INVERTEBRATE TROPOMYOSIN

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Certain muscles of lamellibranchs became of especial interest biochemically when they were shown to contain a component or components having electron-optical and X-ray patterns quite distinct from those of mammalian skeletal muscle (HALL, JAKUS AND SCHMITT[1]; BEAR[2]; SELBY AND BEAR[3]). The fibrils giving rise to these patterns were especially abundant in the slow, smooth part of bivalve adductor muscles, and were named type I fibrils or paramyosin. In order to characterise paramyosin chemically, the author undertook a general investigation of the proteins of adductor muscle during 1955 at the Stazione Zoologica, Naples, using chiefly material from the oyster and from *Pinna nobilis*, both members of the Pseudolamellibranchs.

The adductor of *Pinna* is entirely smooth, but nevertheless consists of a fast part (ABBOTT AND LOWY[4]) which is gray in colour, and a white, slow part. Oyster adductor possesses both slow and fast parts which are more clearly differentiated, the one smooth and white, the other striated, yellow and rather vitreous in appearance. It was shown (BAILEY[5]) that by extracting the smooth muscles with *M* potassium chloride, extremely viscous extracts could be obtained which responded only feebly to tests characteristic of myosin or of actomyosin, and that almost all the protein present (if the muscle residue were initially washed with water) could be crystallised in the form of birefringent needles by dialysing to an ionic strength of 0.2. The same needles could be obtained from striated muscles by varying the procedure slightly, using muscle residue first dried in ethanol-ether and then extracted with salt, as described in step 1 of the method originally developed for rabbit tropomyosin (BAILEY[6]).

The identity of the protein by reference to the known components of muscle was most puzzling. On the one hand, it possessed some of the properties of rabbit tropomyosin, surviving the action of organic solvents, whether *in situ* or after isolation; but unlike tropomyosin, it was quite insoluble in the absence of salt, and was precipitated at much lower concentrations of ammonium sulphate. On the other hand, in addition to its pronounced globulin properties, it resembled myosin in its extreme asymmetry as judged by its high intrinsic viscosity and pronounced double refraction of flow; moreover, it gave a strong nitroprusside reaction. Common to both proteins, however, and also to paramyosin, was the strong α-keratin diffraction pattern, obtained when films of the crystals were dried down and oriented (unpublished observations of Dr. E. BEIGHTON). In spite of these uncertainties, it seemed probable that the protein, whatever its nature, was responsible for the characteristic long spacings of paramyosin: first, because the wide-angle pattern carries one of the two strong meridian spacings observed by BEAR — that at 70 A; second, in the smooth part of oyster adductor and in *Pinna* it comprises 25–30% of the total protein of

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the muscle, an amount large enough to account for the complete dominance of any one pattern.

Subsequent investigations, reported in a preliminary communication (BAILEY[7]), and described more fully here, have shown that the crystalline protein possesses the amino acid pattern of tropomyosin (TM), with minor variations that may reasonably be expected in phyla so distantly removed as the mammals and bivalves. In addition to the water-insoluble TM here described and isolated both from pseudolamellibranch and cephalopod muscle, there exists also a water-soluble variety present in low concentration, which crystallises in somewhat thick plates or as dense sheafs of needles (RÜEGG, unpublished).

Comparable proteins appear to have been recently isolated independently by Drs. A. G. SZENT-GYÖRGYI AND K. LAKI from *Venus mercenaria*, a eulamellibranch (private communication from Dr. K. LAKI; see also a preliminary communication, KOMINZ, SAAD AND LAKI[8]).

MATERIALS

Tropomyosin from Pinna nobilis. This was prepared from the whole adductor by the "ethanol" method (BAILEY[5]). The protein was extracted by *M* KCl from an ethanol-dried residue originally prepared in Naples. After filtration through paper-pulp, the solution was dialysed against 0.4 *M* KCl, when a sheen of crystals began to appear. Further dialysis to a final salt concentration of 0.2 *M* gave a heavy precipitate of crystals which was centrifuged down. After addition of 0.5 *M* KCl, the pH was adjusted from 6.2 to 6.9 to assist solution, and the dialysis procedure was then repeated. After two recrystallisations, the material was entirely homogeneous electrophoretically (0.5 % protein dissolved in 0.4 *M* KCl-phosphate $I = 0.54$, pH 6.9). It contained < 0.05 % tryptophan when assayed by the sensitive method of SPIES AND CHAMBERS[9], using edestin as standard. For analysis, the crystals were washed with acetate buffer pH 5.0, then with water, and dried in ethanol and ether.

