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Note on the Thermal Death-point of Blue-green Algae of Hot-springs at Maha-oya in Ceylon

by

M. W. N. DHARMAWARDHANA
Dept. of Biological Sciences, Vidyodaya University, Ceylon.

Abstract : Hot-springs at Maha-oya and the algal flora found in them are described. Use of vital stains, Methylene Blue and Neutral Red, to determine the thermal death point is explained. Results obtained for hot springs algae are compared with those of paddy field Blue green algae.

Introduction

Maha-oya is a village about 50 kilometres from Bibile, along the main road which cuts diagonally across the dry jungles of Ceylon's eastern lowlands from Bibile in the foothills near the centre of the island to Chenkaladi near the sea-coast. About 5 kilometres from this village, along a cart-track through the jungle lies a cluster of hot-springs, which Seneviratne and Balendran (1968) have described as being situated 7°32.9′ North and 81°21′ East, with their vent in a flat marshy area at an elevation of about 1 to 2 hundred feet above sea-level.

There are 7 springs within an area of about 300 ft^2. each spring with a small masonry well built knee-high around it. (see Figs. 1, 2 & 3) All except the one marked *G* in Fig. 3, are hot-springs. Gas constantly rises to the surface of the water in the hot-wells which fills them and overflows down the outer sides. Algae grow as a thick scum both within and on the outer surfaces of these wells, generally embedded in copious mucilage.

This study was undertaken to determine the upper limit of temperature tolerance for the algae of these hot-springs.

Material and Methods

For detailed examination portions of the algal scum from the walls were collected (i) into hot-spring water in polythene bags stacked within a portable insulated chest and (ii) into a preservative made up of 50% Alcohol, Formalin, Glycerin, Glacial Acetic Acid and Copper Sulphate in the following proportions : 90 : 5 : 2.5 : 2.5 mls and 1.0 gram of $CuSO_4$.

Temperature measurements, with copper constantan thermocouple and with mercury thermometer, were made of

(i) Surface water in each well (thermometer),

(ii) Bottom water temperature in 2 of the wells having highest surface temperatures (thermocouple), and

(iii) overflow water running over the algal scum (thermometer).

From a calibration curve drawn for the thermocouple, the temperature difference between the surface and bottom of these 2 wells was read off and their bottom temperatures obtained by a simple calculation.

Thermal death-points were determined at the site in the following way : A 0.02% solution of the vital stain Methylene Blue was brought up to hot-spring temperature by partially immersing a beaker of it in the spring with the highest surface temperature. About 10 ml. of this warmed dye was transferred into a boiling tube also held partially immersed in the hot-spring, and then about 0.5 gms. (wet weight) of the algal scum was introduced into the tube so that the algae were completely covered by the Methylene Blue solution. After 20 minutes the Methylene Blue solution was drained off and the algae thoroughly washed in hot-spring water until the washings were colourless The stained algae were then transferred into a second tube with about 10 ml.. of hot-spring water and this tube warmed using a spirit lamp. The temperature as recorded by a mercury thermometer, at which the water in the tube became coloured was noted, a control tube of the same amount of dye treated algae in 10 mls. of hot-spring water being maintained. This experiment was repeated 4 times with Methylene Blue and a further 4 times with another vital dye, 0.02%(W/V) solution of neutral red. For comparison thermal death-points of blue green algae from a paddy field in Udahamulla, near the University, were similarly determined in a series of tests.

Results

I. Surface temperature and pH of water in wells.

Hot-spring	Temperature in °C	pH
A	54.0	7.5
B	48.0	7.6
C	52.3	7.4
D	54.0	7.4
E	54.0	7.5
F	55.0	7.5
G	32.5	7.5

II. Temperature of water flowing over algal scum on the outer surface of well.

Hot-spring	Temperature in °C
A	52.0
D	53.0

III. Thermocouple Readings and Computed Bottom Temperatures :

Hot-spring	Depth of water in well*	surface temp.	Galvanometer Deflection	Temp. difference between surface & bottom	Bottom temp.
A	48 cm.	54.0°C	16 units	6.5°C	60.5°C
F	105 cm.	55.0°C	17 units	9.0°C	64.0°C

* Sand shifted by flood-waters in the month previous to this visit was still lying in some of the wells.

