Biochemical Pharmacology, Vol. 25, pp. 551–555. Pergamon Press, 1976. Printed in Great Britain.

EFFECTS OF PURIFIED COMPONENTS OF JELLYFISH TOXIN (*STOMOLOPHUS MELEAGRIS*) ON ADENOSINE TRIPHOSPHATASE ACTIVITIES

PAUL M. TOOM and TIM D. PHILLIPS

Department of Chemistry, University of Southern Mississippi, Hattiesburg, Miss. 39401, U.S.A.

and

R. B. KOCH

Department of Biochemistry, Mississippi State University, Mississippi State, Miss. 39762, U.S.A.

(*Received* 11 *April* 1975; *accepted* 24 *June* 1975)

Abstract—Toxin from the jellyfish *Stomolophus meleagris* was found to stimulate both Na^+-K^+ and mitochondrial Mg^{2+} ATPase activities at low toxin concentrations and to inhibit these ATPase activities at higher (> 0·2 mg/ml) concentrations. Using discontinuous membrane partition chromatography, the toxin was fractionated into six fractions. Of these six fractions, one strongly activated while five inhibited mitochondrial Mg^{2+} ATPase. One fraction strongly activated, a second mildly activated, while the other four inhibited Na^+-K^+ ATPase about 50 per cent. Only two fractions affected oligomycin-insensitive ATPase, and both of these exhibited a mild stimulation. It is concluded that the ability of this toxin to alter membrane permeability to Na^+ is due, at least in part, to components in the toxin which act directly on ATPase.

Toxin from the jellyfish *Stomolophus meleagris* has been found to be composed of numerous proteins [1], many of which exhibit enzymatic activity toward a variety of substrates [2]. The major effects of the toxin on experimental animals appear to be dermonecrotic [3], hemolytic [4] and cardiovascular [4]. Abnormal electrocardiographic patterns from animals injected with this toxin suggest that the toxin is interfering with normal cardiac conduction [4].

When the effect of toxin on the sodium transport across isolated frog skin was studied, low toxin concentrations were found to increase sodium transport, while higher concentrations inhibited the flux of sodium across the skin. Using preparative isoelectric focusing and discontinuous membrane partition chromatography, two fractions were found which increased sodium transport, while a third component was found to inhibit the transport of sodium across isolated skin [3].

Other investigators [5–9] have also observed that various Cnidarian toxins disrupt the normal sodium permeability of membranes. This disruption of sodium flux is so dramatic that it has been suggested that many of the physiological effects produced by these toxins are facilitated by those components in the toxin affecting sodium transport mechanisms.

Results of a previous investigation using isolated frog skin led to the conclusion that toxin from the jellyfish *Stomolophus meleagris* contains components which inhibit active sodium transport by inhibiting Na^+-K^+ ATPase. The purpose of the present investigation was to study the effects (both inhibitory and stimulatory) of both *Stomolophus meleagris* toxin and partially purified components of this toxin on ATPase activities from a brain homogenate fraction.

MATERIALS AND METHODS

Rat brain tissue ATPase homogenates were prepared by dissection, homogenization and fractionation procedures as described by Koch [10]. The fractional pellet obtained by centrifuging at 13,000 *g* for 20 min was resuspended in 0·32 M sucrose, 1 mM EDTA and 10 mM imidazole. The resulting fraction (B) contained a mixture of mitochondrial (Mito.) and nerve-ending particles. These preparations were then appropriately diluted, quick frozen in liquid nitrogen, and stored at −20° until ATPases analysis.

ATPase activity was determined using the coupled enzymatic procedure of Pullman *et al.* [11] and Fritz and Hamrick [12] as reported by Koch [[13]. Total Mg^{2+} and Na^+-K^+ ATPase activities were determined by the use of oaubain (a cardiotonic glycoside which specifically inhibits Na^+-K^+ ATPase) in the reaction mixtures. This activity was further separated into oligomycin-sensitive (Mito. Mg^{2+}) and -insensitive parameters by addition of 0·03 μg oligomycin (oligomycin containing 15% A and 85% B was obtained from Sigma, St. Louis, Mo.).

