

**Bangor University**

## **DOCTOR OF PHILOSOPHY**

**Biosystematic and cytological studies of mosses and liverworts.**

Abderrahman, Salim Moh'd Mansur

*Award date:*  
1981

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Bangor University

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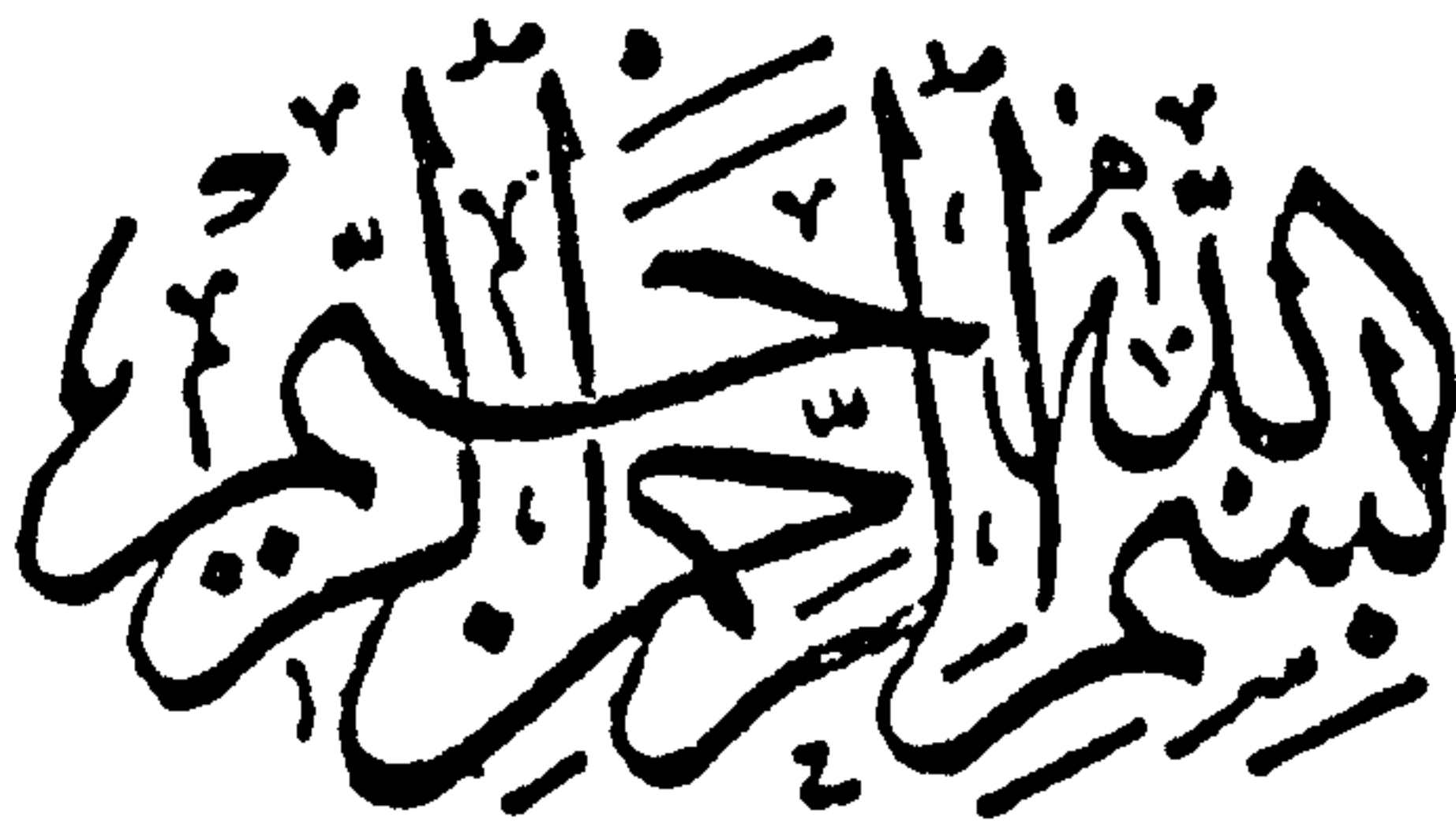
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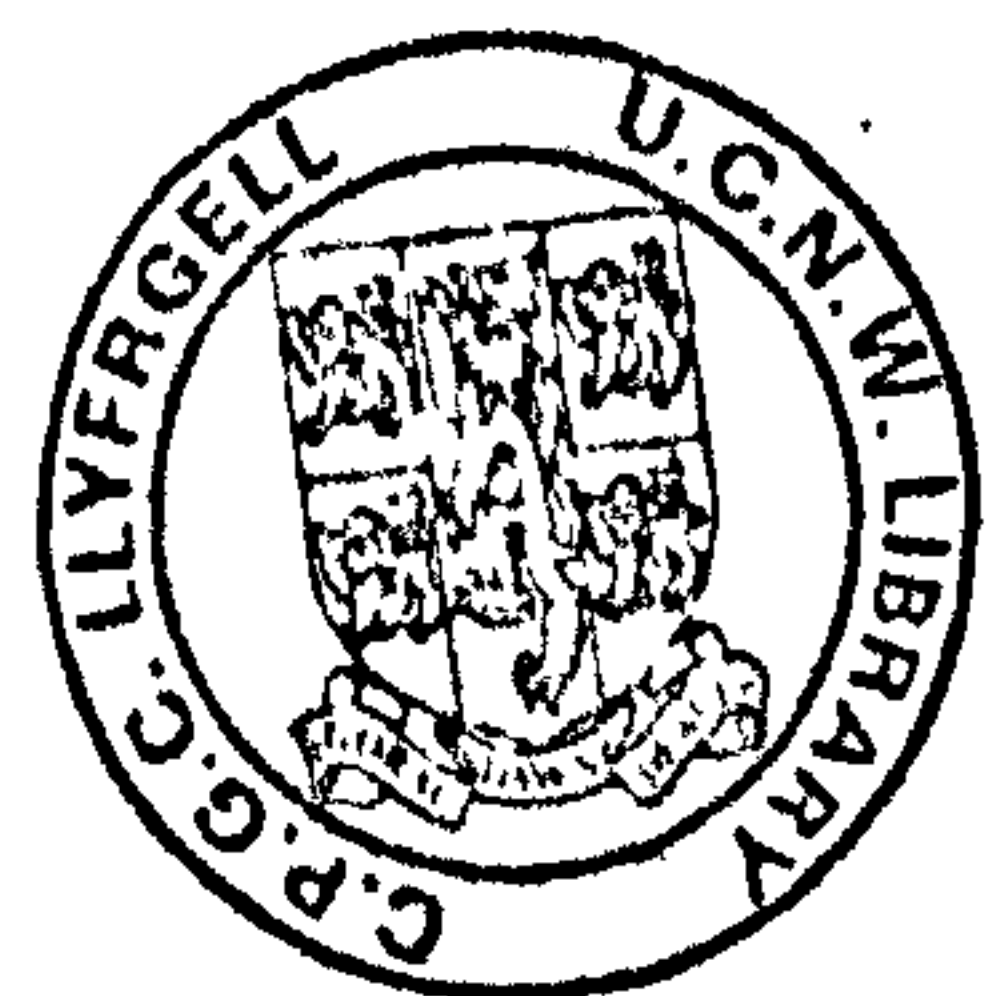
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BIOSYSTEMATIC AND CYTOLOGICAL STUDIES OF  
MOSSES AND LIVERWORTS

A thesis

Presented for the degree of

Philosophiae Doctor

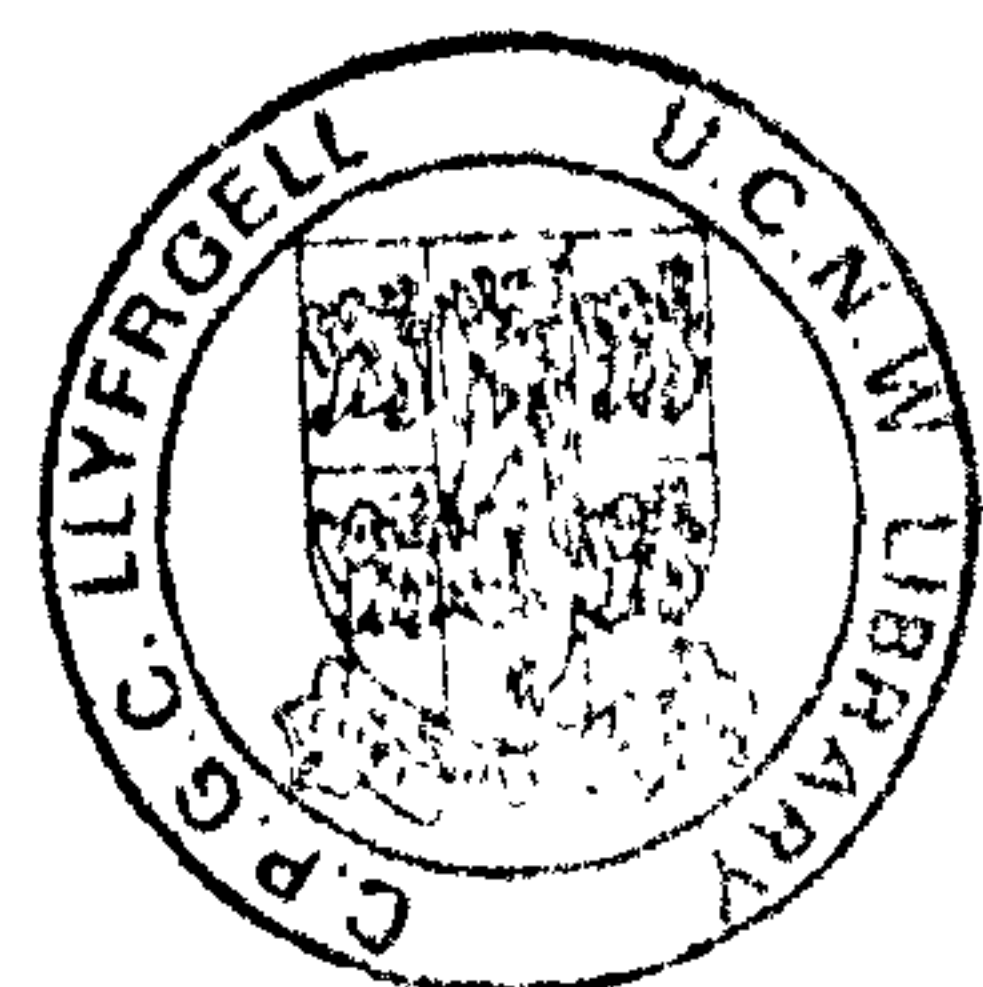
in the University of Wales

by

Salim Moh'd Mansur Abderrahman, B.Sc. Biology & Ed.

(Kuwait University)

School of Plant Biology,  
University College of North Wales,  
Bangor (U.K.).



### Summary

Biosystematic investigations have been carried out on members of the Hypnum cupressiforme aggregate which are variable morphologically and on Atrichum undulatum which is variable cytologically. Cultivation experiments have shown that variation in gametophyte plants of the Hypnum cupressiforme aggregate is partly genotypic and partly environmental and that there are four distinct species within the aggregate.

H. cupressiforme sensu stricto, H. jutlandicum, H. mammillatum and H. vaucheri. Within H. cupressiforme s.s. there are three varieties, var. cupressiforme, var. lacunosum and var. resupinatum and within H. mammillatum are two varieties, var. mammillatum and var. filiforme.

These conclusions are supported to some extent by physiological and electrophoretic studies but the aggregate is cytologically uniform.

In Atrichum undulatum there are haploid, diploid and triploid cytotypes which are indistinguishable both in field material and after cultivation, morphological variation being both genetical and environmental. Only a single taxon can be recognized although there are measurable differences in DNA content between the three cytotypes. There are significant differences in the relation frequencies of the three cytotypes in different parts of Britain.

### Acknowledgements

I am greatly indebted to Dr. A.J.E. Smith for valuable guidance, supervision and help throughout the study; to Professor J.L. Harper for the facilities provided in the School of Plant Biology, to Dr. M. Newton for her help and advice.

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## CHAPTER 1

### Hypnum cupressiforme aggregate

#### Introduction

There are eleven species of Hypnum recognized in Britain (Smith, 1978a; Ando and Townsend, 1980),, Hypnum bambergeri, H. callichroum, H. cupressiforme, H. hamulosum, H. imponens, H. jutlandicum, H. lindbergii, H. mammillatum, H. revolutum, H. uncinulatum and H. vaucheri. There are a further five European species that have not been found in Britain (Corley et al., 1981). Eight of the British species show little morphological or ecological variation and have posed no taxonomic problems, but the other three, H. cupressiforme, H. jutlandicum and H. mammillatum are a very different matter and their treatment has been a matter of controversy. Thus Wilson (1855) says about H. cupressiforme s.l. "extremely variable .....", Dixon and Jameson (1924) say: "Extremely variable", Rilstone (1948) described it as very variable, Nyholm (1954) says: "a strongly variable species .....", Watson (1968) says: "... most variable British moss" and in (1971) he says: "often regarded as the most variable British moss .....", Smith (1978a) says: "a very polymorphic species the nature of variation within which is unknown .....". Ando (1979) says "this species is one of the most variable mosses .....", and Crum and Anderson (1981) state: "Exceedingly common and notoriously variable in Europe .....".

H. cupressiforme s.l. (in the broad sense and which includes H. cupressiforme, H. jutlandicum and H. mammillatum) has been variously divided into varieties and sub-species and in some instances species have been segregated from it. Some of the taxa have been treated uniformly by most authorities whilst others have ranged in taxonomic status

from worthless form to species depending upon the author concerned. Treatment in Britain by Wilson (1855), Braithwaite (1895-1905), Dixon and Jameson (1924), Richards and Wallace (1950), Warburg (1963) and Smith (1978a) is shown in Table 1. The opinion of sixteen British bryologists gathered during preparation of The British and Irish moss Flora (Smith, 1978a) is shown in Table 2. From Table 2 it is evident that if views of the majority of the British bryologists asked are accepted, then var. ericetorum should be treated as a species, var. mammillatum as a sub-species and var. filiforme, lacunosum and resupinatum as varieties with var. lacunosum and var. tectorum being regarded as synonymous.

Treatment by other European bryologists has also varied. The opinions of bryologists in Finland (Koponen et al. 1977), Fennoscandia (Nyholm, 1954), Poland (Ochyra and Szmajada, 1978), Scandinavia (Jensen, 1939; Damsholt et al. 1969), Germany (Düll, 1977) and France (Guillamont, 1949; Doignon 1950, 1963) are shown in Table 3.

Some authorities treat H. vaucheri as a variety of H. cupressiforme (e.g. Wijk et al., 1963; Ando, 1972a; Crum and Anderson, 1981), others treat it as a distinct species (e.g. Ando, 1976; Smith, 1978a).

There is a record of H. vaucheri Lesq. from Yarmouth in Wilson (1855) but there has clearly been some confusion as he says it is intermediate between H. crassinervium (Cirriphyllum crassinerium) and H. piliferum (C. piliferum). He is probably referring to H. vaucheri Rabuh ( $\equiv$  C. tenuinerve (Lindb.) Wijk & Marg.) a plant not recorded from Britain.



Table 1. The treatment of British bryologists to Hypnum cupressiforme s.l.

Wilson (1855)	Braithwaite (1895-1905)	Dixon and Jameson (1924)	Richards and Wallace (1950)	Warburg (1963)	Smith (1978)
var. <u>compressum</u>					
(= var. <u>cupressiforme</u> )					var. <u>cupressiforme</u>
var. <u>minus</u>		var. <u>minus</u>			
(= forma)		(= forma)			
var. <u>longisetum</u>					
(= forma)					
var. <u>nigroviride</u>					
(= forma)					
var. <u>filiforme</u>	var. <u>filiformis</u> (= var. <u>filiforme</u> )	var. <u>filiforme</u>	var. <u>filiforme</u>	var. <u>filiforme</u>	var. <u>filiforme</u> (treated as synonym <u>QMS</u> with <u>H. mammillatum</u> )
var. <u>mammillatum</u>	var. <u>mammillatum</u>	var. <u>mammillatum</u>	var. <u>mammillatum</u>	var. <u>mammillatum</u>	<u>H. mammillatum</u>
(= <u>H. mammillatum</u> )	(= <u>H. mammillatum</u> )	(= <u>H. mammillatum</u> )	(= <u>H. mammillatum</u> )	(= <u>H. mammillatum</u> )	



Table 1. cont'd

Wilson (1855)	Braithwaite (1895-1905)	Dixon and Jameson (1924)	Richards and Wallace (1950)	Warburg (1963)	Smith (1978)
var. <u>resupinatum</u>	var. <u>resupinatum</u>		var. <u>resupinatum</u>	var. <u>resupinatum</u>	var. <u>resupinatum</u>
var. <u>lacunosum</u>			var. <u>lacunosum</u>	var. <u>lacunosum</u>	var. <u>lacunosum</u>
	var. <u>tectorum</u>	var. <u>tectorum</u>	var. <u>tectorum</u>	var. <u>tectorum</u>	
	(= var. <u>lacunosum</u> )	(= var. <u>lacunosum</u> )	(= var. <u>lacunosum</u> )	(= var. <u>lacunosum</u> )	
	var. <u>elatum</u>	var. <u>elatum</u>	var. <u>elatum</u>		
	(= var. <u>lacunosum</u> )	(= var. <u>lacunosum</u> )	(= var. <u>lacunosum</u> )		
	var. <u>ericetorum</u>	var. <u>ericetorum</u>	var. <u>ericetorum</u>	var. <u>ericetorum</u>	H. <u>jutlandicum</u>
	(= H. <u>jutlandicum</u> )	(= H. <u>jutlandicum</u> )	(= H. <u>jutlandicum</u> )	(= H. <u>jutlandicum</u> )	
	var. <u>uncinatulum</u>				
	(= var. <u>uncinatulum</u> )				
	var. <u>longirostris</u>				
	(= forma)				
	var. <u>tenu</u>				
	(= forma)				
	var. <u>implexum</u>				
	(= H. <u>revolutum</u> )				H. <u>vaucheri</u>

Table 2. Showing the opinion of sixteen British bryologists about the treatment of Hypnum cupressiforme s.l.

Taxon	Species	Sub-species	Variety	Forma
resupinatum	3	2	10	1
filiforme	2	-	13	1
mammillatum	5	6	5	-
ericetorum	8	3	5	-
tectorum	1	1	12	2
lacunosum	1	3	10	2

Ten out of the sixteen British bryologists suggest that lacunosum and tectorum belong to a single taxon.

Table 3. The treatment of European bryologists to Hypnum cupressiforme s.l.

---

FINLAND (1)	mammillatum, resupinatum, ericetorum, filiforme, lacunosum, vaucheri
FENNOSLANDIA (2)	filiforme, lacunosum, resupinatum, vaucheri, mammillatum, ericetorum
POLAND (3)	cupressiforme, ericetorum, lacunosum, mammillatum, resupinatum, subjulaceum ( H. cupressiforme forma)
SWEDEN (4)	resupinatum, mammillatum, ericetorum, vaucheri,
DENMARK (5)	mammillatum, jutlandicum
DDR (6)	filiforme, mammillatum, lacunosum, resupinatum, jutlandicum, vaucheri
FRANCE (7)	tectorum, filiforme, mammillatum
FRANCE (8)	tectorum, uncinatum ( H. cupressiforme forma), filiforme, resupinatum, mammillatum, subjulaceum, ericetorum
FRANCE (9)	imbricatum, elatum, tectorum, longirostrum, resupinatum, filiforme, cuspidatum ( H. cupressiforme forma), imponens, mexicanum ( H. cupressiforme forma), ericetorum.

---

1. Koponen, Isoviita & Lamres (1977); 2. Nyholm (1954);
3. Ochyra & Szmajda (1978); 4. Jensen (1939); 5. Damsholt et al. (1969);
6. Dull (1977); 7. Guillamont (1949); 8. Doignon (1950);
9. Doignon (1963)

The taxa that have been recognized in Britain since Wilson (1855) are:

var. cupressiforme

var. elatum

var. ericetorum

var. filiforme

var. lacunosum

var. mammillatum

var. resupinatum

var. tectorum

H. cupressiforme var. elatum was reduced to synonymy with H. cupressiforme var. lacunosum by Limpricht (1904), var. tectorum reduced to a forma of var. lacunosum by Podpera (1954), var. ericetorum and var. mammillatum are treated as species by Nyholm (1954) and Smith (1978a), var. filiforme is reduced to synonymy with H. mammillatum by Smith (1978a).

Clearly the status of the taxa of H. cupressiforme s.l. is controversial and it has been the aim of this study to investigate the nature and range of variation between and within taxa as well as assessing the status of the various taxa.

## CHAPTER 2

### Variation in morphology

#### 2.1 Materials and Methods

For convenience the name H. cupressiforme s.l. (sensu lato) is used to cover all taxa of the aggregate in general descriptions etc.

Several approaches were used in the study of H. cupressiforme s.l.

These were:

1. Studies of field and herbarium specimens
2. Cultivation experiments
3. Phytochemistry
4. Spore wall ornamentation
5. Cytological investigation
6. Drought tolerance
7. Ecology and distribution

#### 2.2 Study of living and herbarium specimens

Material of H. cupressiforme s.l. was collected in the field.

Each gathering was divided into two parts, one of which was cultured. the other preserved as an herbarium specimen. Herbarium material from Bangor (U.C.N.W.), Glasgow (G.L.) and Edinburgh (E) was studied.

Living material was cultivated at the School of Plant Biology, Pen-y-Ffridd Field Station, Bangor. The specimens were grown in an unheated glasshouse in a mist unit on peat-based potting compost in 10 inch (25 cm) flower pots. The pots were covered with transparent polythene hoods to prevent cross-contamination. The plants were unshaded and watered with tap water (pH 7.5). As all the cultures were grown in a limited area (250cm x 120cm) at the same time, it could be assumed that growth was under uniform conditions. It was found that three months growth was adequate for the purposes required.



Samples were taken from living material before and after culture under uniform conditions were dried for later examination in the laboratory. Twenty five samples with five replicates from each of the gatherings was found to give satisfactory results.

The samples were dissected and the plants mounted in gum chloral and examined using a Leitz "laborlux" microscope. Measurements were made with an eyepiece micrometer .

### 2.3 Characters selected for the study of *H. cupressiforme* s.l.

In the past relatively few morphological characters have been used for the discrimination of the taxa within *H. cupressiforme* s.l.

(see for example, Dixon, 1924; Nyholm, 1954; Smith, 1978a). These include gross morphology, branching pattern, shape of pseudoparaphyllia, leaf shape, leaf apex shape, nature of the leaf margin, nerve strength, basal cell shape, angular cell morphology, leaf cell size, capsule shape, posture of capsule, operculum shape and peristome structure. The following characters were studied and found to be of use.

#### 1. Gross morphology

- (a) Plant size
- (b) Branching pattern
- (c) Pseudoparaphyllum shape

#### 2. Leaf morphology and anatomy

- (a) Leaf length
- (b) Leaf curvature
- (c) Leaf apex shape
- (d) Nerve strength
- (e) Nerve length
- (f) Cell size
- (g) Angular cell shape

(h) Angular cell wall thickness

(i) Angular cell size

### 3. Sporophyte

(a) Seta length

(b) Capsule posture

(c) Capsule length

(d) Lid shape

(e) Lid length

(f) Spore size

Other characters sometimes thought to have some taxonomic value include plant colour and nerve width and which show a great degree of variability within H. cupressiforme s.l. were discarded after a preliminary study.

Measurements of gametophyte characters were made on field material before and after culture. Measurements of sporophyte characters were made on herbarium and field gatherings only as culture was not long enough for the growth of sporophyte. Plants were identified using the nomenclature of Smith (1978a) and additionally var. filiforme, which Smith (1978a) treats as a synonym of H. mammillatum, was recognized.

#### 1. Gross morphology

(a) Plant size: plants were scored for three categories of size: large, medium and small. All plants except var. filiforme came within the first two categories and intergraded. 70% of var. filiforme was small but after culture only 34% of plants remained small. It would appear that plant size, which is of some importance in discriminating var. filiforme, is partly an environmental effect and is of questionable taxonomic value.

(b) Branching pattern: plants were scored for three categories of branching pattern : irregular, loosely pinnate and densely pinnate. All plants except H. jutlandicum fell within the first two categories and intergrade. 88% of H. jutlandicum gatherings were densely pinnate and almost the same proportion (84%) remained so after culture. This character appears to be genetically determined and of good taxonomic value.

(c) Pseudoparaphyllia small,  $\pm$  leaf-like structures surrounding branch primordia and found at the base of young branches. Three categories were scored: narrow, medium and wide. H. vaucheri has much wider pseudoparaphyllia than H. cupressiforme s.l. (see Fig. 7d). Other plants came within the first two categories with 63% of them with medium width pseudoparaphyllia. This character is valuable for discriminating H. vaucheri.

## 2. Leaf morphology and anatomy

(a) Leaf length: reference to Figs. 1a-6a and Table 4 shows that there is a considerable degree of overlapping. The amount of variation in leaf length among all plants of H. cupressiforme under uniform conditions is markedly lower than that in the wild, suggesting high environmental effects. In gatherings named var. filiforme the amount of variation is almost the same in plants under both conditions whilst a slight increase in leaf length is shown (see Table 4), indicating very low environmental effects.

(b) Leaf curvature: Three categories were scored: slightly curved, curved and strongly curved. H. mammillatum tends to have strongly curved leaves and almost all plants retained the character after culture suggesting it is genetically determined. This character, moreover, has a good value in discriminating between H. mammillatum and var. cupressiforme. All plants of H. cupressiforme came within the first two categories, this did not change after culture, taxa intergraded with one another and with var. cupressiforme. It would appear that leaf curvature



Fig. 1. Morphology of leaves and capsule of var. cupressiforme:

- |                        |                    |
|------------------------|--------------------|
| (a) leaf;              | (b) angular cells; |
| (c) pseudoparaphyllum; | (d) capsule;       |
| (e) cells;             | (f) capsule lid;   |
| (g) spores.            |                    |

0.1mm

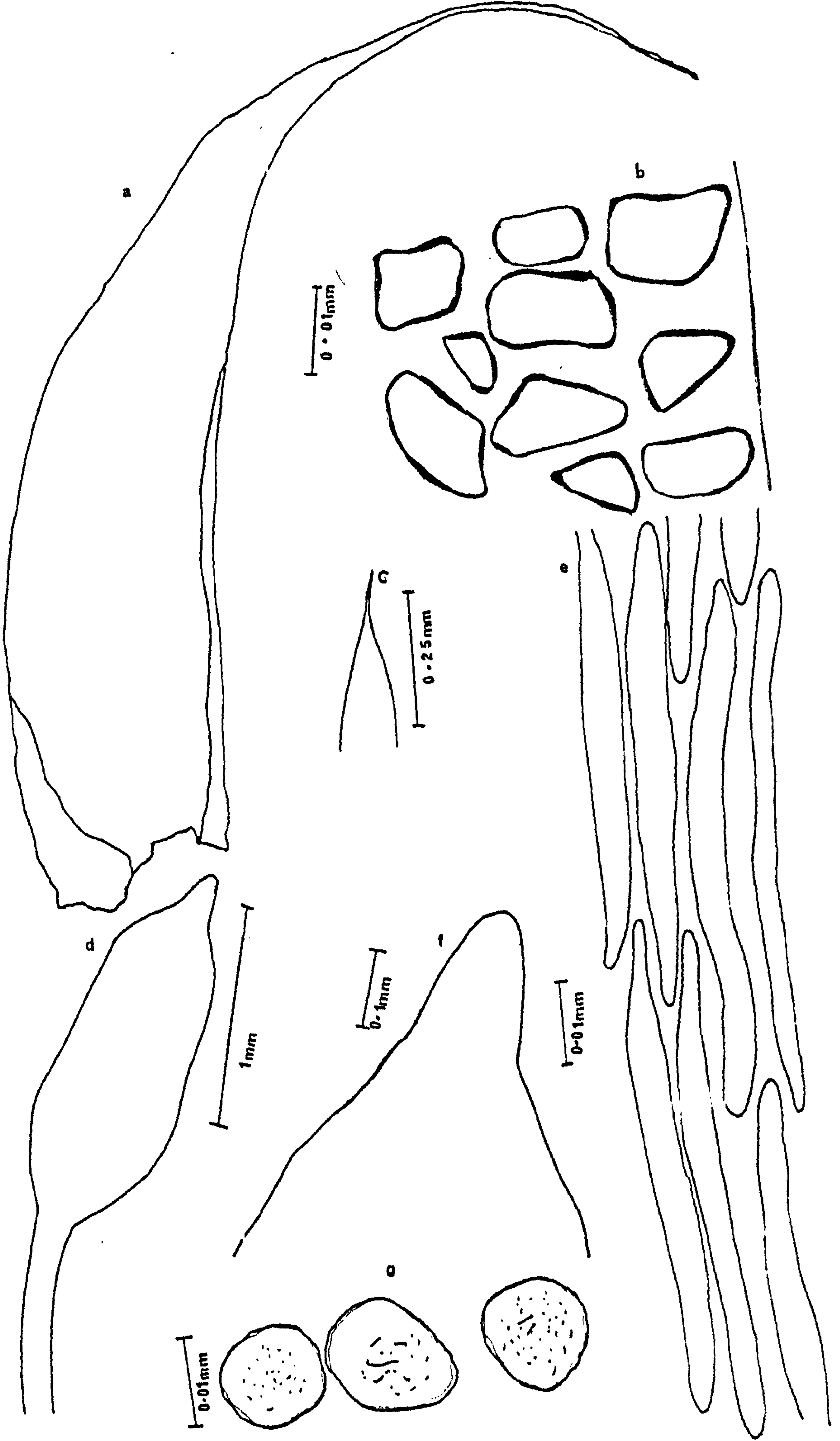


Fig. 2. Morphology of leaves and capsule of var. resupinatum:

- |                        |                    |
|------------------------|--------------------|
| (a) leaf;              | (b) angular cells; |
| (c) pseudoparaphyllum; | (d) capsule;       |
| (e) cells;             | (f) capsule lid;   |
| (g) spores.            |                    |

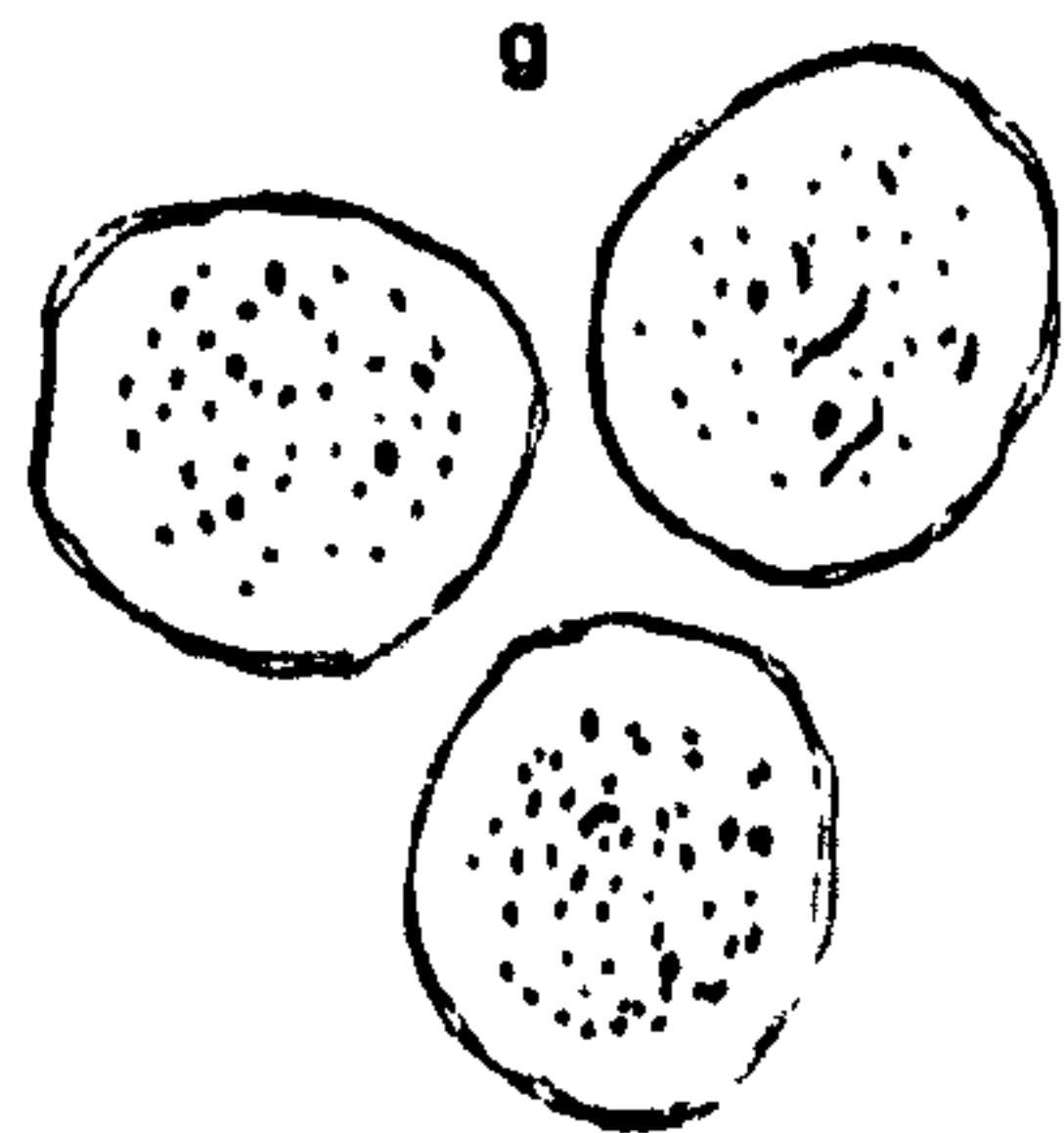
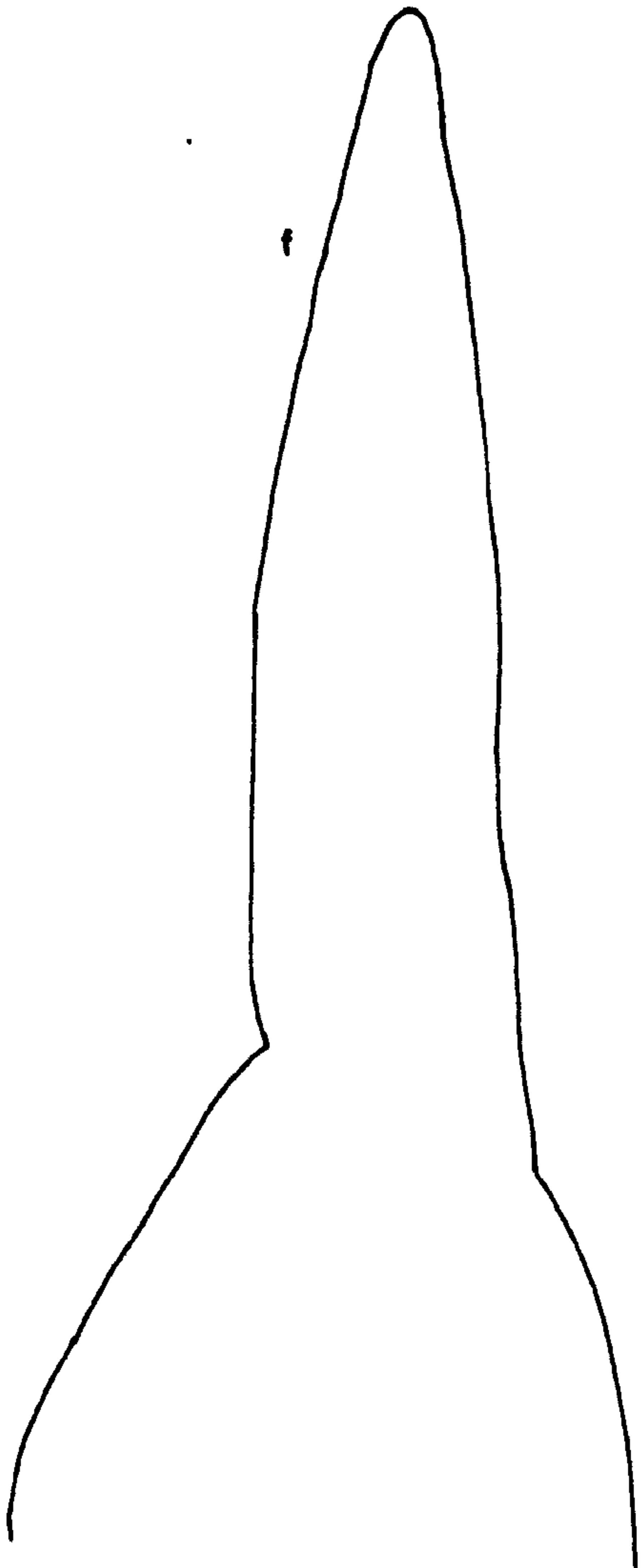
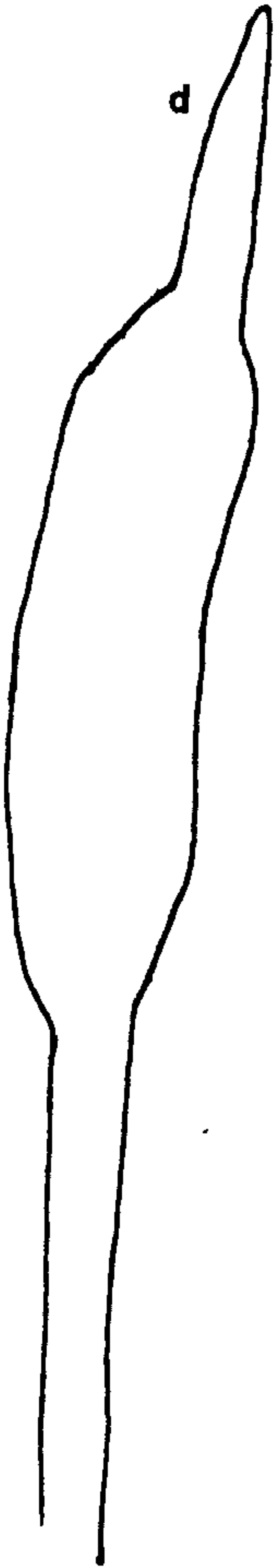
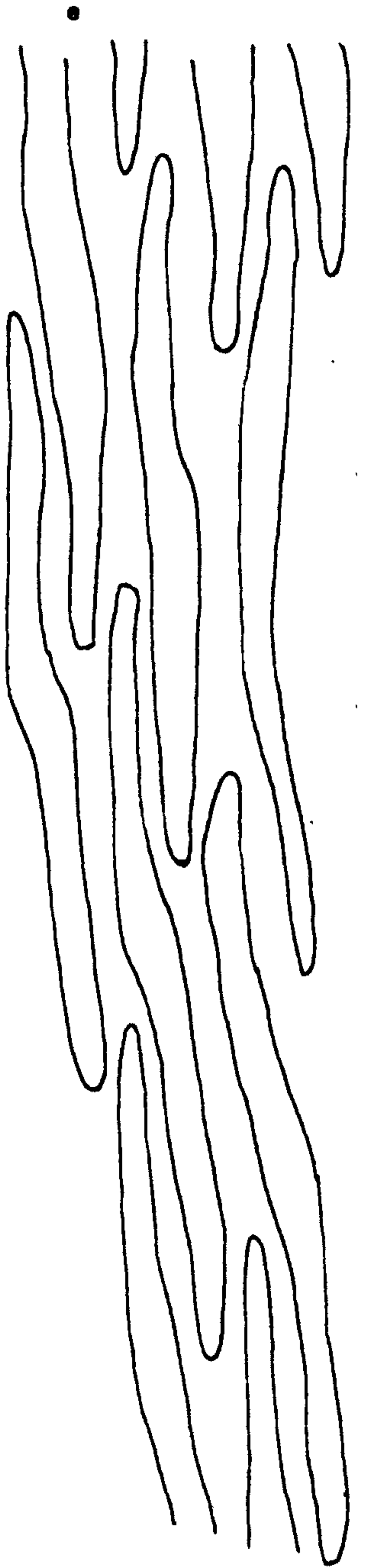
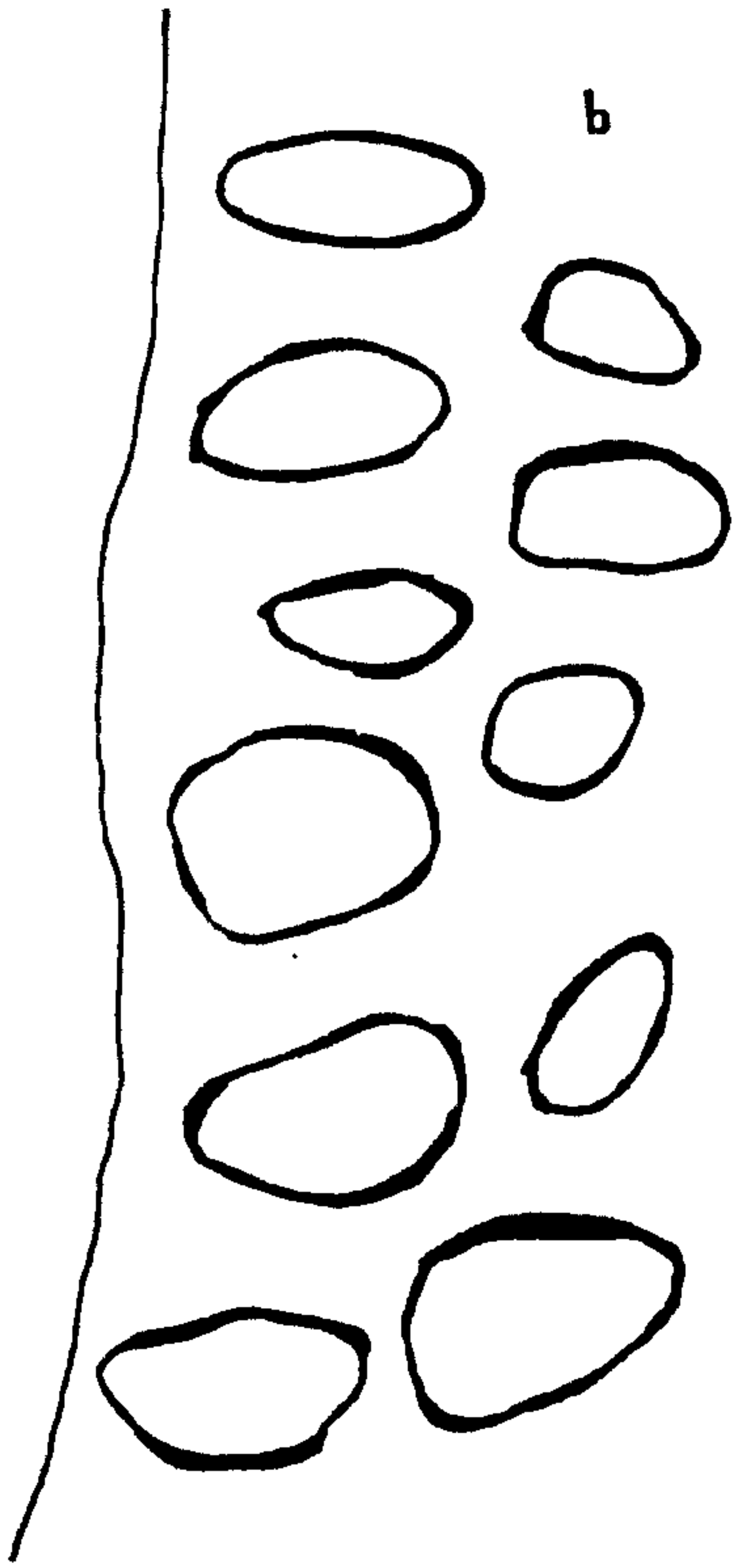
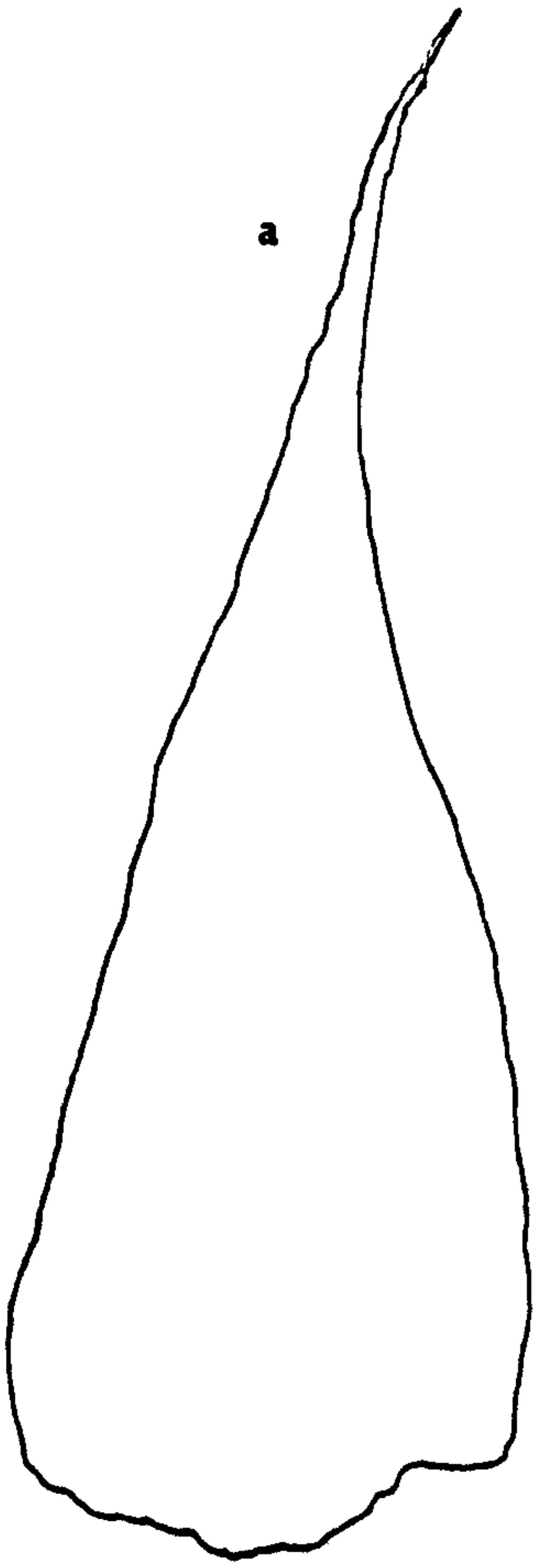


Fig. 3. Morphology of leaves and capsule of var. lacunosum:

- |                         |                     |
|-------------------------|---------------------|
| (a) leaf ;              | (b) angular cells ; |
| (c) pseudoparaphyllum ; | (d) capsule ;       |
| (e) cells ;             | (f) capsule lid ;   |
| (g) spores .            |                     |

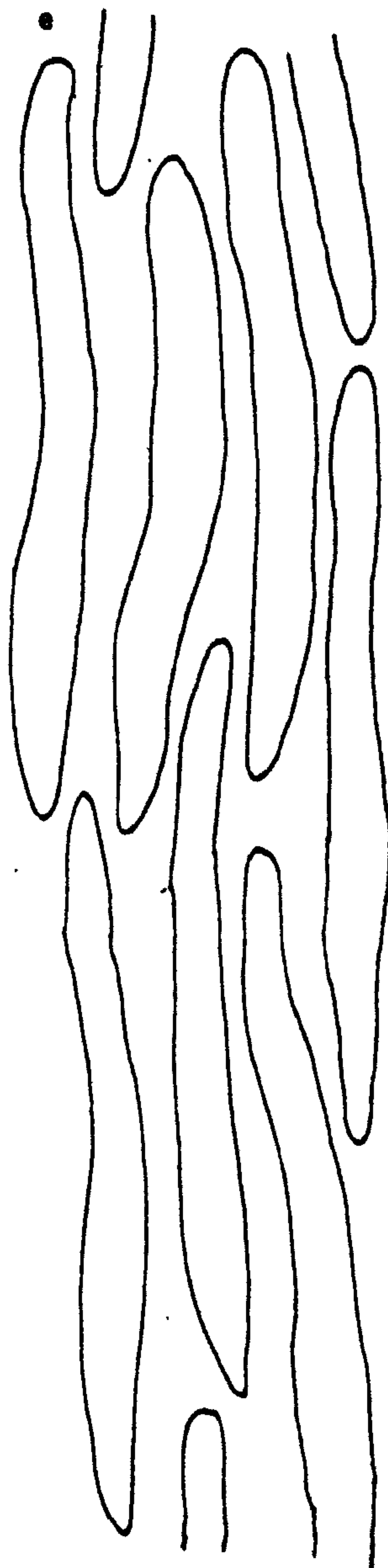
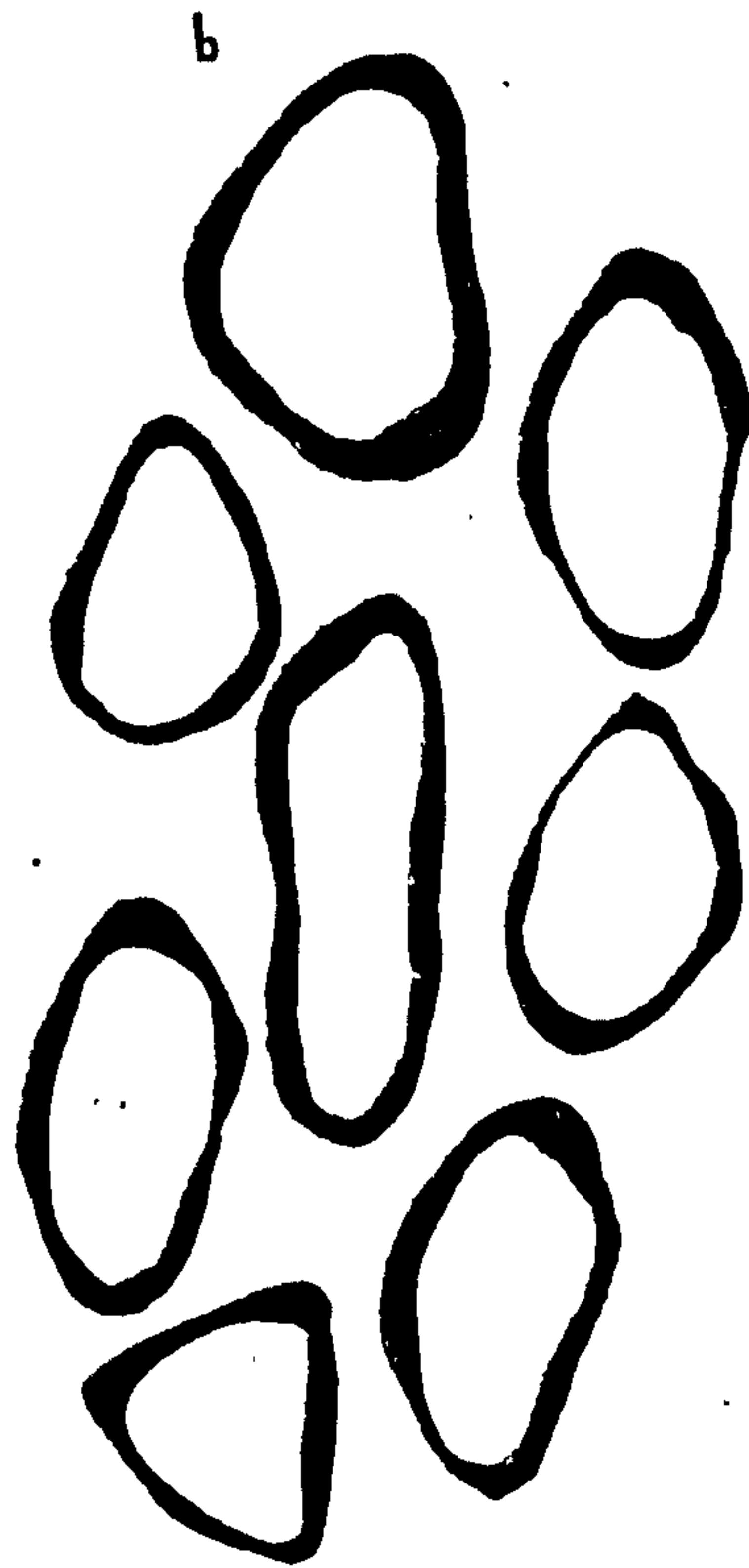
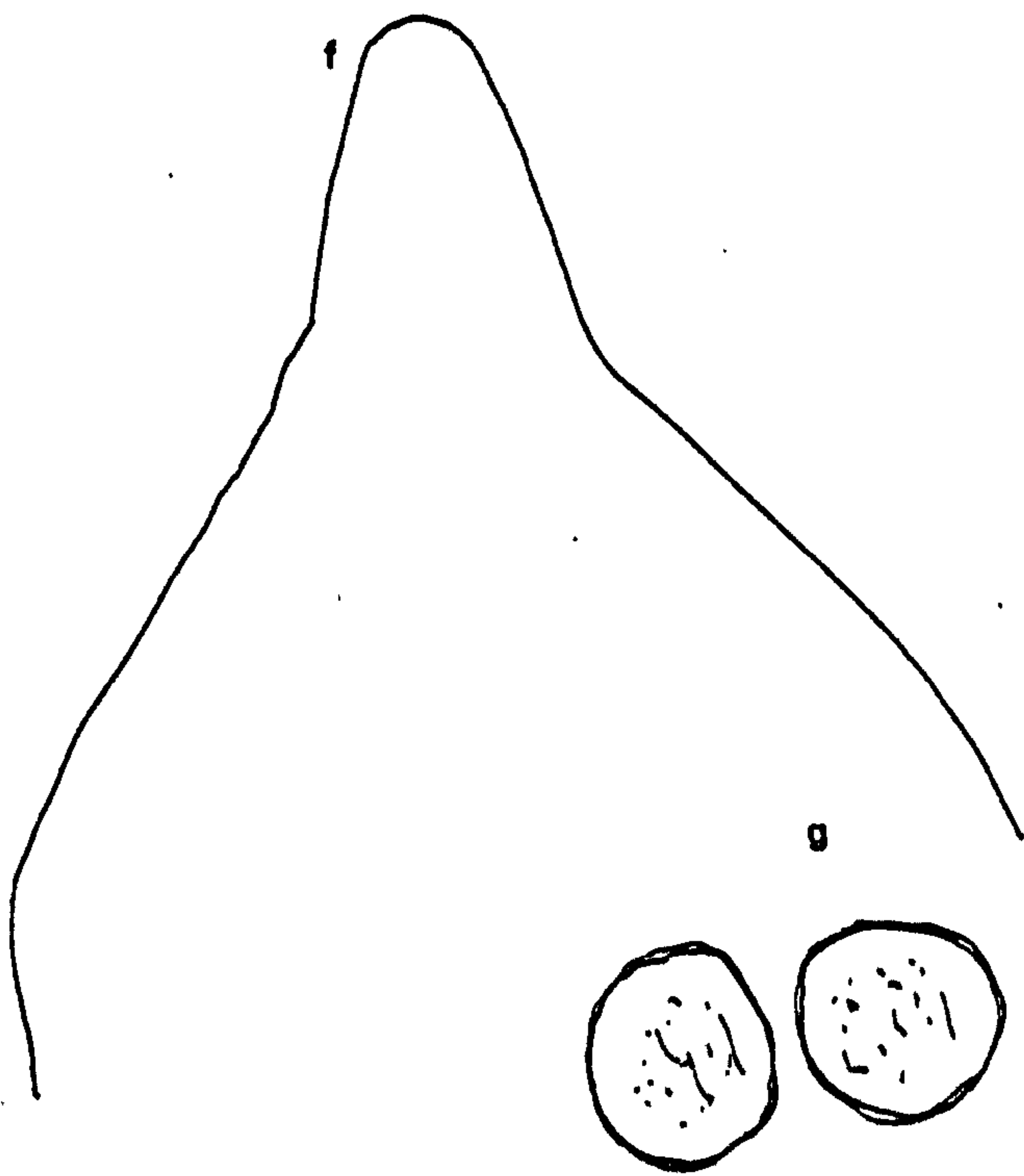
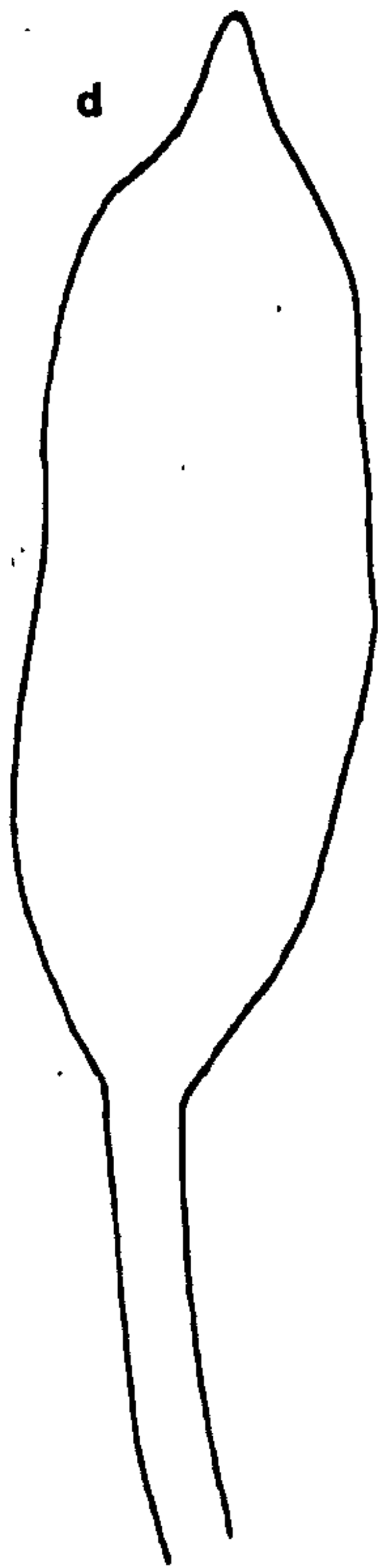
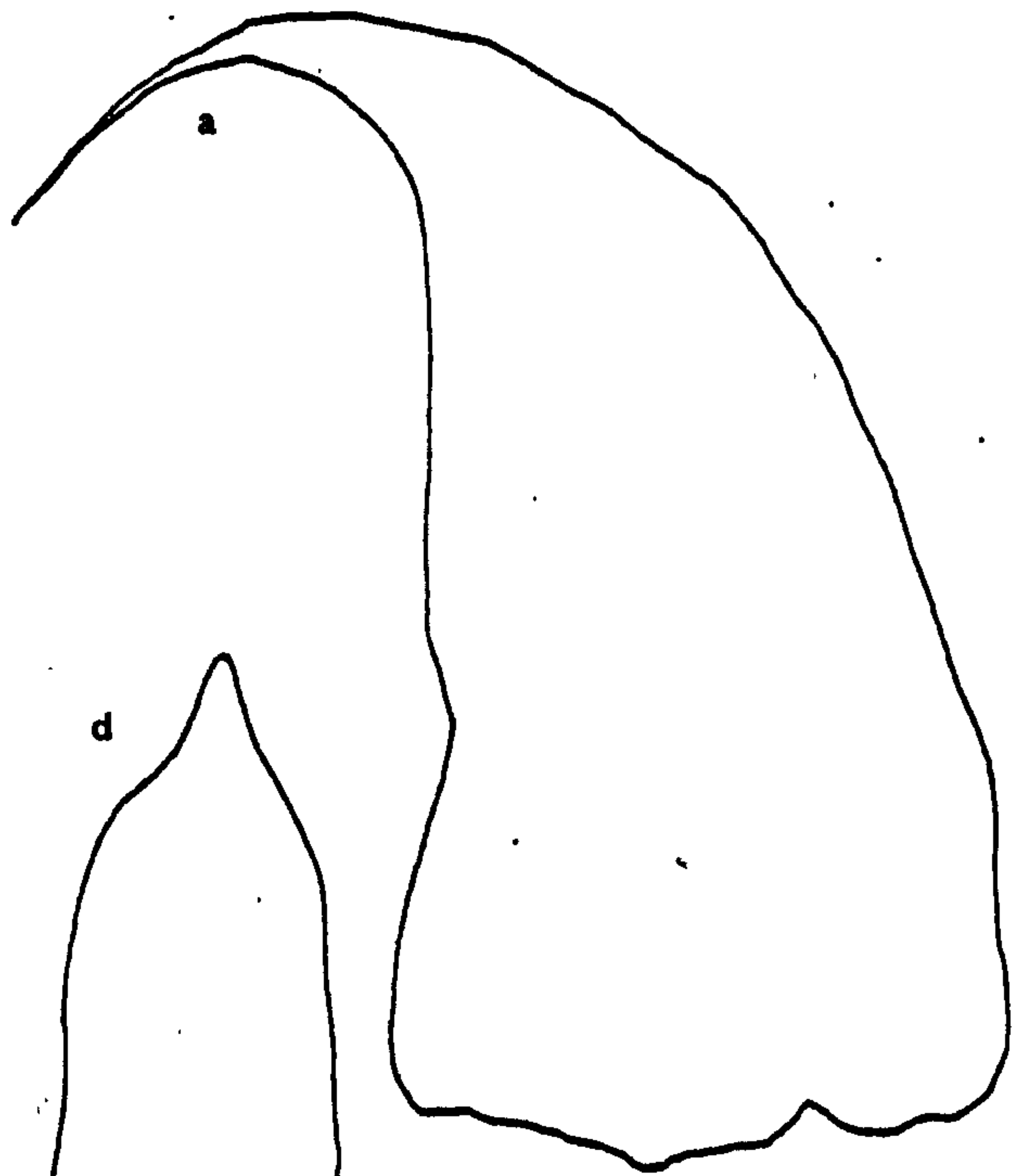


