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TOXICITY STUDIES WITH THE BLUE-GREEN ALGA OSCILLATORIA AGARDHII FROM TWO EUTROPHIC NORWEGIAN LAKES

By

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BERG, KJETIL and NILS EIVIND SØLI: *Toxicity studies with the blue-green alga Oscillatoria agardhii from two eutrophic Norwegian lakes.* Acta vet. scand. 1985, *26*, 363—373. — Extracts of the blue-green alga Oscillatoria agardhii were tested for acute toxicity on laboratory mice and rats. Material originating from lake Gjersjøen proved to be toxic to the animals, samples from the nearby lake Årungen did not. Clinical symptoms culminated in the development of a fatal shock due to decrease in circulating blood volume. Pathological examination revealed heavy pooling of blood in the liver and severe damage to the organ. Blood analyses also indicated liver damage. Effects were the same with extracts from a laboratory clone culture as from a natural water bloom, but the toxin content was higher in the bloom material. Toxicity was not affected by heat, acid or alkali treatment.

acute toxicity; mice; rats; hepatotoxin.

Blooms of blue-green algae constitute a commonly observed phenomenon in eutrophic inland waters. Among the undesirable effects of such mass growth are interference with the normal food chains, problems associated with abnormal taste and odour and production of toxic substances in the water masses (*Skulberg* 1981).

Toxin-producing blue-green algae are known from many parts of the world. The most commonly reported toxic species are Microcystis aeruginosa, Anabaena flos-aquae and Aphanizomenon flos-aquae. These algae have caused deaths in livestock and wildlife in several countries (*Carmichael* 1982, *Codd* 1984).

In Norway, animal deaths believed to be caused by blue-green algal toxins were reported for the first time in 1971. Two episodes of fatal intoxication among livestock occurred in 1978

and 1982, respectively, both in the south-western part of the country (*Forus & Flesjå* 1979, *Haaland et al.* 1983.

In 1978, investigations were started to survey the presence and toxicity of blue-green algae in Norwegian inland waters. As a result, blooms of toxin-producing species have been discovered in many localities (*Berg et al.*, in prep.). Of note was the registration of Oscillatoria agardhii as one of the toxin-producing species. This finding was reported for the first time from a lake in Norway in 1980 (*Østensvik et al.* 1981).

This paper deals with toxicological and pathological studies with extracts of freeze-dried material of Oscillatoria agardhii.

MATERIAL AND METHODS

Material

The test material originated from 2 eutrophic lakes located in close proximity in Akershus, Norway, namely lake Gjersjøen and lake Årungen. Both lakes were known to have regular water blooms of Oscillatoria agardhii, a filamentous blue-green alga. Toxicity tests were carried out with laboratory cultures of isolated strains as well as with material from natural water blooms. Laboratory clones originating from the lakes were NIVA CYA 18 and NIVA CYA 116, respectively (NIVA culture collection of algae)[1]. Material from water blooms in both lakes was collected in 1981.

Preparation of extracts

After collection or harvesting, the samples were concentrated through plankton nets (mesh size 25 µ) and freeze-dried. From the material, extracts were made in sterile 0.9 % NaCl, equivalent to 50 mg freeze-dried algal material per milliliter. The extraction period was 30 min at 20°C with regular stirring. Finally, the extracts were centrifuged for 10 min at 4000 g and the supernatants used for further testing.

Extraction efficiency test

To examine the efficiency of the extraction process used, acute toxicity tests were also performed on mice with crude saline suspensions of freeze-dried algal cells.

[1] Norwegian Institute for Water Research, P. O. Box 333, Blindern, 0314 Oslo 3, Norway.

Toxin stability test

Stability of the toxins was studied by treating the 50 mg/ml extracts in different ways. Heat stability was examined by using a thermal waterbath. The extracts were kept at a temperature of 100°C for 30 min, then cooled to 20°C. Acid stability was tested by lowering the pH to 1.5 with 2.0 mol/l HCl, and raising it to normal values with 2.5 mol/l NaOH after 60 min. Likewise, alkali stability was studied by raising the pH to 12.0 with 2.5 mol/l NaOH and readjusting it with 2.0 mol/l HCl after 60 min. Acute toxicity tests were then performed on mice with the treated extracts. Stability of the freeze-dried material itself was also tested by performing acute toxicity tests on mice after various times of storage.