Tropomyosin from cephalopod muscle, Octopus vulgaris. Since the consistency of the muscle renders mincing difficult, the arm muscle of freshly-killed *Octopus* was frozen at —20° and minced in the frozen state. After washing with several changes of water at 0°, the residue was blended with an equal volume of water and spun down. Crystals of tropomyosin could be prepared from this residue by the "ethanol" method as described above, or direct from the wet muscle. In the latter case, an equal volume of 2 *M* KCl added to the mince gave, after standing overnight at 0°, a very turbid extract which was dialysed to $I = 0.4$ at pH 7. At this stage, the turbidity could be removed by filtration through paper-pulp, and the filtrate now deposited, at $I = 0.2$, a sheen of crystals thickening to a suspension at $I = 0.1$.

The cephalopod material seems to be slightly more soluble than that of *Pinna*, but in both cases, water-soluble material remains in the mother-liquor after the first recrystallisation. The water-soluble protein of *Octopus* could be precipitated by half-saturation with ammonium sulphate, and when dialysed under the conditions recommended for rabbit TM (BAILEY[6]), gave amorphous material, some plates and small rosettes of needles. This protein is probably similar to that isolated from squid by YOSHIMURA[10].

For analysis, a 3-times crystallised specimen of the water-insoluble variety was used, prepared by the "ethanol" method. This too was electrophoretically pure and free from tryptophan.

METHODS

General

Total N and moisture determinations were carried out according to the directions of CHIBNALL, REES AND WILLIAMS[11]. For amino acid analysis, the protein was boiled for 22 h in a 1 : 1 mixture of formic acid–HCl, taken to dryness, and subjected to the gradient-elution chromatographic procedure of MOORE AND STEIN[12] on a column of Dowex-50-X4.

The yields of serine and threonine were corrected for destructive losses using the factors determined by REES[13].

The determination of cystine + cysteine as cysteic acid was carried out chromatographically after treatment of the protein with performic acid and subsequent hydrolysis (SCHRAMM, MOORE AND BIGWOOD[14]). Sulphydryl groups were estimated on freshly-prepared protein dissolved in guanidine-HCl by the method of TSAO AND BAILEY[15], and carbohydrate by the method of TILLMANS AND PHILIPPI[16].

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N-terminal groups: a new method for detection of N-terminal arginine

Protein material was treated with fluorodinitrobenzene (FDNB) under the conditions prescribed by SANGER[17]. After hydrolysis under various conditions (see later) the ether-soluble DNP-acids were separated from artifacts on unbuffered silica-chloroform columns, and after estimation (BAILEY[18]) were run on paper in the phthalate–*tert*-amyl alcohol system of BLACKBURN AND LOWTHER[19].

A new, simple method has been developed for the detection of N-terminal arginine, which after hydrolysis of DNP protein remains in the aqueous phase together with large amounts of ε-DNP lysine. The principle was suggested by current investigations of Drs. F. P. SPAHR AND A. C. CHIBNALL on the use of methoxycarbonyl chloride in the identification of N-terminal acids as the *N*-methoxycarbonyl (MC) derivative. ε-DNP lysine is converted to ε-DNP-α-MC lysine, which becomes ether-soluble, leaving DNP-arginine in solution. (The method can obviously be developed for the estimation of lysine groups reacting with FDNB.)

After extraction of the ether-soluble DNP-amino acids, the aqueous phase (equivalent to 0.2 g protein) is taken to dryness, dissolved in 0.1 *N* NCl and passed down a column of talc (see BAILEY AND BETTELHEIM[20]). This effects a partial separation of free amino acids from the DNP-material which is retarded. The latter is eluted with ethanol–HCl (4 vol. ethanol:1 vol. *N* HCl) and taken to dryness. It is dissolved in 2–3 ml of a bicarbonate–carbonate mixture pH 8.9, prepared by mixing 20 ml $NaHCO_3$ with 5 ml Na_2CO_3 (each 10%). Four portions, each of 0.02 ml of MC chloride are added at intervals of 10 min at 20°, shaking vigorously after each addition. The mixture is then acidified with HCl, extracted four times with ether to remove ε-DNP-α-MC lysine, and the aqueous phase taken to dryness.