IV. Thermal Death-Points (TDP) :

Source of Blue-green Algae	TDP in'C — Methylene Blue	TDP in 'C — Nuetral Red
Hot-springs (A+F)	72.2 71.5 72.6 70.4	73.0 72.5 71.8 72.5
Paddy-field	55.0 54.0 60.0 58.0	53.0 55.0 57.0 61.0

V. The algal flora of the Hot-springs :

Only Blue-greens were found in the collections made from the Hot-springs. Both filamen ous and unicellular colonial types were present, the former more abundaut outside, the latter inside the wells. The following families were most abundant :

(i) **Chroococcaceae**

Chroococcus limenticus Lemm. was the most prominent of these. Other types found were *Gleocapsa* sp. and *Cyanidium* sp.

(ii) **Oscillatoriaceae**

The filaments, embedded in mucilage, formed flat sheets, pale green in colour to the naked eye. *Oscillatoria* sp. was the commonest type ; and showed many concave discs (Fig. 4). *Lyngbya contorta Lemm. and L. circumcreta Lemm.* (Fig. 5). *Phormidium* sp., *Microcoleus* sp., *Schizothrix* sp. were other species found.

(iii) **Rivulariaceae**

These were found as bluish-green gelatinous clumps, types identified : *Calothrix sp.* (Fig. 4) and *Raphidiopsis curvata* Fritsch and Reich (Fig. 5).

(iv) **Nostocaceae**

Types identified were *Nostoc* sp. and *Anabaena* sp. (Fig. 4) *Anabaenopsis* sp. (Fig. 5).

(v) **Scytonemataceae**

Types identified. *Scytonema* sp., *Scytonematopsis* sp.

(vi) **Stigonemataceae**

Types identified. *Mastigocladus* sp.

Discussion

Of the 20 families of Blue-greens listed by Fritsch (1945), 6 are well represented in the Maha-oya hot springs ; and their growth was rich as might be expected from the fact that the pH of these waters ranges from 7.4 to 7.6 (Brock & Brock 1966). Copeland (1936) has recorded a similar algal flora of Blue-greens in the Yellowstone hot-springs. The absence from Maha-oya of Diatoms, another group of algae generally present in hot springs is note-worthy and is probably connected with the especially high temperature prevalent at Maha-oya, these being more than 10°C above the highest temperatures (40°) tolerated by diatoms (Fogg 1956).

The thermal death-point (70-73°C) of the Blue-green algae of these Maha-oya hot-springs lies within the ranges, extending from 65° to 85°C, which Fritsch (1945) states have been reported by various other workers in this field. Of special interest, however, is the fact that the TDP. of Blue-green algae collected from paddy fields the shallow waters of which often attain relatively high temperatures was found to be very much lower (53-61°C.). Absence of the nucleus which Prescott (1969) claimed is correlated with the ability to withstand high temperatures, is a character shared by the Blue-greens from both habitats and cannot by itself account for this difference. Fogg (1956) and Marre (1962) have attributed the heat-tolerance of hot-spring Blue-green algae to (1) the thermostability of their protoplasmic structure, and (ii) their low katabolic rate ; and Fogg claims that there are more cross-linked primary bonds in their protoplasmic proteins, most of the H-bonds being replaced by them. A comparative analysis of protein structure of Blue-greens from hot-springs and paddy-fields is likely therefore to provide revealing results. But it is worth noting here that contrary to their condition in paddy-fields, all the different species of Blue-green algae from hot springs were embedded in thick secretions of mucilage.

Another characteristic feature ofthe Blue-greens from the Hot-springs was their markedly small size. This may be a form of stunting.

It was noted that some of the Blue-greens from hot springs which had been stored in polythene bags at room temperature (29°C) showed signs of decay and death within 24 hours. And it is perhaps to be explained as due to a degree of adaptation to life at high temperature which includes a limit of lower temperature tolerance well above that of the surrounding atmosphere.

