Aust. J. Biol. Sci., 1978, **31**, 209–18

Isolation, Characterization and Pathology of the Toxin from a *Microcystis aeruginosa* (= *Anacystis cyanea*) Bloom

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Abstract

The nature of the toxicity of a bloom of blue-green alga, *M. aeruginosa* (= *Anacystis cyanea*), that occurred in a man-made lake was investigated. Crude algal bloom extracts were toxic to laboratory mice when injected intraperitoneally. The lethal dose (LD_{100}) of these extracts was 15–30 mg of lyophilized algal bloom per kilogram body weight. The toxin was purified by a procedure that included ammonium sulphate fractionation, solvent extraction, acid precipitation, Sephadex G25 and DEAE–Sephadex chromatography, and high-voltage electrophoresis at pH 6·5. The preparation gave a single spot on high-voltage electrophoresis at pH 9·0, had no free amino group, and was characterized by a simple amino acid composition of equimolar quantities of L-methionine, L-tyrosine, D-alanine, D-glutamic acid, *erythro* β-methyl aspartic acid and methylamine.

The LD_{50} for the purified toxin was estimated to be 0·056 mg/kg of mice, and the approximate LD_{100} is 0·070 mg/kg, based on the total material found from amino acid analysis.

Parenteral administration of the purified toxin to mice produced extensive liver lobular haemorrhage and death within 1–3 h. Repeated inoculation of sublethal doses daily over some weeks produced progressive hepatocyte degeneration and necrosis and the development of fine hepatic fibrosis.

Introduction

There is a long history of the occurrence of toxic algal blooms in lakes and dams in Australia and many other parts of the world (McBarron and May 1966; Gentile 1971; May 1972; Shilo 1972).

Of those blue-green algae reported to produce toxic effects, *Microcystis aeruginosa* (= *Anacystis cyanea*) is probably the most frequent offender. The isolation and nature of the toxin from *M. aeruginosa* NRC-1, a strain cultured from a naturally occurring bloom, were first investigated by Bishop *et al.* (1959). The endotoxin was shown to be a peptide which on acid hydrolysis gave L isomers of valine, leucine, ornithine, aspartic acid, glutamic acid and alanine, and the D isomer of serine. The toxin from this same strain was also investigated by Murthy and Capindale (1970), using a different method of isolation. The toxin was again shown to be a peptide. The product of acid hydrolysis of the toxin included at least 14 different amino acids, and the overall composition indicated a different compound from that previously described by Bishop *et al.* (1959). The isolation and nature of the endotoxin from this same strain of *M. aeruginosa* have since been investigated by Rabin and Darbre (1975), who found the toxin to be a peptide of molecular weight 1750 ± 450. On acid hydrolysis it was shown to contain most of the common amino acids and ornithine.

The pathological effects caused by algal blooms in which *M. aeruginosa* is the predominant species have been observed by a number of workers (Ashworth and Mason 1946; Konst *et al.* 1965; McBarron and May 1966). Enlargement and congestion of the liver with necrosis of the hepatic cells were found after oral administration or parenteral injection of toxic algal extracts.

The occurrence of toxic water blooms in the New England region prompted an investigation of the factors causing these blooms and of ways of controlling or neutralizing their toxicity. Here we report on the isolation, chemical composition, and pathology of the toxic principle derived from a bloom of *M. aeruginosa* that occurred on Malpas Dam, a domestic water reservoir in New England, northern New South Wales.

An initial attempt to isolate the toxin by the procedure of Murthy and Capindale (1970) resulted in non-toxic material and so the procedure was altered to yield a high recovery of toxicity (Runnegar and Falconer 1975).

Materials and Methods

Enzymes

L-Amino-acid oxidase (EC 1.4.3.2) of *Crotalus adamanteus* venom lot 471–4 and D-amino-acid oxidase (EC 1.4.3.3) of porcine kidney lot 469–4 were from P-L Biochemicals Inc. (Wisconsin, U.S.A.). L-Glutamate decarboxylase (EC 4.1.1.15), type IV, of *Clostridium welchii* lot 129B 6871 was from Sigma Chemical Co. (St Louis, Mo., U.S.A.). A unit of activity of each of these enzymes converts one micromole per minute at 37°C at the appropriate pH.