Fig. 4. Morphology of leaves and capsule of H. mammillatum:

- |                         |                     |
|-------------------------|---------------------|
| (a) leaf ;              | (b) angular cells ; |
| (c) pseudoparaphyllum ; | (d) capsule ;       |
| (e) cells ;             | (f) capsule lid ;   |
| (g) spores .            |                     |

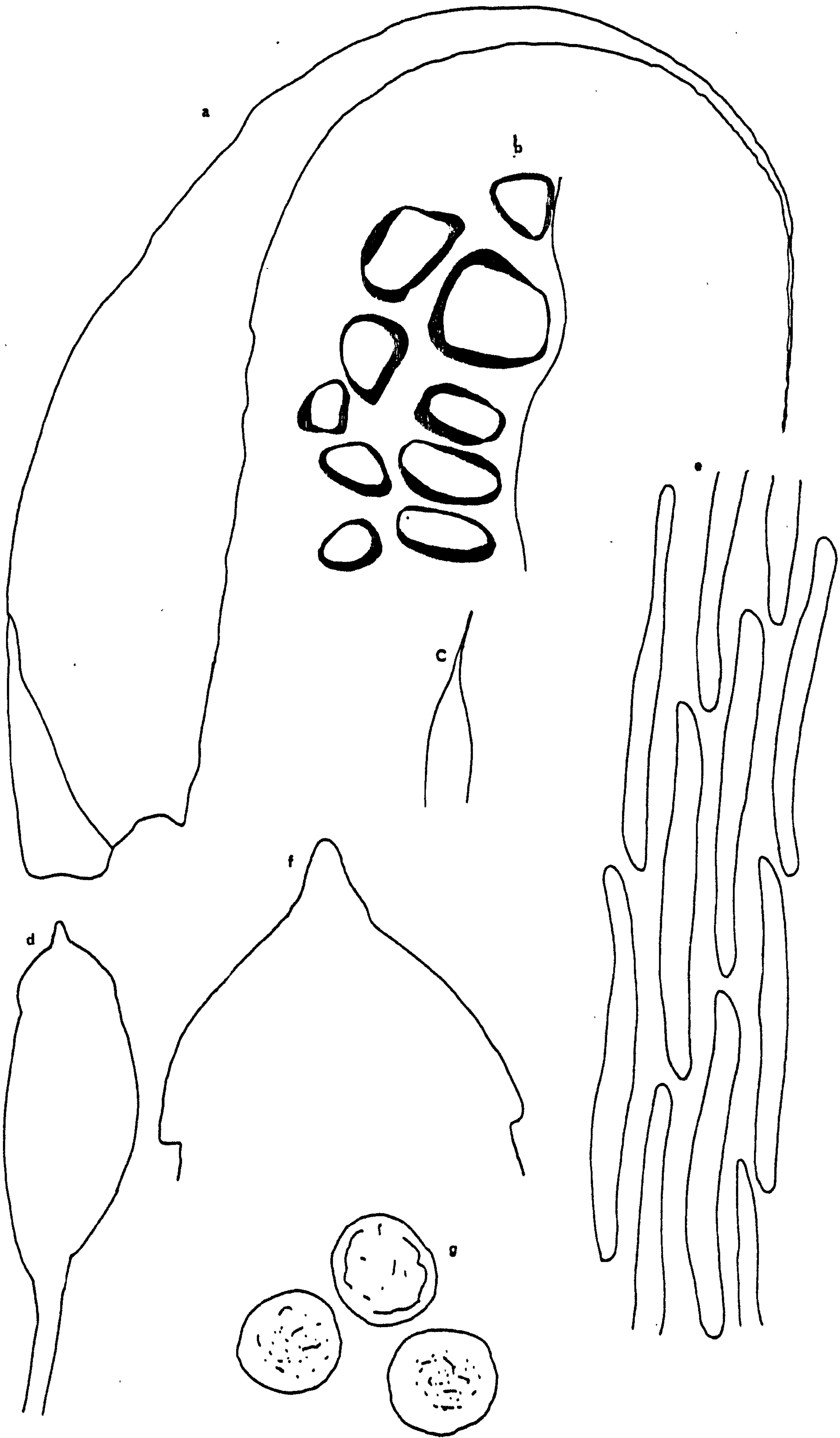




Fig. 5. Morphology of leaves and capsule of var. filiforme:

- |                         |                     |
|-------------------------|---------------------|
| (a) leaf;               | (b) angular cells ; |
| (c) pseudoparaphyllum ; | (d) capsule ;       |
| (e) cells;              | (f) capsule lid ;   |
| (g) spores.             |                     |

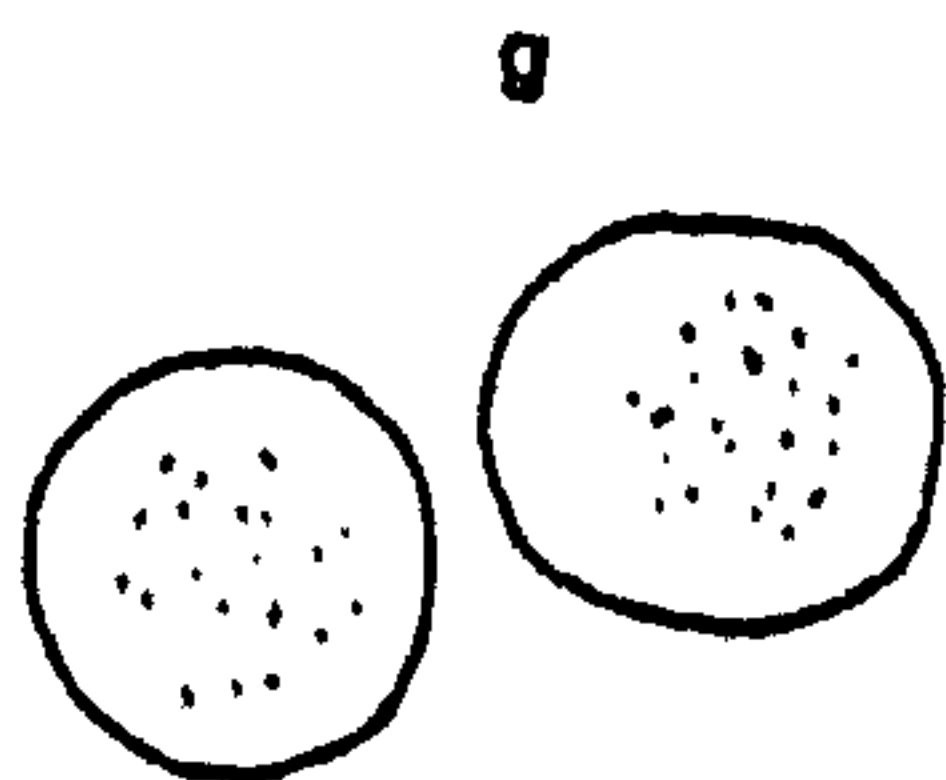
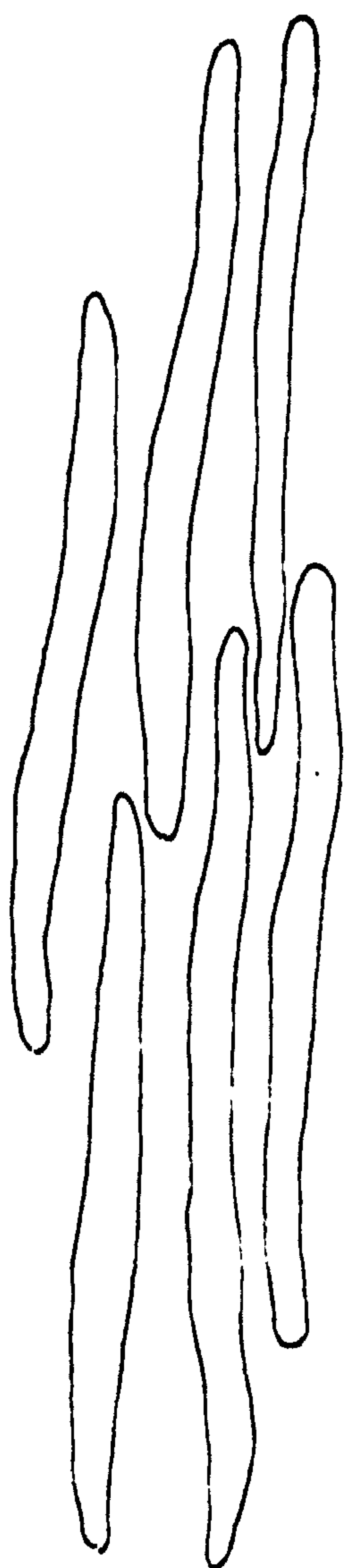
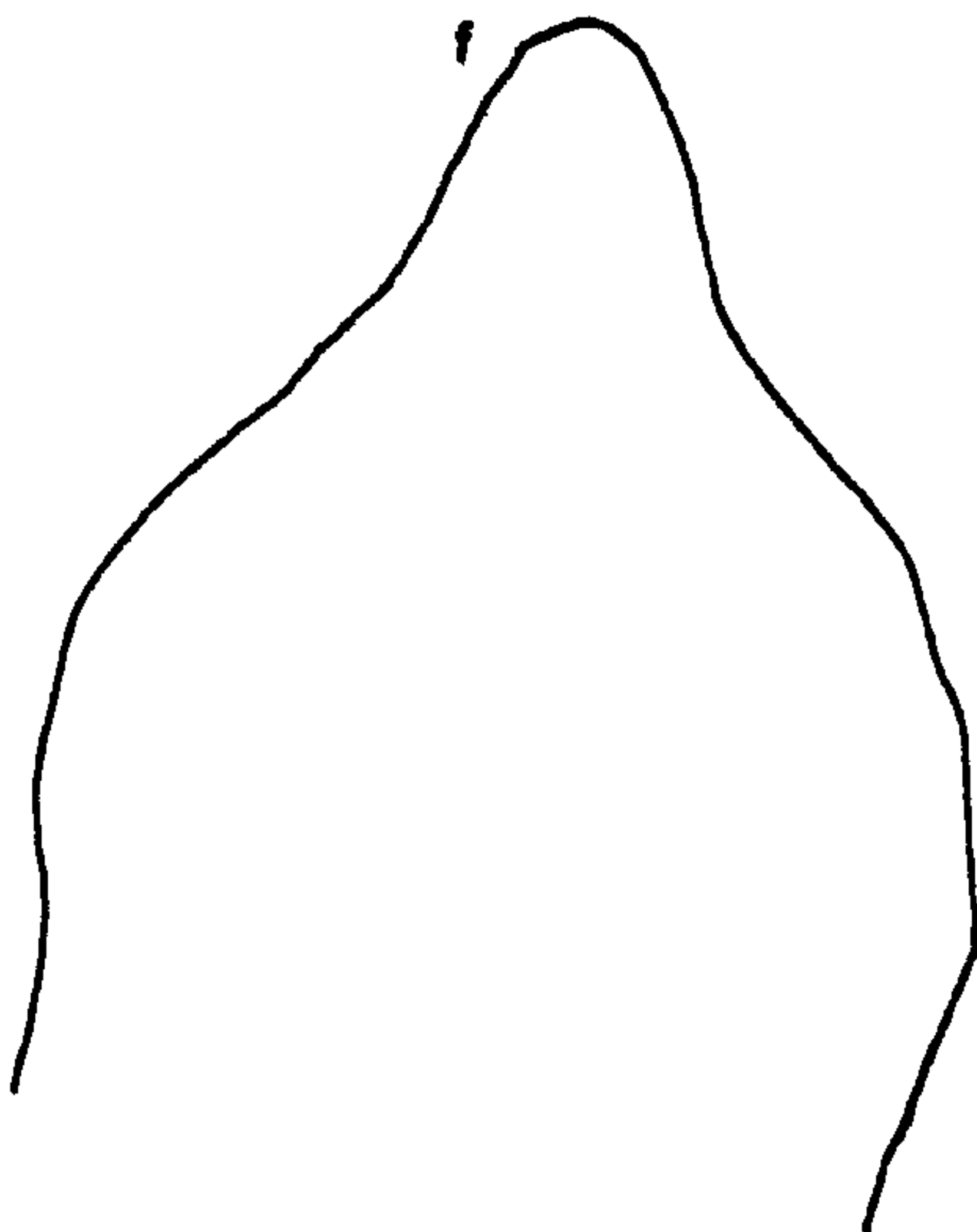
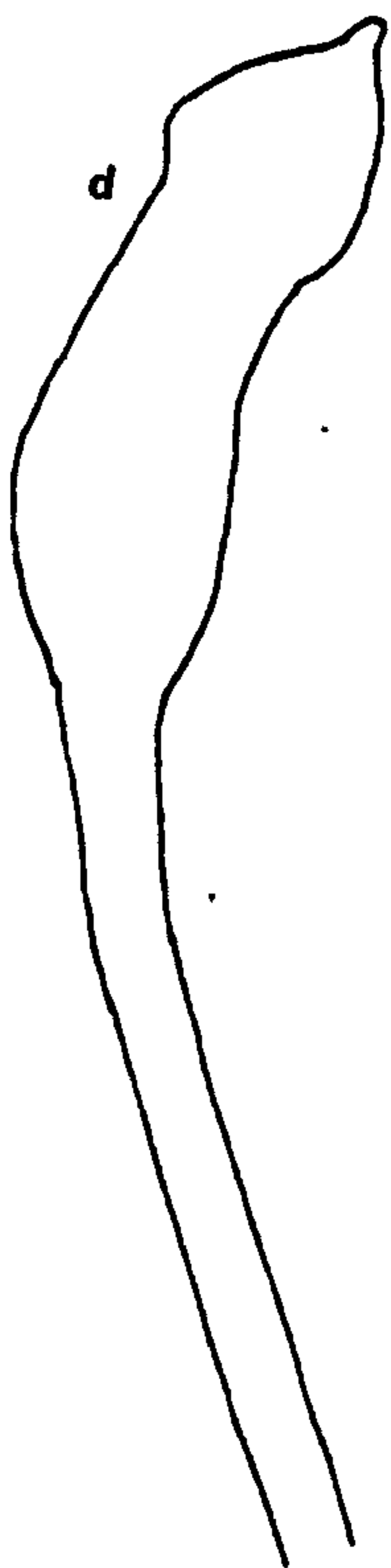
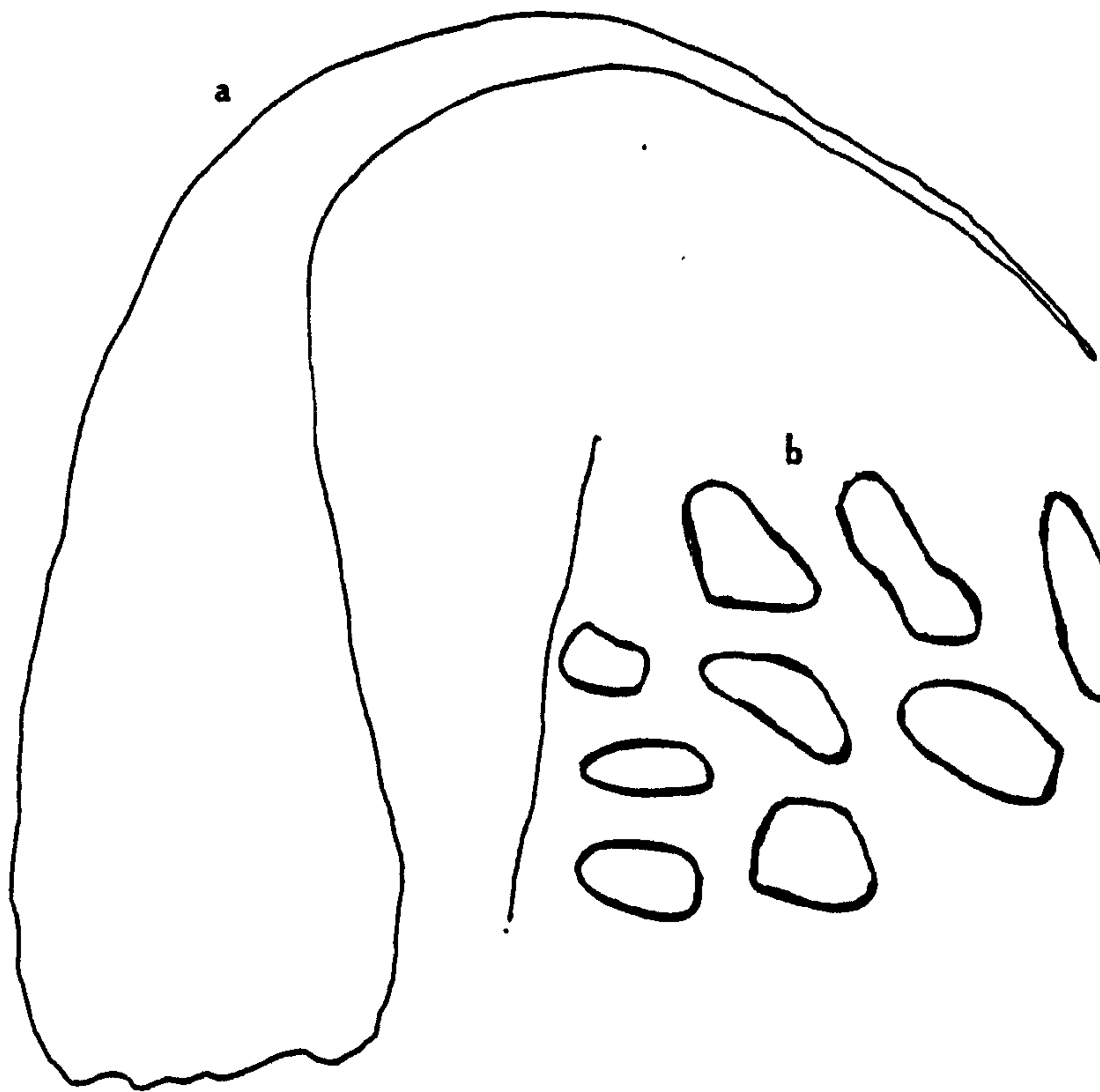
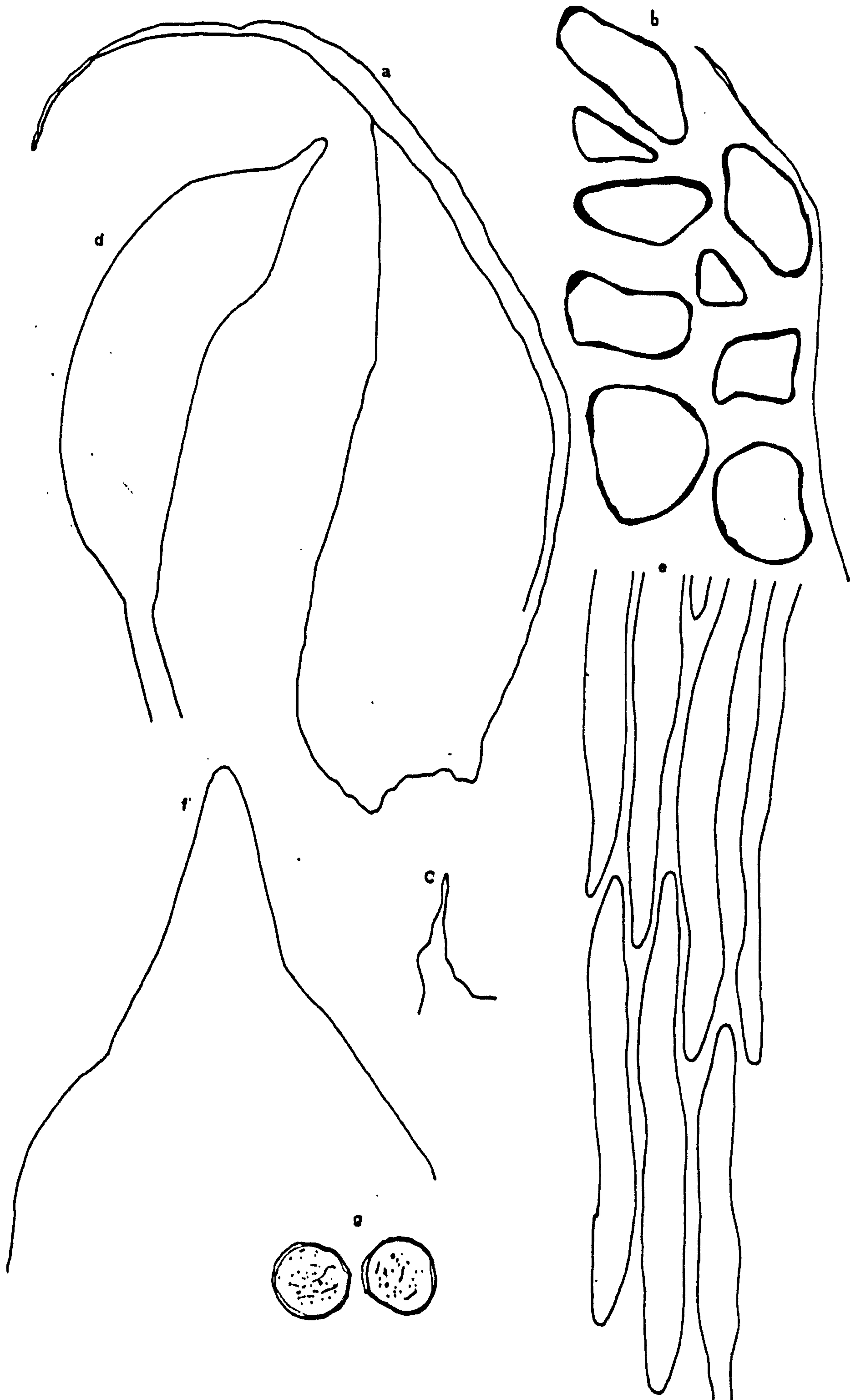


Fig. 6. Morphology of leaves and capsule of H. jutlandicum:

- |                         |                    |
|-------------------------|--------------------|
| (a) leaf;               | (b) angular cells; |
| (c) pseudoparaphyllum ; | (d) capsule ;      |
| (e) cells;              | (f) capsule lid;   |
| (g) spores.             |                    |



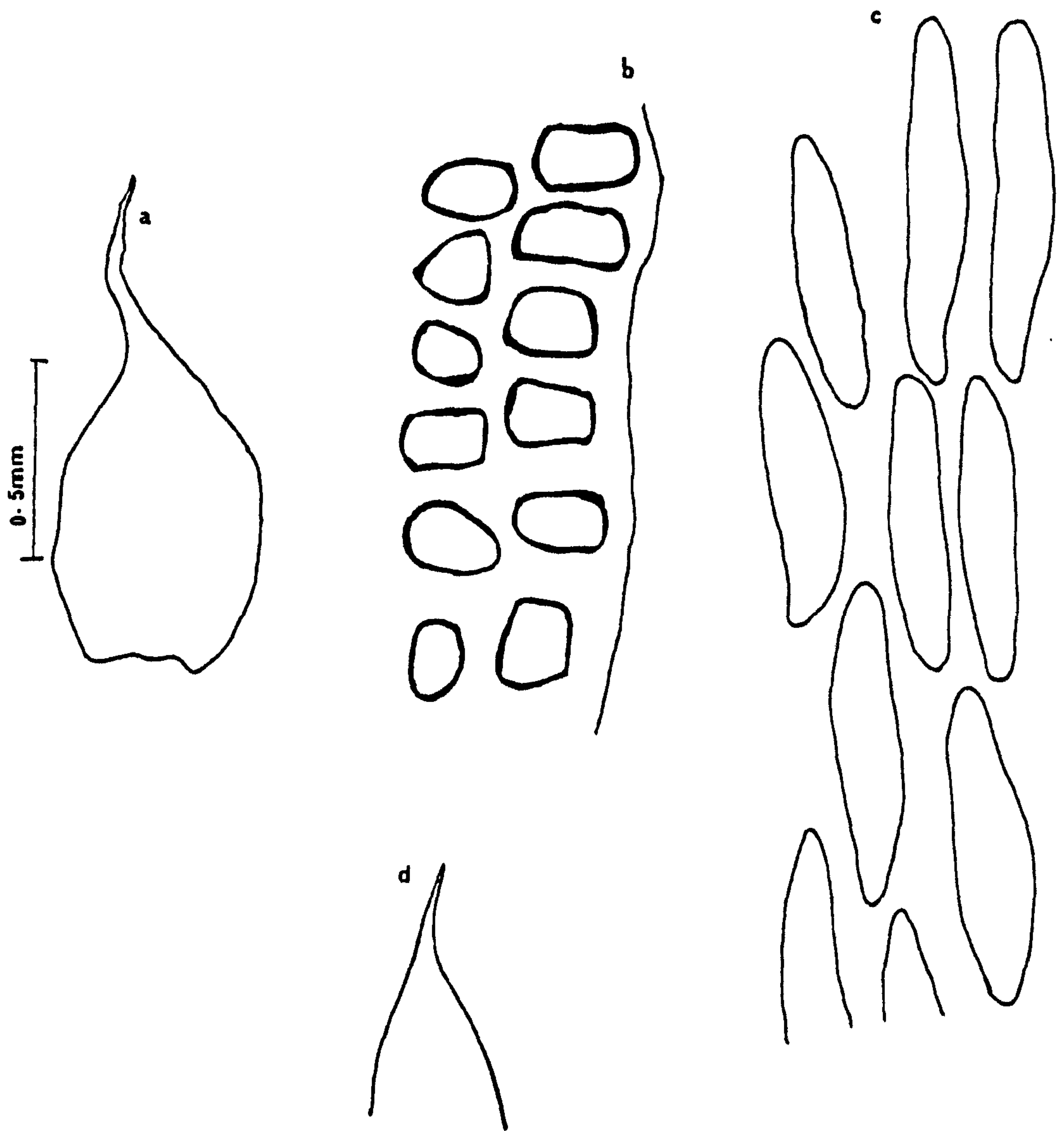


Fig. 7. Morphology of leaves of H. vaucheri:

(a) leaf ;

(b) angular cells ;

(c) cells;

(d) pseudoparaphyllum.

which is of importance in discriminating H. mammillatum from other plants is not environmentally influenced and of good taxonomic value for H. mammillatum.

(c) Leaf apex shape: two categories were scored: curved downwards and  $\pm$  straight. All plants except var. resupinatum have leaves curved downwards at the stem apices, and were distinguishable. 96% of the gatherings named var. resupinatum had  $\pm$  straight leaves at the stem apices, but only 50% of the gatherings remained thus which suggests that leaf apex shape is not a reliable character in taxon discrimination as it is phenotypically variable. This character is of little taxonomic value.

(d) Nerve strength: three categories were scored: not detectable, distinct and very distinct. All plants except H. vaucheri and some gatherings of H. mammillatum had distinct nerves. H. vaucheri tends to have a much more distinct nerve than other plants. 10% of the gatherings of H. mammillatum had no nerves. This character is of good taxonomic value for discriminating H. vaucheri but is of no use within H. cupressiforme s.l.

(e) Nerve Length: H. mammillatum, which possesses very short nerves in comparison with other plants, shows a sharp decrease in the amount of variation under uniform conditions (see Table 4). Other plants show a considerable degree of overlapping. In plants referable to H. mammillatum nerve length is evidently affected by environmental factors and, as with other taxa, is of little taxonomic value.

(f) Cell Size: This is a variable character. Plants corresponding to H. mammillatum and var. filiforme have shorter cells (30-75  $\mu$ m and 28-44  $\mu$ m) compared with other plants (28-90  $\mu$ m). As can be seen from Fig. (4e) and Fig. (5e) these two taxa are more or less distinct from the others of H. cupressiforme s.l., both before and after culture (see Table 4).



It is evident that cell size is markedly affected by environmental conditions (Table 4), an interesting point as cell size is often regarded as a stable character.

(g) Angular cell shape: this character was scored for three categories: irregular, irregularly quadrate and irregularly rounded. All plants except var. resupinatum had irregular and/or irregularly rounded angular cells. Almost all plants named var. resupinatum had regularly quadrate angular cells (see Fig. 2b), but only 55% of them retained this after culture which suggests that this character is genotypically variable and of little taxonomic value.

(h) Angular Cell wall thickness: three categories were scored: thin, medium and thick. Three groups were formed based on these categories, the first groups with thin walls consisted of var. filiforme (see Fig. 5b). Var. resupinatum and H. jutlandicum formed a second group with medium cell walls (see Fig. 2b; 6b). H. mammillatum and var. cupressiforme formed a third group with thick walls (see Fig. 4b; 1b). Var. lacunosum had a range of cell wall thickness. Observations after culture show that 52%, 65%, 80% and 72% of the gatherings of var. filiforme, var. resupinatum, H. jutlandicum and H. mammillatum respectively retained their original wall type. Clearly this character is genetically variable.

(i) Angular cell size: this is a variable character. All plants except H. vaucheri overlapped and were indistinguishable. H. vaucheri has very short angular cells in comparison with other plants (see Fig. 7b). The amount of variation among plants corresponding to H. mammillatum, var. cupressiforme and var. resupinatum produced under uniform condition is almost the same of that in wild plants (see Table 4) suggesting genetic uniformity and little environmental effects. On the other hand the amount

of variation in the other three taxa showed a sharp decrease after culture suggesting marked environmental effects. Size of angular cell is a useful character for recognizing H. vaucheri.

### 3. Sporophyte

a) Seta Length: This is a variable character, There is a tendency for var. lacunosum to have a relatively short seta and for H. jutlandicum to have a relatively longer one. Other plant show a complete overlap and discrimination between them using this character is not possible.

b) Capsule posture: three categories were scored: inclined, intermediate and erect. Three groups were formed, the first group with inclined capsules was made up of var. cupressiforme, var. filiforme and H. jutlandicum (see Figs. 1d, 5d, 6d). It was found that 82%, 82% and 89% of these plants respectively had inclined capsules. A second group was made up of var. lacunosum in which 84% of the gatherings fall within the second category (see Fig. 3d). The third group with erect capsules contained H. mammillatum and var. resupinatum in which 86% and 84% of their gatherings respectively had erect capsules (see Fig. 4d, 2d).

c) Capsule Length: there is a tendency for H. jutlandicum to have the largest capsules (Fig. 6d) and for var. filiforme and var. resupinatum to have the smallest ones (see Figs 2d, 5d). Other plants overlapped and were indistinguishable. This character may be of some taxonomic value.

d) Lid shape: three categories were scored: lid mammillate, rostrate and subulate. Lid shape is a good character for separating H. mammillatum and var. resupinatum from other taxa of H. cupressiforme s.l. H. mammillatum has a mamillate lid and var. resupinatum a subulate one (see Figs. 4f, 2f). Other plants have a rostrate lid and were indistinguishable from one another.



Table 4. Showing the mean value, the variability in leaf length, nerve length, cell size and angular cell size before and after culture in H. cupressiforme aggregate.

Taxon	Character	Before culture				After culture			
		$\bar{X}$	S.D.	S.E.	C.V	$\bar{X}$	S.D.	S.E.	C.V.
<u>H. cupressiforme</u> Var. <u>cupressiforme</u>	1 Leaf length	1.8	0.6 37	0.07	20.1	2.2	0.2	0.05	9.6
	2 Nerve length	5.4	1.1	0.22	20.4	6.3	0.69	0.13	10.7
	3 Cell size	68	3.8	2.7	20	67.8	8.4	1.6	11.8
	4 Angular cell size	0.63	0.22	0.12	9.5	0.72	0.162	0.12	8.7
<u>H. cupressiforme</u> Var. <u>resupinatum</u>	Leaf length	1.00	0.27	0.06	26.9	1.2	0.2	0.05	17.4
	Nerve length	5.5	0.74	0.15	13.5	5.9	0.6	0.12	10.1
	Cell size	40.0	2.5	1.12	13.8	42	4.9	0.98	11.6
	Angular cell size	0.68	0.22	0.02	13.5	0.8	0.183	0.017	10.1
<u>H. cupressiforme</u> Var. <u>lacunosum</u>	Leaf length	2.2	0.58	0.16	26.2	2.3	0.30	0.07	15.2
	Nerve length	4.2	0.73	0.15	17.2	5.8	0.45	0.09	7.9
	Cell size	59.9	1.67	3.5	29.4	55.3	11.9	2.4	21.6
	Angular cell size	0.8	0.24	0.03	21.9	1.00	0.14	0.03	14.2
<u>H. mammillatum</u> Var. <u>filiforme</u>	Leaf length	1.3	0.3	0.07	25.5	1.37	0.30	0.07	23.6
	Nerve length	4.9	0.9	0.18	18.2	5.3	0.30	0.07	6.4
	Cell size	32.8	2.3	0.75	11.4	43.8	13.4	2.7	30.5
	Angular cell size	0.7	1.6	0.05	33.2	0.8	0.1	0.02	13.3

Note that character 1 in nm; for 2, 1 unit = 0.1 mm; for 3 & 4, 1 unit = 26  $\mu$ m.

Table 4 cont'd

Taxon	Character	Before culture				After culture			
		$\bar{X}$	S.D.	S.E.	C.V.	$\bar{X}$	S.D.	S.E.	C.V.
<u>H. mammillatum</u>	Leaf length	1.7	0.42	0.08	23.5	2.12	0.22	0.04	10.2
	Nerve length	3.00	0.67	0.13	21.6	3.9	0.54	0.12	13.5
	Cell size	46.5	0.88	1.7	18.8	46.9	5.3	1.1	11.3
	Angular cell size	0.7	0.2	0.21	14.5	0.70	0.11	0.23	15.6
<u>H. jutlandicum</u>	Leaf length	1.62	0.59	0.118	36.4	1.6	0.4	0.08	22.6
	Nerve length	5.75	0.95	0.2	16.5	6.2	0.53	0.09	7.1
	Cell size	48.8	1.1	2.1	21.1	55.9	5.3	1.1	9.5
	Angular cell size	1.1	0.18	0.07	34.8	0.99	0.11	0.10	11.1

e) Lid length: this is a variable character. All taxa overlapped and were indistinguishable from one another. It is clearly of no taxonomic value (see Figs. 1f-6f).

f) Spore Size: spore size of H. cupressiforme s.l. ranges from 11 to 24  $\mu$ m. The spores of var. filiforme tend to be smaller (11-16nm) (see Fig. 5g) than those of other plants, but otherwise the character has little systematic value.

As no one character by itself, except for lid shape in the case of H. mammillatum is discriminatory, it was clearly necessary to see if all the characters taken in combination would separate the taxa.

## 2.4 Spore Wall Ornamentation

### Introduction

The spores of H. cupressiforme range from 11 to 24  $\mu$ m. Surface structures are not easily visible with the light microscope. The surface of moss spores is often described simply as either smooth or papillose (Koponen, 1977). Although Erdtman (1965) noted the presence of large and small spores in some Macromitrium species (e.g. M. crenulatum and M. criomitrium) he made no mention of possible difference in surface structure. The limits of light microscopy in the study of moss spores and the possibilities of using the Scanning Electron Microscope (SEM) have been shown by Boros & Jarai-Komlodi (1975). McClymont (1955) showed the importance of observing ornamentation in fully mature spores. He studied spores from a range of Bruchia species and could recognize two distinct types of ornamentation. A similar observation with Orthotrichum species was made by Hirohama (1977). The spores of British species of Polytrichum, which all look similar under the light microscope, can be distinguished under the SEM by ornamentation (Dickson, 1969, 1973). Denizot (1971) showed in a similar way that Lunularia species are clearly ornamented

at the higher magnification of SEM. The study of the ornamentation of spore walls of the Bryum capillare group under SEM shown. Considerable differences between one species and another which are of taxonomic significance (Syed, 1973). Sorsa & Koponen (1973) studied the spores of forty two species of the family Mniaceae by both light and SEM. Smith 1974 described three major groups of spores in the family Polytrichaceae and suggests that the differences between Polytrichum and Polytrichastrum are fundamental. Eight North American species of Eucalypta can be distinguished on the basis of the spore morphology (Vitt and Hamilton, 1974; Horton & Murray, 1976). Ramsay (1979) gathered SEM data on the spores of eighteen Macromitrium species in Australia and examined several populations of some species (e.g. six of M. watsii, five of M. daemelli and seven of Macrocoma tenue) as well as several capsules from each population to determine the reliability of surface ornamentation as a taxonomic character, she found that the general pattern that emerged was that although the papillae on the perine areas are densely distributed on the small spores and more widely spaced on the large, there appears to be no absolute distinction between the two types. From the investigation mentioned above, spore morphology in mosses may be useful in their classification and in some cases in identification in spite of the note of Koponen (1977) that ultrastructure is useful mainly in the classification of taxa above specific level.

#### Materials and Methods

For SEM work, untreated spores are often taken directly from herbarium specimens (Taylor et al. 1974, Saito and Hirohama, 1974), and this can give satisfactory results.

#### Results and Discussion

Although there are differences in size between the spores of some taxa of H. cupressiforme s.l., spore wall ornamentation of H. mammillatum, H. jutlandicum, var. cupressiforme, var. lacunosum, var. resupinatum and var. filiforme shows no clear differences between them (see Fig. 8)



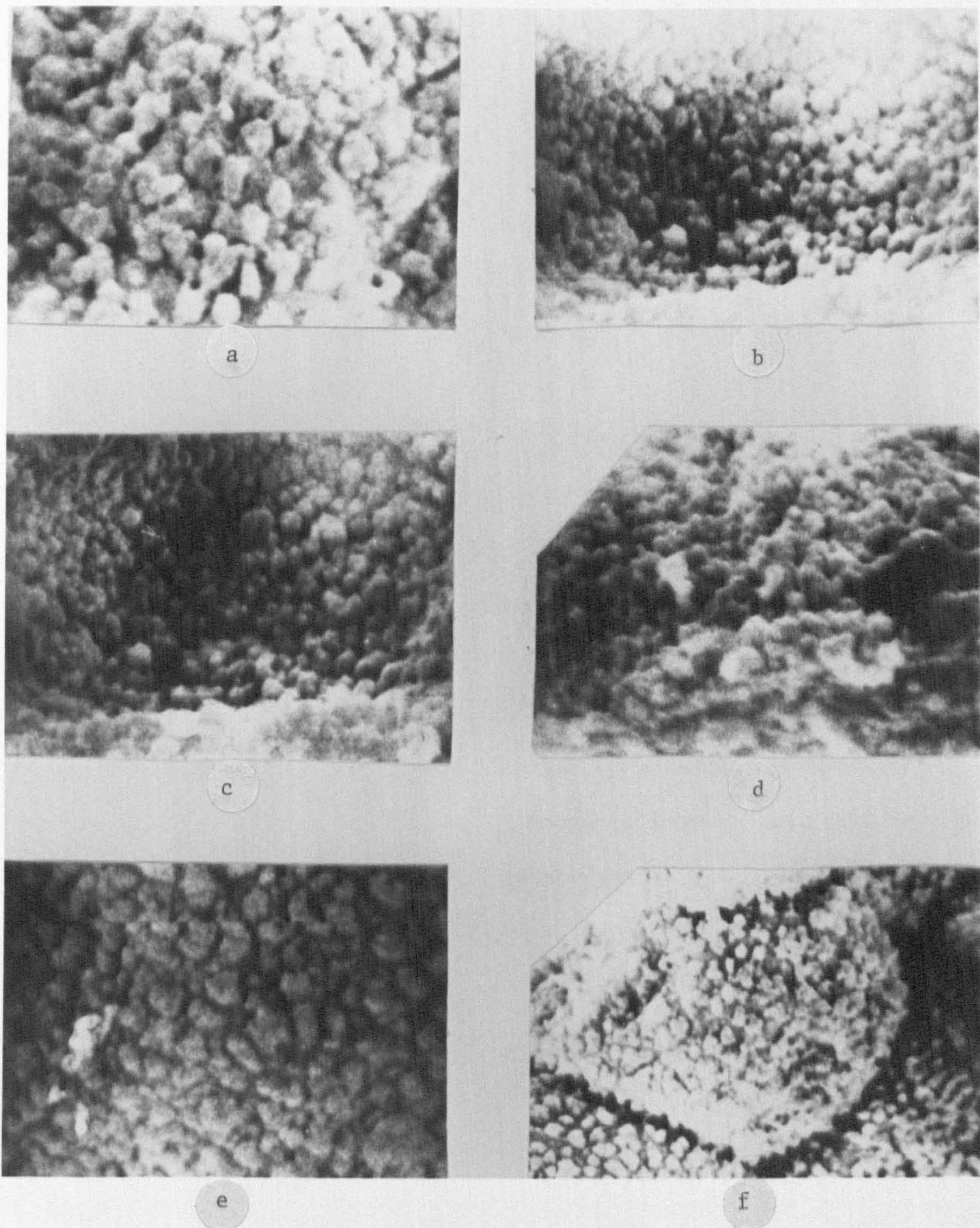


Fig. 8. Spore wall ornamentation of H. cupressiforme s.l.

(a) var. cupressiforme;

(b) var. lacunosum

(c) H. mammillatum;

(d) var. resupinatum

(e) H. jutlandicum;

(f) var. filiforme

All x 15000



Spore size has been discussed before (p. 17) and used in the numerical analysis. Photomicrographs in plate 8 show that the spore walls are papillose and of the simple type in all the taxa examined. It would appear that the papillae are of almost equal size, with irregular rounded tips and more or less evenly distributed on the walls.

Spore wall ornamentation appear to be of no taxonomic value in the H. cupressiforme complex. More transmission electron microscopic studies are necessary before any significant conclusions can be drawn.

## 2.5 Cytological investigation

Cytological observations on H. cupressiforme s.l. were carried out on six taxa: H. mammillatum, H. jutlandicum, var. cupressiforme, var. resupinatum, var. filiforme, and var. lacunosum.

Observations on somatic chromosomes were made on meristematic tissue of gametophytes. About 0.5cm of shoot apices were fixed in (1:1:1) acetic acid : absolute alcohol : chloroform for 2 hours or more (Ramsay, 1969). Fixed material was stained in a saturated solution of aceto-orcein in 45% acetic acid, for 12 hours or more at room temperature. The apex was transferred to a drop of aceto-orcein on a slide. The shoot was macerated with a brass rod and a cover-slip added. The cover-slip was tapped a few times with a needle handle. Pressure applied to cover-slip to spread the chromosomes. After removing surplus stain by filter paper, the cover-slip was sealed with rubber solution.

Chromosome numbers and source of the specimens from which they were obtained are listed in Table 5. Table 6 gives chromosome counts from H. cupressiforme s.l. by other authors. The results in Fig. 11, are similar to those obtained by Ramsay (1969) from British material. Gametophytic chromosomes from the six examined taxa are similar in number, morphology and position of the centromere. The longest chromosome in gametophyte mitosis is quite distinct. It is almost one and a half times length of the second



Table 5. Showing chromosome numbers and source of Hypnum cupressiforme s.l. used in the present study.

Taxon	Locality	( <u>n</u> = )
<u>H. mammillatum</u>	Cwm-y-Glo, Caernarfonshire	10
	Nant Gwynant, Caernarfonshire	10
	Aber Valley, Aber, Caernarfonshire	10
	Treborth Botannical Garden, Bangor.	10
	Betws-y-Coed	10
<u>H cupres siforme</u>		
Var. <u>cupressiforme</u>	Treborth Botannical Garden, Bangor	10
	Aber Valley, Aber.	10
	Tyn-y-Mynydd, Anglesey	10
	Nant Gwynant	10
<u>H cupressiforme</u>		
Var. <u>lacunosum</u>	Aberffraw, Anglesey	10
	Bwrdd Arthur, Anglesey	10
<u>H cupressiforme</u>		
Var. <u>resupinatum</u>	Maes-y-Geirchen, Bangor	10
	Glanrafon Hill, Bangor	10
	Penmynydd, Menai Bridge, Anglesey (Smith)*	10
<u>H. jutlandicum</u>	Old Quarries, near Llanberis	10
	Quarry near Llanberis	10
	Pengilfach, near Llanberis	10
<u>H. mammillatum</u>		
Var. <u>filiforme</u>	Aber valley, Aber	10
	Treborth Botannical Gardens	10

\*All gatherings collected by the author unless otherwise stated

Table 6. Showing the chromosome counts reported in the literature for  
H. cupressiforme s.l.

Taxon	Chromosome Number	Source	Author
<u>Hypnum</u>	10	Finland	Vaarama, 1950
<u>cupressiforme</u>	16	Rocky Mountains, N. America	Anderson & Crum, 1958
	10	India	Chopra & Kumar, 1967
	11	Ukraine	Visotskya, 1967
	10	Great Britain	Ramsay, 1969
	10	Caucasus	Visotskya, 1969
	10	U.S.S.R.	Visotskya, 1970
	10	U.S.S.R.	Fetisova and Visotskya, 1970
	10	U.S.S.R.	Lazarenko et al. 1970
	11	U.S.S.R.	Lazarenko et al. 1971
	10	Japan	Inoue, 1971
	10	France	Wigh, 1972
	10	Sweden	Tsutsumi et al., 1973
	10, 10+1.	Austria	Bryan, 1973
<u>H. cupressiforme</u>			
Var. <u>cupressiforme</u>	10	Great Britain	Smith and Newton, 1966
	10	Tasmania	Ramsay, 1974
<u>H. mamillatum</u>	10	Great Britain	Smith and Newton, 1966
	10	Turkey	Nyholm and Wigh, 1973
<u>H. cupressiforme</u>			
Var. <u>resupinatum</u>	10	Great Britain	Smith and Newton, 1966

longest with sub-terminal centromere. Four metacentric and three sub-terminal chromosomes followed, while the two smallest, of almost equal size, are metacentric.

British material of H. cupressiforme s.l. has been studied by Smith and Newton (1966), Newton (1968) and Ramsay (1969). Meiotic studies of H. mammillatum, var. cupressiforme and var. resupinatum by Smith and Newton (1966) showed that there are ten bivalents and that variation in size of the chromosome complement is not very marked. Meiosis in H. mammillatum, var. cupressiforme and var. resupinatum was studied by Newton (1968) who found ten bivalents, generally large showing a wide range of size. Ramsay (1969) showed that there are ten bivalents in the meiotic complement (Fig. 9g). Of mitotic chromosome from gametophyte cells the longest is quite distinct.

The haploid number,  $n = 10$  has been reported by several authorities (Vaarama, 1950; Chopra and Kumar, 1967; Ramsay, 1967; Vysotska, 1970; Inoue, 1971; Bryan, 1973; Tsutsumiet al. 1973);  $n = 11$  was reported by Lazarenko et al (1971) from the U.S.S.R. and  $n = 10 + 1m$  by Bryan (1973) from a single gathering from Austria. The count  $n = 16$  was reported by Anderson and Crum (1958) from a single population from the Canadian Rocky Mountains, but Smith and Newton (1966) say: "Professor P.W. Richards (personal communication) suggests from field observations that the north American plant called H. cupressiforme may not be conspecific with the European plant and the difference in chromosome number supports this".

Vaarama (1950) examined meiosis in Finnish material and found ten bivalents, three large, in addition to three medium size and four smaller ones. This differs from British material (see Fig. 9h).

Indian H. cupressiforme has  $n = 10$  (Chopra and Kumar, 1967). The complement contains three large, three medium-sized and four small

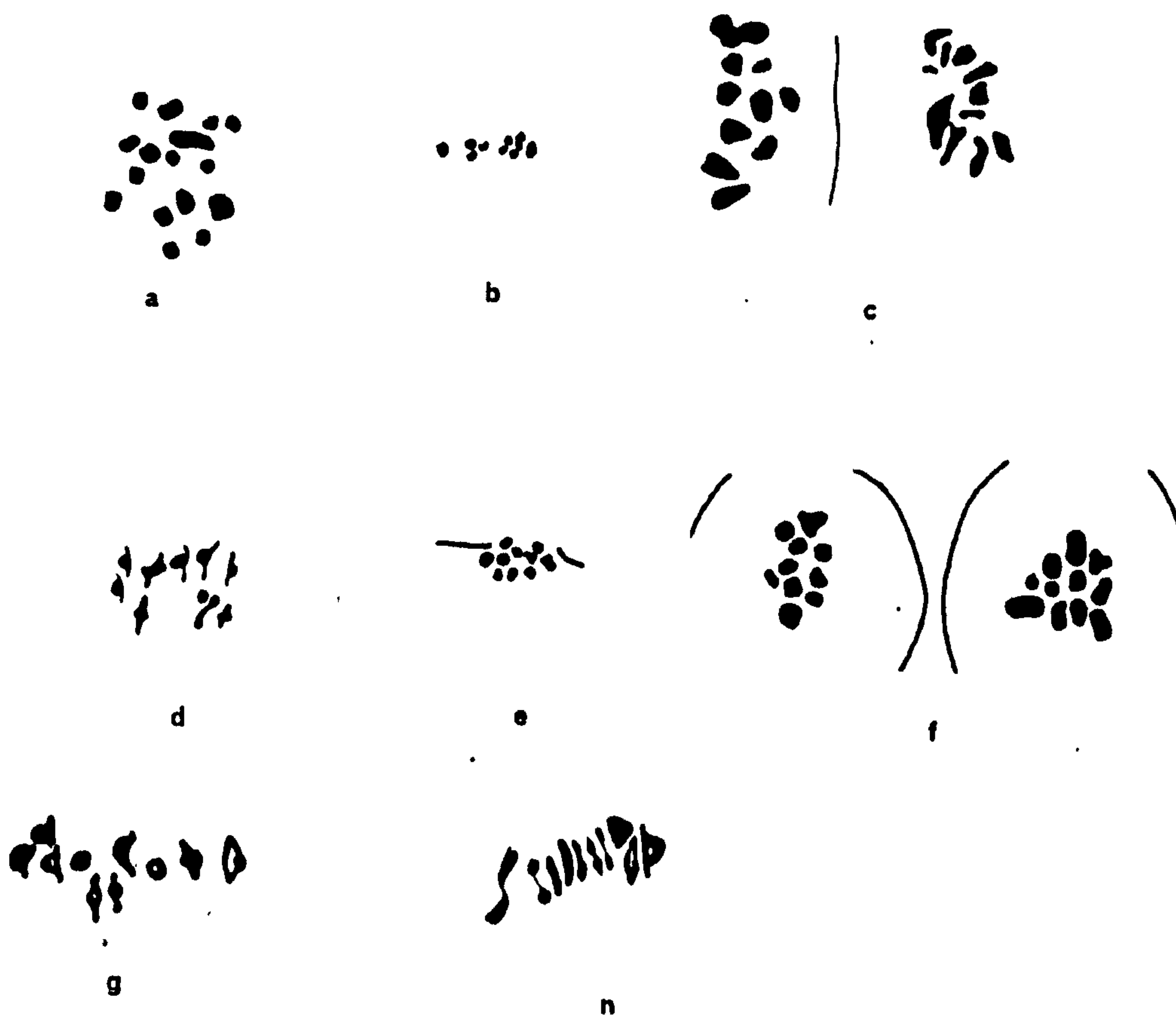


Fig. 9. Illustrations of meiotic chromosomes of H. cupressiforme s.l.

by:

- |                              |   |
|------------------------------|---|
| (a) Anderson & Crum (1958) ; | (b) Chopra & Kumar (1967) ;                                 |
| (c) Bryan (1973) ;           | (d) Ramsay (1974) ;   |
| (e) Vysotska (1970) ;        | (f) Lazarenko, Visotskaya, Lesnyak &<br>Mamatkulov (1970) ; |
| (g) Ramsay (1969) ;          | (h) Vaarama (1950) .  |



bivalents. This agrees with Finnish material (Vaarama, 1950) but is different from British material (see Fig. 9b).

Bryan (1973) reported  $\underline{n} = 10$  from all her gatherings from Austrian material except one which had  $\underline{n} = 10 + 1m$ . She found the first chromosome to be a large conspicuous bivalent and in this it resembles British material (see Fig. 9c).

Ramsay (1974) examined material from Tasmania and found ten bivalents at metaphase-I. The smallest bivalent disjoined early in some and in others was late separating. Such behaviour of the smallest bivalent was not reported from the British material but otherwise Tasmanian and British plants are very similar (Fig. 9d).

Russian material was studied by Lazarenko et al. (1971) who reported  $\underline{n} = 11$ . It would appear from their illustration (Fig. 9f) that there are eleven bivalents, three large, seven of similar medium size and the last one small.  $\underline{N} = 10$  was reported by Vysotska (1970). It would appear from her illustration (Fig. 9e) that there are three large and seven smaller of almost equal size. The first report disagrees with that of the British material in both number and morphology of chromosomes (see Fig. 9f), but as in both report the presence of three large bivalents they agree with those of Vaarama (1950) and Chopra and Kumar (1967) from Finnish and Indian material respectively.

Mitotic karotypes illustrated by various authors are as follows:

Ramsay (1969) found ten chromosomes in the gametophyte cells with the longest quite distinct, being one and a half times the length of the second longest. It had a subterminal centromere. The next seven consist of four metacentric and three with subterminal centromeres, while the two smallest (of almost equal size) are metacentric (Fig. 10a).



Fig. 10. Illustrations of mitotic chromosomes of H. cupressiforme s.l.

by:

- |                            |                   |
|----------------------------|-------------------|
| (a) Ramsay (1969) ;        | (b) Wigh (1972) ; |
| (c) Nyholm & Wigh (1973) ; | (d) Inoue (1971). |



Fig. 11. Illustration of gametophytic mitoses of H. cupressiforme.



Gametophyte mitosis of Swedish material was examined by Tsutsumi et al. (1973) who reported  $\underline{n} = 10$  according to the formula  $\underline{n} = 10 : V + 3V + 2J + 2v + j + v$ . It would appear from this formula that the first six chromosomes are large. The first four with median or submedian centromeres and the other two are with sub-terminal ones. The rest are small consisting of two median or submedian, one terminal and the last median or submedian. Comparative formula at British and Swedish material would be:

British material  $\underline{n} = 10 : J + 4V + 3J + 2v$

Swedish material  $\underline{n} = 10 : V + 3V + 2J + 2v + j + v$

Hungarian material studied by Wigh (1972) had  $\underline{n} = 10$  with three long chromosomes of similar size and seven others difficult to classify (see Fig. 10b). The three large chromosomes are similar to those found by Vaaroma (1950) and Chopra and Kumar (1967).

Wigh (1972) found the chromosomes of var. lacunosum from Czechoslovakia to be similar to those of his Hungarian populations of H. cupressiforme.

Turkish material reported on by Nyholm and Wight (1973) had  $\underline{n} = 10$  with three long chromosomes and seven small (Fig. 10c).

In Japan, Inoue (1971) studied gametophyte mitosis of H. cupressiforme and reported  $\underline{n} = 10$  with four median or submedian (Fig. 10d). The first is the longest. The next two chromosomes had subterminal centromeres, while the remaining for small chromosomes were two median or submedian one subterminal and the last one is an m-chromosome according to the formula:

$\underline{n} = 10 : V + 3V + 2J + 2v + j + m$

Comparative formulae of British and Japanese material are:

British material  $\underline{n} = 10 : J + 4V + 3J + 2v$

Japanease  $\underline{n} = 10 : V + 3V + 2J + 2v + j + m$

The latter report agrees with that of Tsutsumi et al. (1973) except that the last chromosome which was reported from the Swedish material as median or submedian is considered by Inoue (1971) to be an m-chromosome. This agrees with the British material in the presence of the longest chromosome.