The method has been tested with DNP-fibrin (bovine) and DNP-tropomyosin (rabbit). In the final aqueous phase there is present from these proteins a brown impurity which can readily be separated from DNP-arginine (if present) by passing down a short column of acid silica with methyl ethyl ketone as ambient phase (SANGER[17]). The brown material runs fast and separates from the slower DNP-arginine, which after elution is estimated spectrophotometrically at 390 mμ. It can finally be characterised by paper chromatography against authentic DNP-arginine, and doubly checked by applying the Sakaguchi reaction.

As a control experiment, DNP-arginine (equiv. to 4.6 μg α-amino N) was swamped with the water-soluble DNP acids from DNP-fibrin (equiv. to 147 μg α-amino N as lysine). Such a proportion of arginine to lysine might be obtained for a *Pinna* tropomyosin possessing 1 N-terminal arginine/50,000 g protein. No trace of DNP-arginine could be detected in the fibrin hydrolysate by itself, but the added arginine was quantitatively recovered.

RESULTS

According to the source and method, tropomyosin may contain considerable amounts of nucleic acid (HAMOIR[21,22]; SHENG AND TSAO[23]), though there is little positive evidence that the two are associated *in situ* (PERRY[24]; SHENG AND TSAO[23]). Our present samples appeared to be virtually free from nucleic acid, whether judged by the carbohydrate content (< 0.2% as mannose), or the UV absorption of soluble material after hydrolysing the protein in boiling *N* HCl for 2 hours.

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TABLE I

AMINO ACID COMPOSITION OF THE GLOBULIN TROPOMYOSINS FROM *Pinna* ADDUCTOR AND *Octopus* ARM MUSCLE

	Pinna			*Octopus*		
	N as % total N	*g amino acid/100 g protein*	*Residues/10^5 g protein*	*N as % total N*	*g amino acid/100 g protein*	*Residues/10^5 g protein*
Pro	nil	nil	nil	nil	nil	nil
Try	nil	nil	nil	nil	nil	nil
Cys/2	0.19	0.30	2.5	—	—	—
Asp	8.37	14.72	111	7.01	12.06	91
Thre	1.68	2.64	22	2.86	4.40	37
Ser	3.70	5.13	49	4.01	5.44	52
Glu	13.05	25.37	173	12.4	23.5	160
Gly	0.93	0.92	12	1.26	1.22	16
Ala	7.69	9.05	102	6.08	7.0	78.5
Val	2.70	4.16	35.5	2.50	3.79	32
Met	1.17	2.31	15.5	1.03	1.98	13
Ileu	2.14	3.70	28	2.64	4.47	34
Leu	7.84	13.55	103	8.02	13.60	104
Tyr	1.07	2.56	14	0.63	1.47	8
Phe	0.63	1.37	8.5	0.50	1.09	6.5
Amide N	8.80	1.63	116	7.14	1.29	92
Lys	9.78	9.45	64.5	10.2	9.61	66
His	1.01	0.69	4.5	2.55	1.70	11
Arg	24.04	13.80	79	21.7	12.2	70
Sum	94.8	109.7*	824*	90.53	103.53*	779*

* Does not include amide N.

Note: the second place in col. 1 and 2 is retained for the sequence of calculation. The figures are rounded off in the last column.

In Table I is presented the amino acid composition of the water-insoluble TM of *Pinna* and of *Octopus*. In Table II, the figures are compared with those for squid by YOSHIMURA[10] and for rabbit (KOMINZ, HOUGH, SYMONDS AND LAKI[25]), these too being derived by the Moore-Stein chromatographic method. From its method of preparation, which rigorously follows that of BAILEY[6], the squid TM appears to be the water-soluble variety, obtained by salting-out between 40 and 70% saturation with ammonium sulphate, whereas the water-insoluble type is already precipitated at 30% saturation. The values which YOSHIMURA[10] reports for squid are based on an estimated N content of 16.7%, though the N content calculated from yields of amino acids is 18.1%. The original figures have therefore been recalculated using this latter figure.