Collection and Preparation of Algal Bloom

An algal bloom consisting of *M. aeruginosa* occurred at Malpas Dam Reservoir in October 1973. Malpas Dam is a man-made water reservoir for the town of Armidale, N.S.W. The wind and the geometry of the dam greatly concentrated the bloom on the south-eastern edge of the dam. About 40 litres of the bloom was collected from the surface of the water by partially draining out the water with a hand sieve and retaining the clumps of algae. This material (apart from a small sample retained for preliminary testing of toxicity) was divided into 200-ml portions which were stored frozen at −15°C until lyophilized. The lyophilized bloom was stored at −15°C.

Assays of Toxicity

Male white laboratory mice weighing 20–40 g were used to test the toxicity of crude bloom extracts and material from the various stages in the purification procedure. Aliquots of test material were administered by intraperitoneal injection from an Armstrong precision syringe (0·1–0·5-ml aliquots). The minimum dose sufficient to kill each member of a group of mice was found and from this an approximate LD_{100} was determined. Generally about six groups, each containing at least four mice, were used and each group received one dose from a range of doses (expressed as milligrams per kilogram of mice). These approximate LD_{100} values were used as a measure of potency of the bloom and the purified toxin, and were used to estimate the recovery of toxic material during the preparation, in which total toxicity was expressed as the approximate maximum weight of mice to which the preparation would be lethal.

Purification of Toxin

The isolation scheme for the toxin to step 6 inclusive is shown in Table 1.

Step 1

Freeze-dried algal bloom (25 g) was extracted (by magnetic stirring) at 5°C for 1 h with a buffer consisting of 375 ml 0·1 M Na_2CO_3 and 250 ml 0·05 M $NaHCO_3$. The suspension was centrifuged at 35000 *g* for 40 min, and the precipitate discarded. The supernatant was kept at 5°C.

Step 2

Solid ammonium sulphate was added to the supernatant of step 1 to give 40% saturation; the suspension was centrifuged at 35 000 *g* for 40 min, the supernatant was discarded and the precipitate suspended in 100 ml of 0·05 M $NaHCO_3$ and dialysed overnight against 4 litres of 0·05 M $NaHCO_3$ in Visking cellulose dialysis tubing size 32/32. Toxic activity remained inside the tubing. The small amount of insoluble material after dialysis was removed by centrifugation at 35 000 *g* for 30 min, and the supernatant was frozen at −15°C.

Step 3

The powder obtained after freeze-drying the supernatant from step 2 was extracted twice at 5°C with 150 ml wet n-butanol [135 ml anhydrous butanol (A.R. grade) and 15 ml H_2O] for 2 h. The butanol extract obtained by filtering the suspension through Whatman No. 41 paper was dried by rotary evaporation at 40°C. A brown gummy residue was left which was dissolved in 0·1 M NH_4HCO_3 and the volume made up to 10 ml.

Step 4

The toxin sample (10 ml) was applied to a Sephadex G25 column (1·3 by 60 cm) and eluted with 0·1 M NH_4HCO_3; 3-ml fractions were collected. The toxic fractions were pooled (about 50 ml).

Step 5

HCl (1 M) was added to the toxin eluted from Sephadex G25 till the pH dropped to 3·0. The suspension was centrifuged at 35 000 *g* for 30 min. The supernatant was discarded and the precipitate was dissolved in 60 ml 0·1 M NH_4HCO_3.

Step 6

The toxin solution (about 120 ml), the product of two extractions (steps 1–5), was applied to a DEAE–Sephadex A25 column (1·7 by 85 cm) equilibrated with 0·1 M NH_4HCO_3. After thorough washing with 0·1 M NH_4HCO_3, the toxin was eluted with 0·3 M NH_4HCO_3, pH 8·0. The toxic fractions were pooled, dialysed against H_2O, and then freeze-dried. The white powder obtained was stored dry at −15°C.