Acute toxicity tests

Assays for acute toxicity were performed by intraperitoneal injections on pairs of mice (Bom: NMRI, females, 20—25 g) and rats (Mol: WIST, males and females, 200—250 g). Injection volumes were standardized to 1.0 ml on mice and 1.0 ml per 100 g body weight on rats, the extract concentration to 50 mg/ml. After the injections, the animals were observed continously for 4 h, thereafter regularly for the next 4 h. Surviving animals were kept in observation cages for 4 days. Clinical symptoms and survival times were noted, and pathological examinations performed on a number of animals.

Acute toxicity of the tested material was expressed in terms of mouse units (MU) per gram freeze-dried material. A mouse unit was defined as the minimum dose necessary to kill a 20 g mouse within 4 h. The toxin content was determined by dilution of the extracts. From this value, the approximate minimum lethal dose (MLD_{100}) per kilo body weight was estimated.

Blood parameter assays

A lethal dose giving survival times of 2—4 h was administered to a group of rats, the animals afterwards being sacrificed at regular time intervals (2—4 animals each time). Blood samples were collected and plasma concentrations of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (AP), gamma-glutamyl transpeptidase (γ-GT), glu-

cose and bile acids measured. Livers were weighed and relative liver weights calculated.

ASAT, ALAT, AP and γ-GT values were measured on an automatic Gemsaec fast analyzer (reagents from J. T. Baker Chemicals B.V., P.O. Box 1, Deventer, Holland). Glucose was measured on a Seralyzer® Reflectance Photometer using Seralyzer®, Glucose Reagent Strips (Ames Division, Miles Laboratories, Inc., P.O. Box 70, Elkhart, Ind. 46515, USA), and bile acids on a spectrophotometer using an Enzabile® kit (Diagnostics Division, Nyegaard & Co., P.O. Box 4220 Torshov, 0401 Oslo 4, Norway).

RESULTS

Acute toxicity in mice

Material from lake Gjersjøen: Extracts both of the harvested culture as well as of the natural water bloom of Oscillatoria agardhii var. were toxic in mice. The material from the water bloom was, however, the more toxic, with an approximate MLD_{100} of 200 mg/kg, the corresponding figure for the laboratory culture being 1000 mg/kg.

Toxicological findings were the same with both types of extracts. Following intraperitoneal injection of a lethal dose, there was a latent period of around 30 min. The main symptoms developing were coordination failure, paralysis of hind quarters and lethargy. Shorter periods of tremor and convulsions were seen. Piloerection, pallor and coldness of ears, legs and tail were other constant findings. As the animals became weaker, there were obvious signs of respiratory distress, and a coma-like state developed. Relatively independent of the doses given, survival times varied between 60 and 180 min.

Necropsies revealed a pale carcass, with an enlarged, dark red liver. The relative liver weights were nearly doubled compared with control mice, increasing from the normal around 5 % of body weight up to around 10 %. Kidneys and lungs were pale, but did not show macroscopic pathological changes.

Histological examinations were carried out on liver, kidneys and lungs. Liver sections showed increased amounts of blood in the sinusoids, particularly in the centrolobular regions. Widespread haemorrhages were also seen, and there was a marked dissociation of hepatocytes in the same areas. The hepatocytes were partly swollen, with a pale, eosinophilic cytoplasm. The

cells were irregular in shape, and isolated fragments of cytoplasm were seen. The nuclei showed degenerative changes such as pyknosis, karyorhexis adn karyolysis.

Numerous weakly eosinophilic bodies were seen in the sinusoidal lumens (Councilman bodies). Hepatocyte-like cells were also found in the sinusoidal lumens and the larger blood vessels in the liver. The kidney sections revealed moderate signs of acute degeneration of tubular epithelium. The lungs showed evidence of developing interstitial oedema, and the alveolar septa were somewhat rich in cells. Weakly eosinophilic bodies were found in the lumens of the blood vessels in the lungs, some vessels being almost occluded by these bodies. No regular thrombi were seen.