Varying dosage levels of toxin preparations dissolved in glass-distilled deionized water were added to reaction mixtures by release from Hamilton microsyringes under the liquid surface of the vortices of rapidly stirred reaction mixtures as described by Koch [13]. The 3-ml reaction mixtures used in the analyses contained: 4·3 mM ATP, 5 mM Mg^{2+}, 100 mM Na^+, 20 mM K^+, 135 mM imidazole buffer (pH 7·5), 0·2 mM NADH, 0·5 mM PEP (phospho-enol-pyruvate), 0·02% bovine serum albumin, 9 units of pyruvate kinase, 12 units of lactic dehydrogenase and 25 μl of brain homogenate fraction B. Reaction temperature was maintained at 37° and absorbance

changes were measured at 340 nm over a period of 10 min using a Cary model 17 spectrophotometer with lambda temperature bath. A typical ATPase assay is presented in Fig. 1C.

Protein assays on brain preparations were carried out by the modified Lowry procedure described by Hartree [14]. Fractionation of the toxin was carried out by means of discontinuous membrane filtration techniques described previously [3]. Partially purified components were separated into molecular weight ranges of: less than 1000 (fraction A); 1000–10,000 (fraction B); 10,000–50,000 (fraction C); 50,000–100,000 (fraction D); 100,000–300,000 (fraction E); and greater than 300,000 (fraction F).

RESULTS

As can be seen in Fig. 1, the toxin exhibited no ATPase activity itself (Fig. 1A) nor did the toxin interfere with the assay procedure by inhibiting any of coupled reactions (Fig. 1B). However, whole toxin from *Stomolophus meleagris* caused biphasic, dosage responses on Na^+-K^+ ATPase and on Mito. Mg^{2+} and oligomycin-insensitive Mg^{2+} ATPases (Fig. 2). As can be seen from Fig. 2, both Na^+-K^+ ATPase and Mito. Mg^{2+} ATPase were markedly stimulated by low concentrations of toxin, but were inhibited at high toxin concentrations. The opposite effect was observed on oligomycin-insensitive ATPase, where low toxin concentrations inhibited and high toxin concentrations slightly stimulated activity.

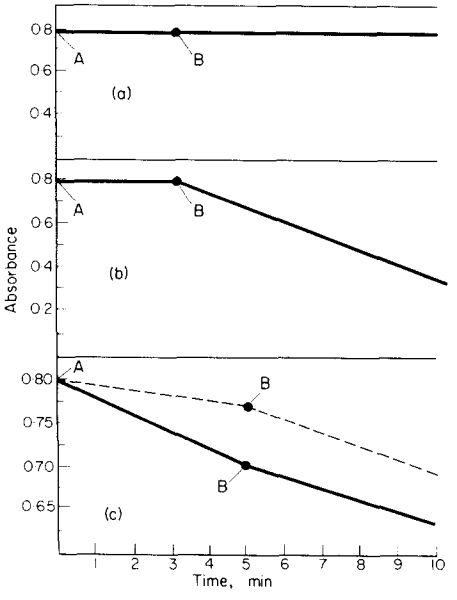

Fig. 1. (1A) ATP hydrolysis in absence of rat brain homogenate. (A) Addition of reaction mixture containing all substrates and enzymes except rat brain homogenate; (B) addition of 1·0 mg toxin. (1B) Effect of toxin on enzymes of the coupled ATPase assay system. (A) Addition of all enzymes and substrates described in Materials and Methods except rat brain homogenate; (B) addition of 5 μg ADP; (C) addition of 0·1 mg toxin. (1C) Total ATPase assay ( ). (A) Addition of all enzymes and substrates described in Materials and Methods; (B) addition of 0·5 mg unfractionated toxin. Oaubain-insensitive ATPase ( ). (A) Addition of all enzymes and substrates described in Materials and Methods plus 0·735 mm oaubain; (B) addition of 0·5 mg unfractionated toxin.

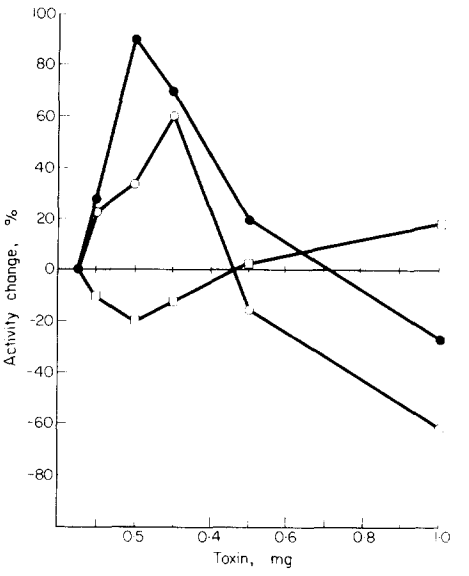

Fig. 2. Effect of whole toxin on ATPase activities. ● ●, Na^+-K^+ ATPase; ○ ○, Mito. Mg^{2+} ATPase; □ □, oligomycin-insensitive ATPase.

