Mem. Inst. Butantan
Simp. Internac.
33(3):943-950, 1966

38. 5'-NUCLEOTIDASE ACTIVITY IN SNAKE VENOMS

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5'-nucleotidases, the enzyme capable of hydrolyzing nucleoside 5'-monophosphates to nucleosides and orthophosphate are widely distributed in nature. 5'-nucleotidase activity has been found in animal tissues (1-3), bull semen (4), micro-organisms (5, 6) and in venoms of different species of snakes (7, 8). In human tissues, the highest activity has been found in the posterior pituitary gland (9, 10). Venoms generally exhibit high 5'-nucleotidase activity. This enzyme is found with phosphodiesterase and non-specific phosphatases in venoms which have been largely used in recent years to purify phosphodiesterase and 5'-nucleotidase as these enzymes are valuable tools in the study of structure and nucleotide sequence of nucleic acids (11-14).

After the findings of Gulland and Jackson (7) showed the presence of 5'-nucleotidase in venoms, many attempts had been made to isolate it from venoms in order to study the substrate specificity of the enzyme, its metal requirement, and optimum pH (13-15). We have recently studied 5'-nucleotidase activity in venoms of different species of snakes from widely separated parts of the world and the effects of various metallic ions, inhibitors, and activators on the enzyme activity. The results are presented in this paper.

Methods

In the present study a 20 mg percent venom solution made in glass-distilled water, the pH of which was previously adjusted to 7.4 with 1N $NaHCO_3$ was used. The assay involved the measurement of orthophosphate liberated from adenylic acid (5'-AMP) when a reaction mixture containing 0.1 ml of the venom solution, 5 μmoles of 5'-AMP (Na salt), 1 μmole of Mg^{++} and 20 μmoles of glycine buffer, pH 8.5, in a total volume of 0.5 ml, was incubated at 37°C for 20 min. The reaction was terminated by adding an equal volume of 10% cold trichloroacetic acid (TCA), and allowing it to stand at 0°C for 15 min and then centrifuging it at 1200 × g at 0°C for 15 min. The clear liquid (TCA extract) was used for the orthophosphate determination. The methods of Fiske and Subba Row (16), Cleland and Slator (17) were used to measure Pi and protein.

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Results

Fig. 1 shows the 5'-nucleotidase activity of six venoms obtained from three different species of snakes. The rate of hydrolysis of 5'-AMP was linear to the enzyme concentration in each case, until 65 to 70% of the substrate was hydrolyzed. Of all the venoms tested for 5'-nucleotidase activity the venom of *Crotalus adamanteus* exhibited highest activity and the venom of *Bothrops jararaca* the least. The venom of *Vipera russelli* showed slightly less activity than the venom of *C. adamanteus.* The Km values calculated for the venoms of *V. russelli* and water moccasin by Lineweaver and Burk's equation (18) from the plots 1/V against 1/S (Fig. 2) were found to be 2.2×10^{-3} and 1.66×10^{-3} M.