It would seem that there is considerable variation in chromosome morphology within H. cupressiforme and that cytologically there are two groups, plants with one large bivalent or chromosome:

Ramsay (1969) reported from British material

Bryan (1973) reported from Austrian material

Ramsay (1974) reported from Tasmanian material

Tsutsumi et al. (1973) reported from Swedish material

Inoue (1971) reported from Japanease material

and plants with three large bivalents or chromosomes:

Vaarama (1950) reported from Finnish material

Chopra and Kumar (1967) reported from Indian material

Lazarenk et al. (1971) reported from Russian material

Vysostska (1970) reported from Russian material

Wigh (1972) reported from Czechoslovakian material

Wigh (1972) reported from Hungarian material

Nyholm and Wigh (1973) reported from Turkish material

Whilst the Japanese system of chromosome formulae is stylized and their labelling chromosomes H or h (heterochromatic) is open to criticism (Ramsay, 1969; Newton, 1977; Smith, 1978b), it is nevertheless a useful way of rapidly comparing Karyotypes. Karyotype formulae of mitotic

chromosomes illustrated by various authors are as follows:

Inoue (1971)  $\underline{n} = 10 : V + 3V + 2J + 2v + j + m$

Tsutsumi (1973)  $\underline{n} = 10 : V + 3V + 2J + 2v + j + v$

Ramsay (1969)  $\underline{n} = 10 : J + 4V + 3J + 2v$

Wigh (1972)  $\underline{n} = 10 : 3(J \text{ or } V) + 7 (j \text{ or } v)$

Wigh (1973)  $\underline{n} = 10 : 3(J \text{ or } V) + 7 (j \text{ or } v)$

## 2.6 Variability within taxa of H. cupressiforme s.l.

### 1. Var. cupressiforme

Leaf length increased in culture (see Table 4, Fig. 12a), but the variation in length decreased. The increase in length is similar to that in H. mammillatum. All the other taxa show decrease in length variation in culture. This character is highly influenced by environmental factors.

Nerve length varies in a similar way (see Table 4, Fig. 12b). The increase in its length is also similar to that in H. mammillatum. All the other taxa also show decrease in nerve length variation in culture, suggesting that nerve length is highly influenced by environmental conditions.

Cell size is almost the same under both conditions as it is in H. mammillatum (see Table 4, Fig. 12d). The variation in size decreased in culture similar to all the other taxa except var. filiforme where it increased, suggesting that cell size is slightly affected by environmental factors and the variation is partly genetic in nature.

A slight increase and a slight decrease in angular cell size (see Table 4, Fig. 12c), and variation in culture similar to that in var. resupinatum, suggesting that angular cell size is slightly affected by environmental conditions and the variation is partly genetic in nature.

Variation in other characters did not indicate that any differences



between wild population are genetic.

## 2. Var. resupinatum

There is a slight increase in leaf length and a decrease in length variation (Table 4, Fig. 13a). All the other plants show decrease in length variation in culture, suggesting that leaf length is highly influenced by environmental factors.

Nerve length increased slightly in culture and the variation in length decreased slightly (Table 4, Fig. 13b), suggesting that nerve length is slightly influenced by environmental conditions and the variation is partly genetic in nature.

A slight increase in cell size and a slight decrease in size variation in culture (Table 4, Fig. 13d), suggesting that cell size is slightly influenced by environmental factors and the variation is partly genetic in nature.

Angular cell size slightly increased and the variation in size slightly decreased in culture (Table 4, Fig. 13c). This is similar to that in var. cupressiforme, suggesting that angular cell size is slightly influenced by environmental factors and the variation is partly genetic in nature.

About half the plants remained after culture with  $\frac{+}{-}$  straight leaves at the stem apices suggests that the apex shape is not a reliable character in taxon discrimination and it is environmentally variable. Other characters did not show remarkable differences before and after culture.

## 3. Var. lacunosum

Leaf length is almost the same in field and cultured material (see Table 4 Fig. 14a). The variation in length decreased in culture. Leaf length in H. jutlandicum and var. filiforme is almost the same in both cultures

(Table 4). All the other taxa show decrease in length variation in culture, suggesting that leaf length is slightly influenced by environmental factors and the variation is partly genetic in nature.

A slight increase in nerve length is similar to that in var. resupinatum, H. jutlandicum and var. filiforme (Table 4, Fig. 14b). The decrease in length variation is similar to all the other taxa, suggesting that nerve length is slightly influenced by environmental factors and the variation is partly genetic in nature.

Cell size decreased slightly and this is unique (Table 4, Fig. 14d). The variation in size decreased similar to all the other taxa except var. filiforme where it increased, suggesting that cell size is slightly influenced by environmental conditions and the variation is partly genetic in nature.

An increase in angular cell size and a decrease in size variation in culture is similar to that in var. cupressiforme and var. resupinatum (Table 4, Fig. 14c) suggesting that angular cell size is highly influenced by environmental factors.

Other characters did not show a remarkable difference between wild population and probably are genetically determined.

#### 4. Var. filiforme

Leaf length is almost the same in both cultures (see Table 4, Fig. 15a). This is similar to that in var. lacunosum and H. jutlandicum. The variation in length is slightly decreased in culture. All the other taxa show a decrease in length after culture, suggesting that this character is slightly affected by environmental conditions and the variation is partly genetic in nature.

A slight increase in nerve length is similar to that in var. resupinatum, var. lacunosum and H. jutlandicum (Table 4, Fig. 15b). The variation in



length decreased in culture as in all the other taxa, suggesting that nerve length is slightly influenced by environmental factors and the variation is partly genetic in nature.

A sharp increase in cell size is unique (Table 4, Fig. 15d). The variation in size increased sharply and this is also unique, suggesting that this character is highly influenced by environmental factors.

The size of angular cells is almost the same in both culture similar to that in H. mammillatum and H. jutlandicum (Table 4, Fig. 15c). The variation in size decreased in culture similar to all the other taxa except H. mammillatum where it is almost the same, suggesting that this character is slightly influenced by environmental conditions and the variation is partly genetic in nature.

##### 5. H. mammillatum

Leaf length increased in culture and the variation in length decreased (Table 4, Fig. 16a). This is similar to that in var. cupressiforme. Var. resupinatum showed a slight increased in length, suggesting that leaf length is highly influenced by environmental conditions. Nerve length varies in a similar way (Table 4, Fig. 16b). Cell size before and after culture is almost the same (see Table 4, Fig. 16d). The variation in size decreased in culture similar to all the other taxa except var. filiforme where it increased, suggesting that cell size is slightly affected by environmental factors and the variation is partly genetic in nature. Angular cell size was found to be almost the same size before and after culture with the same amount of variation (Table 4, Fig. 16c), suggesting that the variation is genetic in nature.



Fig. 12. Histograms illustrating variation of var. cupressiforme before culture (a-d) and after culture (e-h):

- |                        |                   |
|------------------------|-------------------|
| (a) leaf length;       | (b) nerve length; |
| (c) angular cell size; | (d) cell size.    |

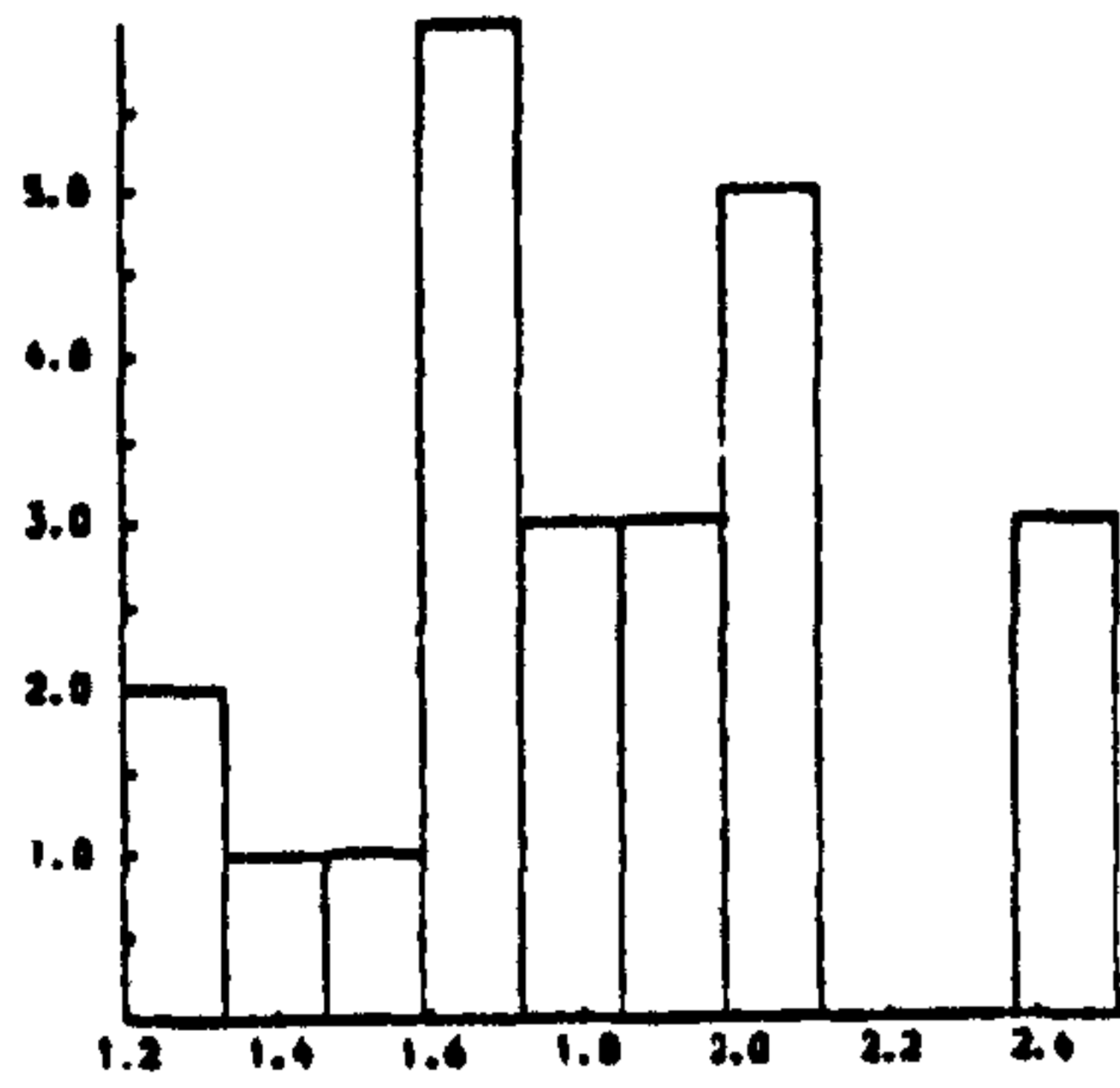
Note that the Y axis is not identical in all cases.

Character (a) in mm; for (b), 1 unit = 0.1 mm; for (c) & (d), 1 unit = 26  $\mu$ m.

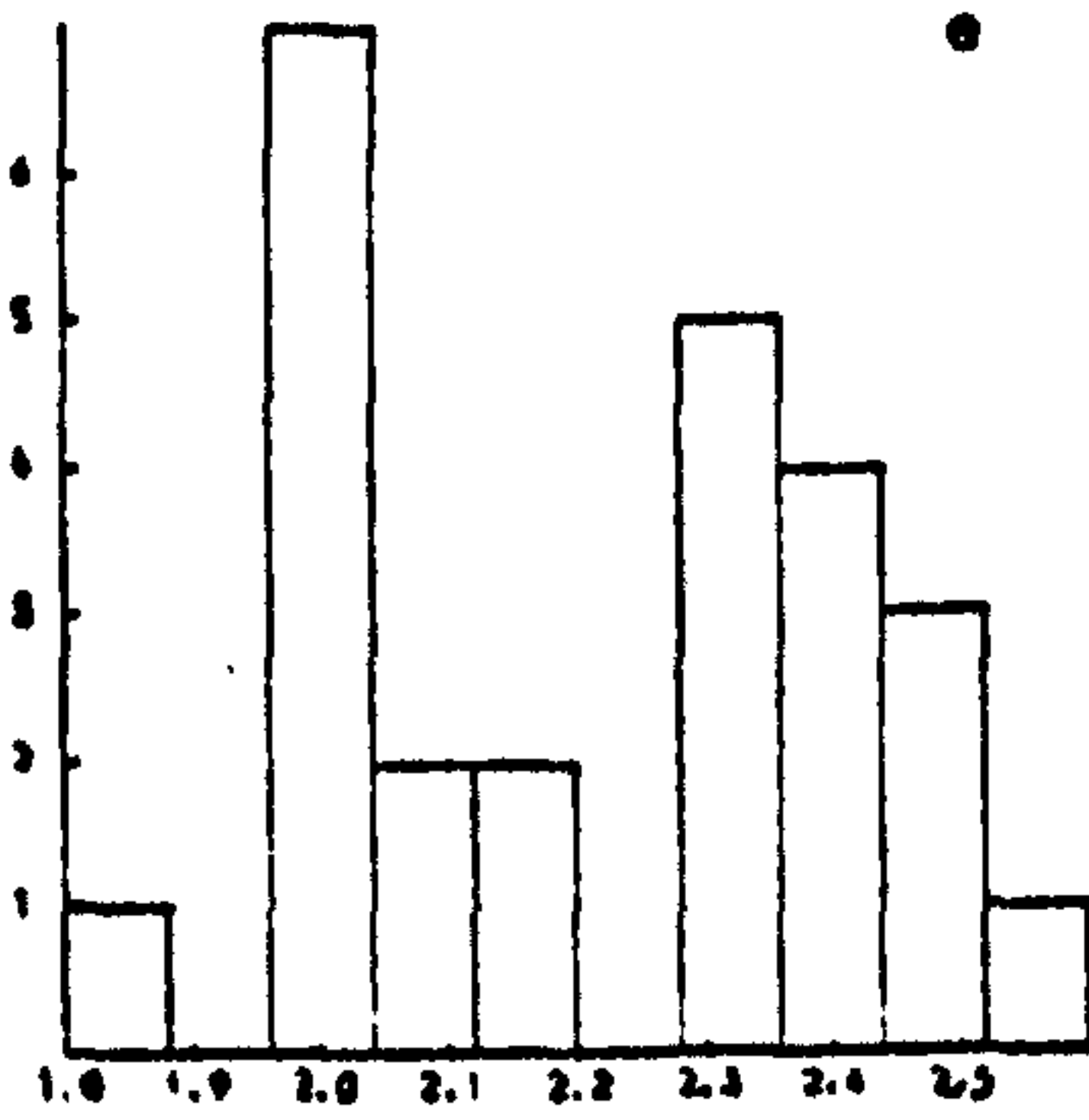
# BEFORE CULTURE

# AFTER CULTURE

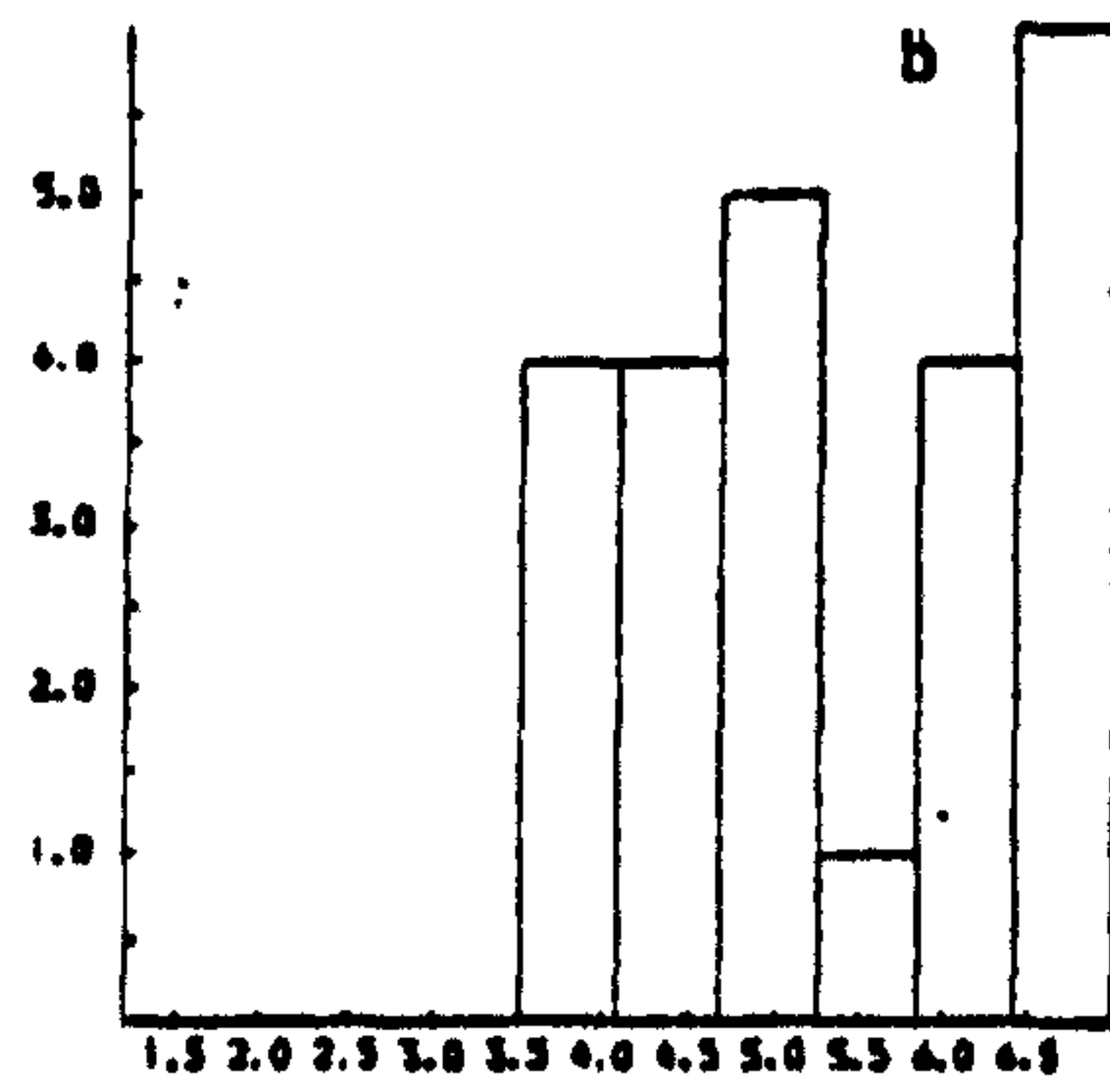
a



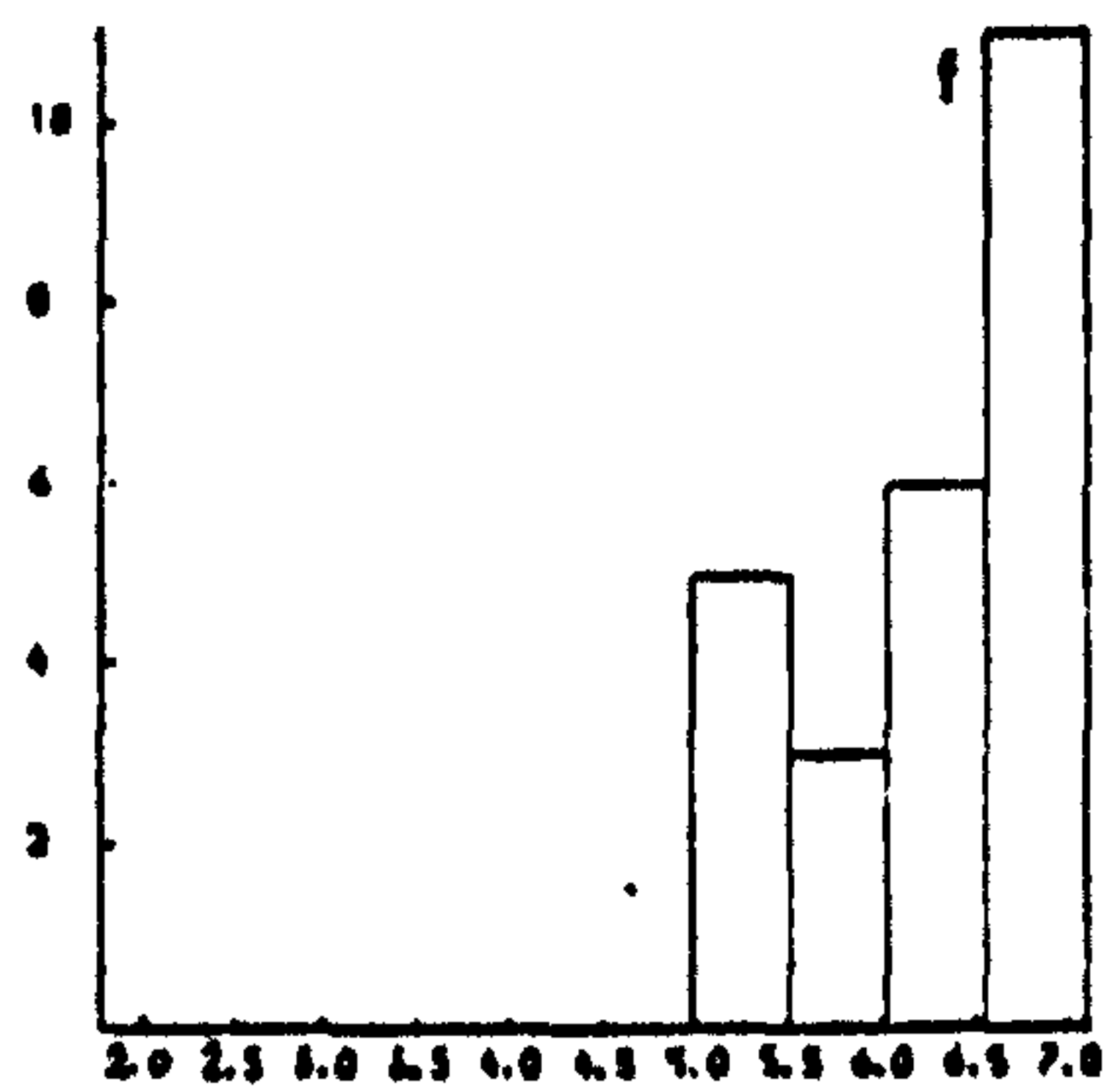
e



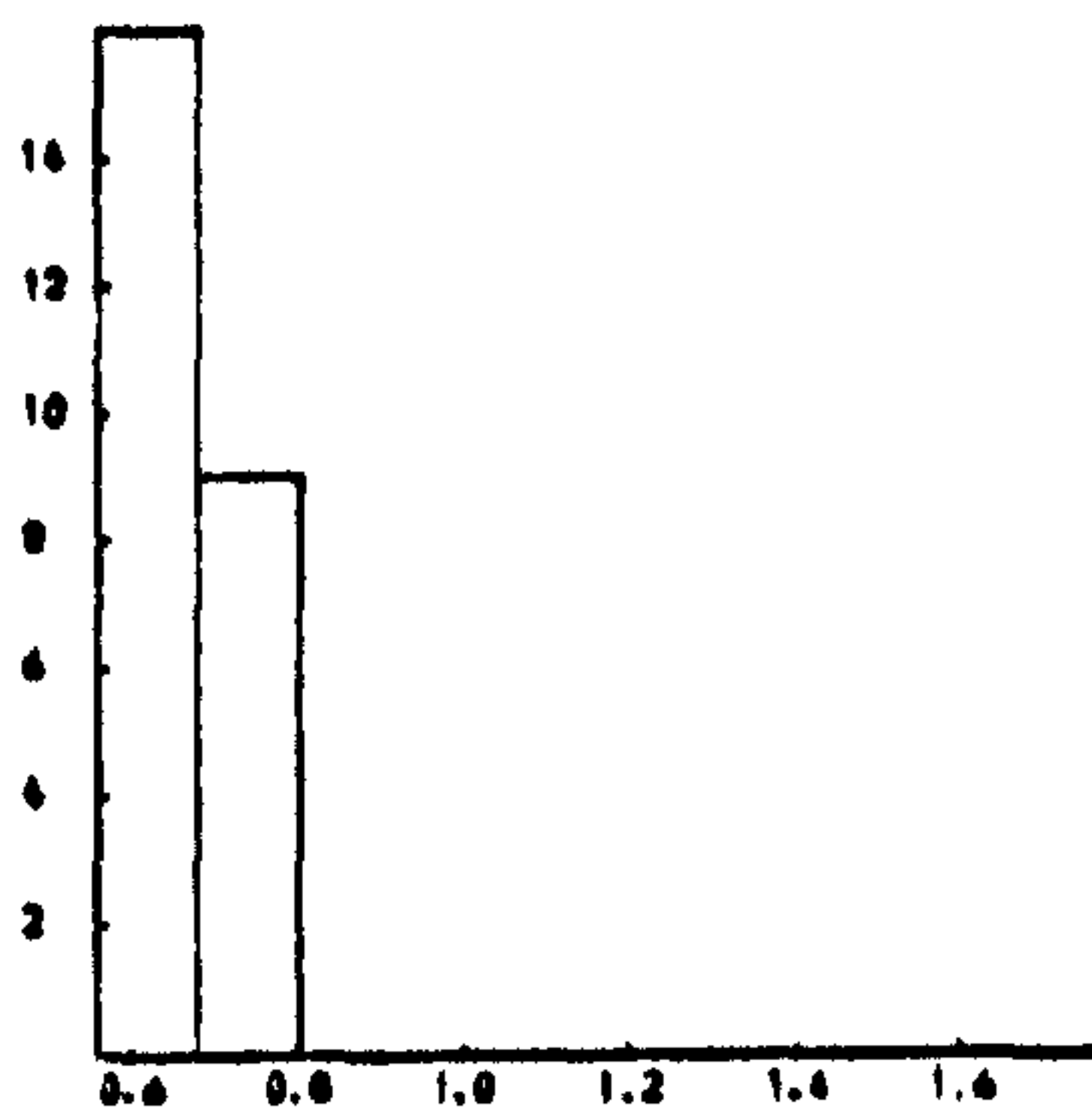
b



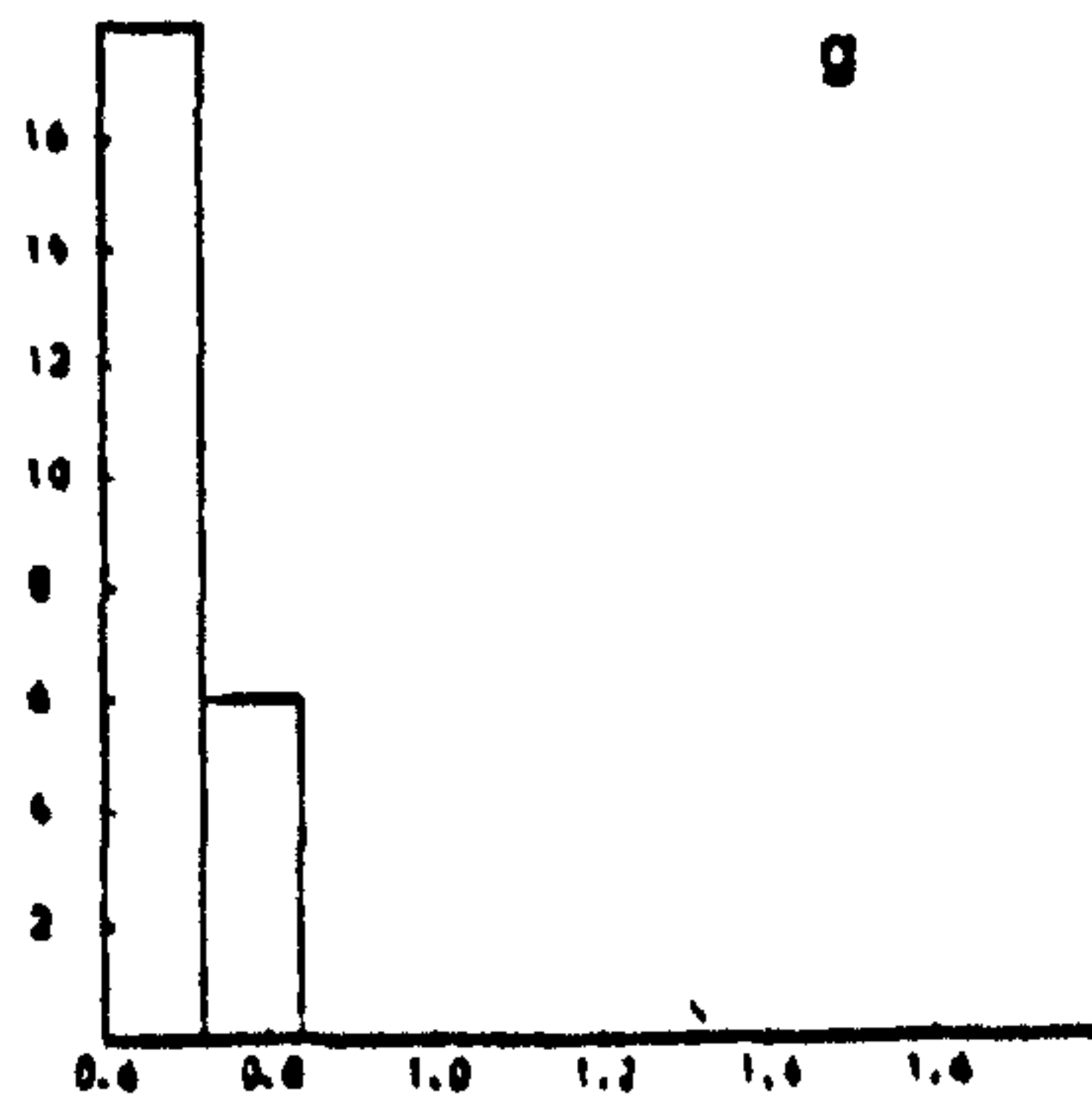
f



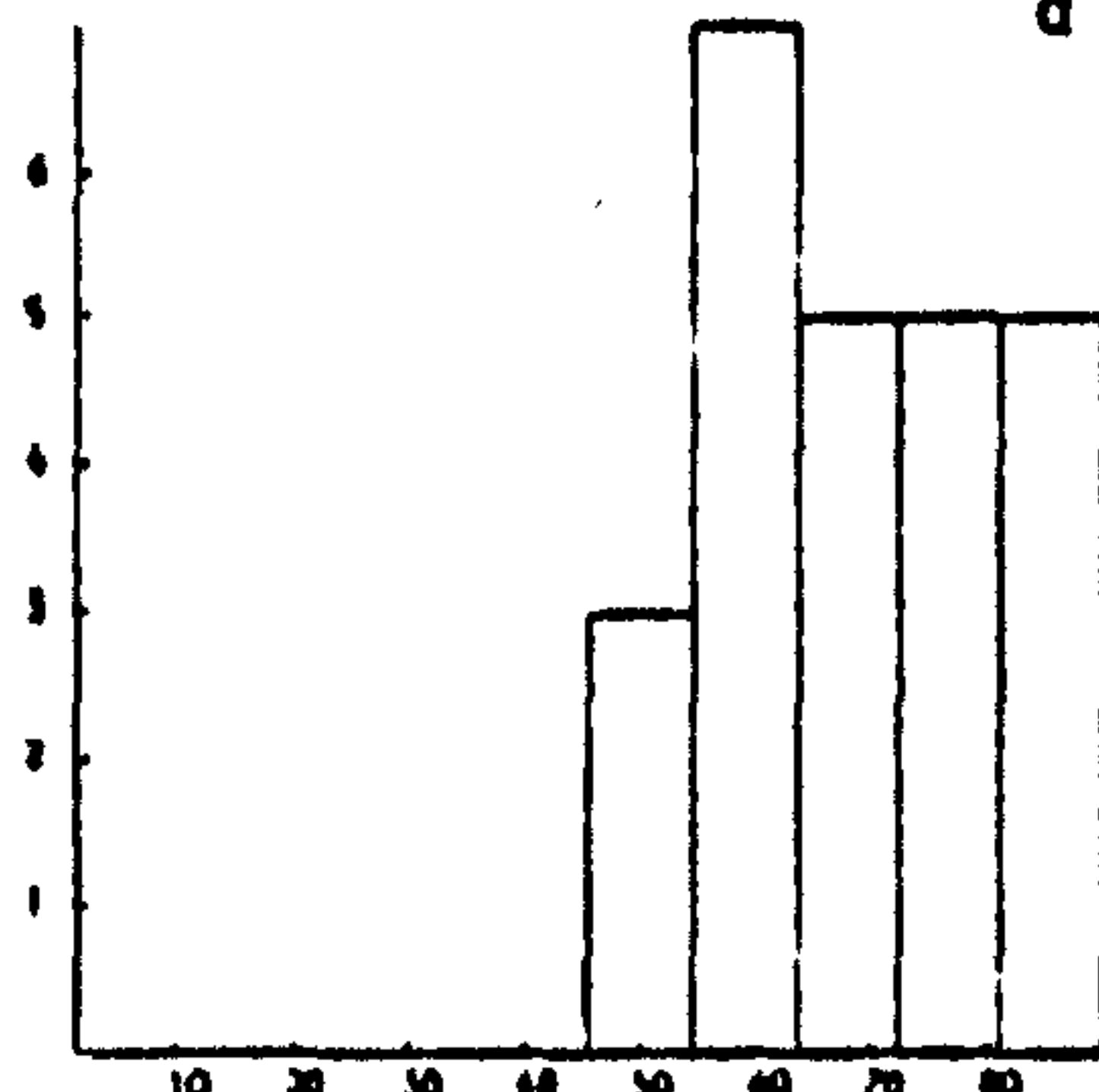
c



g



d



h

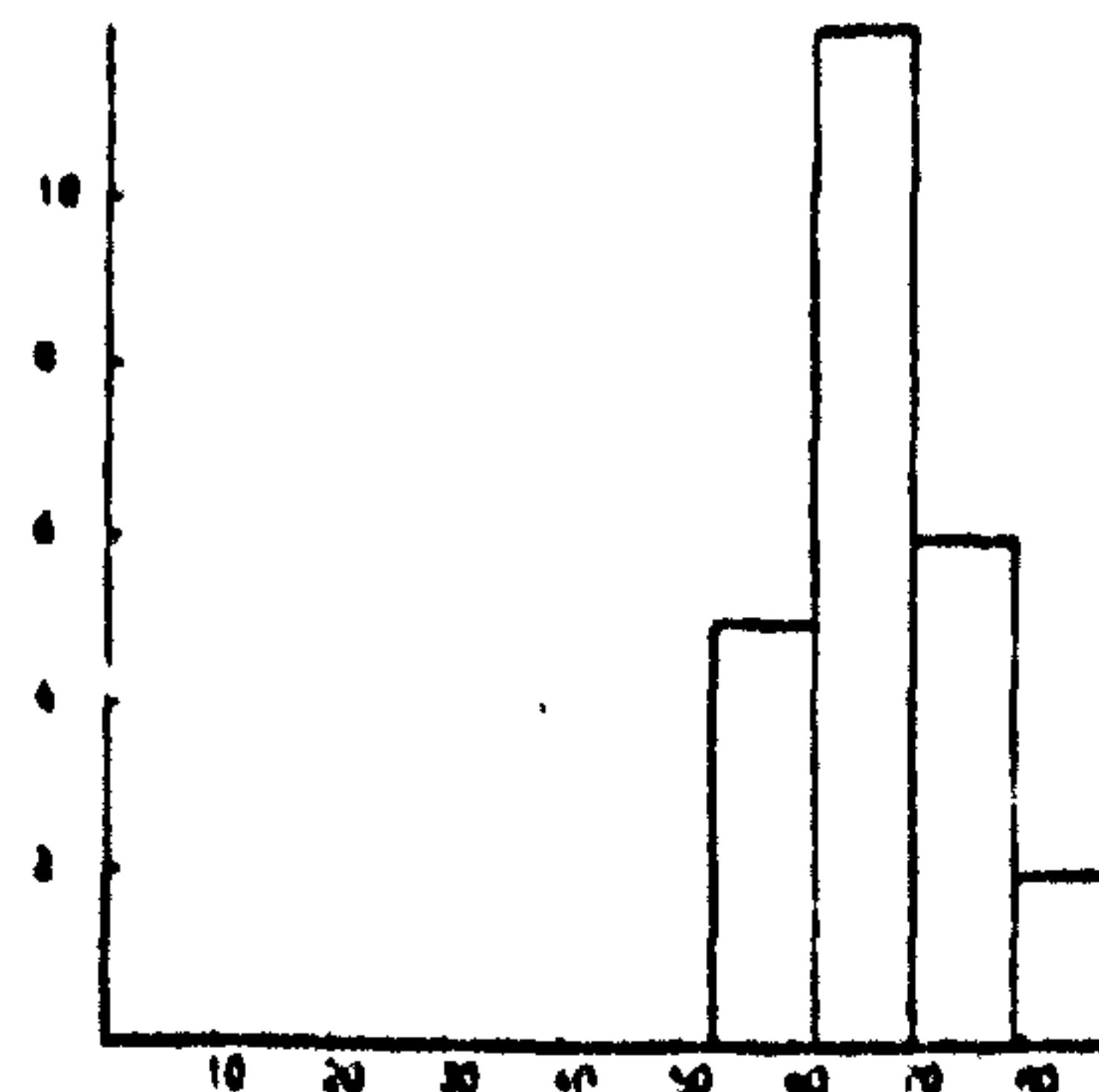


Fig. 13. Histograms illustrating variation of var. resupinatum  
before culture (a-d) and after culture (e-h):

- |                         |                    |
|-------------------------|--------------------|
| (a) leaf length;        | (b) nerve length ; |
| (c) angular cell size ; | (d) cell size .    |

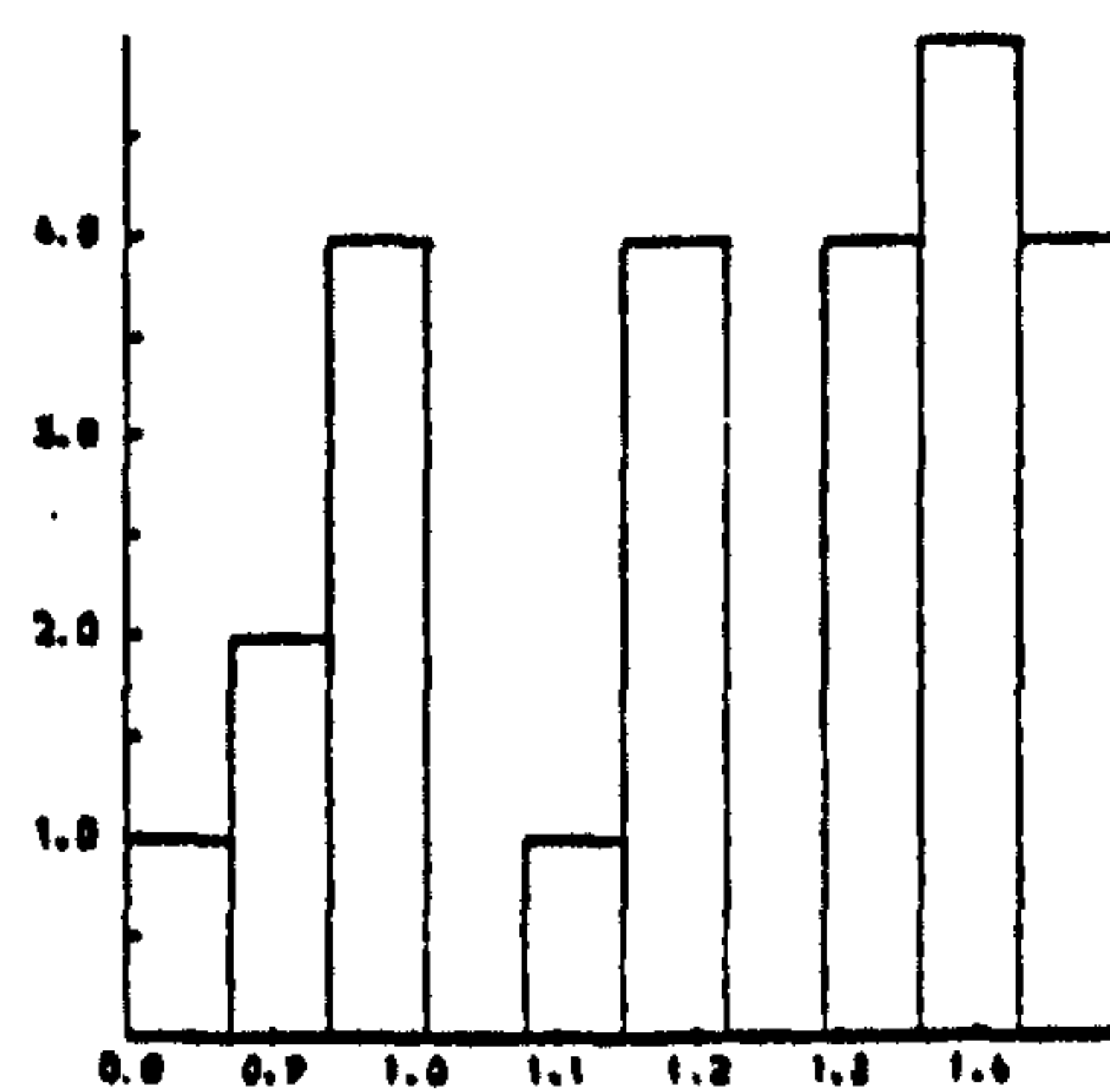
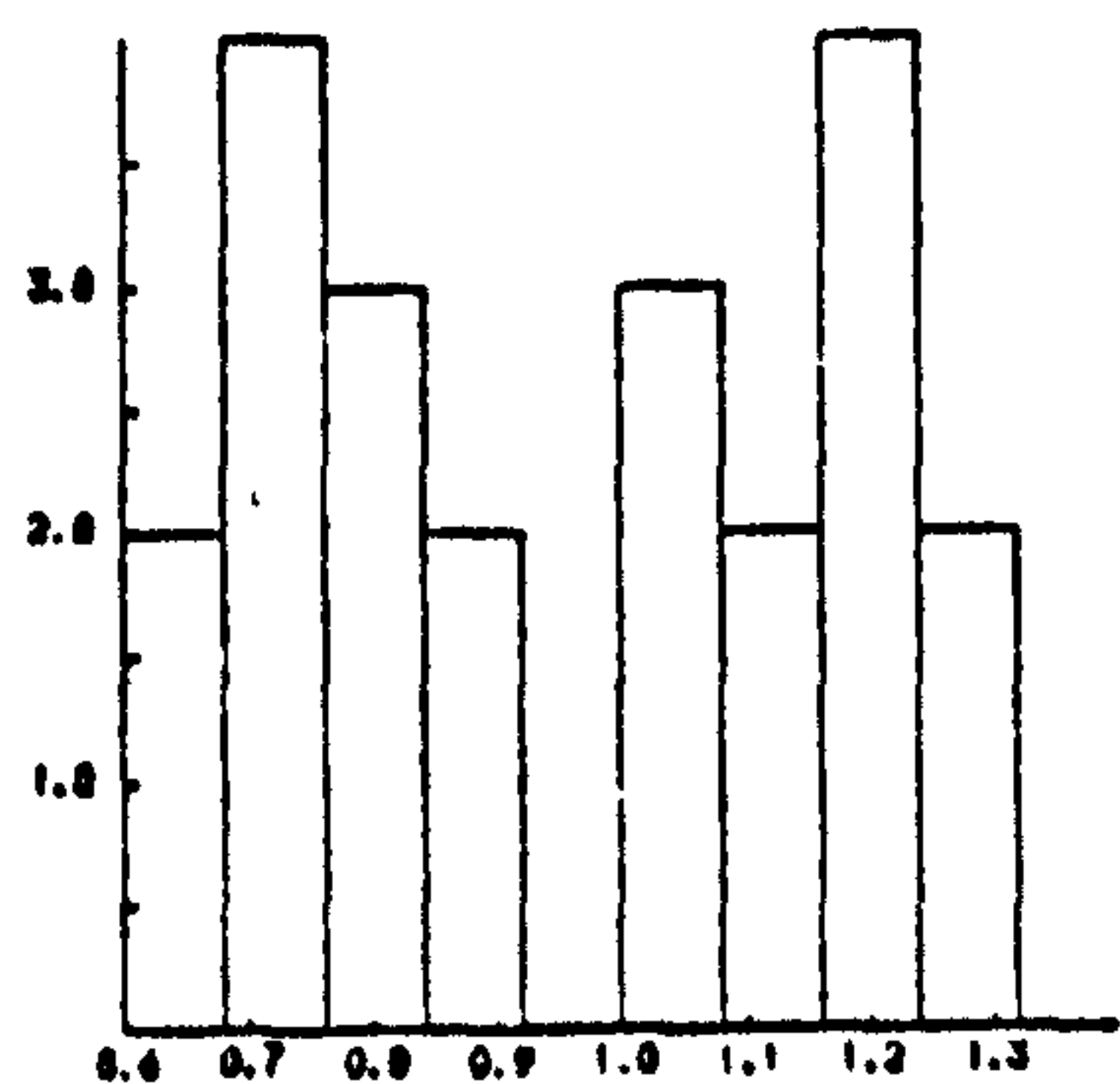
Note that the Y axis is not identical in all cases.

Character (a) in mm; for (b), 1 unit = 0.1 mm; for (c) & (d), 1 unit = 26  $\mu$ m.

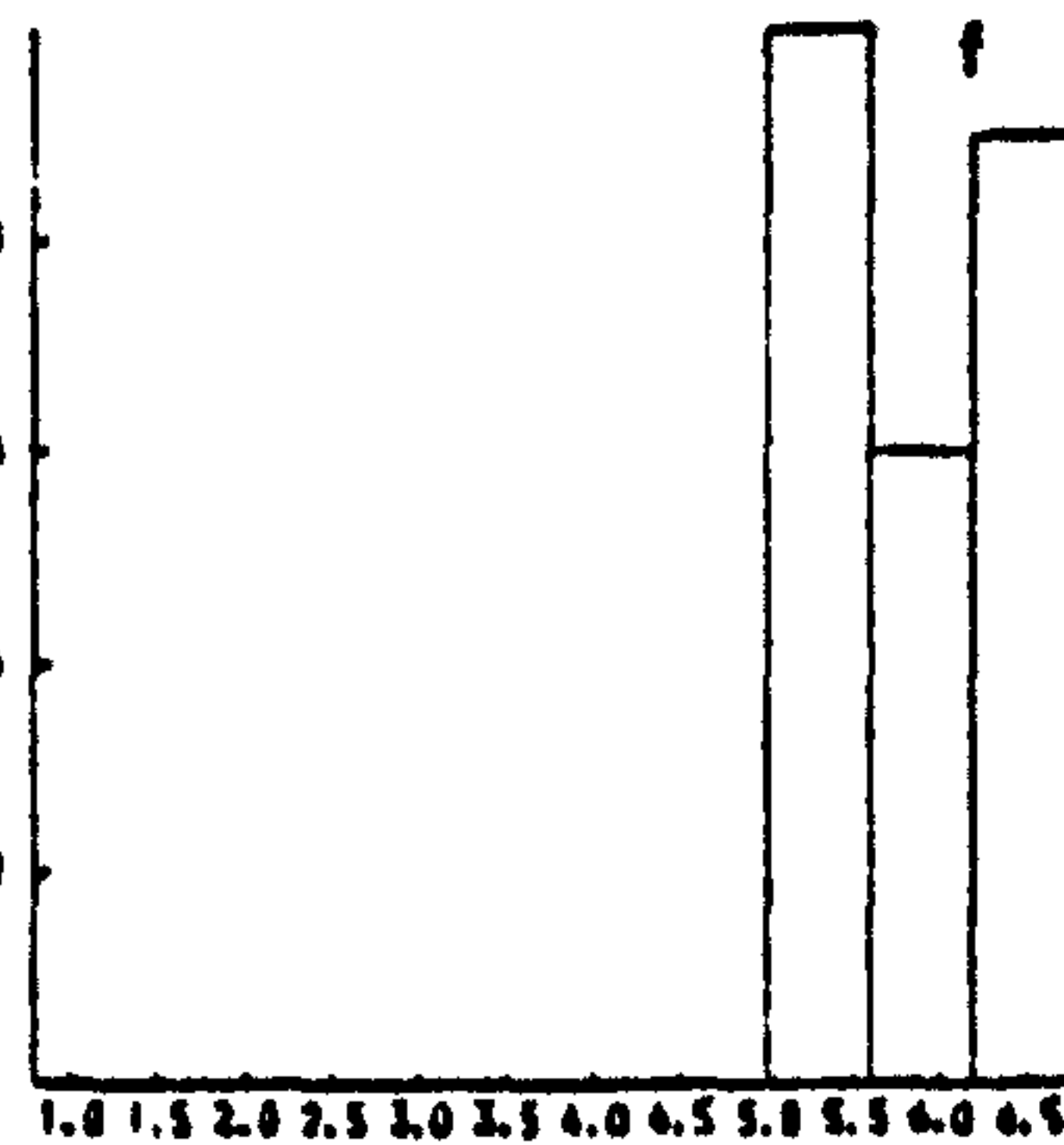
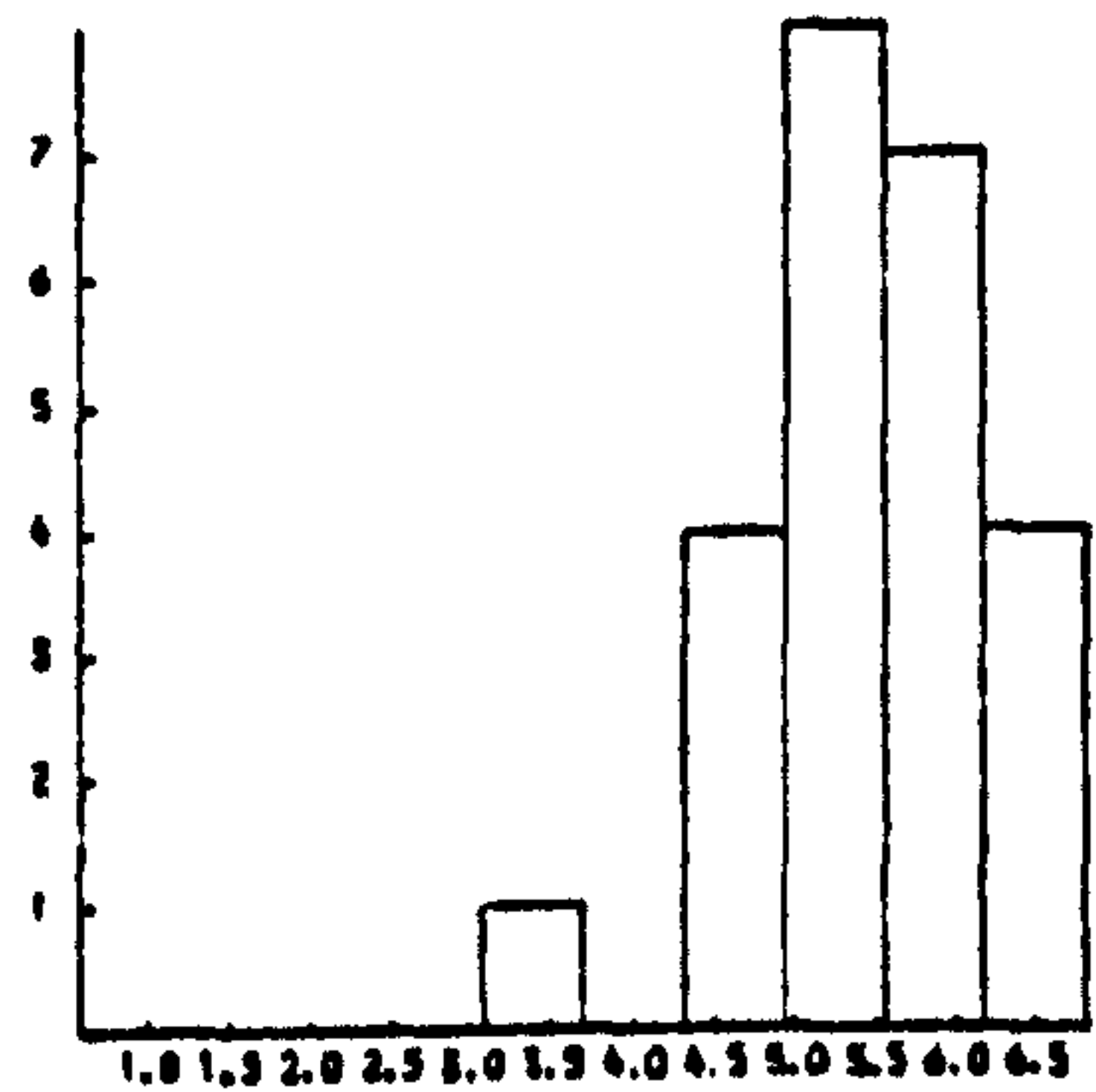
# BEFORE CULTURE

# AFTER CULTURE

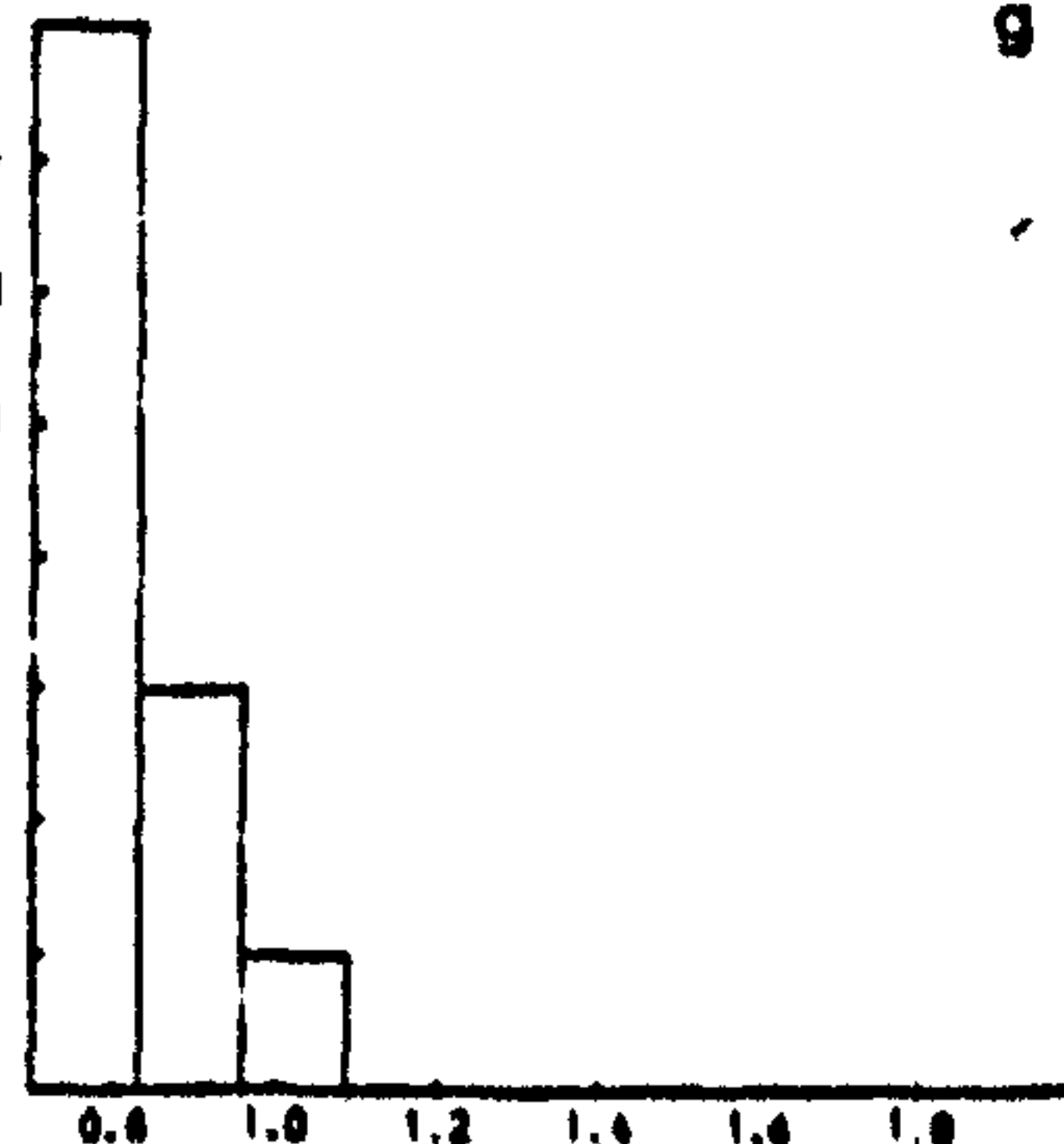
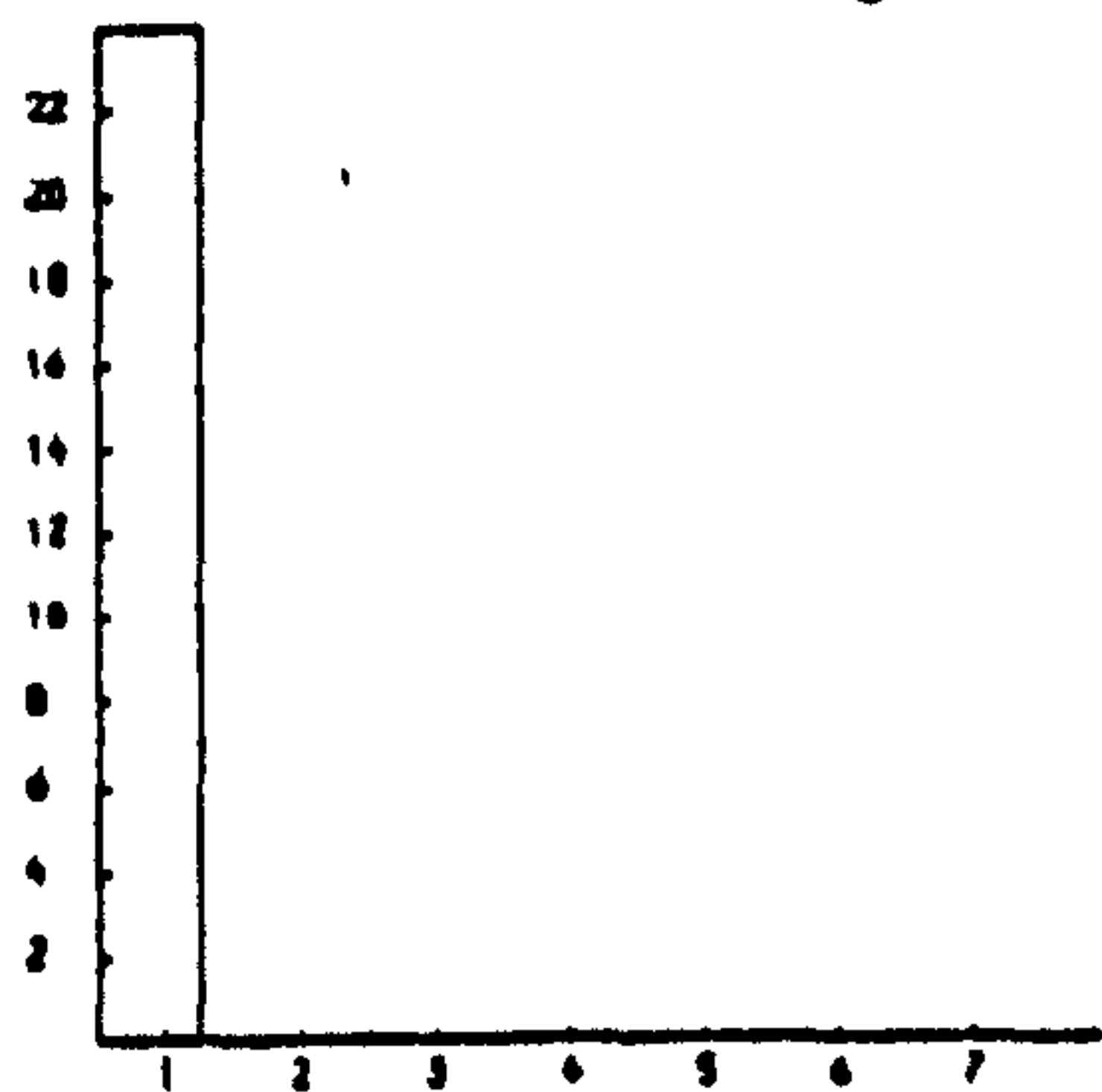
a