The amino acid pattern of rabbit TM is characterised by the absence of tryptophan and proline, by the presence of quite small amounts of glycine, phenylalanine, histidine and cystine, and the very large amount of dicarboxylic acid (particularly glutamic) and of total base (particularly lysine). If rabbit TM be compared with *Pinna* TM, it will be seen at once that the amino acid pattern with respect to the monocarboxylic acids is extraordinarily similar and at once demonstrates a relationship between the two proteins. The major divergence is found in the dicarboxylic acids and the bases, in which aspartic has tended to replace glutamic, and arginine the lysine. Curiously enough, there is on the whole a greater divergence between the

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TABLE II

COMPARATIVE ANALYSES OF SOME TROPOMYOSINS

	g/100 g protein				*Residues/10^5 g*			
	Octopus	*Squid**	*Pinna*	*Rabbit***	*Octopus*	*Squid**	*Pinna*	*Rabbit***
Cys/2	—	0.7	0.30	0.78	—	6	2.5	6.5
Thre	4.40	3.91	2.64	3.33	37	33	22	28
Ser	5.44	5.81	5.13	4.20	52	55	49	40
Gly	1.22	2.13	0.92	0.94	16	28	12	12.5
Ala	7.0	8.47	9.05	9.78	78.5	95	102	110
Val	3.79	2.90	4.16	4.45	32	25	35.5	38
Met	1.98	2.58	2.31	2.38	13	17	15.5	16
Ileu	4.47	2.58	3.70	3.80	34	20	28	29
Leu	13.6	12.4	13.55	12.44	104	95	103	95
Tyr	1.47	1.45	2.56	2.72	8	8	14	15
Phe	1.09	1.28	1.37	0.58	6.5	8	8.5	3.5
Asp	12.06	13.2	14.7	11.84	91	99.5	111	89
Glu	23.5	31.2	25.4	31.0	160	212	173	211
Lys	9.6	12.5	9.45	16.06	66	85	64.5	110
His	1.70	1.93	0.69	0.85	11	12	4.5	5.5
Arg	12.2	12.0	13.8	7.3	70	69	79	42
Amide N	1.29	1.32	1.63	0.9	92	94.5	116	64
Average residue wt.					115	115	115	114
As % of total residues:								
Free acid groups					18.4	25.0	19.3	26.9
Base groups					16.9	19.1	17.0	18.0
Excess acid groups					1.5	5.9	2.3	8.9
Amide					10.6	10.9	13.3	13.0
Experimental N content					18.1	(16.7)	18.5	16.7
Calculated N content					18.3	18.1	18.5	16.75

* Results of YOSHIMURA[10] recalculated.

** Results of KOMINZ, HOUGH, SYMONDS AND LAKI[25].

Note: *Octopus* arm muscle TM and *Pinna* adductor TM are water-insoluble; squid and rabbit the water-soluble variety.

figures, if, on the one hand, squid TM is compared with *Octopus* TM, and, on the other, if either is compared with *Pinna* (*e.g.* glycine, isoleucine, histidine). Moreover, in the case of squid, the glutamic-aspartic and the lysine-arginine figures more closely resemble rabbit TM than do squid and *Octopus*. These peculiarities, however, may be a reflection of physico-chemical differences, since both rabbit and squid TMs are the water-soluble variety, whilst *Octopus* and *Pinna* are the less soluble types. Some support for this view is also forthcoming from the comparison of the acid-base balance of the several proteins (Table II), the number of free acid groups in excess of base being considerably greater in squid and rabbit than in the other two. Such generalisations must of course be offered with caution until more analyses can be compared: some differences may certainly be due to differing N yields, differences of technique and of hydrolysis conditions.

Some difficulty has been met in characterising the cysteic acid peak in hydrolysates of oxidised *Pinna* TM and the figure given is derived by augmenting it with an added amount of cysteic acid whose recovery had previously been determined.

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The figure is commensurate with the –SH content of *Pinna* TM, which was found to be equivalent to approx. 0.25 g (as cysteine)/100 g protein.

Finally, it will be noticed there is excellent agreement between the calculated N content of the author's TMs and the experimentally determined value: it would be very desirable that wherever analyses are reported this check should be made. The average residue weights of all the TMs in Table II, calculated from (sum amino acid wts./sum amino acid residues) are virtually identical.

N-terminal residues in Pinna *tropomyosin.* As in rabbit TM, the Sanger FDNB method has not disclosed the presence of a N-terminal group. The total ether-soluble DNP material (conditions A, Table III) amounts only to 1 group/500,000 g protein. Short hydrolysis (conditions C) did not reveal the presence of a labile acid such as DNP-proline (PORTER AND SANGER[26]). The lysine content by the FDNB method (PORTER[27]) approximates the value found by the Moore-Stein method. Several separate experiments (conditions D) have failed to detect terminal DNP-arginine by the new method. It was usually possible to obtain mere traces of DNP-material (1 group/10^6–$2 \cdot 10^6$ g protein) which split up after paper chromatography into several spots, none identical with the arginine control.