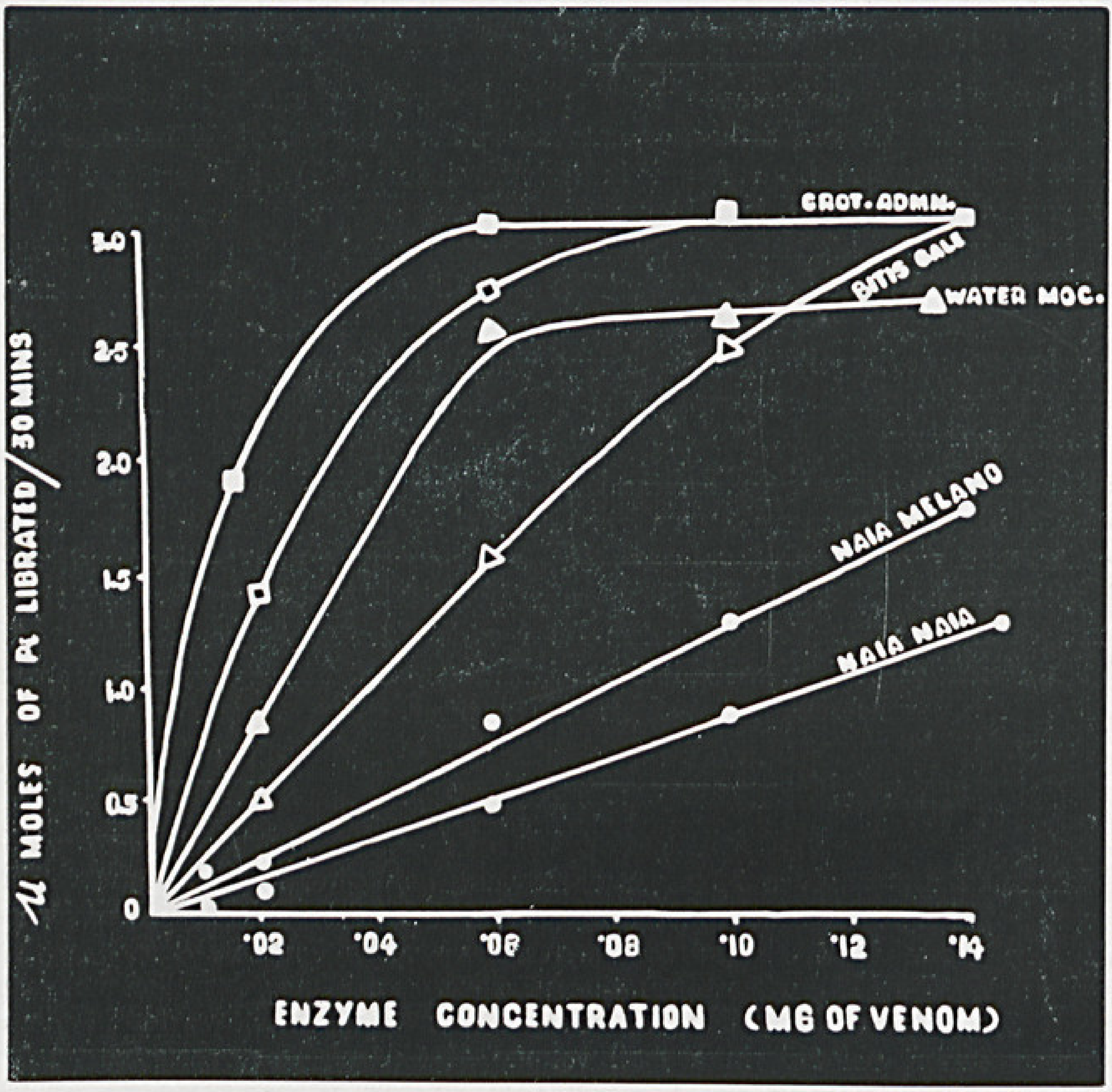

Fig. 1

Effects of metallic ions, ortho- and pyrophosphates and reducing agents on venom 5'-nucleotidase activity.

Maximum activity was found in the presence of Mg^{++}; and in its absence 60-70% activity was noted. In the presence of EDTA, the activity dropped to 10-15%. However, when Mg^{++} was added a twofold increase in activity was found. With dialyzed venom which showed only 20-22% of the activity of