Effects of various fractions from the toxin on ATPase activities. As can be seen from Table 1, the majority of the toxin possessed a molecular weight of less than 100,000. Of the six fractions separated, 60 per cent of the total toxin mass was found in two fractions, A and C.

Fraction A (which represents the low molecular weight components of the toxin) caused the most unusual responses on the ATPase activities. This fraction stimulated Na^+-K^+ ATPase approximately 30 per cent, while inhibiting Mito. Mg^{2+} ATPase approximately 40 per cent. No change in activity toward oligomycin-insensitive Mg^{2+} ATPase was detected with this fraction (Fig. 3).

Fraction B (mol. wt range, 1000–10,000) strongly activated both Na^+-K^+ and Mito. Mg^{2+} ATPases (Fig. 4). As was the case with fraction A, no change in oligomycin-insensitive ATPase was observed following the addition of toxin.

In contrast to fractions A and B, fraction C (mol. wt range, 10,000–50,000) strongly inhibited both Na^+-K^+ ATPase and Mito. Mg^{2+} ATPase (Fig. 5). This fraction also exhibited a slight stimulation of oligomycin-insensitive ATPase.

Fraction D (mol. wt range, 50,000–100,000) exhibited properties nearly identical to those of fraction

Table 1. Fractionation summary of *S. Meleagris* toxin

Fraction	Molecular wt range	% of Total
A	< 1000	30
B	1,000–10,000	2
C	10,000–50,000	30
D	50,000–100,000	18
E	100,000–300,000	11
F	> 300,0000	9

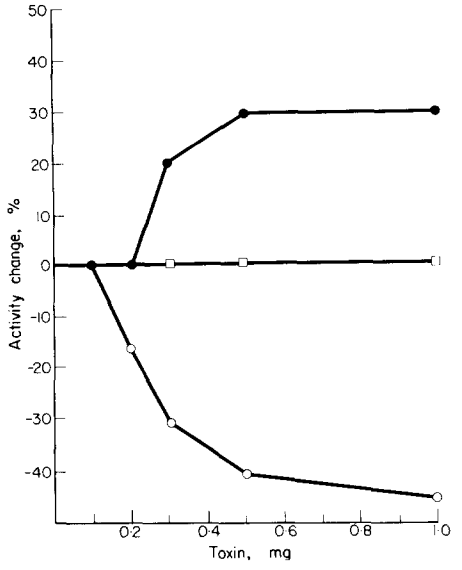

Fig. 3. Effect of fraction A (mol. wt. < 1000) on ATPase activities. ●—●, Na^+-K^+ ATPase; ○—○, Mito. Mg^{2+} ATPase; □—□, oligomycin-insensitive ATPase.

C. Both Na^+-K^+ ATPase and Mito. Mg^{2+} ATPase were strongly inhibited by this fraction, while oligomycin-insensitive ATPase was slightly stimulated (Fig. 6).

Fraction E (mol. wt range, 100,000–300,000) was found to affect only Na^+-K^+ ATPase. This fraction exhibited a nearly linear rate of inhibition with increasing toxin concentrations (Fig. 7).

Fraction F (mol. wt, over 300,000) resembled fractions C and D in its actions on the three ATPases, that is, inhibition of both Na^+-K^+ and Mito. Mg^{2+} ATPases and slight activation of oligomycin-insensitive ATPase (Fig. 8).

When one compares the effects of the six fractions on Na^+-K^+ ATPase (Figs. 3–8), it can be seen that fraction B has the strongest activating effect, while

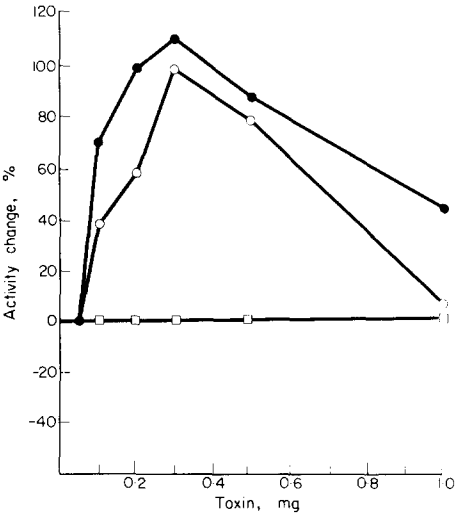

Fig. 4. Effect of fraction B (mol. wt. 1000–10,000) on ATPase activities. ●—●, Na^+-K^+ ATPase; ○—○, Mito. Mg^{2+} ATPase; □—□, oligomycin-insensitive ATPase.