One final matter of interest is the presence in this flora of *Raphidiopsis curvata* a species which has been recorded by Fritsch and Reich in 1929 from Ohio in the U.S.A. (Smith, 1950).

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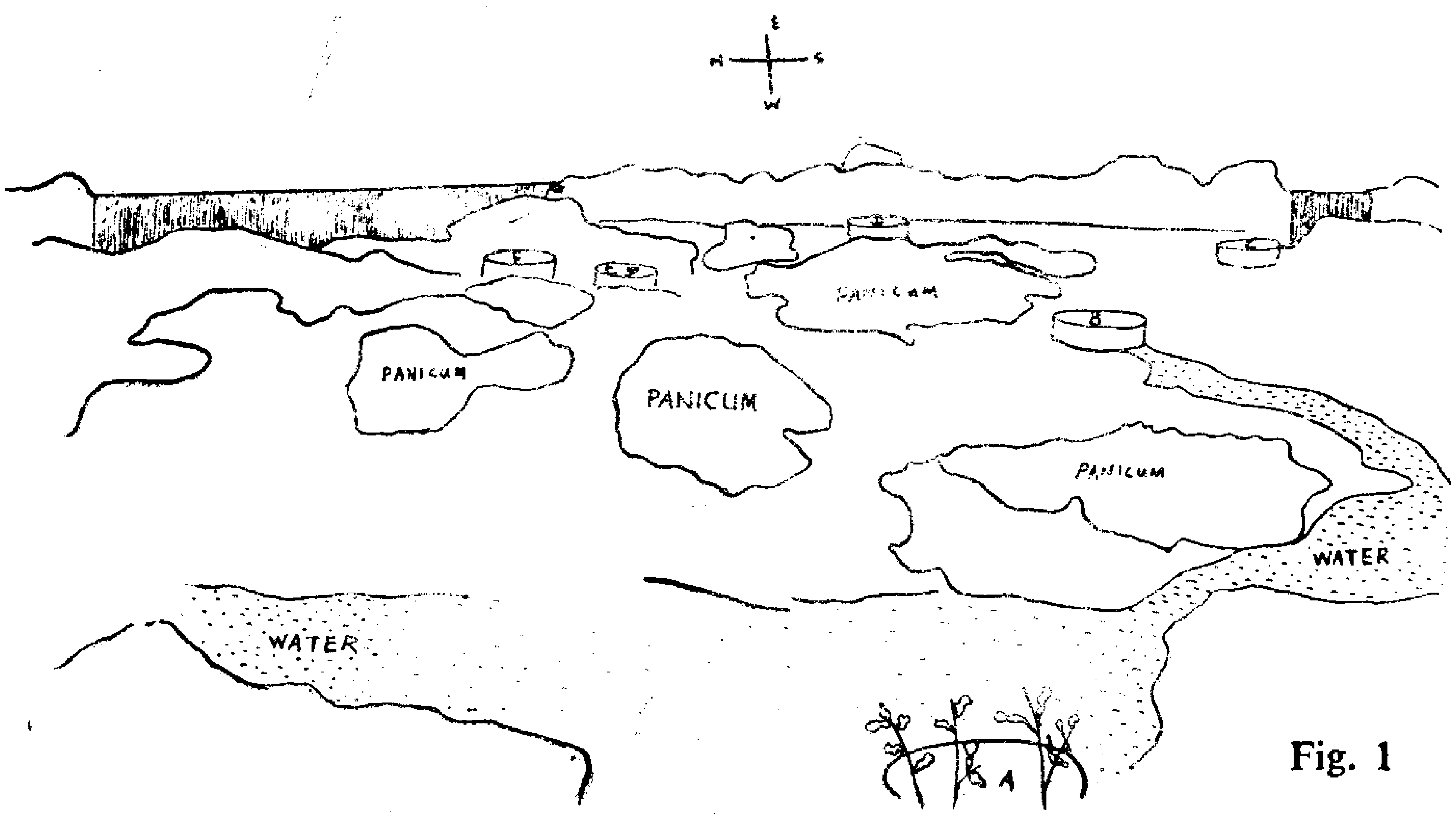