Material from lake Årungen: Neither the extract from the laboratory clone nor from the water bloom in the lake produced toxicological effects in mice. No clinical signs of illness were observed, and necropsies as well as histological examinations revealed normal findings.

Acute toxicity in rats

Material from lake Gjersjøen: Extracts of both cultured algae and natural water bloom of Oscillatoria agardhii var. were toxic to rats. MLD_{100}'s were the same as for mice, approximately 200 mg/kg for the natural bloom and 1000 mg/kg for the laboratory material. Both types of extract gave the same kind of response. Clinical symptoms were much the same as those seen in mice. Periods of tremor and convulsions were not typical, however. Survival times were not as constant as in mice. Given a dose four times the MLD_{100} or more, survival times were 2—4 h. With lower doses, survival times increased to 24—48 h.

Pathological examinations revealed the same types of changes as seen in mice. Again, there was an enlarged, dark red liver, and paleness of the carcass and other internal organs. Histological sections gave the same picture as that already described, with dissociation and degeneration of the centrolobular areas in the liver as the most important findings.

Plasma ASAT, ALAT and bile acid values increased markedly during the interval 30 min to 90 min after injection, to levels 10—20 times the normal plasma concentrations (Fig. 1). No significant changes were seen in AP or γ-GT concentrations.

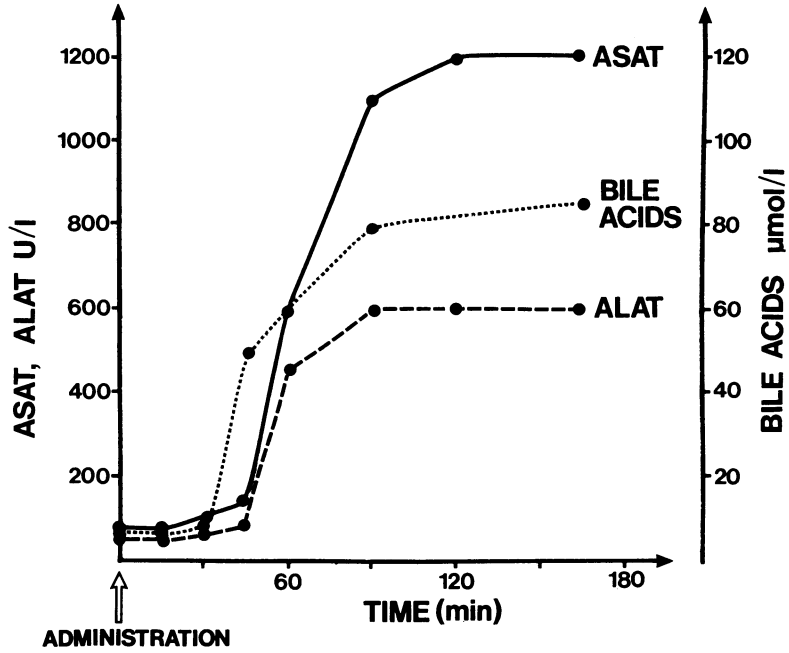

Figure 1. Mean plasma values (n = 2—4) of ASAT, ALAT and bile acids in intoxicated rats. Animals injected with 1.0 ml/100 g (50 mg/ml) Oscillatoria agardhii-extract from lake Gjersjøen.

Blood glucose levels were two to three times the normal values. During this period, relative liver weights showed a significant increase, from the normal 3.5—4.0 % of body weight to 7.0—7.5 % (Fig. 2).

When doses giving survival times of more than 24 h were administered, ASAT and ALAT values rose to even higher levels. Values found after 24 h were 100—200 times the normal plasma concentrations. AP and γ-GT levels also increased significantly by this time, with findings 5—10 times the normal values. Blood glucose concentrations were markedly reduced compared with control animals (Table 1). With longer survival times, there were smaller increases in relative liver weights.