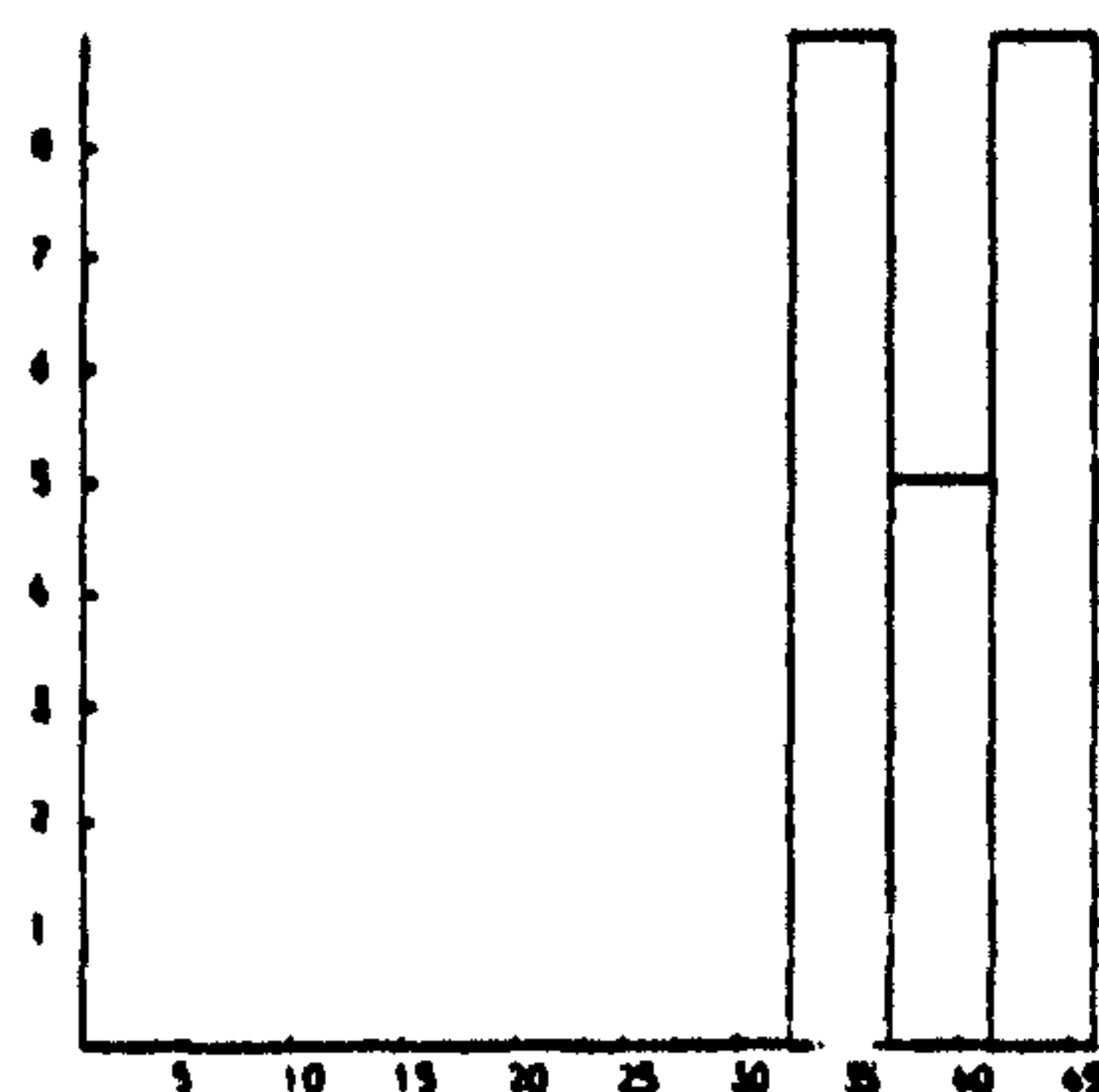
b



c



d



h

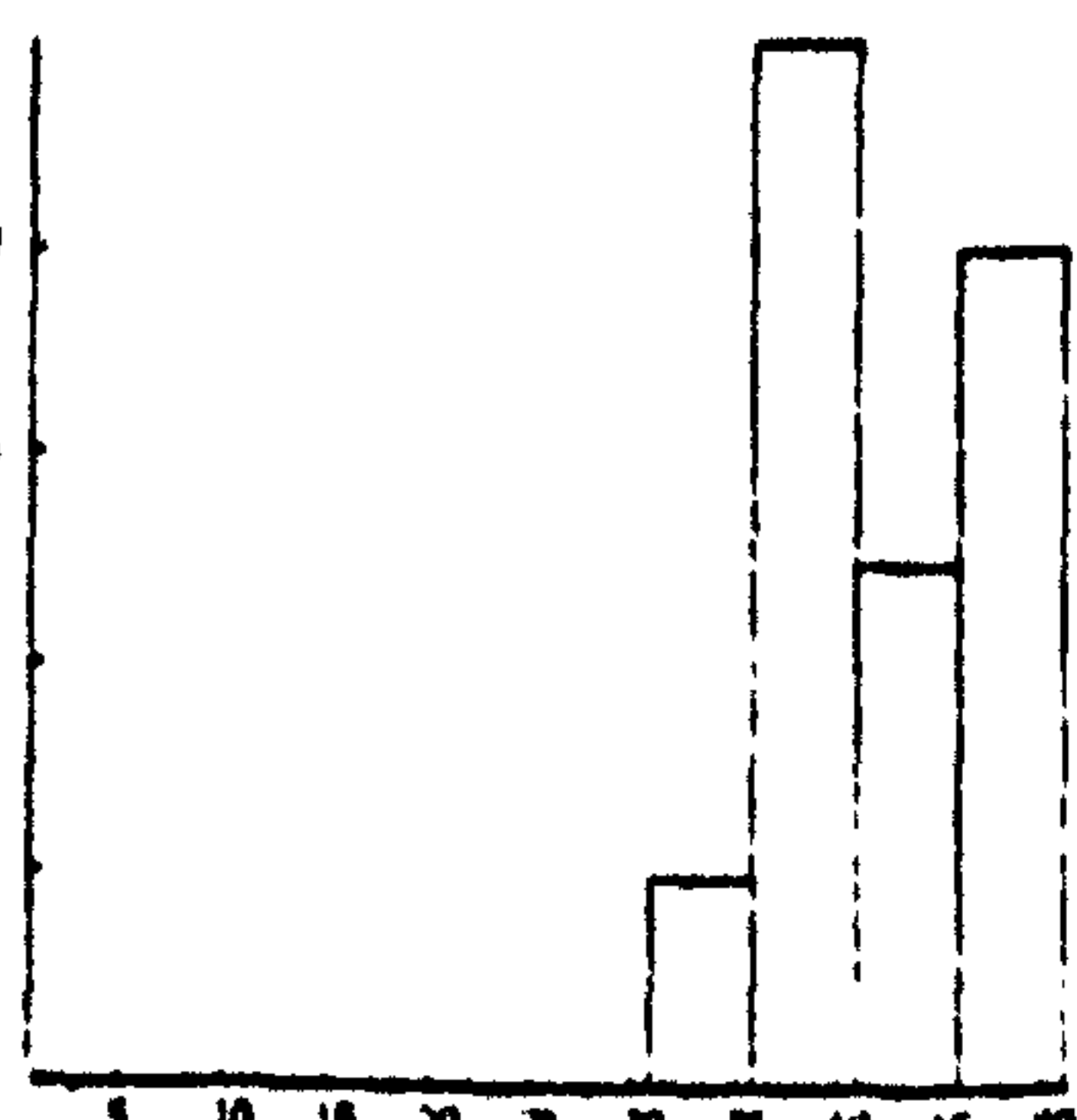


Fig. 14. Histograms illustrating variation of var. lacunosum  
before culture (a-d) and after culture (e-h):

- |                        |                   |
|------------------------|-------------------|
| (a) leaf length;       | (b) nerve length; |
| (c) angular cell size; | (d) cell size.    |

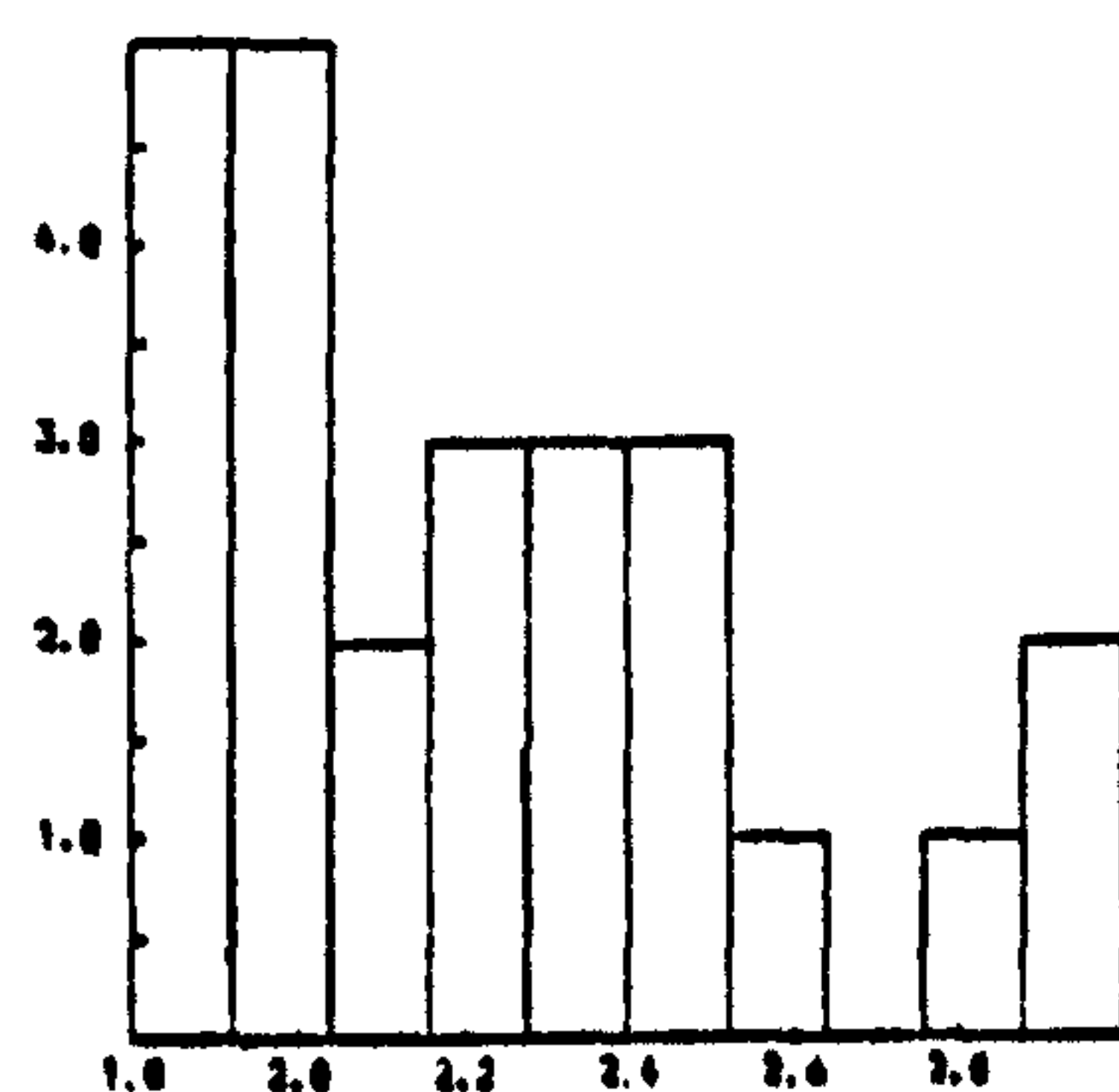
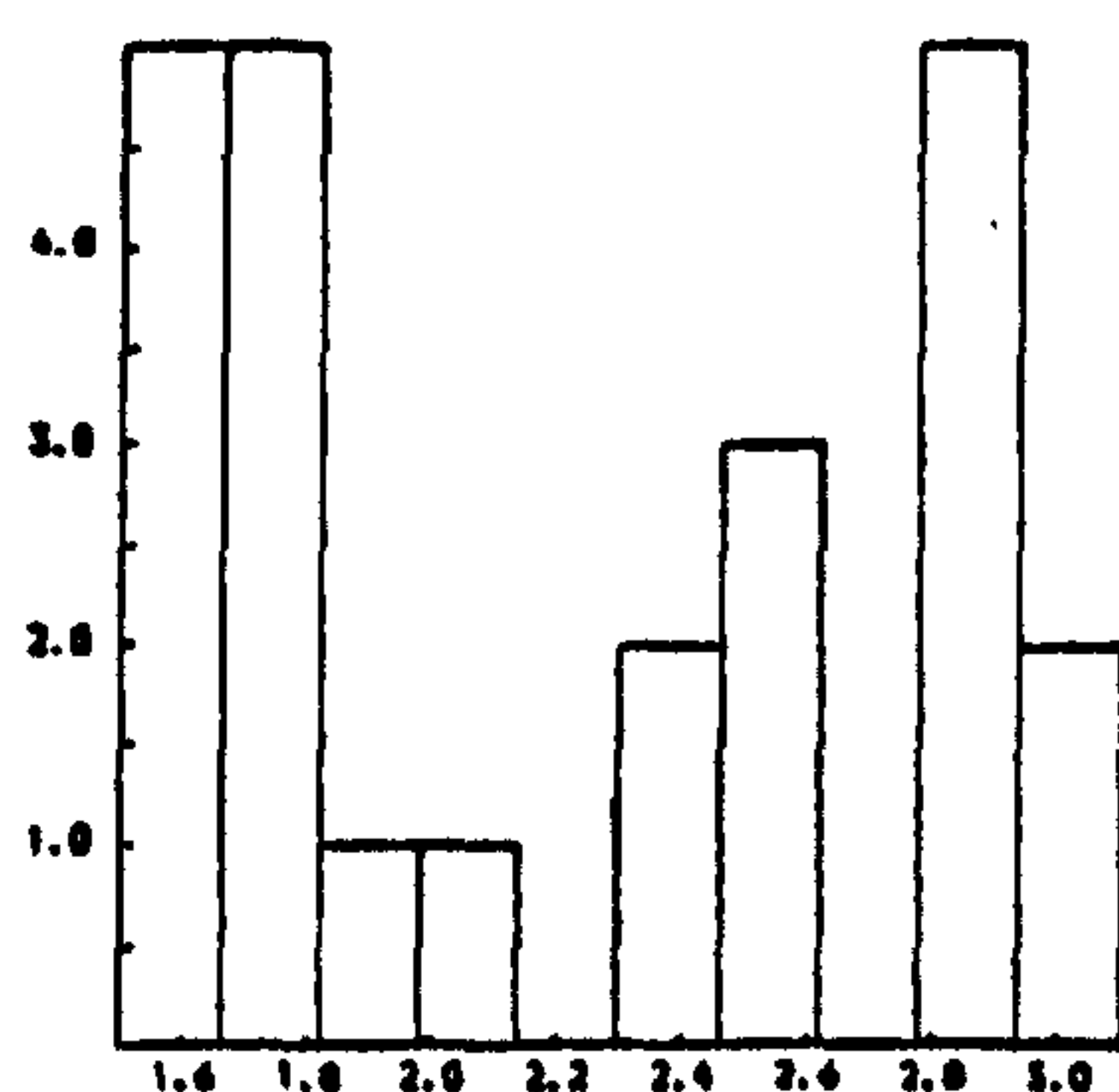
Note that the Y axis is not identical in all cases.

Character (a) in mm; for (b), 1 unit = 0.1 mm; for (c) & (d), 1 unit = 26  $\mu$ m.

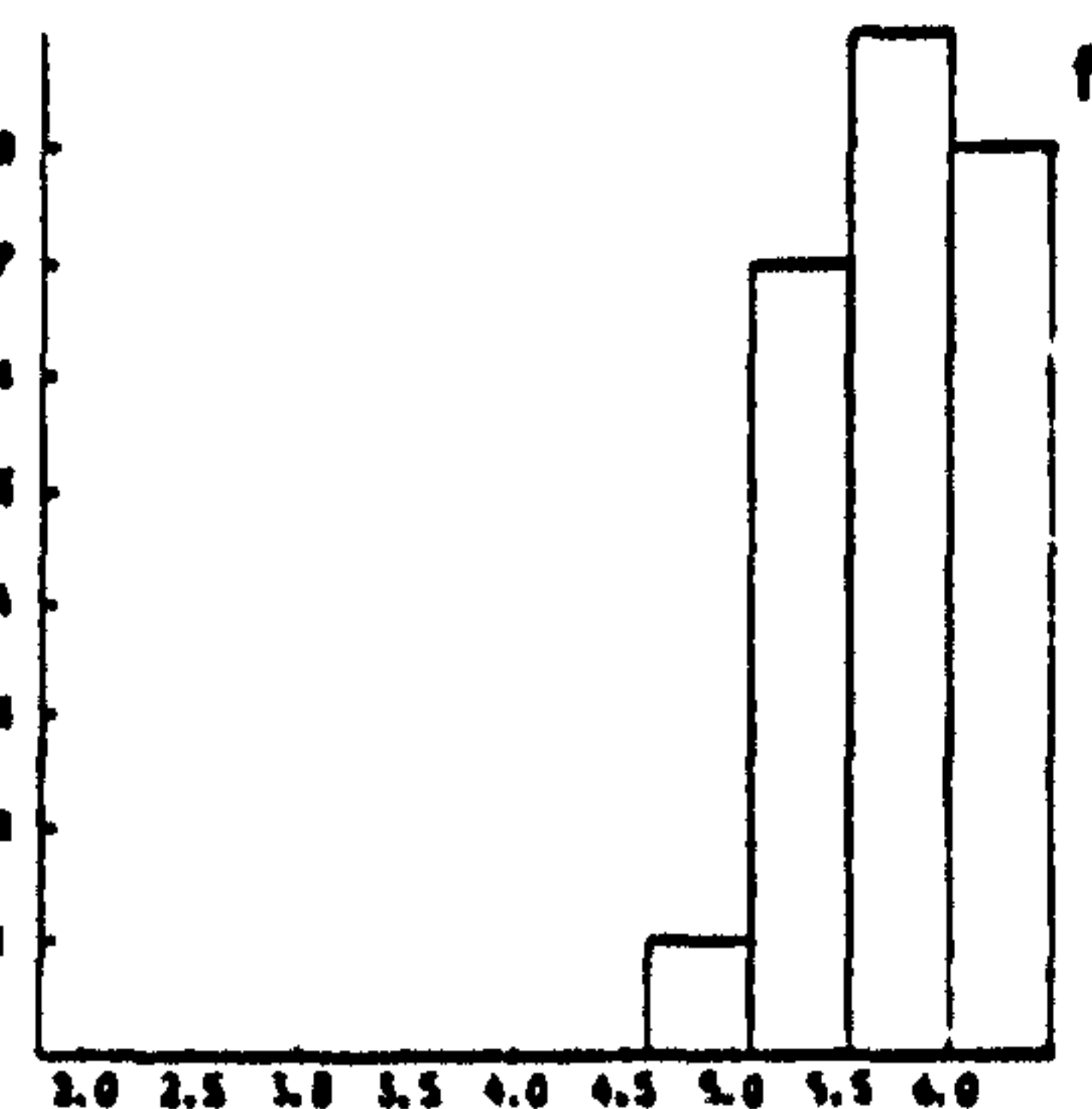
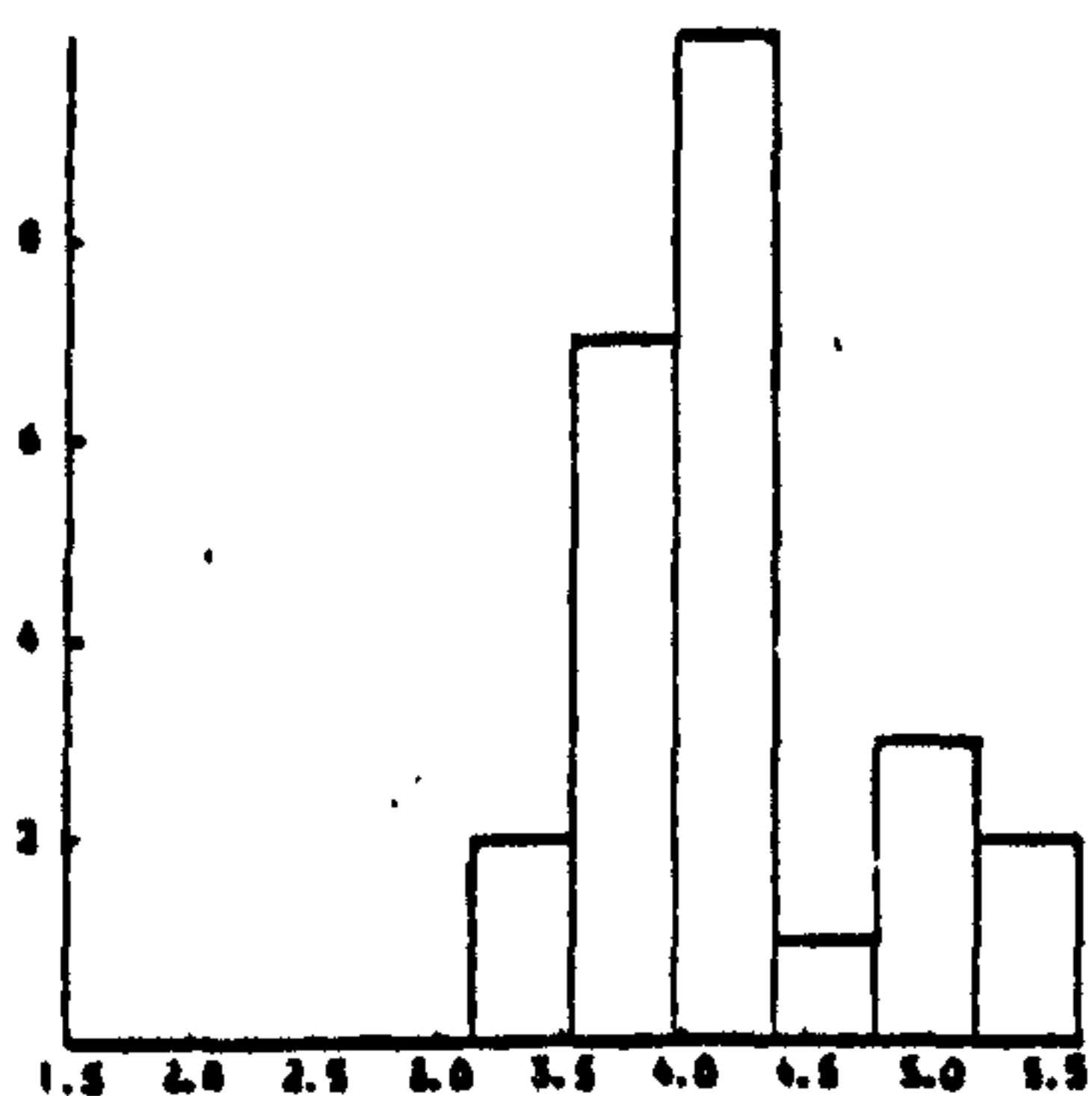
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# AFTER CULTURE

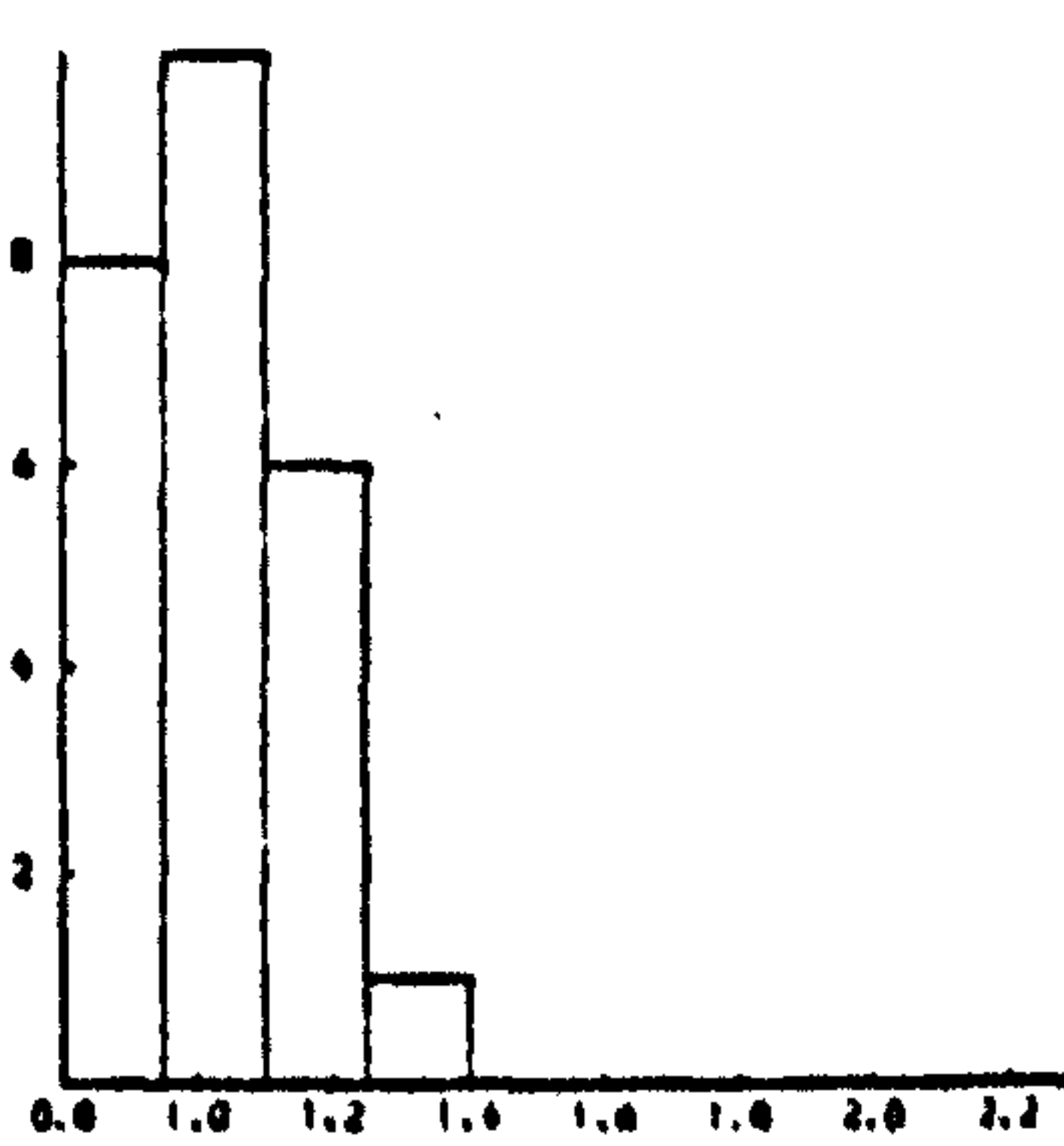
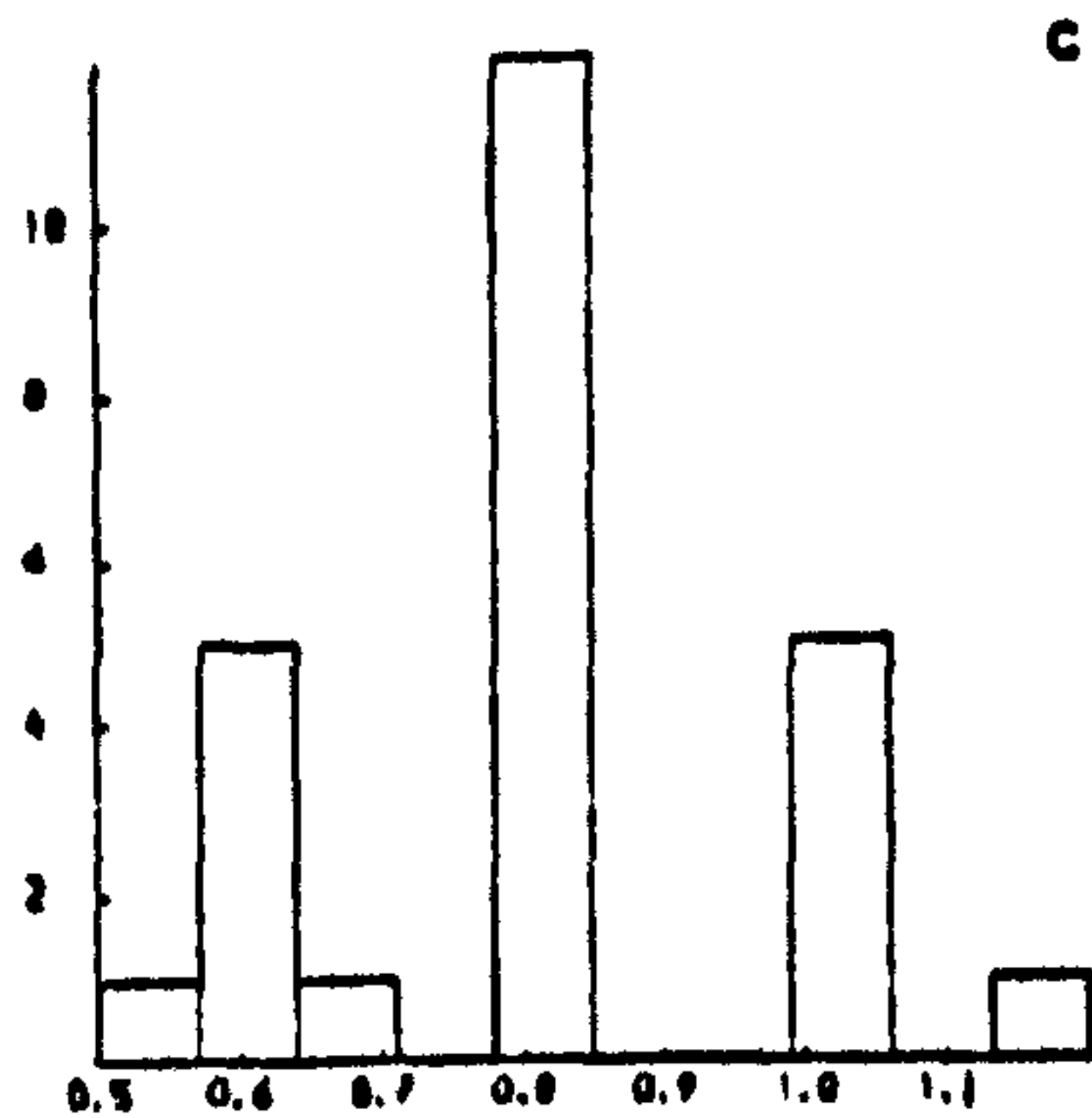
a



b



c



d

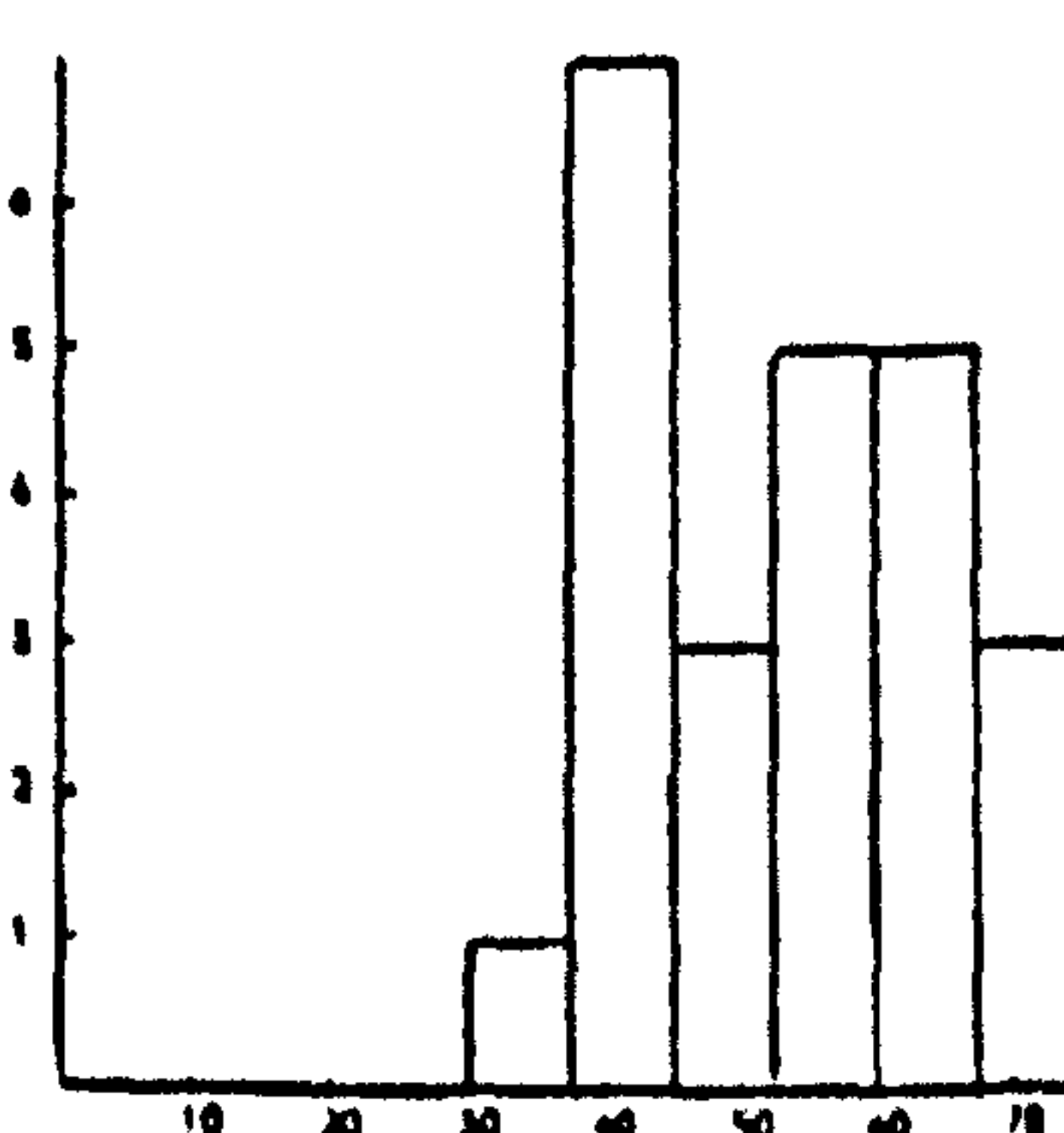
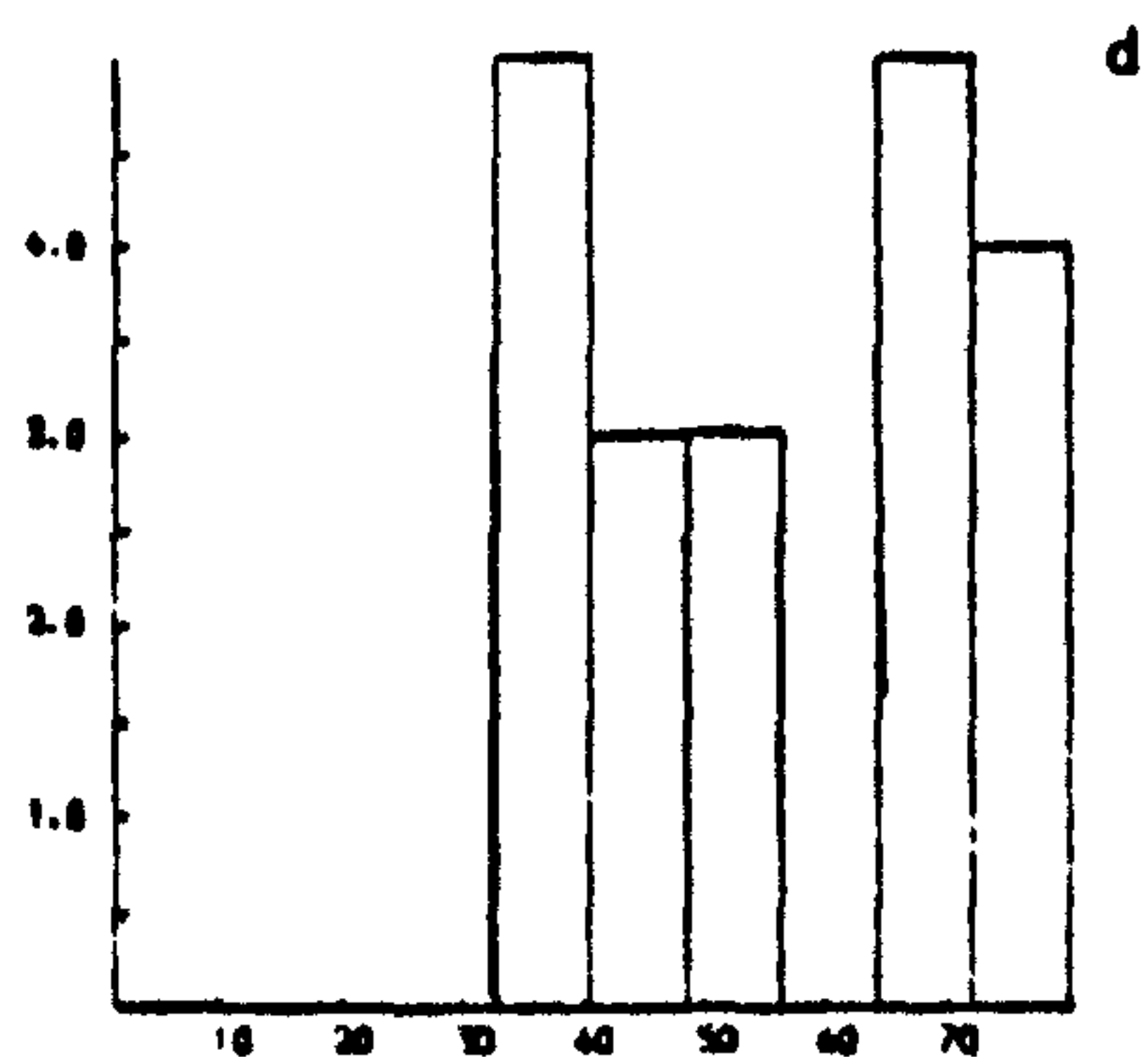




Fig. 15. Histograms illustrating variation of var. filiforme  
before culture (a-d) and after culture (e-h):

- |                         |                   |
|-------------------------|-------------------|
| (a) leaf length;        | (b) nerve length; |
| (c) angular cell size ; | (d) cell size .   |

Note that the Y axis is not identical in all cases.

Character (a) in mm; for (b), 1 unit = 0.1 mm; for (c) & (d), 1 unit = 26  $\mu$ m.

# BEFORE CULTURE

# AFTER CULTURE

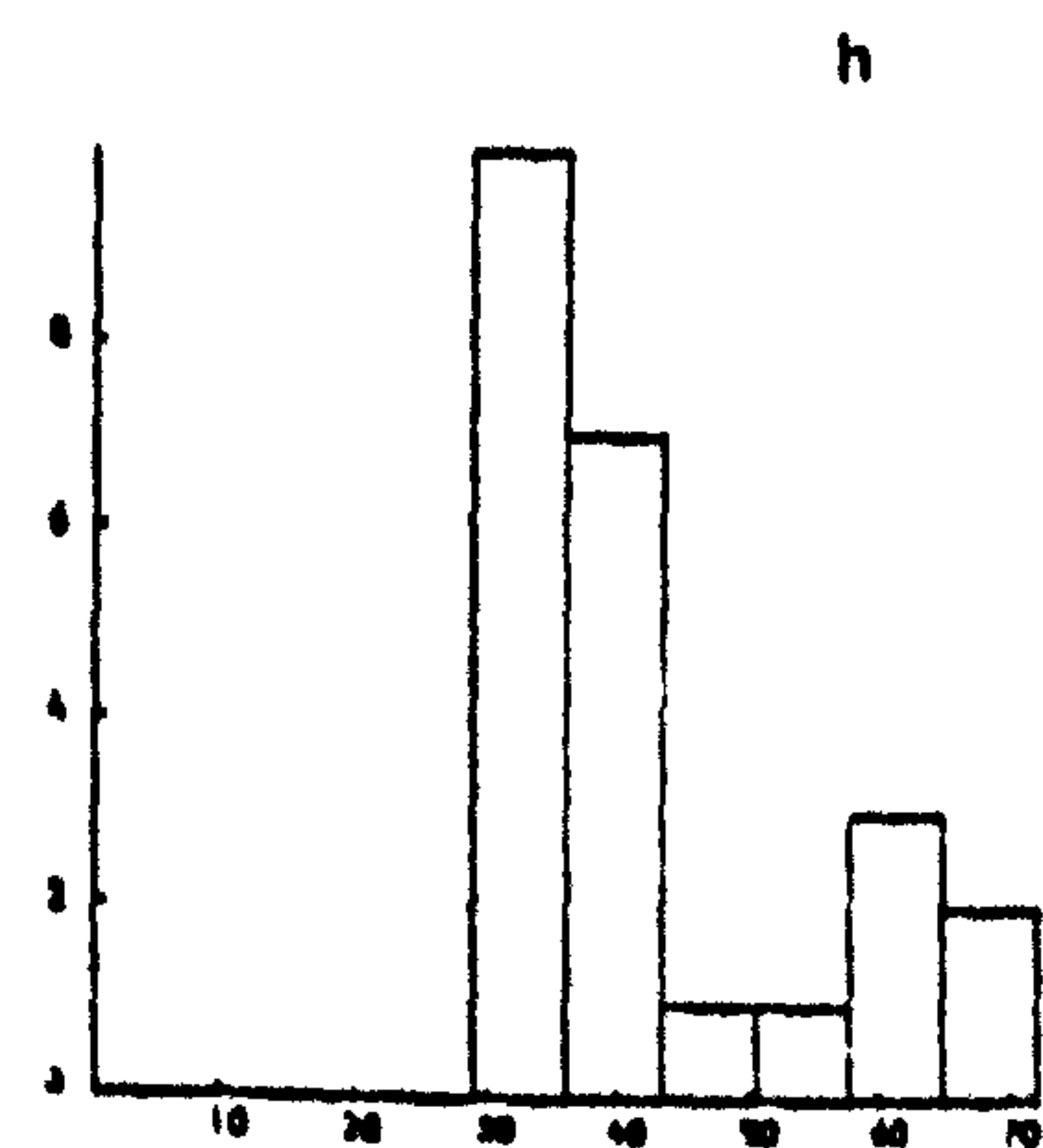
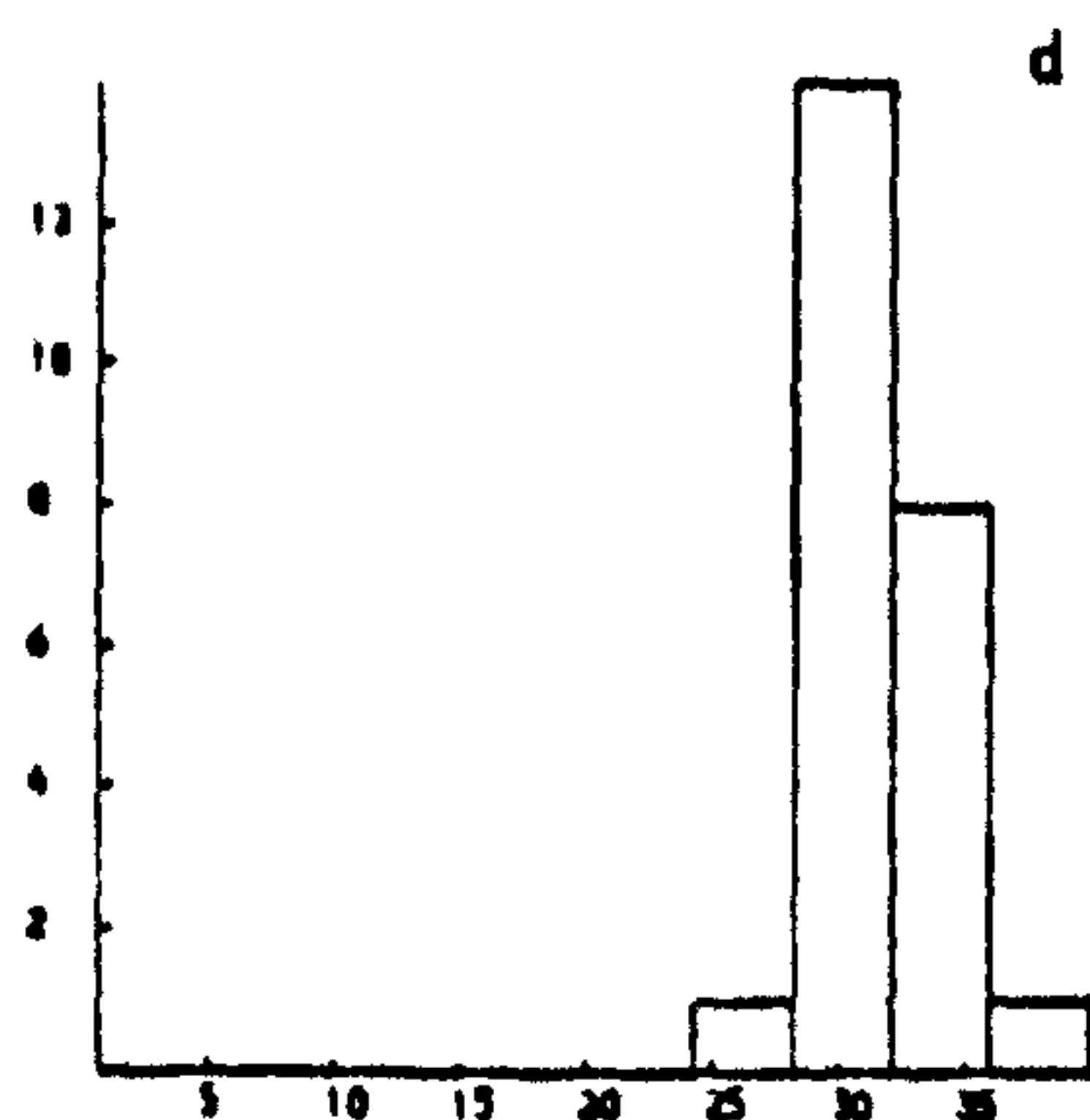
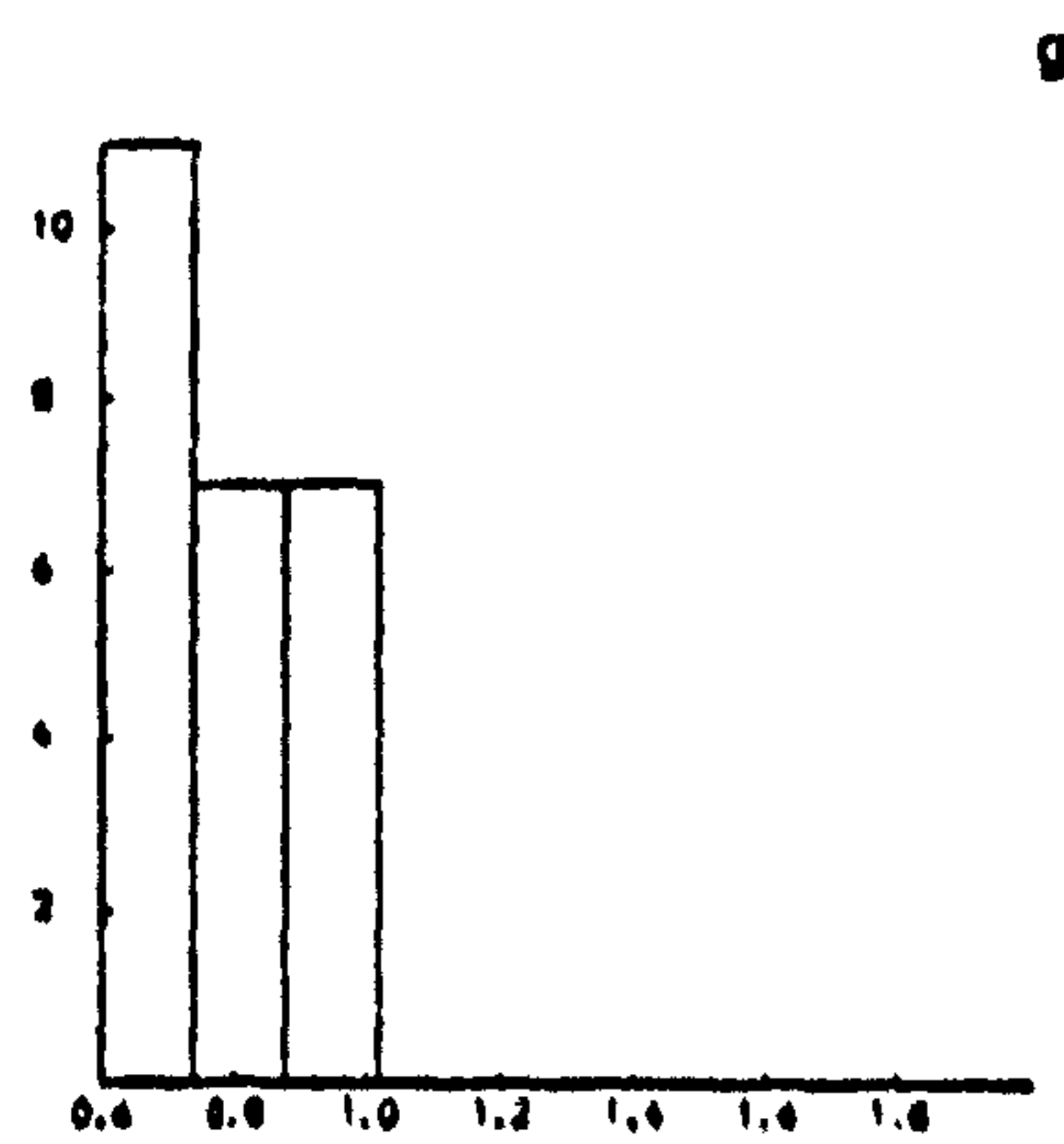
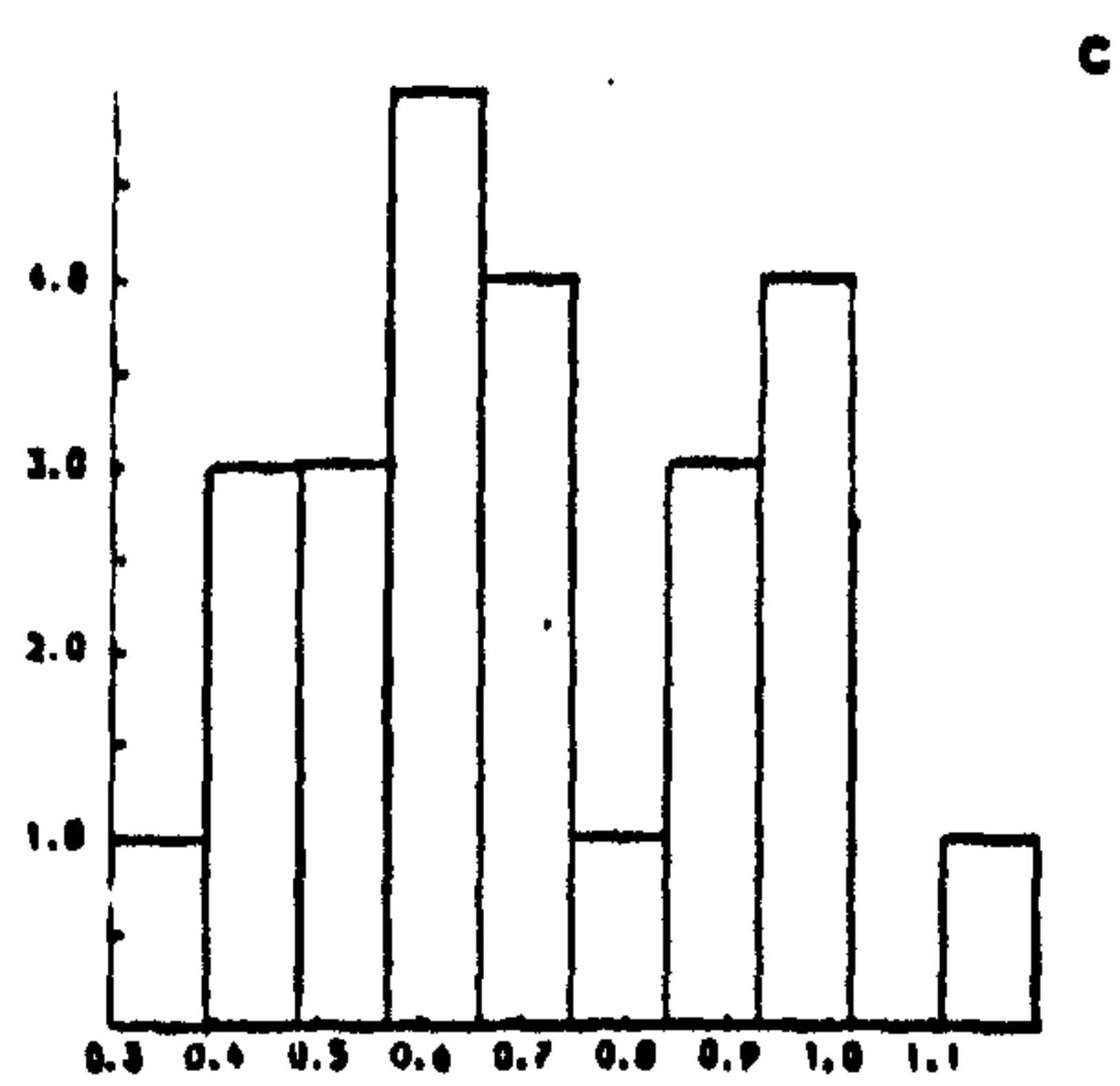
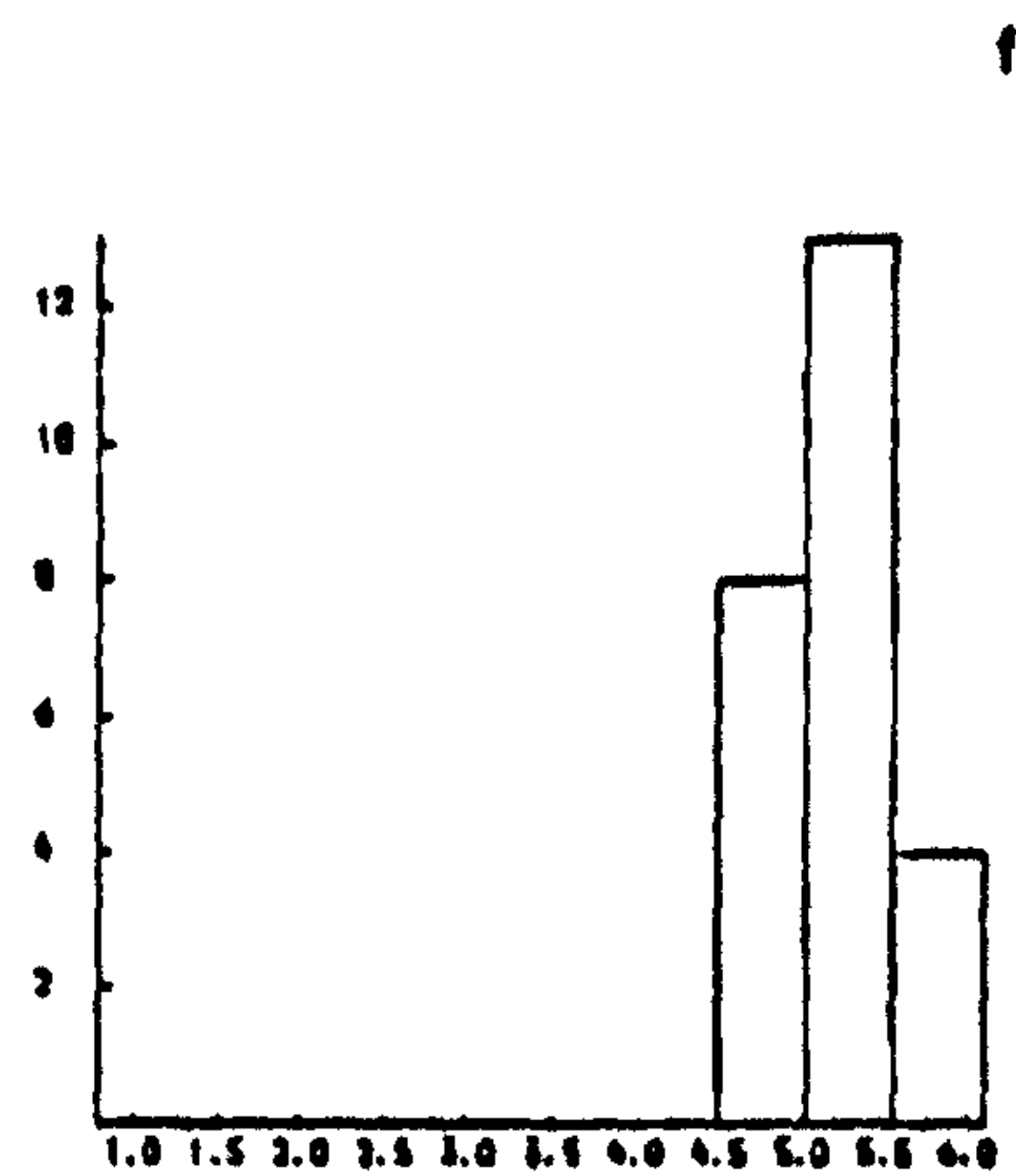
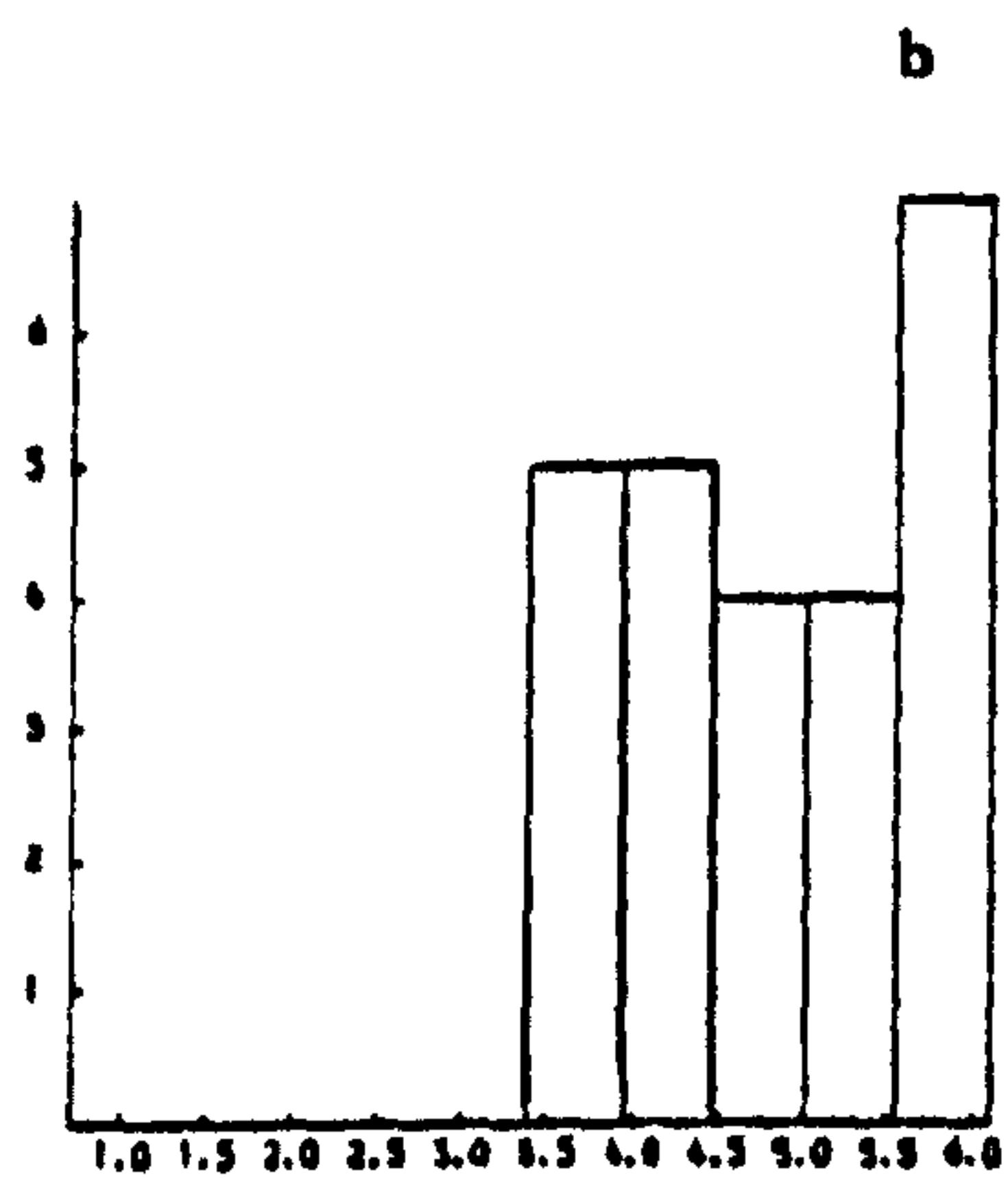
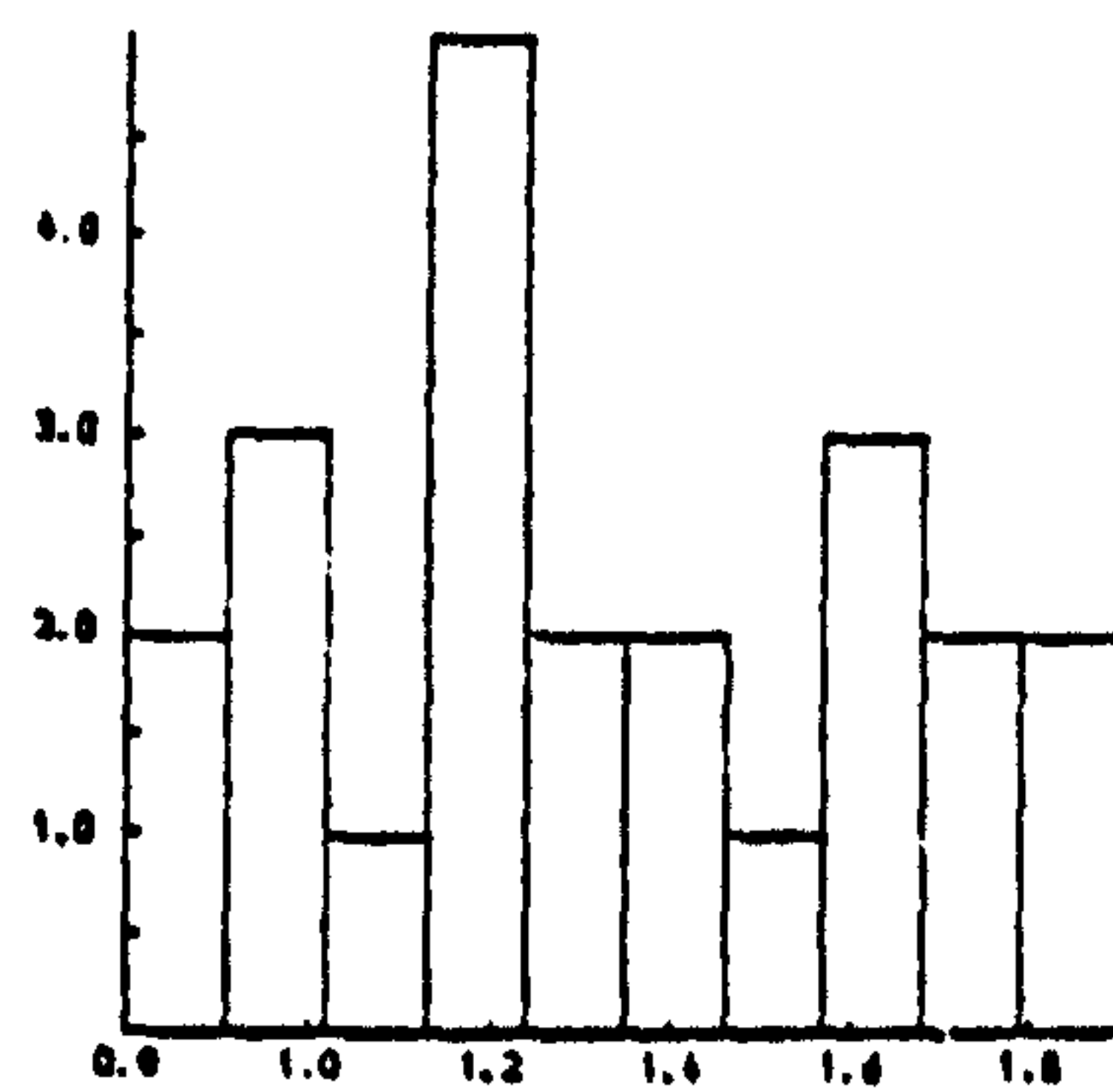
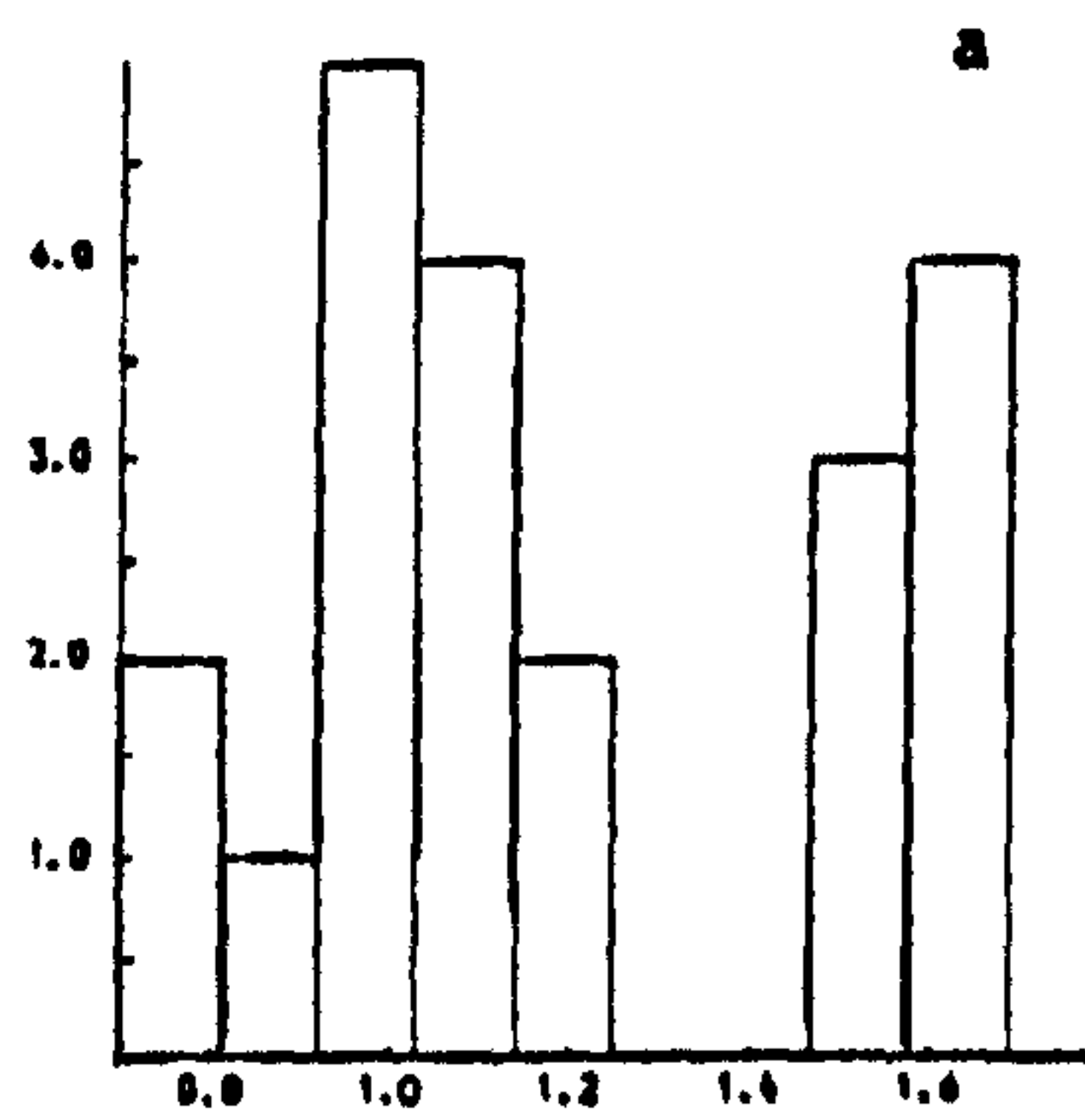