E
N
S
W
PANICUM
PANICUM
PANICUM
PANICUM
PANICUM
B
WATER
WATER
A

Fig. 1

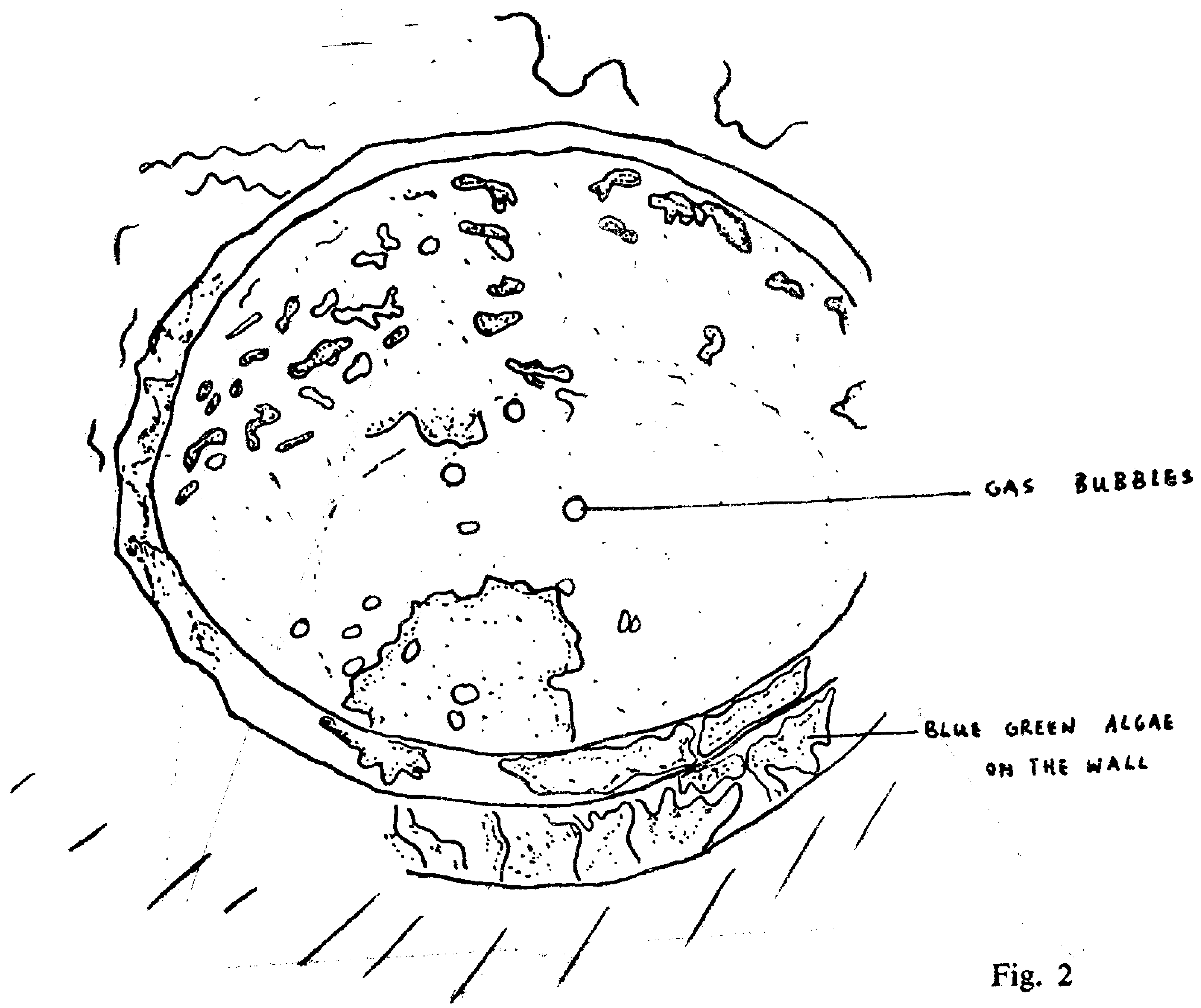

GAS BUBBLES
BLUE GREEN ALGAE
ON THE WALL

Fig. 2