Step 7

The material from step 6 was applied to Whatman 3 MM paper at a loading of 0·4 mg/cm and electrophoresis was performed at pH 6·5 in 1% (v/v) pyridine–acetic acid. The region of toxic activity stained heavily with the chlorine stain, and was eluted with 1% (w/v) aqueous ammonia. This material generally ran as a single compact spot on paper electrophoresis at pH 9·0 in 1% (w/v) ammonium carbonate, otherwise it was submitted to electrophoresis at this pH and the toxic material was eluted.

High-voltage Paper Electrophoresis

This was performed on Whatman 3 MM paper at pH 1·9, 3·5 and 6·5 (Ambler 1963) and pH 9·0 [1% (w/v) ammonium carbonate as buffer], in tanks with varsol as coolant, using a voltage gradient of 60 V/cm. Material was located by guide strips using 0·25% (w/v) ninhydrin, 1% (v/v) collidine in ethanol, and then for the toxin, by the chlorination method of Reindel and Hoppe (1954) applied on the same strips. Material was eluted from paper by 1% (w/v) aqueous ammonia solution. Amines were well separated after only 20 min at pH 3·5.

Amino Acid Analysis

The toxin was hydrolysed with 5·7 M HCl (0·4 ml+0·5 µl β-mercaptoethanol) in sealed evacuated tubes at 108°C for 24 h. Hydrolyses were also performed with oxidation of the toxin prior to acid hydrolysis to convert methionine and methionine sulphoxide to methionine sulphone by using a 50-fold molar excess of performic acid over total thioether at 0°C (Hirs 1956). Hydrolysis with 3 M p-toluenesulphonic acid containing 0·2% (w/v) 3-(2-aminoethyl)-indole was performed in sealed evacuated tubes for 24 h at 115°C (Liu and Chang 1971) to ascertain if tryptophan was present.

Hydriodic acid hydrolysis was performed with a constant-boiling mixture in sealed evacuated tubes at 130°C for 24 h. This latter procedure results in the reduction of β-hydroxy amino acids and demethylation of methionine. Alkaline hydrolysis was performed under vacuum in 4·2 M NaOH at 108°C for 24 h in polypropylene liners sealed inside glass tubes.

Amino acids and methylamine were identified directly both by a Beckman model 120C amino acid analyser and by paper electrophoresis at pH 1·9 and 3·5, and indirectly after dansylation of the hydrolysate from HCl hydrolysis (Gray 1967) as the dansyl derivatives by thin layer chromatography (Woods and Wang 1967).

Amide Analysis

Toxin (20 nmol) was analysed for ammonia after hydrolysis with 250 μl constant-boiling hydriodic acid at 108°C for 1¾ and 6 h (Inglis *et al.* 1974). Blanks for the toxin without hydrolysis, the hydriodic acid or the loading 0·1 M HCl were also performed, since all contain some ammonia.

Determination of Configuration of Amino Acids

Amino acid oxidase

Toxin (100 nmol) hydrolysed by constant-boiling HCl alone (no β-mercaptoethanol) was split into three equal aliquots. The first of these was incubated with L-amino-acid oxidase (5 μl of 8·8 mg/ml, 0·30 units) for 2½ h at 37°C in 100 μl 0·2 M *N*-ethylmorpholine acetate buffer at pH 7·5. The second fraction was incubated with D-amino-acid oxidase (20 μl of 4 mg/ml, 0·38 units) for 2½ h at 37°C in 100 μl 0·2 M *N*-ethylmorpholine acetate buffer at pH 8·4. The third aliquot acted as a control without enzyme. Additional controls of enzyme alone and enzyme with synthetic mixtures of either L or D amino acids were also performed. The samples were dried down and their compositions determined both qualitatively by paper electrophoresis and quantitatively by amino acid analysis.