TABLE III

APPLICATION OF THE FLUORODINITROBENZENE METHOD FOR THE DETECTION OF N-TERMINAL GROUPS IN *Pinna* TROPOMYOSIN

Conditions of hydrolysis	*Findings*
(A) 8 h boiling 5 *N* HCl	Ether-soluble acids, 1 group/480,000 g protein
(B) 12 h boiling 5 *N* HCl	Lysine, 9.0 g/100 g protein (*cf.* Table I)
(C) 2 h conc. HCl at 105°	No proline detected
(D) 10 h conc. HCl at 105°	Negligible traces DNP-material by methods designed to detect N-terminal arginine

DISCUSSION

Since the original isolation of TM from the skeletal muscle of mammals and fish, and from mammalian heart (BAILEY[6]), TM has been shown to have a ubiquitous occurrence in the muscles of many phyla. SHENG AND TSAO[23] have isolated it from crustacean and amphibian skeletal muscle, and from the smooth muscle of duck gizzard and bovine uterus. Preliminary reports indicate that TMs from many different sources have been analysed and compared (KOMINZ AND SAAD[28]; KOMINZ, SAAD AND LAKI[8]). TSAO, TAN AND PENG[29] have examined the molecular characteristics of pig heart, duck gizzard, crustacean (prawn) and cephalopod (*Sepia*) TM, finding for the monomer particle weights from 68,000–150,000. As far as one can judge, these TMs of TSAO's group are soluble in water or at very low ionic strength (pH 6.5–7), and are generally salted-out by ammonium sulphate in the range 40–70% saturation. TSAO, TAN AND PENG[29] have also obtained needle-like crystals of TM from the adductors of *Anodonta pacifica* and *Cristaria plicata*, the method following, like that of YOSHIMURA (see above), the original method of BAILEY. Here too, it seems likely that these represent the more soluble variety.

The large amount of the globulin form of TM in muscles where it can be more readily estimated (*i.e.* in the smooth adductors) suggests that it must serve an important physiological function as yet unknown. ABBOTT AND LOWY[4] from their

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studies on *Pinna* and other smooth molluscan muscles have noted that their common property lies not in the intrinsic speed of contraction (the gray part of *Pinna* being as fast as some vertebrate striated muscles) but in their slow relaxation. In *Pinna*, the fully active state lasts only 200 msec, but the complete decay of tension has a duration of 12 sec. It is perhaps towards this economy of lamellibranch muscle that TM contributes, a role which SHENG AND TSAO[23] have called—though not with particular reference to adductors—the "holding" function. This view might be strengthened if the striated parts of adductors were found to contain much less TM than smooth, and unfortunately, the method applicable to the latter is unsuitable for the former; but at least, there is a strong case for the proposition that TM is the paramyosin of BEAR's group, and whereas the paramyosin spacings are prominent in the smooth part, they are replaced by those of actin in the striated, as is the case in mammalian skeletal muscle where the amount of TM is rather low.

From a comparative standpoint, the analytical and physical data which are being collected by the several groups of workers mentioned above should provide interesting material for the study of the range of variations in a protein of very definite type. Like collagens, the TMs are characterised by the complete absence of certain amino acids, and unduly large amounts of others, but from a comparative aspect they have the added advantage over the collagens in their wide range of solubility, so that generalisations between composition and physico-chemical properties are more likely to emerge. In addition to their overall composition there appears to be a common structural feature in that all the TMs thus far examined (rabbit (BAILEY[6]); *Pinna* adductor (this paper); pig heart, duck gizzard, prawn body muscle (JEN AND TSAO[30])) do not reveal a N-terminal group by Sanger's FDNB method.

The emphasis of the present study lies in the fact that the unknown globulin so easily isolated from smooth adductor muscles, and present in such large amounts, must now be classed as a tropomyosin, and such material may be very favourable for elucidating the role of tropomyosins in general. Physical data on *Pinna* protein (ultracentrifuge, light-scattering etc.) will be considered by Dr. C. M. KAY in a separate communication.

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SUMMARY

1. The crystalline globulin first isolated by the author[5] from the smooth adductors of *Pinna nobilis* and oyster, and probably responsible for the paramyosin diffraction pattern of these muscles, is now shown by amino acid analysis to be a tropomyosin.