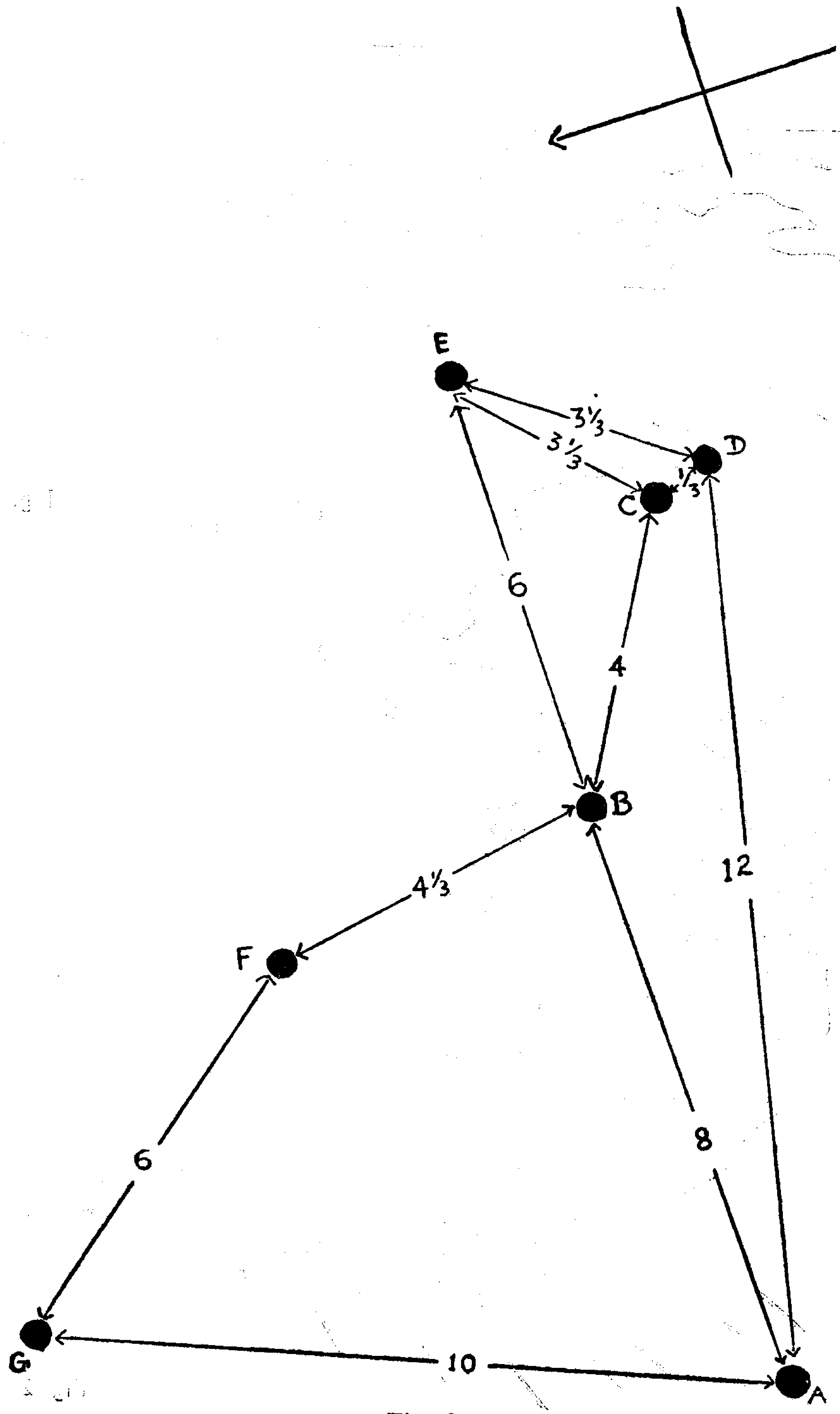
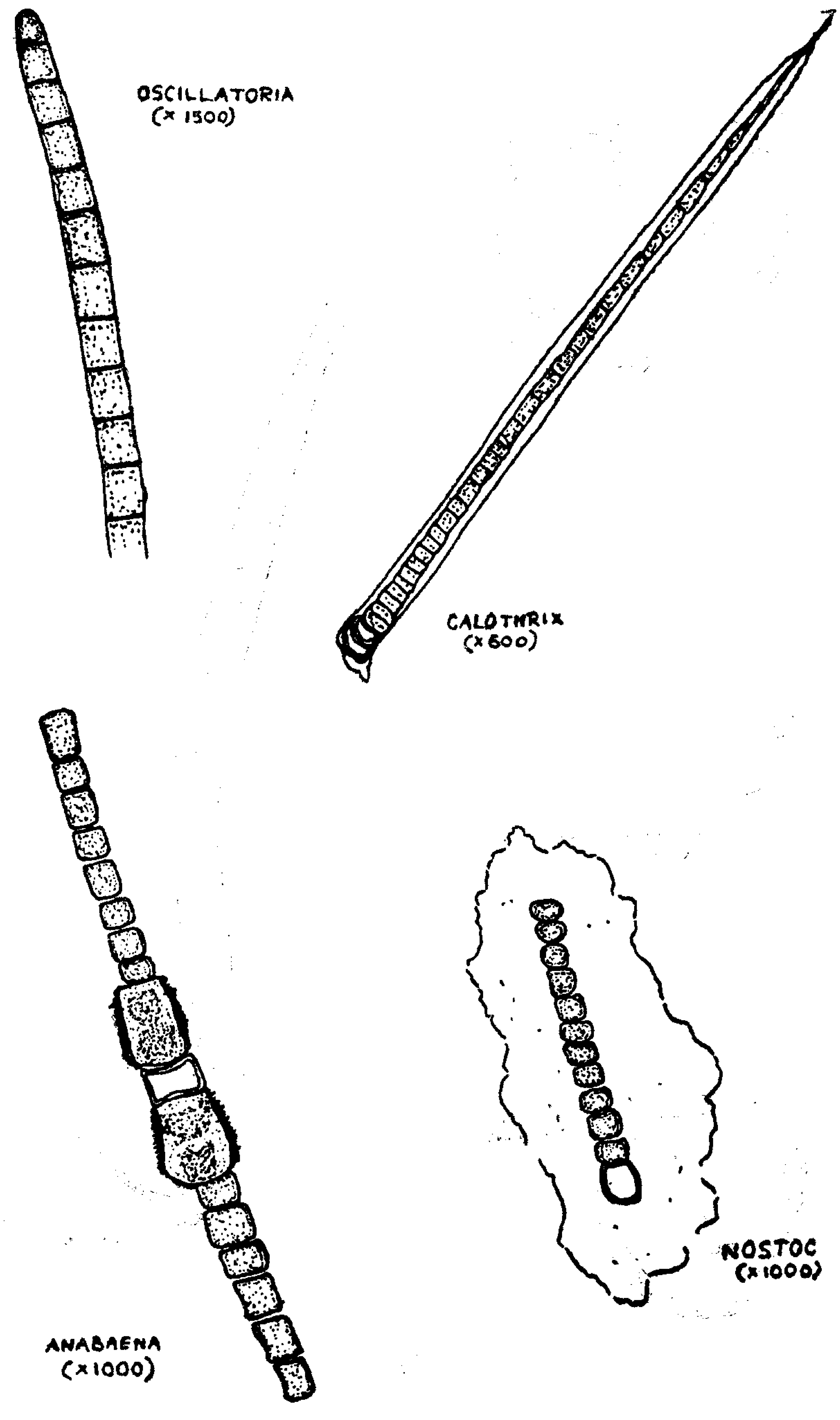