L-*Glutamate decarboxylase*

Toxin hydrolysate (80 nmol) was split into two equal aliquots. The first was incubated with L-glutamate decarboxylase (20 μl of 3 mg/ml, 0·05 units) for 16 h at 37°C in 100 μl of 0·2 M pyridine–acetic acid buffer at pH 5·0. The second fraction acted as a control without enzyme, and additional controls of enzyme alone and enzyme with either L or D glutamic acid were also performed.

Periodate Oxidation

Toxin hydrolysate (40 nmol) was incubated with 50 μl of 0·01 M sodium metaperiodate for 1½ h at 37°C, then dried and dissolved in 20 μl 50% (v/v) aqueous pyridine, centrifuged, and an aliquot applied to paper for electrophoresis at pH 1·9 or 3·5. Serine, threonine, 3-hydroxy aspartic acid and 3-hydroxy glutamic acid were completely oxidized by this procedure.

Derivatization and Identification of β-Methyl Aspartic Acid

Toxin was hydrolysed *in vacuo* with 2 M HCl at 100°C for 2 h, and applied as a band 7 cm wide to Whatman 3 MM paper. The unknown amino acid was separated from the other products by flat-plate electrophoresis at pH 3·5, eluted with 50% (v/v) aqueous pyridine and dried over phosphorous pentoxide and sodium hydroxide pellets. The amino acid (*c.* 20 nmol) was esterified by thionyl chloride in methanol (100 μl of 1 : 5 mixture) for 24 h at room temperature, and the reagents removed under vacuum. The dansyl derivative was prepared using a 10-fold excess of dansyl chloride in acetone (40 μl) and an equal volume of 0·1 M sodium bicarbonate at 37°C for 2 h; it was then separated from salt and excess dansyl sulphonic acid by extraction from water (100 μl) with ethyl acetate (three times 300 μl) and, after drying, it was transferred in methanol to the solids probe of an AEI MS30 mass spectrometer. Mass spectra were obtained with a beam current of 100 μA and energy 70 eV. Samples were introduced at a source temperature of 100°C. As little as 2 nmol produced a good molecular ion.

Pathogenicity for Mice

Acute toxicity induced by a single lethal dose and chronic toxicity following repeated sublethal doses were both studied. Toxin used was purified to step 6 as described above.

Acute toxicity

Fourteen mice were stratified according to body weight and divided into two equal groups with weight ranges of 25–36 g. Mice in one group were given the approximate LD_{100} for a 30-g mouse, and in the other group twice this quantity was given by a single intraperitoneal injection. All mice were autopsied and histological studies were made of those which were examined within minutes of death. Sections of liver, kidney, heart, lung, spleen and brain were fixed in 10% formal saline, routinely sectioned and stained with haematoxylin and eosin.

Chronic toxicity

Four groups of mice of similar weight range were inoculated daily by intraperitoneal injection for 6 weeks with 0·75, 0·50 and 0·25 of the approximate LD_{100} for a 30-g mouse; injections were made up in saline and the fourth group received saline only. All treatments were given with due regard to sterility. Two mice from each group were killed at the end of each week. Histopathological sections were prepared as described above.

Results and Discussion

Purification and Chemical Composition of the Toxin

The toxicity of the original bloom as extracted in step 1 of the purification procedure (Table 1) was in the range LD_{100} 15–30 mg/kg; similar values were obtained by sonic disintegration of the bloom. The purification procedure up to step 6 has been repeated at least 10 times with results similar to those shown in Table 1. The toxin after step 6 had an approximate LD_{100} of 0·2 mg/kg.

Table 1. Partial purification of the toxin from a bloom of the blue-green alga *M. aeruginosa*

25 g of lyophilized algal bloom was extracted and steps 1–6 of the purification were carried out as detailed in Methods

	Step	Total toxicity (kg of mice)	Recovery[A] (%)
1	Na_2CO_3–$NaHCO_3$ extraction	970	100
2	$(NH_4)SO_4$ fractionation	550	56
3	Butanol extraction	475	49
4	G25 Sephadex	358	37
5	Acid precipitation	280	29
6	DEAE–Sephadex	213	22

[A] Relative to step 1.