2. The amino acid composition of *Pinna* tropomyosin and also of a comparable type from cephalopod muscle has been compared with that of rabbit. The invertebrate tropomyosins differ mainly from the latter in the replacement of lysine groups by arginine and in the higher amide N.

3. The water-soluble type of tropomyosin which is found as a minor component in invertebrate material, appears, like rabbit tropomyosin, to possess a greater ratio of acid groups to basic groups than does the globulin type of tropomyosin.

4. No N-terminal groups can be demonstrated in *Pinna* tropomyosin by the Sanger fluorodinitrobenzene method.

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5. A new method is proposed for the identification of N-terminal arginine where it occurs as the DNP-derivative in presence of large amounts of ε-DNP lysine.

REFERENCES

1 C. E. HALL, M. A. JAKUS AND F. O. SCHMITT, *J. Appl. Phys.*, 16 (1945) 459.
2 R. S. BEAR, *J. Am. Chem. Soc.*, 66 (1944) 2043.
3 C. C. SELBY AND R. S. BEAR, *J. Biophys. and Biochem. Cytol.*, 2 (1956) 55.
4 B. C. ABBOTT AND J. LOWY, *J. Marine Biol. Assoc. U.K.*, 35 (1956) 521.
5 K. BAILEY, *Pubbl. staz. zool. Napoli*, 29 (1956) 96.
6 K. BAILEY, *Biochem. J.*, 43 (1948) 271.
7 K. BAILEY, *Biochem. J.*, 64 (1956) 9P.
8 D. R. KOMINZ, F. SAAD AND K. LAKI, *Nature*, 179 (1957) 206.
9 J. R. SPIES AND D. C. CHAMBERS, *Anal. Chem.*, 21 (1949) 1249.
10 K. YOSHIMURA, *Mem. Fac. Fisheries, Hokkaido Univ.*, 3 (1955) 159.
11 A. C. CHIBNALL, M. W. REES AND E. F. WILLIAMS, *Biochem. J.*, 37 (1943) 354.
12 S. MOORE AND W. H. STEIN, *J. Biol. Chem.*, 211 (1954) 893.
13 M. W. REES, *Biochem. J.*, 40 (1946) 632.
14 E. SCHRAMM, S. MOORE AND E. J. BIGWOOD, *Biochem. J.*, 57 (1954) 33.
15 T.-C. TSAO AND K. BAILEY, *Biochim. Biophys. Acta*, 11 (1953) 102.
16 J. TILLMANS AND K. PHILIPPI, *Biochem. Z.*, 215 (1929) 36.
17 F. SANGER, *Biochem. J.*, 39 (1945) 507.
18 K. BAILEY, *Biochem. J.*, 49 (1951) 23.
19 S. BLACKBURN AND A. G. LOWTHER, *Biochem. J.*, 48 (1951) 126.
20 K. BAILEY AND F. R. BETTELHEIM, *Biochim. Biophys. Acta*, 18 (1955) 495.
21 G. HAMOIR, *Biochem. J.*, 48 (1951) 146.
22 G. HAMOIR, *Biochem. J.*, 50 (1951) 140.
23 P.-K. SHENG AND T.-C. TSAO, *Acta Physiol. Sinica*, 19 (1954) 203 (in Chinese); *Scientia Sinica*, 4 (1955) 157 (in English).
24 S. V. PERRY, *Biochem. J.*, 55 (1953) 114.
25 D. R. KOMINZ, A. HOUGH, P. SYMONDS AND K. LAKI, *Arch. Biochem. Biophys.*, 50 (1954) 148.
26 R. R. PORTER AND F. SANGER, *Biochem. J.*, 42 (1948) 287.
27 R. R. PORTER, *Methods in Medical Research*, 3 (1950) 256.
28 D. R. KOMINZ AND F. SAAD, *Federation Proc.*, 15 (1956) 112.
29 T.-C. TSAO, P.-H. TAN AND C.-M. PENG, *Acta Physiol. Sinica*, 19 (1955) 389 (in Chinese); *Scientia Sinica*, 5 (1956) 91 (in English).
30 M.-H. JEN AND T.-C. TSAO, *Scientia Sinica*, (in the press).

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CHROMATOGRAPHY OF RATTLESNAKE VENOM

A SEPARATION OF THREE PHOSPHODIESTERASES

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INTRODUCTION

Snake venoms have been studied for more than twenty years (see reviews by SLOTTA[1] and ZELLER[2]) but so far most of the enzymes from this biologically interesting material have not been obtained in pure states. Also, with the exception of *l*-amino

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