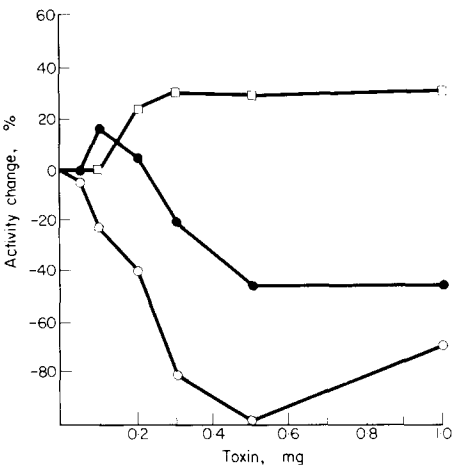

Fig. 5. Effect of fraction C (mol. wt. 10,000–50,000) on ATPase activities. ●—●, Na^+-K^+ ATPase; ○—○, Mito. Mg^{2+} ATPase; □—□, oligomycin-insensitive ATPase.

fraction A activates this ATPase slightly. Each of the other four fractions inhibits Na^+-K^+ ATPase about 50 per cent at the higher concentrations of toxin tested.

Comparison of the effects of the fractions on mitochondrial ATPase (Figs. 3–8) reveals that only fraction B activates this ATPase, while all other fractions strongly inhibit mitochondrial ATPase, except fraction F, which has no effect.

Only two fractions exhibit an effect on the oligomycin-insensitive ATPase (Figs. 3–8). Both fractions C and D slightly activate this ATPase activity.

DISCUSSION

It has been reported that toxin from the jellyfish *Chironex fleckeri* possesses ATPase activity [15]. Since ATPase activity could not be detected either in whole toxin or in any of the fractions in the present investigation, these two jellyfish toxins obviously differ in

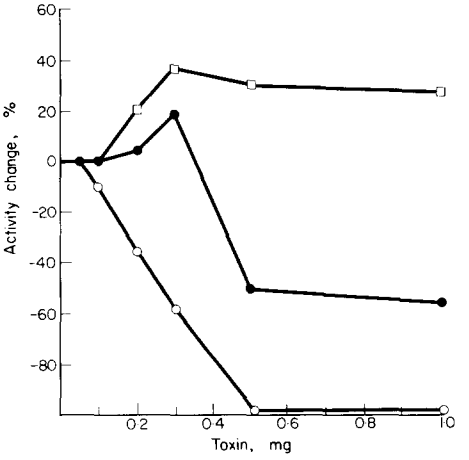

Fig. 6. Effect of fraction D (mol. wt. 50,000–100,000) on ATPase activities. ●—●, Na^+-K^+ ATPase; ○—○, Mito. Mg^{2+} ATPase; □—□, oligomycin-insensitive ATPase.

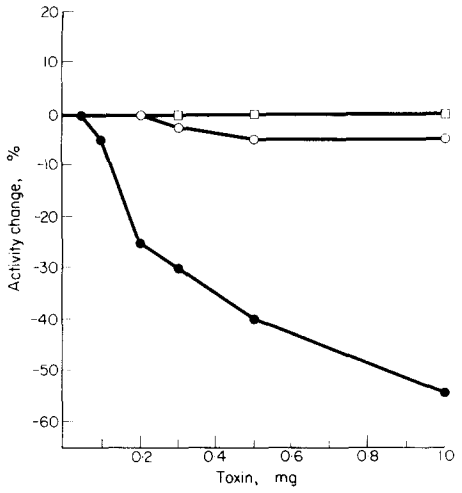

Fig. 7. Effect of fraction E (mol. wt. 100,000–300,000) on ATPase activities. ●—●, Na^+-K^+ ATPase; ∅—∅, Mito. Mg^{2+} ATPase; □—□, oligomycin-insensitive ATPase.

this respect. However, our finding of activators of Na^+ K^+, Mito. Mg^{2+} and oligomycin-insensitive ATPases in the toxin of *Stomolophus meleagris* suggests that many of the pharmacological symptoms resulting from stings of these two jellyfish would be similar, since both venoms result in increased hydrolysis of ATP at low toxin concentrations.