Material from lake Årungen: None of the extracts of material originating from lake Årungen, neither cultured algae nor natural water bloom, exerted any toxicological effect in rats. Clinical signs of illness were not seen, and no pathological chan-

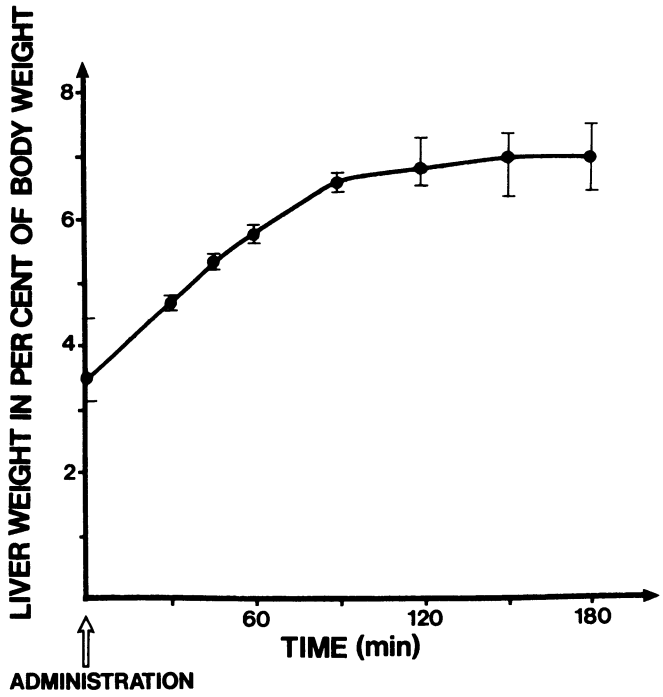

Figure 2. Mean liver weight (n = 2—4) in percentage of body weight in intoxicated rats. Animals injected with 1.0 ml/100 g (50 mg/ml) Oscillatoria agardhii-extract from lake Gjersjøen. Vertical bars show range of values.

Table 1. Mean plasma values (n = 2—4) in control and intoxicated rats. Control: animals not injected. 2 h: animals injected with 1.0 ml/100 g (50 mg/ml) Oscillatoria agardhii-extract from lake Gjersjøen. 24 h: animals injected with 1.0 ml/100 g (30 mg/ml) Oscillatoria agardhii-extract from lake Gjersjøen. Range of values in brackets.

	Mean plasma values		
Parameter	Control	2 h	24 h
ASAT (U/l)	80 (75—97)	1200 (850—1505)	20000 (16600—22100)
ALAT (U/l)	50 (44—61)	600 (465—910)	6000 (2100—7000)
AP (U/l)	200 (40—410)	200 (60—520)	1000 (730—1220)
γ-GT (U/l)	5 (0—9)	5 (4—10)	100 (70—130)
Glucose (mmol/l)	10 (9—12)	25 (22—27)	3 (0—5)
Bile acids (μ mol/l)	7 (4—13)	80 (64—102)	not measured

ges were found. There were no increases in liver weights compared with control animals, and no changes in plasma concentrations of the measured enzymes.

Extraction efficiency

With a crude suspension of material from the natural bloom in lake Gjersjøen, MLD_{100} was 80 mg/kg. This means that the extraction process used released about 40 % of the toxin present. A similar suspension of material from the lake Årungen bloom did not exert any toxic effect in the test animals.

Stability of the toxins

The same types of toxic response as already described were seen in mice given heat, acid or alkali treated extracts of the lake Gjersjøen material. The toxin content was not altered by any of the treatments. Tests performed after 1, 2 and 3 years of storage of the freeze-dried material did not reveal any change in toxicity levels compared with tests carried out when the algae were freshly freeze-dried. No toxic effects were seen in mice injected with any of the differently treated extracts of material originating from lake Årungen.

DISCUSSION

Acute toxicity tests performed on laboratory animals revealed the presence of stable, fast acting and highly toxic components in Oscillatoria agardhii var. from lake Gjersjøen. This toxicity was present in the algae as well in the natural population in the lake as in a laboratory clone culture. The gradual weakening, nervous symptoms and signs of an insufficient blood supply, are clinical findings characteristic of the development of a fatal shock due to decrease in circulating blood volume.

With an MLD_{100} of 80 mg/kg, the algal bloom material collected from lake Gjersjøen in 1981 must be considered as being highly toxic. From information available (*Carmichael et al.* 1977, *Carmichael* 1981, *Carmichael*, personal communication), a lethal oral dose of around 60 g dry weight can be estimated for a 100 kg calf. During the bloom, this algal mass would represent a water volume of 4—5 l, which an animal of this size could consume during a relatively short period of time.