The LD_{50} of the toxin from step 7 was 56 μg/kg (95% confidence interval 43–60 μg/kg), and the approximate LD_{100} was 70 μg/kg based on the yield of amino acids from acid hydrolysis.

The toxin was insoluble at pH 3·5 and so failed to move from the origin on paper electrophoresis at pH 3·5. At high loading (>0·4 mg/cm) the material tailed on electrophoresis at pH 6·5, whilst at lower loading it moved towards the anode with the leading edge having 0·6 times the mobility of aspartic acid.

The toxin was not stained on paper by ninhydrin, indicating the absence of free amino groups, nor was a free amino group available for dansylation, but the hydroxyl group of tyrosine did dansylate. Selective tritiation (Holcomb *et al.* 1968) indicated the absence of the free carboxyl groups of alanine, methionine, or tyrosine (the only

monocarboxylic amino acids present) whilst both dicarboxylic amino acids incorporated tritium indicating the presence of free alpha carboxyl groups in these amino acids.

Amino acid analysis showed the presence of five amino acids and methylamine in approximately equimolar quantities (Table 2), and analyses for ammonia showed the absence of primary amides. Methionine and tyrosine were oxidized by L-amino-acid oxidase, an enzyme which does not react with L-glutamic acid or L-alanine at an appreciable rate (Lichtenberg and Wellner 1968); alanine only was oxidized by D-amino-acid oxidase. Since the latter enzyme does not oxidize D-glutamic acid (Dixon and Kleppe 1965), the configuration of this amino acid was identified by lack of reaction with L-glutamate decarboxylase which converted only 7% to γ amino butyric acid, a quantity commensurate with the amount of racemization on acid hydrolysis with constant-boiling hydrochloric acid at 108°C. Thus alanine and glutamic acid are in the D configuration and methionine and tyrosine are in the L configuration.

Table 2. Amino acid analyses of the toxin from *M. aeruginosa*

Values given are ratios of the relative proportions of the amino acids

	3 M p-toluene-sulphonic acid or 5·7 M HCl	Performic acid oxidation plus 5·7 M HCl	Constant boiling HI	4·2 M NaOH
β-Methylaspartic acid	1·0	0·9	0·9	1·0
Glutamic acid	1·0	1·0	1·0	0·8
Alanine	1·1	1·1	1·1	1·2
Methionine	0·6	0·7[A]	0·8[B]	0·7[C]
Tyrosine	0·9	0·8	0·9	1·0
Methylamine	0·9	0·9	1·0	0·7[D]

[A] Estimated as methionine sulphone. [B] Estimated as homocysteine thiolactone.
[C] 70% as methionine sulphoxide. [D] Distilled off and estimated separately.

The unknown acidic amino acid exhibited two separable forms which were both converted to the mixture again on acid hydrolysis. If the toxin was subjected to partial acid hydrolysis (2·0 M HCl, 2 h, 100°C, *in vacuo*), only the form of lower anionic mobility at pH 3·5 was produced.

Both forms had larger anionic mobilities than glutamic acid at pH 3·5, although only one form had a larger anionic mobility than aspartic acid at pH 3·5. On amino acid analysis two partially resolved components eluted in the position of aspartic acid, the component of lower anionic mobility at pH 3·5 eluting first. The dansyl derivatives were unresolved from each other in solvents I and II of Woods and Wang (1967) and moved with dansyl glutamic acid. Separation was effected by solvent IV of Crowshaw *et al.* (1967), the component of lower anionic mobility at pH 3·5 running near dansyl aspartic acid and the faster component still running with dansyl glutamic acid.

The unknown amino acid was inert to periodate oxidation and performic acid oxidation (3 h, room temperature), indicating the absence of vicinal hydroxyl and amino acid groups, and unsaturated C–C bonds respectively.

