. While the overall activity of the Crotalidae family was quite low, the venoms of the genera *Agkistrodon* showed considerably higher activity than the other members of the family.

Many enzymes present in 1 or 2 families of poisonous snakes have been found to be absent in other families. For example, it has been found that while cholinesterase is present in the venoms of the family Elapidae, it is absent in the venoms of Crotalidae and Viperidae[5–8]. In like manner, TU et al.[9–11] have found that while the venoms of Crotalidae and Viperidae hydrolyze *p*-toluenesulfonyl-L-arginine methyl ester (TAME) and *N*-benzoyl-L-arginine ethyl ester (BAEE), the venomes of Elapidae and Hydrophiidae are inactive toward these substrates. The ability of most venoms presently investigated to hydrolyze L-leucyl-β-napthylamide indicates that the ability to hydrolyze this substrate is indeed quite common in venoms of poisonous snakes[12,13].

Résumé. Pour déterminer le caractère des exo-peptidases dans le venin des serpents on a employé comme substrat du L-leucyl-β-napthylamide. Des venins de 49 espèces et sous-espèces des 4 familles de serpents venimeux ont été étudiés. La capacité d'hydroliser ce substrat a été rencontrée assez souvent dans les venins des serpents venimeux quelles que joient leurs origines géographiques ou leurs différences génétiques.

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