short of evidence or if he considered the problem to be unsuitable for solution by the scientific method of approach. This attitude of mind gave all the more point to his opinions when he felt inclined to express them. His dignified presence and gift of clear and concise expression inspired confidence and enabled him to put his views with great force in all circumstances.

Between 1927 and 1939 it was frequently necessary for Pyman to visit not only most of the countries in Europe but also the United States of America and Canada. Wherever he went it was evident that scientists in other countries held him in the same high regard as his colleagues at home. To be able to travel widely was a source of great satisfaction to him. He also enjoyed the opportunities it gave him to indulge his taste for good music. Few things gave him greater pleasure than an evening at the

Opera House in Frankfurt, Berlin, Copenhagen, Stockholm or Paris.

Pyman was for many years a regular attendee at the Annual Meetings of the Society of Chemical Industry, The British Medical Association and The British Association, and when these Societies held their meetings in Nottingham, he was called upon to play a leading part in their functions. He did so very willingly because he always appreciated the interdependence of academic science and industry.

He made friends easily and developed his capacity for friendship more and more as he grew older. All his friends and colleagues will remember gratefully the warm hospitality that he and his wife were always ready to give, and his students and all those who worked under his guidance will cherish the memory of his inspiring leadership.

L. ANDERSON

Notes on the Toxins of Egyptian Scorpions

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This note records observations on scorpion venoms, including some details of the unpublished method of Dr & Mrs Hassan for the purification of the toxins, and an examination of the stability and absorption spectra of the purified products.

PREPARATION OF PURIFIED TOXINS

The species of scorpion used were: *Buthus quinque striatus*, *B. leptochelys*, *B. acute carinatus*, *B. hottentotta minax*. Stings and last segment of the tails (the telsons) of scorpions were detached and dried over calcium chloride. The dried telsons were ground in a mortar for about 30 min. with quartz sand, wetting with small volumes of 0.1 N-HCl. The finely ground mass was extracted with 5 × 30 ml. lots of 0.1 N-HCl, the liquid being separated by decantation. The combined extracts were neutralized with N-NaOH, a sat. aqueous solution of picric acid added and the liquid allowed to stand for 24 hr. The supernatant liquid was poured off the precipitate which had formed. This precipitate, containing the toxin, was repeatedly extracted with 80 % (v/v) acetone in water, until no more soluble picrotoxin was removed. The toxin was then precipitated as hydrochloride by adding a few drops of conc. hydrochloric acid and an excess of acetone to the combined 80 % acetone extracts. The toxic precipitate was centrifuged off, washed successively with dry acetone and dry ether, and kept in vacuum over phosphorus

pentoxide. The potency of this toxin, measured as the minimal lethal dose (M.L.D.) for the rat, was about 25–50 µg./100 g. body weight.

PROPERTIES OF PURIFIED TOXIN PREPARATIONS

In the sequel, unless otherwise stated, 'toxin' refers to a purified product prepared in the above manner. Toxin prepared as described dissolved easily in distilled water to give a clear solution. The following properties of the toxin were observed, using an aqueous solution containing 1 mg./ml.: Millon, xanthoproteic and biuret reactions were positive. There was no precipitation on boiling or half-saturation with ammonium sulphate. (Half-saturation with ammonium sulphate did produce a small flocculent precipitate, which was non-toxic, from a solution containing 4 mg. toxin/ml.) Toxin was completely precipitated by full saturation with ammonium sulphate, by absolute ethanol and by acetone. Picric acid gave a precipitate which dissolved on heating and reappeared on cooling. The general behaviour was that of a proteose. Incineration of 20 mg. of dried toxin left no residue.