The mass spectra of the dansyl methyl ester showed a strong molecular ion at 408, which together with the other evidence suggested β-methyl aspartic acid.

The diastereoisomers of the unknown amino acid and those from a sample of a racemic mixture of DL β-methyl aspartic acid (kindly prepared by Dr F. H. C. Stewart, Division of Protein Chemistry, CSIRO) were identical in electrophoretic mobility at pH 1·9, 3·5 and 6·5, and co-chromatographed on ion-exchange amino acid analysis and as the dansyl derivatives on thin-layer chromatography. The differing water solubilities of these two synthetic diastereoisomeric racemates indicated that the component of lower anionic mobility of pH 3·5 had the erythro configuration, since this was the more water-soluble racemate (Bocheńska and Biernat 1972).

A minimum molecular weight of 654 can be calculated for the toxin from the amino acid analysis, and if this approximates to the actual molecular weight, the mobility at pH 6·5 indicates an overall negative charge of two (Offord 1966). If the methylamine derives from amide linkage to a carboxyl residue, this suggests a linear structure with a blocked *N*-terminal residue and one carboxyl group blocked by methylamine. However, the possibility of a cyclic structure or a dimeric molecule cannot be excluded.

There is evidence in the literature (Hughes *et al.* 1958; Shilo 1972) that not all blooms of *M. aeruginosa* have the same toxicity, and that even individual blooms will vary in toxicity with time. In our case, a further small bloom that occurred 6 months later was shown to have similar toxicity (approximate LD_{100} of 25 mg/kg). These blooms are as toxic as any *M. aeruginosa* blooms described in the literature: the maximal toxicity reported for such blooms is an LD_{100} of 40 mg/kg (Hughes *et al.* 1958).

The variation in toxicity in blooms, and the differences found in amino acid composition and physical properties by the different workers studying *M. aeruginosa* might be rationalized by the possibility of a variability in structure having little consequence on the pathological effects of the toxin. Such structural variability has been noted in peptide antibiotics synthesized by nucleic acid-independent enzyme systems, e.g. tyrocidins, gramicidins, actinomycins and bacitracins.

This hypothesis is supported by the general similarity between the main amino acids in the toxin preparations of Bishop *et al.* (1959) and Murthy and Capindale (1970) and the amino acids in this preparation, and the similar solubility characteristics of the toxins. However, the toxin isolated by Rabin and Darbre (1975) was quite unlike the other preparations in amino acid composition and in being soluble in acidic media.

Pathogenicity

Acute toxity

Survival. All mice in the group receiving twice the approximate LD_{100} died between 1 h 20 min and 3 h 15 min after inoculation. In the group receiving the approximate LD_{100}, one was accidently killed at the commencement of the experiment, one mouse died 1 h 40 min after inoculation, a second mouse died at 4 h, three mice died the following day and the remaining mouse survived for $2\frac{1}{2}$ days.

As mice became affected they huddled together but soon became prostrate. Terminally the respiration became gasping and the mice gave a few convulsive muscular movements. At death there were no discharges or scouring, but ears, tails and conjunctiva were blanched.

Autopsy. The general appearance of the carcasses was that of paleness. In contrast the livers of most mice were intensely and uniformly congested, appearing dark red. In some of those surviving a day or more, their livers showed a dark red mottling, or individual pin-point haemorrhages were discernable. In the latter cases the liver parenchyma appeared a pale greyish yellow. In all cases the hearts and lungs and all other organs appeared normal.

Histopathology. There was massive liver haemorrhage involving the mid and peripheral zones of the lobules. In mice dying within 2 h there was little discernible change in the hepatocytes but those surviving beyond this time showed progressive hepatocyte degeneration, vacuolation and necrosis. In the longer-surviving mice there were small accumulations of neutrophils in the liver and precipitated material present in the glomerular spaces and lumen of the convoluted tubules of the kidneys, but otherwise in all mice other organs showed little change.