The activation of both Na^+-K^+ and Mito. Mg^{2+} ATPases at low toxin concentrations and the inhibition of these two ATPases at high toxin concentrations are noteworthy when one compares results of this study to previously published results. Similar observations were made by Rayner and Szekerczes [16] when they studied the effects of partially purified Ciguatoxin on these ATPase activities. When one compares the results obtained in this study to the results reported previously on the application of toxin to short-circuited frog skin [3], essentially identical results are observed, namely, an initial increase in activity (Na transport or ATPase) followed by an inhibition of activity at higher toxin concentrations. This same trend was also observed when the effect of toxin on spontaneous nerve impulses was studied, where a progressive increase in propagated action potentials was first observed, followed by a rapid, irreversible block of spontaneous activity.* The similar biphasic results obtained in our present study suggest that the underlying cause for these pharmacological findings is at least partially attributable to actions of the toxin on ATPase activity.

When one compares the effects of the partially purified fractions on the three ATPases studied in this investigation, it is readily apparent that the effects of whole toxin on ATPase activity are actually the combined effects of numerous components within the venom acting in different ways on different ATPases. All fractions except fraction A (mol. wt below 1000) caused similar effects toward both Na^+-K^+ and Mito. Mg^{2+} ATPases, suggesting that the components in the venom affect these two ATPases similarly, but to a different extent. The activation of Na^+-K^+ ATPase and the inhibition of Mito. Mg^{2+} ATPase by fraction A suggest that possibly two different components having molecular weights of less than 1000 are present in this fraction. Additional purification of this fraction is essential before more meaningful conclusions can be drawn.

Fraction E can be shown to inhibit Na^+-K^+ ATPase with essentially no effects on the two Mito. Mg^{2+} ATPases. This same fraction (mol. wt range, 100,000–300,000) has also been shown to suppress Na^+ transport across frog skin [3] to a greater extent than any of the other fractions, an effect which is parallel to the action of the fraction on Na^+-K^+ ATPase. Clearly, the results of this investigation confirm the earlier observation of a cardiac glycoside-like activity in this fraction [3].

The strongest activation of both Na^+-K^+ ATPase and Mito. Mg^{2+} ATPase was found in fraction B (mol. wt 1000–10,000). This same fraction has previously been shown to stimulate greatly Na^+ transport across frog skin [3] and to produce increased amplitude and frequency of spontaneous nerve impulse firings.* The results of the present investigation would suggest that such pharmacological effects are also brought about through a direct action on ATPase, rather than by permeability changes of the membrane.

When one compares the effects of the different fractions on Na^+-K^+ ATPase, it can be seen that the two low molecular weight fractions activate this ATPase at all toxin concentrations, while the higher molecular weight fractions all inhibit it. Thus, even assuming that the fractionation procedure employed in this investigation allowed large amounts of contamination from one fraction to another, it can still be concluded that this toxin has at least one component which activated Na^+-K^+ ATPase and at least one component which inhibited that ATPase.

It should also be pointed out that the inhibition of Na^+-K^+ ATPase observed with 1·0 mg of the crude toxin is at the predictive value if one calculates from the purified fractions (knowing their effect on the enzyme activity and the proportion they represent

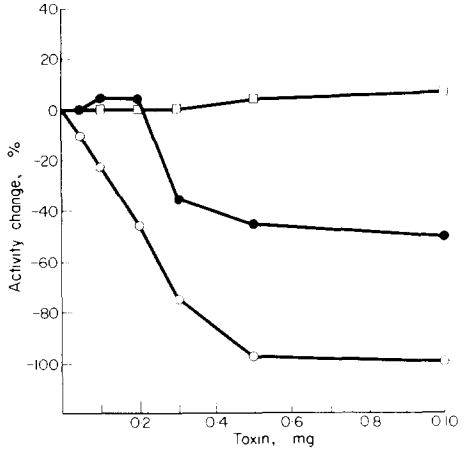

Fig. 8. Effect of fraction F (mol. wt. >300,000) on ATPase activities. ●—●, Na^+-K^+ ATPase; ○—○, Mito. Mg^{2+} ATPase; □—□, oligomycin-insensitive ATPase.

* P. M. Toom and T. D. Phillips, manuscript in preparation.

of the total). However, such prediction does not hold for the stimulatory effect observed at low toxin concentration, since similar calculations predict that no stimulation should occur between 0·25 and 0·35 mg of the crude toxin. Since a marked stimulation was observed it could well be that the binding affinity of the stimulator to the membrane-bound enzyme is lower in the fractionated form than in the crude venom.