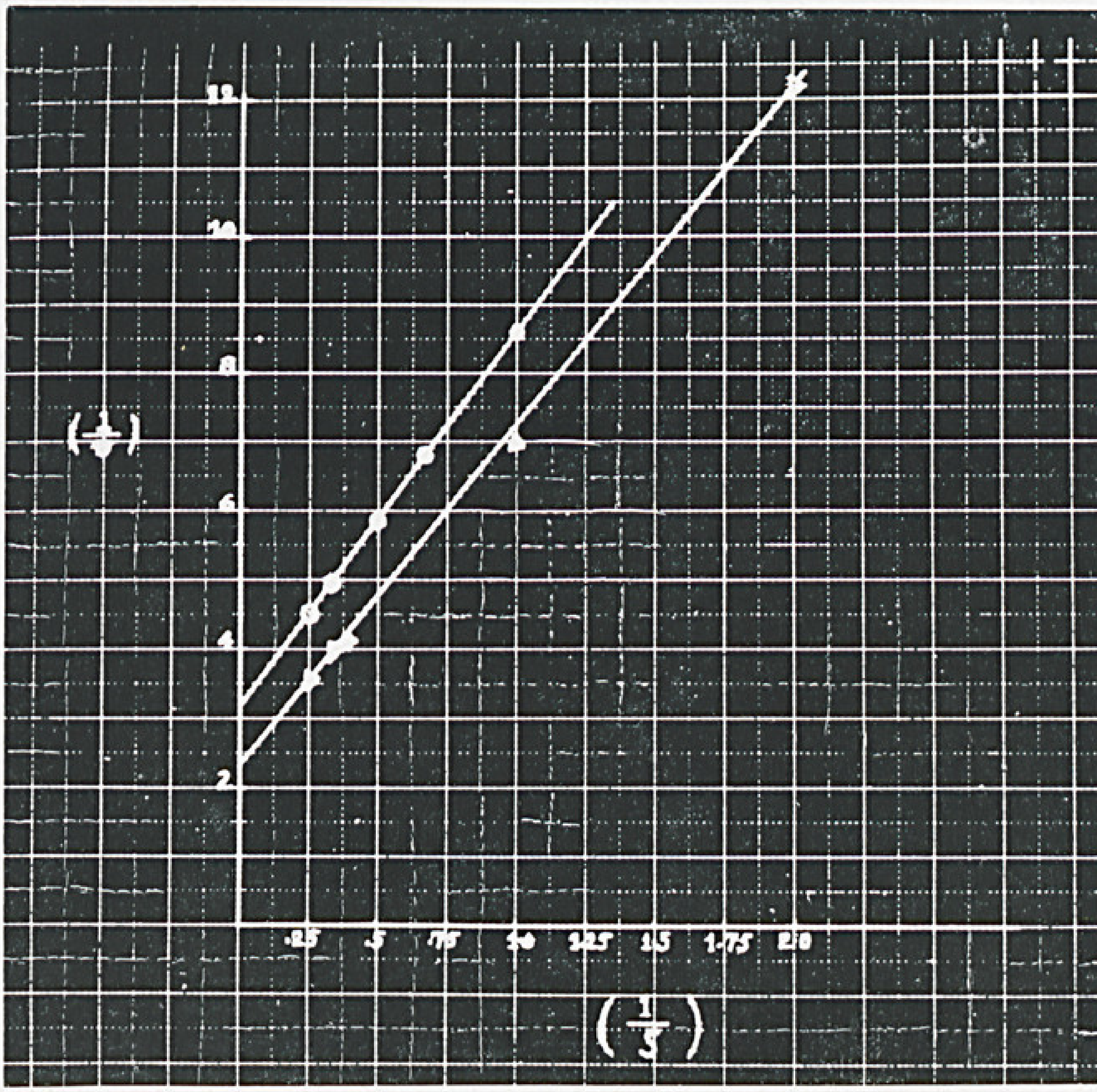

Fig. 2

the undialyzed venom, a three- to fourfold increase in activity was observed when the activity was measured in the presence of both EDTA and Mg^{++}. Zn^{++}, Mn^{++}, and Ni^{++} inhibited the enzyme activity strongly. Co^{++} at low concentrations stimulated the enzyme activity and at high concentrations inhibited its action (Table I).

Orthophosphate and pyrophosphate both inhibited 5'-nucleotidase activity. Cystein and glutathione enhanced the enzyme activity, but methionine and cystine inhibited its activity. NaF acted as an inhibitor but was less active than the orthophosphate and pyrophosphate (Table I).

Effects of pH on venom 5'-nucleotidase

From the pH-activity curve (now shown) it was concluded that venom 5'-nucleotidases have no well-defined pH optimum because the venom of *V. russelli* exhibited maximum activity over the pH range 7.6 to 8.4, and *C. adamanteus* venom was equally active over the pH range 8.0 to 10.0. At pH below 7.4

TABLE I — EFFECTS OF METALLIC IONS, ACTIVATORS AND INHIBITORS ON VENOM 5'-NUCLEOTIDASE ACTIVITY

Reagents		μmoles added	Relative Activity	Activation (%)	Inhibition (%)
Complete	system	—	100	—	—
"	$- Mg^{++}$	—	70	—	—
"	$+ Mn^{++}$	0.05		—	75
		0.10		—	94
"	$+ Ni^{++}$	1.00		—	70
		2.00		—	82
"	$+ Co^{++}$	0.10		20	
		1.00		—	12
"	$+ Zn^{++}$	5.00			22
		0.05		—	80
		0.10		—	95
"	+ cysteine	1.00		23	—
		5.00		48	—
"	+ glutathione	1.00		18	—
		5.00		46	—
		1.00		—	12
"	+ Pi (Na-phosphate)	2.00		—	37
		5.00		—	88
"	+ PP (Na-pyrophosphate)	1.00		—	52
		2.00		—	71
		5.00		—	96
"	+ NaF	1.00		—	5
		2.00		—	12
		5.00		—	66

The complete system contained 20 μmoles of glycine buffer, pH 8.5, 1 μmole of Mg^{++}, 5 μmoles of AMP (Na-salt) and 0.1 ml of a 0.02% venom solution. The reaction mixture was incubated for 20 mins. at 37°C. The amount of metallic ions, activators and inhibitors added are shown in column 2. The degree of activation and inhibition of 5'-nucleotidase is expressed as the percentage of the activity of the complete system, which has been arbitrarily taken as 100%.

and above 8.4, the 5'nucleotidase activity of *V. russelli* venom declined rapidly. A sharp drop in 5'-nucleotidase activity of *C. adamanteus* venom was also noted at pH below 8.0 and above 10.0.