Chronic Toxicity

Survival. Of the 16 mice inoculated daily with 0·75 of LD_{100}, four died the following day, two died on the second day, two died on the third day and one died on the fourth day. One died naturally at the end of the second week and one died during the third week; the balance were killed according to the schedule, the last at the end of the fourth week. Of the 14 mice inoculated daily with 0·50 of LD_{100}, two died naturally during the fourth week and three died during the fifth week. The last was killed according to the schedule at the end of the sixth week. All of the 12 mice inoculated daily with 0·25 of LD_{100} were killed according to the schedule, the last at the end of the sixth week.

Autopsy. Mice dying within 2 or 3 days of the commencement of the experiment in the group receiving the highest dose rate showed changes essentially the same as those described above under acute toxicity. In animals killed at the end of the first week significant changes were present only in those receiving the highest dose rate. In these the livers were pale but with a fine lobular surface pattern. There were no haemorrhages. At each successive week liver changes became more obvious and were also seen, although less severely, at the lowest dose rate. Livers became progressively enlarged, increasing to 7–10% of body weight (averaging 5·3% in controls), and were pale with surface dimpling. Severely affected mice at the two higher dose rates showed weight loss and some developed subcutaneous oedema and mild jaundice.

Histopathology. There was a progressive and generalized hepatocyte degeneration with scattered necrosis accompanied by progressive fibrosis and mononuclear cellular infiltration. There was no bile ductule proliferation. The severity of the change was in proportion to the injected dose, being quite mild in the group receiving 0·25 of LD_{100}. In this group the presence of quite numerous mitotic figures in hepatocytes was an early finding.

The liver is clearly the prime target organ of the toxin, changes in other organs being relatively slight or absent. The striking feature in mice dying from acute poisoning was the extensive liver haemorrhages preceding recognizable hepatocyte degeneration and necrosis. Such necrosis was never massive. In contrast, repeated sublethal doses were only slowly toxic, taking usually some weeks to produce sickness and death from progressive liver degeneration.

Ashworth and Mason (1946) and Konst *et al.* (1965) both working with *M. aeruginosa* also demonstrated its marked hepatotoxic properties in mice and in par-

ticular noted a severe necrotizing action. The former authors also noted liver haemorrhages associated with sinusoidal endothelial destruction whilst the latter author described replacement haemorrhages. These experimental findings are in keeping with those reported in naturally occurring losses in domestic stock (MacDonald 1960; Schwimmer and Schwimmer 1967; McBarron and May 1966).

Both the pathological effects and the peptide nature of this toxin differ from those of two toxins from other genera of blue-green algae which have been studied. *Anabaena flos-aquae* (Gorham 1962; Gentile 1971; Huber 1972; Carmichael *et al.* 1975) has been shown to produce a toxin VFDF, a tertiary amine of molecular weight less than 300 which causes death within minutes due to respiratory arrest.

Aphanizomenon flos-aquae is another freshwater blue-green alga that produces a toxin which has been well characterized (Alam *et al.* 1973). This toxin is most likely a substituted guanidine derivative, similar if not identical to saxitoxin (Bordner *et al.* 1975) and acts as a nerve and muscle blocking agent.

The toxin described in this paper has the simplest amino acid composition, and highest toxicity, of any toxic material isolated from *M. aeruginosa*. The pure toxin was approximately 20 times more toxic than hydrogen cyanide or strychnine on a weight basis (Merck Index 1960). The occurrence of the toxic alga as a bloom in lakes man-made for domestic and agricultural water supply therefore deserves continuing attention.

Acknowledgments

Financial support by the Australian Research Grants Committee is gratefully acknowledged. The authors would like to thank Mrs Valerie May and Dr D. Moriarty for confirming the identification of the algal species as *Microcystis aeruginosa*, Mr K. Quinn for the preparation of the histopathological sections, Dr C. M. Roxburgh for amino acid analyses, Mr P. Davis for mass spectroscopy, and Mr R. E. Guthrie for technical assistance.

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Manuscript received 5 September 1977