Fig. 16. Histograms illustrating variation of H. mammillatum before culture (a-d) and after culture (e-h):

- |                         |                    |
|-------------------------|--------------------|
| (a) leaf length ;       | (b) nerve length ; |
| (c) angular cell size ; | (d) cell size .    |

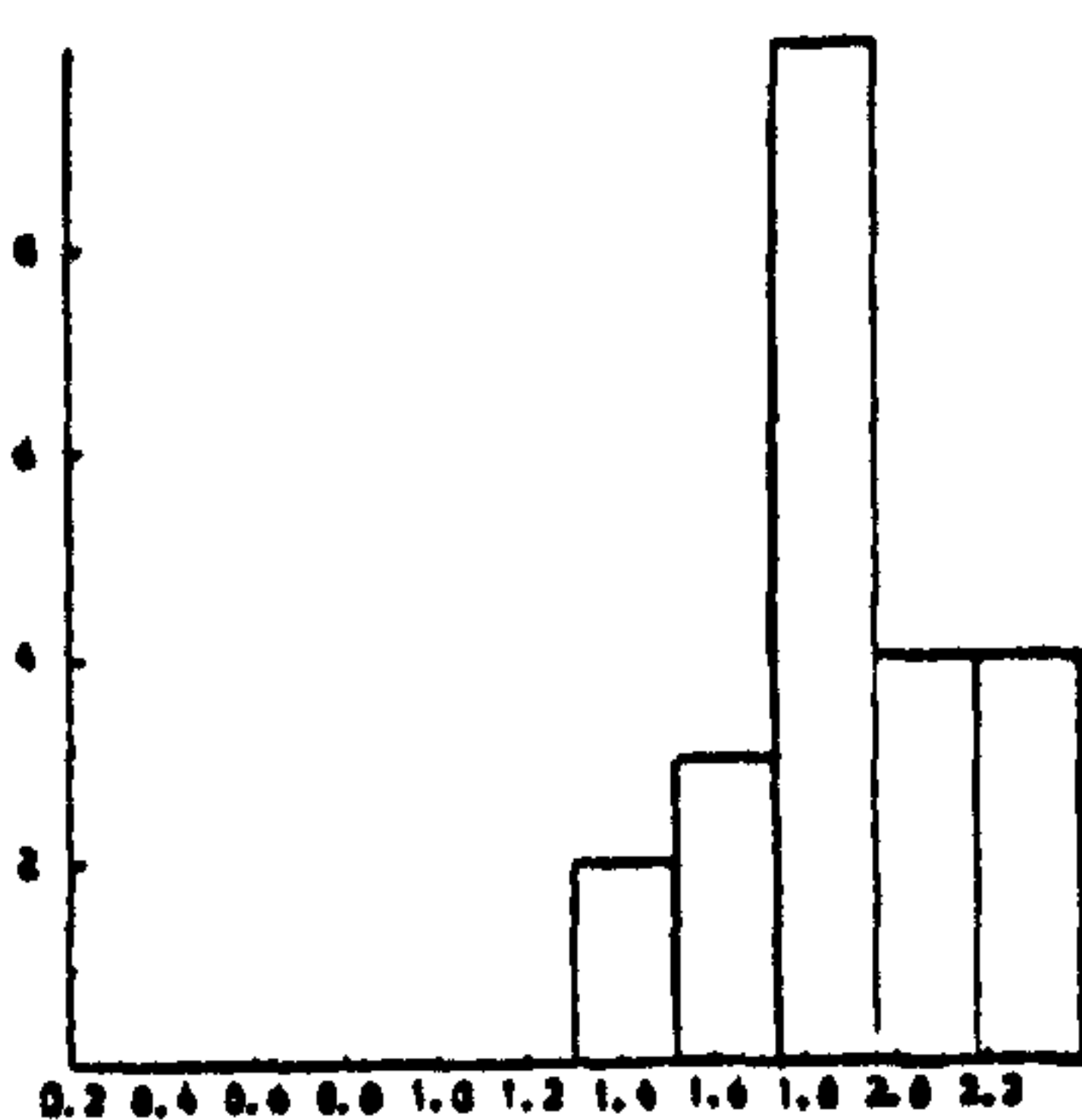
Note that the Y axis is not identical in all cases.

Character (a) in mm; for (b), 1 unit = 0.1 mm; for (c) & (d), 1 unit = 26  $\mu$ m.

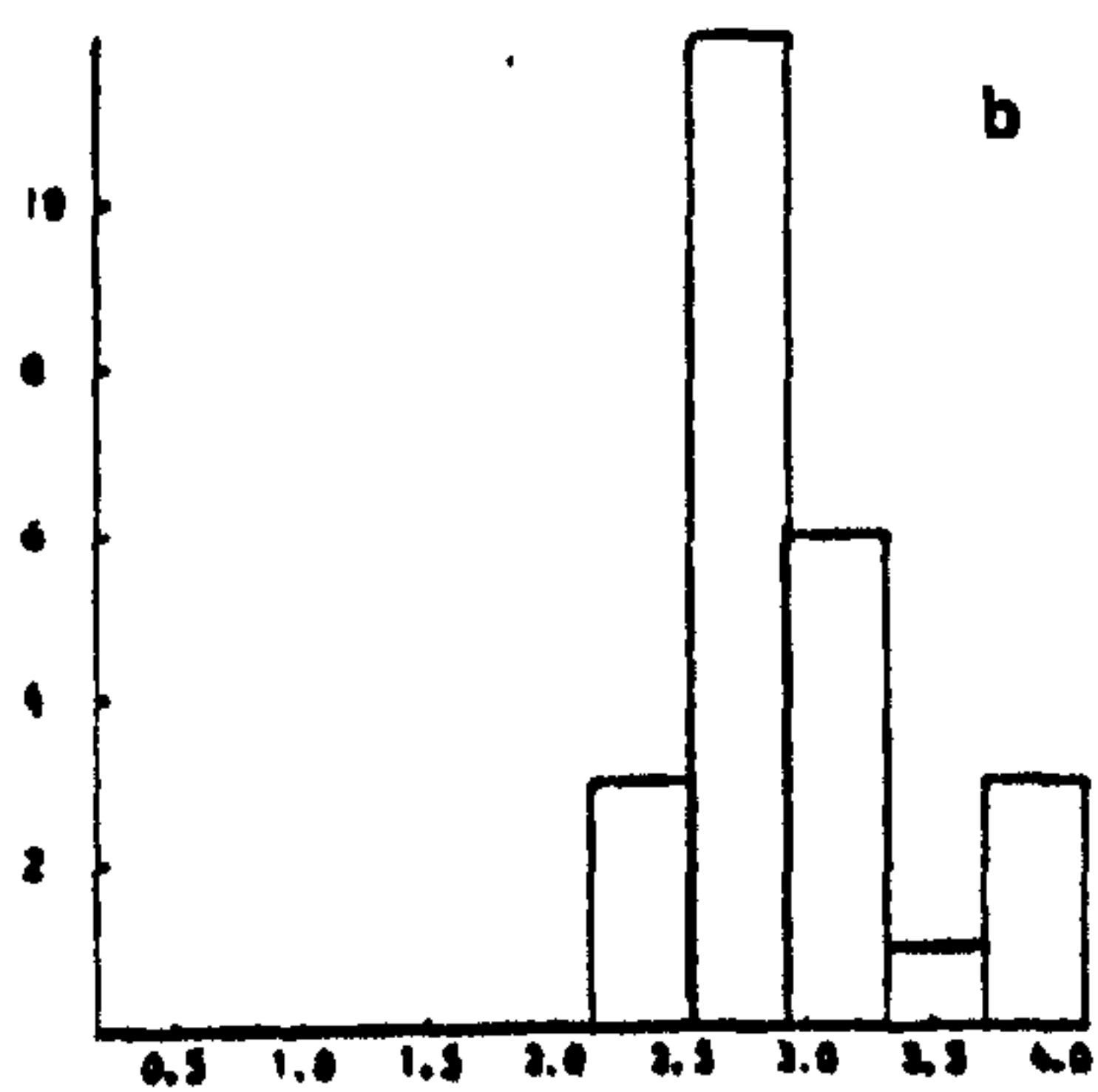
# BEFORE CULTURE

# AFTER CULTURE

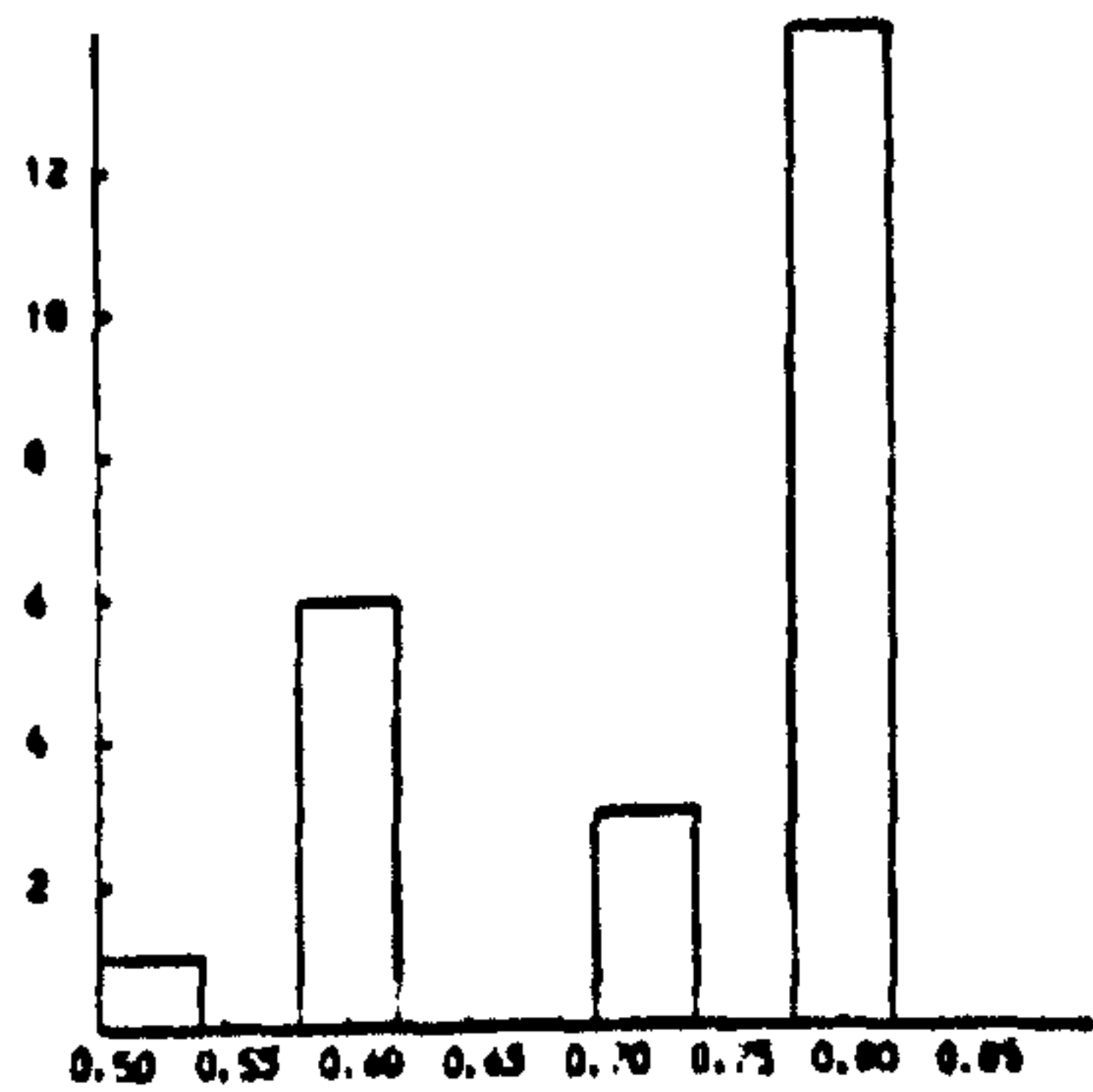
a



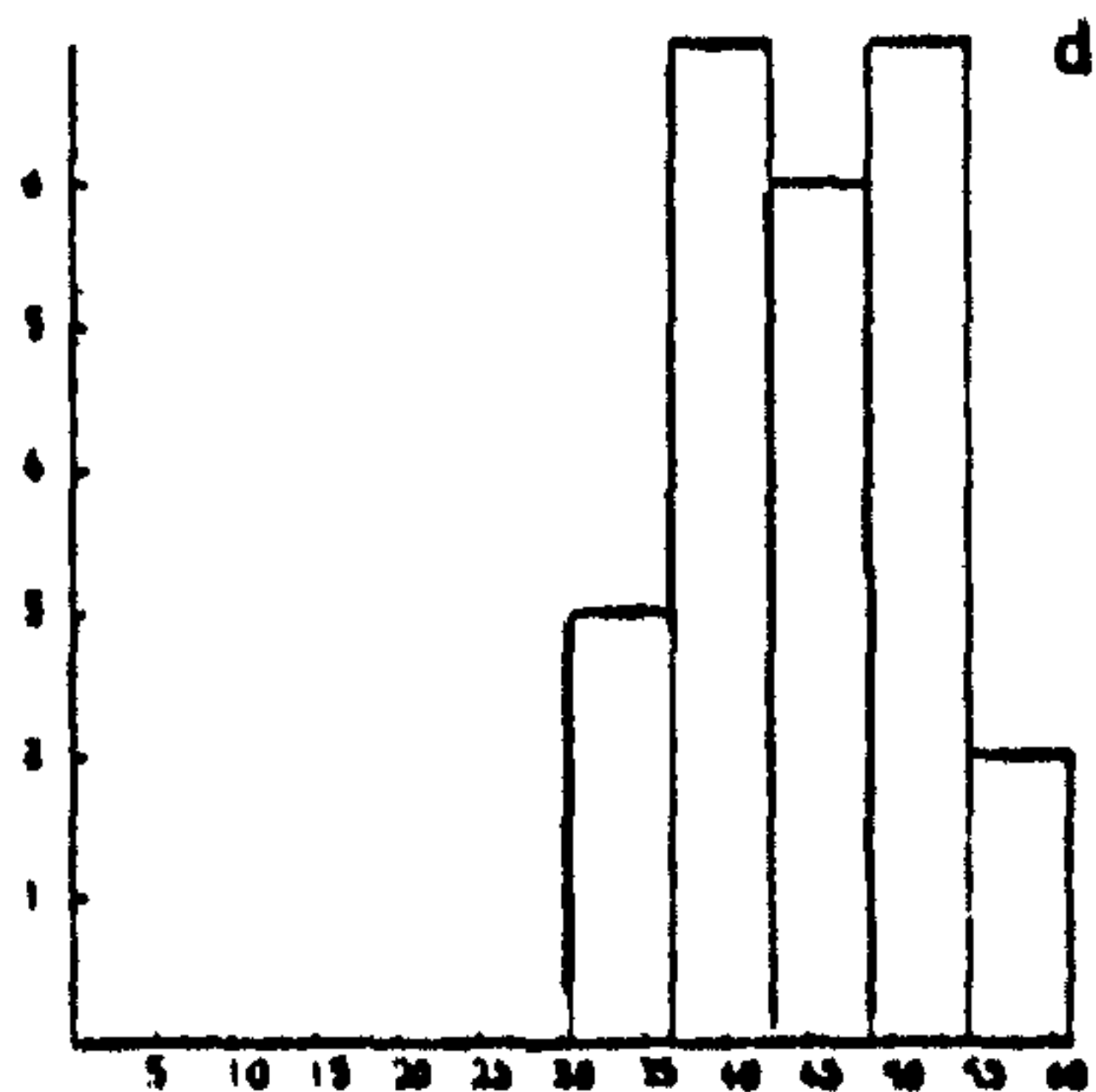
b



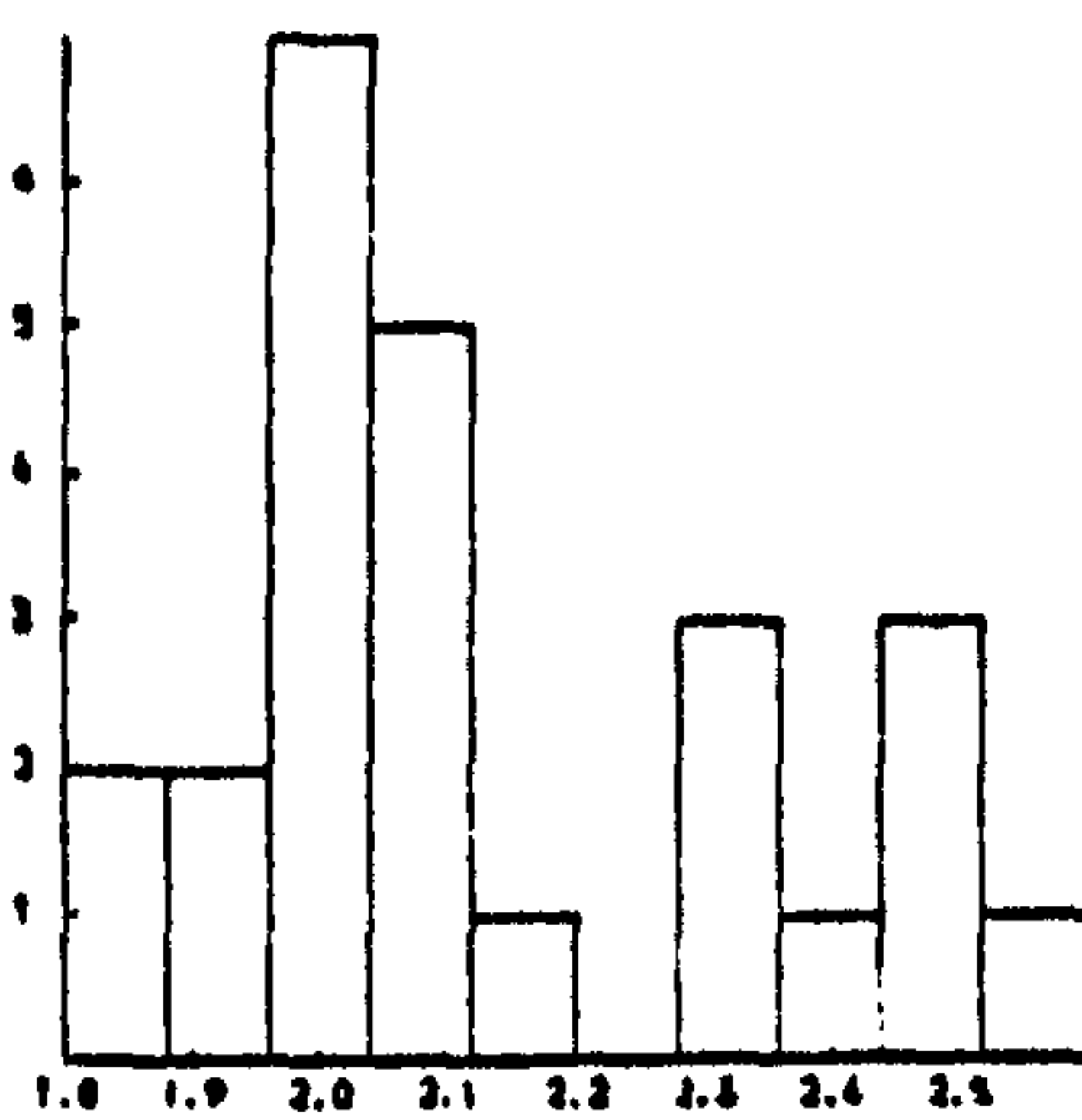
c



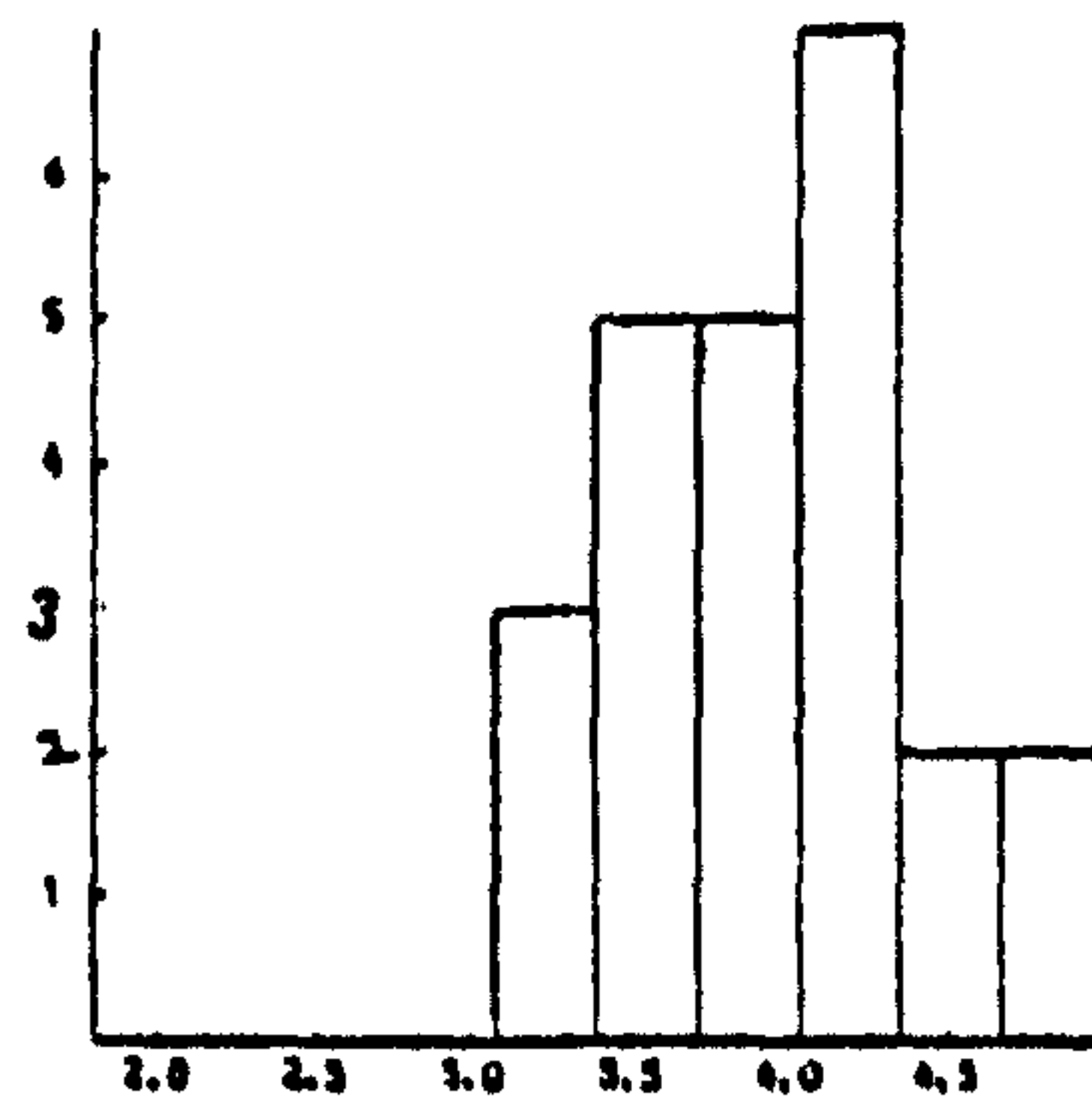
d



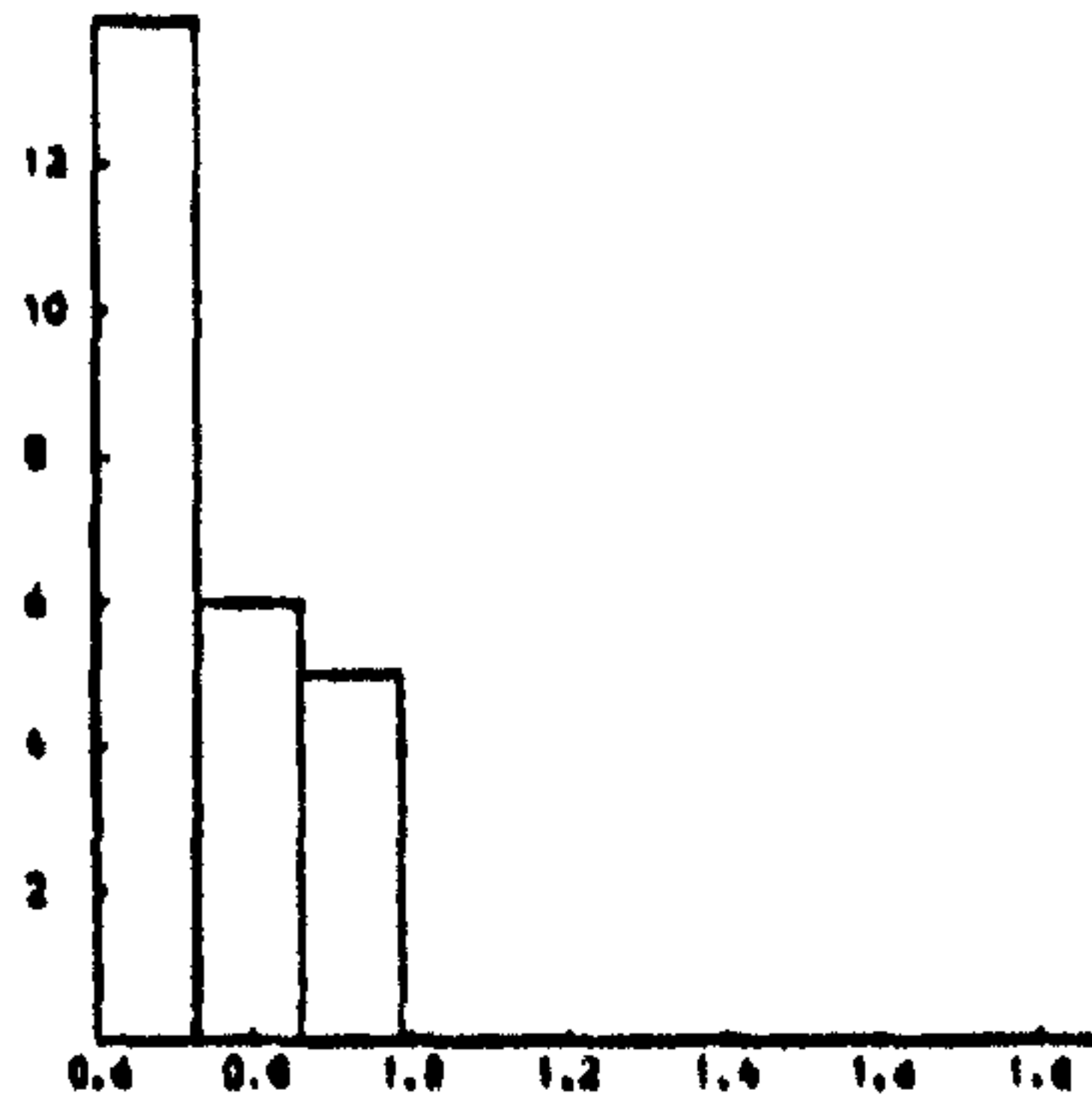
e



f



g



h

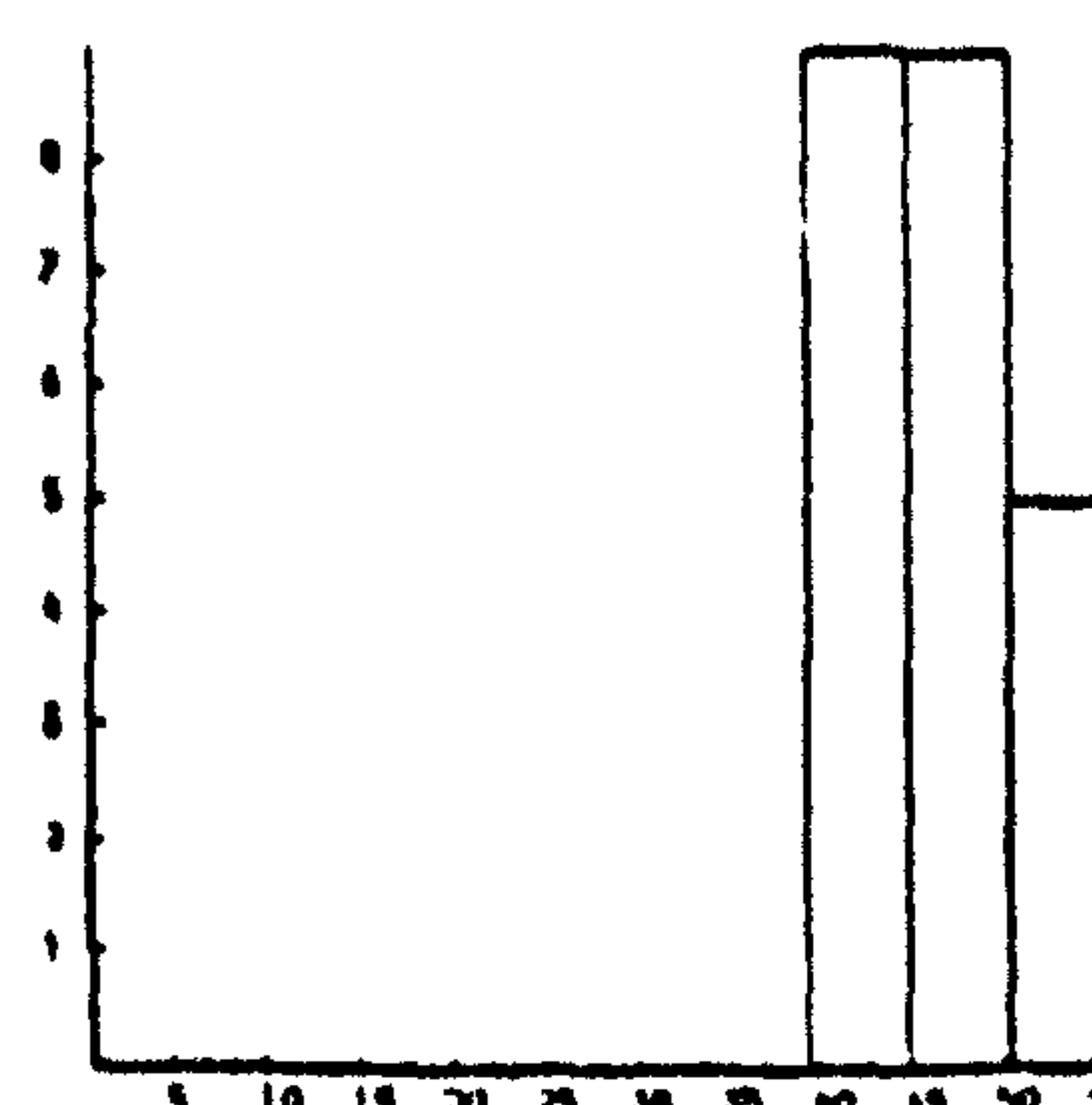




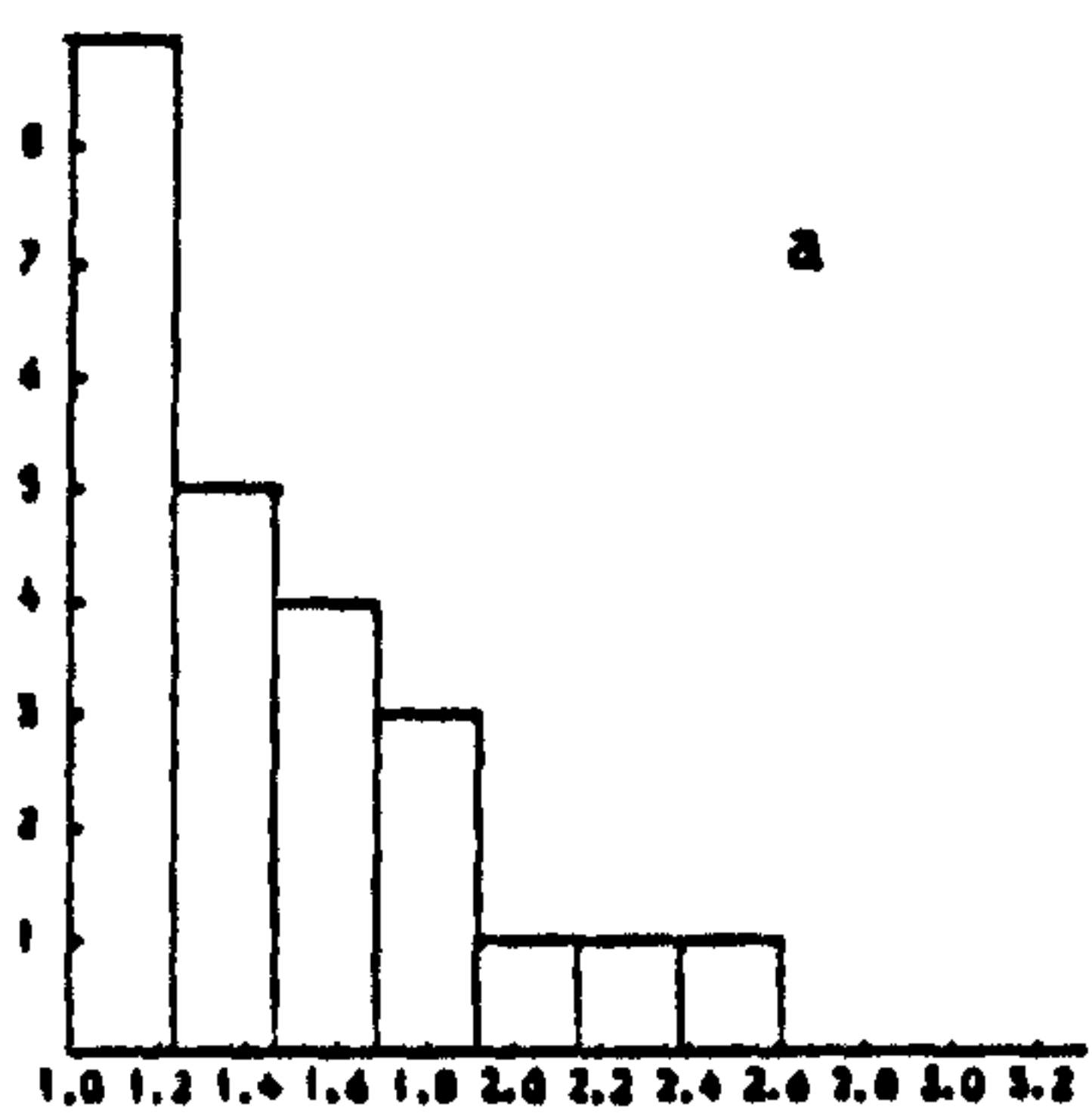
Fig. 17. Histograms illustrating variation of H. jutlandicum before culture (a-d) and after culture (e-h):

- |                         |                    |
|-------------------------|--------------------|
| (a) leaf length ;       | (b) nerve length ; |
| (c) angular cell size ; | (d) cell size.     |