Fig. 3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(× 1500)
CALOTHRIX
(×600)
ANABAENA
(×1000)
NOSTOC
(×1000)

Fig. 4

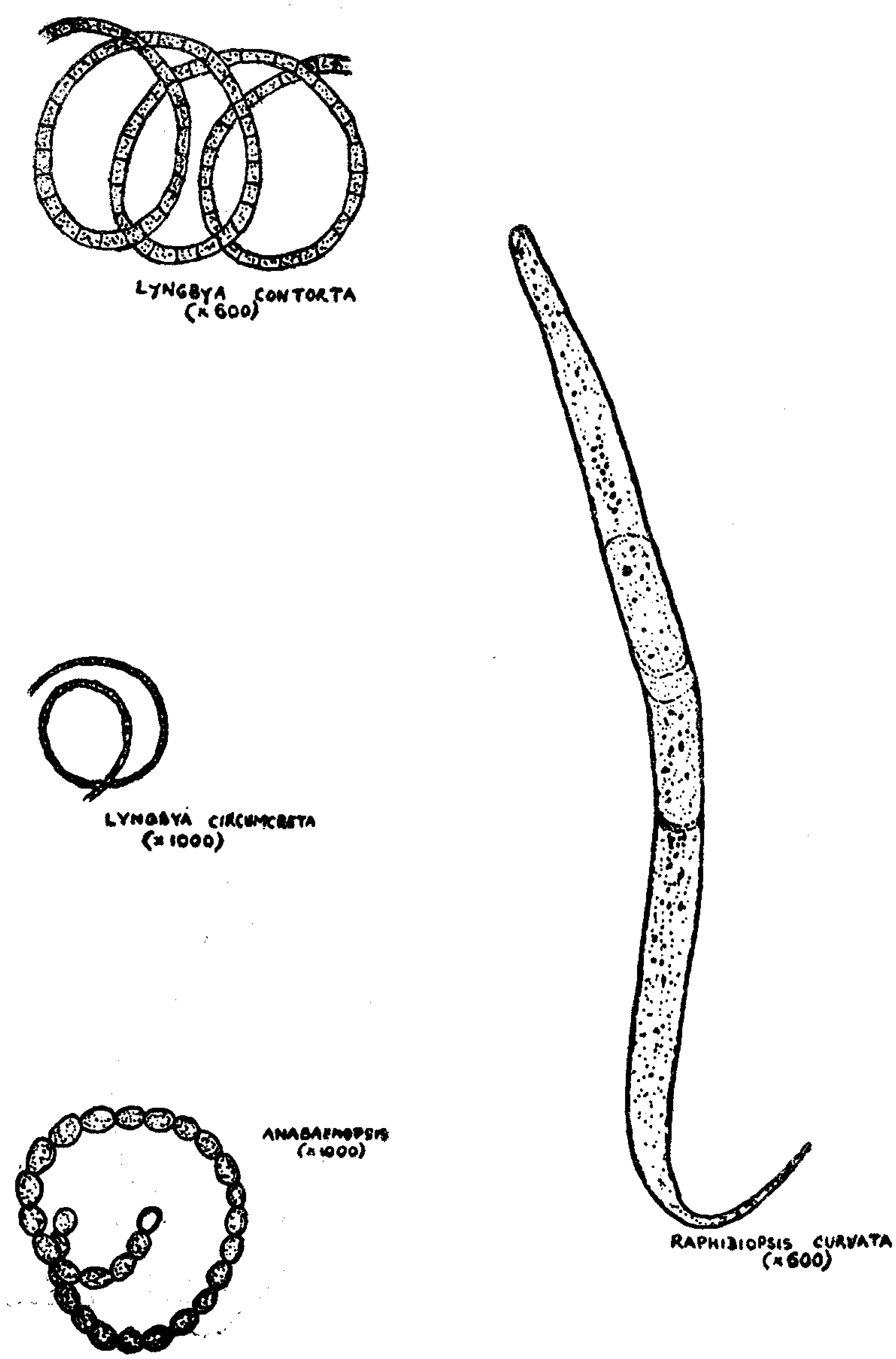
LYNGBYA CONTORTA
(×600)
LYNGBYA CIRCUMCRETA
(×1000)
ANABAENOPSIS
(×1000)
RAPHIDIOPSIS CURVATA
(×600)

Fig. 5