Substrate specificity of venom 5'-nucleotidase

The ability of venom 5'-nucleotidase to hydrolyze other nucleotides is shown in Table II. The results indicated that venom 5'-nucleotidase can hydrolyze almost all nucleoside 5'-monophosphates and among them, 5'-AMP was hydrolyzed maximally (100%) and 5'-CMP by 76%. Next to it in order of effectiveness as

TABLE II — SUBSTRATE SPECIFICITY OF VENOM 5'-NUCLEOTIDASE

Substrates	Relative Activity
5'-AMP	100
5'-UMP	36
5'-CMP	76
5'-GMP	28
5'-IMP	22
5'-dAMP	35
5'-dTMP	58
5'-dCMP	54
5'-dGMP	26
2'-AMP	5
3'-AMP	8
cyclic-2', 3'-AMP	0
ribose-5'-phosphate	0
α — glycerophosphate	6
β — glycerophosphate	8

The reaction mixture contained 20 μmoles of glycine buffer, 8.5, 1 μmole of Mg^{++}, 5 μmoles of substrate (Na salt), and 0.1 ml of a 0.02% venom solution in a total volume of 0.5 ml, incubated for 20 mins. at 37°C.

substrates were 5'-UPM, 5'-GMP and 5'-IMP which were hydrolyzed by 36, 28 and 22%, respectively. Venom 5'-nucleotidase can also hydrolyze deoxynucleoside 5'-monophosphates viz 5'-dTMP could be hydrolyzed by 58%, 5'-dCMP, 5'-dAMP and 5'-dGMP by 54, 35 and 26%, respectively. Neither ribose-5-phosphate nor cyclic-2',3'-AMP could be hydrolyzed. Very limited hydrolysis was noted with α- and β-glycerophosphates.

Discussion

The twofold increase in the activity of cobra venom 5'-nucleotidase observed by Kaye (19) after dialysis, was not noted by us with the venom of *V. russelli*, but a three- to fourfold increase in the activity of the dialyzed venom was noted when measured in the presence of EDTA and Mg^{++}. Probably the venom used by us did not contain any free Zn ions or other poisonous metals that can be removed by dialysis, but contained traces of metals in bound form that can be removed by EDTA. This perhaps explains the fourfold increase in activity noted by Kaye (19) and us when the activity was measured in the presence of EDTA and Mg^{++} and not just in the presence of Mg^{++} alone. A 90% inhibition of enzyme activity was observed with orthophosphate and pyrophosphate whereas NaF inhibited only 65% of the activity. Activation by glutathione (reduced form) and cysteine amounted to 46 and 48% in each case (Table II).

Because venoms rich in 5'-nucleotidase activity did not hydrolyze α- and β-glycerophosphates at pH 5.0 and 9.0 and their activity was inhibited by Ni^{++}, which did not affect the hydrolysis of glycerophosphates by nonspecific phosphomonesterases, the hydrolysis of nucleoside 5'-monophosphates by 5'-nucleotidase cannot be related to the action of any nonspecific phosphomonesterase present in the venoms. It can not either be linked to venom adenosine triphosphatase since the hydrolysis of ATP by venoms was not altered by Co^{++}, which enhanced the hydrolysis of 5'-AMP by venoms at low concentrations and inhibited at higher concentrations. Venom 5'-nucleotidase was not identical to 5'-nucleotidases obtained from other sources since the hydrolysis of 5'-AMP by them was not affected in the same way by the same metallic ions and also because the pH at which maximum hydrolysis of the substrate attained, was also different. Although venom 5'-nucleotidase hydrolyzed all deoxynucleoside 5'-monophosphates such as 5'-dAMP, 5'-TMP, 5'-dCMP, and 5'-dGMP the affinity of the enzyme for 5'-AMP was much greater than for 5'-dAMP. Other deoxy compounds were also hydrolyzed but to lesser degrees than the corresponding ribose compounds. The hydrolysis of deoxy nucleotides by bacterial 5'-nucleotidase was first reported by Carter (20). It did not however hydrolyze 2'-AMP, 3'-AMP or cyclic-2',3'-AMP, nor catalyze the hydrolysis of ribose-5-phosphate. 5'-nucleotidases obtained from different sources appear to be different in many ways, particularly in regard to their behaviour towards metallic ions and inhibitors, and pH, their substrate specificity seems to be the same, and therefore should be considered as isoenzymes.

The existence of an enzyme capable of hydrolyzing a specific substrate (adenylic acid), and as widely distributed in nature as its own substrate possibly implies the involvement of this enzyme in regulating the concentrations of orthophosphate for glycogenolysis and adenylic acid (other monophosphates) for nucleic acid synthesis in the cell. It is difficult to understand the reason for the high 5'-nucleotidase activity in snake venoms since the amount of enzyme present is not sufficient to inflict any toxic effect. It may be possible that the enzyme 5'-nucleotidase, like other enzymes present in the venoms, also contribute to a limited extent towards the total toxicity of the venoms (21).

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