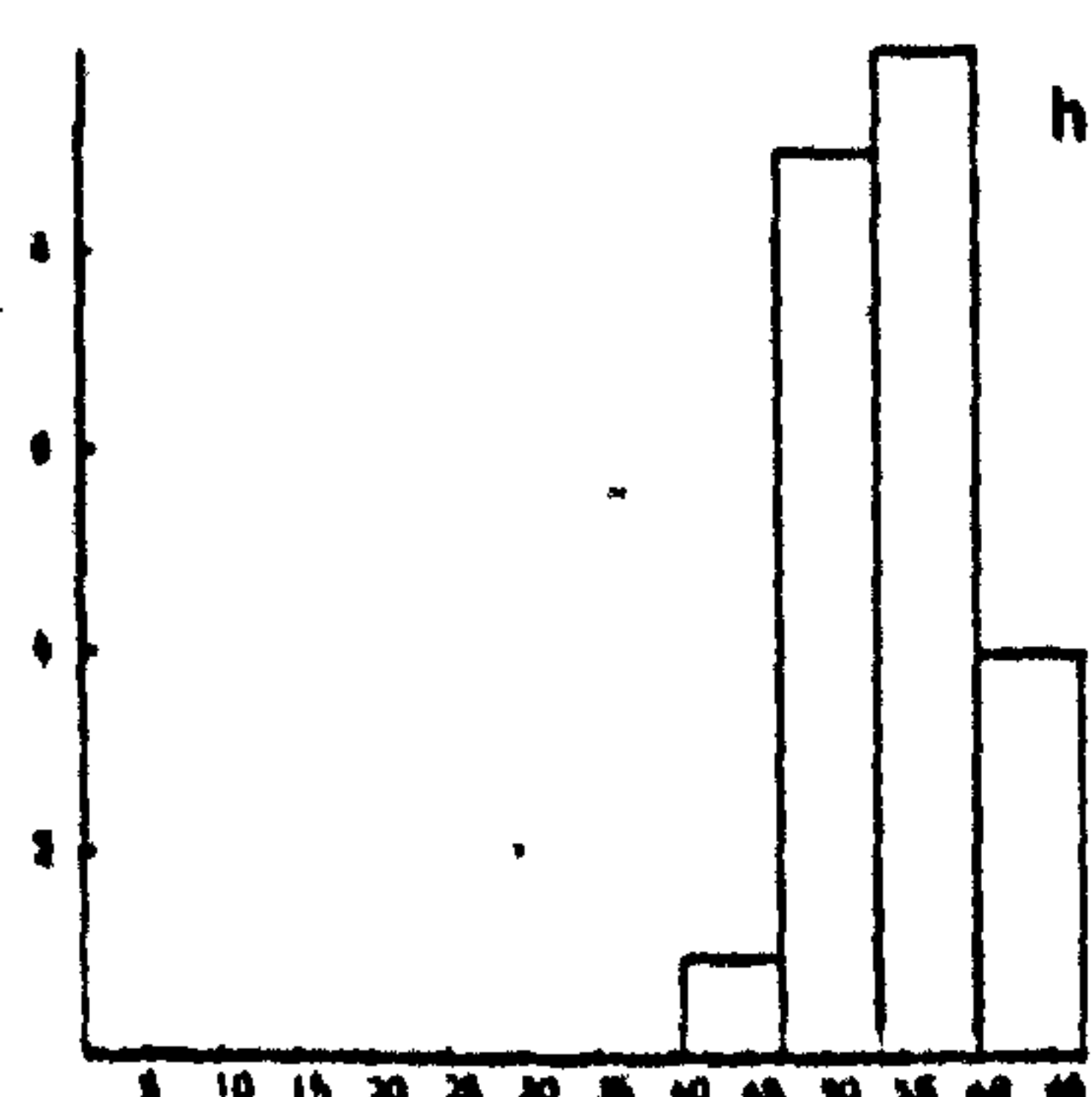
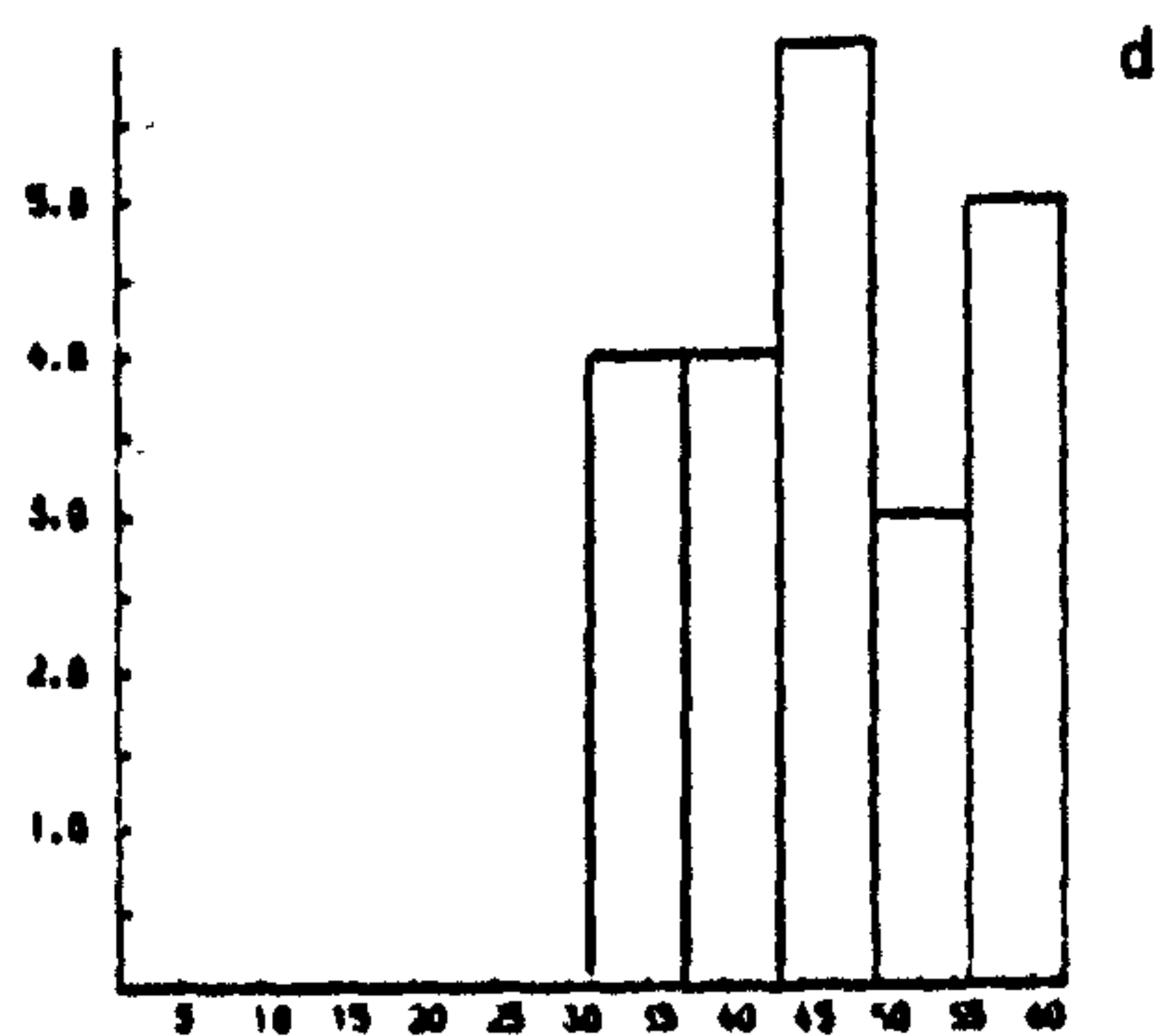
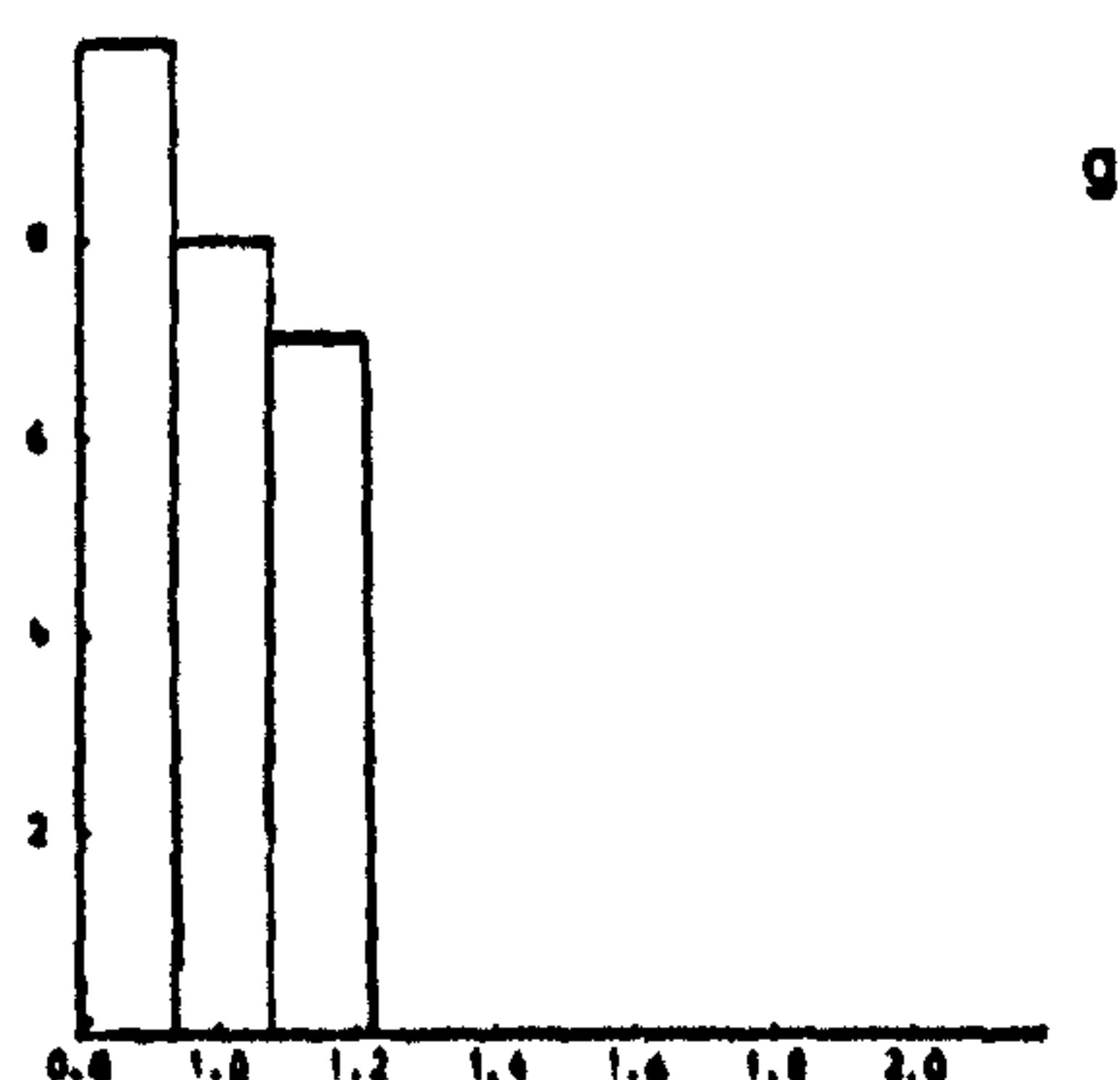
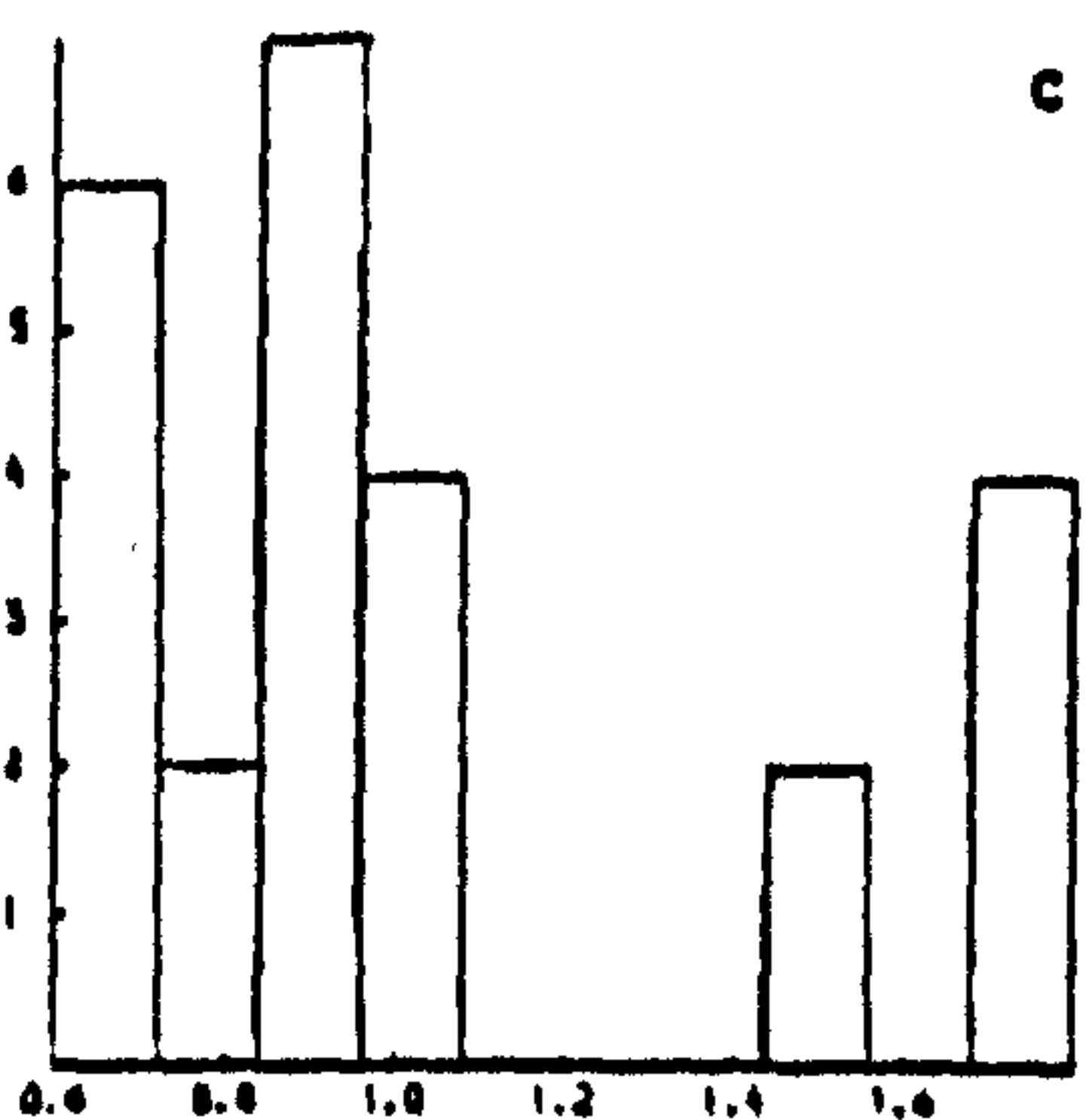
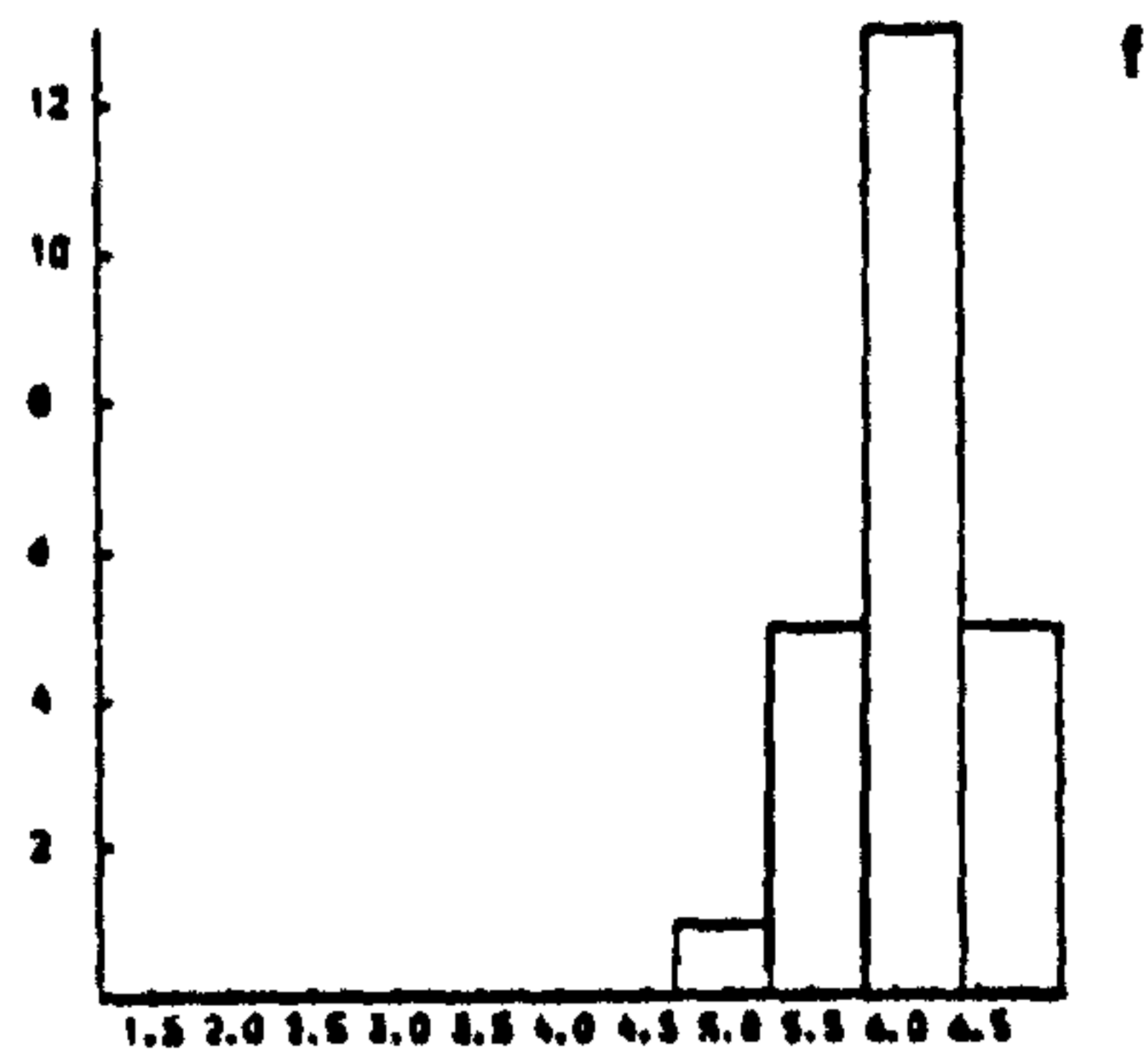
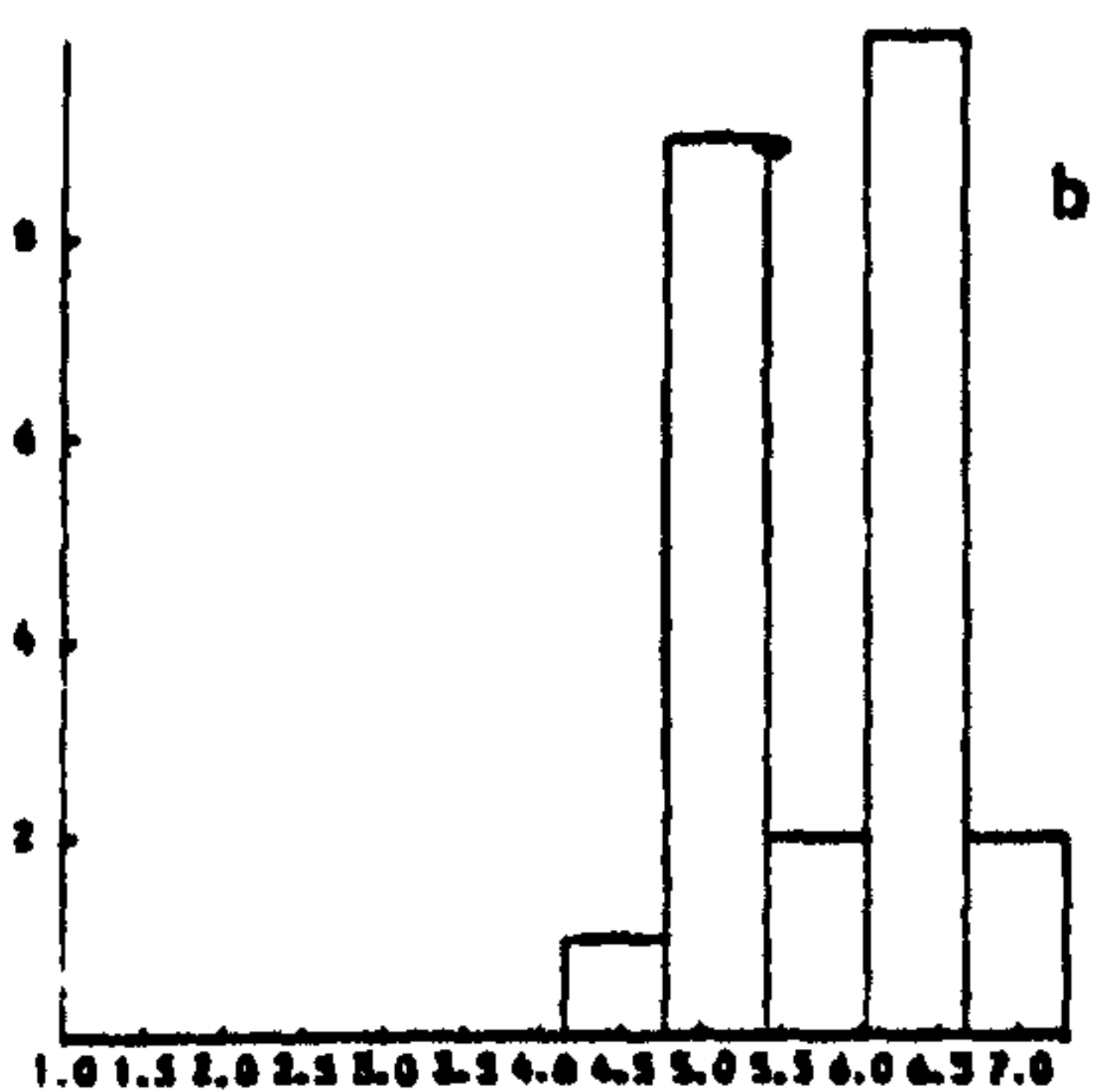
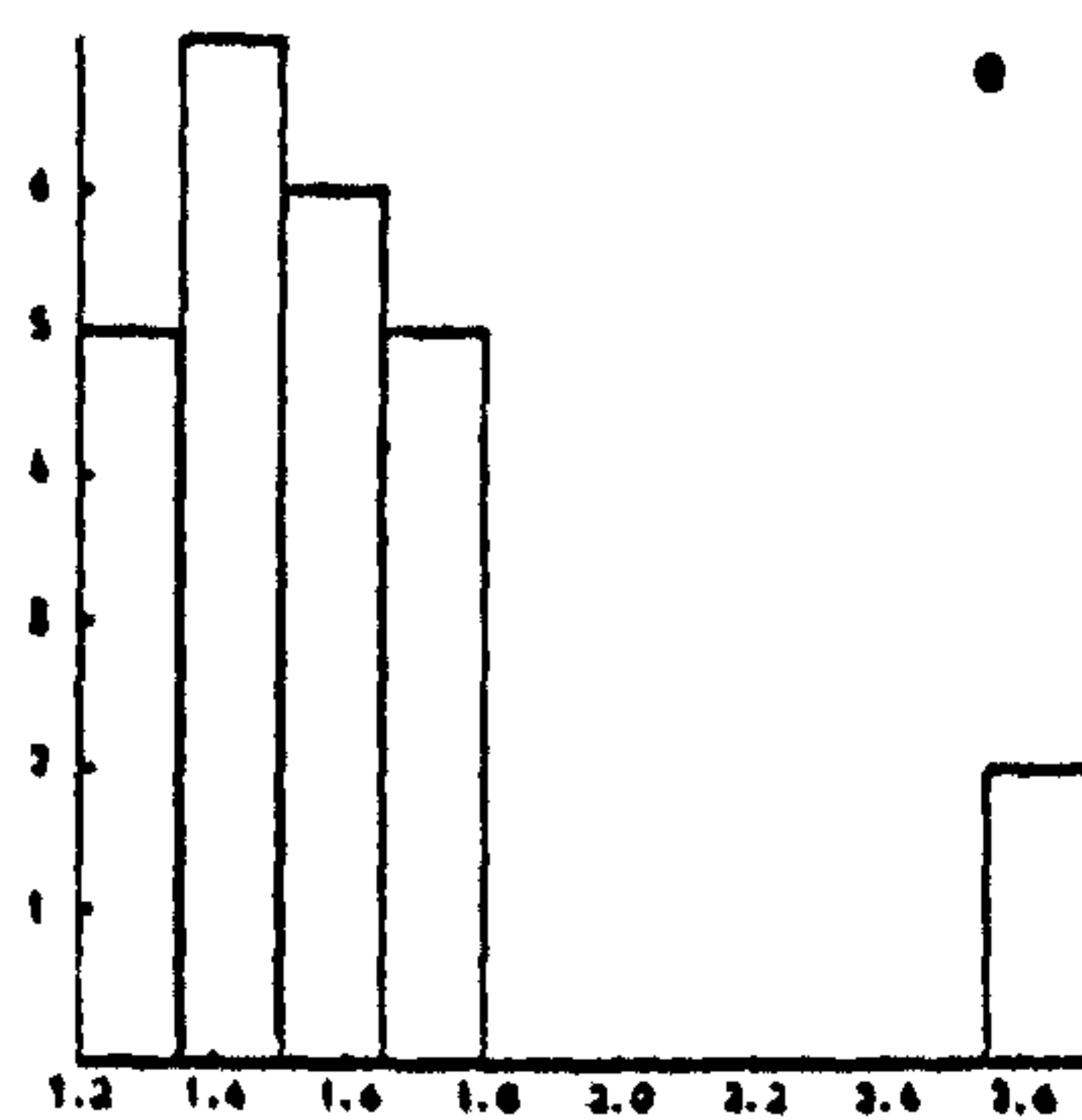
Note that the Y axis is not identical in all cases.

Character (a) in mm; for (b), 1 unit = 0.1 mm; for (c) & (d), 1 unit = 26  $\mu$ m.

# BEFORE CULTURE



# AFTER CULTURE



6. H. jutlandicum

Leaf length before and after culture is almost the same, as in var. lacunosum and var. filiforme (Table 4, Fig. 17a). The variation in length decreased in culture as in all the other taxa, suggesting that leaf length is slightly affected by environmental conditions partly genetic in nature.

A slight increase in nerve length is similar to that in var. resupinatum, var. lacunosum and var. filiforme (see Table 4, Fig. 17b). The variation in length decreased in culture as it does in all the other taxa, suggesting that this character is highly influenced by environmental factors.

An increase in cell size and a decrease in size variation in culture is similar to that in var. resupinatum (Table 4, Fig. 17d). The decrease in size variation is similar in all the other taxa except var. filiforme where it increased.

Angular cell size is almost the same before and after cultures as it is in H. mammillatum and var. filiforme (table 4, Fig. 17c). The variation in size decreased as in all the other taxa except H. mammillatum where it is the same, suggesting that this character is partly genetic in nature.

2.7 Numerical approach

As quantitative characters overlap between taxa of H. cupressiforme, it might be that the qualitative characters are the important ones and to investigate this a numerical technique incorporating both types of characters used. Ordination methods have been found useful in a variety of taxonomic contexts (Sneath and Sokal, 1973).

A commonly used ordination technique, principal component, analysis (PCA), is sometimes used in a taxonomy (e.g. Smith, 1970; Rahman, 1972; Lewis and Smith, 1977). The choice of a numerical method was limited by the type of variation found in H. cupressiforme, continuous and multistate discrete. PCA can be applied to continuous data but not to multistate discrete characters. Discrete characters with only two states can be accommodated to give satisfactory results by coding 0 for the first state and 1 for the second as if the characters are continuous. Sneath and Sokal (1973) have shown multistate discrete characters can be accommodated as dichotomous variables. However, they advise caution when applying this method; in small problems it may be possible to calculate the best dichotomy directly by simple enumeration, but in large problems it is computationally impractical, for example, if the colours green and blue have been divided into numerous different shades using a colour chart. PCA cannot be used satisfactorily where there are multistate discrete characters (Hill and Smith, 1976). On the other hand correspondence analysis (CA) can be applied satisfactorily to data with multistate discrete characters. Smith and Hill (1975) used CA in an investigation of European Ulotia species. But CA cannot be applied to a continuous data. Therefore a technique which combines PCA and CA is needed. An extension of PCA combining these two requirements is described by Hill and Smith (1976) and programmed by Hill 1976, in ALGOL for use on the U.C.N.W. Computer, the program being called MIXORD.

MIXORD was applied as follows:

- (i) to herbarium material including H. vaucheri
- (ii) to herbarium and field material without H. vaucheri
- (iii) to wild material before culture
- (iv) to wild material after culture.



As indicated below H. vaucheri is very distinct and the second analysis was run after the removal of H. vaucheri data which may have affected the discrimination of the H. cupressiforme taxa.

In Figs. 18-26 it can be seen that there are four distinct groups. These correspond to H. vaucheri (Figs. 18,20), H. mammillatum (Figs. 18, 20, 21, 22, 24, 25), H. jutlandicum (Fig. 24) and var. filiforme (Figs. 22, 23, 26). Two groups remain distinct after culture and these correspond to H. mammillatum and H. jutlandicum (see Figs. 27, 28), but var. filiforme overlaps after culture with other plants (see Figs. 27, 28, 29).

Reference to Figs. (18,20) show H. vaucheri to be a homogenous and distinct group. Three gametophytic characters, pseudoparaphyllia, nerve strength and angular cells are discriminatory. Ando (1976) has listed twelve gametophytic and sporophytic characters distinguishing H. vaucheri from H. cupressiforme. As I was unable to examine the sporophyte of H. vaucheri I found the other gametophytic characters, epidermal cells of stem, leaf margin, laminal cells, lower laminal cells and perichaetial leaves showed a considerable degree of overlapping. On the above evidence H. vaucheri should be treated as a good species and not a variety or subspecies of H. cupressiforme.

H. mammillatum shows some overlap with the other taxa examined in quantitative characters. Two quantitative and two qualitative characters are to some extent discriminatory. These are nerve length and cell size. H. mammillatum has much shorter nerve whilst cell size tends to be short but shows some overlap with the other taxa (see Table 4). The mamillate lid and strongly curved leaves are discriminatory. H. mammillatum has been treated by several authors as a variety of H. cupressiforme (Wilson, 1855, Braithwaite, 1895-1905; Dixon and Jameson, 1924; Jensen, 1939;

Richards & Wallace, 1950; Warburg, 1963; Koponen, 1977). Others have treated it as a distinct species (Nyholm, 1954; Damsholt et al, 1969; Smith, 1978a). Figs. 18,20,21,22,24,25 and Figs. 27, 28 after culture show H. mammillatum as a homogeneous and distinct group and therefore it would be more appropriate to treat it as a species than a variety. Overlapping between H. mammillatum and var. filiforme after culture occurred. The reason for this intergrading is because of the presence of intermediate plants and the intergradation of various characters. Usually H. mammillatum and var. filiforme are distinct in the shape of the capsule lid, but the presence of plants with lids of an intermediate nature, make this character of little value.

In Britain var. filiforme was treated as a variety of H. cupressiforme by Wilson (1855), Braithwaite (1895-1905), Dixon & Jameson (1924), Richards and Wallace (1950). Smith (1978a) treats this taxon as synonym of H. mammillatum. In Europe it has been variously treated as a variety of H. cupressiforme (Nyholm, 1954, Doignon, 1963; Dull 1977; Koponen et al; 1977), and as a distinct species by Guilmont (1949). Because of the occurrence of intermediate plants between it and H. mammillatum it would seem that var. filiforme is closely related to H. mammillatum rather than to H. cupressiforme. It should be treated as a variety of H. mammillatum.

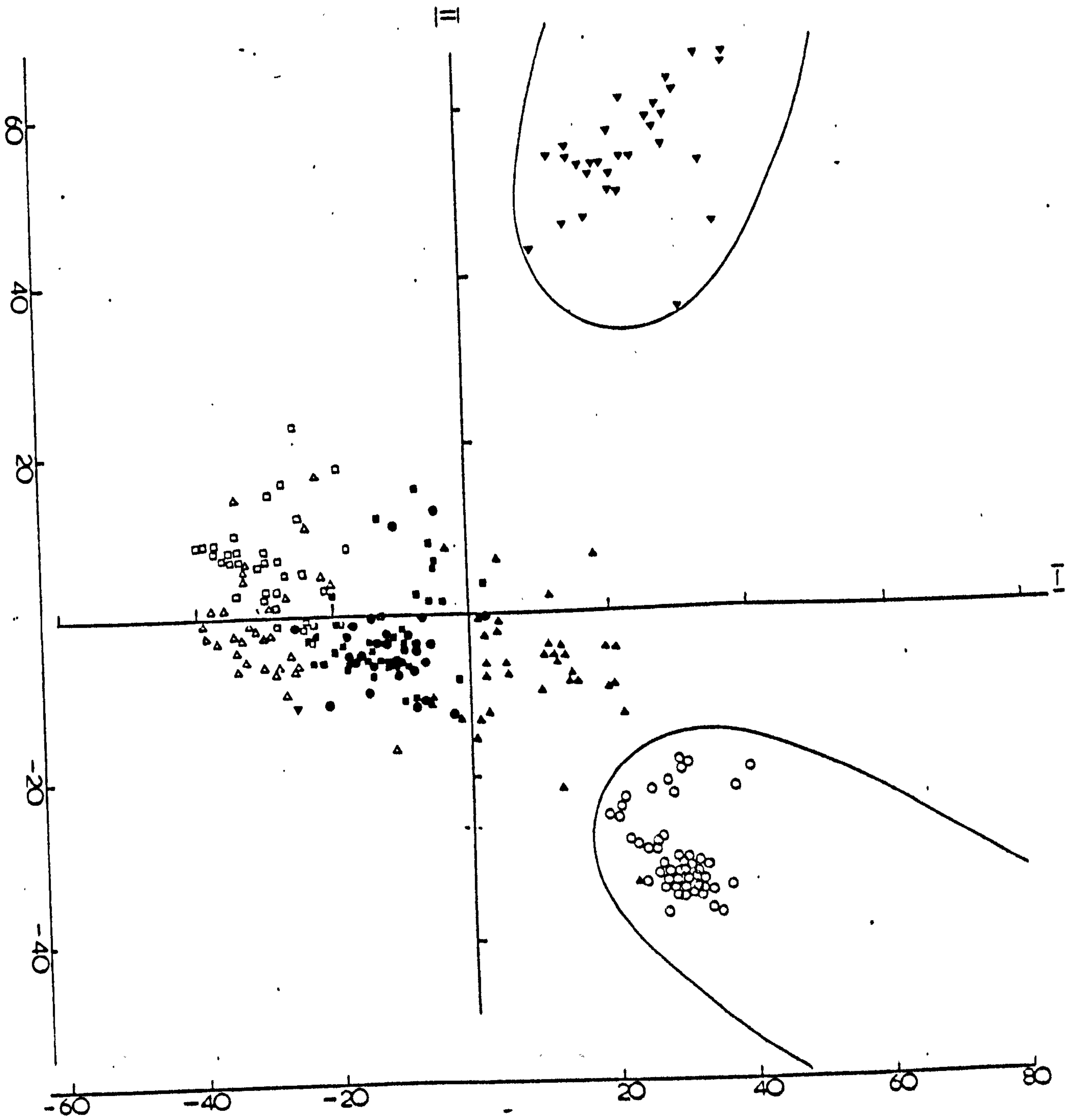
It is evident that H. jutlandicum is distinct both before and after culture (Figs. 24, 27, 28), supporting the opinion of the sixteen British bryologists as given in Table 2. The distinguishing features are densely pinnate branches and a very long seta. Holmen and Warncke (in Damsholt et al., 1969) introduced the name H. jutlandicum to replace H. ericetorum (B.S.G) Loesq. which is a later homonym of H. ericetorum Brid.

Fig. 18. Ordination of morphological characters for H. cupressiforme  
s.l. herbarium and field material. Key to symbols:

- (▼) Specimens referred to H. vaucheri
- (▲) Specimens referred to var. cupressiforme
- (□) Specimens referred to var. resupinatum
- (■) Specimens referred to var. lacunosum
- (●) Specimens referred to var. filiforme
- (○) Specimens referred to H. mammillatum
- (△) Specimens referred to H. jutlandicum

Note that axis I plotted against II.







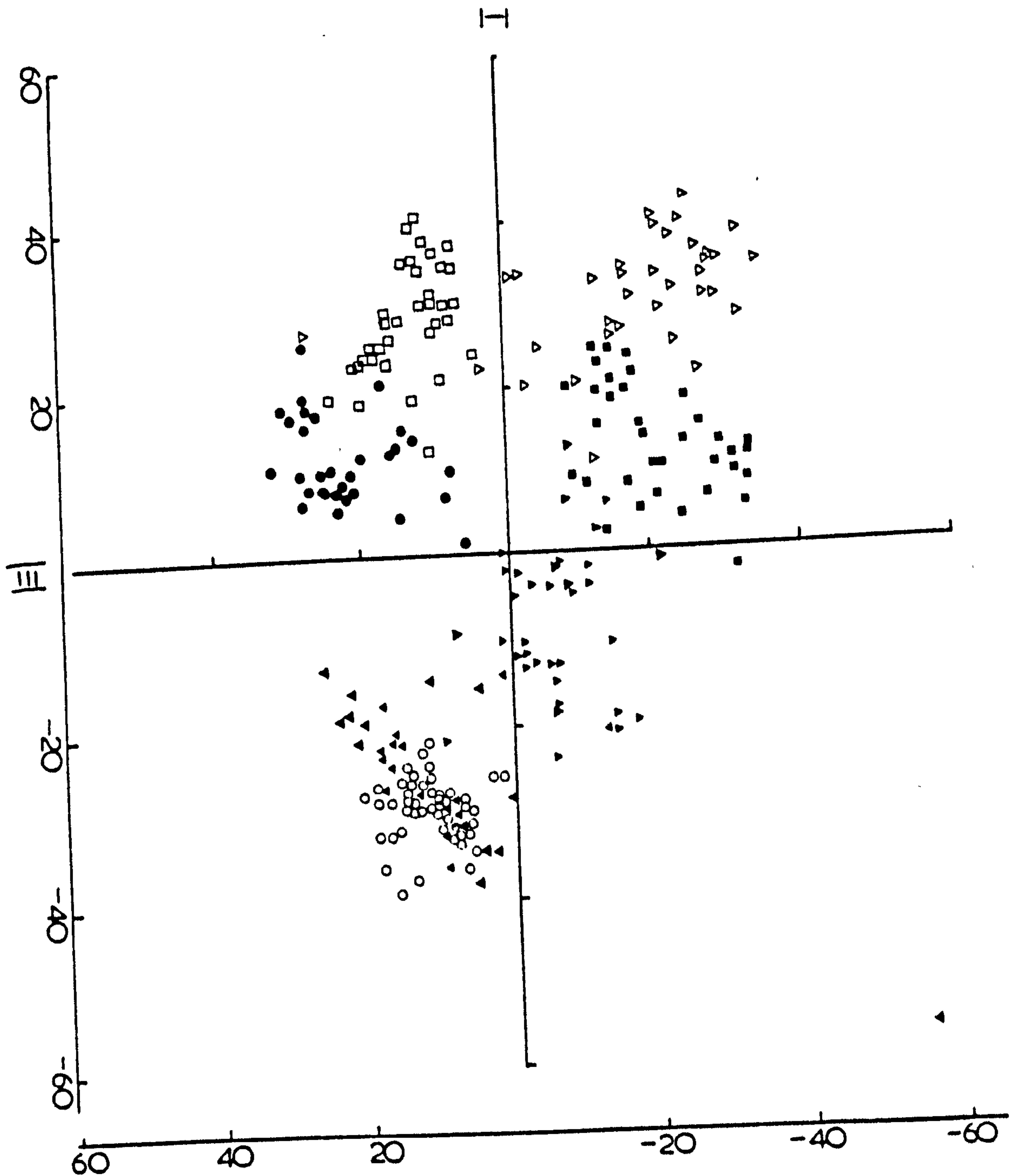


Fig. 19. Ordination of morphological characters for H. cupressiforme s.l. herbarium and field material. Key to symbols:

- (▼) Specimens referred to H. vaucheri
- (▲) Specimens referred to var. cupressiforme
- (□) Specimens referred to var. resupinatum
- (■) Specimens referred to var. lacunosum
- (●) Specimens referred to var. filiforme
- (○) Specimens referred to H. mammillatum
- (△) Specimens referred to H. jutlandicum

Note that axis I plotted against III.

Fig. 20. Ordination of morphological characters for H. cupressiforme  
s.l. herbarium and field material. Key to symbols:

- (▼) Specimens referred to H. vaucheri
- (▲) Specimens referred to var. cupressiforme
- (□) Specimens referred to var. resupinatum
- (■) Specimens referred to var. lacunosum
- (●) Specimens referred to var. filiforme
- (○) Specimens referred to H. mammillatum
- (△) Specimens referred to H. jutlandicum

Note that axis II plotted against III.

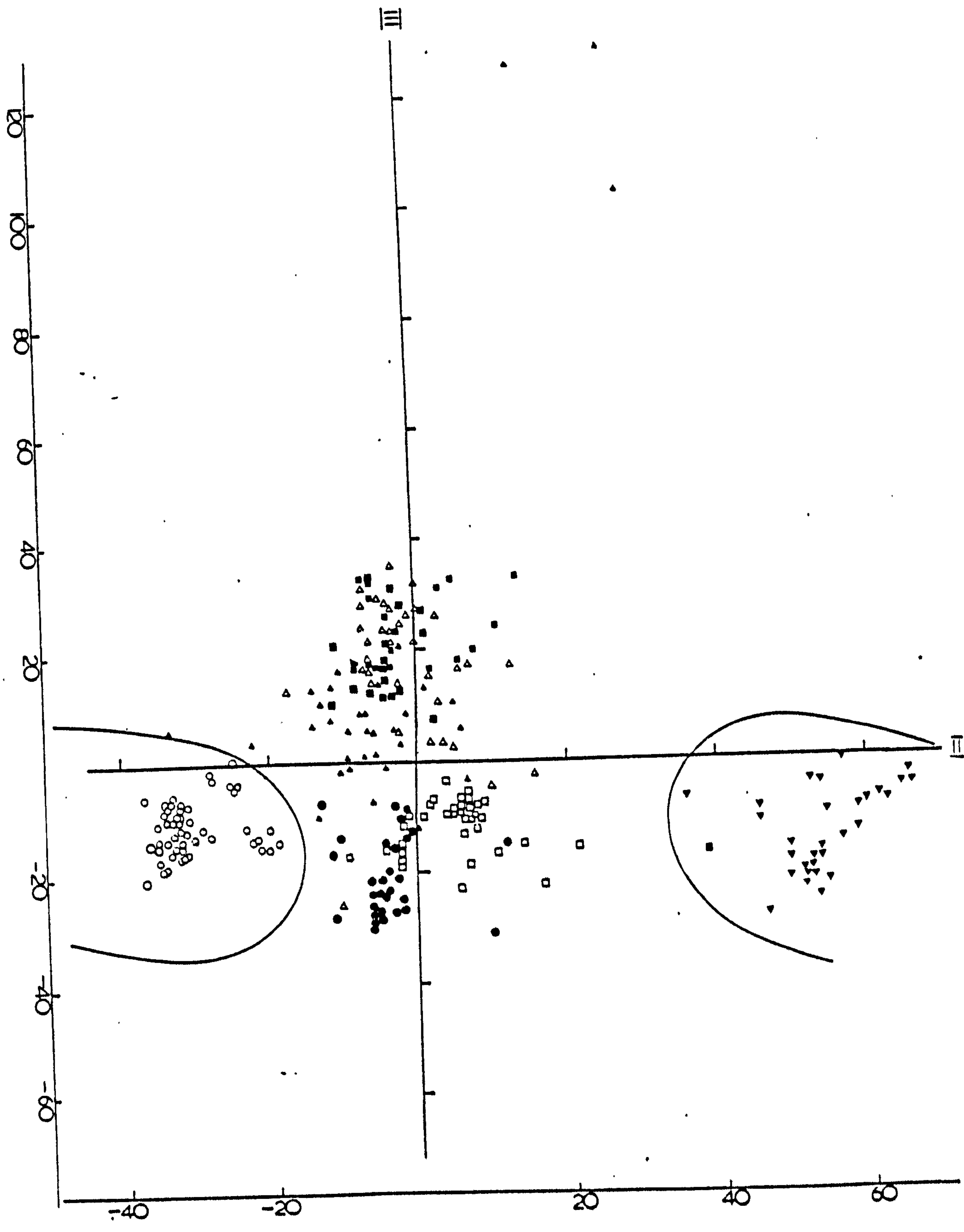
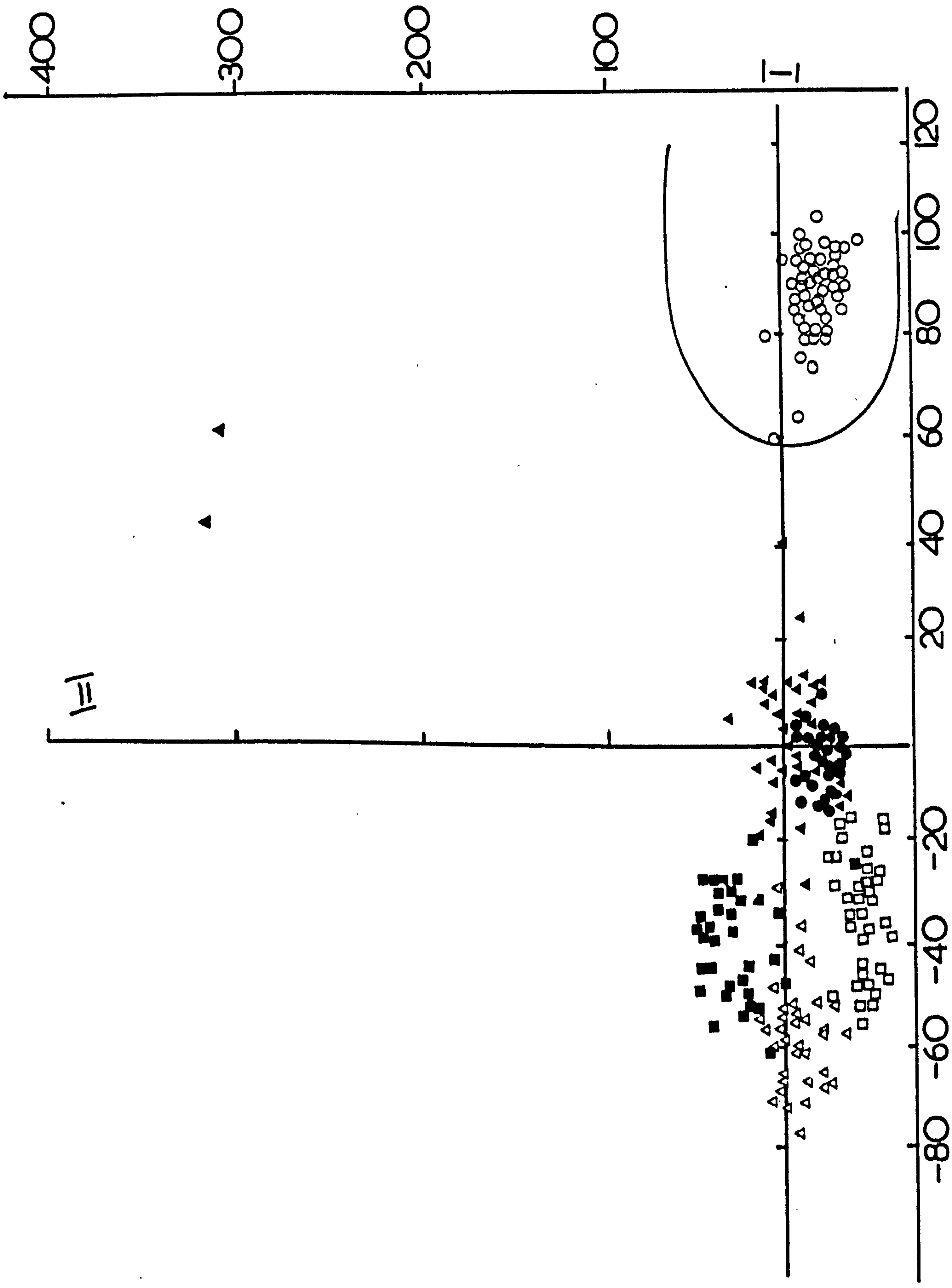


Fig. 21. Ordination of morphological characters for H. cupressiforme  
s.l. herbarium and field material. Key to symbols:

- (▲) Specimens referred to var. cupressiforme
- (◻) Specimens referred to var. resupinatum
- (■) Specimens referred to var. lacunosum
- (●) Specimens referred to var. filiforme
- (○) Specimens referred to H. mammillatum
- (△) Specimens referred to H. jutlandicum

Note that axis I plotted against II. Gatherings of H. vaucheri have been omitted.





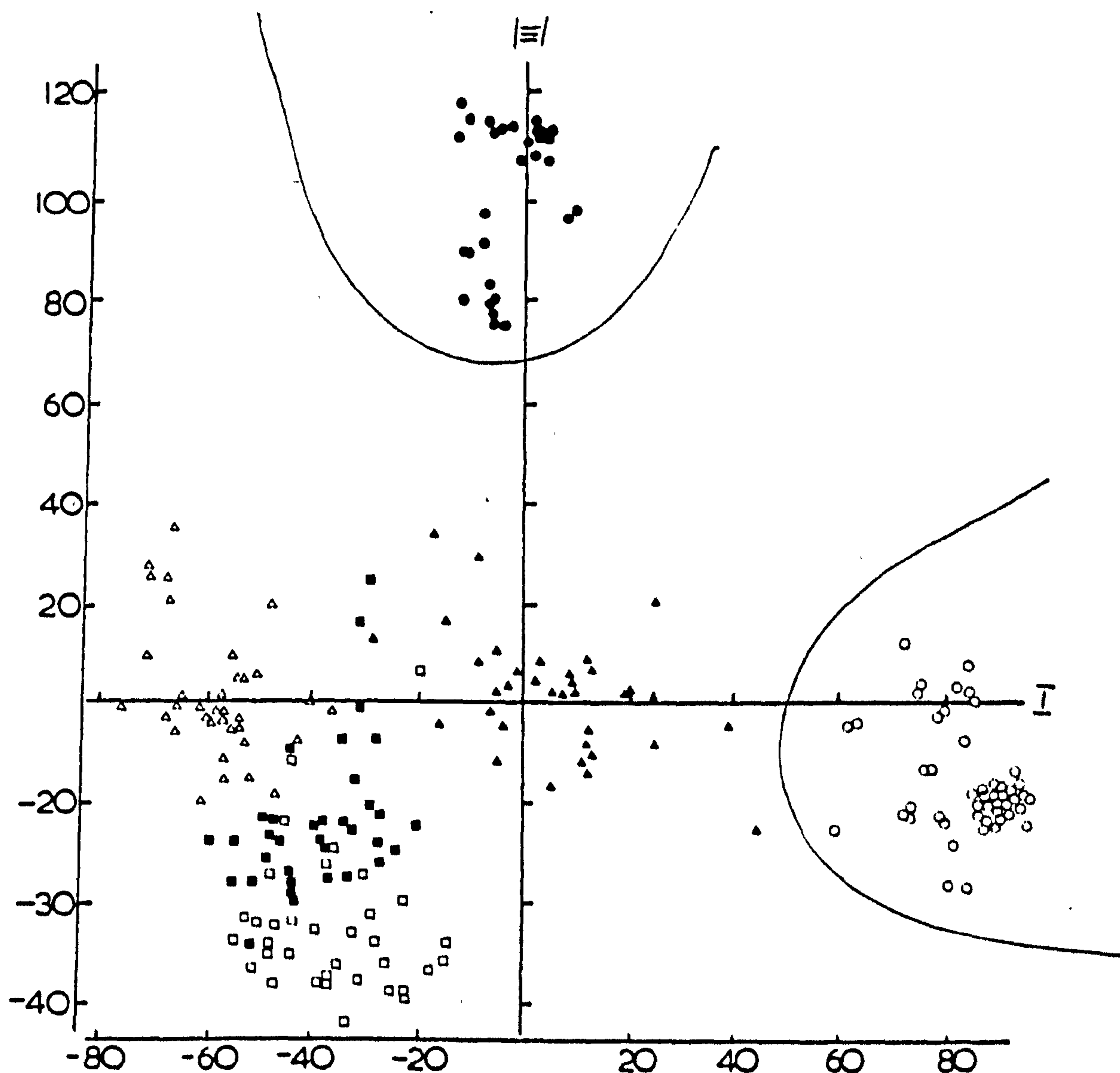


Fig. 22. Ordination of morphological characters of H. cupressiforme s.l. herbarium and field material. Key to symbols:

- (▲) Specimens referred to var. cupressiforme
- (□) Specimens referred to var. resupinatum
- (■) Specimens referred to var. lacunosum
- (●) Specimens referred to var. filiforme
- (○) Specimens referred to H. mammillatum
- (△) Specimens referred to H. jutlandicum

Note that axis I plotted against III. Gatherings of H. vaucheri have been omitted.

Fig. 23. Ordination of morphological characters for H. cupressiforme  
s.l. herbarium and field material. Key to symbols:

- (▲) Specimens referred to var. cupressiforme
- (□) Specimens referred to var. resupinatum
- (■) Specimens referred to var. lacunosum
- (●) Specimens referred to var. filiforme
- (○) Specimens referred to H. mammillatum
- (△) Specimens referred to H. jutlandicum

Note that axis II plotted against III. Gatherings of H. vaucheri have been omitted.

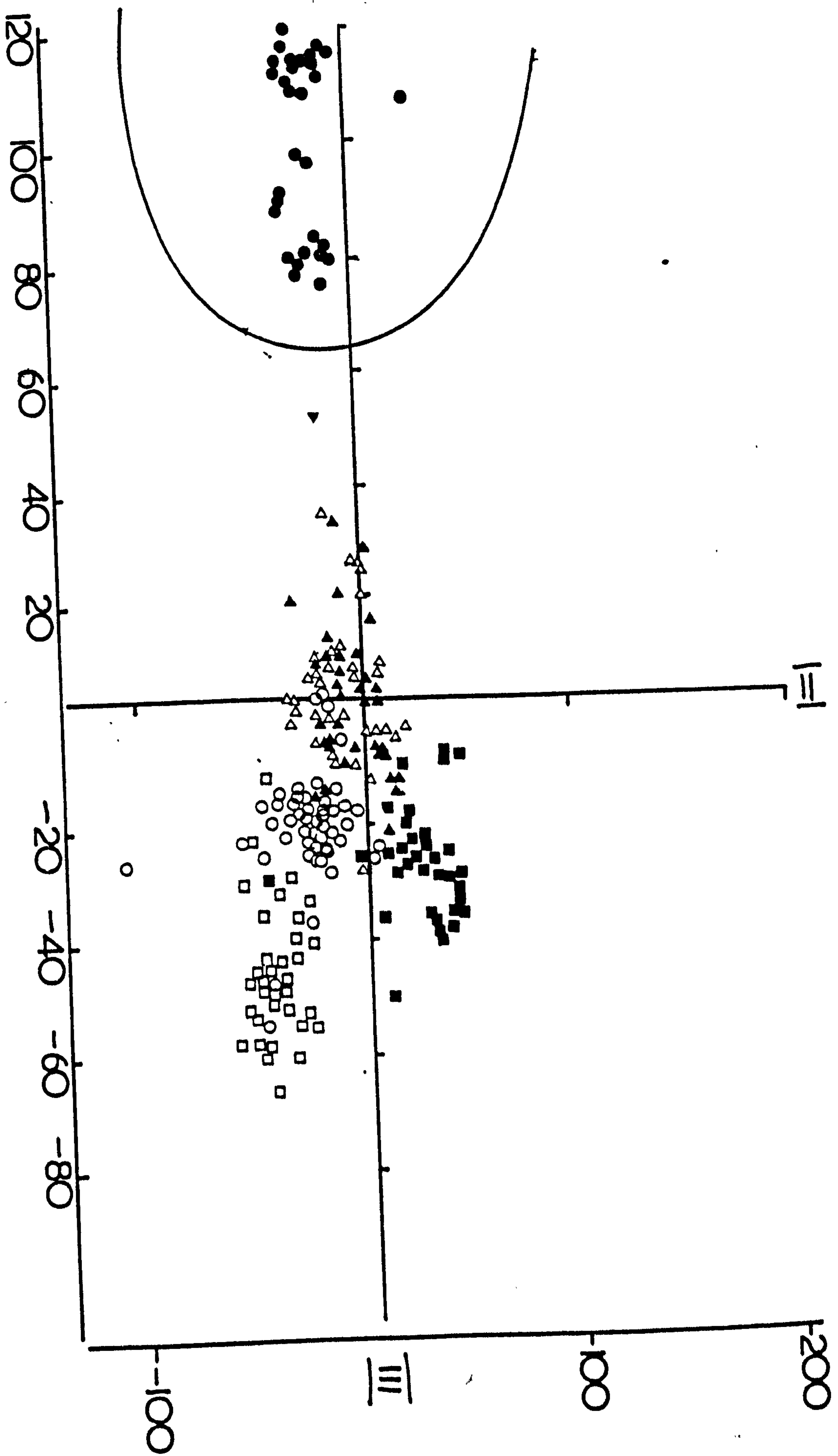




Fig. 24. Ordination of morphological characters of H. cupressiforme s.l. field material. Key to symbols:

- (▲) Specimens referred to var. cupressiforme
- (□) Specimens referred to var. resupinatum
- (■) Specimens referred to var. lacunosum
- (●) Specimens referred to var. filiforme
- (○) Specimens referred to H. mammillatum
- (△) Specimens referred to H. jutlandicum

Note that axis I plotted against II. Gatherings of H. vaucheri have been omitted.

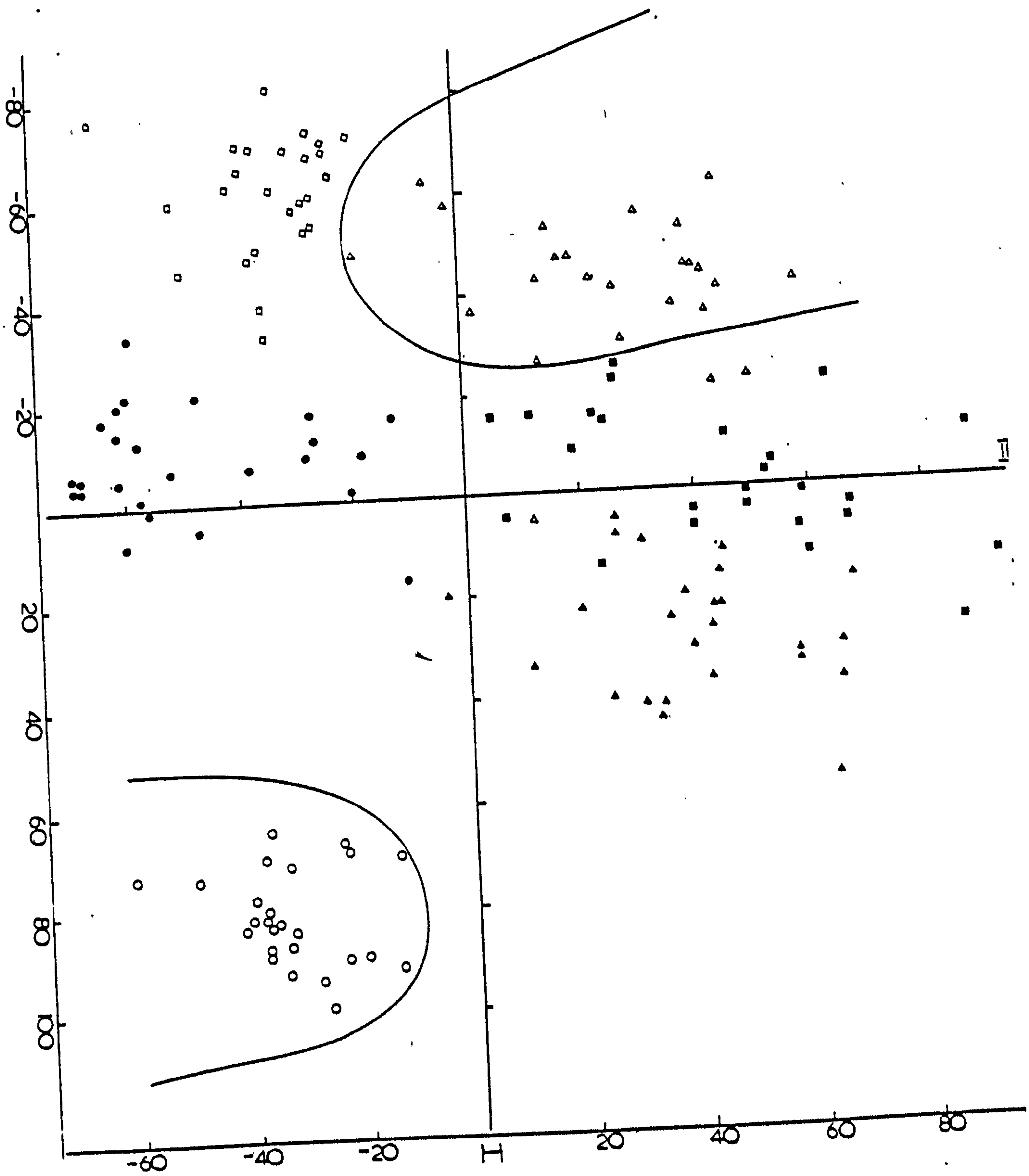


Fig. 25. Ordination of morphological characters of H. cupressiforme s.l. field material. Key to symbols:

- (▲) Specimens referred to var. cupressiforme
- (□) Specimens referred to var. resupinatum
- (■) Specimens referred to var. lacunosum
- (●) Specimens referred to var. filiforme
- (○) Specimens referred to H. mamillatum
- (△) Specimens referred to H. jutlandicum

Note that axis I plotted against III. Gatherings of H. vaucheri have been omitted.

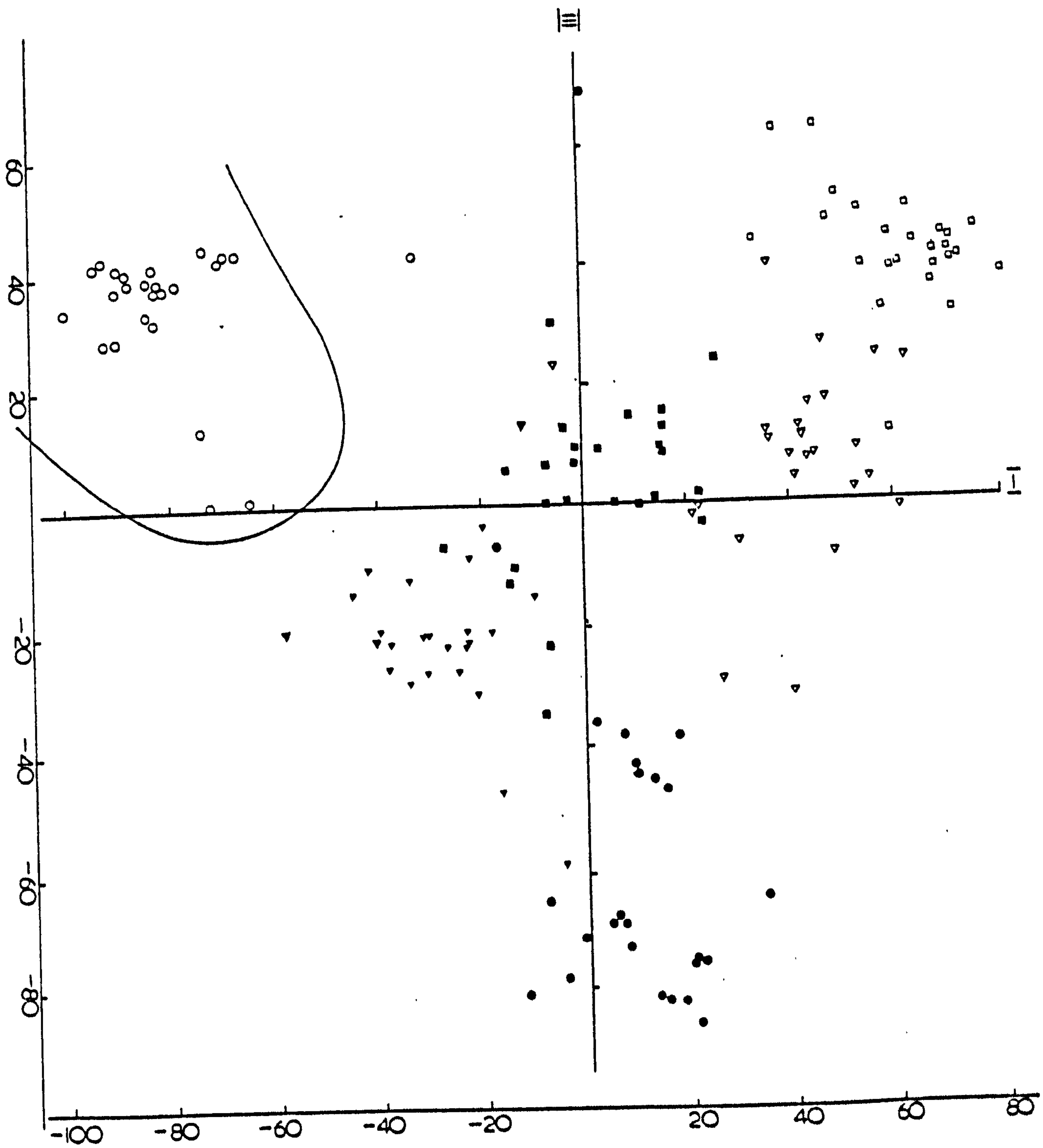
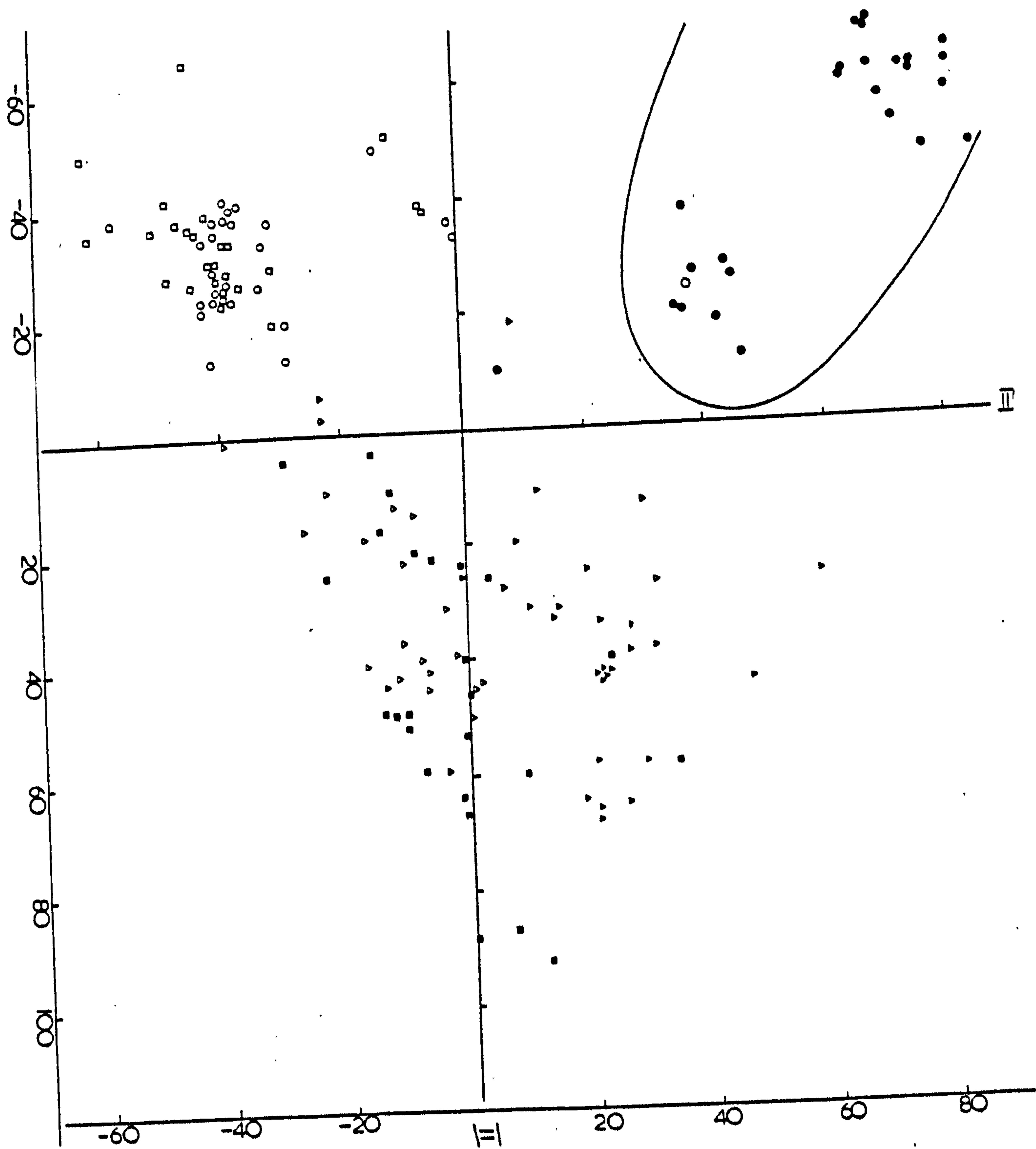




Fig. 26. Ordination of morphological characters of H. cupressiforme s.l. field material. Key to symbols:

- (▲) Specimens referred to var. cupressiforme
- (□) Specimens referred to var. resupinatum
- (■) Specimens referred to var. lacunosum
- (●) Specimens referred to var. filiforme
- (○) Specimens referred to H. mammillatum
- (Δ) Specimens referred to H. jutlandicum

Note that axis II plotted against III. Gatherings of H. vaucheri have been omitted.