Absorption spectra of toxins and venoms. Absorption spectra of toxins purified as described from the four species mentioned were examined, and also the venom from two of the species (*Buthus quinque*

striatus and *B. hottentotta minax*). Fresh venom when required was collected by the method of Jousset de Bellesme (1872), in which the gland is stimulated electrically and the venom collected through a capillary tube fitted to the sting. Venom so collected was milky in appearance, was soluble in 0.9 % saline and clotted on standing. Data on the absorption spectra are recorded in Table 1, from which it is seen that the toxins prepared from the four species all show the same absorption band at 278m μ . The two venoms both showed a band at 260m μ , the shift to 278m μ apparently occurring during the process of purification.

spectra. With 0.5 % formalin toxicity decreased from M.L.D. $\equiv$ 0.05–0.08 mg. in 15 days; with 1.0 % formalin the respective values were 0.05–0.10 mg. in 15 days, without significant change in the absorption band. Even with 5 % formalin the M.L.D./100 g. rat had increased only from 0.05 to 0.16 mg. during 15 days; the absorption band was then affected.

The action of formalin at 55° was much more rapid, the M.L.D. increasing from 0.05 to 0.5 mg./100 g. rat in 24 hr. with 0.5 % formalin, or within 1 hr. with 5 % formalin, the characteristic absorption band becoming inflected.

Table 1. *Absorption spectra data on scorpion toxins and venoms*

Species	Substance examined	Cell (mm.)	$\lambda_{\max}$ (m μ)	$E_{\max}$	$\lambda_{\min}$	$E_{\min}$	M.L.D./100 g. rat (μ g.)
<i>B. quinque striatus</i>	Toxin HCl 0.2 %	5	278	1.16	253	0.67	25
<i>B. leptochelys</i>	"	5	278	1.44	253	0.97	33
<i>B. acute carinatus</i>	"	5	278	1.43	253	0.96	27
<i>B. hottentotta minax</i>	"	5	278	1.10	253	0.80	20
<i>B. quinque striatus</i>	Venom 0.5 ml. + 1.5 ml. saline	10	262.5	0.6	—	—	(0.3 ml.)
<i>B. hottentotta minax</i>	Venom 1 drop + 0.75 ml. saline	5	262.5	1.0	219	0.2	(0.1 ml.)

Effects of pH and of temperature. The toxins were stable when kept in solution in 0.1N-HCl at 0° for at least 6 days, neither the absorption band nor the M.L.D. altering during this time. They were unstable in 0.1N-NaOH, the M.L.D. being at least 10 times greater after 24 hr. at 0° and the characteristic absorption band becoming inflected. Toxin in distilled water at 55° was unaltered in 24 hr., but after 6 days had only one-tenth its former activity and the absorption band had changed.

Effect of formalin. Solutions (0.2 %) of toxin in water with different concentrations of formalin were allowed to stand at 0° and examined at intervals for toxicity and for changes in the absorption

Effect of acetaldehyde. When acetaldehyde (5 %) was used instead of the formalin at 55° the potency of the toxin decreased from 0.05 to 0.08 mg./100 g. rat in 1 hr. without alteration of the absorption band, but after 24 hr. under these conditions the M.L.D. had increased to 0.5 mg. and the characteristic absorption band was no longer present.

It appears from the parallelism in the changes in toxicity with the changes in the absorption band produced by various treatments that this absorption band is related to the toxin.

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REFERENCES

- Jousset de Bellesme (1872). In Winterstein (1924). *Handbuch der vergleichende Physiologie*, 2 (ii), 96. Jena: G. Fischer.
- Wilson, W. H. (1904). *Rec. Egypt Gov. Sch. Med.* 11, 13.
- (1921). *Bull. Inst. Egypte*, 111, 67.

Analysis of a Partial Hydrolysate of Gramicidin by Partition Chromatography with Starch

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Following the analysis of the constituent amino-acids of gramicidin previously reported (Gordon, Martin & Synge, 1943b), a beginning has been made with a study of the peptides arising from the partial hydrolysis of gramicidin by acid. In the present paper are described the use of a new technique (partition chromatography employing starch) which

has proved valuable for this work and may have wider applicability, and the isolation of the dipeptide *l*-valylglycine from gramicidin by partial hydrolysis with acid. Some further evidence concerning the amino-acid composition of gramicidin and the optical nature of the constituent valine residues has been obtained.