On the other hand, the studies of the different fractions on oligomycin-insensitive ATPase show that only two fractions affect this enzyme activity, and that both of these fractions exhibit only a slight stimulation. Thus, it would appear that the contribution of these components on this enzyme to the overall symptoms which accompany stings from these jellyfish would be minimal.

The effects of the fractions on Mito. Mg^{2+} ATPase were the most pronounced and exhibited both stimulation and inhibition responses. One fraction strongly activated and four fractions strongly inhibited this enzyme activity. Similar differential stimulation–inhibition responses of the three types of ATPase activities have also been observed for single components in studies with some chlorinated hydrocarbon and pesticide compounds on the ATPase system [17–19]. A polychlorinated biphenyl (PCB 1221) was shown to stimulate Mito. Mg^{2+} ATPase at low concentrations and inhibit at higher concentrations [17]. Plictran (a miticide) had the opposite effect on oligomycin-insensitive Mg^{2+} ATPase, i.e. inhibition at low concentrations followed by stimulation at higher concentrations [18]. Dieldrin was the only chlorinated hydrocarbon insecticide which was observed to cause a stimulation of Na^+-K^+ ATPase activity [19]. This stimulation was remarkable in that it showed increasing stimulation with increase in reaction time.

It has been shown that toxins from the coelenterates *Physalia physalis* and *Chrysaora quinquecirrha* affect mitochondrial function by producing a loss of ground substance, swelling of cristae and relaxation of membranes, as well as a decreased respiratory control index [20]. The strong inhibition of mitochondrial ATPase by our fractions supports these findings. Since the apparent major action of ATPase is the formation of ATP in oxidative phosphorylation, the long-term effects of the toxin observed in previous studies [3, 4] might be attributed to the inhibition of ATP production by Mito. Mg^{2+}.

There is an ever growing body of evidence that Cnidarian toxins possess the ability to alter membrane permeability to Na^+. The results of this investigation strongly support this view and further suggest that this alteration in Na^+ permeability is due to components in the venom acting directly on ATPases.

Acknowledgements—This work was supported by United States Public Health Service Grant 1 RO1 ES GM 00858-01 and by the University of Southern Mississippi Faculty Research Council.

REFERENCES

1. P. M. Toom and D. S. Chan, *Toxicon* **10**, 605 (1972).
2. P. M. Toom and D. S. Chan, *Comp. Biochem. Physiol.* **43B**, 435 (1972).
3. P. M. Toom and T. D. Phillips, *Toxicon*, **13**, 261 (1975).
4. P. M. Toom, J. B. Larsen, D. S. Chan, D. A. Pepper and W. Price, *Toxicon*, **13**, 159 (1975).
5. J. B. Larsen and C. E. Lane, *Toxicon* **4**, 199 (1966).
6. G. W. Gould and J. W. Burnett, *J. invest. Derm.* **57**, 266 (1971).
7. J. W. Burnett and R. Goldner, *Toxicon* **8**, 179 (1970).
8. J. J. Watrous and R. Blanquet, *Physiol. Chem. Physics* **6**, 41 (1974).
9. J. J. Watrous, *Toxicon* **12**, 657 (1974).
10. R. B. Koch, *J. Neurochem.* **16**, 145 (1969).
11. M. E. Pullman, H. S. Penefsky, A. Datta and E. Racker, *J. biol. Chem.* 235 (1960).
12. P. J. Fritz and M. E. Hamrick, *Enzymologia* **30**, 57 (1966).
13. R. B. Koch, *Chem. Biol. Interact.* **4**, 195 (1971–72).
14. E. F. Hartree, *Analyt. Biochem.* **48**, 422 (1972).
15. R. Endean and L. Henderson, *Toxicon* **7**, 303 (1969).
16. M. D. Rayner and J. Szekerczes, *Toxic. appl. Pharmac.* **24**, 489 (1973).
17. D. Desaiah, L. K. Cutkomp, H. H. Yap and R. B. Koch, *Biochem. Pharmac.* **21**, 857 (1972).
18. D. Desaiah, L. K. Cutkomp and R. B. Koch, *Life Sci.* **13**, 1693 (1973).
19. D. Desaiah and R. B. Koch, *Biochem. biophys. Res. Commun.* **64**, 13 (1975).
20. G. J. Calton, J. W. Burnett, G. Joel and S. R. Max, *Proc. Soc. exp. Biol. Med.* **143**, 971 (1973).