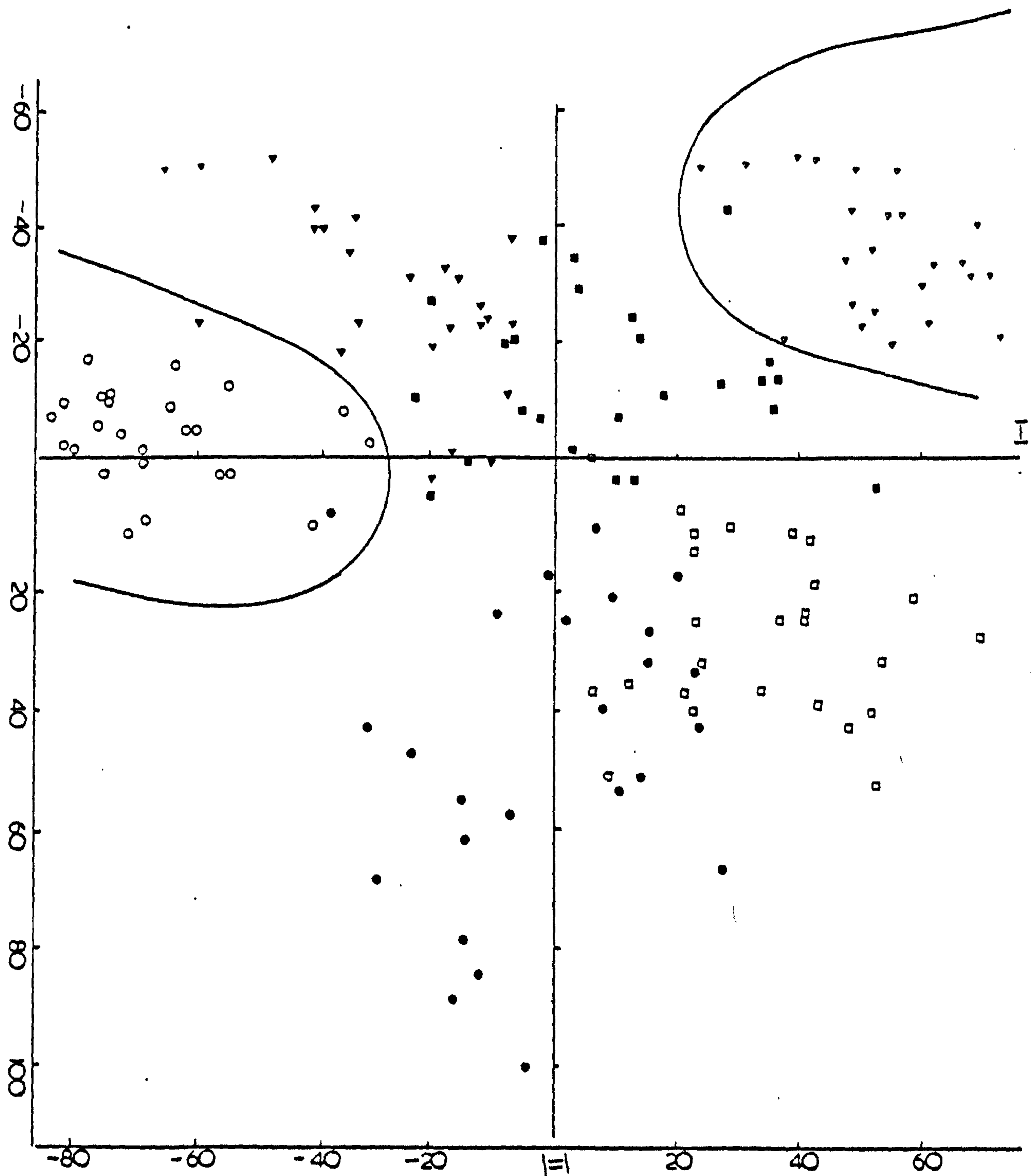


Fig. 27. Ordination of morphological characters of H. cupressiforme s.l. after culture. Key to symbols:

- (▲) Specimens referred to var. cupressiforme
- (□) Specimens referred to var. resupinatum
- (■) Specimens referred to var. lacunosum
- (●) Specimens referred to var. filiforme
- (○) Specimens referred to H. mammillatum
- (Δ) Specimens referred to H. jutlandicum

Note that axis I plotted against II. Gatherings of H. vaucheri have been omitted.

Fig. 28. Ordination of morphological characters of H. cupressiforme s.l. after culture. Key to symbols:

- (▲) Specimens referred to var. cupressiforme
- (□) Specimens referred to var. resupinatum
- (■) Specimens referred to var. lacunosum
- (●) Specimens referred to var. filiforme
- (○) Specimens referred to H. mammillatum
- (△) Specimens referred to H. jutlandicum

Note that axis I plotted against III. Gatherings of H. vaucheri have been omitted.



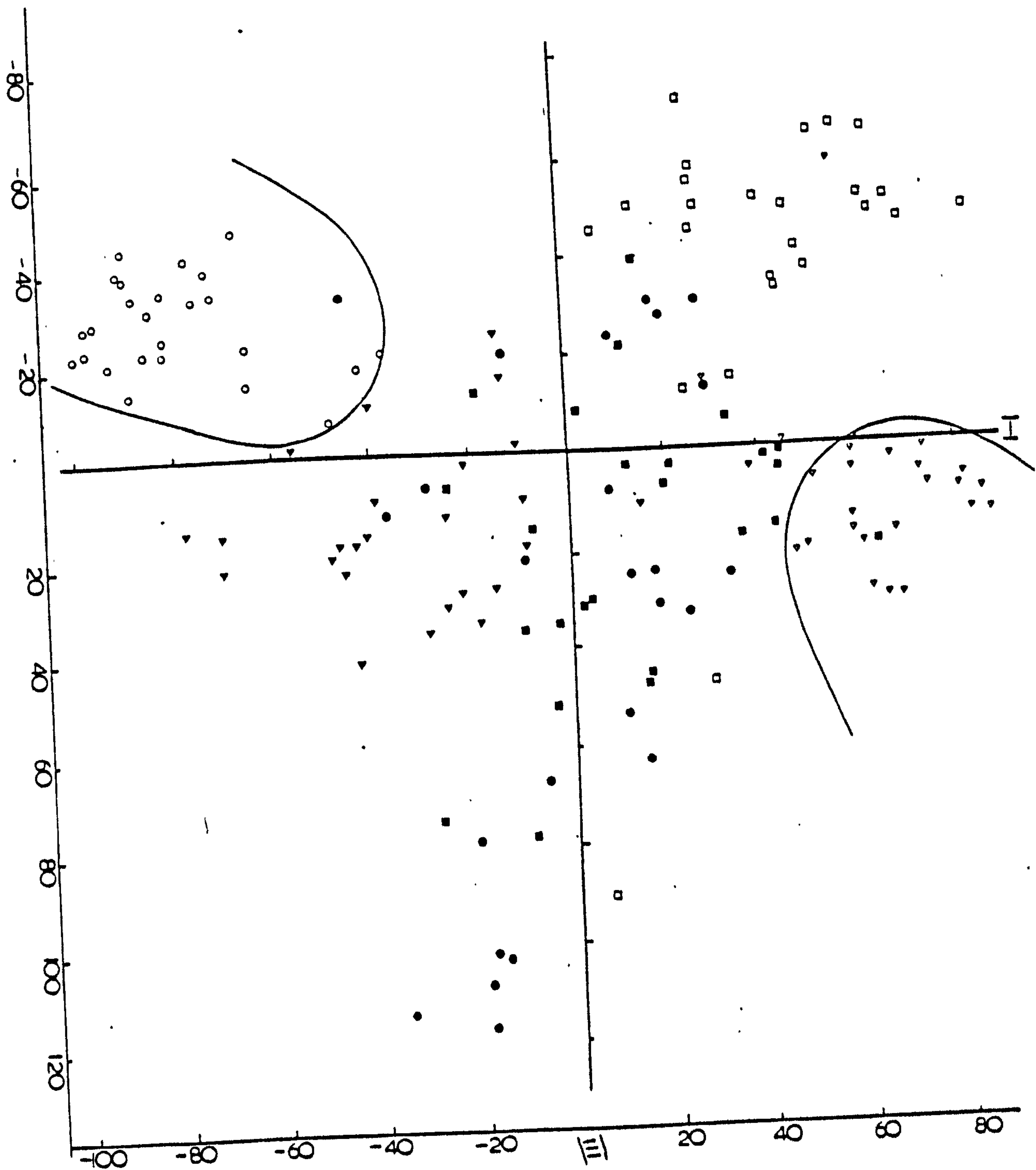
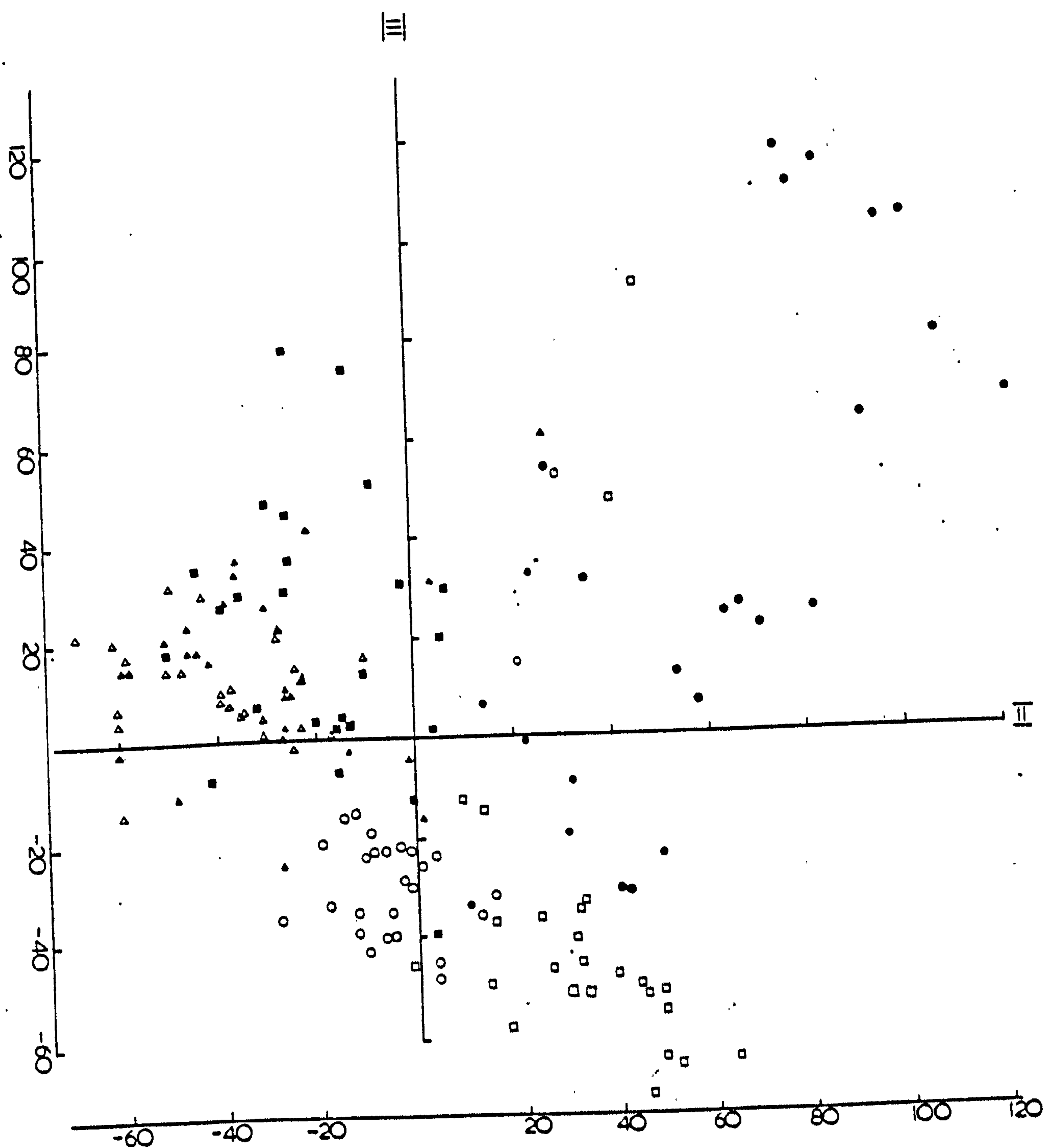


Fig. 29. Ordination of morphological characters of H. cupressiforme s.l. after culture. Key to symbols:

- (▲) Specimens referred to var. cupressiforme
- (□) Specimens referred to var. resupinatum
- (■) Specimens referred to var. lacunosum
- (●) Specimens referred to var. filiforme
- (○) Specimens referred to H. mammillatum
- (△) Specimens referred to H. jutlandicum

Note that axis II plotted against III. Gatherings of H. vaucheri have been omitted.



The situation concerning the remaining three taxa of H. cupressiforme s.l. is much less clear and it is clear from Figs. 18-29 that there is considerable overlap. The erect capsule with subulate beak of var. resupinatum is distinctive although the upward pointing leaves, usually regarded as a character, grade into the type found in var. cupressiforme in culture. Var. resupinatum is probably best treated as a variety of H. cupressiforme.

Var. lacunosum is also distinctive in its sporophyte, with a short seta and <sup>+</sup> erect capsule although overlapping in gametophyte characters with var. cupressiforme. Again it is probably best treated as a variety of H. cupressiforme.



### CHAPTER 3

#### Variation in Non-Morphological Characters

##### 3.1 Introduction

Mosses have a comparatively simple organization, and therefore the number of morphological diagnostic characters available for taxonomic work is rather low and, moreover, these characters show a relatively large degree of variability between and within taxa (Krzakowa, 1977). Thus recent modern taxonomic methods such as phytochemistry and isozyme studies may provide additional means to investigate variability.

Several phytochemical methods of analysing plants have proved of value in both orthodox taxonomy and biosystematics. These methods seem to be the best alternative where genetical experiments are not possible (Smith, 1978b). Bryophyte phytochemistry is reviewed by Huneck (1974) and Suire (1975). Use was made by Koponen (1968) of the so called "Colour Substances" in cell walls of members of Mniaceae to group species. A comparison was made of a cyclic sugar alcohol, in two Plagiochia and two Jamesoniella species (Lewis, 1970). The study of the distribution of unsaturated fatty acids in mosses was applied by Anderson et al. (1974). Lewis and Smith (1977) found that seven British species of Pohlia formed three distinct groups on the basis of their carotenoid pigments. Isozymes were used in studies on interspecific variability of four Pellia species (Krzakowa, 1977). Investigation of peroxidase isozymes in natural populations of Plagiochila asplenioides revealed very distinct isozyme polymorphism and geographical races in this species. Investigation of isozymes of twenty-one populations of Conocephalum conicum showed differentiation of this species into three groups (Krzakowa, 1977). In an attempt to find more characters in the H. cupressiforme s.l. carotenoid

pigments and amino acids were analysed and isozymic studies carried out.

### 3.2 Analyses of Carotenoid Pigments

Differences in colour occur between some populations of H. cupressiforme s.l. and these were investigated by analysis of carotenoid pigment content.

#### Materials and Methods

The pigment content of six taxa of H. cupressiforme s.l. was investigated using thin layer chromatography (Davies, 1965). The procedure followed in this analysis is given in Appendix 1. Two developing solvents were used, 20% ethyl acetate in methylene chloride and 15% methanol in benzene.

#### Results and Discussion

The chromatograms are shown in Figs. 30 and 31. With ethyl acetate as the solvent, the taxa examined fall into three groups on the basis of the number and position of pigments:

- |  |         |
|--|---------|
| 1. <u>H. mammillatum</u> , var. <u>filiforme</u> and <u>H. jutlandicum</u> | 7 spots |
| 2. var. <u>cupressiforme</u> and var. <u>resupinatum</u>                   | 5 spots |
| 3. var. <u>lacunosum</u>   | 9 spots |

Using benzene as the solvent:

- |  |         |
|--|---------|
| 1. <u>H. mammillatum</u> and var. <u>filiforme</u>       | 7 spots |
| 2. var. <u>cupressiforme</u> and var. <u>resupinatum</u> | 5 spots |
| 3. var. <u>lacunosum</u>                                 | 9 spots |
| 4. <u>H. jutlandicum</u>                                 | 8 spots |

There is a close similarity in the number and position of spots in H. mammillatum and var. filiforme and in var. cupressiforme and var. resupinatum. This supports the suggestion that var. filiforme is close

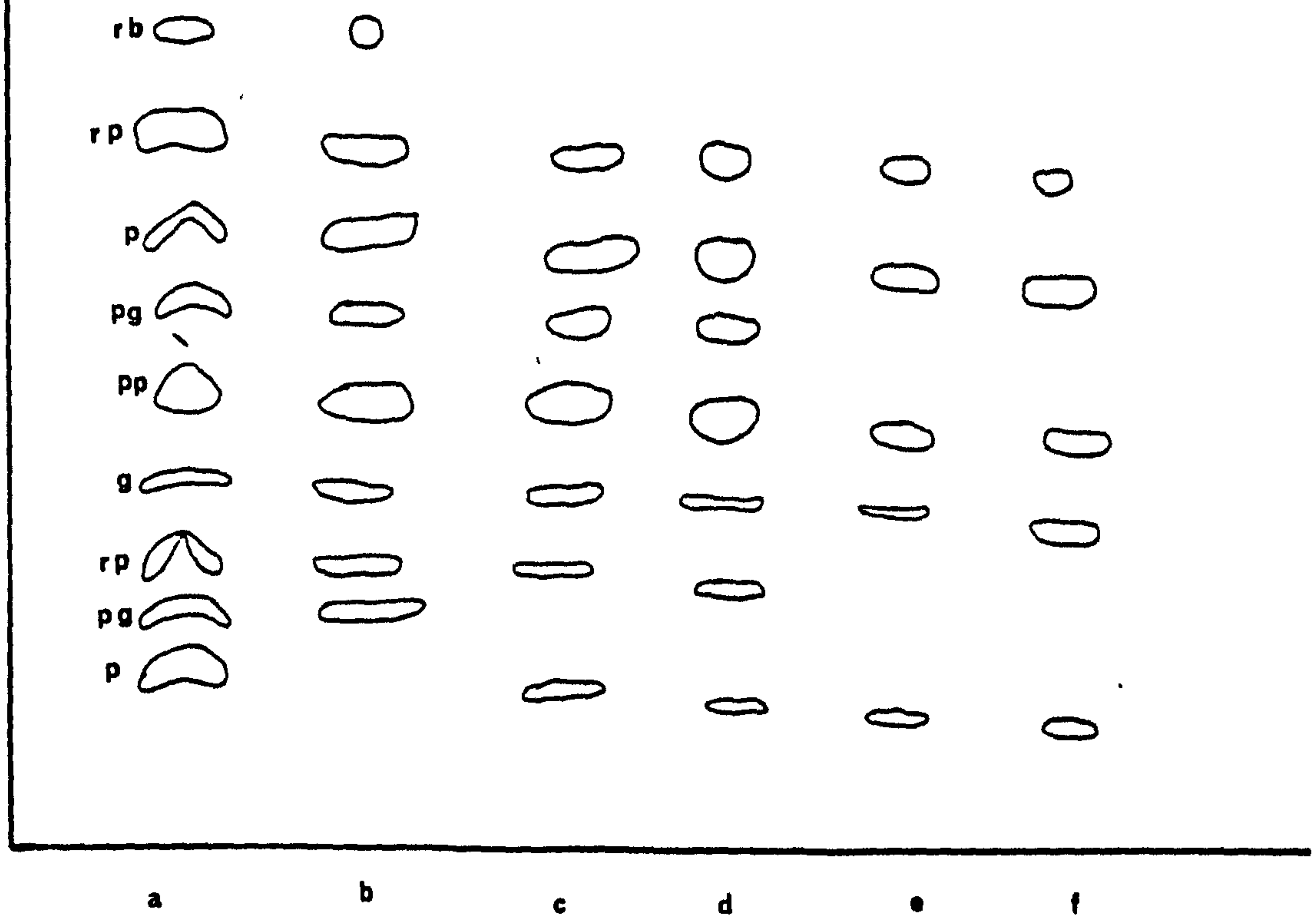


Fig. 30. Chromatographic patterns and spot colours of H. cupressiforme s.l. after spraying with antimony trichloride. Developing solvent: 15% methanol in benzene. Key to symbols:

p = purple

pp = pale purple

rp = reddish

g = grey

rb = reddish brown

pg = pale grey

Where,

(a) var. lacunosum;

(b) H. jutlandicum;

(c) H. mamillatum;

(d) var. filiforme;

(e) var. cupressiforme;

(f) var. resupinatum.

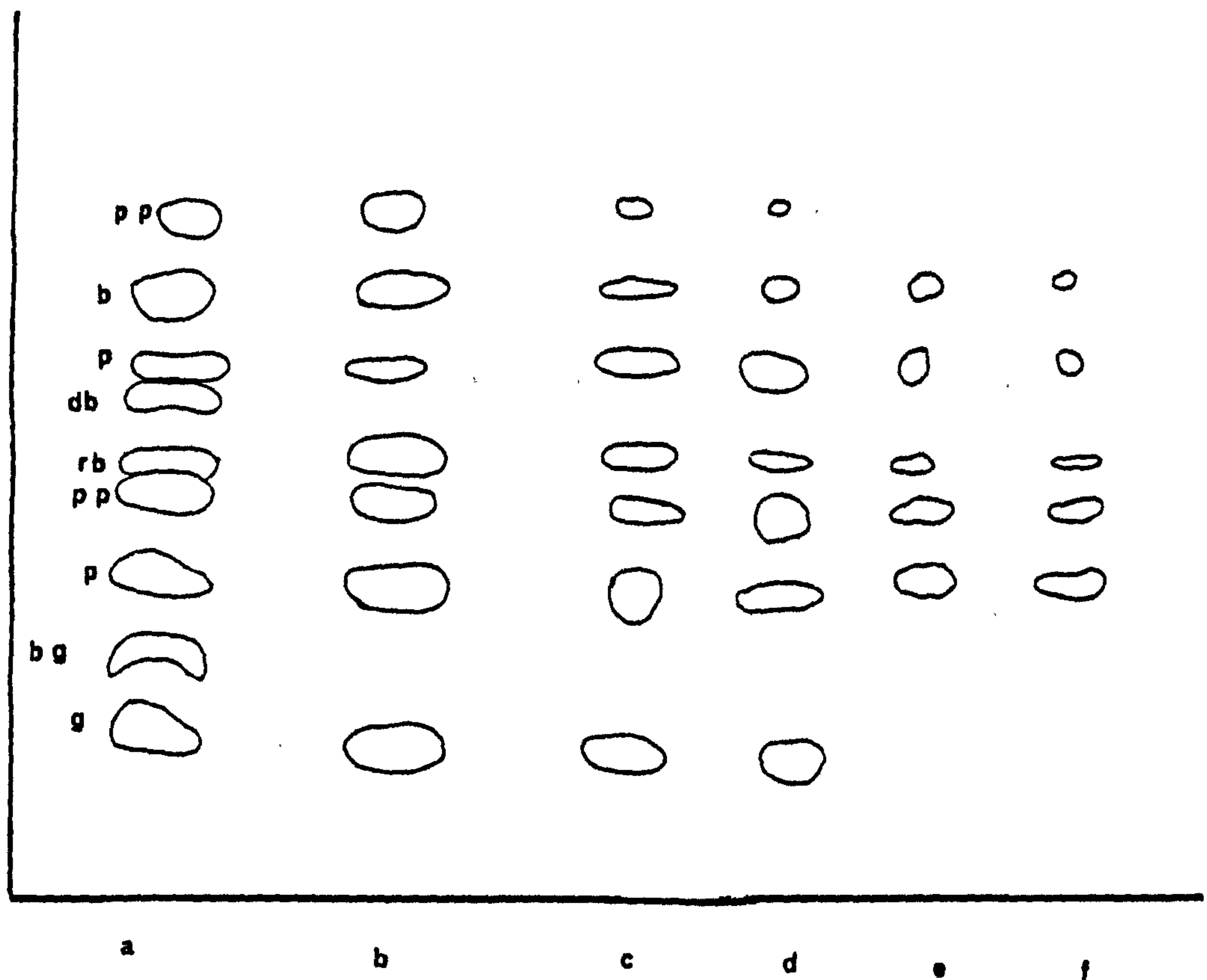


Fig. 31. Chromatographic patterns and spot colours of  
*H. cupressiforme* s.l. after spraying with antimony trichloride.  
 Developing solvent 20% ethyl acetate in methylene chloride.

Key to symbols:

p = purple

pp = pale purple

rb = reddish brown

b = blue

g = grey

bg = bluish grey

db = dirty brown

Where,

(a) var. lacunosum;

(b) H. mamillatum;

(c) var. filiforme;

(d) H. jutlandicum;

(e) var. cupressiforme;

(f) var. resupinatum.



to H. mammillatum and these two are quite distinct from the other H. cupressiforme s.l. taxa, as is also H. jutlandicum, supporting the argument that they should be given specific status. Var. resupinatum is obviously close to var. cupressiforme but the affinity of var. lacunosum is less clear with the e.a. solute. There are four spots additional to those of H. cupressiforme but the other spots are similar indicating similarity. With the benzene solute var. lacunosum is distinctive, suggesting it is less clearly related to var. cupressiforme and var. resupinatum.

### 3.3 Isozymes

Isozymes are considered to be the product of genes which segregate in a Mendelian manner and they are therefore very useful as genetic markers at the molecular level (Conklin and Smith, 1971). Systematists exploiting electrophoretic methods of isozyme separation have discovered particularly important characters in distinguishing species and clustering them into species groups (Krzakowa, 1981). In recent years, studies on genetic variation in natural hepatic populations based on electrophoretic allozymic variation, have shown that their patterns of variability are of the same characters as those of higher plants (Krzakowa, 1977; Krzakowa, and Szweykowski, 1979; Krzakowa and Szweykowski, 1977a). Taylor and Elliott (1972) found two months of mild conditions were required for Eurhynchium to re-establish normal activities of dehydrogenases after a period of dryness or intense cold. Krzakowa (1977) used peroxidase isozyme patterns as evidence for geographical races in Pellia endiviifolia. Isozyme electrophoresis has been used in investigation of interspecific differences (Krzakowa and Szweykowski, 1977a, b; Krzakowa and Szweykowski, 1980). Krzakowa (1981) used isozyme differences as characters separating Pellia megaspora from the other Pellia taxa. She concluded that there are



two groups of Pellia peroxidase isozyme phenotypes, one composed of Bepiphylla, P. neesiana and P. borealis. The other of P. endiviifolia from Europe, P. endiviifolia from Japan and P. megaspora. The phenotypes within the second group differed supporting the suggestion that there are three taxa present.

#### Materials and Methods

The method used is a modification of that of Gliddon (1976). Electrophoresis of the Xanthein Dehydrogenase (XDH) system was carried out on horizontal-slab, polyacrylamide-gels. The gel boxes used (LKB) allow separation over a distance of up to 10 cm., with a choice of sixteen or twenty-four samples being run concurrently. The gels were polymerized in forms and transferred to the boxes. The gel surface was cooled by a plate in which water at 3°C was circulated. Temperature control was achieved by means of an accurate electronic thermostat, controlling heater immersed in the bath. The cooling facilities, with the accurate thermostat combined with the large reservoir of cooling water and high circulation rates, gave excellent temperature control and therefore allowed good reproducibility of results.

Electrical connection of the gel was via wicks of absorbent material. Both electrode compartments had platinum wire electrodes extending the full width of the gel to minimise differences in the electrical field across the gel. Pockets for the application of samples were made by inserting a toothed plastic formen into the unpolymerized gel mixture and removing it after the gel has polymerized.

#### Preparation of Gels

The gels were polymerized, at a maximum one day prior to use. The gel strength was 7.5% acrylamide and the components of the mixture were:

Acrylamide monomer (BDH)	4.6 gm
N-methylene bis-acrylamide (Eastman)	0.2 gm
N,N,N <sup>1</sup> ,N <sup>1</sup> tetramethyl-ethylene diamine (Sigma)	0.14 ml
10% Ammonium persulphate solution	0.6 ml

All ingredients were dissolved in 65 ml of the appropriate buffer, Tris/citric acid pH 7.0. The solution was made up, as above, adding the ammonium persulphate last and poured into the gel formen. The gel was allowed to polymerize at room temperature for 45 minutes. The appropriate buffer was then added, the gel placed on the cooling-plate and cooled for at least one hour after which it was ready for use.

#### Buffer System

A buffer system was chosen for the gel and electrode buffer that would give satisfactory results for different taxa. This is to minimise differences in treatment between plants. The buffer system used was as follows:

Tris/citric acid, pH 7.0

16.35 gm	Sigma 7-9 buffer
----------	------------------

9.04 gm	Citric acid (anhydrous)
---------	-------------------------

made up to 10 litres. The buffers are used at the same strength for both gel and electrode buffer.

#### Preparation of Plant Material

About 10 mg of fresh shootwere taken, washed in distilled water and placed in a grinding tube with 25 µl of grinding solution. The grinding solution was freshly made up of at least every two weeks and kept in the dark at 2-4°C. The grinding solution was made up as follows:

0.1% Triton X-100	(W/V)
0.1% mercaptoethanol	(V/V)
10% sucrose	(W/V)

in the appropriate buffer, Tris/citric acid, pH 7. The purpose of the detergent was to improve cell disruption during grinding and the mercaptoethanol was used to protect the cell soluble enzymes from the effects of oxidizing agents (e.g. phenols) released in grinding. The sucrose made the extract more dense and this caused it to layer when applied to the gel. The plant material was ground up in the grinding solution by hand over ice. The ground samples were then centrifuged at 10,000 xg for 3 minutes at 0°C. 15 µl of the supernatant was then removed with a Finpipette and applied to the sample pockets on the gel.

#### Enzyme Stain

Details of the stain applied and which gave satisfactory results for the different taxa examined are as follows:

Xanthine Dehydrogenase (XDH)

Stain buffer: 0.1 M pyrophosphate/glycine, pH 9.0.

Stain : 100 mg Hypoxanthine (Sigma) dissolved in 2 ml  
1 M NaOH. .

1 g Glycine (to neutralize NaOH)

40 mg NAD

30 mg NBT

5 mg PMS after 30 minutes incubation at 37°C in  
100 ml incubated at 37°C until a satisfactory bands appeared.

#### Results and Discussion

Four different (XDH) isozyme phenotypes were distinguished (see Fig. 32). These are:-

1. var. cupressiforme, var. lacunosum and var. resupinatum with a single large band.



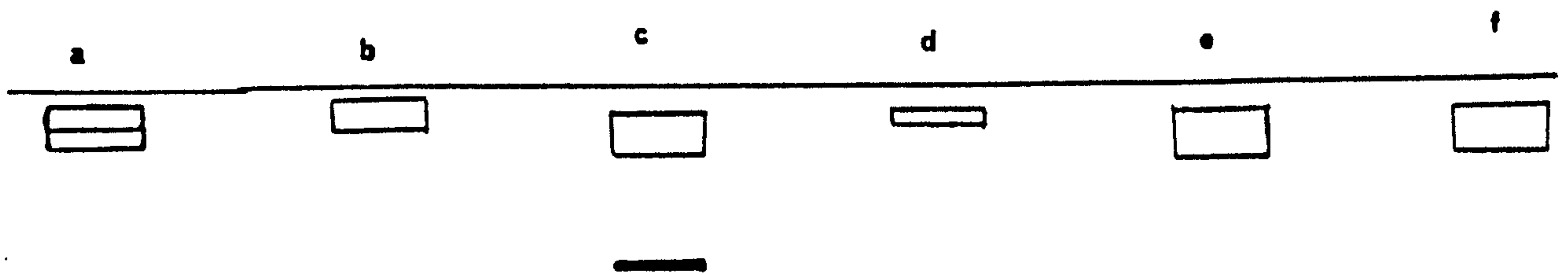


Fig. 32. Diagram showing the observed xanthine dehydrogenase isozyme band patterns in H. cupressiforme.

- |                             |                                 |
|-----------------------------|---------------------------------|
| (a) <u>H. mammillatum</u> ; | (b) var. <u>cupressiforme</u> ; |
| (c) var. <u>filiforme</u> ; | (d) <u>H. jutlandicum</u> ;     |
| (e) var. <u>lacunosum</u> ; | (f) var. <u>resupinatum</u> .   |



2. H. jutlandicum with a single small band.
3. H. mammillatum with two adjacent small bands.
4. var. filiforme with two bands, one large and one small.

Three varieties, var. cupressiforme, var. lacunosum and var. resupinatum are apparently closely related genetically. H. mammillatum and H. jutlandicum are clearly distinct. Whilst there is some similarity between H. mammillatum and var. filiforme in that one band of each coincide, there is another in each taxon which do not, supporting the hypothesis that there are two genetically distinct taxa involved.

### 3.4 Analyses of Amino Acids

According to Davies and Heywood (1963) the techniques of paper chromatography have been applied extensively to amino acid analysis, but the number of acids which have been characterized is quite small, around fifty, and the occurrence of perhaps half of these may be so general that they are of no particular systematic value. Refined techniques have made the identification of individual components feasible and these may have potential taxonomic significance, but so far the results have been disappointing although the restricted distributions shown by some of these acids may prove valuable. Huneck's (1974) and Suire's (1975) reviews contain many tables which show the presence and absence of different chemicals in mosses. The aim of this study was to investigate the presence of different amino acids in the six taxa of H. cupressiforme, and the value of amino acid for the status of these taxa.

### Materials and Methods

The six taxa of H. cupressiforme s.l. were grown under glass in a moist unheated unit in Pen-y-Fridd Station, Bangor. Tap water of pH 7.5 was added daily in order to maintain a moist atmosphere under the glasshouse. Material was removed from this unit after at least two weeks. Leaves were dissected from the stem and cut into fine sections, which were homogenized and extracted with 2 ml 50% aqueous ethyl alcohol at room temperature. After centrifugation the supernatant was evaporated under reduced pressure. The residue was dissolved in water (10 ml) and the resulting solution was added to the column of the analyser in 1 ml quantities (Hider and John, 1973). A Technicon Auto Analyser utilizing a standard Dowex 50 column at 60° was eluted with sodium citrate buffer. The limit of sensitivity for amino acids was 10 n mol.

### Results and Discussion

The following thirteen amino acids were found in every taxon: Aspartic acid, Tyreonine, Serine, Glutamic acid, Glycine, Alanine, Cystine, Valine, Tyrosine, Phenyl alanine, Lysine, Histidine and Arginine.

Analysis of amino acids of six taxa of H. cupressiforme s.l. suggests that there are no differences between them in the type of amino acids. This indicates that this character has no systematic value in distinguishing between the taxa examined. More biochemical studies are needed before any conclusions can be reached.



## CHAPTER 4

### Physiology and Ecology of H. cupressiforme aggregate

#### 4.1 Drought Tolerance

##### Introduction

One of the most important factors capable of causing stress is drought. Bryophytes differ from higher plants in that individual shoots exert little control over water loss to dry air, while the cytoplasm itself shows a high degree of desiccation tolerance (Hinshiri and Proctor, 1971). It is well established that many bryophytes can withstand dehydration to approximately 10% of their fresh weight and subsequently return to a near normal rate of respiration and photosynthesis or dehydration (Tucker et al., 1975). Recovery is not instantaneous, taking a period of hours or days and it may never be complete (Dilks and Proctor, 1974). Drought tolerance can be assessed from the effects of desiccation on rates of oxygen evolution. The highest degree of drought tolerance is generally found in bryophytes from dry habitats (Clausen, 1952, 1964; Dilks and Proctor, 1974). Intraspecific adaptation to desiccation has been investigated by Lee and Stewart (1971) who reported that resistance was less in plants from wet than from dry habitats in Calliergon cuspidatum, Climacium dendroides and Hypnum cupressiforme. Comparative experiments on plants cultivated under similar conditions are particularly important in this type of investigation, as desiccation resistance varies seasonally within the population (Hosokawa and Kubota, 1957; Dilks and Proctor, 1976). There have been relatively few detailed studies of seasonal differences but some were found by Ochi (1952) in Dicranium japonicum and Polytrichum formosum material collected in September, January and April. Hosokawa and Kubota (1957) found that all of their species from Japanese beech

forests survived desiccation longer when collected in winter (November) than in summer (August). In addition, Hinshiri and Proctor (1971) reported that Anomodon viticulosus was more tolerant to desiccation in April than in January. Lee and Stewart (1971) also found that Calliergon cuspidatum from a wet habitat was more tolerant to desiccation in winter than in summer, while for material from a dry habitat the reverse was true. Six bryophyte species showed clear evidence of seasonal variation in desiccation tolerance, as measured by net assimilation rate following 24 hours re-moistening. These species, Plagiochila spinulosa, Hylocomium splendens, Scorpiurium circinatum, Tortula ruraliformis, Racomitrium aquaticum and Andreaea rothii, showed low desiccation tolerance in autumn (October) and winter (January) and increased tolerance in spring and summer (Dilks and Proctor, 1976). The aims of this study were to investigate whether there is any correlation between net assimilation rate and resistance to desiccation in four taxa of H. cupressiforme s.l., to study the seasonal variation in each taxa and to determine whether it is influenced by genetic or environmental factors.

#### Materials and Methods

The material used in the experiments was collected on 4th October, 1979 and 10, 12th January and 5th July, 1980, each from two separate areas, the material was kept after collection for at least one week in polythene bags in the laboratory. Details of the collection sites are as follows:-

Hypnum mammillatum. On fallen trees, in shaded humid habitat, Treborth, near Bangor.

On tree trunks, in shaded humid habitat, Aber Valley.



H. mammillatum  
var. filiforme. On vertical tree trunks, in shaded habitats, Treborth  
and Aber Valley.

H. cupressiforme  
var. lacunosum. Limestone, in exposed habitat, Bwdyrr Arthur,  
Anglesey.

Sandy in exposed habitat, Aberffraw, Anglesey.

H. cupressiforme  
var. cupressiforme. On trees, in shaded habitat, Aber Valley and  
Treborth.

H. cupressiforme  
var. resupinatum. On rocks, in shaded habitat, Glanrafon Hill, Bangor  
and Maesgeirchen.

The method used to desiccate the mosses was similar to that described by Proctor (1972). Cut shoots were placed in desiccators over hydrated calcium chloride  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ , giving relative humidities of approximately 32% at laboratory temperature.

The ability of the plants to recover from desiccation was assessed by dehydrating the plants for up to 150 days and then returning them to moist conditions for 24 hours before measuring  $\text{O}_2$  evolution with the Gilson respirometer. The dark respiration was estimated at  $20^\circ\text{C}$  by placing the material in the bottom of a Warburg flask with  $2\text{ cm}^3$  of water and  $0.5\text{ cm}^3$  of 10% potassium hydroxide in the central-well. The flasks were covered with aluminium foil to exclude light. A second set of Warburg flasks were prepared with no KOH in the central-well and were illuminated from below with a bank of xenon lights which gave a light intensity of  $260\text{ watts/m}^2$ . A measure of photosynthesis was then obtained. All results are based on oven-dry weight. The results shown are the mean of five replicates. To examine the recovery of the plants after dehydration they were transferred to an unheated mist unit at Pen-Y-Fridd Field Station, Bangor and re-examined after at least two months recovery.

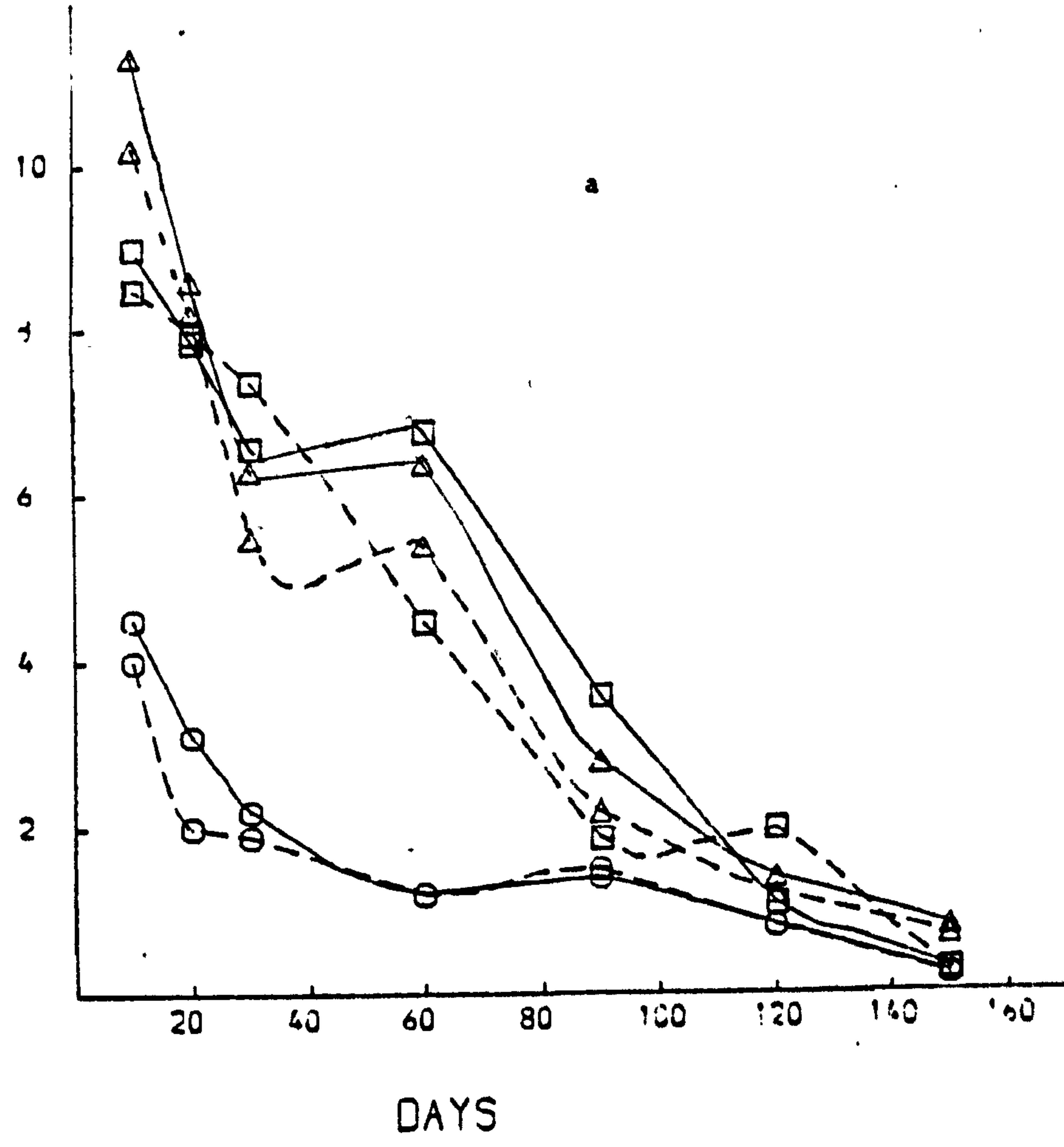
## Results

Drought resistance in all the taxa examined declined with increasing desiccation time as shown by the decrease in oxygen evolution with increased desiccation (Figs. 33a - 37a). Drought resistance appeared to be lowest in material collected in October as shown by the low photosynthetic oxygen evolution which was 50% lower than in material collected in January. Oxygen evolution in October material was between 2.2 - 4.3 (average 3.9)  $\mu\text{l. O}_2/\text{gm. dry weight/hour}$  after ten days of continuous desiccation. A higher resistance to desiccation was found in material collected in January where the photosynthetic oxygen evolution was between 3.9 - 8.6 (average 6.5  $\mu\text{l.}$ ) after ten days of continuous desiccation. The highest resistance was found in material collected in July where the oxygen evolution was 20% higher than that in January. In July the  $\text{O}_2$  evolution varied between 5.2 - 10.8 (average 8.8  $\mu\text{l.}$ ) (Figs. 33a - 37a). After ninety days of continuous desiccation the  $\text{O}_2$  evolution from material collected in October was also 50% lower than material collected in January and was between 0.7 - 1.8  $\mu\text{l.}$  with an average 1.35  $\mu\text{l.}$  A higher resistance was found in material collected in January where the  $\text{O}_2$  evolution was between 1 - 4.1  $\mu\text{l.}$  with an average 3.2  $\mu\text{l.}$  of oxygen (Figs. 33a - 37a).

The highest resistance was found in material collected in July where the  $\text{O}_2$  evolution was 20% higher than that in January and varied between 1.9 - 4.5  $\mu\text{l.}$  (Figs. 33a - 37a). In plants collected in January there was a rapid decrease in oxygen evolution after ninety days of continuous desiccation H. mammillatum and var. filiforme (Figs. 36a, 37a) and after sixty days in var. cupressiforme (Figs. 33a). This rapid decrease in  $\text{O}_2$  evolution was found in plants collected in October after

Fig. 33. Changes in oxygen evolution of var. cupressiforme dehydrated for up to 150 days over a hydrous  $\text{CaCl}_2$  and rehydrated 24 hours before measurements commenced. Material was collected from Aber Valley (—) and Treborth (---) in July ( $\Delta$ ), January ( $\square$ ) and October ( $\circ$ ) and the hydration treatment started immediately (Fig. a) or material was cultivated in glasshouse for two months before the start of dehydration (Fig. b).

Q<sub>2</sub> EVOL. UL. / GM. LK Y WT. / HR.



Q<sub>2</sub> EVOL. UL. / GM. DRY WT. / HR.

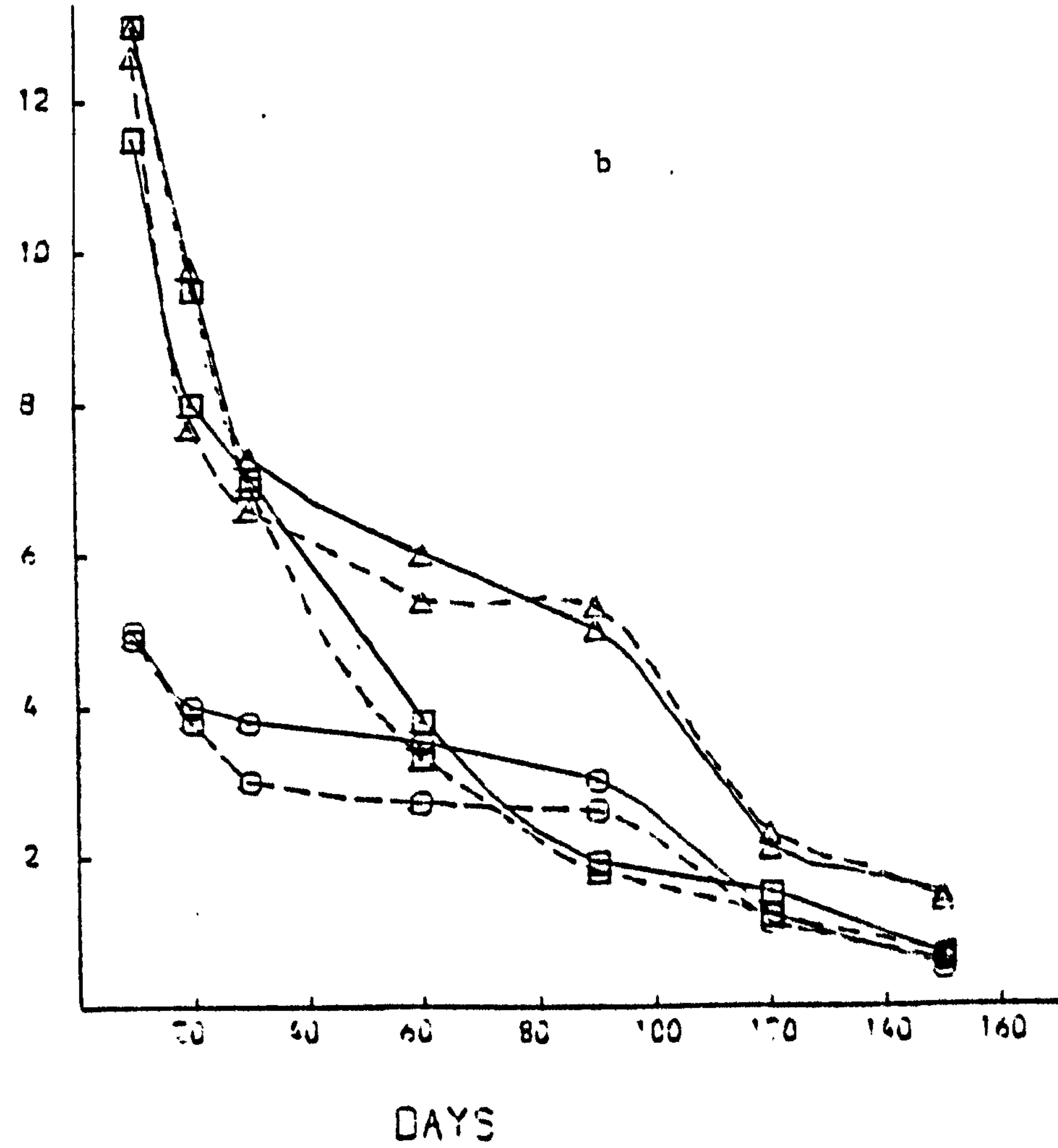




Fig. 34. Changes in oxygen evolution of var. resupinatum dehydrated for up to 150 days over a hydrous  $\text{CaCl}_2$  and rehydrated 24 hours before measurements commenced. Material was collected from Glanrafon Hill (—) and Maesgeirchen (---) in July ( $\Delta$ ), January ( $\square$ ) and October ( $\circ$ ) and the hydration treatment started immediately (Fig. a) or material was cultivated in glasshouse for two months before the start of dehydration (Fig. b).

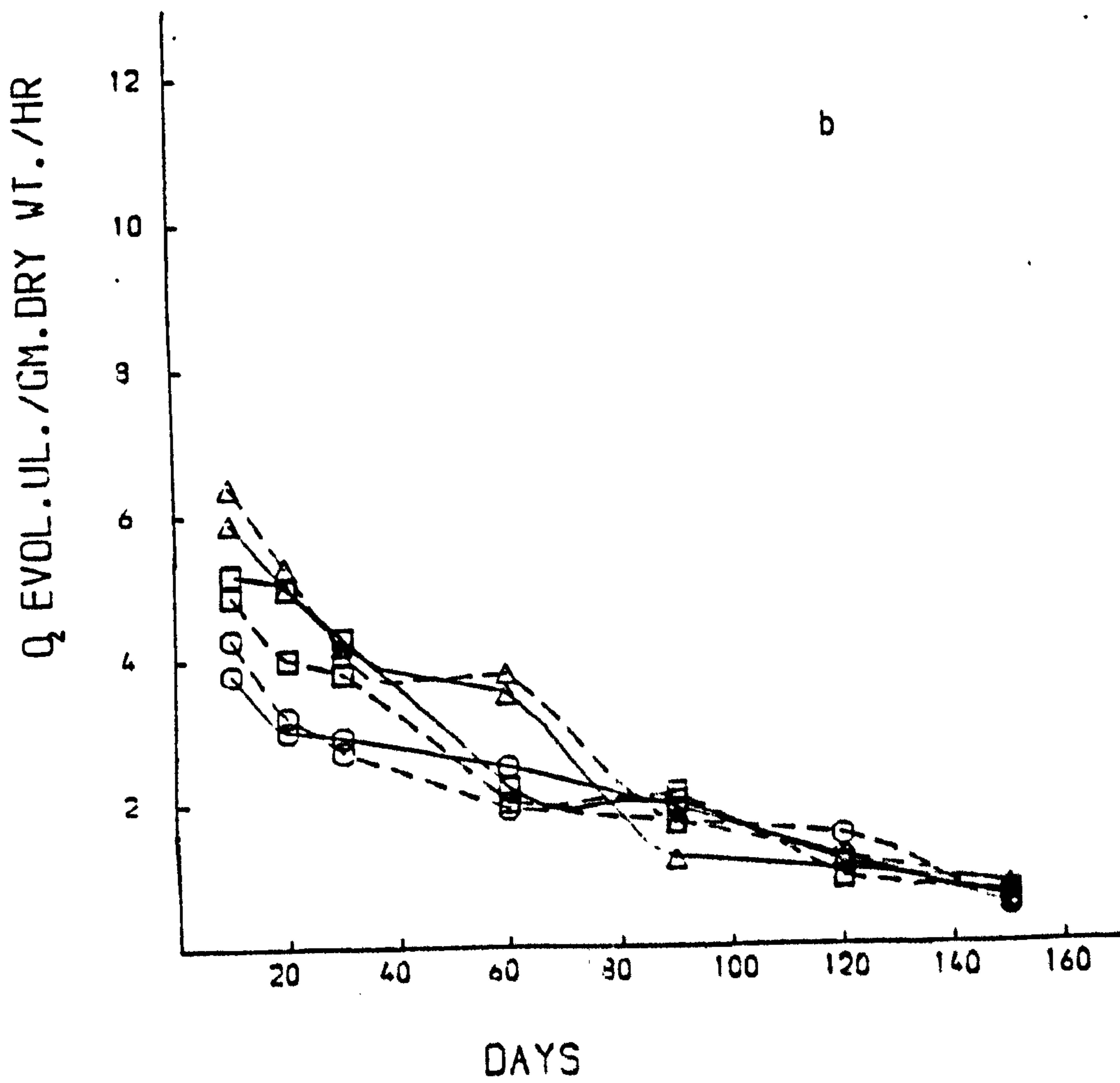
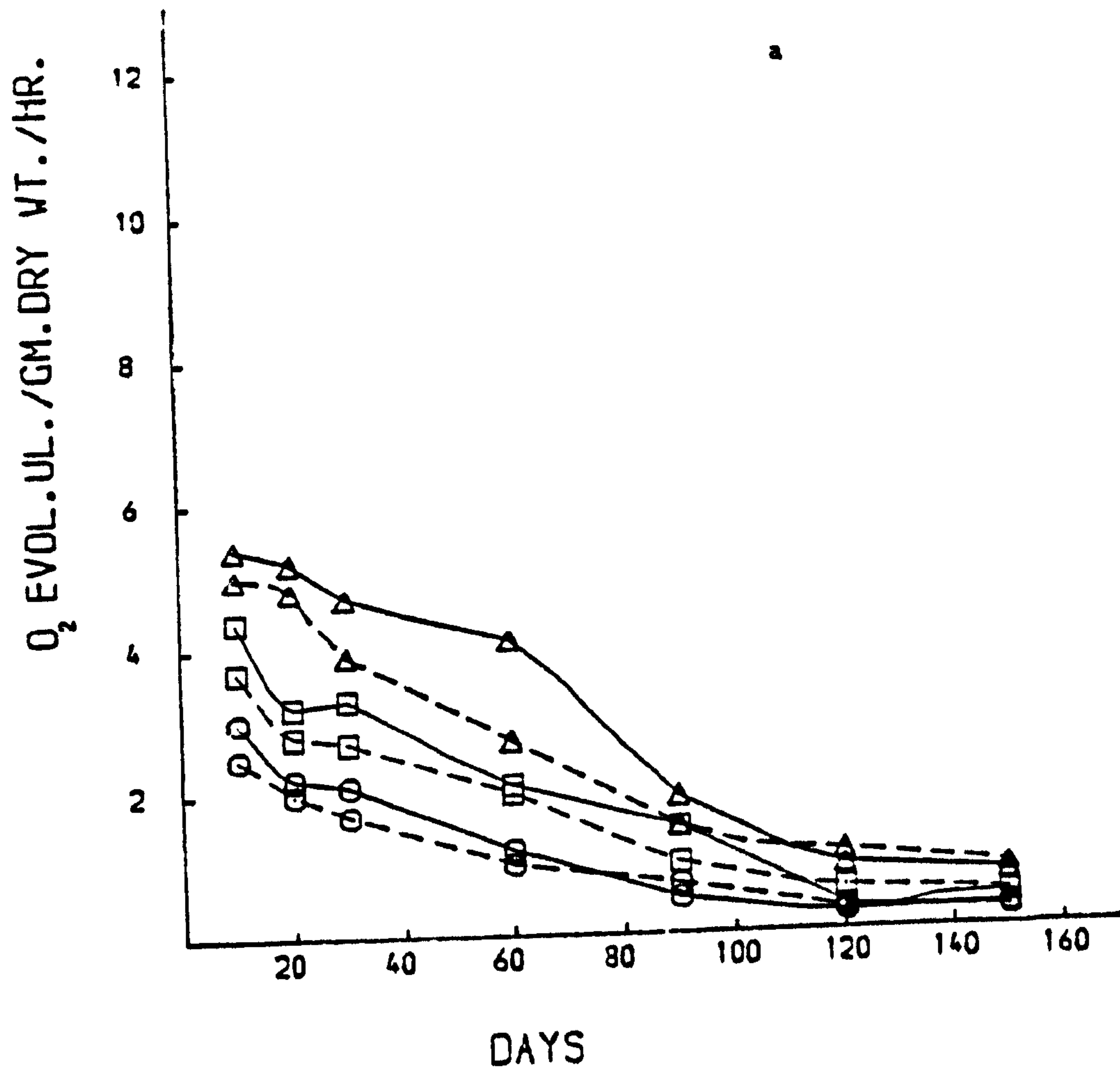
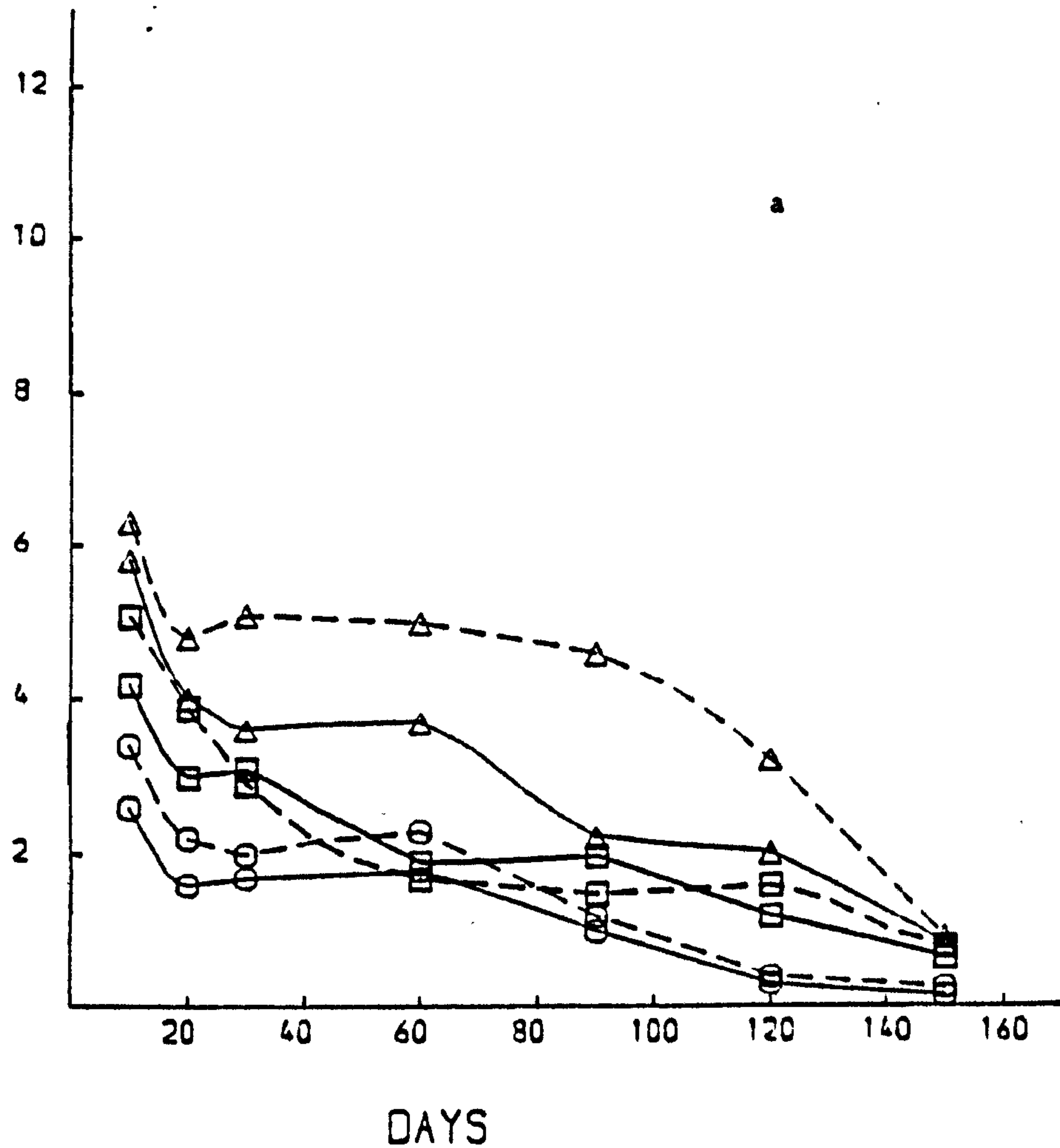


Fig. 35. Changes in oxygen evolution of var. lacunosum dehydrated for up to 150 days over a hydrous  $\text{CaCl}_2$  and rehydrated 24 hours before measurements commenced. Material was collected from Bwrdd Arthur (—) and Aberfraw (---) in July ( $\Delta$ ), January ( $\square$ ) and October ( $\circ$ ) and the hydration treatment started immediately (Fig. a) or material was cultivated in glasshouse for two months before the start of dehydration (Fig. b).