Toxicity levels seem higher in natural populations than in laboratory cultured clones. This indicates that the laboratory growth conditions were probably not optimal for toxin production.

In contrast to the findings from lake Gjersjøen, extracts of Oscillatoria agardhii originating from lake Årungen did not give any toxicological response in mice or rats. This was true for both cultured and natural bloom material. The two algal strains involved, Oscillatoria agardhii var. and Oscillatoria agardhii, are closely related. Both are euplanktonic and bloom-forming, and the filaments are of the same size. Oscillatoria agardhii var. forms brownish-red pigments, Oscillatoria agardhii bluish-green. It is difficult to explain the difference in toxicity only in terms of variation in climate conditions or nutrient availability. The lakes are located only 5 kilometers apart, and the prevailing climatic conditions in the two localities are essentially identical. Both lakes are highly eutrophic, and are in similar catchment areas. Genetic variations in toxin-producing ability, permanent or temporary, might be involved (*Hauman* 1981). However, this and many other aspects of toxin-producing blue-green algae are so far poorly understood.

Necropsies carried out on intoxicated animals indicated severe liver damage and heavy pooling of blood in the organ. The increase in liver weight represents around 2/3 of the total blood volume of the animals, and is assumed to be the direct cause of the development of fatal circulatory shock. Histological sections revealed serious liver damage. Widespread degeneration of hepatocytes was seen in the centrolobular areas. There was also a marked dissociation of hepatocytes and breakdown of the liver cord structure. The weakly eosinophilic bodies found in sinusoidal lumens and lumens of blood vessels in liver and lungs are thought to be isolated cytoplasmatic fragments of damaged hepatocytes.

The changes in various blood enzyme value correspond with the clinical and pathological findings. Elevation in plasma levels of ALAT is a characteristic indication of liver injury. With deeper and more irreversible damage, ASAT levels also tend to rise, usually to higher levels than the ALAT concentrations. Increases in AP and γ-GT concentrations indicate stasis of bile flow. Rise in bile acid values is caused by recirculation of these compounds to the blood. The increase in blood glucose levels

shortly after injection is thought to be a result of stress. Low concentrations after 24 h is an indication of liver damage.

So far, toxic Oscillatoria agardhii has only been reported from a few European localities (*Østensvik et al.* 1981, *Leeuwangh et al.* 1983, *Berg et al.* in prep.). Our results with extracts from this species resemble findings in similar experiments with material from hepatotoxic Microcystis aeruginosa (*Falconer et al.* 1981, *Østensvik et al.* 1981, *Runnegar & Falconer* 1982, *Jackson et al.* 1984). A number of freshwater blue-green algal species are known to have toxin-producing properties. The chemical structures of these toxins are considered to be of alkaloid and oligopeptide nature. Their effects are generally classified into 2 groups, neurotoxic and hepatotoxic, respectively. Neurotoxin-producing species have so far mainly been reported from North America (*Carmichael* 1982). The present investigation indicates that Oscillatoria agardhii var. from lake Gjersjøen like several other blue-green algal species produces toxins that are mainly hepatotoxic. Direct comparison of the different toxins will require further detailed chemical studies.

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SAMMENDRAG

Toksisitetsforsøk med blågrønnalgen Oscillatoria agardhii fra to eutrofe norske innsjøer.

Ekstrakter av blågrønnalgen Oscillatoria agardhii ble undersøkt m. h. p. akutt toksisitet overfor laboratoriemus og rotter. Materiale som stammet fra Gjersjøen var giftig for dyrene, prøver fra den nærliggende Årungen var det ikke. Kliniske symptomer viste utvikling av et irreversibelt sjokk fremkalt av et redusert sirkulerende blodvolum. Patologiske undersøkelser avslørte kraftig blodstuvning i leveren, og alvorlig skade på organet. Blodanalyser indikerte også leverskade. Virkningen var den samme med ekstrakter fra en laboratoriedyrket kultur som fra en naturlig vannblomst, men toksininnholdet var høyere i vannblomstmaterialet. Giftigheten ble ikke påvirket av varme-, syre- eller basebehandling.

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