$O_2$  EVOL. UL. / GM. DRY WT. / HR.



$O_2$  EVOL. UL. / GM. DRY WT. / HR.

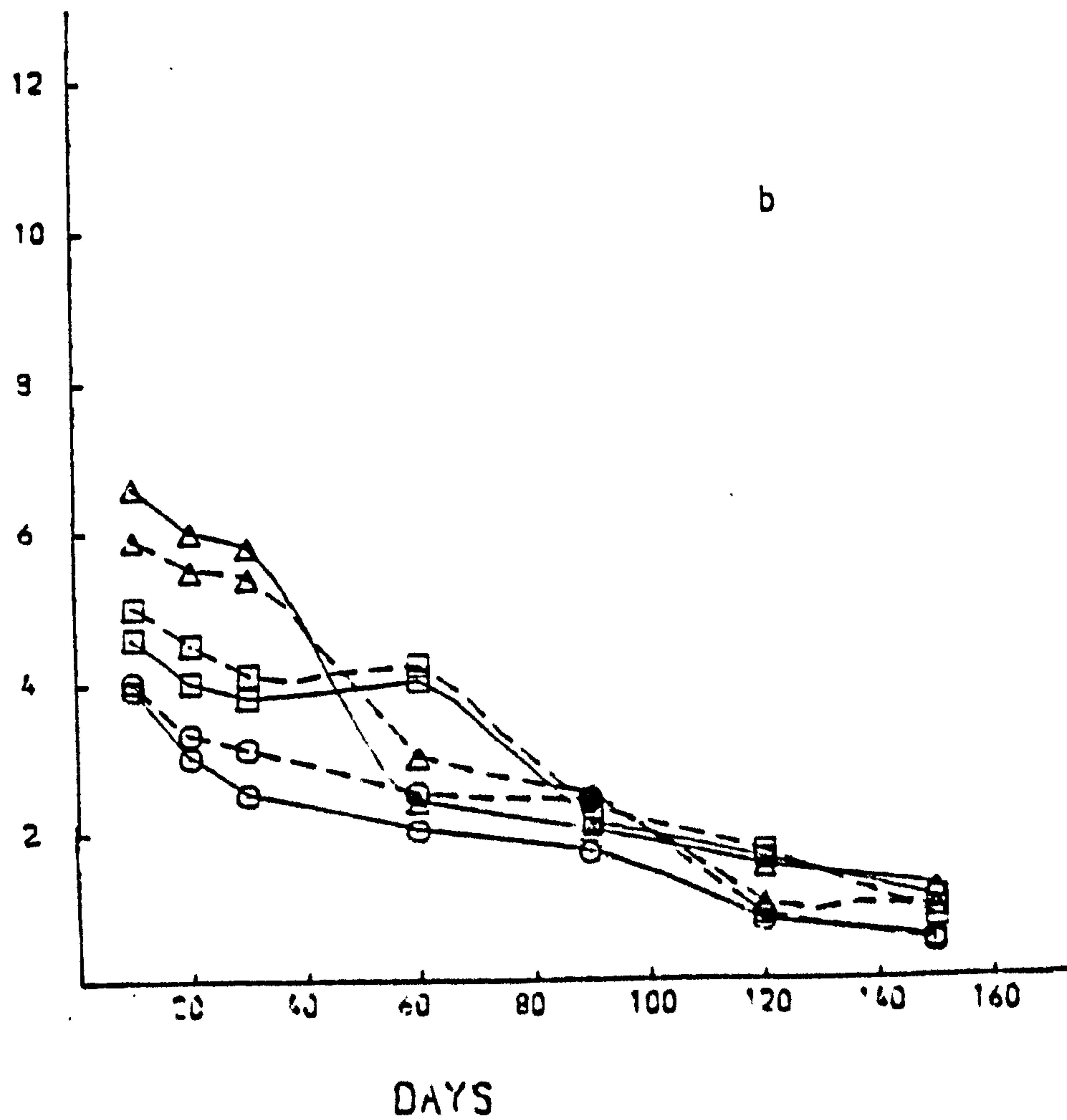
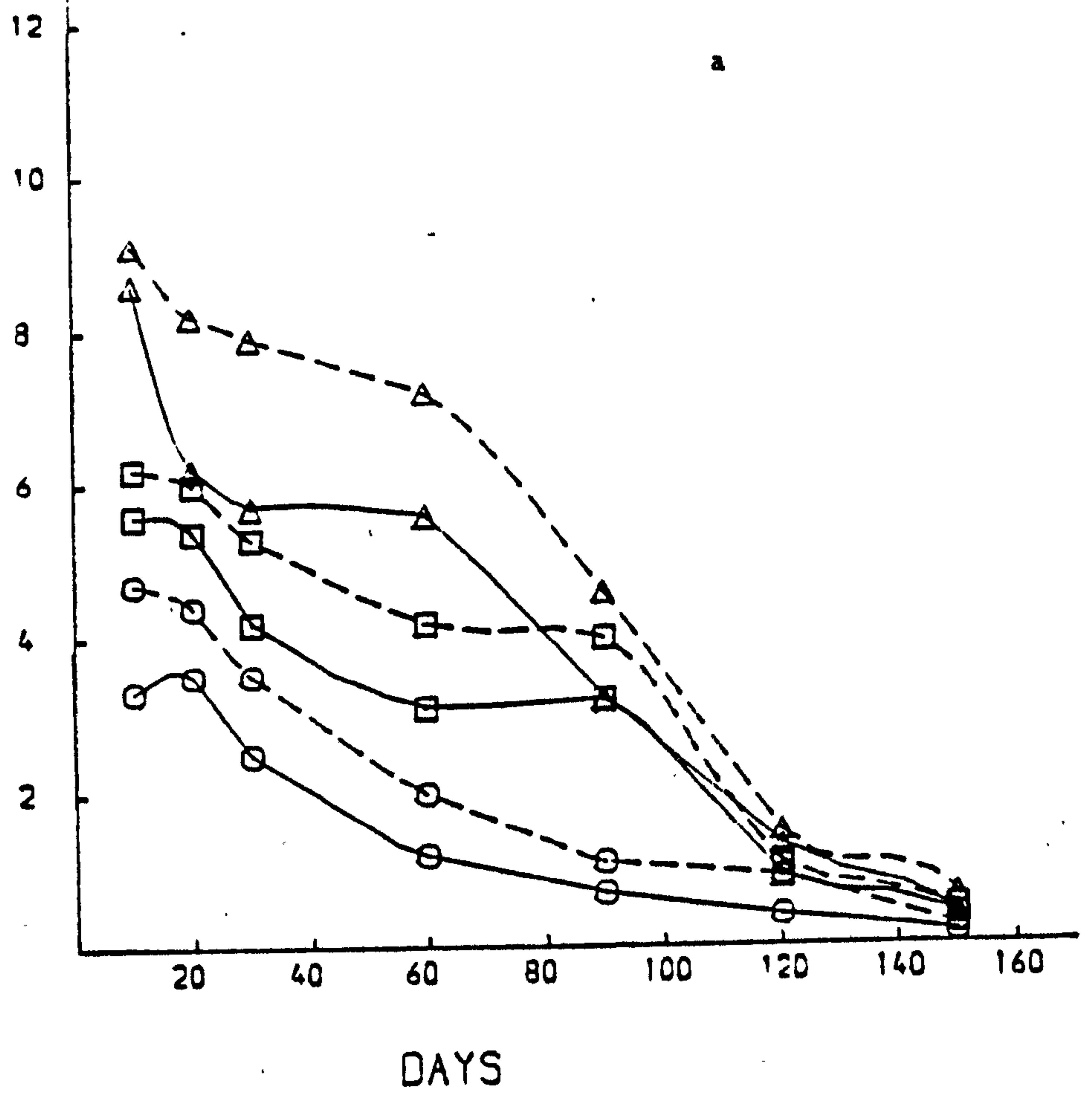




Fig. 36. Changes in oxygen evolution of H. mammillatum dehydrated for up to 150 days over a hydrous  $\text{CaCl}_2$  and rehydrated 24 hours before measurements commenced. Material was collected from Treborth (—) and Aber Valley (---) in July ( $\Delta$ ), January ( $\square$ ) and October ( $\circ$ ) and the hydration treatment started immediately (Fig. a) or material was cultivated in glasshouse for two months before the start of dehydration (Fig. b).

$O_2$  EVOL. UL. / GM. DRY WT. / HR.



$O_2$  EVOL. UL / GM. DRY WT. / HR.

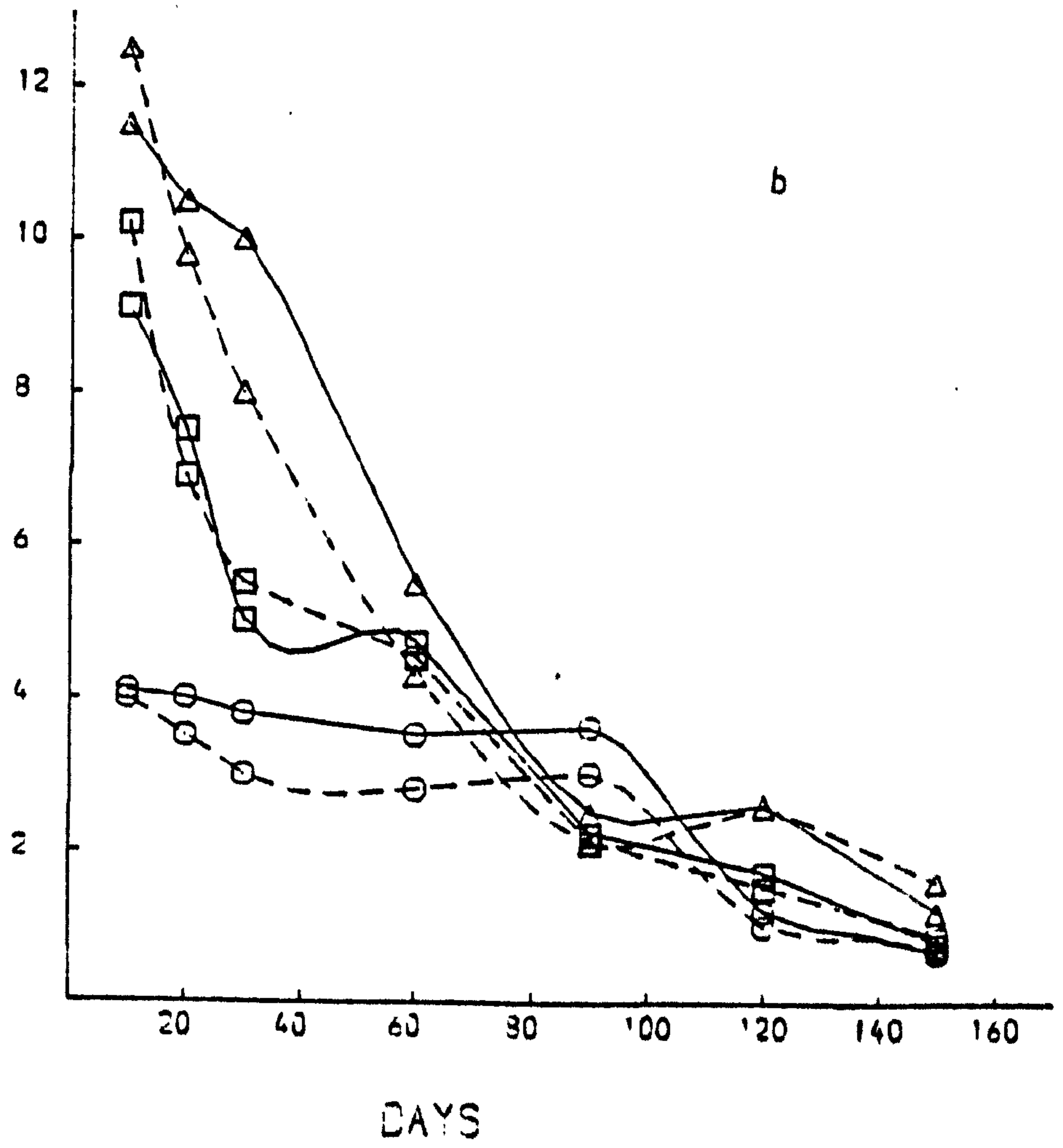
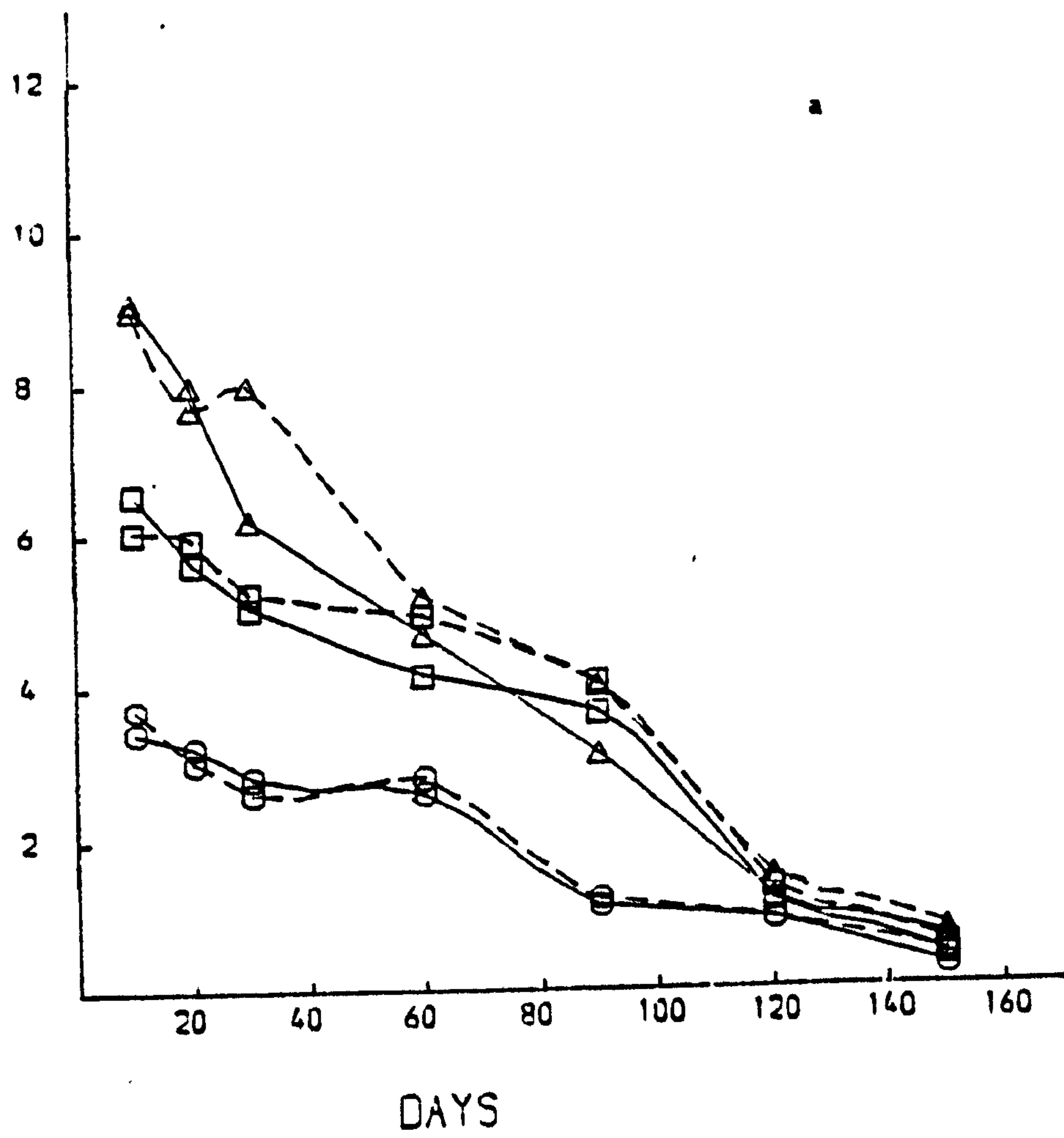
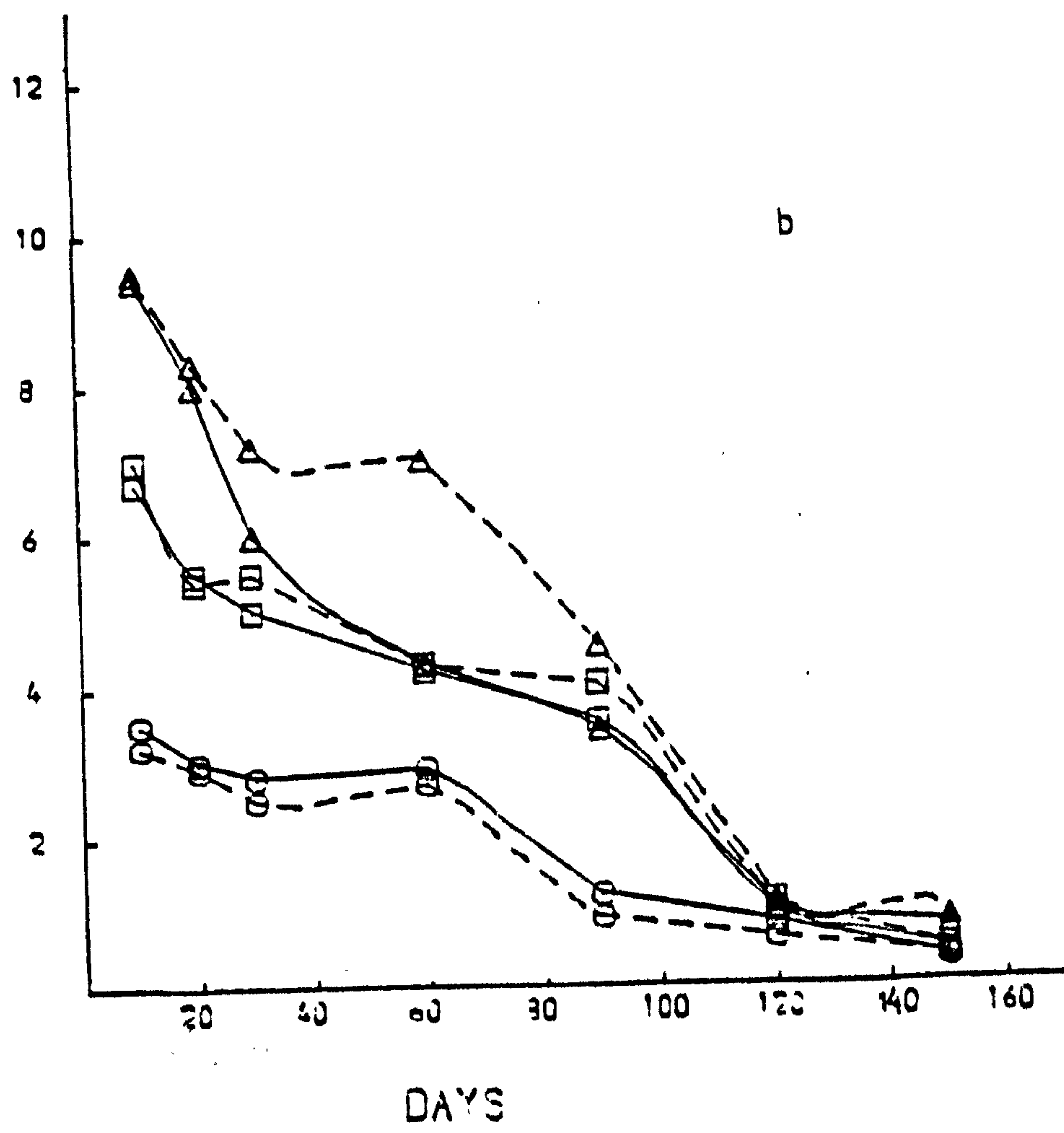


Fig. 37. Changes in oxygen evolution of var. filiforme dehydrated for up to 150 days over a hydrous  $\text{CaCl}_2$  and rehydrated 24 hours before measurements commenced. Material was collected from Treborth (—) and Aber Valley (---) in July ( $\Delta$ ), January ( $\square$ ) and October ( $\circ$ ) and the hydration treatment started immediately (Fig. a) or material was cultivated in glasshouse for two months before the start of dehydration (Fig. b).

$O_2$  EVOL. UL. / GM. DRY WT. / HR.



$O_2$  EVOL. UL. / GM. DRY WT. / HR.





twenty days desiccation in var. cupressiforme while material of var. cupressiforme collected in July showed a similar pattern after sixty days desiccation (Fig. 33a). This pattern is not as readily apparent in the other taxa (Figs. 34a - 37a).

After culture under similar conditions all plants were most sensitive to desiccation in October, became less sensitive in January and they reached their maximum drought tolerance in July (Figs. 33b - 37b). The lowest value for  $O_2$  evolution from material collected in October after ten days desiccation was 3.9 - 5  $\mu$ l. (average 4.5  $\mu$ l.) which was 25% lower than that of material collected in January, where oxygen evolution between 4.3 - 11.7  $\mu$ l with an average 7.6  $\mu$ l. The highest resistance was found in material collected in July when the  $O_2$  evolution was 15% higher than that in January and varied between 5.9 - 12.3  $\mu$ l. (average 10  $\mu$ l.). After 120 days desiccation the  $O_2$  evolution was lowest in material collected in October which was 15% lower than that in January, and varied between 0.8 - 1.9  $\mu$ l. with an average 1.4  $\mu$ l. The resistance increased during January where  $O_2$  evolution was between 1.7 - 2.1  $\mu$ l. with an average 1.9  $\mu$ l. The highest resistance was found in material collected in July where  $O_2$  evolution was 20% higher than that in January (Figs. 33b - 37b).

All taxa showed in each season a different pattern of change in photosynthetic oxygen evolution both before and after culture under glasshouse conditions, except var. filiforme where an almost similar pattern was found (Fig. 37a, b). The same pattern of change in oxygen evolution was found for material of each taxon collected from separate localities except var. lacunosum where material collected from a sandy

habitat showed a higher drought tolerance than that from a limestone habitat (Fig. 35a). This difference was not detectable in plants that had been cultivated in the glasshouse (Fig. 35b).

### Discussion

In all experiments dark respiration was much slower than photosynthesis even though there is often an initial stimulation of respiration on remoistening. This agrees with observations of Romose (1940); Hinshiri and Proctor (1971); Dilks and Proctor (1974). Therefore, this had little effect on the pattern of photosynthetic oxygen evolution under our conditions.

The seasonal differences observed in response to desiccation (Figs. 33a - 37a), agrees with the findings of Dilks and Proctor (1976) on six bryophytes. The occurrence of seasonal variation in resistance to desiccation was already noticed by Ochi (1952) in Dicranum japonicum and Polytrichum attenuatum. In October (autumn), resistance to drought reaches its lowest level where  $O_2$  evolution is 50% and 70% lower than that in material collected in January and October after 10 and 90 days desiccation, increasing during January (winter) and reaches its highest resistance in July (summer), where  $O_2$  evolution is 70% and 20% higher than that in material collected in October and January after 10 and 90 days desiccation. This may relate to the physiological state of the plants at a specific time of year and as all taxa might have a similar growth cycle, little difference can be detected between plants. These results are similar to those obtained by Dilks and Proctor (1976) on six bryophytes. In contrast these results disagree with those obtained by Hosokawa and Kubota (1957) who reported that the resistance of epiphytic mosses to desiccation was higher in winter and lower in summer.



The causes of the seasonal changes in tolerance to desiccation are not known. However, Schwabe and Nachmony-Bascomb (1963) found that the response in Lunularia was unequivocally photoperiodic. The high level of photosynthetic oxygen evolution during the summer suggests high resistance to drought during this season. The absence of the rapid decrease pattern in oxygen evolution during January in var. lacunosum and var. resupinatum indicates high resistance to drought during winter, similarly the absence of this pattern in H. mammillatum, var. lacunosum, var. resupinatum and var. filiforme during October and July suggests high resistance during autumn and summer. The presence of the rapid decrease pattern in oxygen evolution after 90 days of continuous desiccation in material collected in January in H. mammillatum and var. filiforme suggests similar physiologies in these two taxa and low resistance during winter. The fact that all plants when grown under similar conditions showed their lowest resistance to drought in October (autumn), and highest resistance in the summer, suggests a degree of genetic uniformity between the plants. The production of a different pattern of oxygen evolution in each season after culture by all plants except var. filiforme (Fig. 37a, b), suggests that there is a physiological adaptation in var. filiforme. This agrees with Longton (1979) who reported that the morphological variation of H. cupressiforme is in part genetically determined.

A similar pattern of oxygen evolution was produced from material collected from two separate localities of each taxon in each season except for var. lacunosum collected during July from sand dunes which showed a higher drought resistance than that from a limestone habitat. This agrees with the results of Lee and Stewart (1971) who demonstrated in Calliergor cuspidatum, Climacium dendroides and H. cupressiforme, thus the

pattern of oxygen evolution in var. lacunosum is influenced by the type of habitat (at least in summer). The absence of this situation after culture suggests that it is not genetically determined.

#### 4.2 The Ecology and distribution of H. cupressiforme s.l.

The order Hyprobryales is among several orders of mosses described by Miller(1979) which have the potential to adapt to changing climate. Longton (1979) says "Richards also referred to unpublished work by Chamberlain which indicated that many characters distinguishing the varieties of H. cupressiforme are genetically fixed so that these taxa may also be regarded as ecotypes".

The six taxa studied are morphologically and ecologically variable. H. cupressiforme s.l. is widely distributed in different parts of the northern and southern hemisphere except the tropics. The ecology and distribution of H. cupressiforme s.l. has been discussed by Ando (1972b, 1979) and Doignon (1950, 1951). The wide distribution and range of habitat suggest a high degree of ecological tolerance. There is, however, some correlation between habitat and morphological form.

All forms of H. cupressiforme are dioecious and there is some evidence (Gemmell, 1950) that dioecious species are more frequent and genetically variable than monoecious ones. This would appear to be particularly true of H. cupressiforme s.l. with its wide ecological tolerance and range of morphological forms. H. cupressiforme s.l. is the commonest and most variable species in the British moss flora (Smith, personal communication). Habitat relationships of the taxa of H. cupressiforme s.l. in Britain are as follows:-

##### 1. H. cupressiforme var. cupressiforme

This variety occurs on rocks, walls, bark, logs and sometimes on hand-packed soil; never on peat or leaf litter. It is found both in



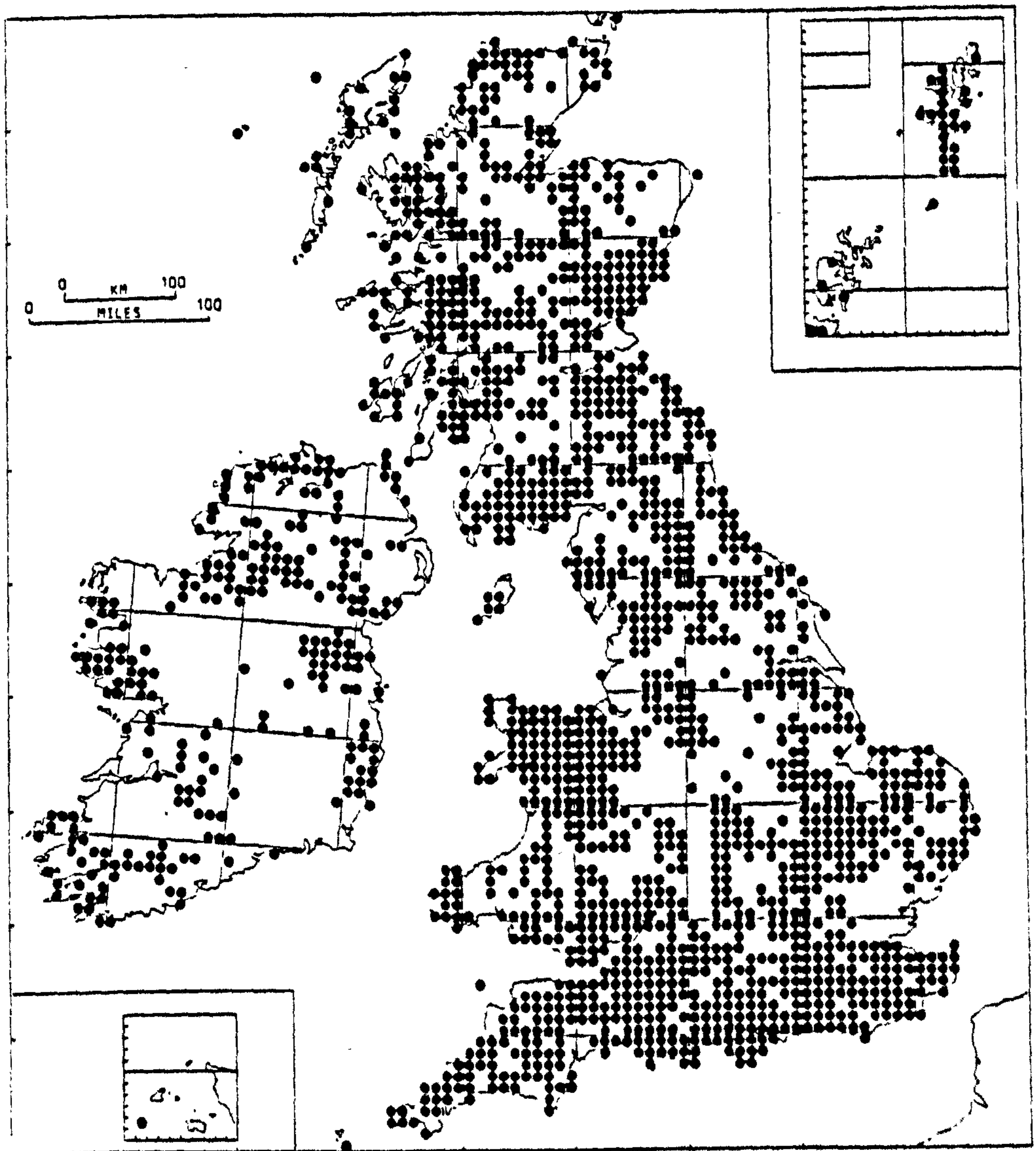


Fig. 38. Distribution of var. cupressiforme in Britain and Ireland.

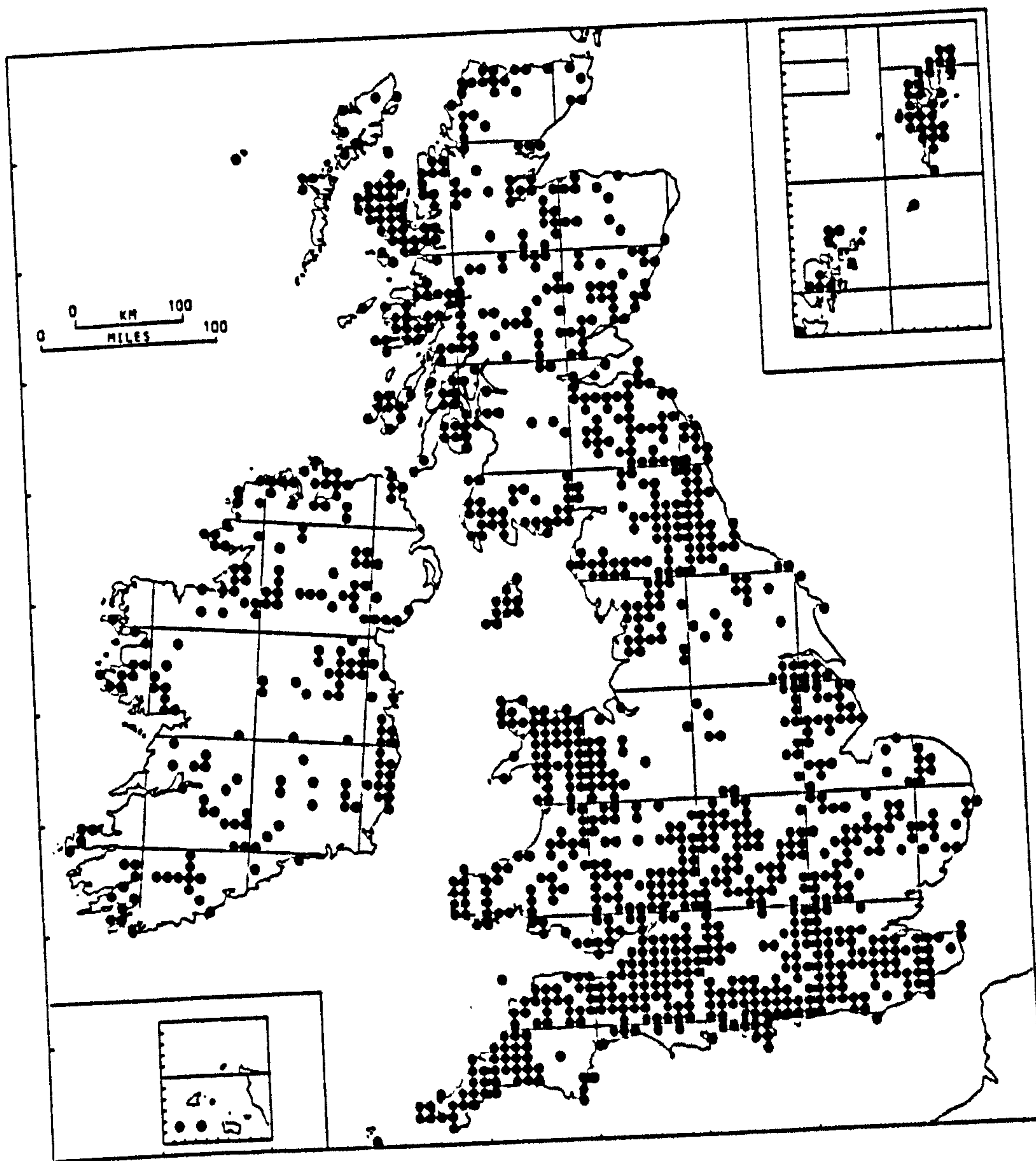


Fig. 39. Distribution of var. resupinatum in Britain and Ireland.

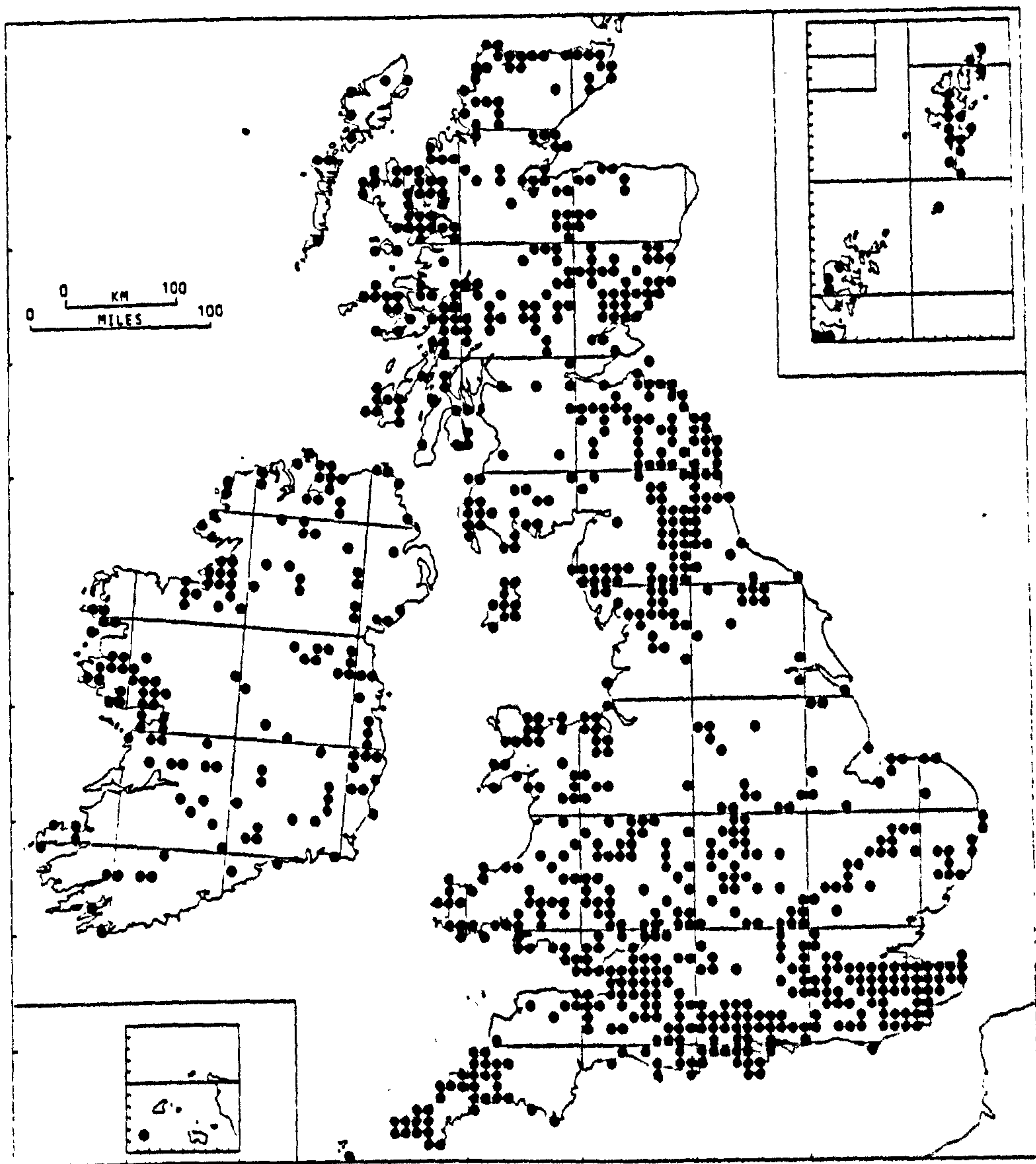


Fig. 40. Distribution of var. lacunosum in Britain and Ireland.



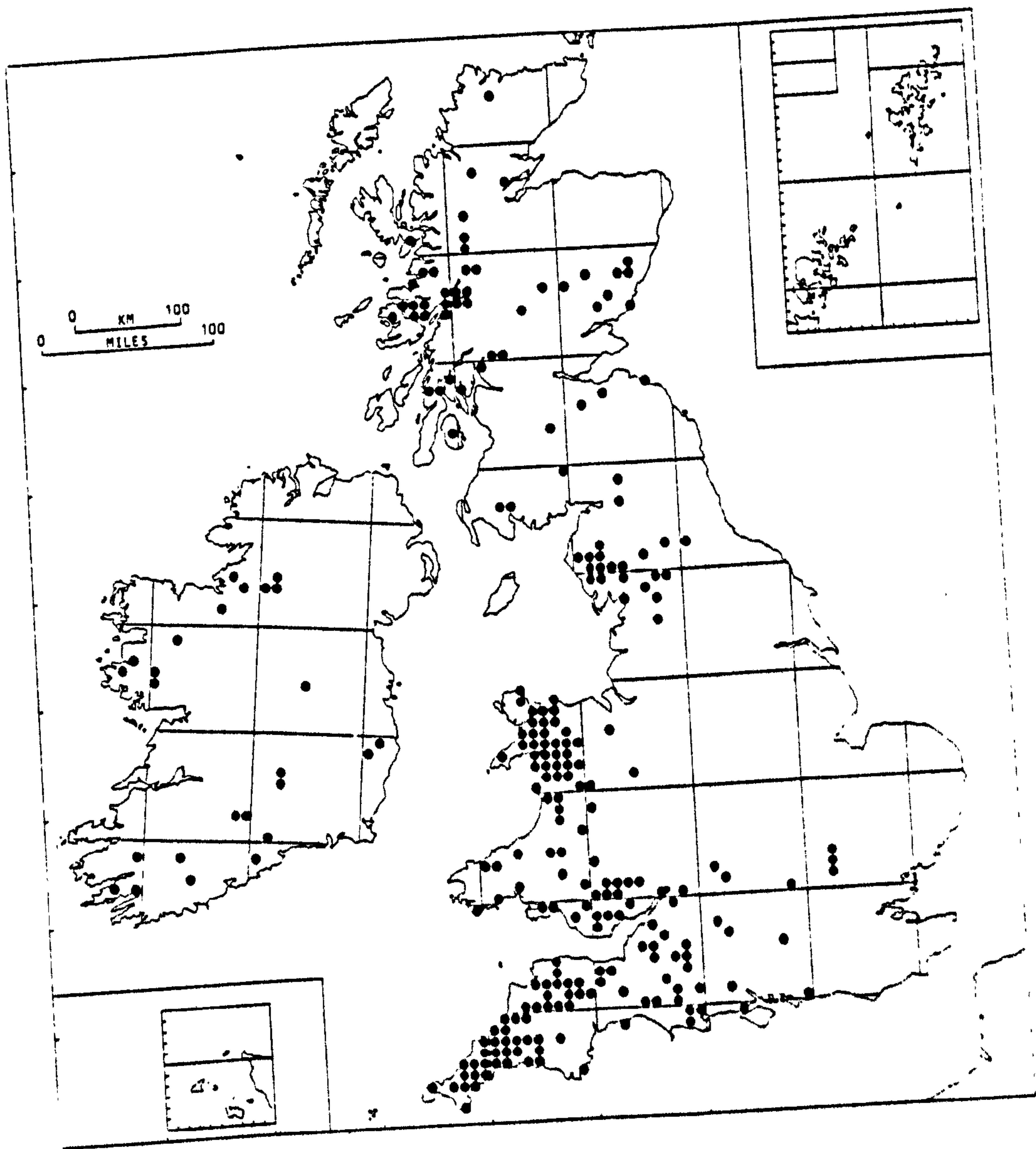


Fig. 41. Distribution of *H. mammilatum* in Britain and Ireland.



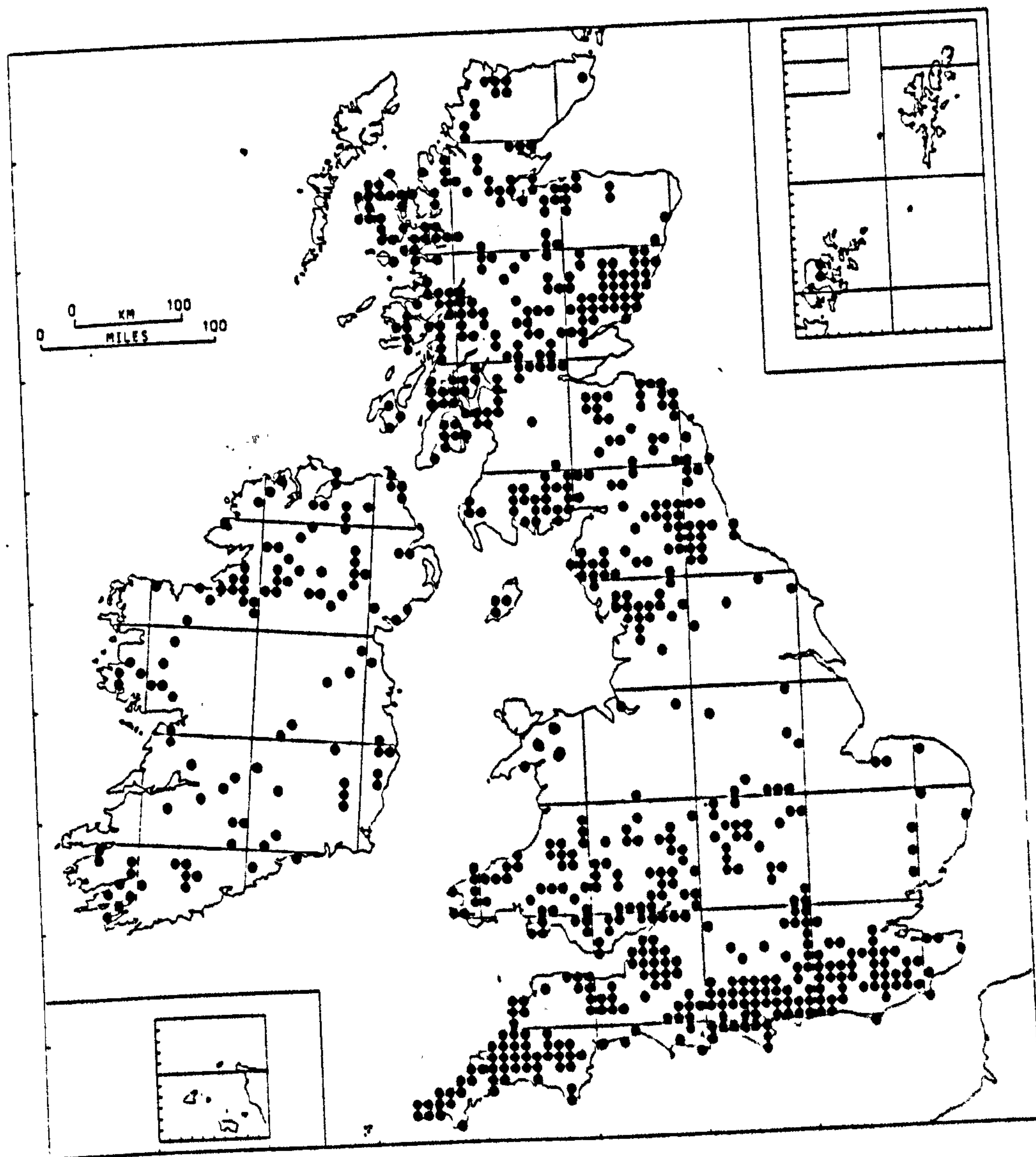


Fig. 42. Distribution of var. filiforme in Britain and Ireland.

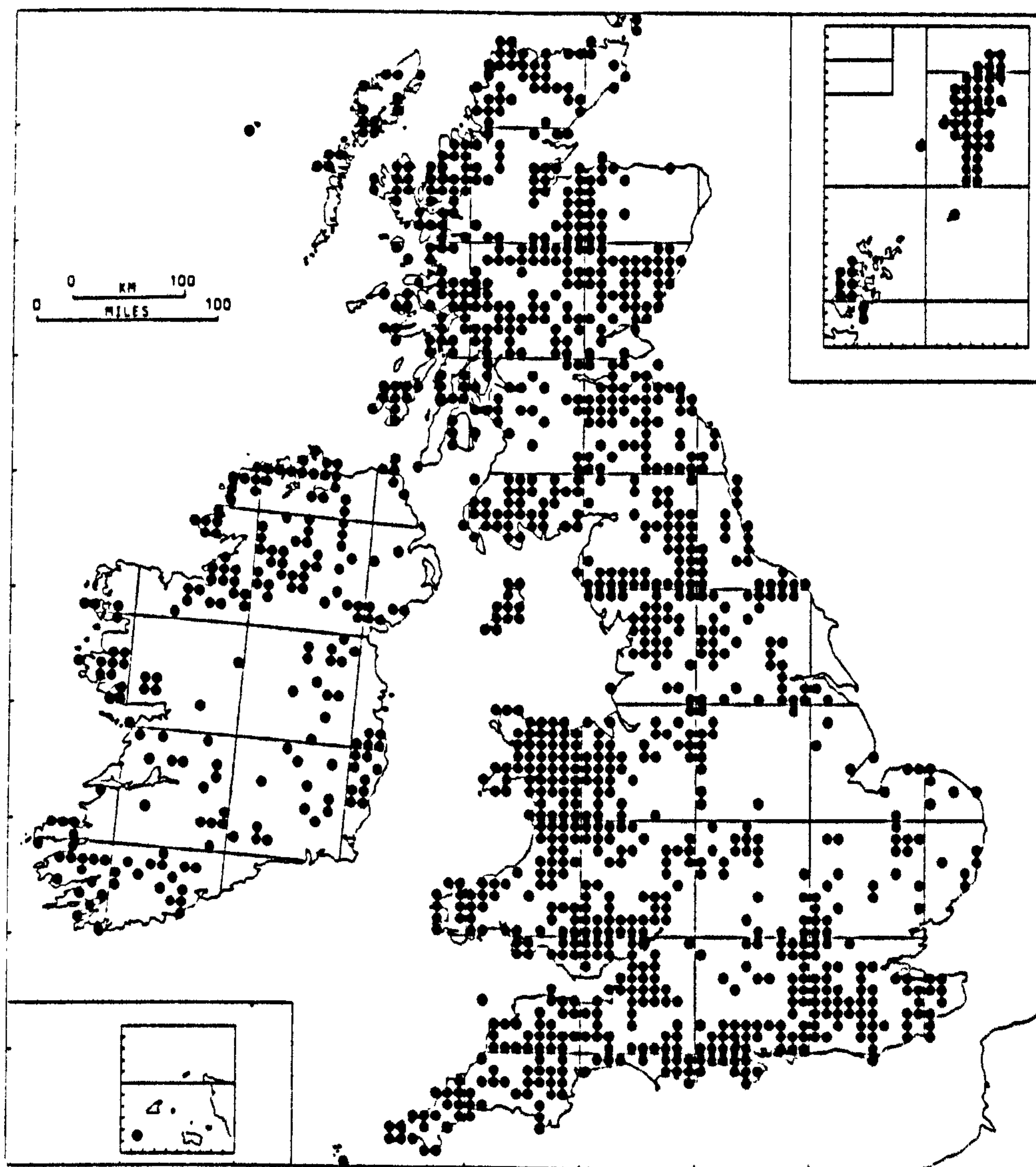


Fig. 43. Distribution of *H. jutlandicum* in Britain and Ireland.

exposed and sheltered habitats, especially where acidic, and the altitudinal range is from sea level to 1230 m.

var. cupressiforme is widely distributed in the British Isles. It is less frequent in the Midland. It would appear from Fig. (38) that in Ireland it is distributed near the coasts.

2. H. cupressiforme var. resupinatum

This plant is found, usually in shaded situations, on tree branches and on rocks and in rock-revices. It is the variety most commonly encountered in maritime habitats (e.g. Cornwall, S.W. Ireland). Apparently neutral to calcifuge, a lowland plant.

Fig. (39) shows the distribution of var. resupinatum. It would appear it is less widespread than var. cupressiforme and especially in the north-east and some parts of the Midlands. It is also less abundant in Scotland than var. cupressiforme. It is scattered in many places in Ireland.

3. H. cupressiforme var. lacunosum

A calcico<sup>le</sup> from chalk and limestone grassland, dune slacks, calcareous rocks and walls, very rarely trees. Plants from acid habitats are smaller and intergrade with var. cupressiforme. Ranging from sea level to 1000 m.

Fig. (40) shows that var. lacunosum is less common than both var. cupressiforme and var. resupinatum. It is less frequent in the east and the north-east of England. It is scattered in Scotland and Ireland.

4. H. mammillatum

In similar habitats to var. cupressiforme, probably as common as, if not more so in western parts of Britain and Ireland, but certainly overlooked, apparently rare in drier and exposed habitats and in lowland



Britain. Ranging from sea level to ca. 1000 m.

Fig. (41) shows the distribution of H. mammillatum. It would appear it is widely distributed in the south-west of England, north-west of Wales and scattered in Scotland and Ireland.

5. Var. filiforme

Forms thin and sometimes extensive mats, usually on vertical tree trunks, more rarely on vertical rock faces, in shaded humid habitats, common in woodland habitats in western and northern Britain and in Ireland, rare elsewhere. Neutral or calcifuge. A mainly lowland plant.

Fig. (42) shows the distribution of var. filiforme. It would appear that it is frequent in the south and north of Britain, less widely distributed in the Midlands and the east coast of England. It is scattered in Ireland.

6. H. jutlandicum

On peaty soil and leaf litter on heaths, moorland and coniferous woodland, occasionally on tree boles and porous rocks. Strongly calcifuge.

Fig. (43) shows the distribution of H. jutlandicum. It would appear it is widely distributed except in south-east and central of Ireland. It is abundant along the coasts and in the north of Ireland.



## CHAPTER 5

### Conclusion and Description of Taxa

#### 5.1 Conclusion

Cultivation experiments with the gametophyte plants of the Hypnum cupressiforme aggregate have shown that variation is partly genotypic and partly environmental. There are four distinct species within the aggregate, H. cupressiforme sensu stricto, H. jutlandicum, H. mammillatum and H. vaucheri. H. vaucheri is clearly distinct from other taxa in three main characters, wider pseudoparaphyllia, shorter angular cells and very distinct nerve. These results are in line with the conclusions of Ando (1976) who listed twelve gametophytic and sporophytic characters distinguishing H. vaucheri from H. cupressiforme. I was unable to examine the sporophyte of H. vaucheri and other characters showed a considerable degree of overlapping with H. cupressiforme aggregate. H. jutlandicum is also a discrete taxon both before and after culture. The branching system and seta length are discriminatory characters, it being densely pinnate branched and having very long setae (up to 4.5 cm). The nerve of H. mammillatum is much shorter than in other taxa and although cell size shows some overlap with the other taxa it can be used to distinguish it from H. cupressiforme which possesses longer cells. The mamillate lid and a strongly curved leaf are also discriminatory characters. H. mammillatum is a discrete taxon and best treated as a distinct species. Plants of var. filiforme may intergrade with H. mammillatum. Usually the lid of capsule and plant shape are distinctive, but intermediate plants occur. It seems that var. filiforme is closely related to H. mammillatum and best treated as a variety of it rather than of H. cupressiforme.

The remaining three taxa appeared to overlap with each other especially in culture. The subulate beak and erect capsule of var. resupinatum are distinctive, although the upward pointing leaves, usually regarded as a

discriminatory character, are not maintained in culture. It is best treated as a variety of H. cupressiforme. Var. lacunosum with a short seta and erect capsule appeared to be distinct. On the other hand it shows a considerable degree of overlapping in the gametophyte characters with the other taxa, and is best treated as a variety of H. cupressiforme.

This taxonomic treatment does not receive any support from cytological data. The aggregate is cytologically uniform. The only general information on moss variability in relation to breeding system is given by Gemmell (1950) who mentioned that dioecious species are the more widespread and that there are more varieties described from dioecious species than monoecious species. He suggests that this is related to greater variability from outbreeding. H. cupressiforme is dioecious and hence outbreeding. It would seem from the degree of variability and the taxonomic complexity within the H. cupressiforme complex that evolution is actively occurring.

## 5.2 Description of Taxa

### Hypnum cupressiforme Hedw.

Dioecious. Plants slender to robust, stems prostrate, irregularly pinnately to pinnately branched, branches prostrate to ascending; pseudoparaphyllia lanceolate-subulate. Leaves appressed and overlapping and straight to strongly curved and pointing in one direction, concave, narrowly lanceolate, in mid-stem 0.9 - 2.8 mm long, apex acuminate to filiform, margin plane or recurved below, sub-entire or denticulate towards apex; nerve very short and double or wanting; basal cells narrowly rhomboidal with rounded ends, incrassate, often porose, angular cells thick-walled to strongly incrassate, irregular-quadrate in shape, hyaline to yellowish, middle angular cells mostly 12 - 23  $\mu$  wide, cells in mid-leaf 30 - 102  $\mu$ m. Capsule erect or inclined, obloid to sub-cylindrical, straight or curved, 1.1 - 3.4 mm long; seta 1.2 - 4.5 cm; lid with rostellate to subulate beak, lid 1.2 - 2.2 mm, beak 0.2 - 0.5 mm; peristome teeth longitudinally striate below, papillose or smooth above, bordered or not.

### Var. cupressiforme

Plants slender to medium sized. Leaves weakly falcato-secund to falcato-secund, 1.2 - 2.5 mm long, ovate to lanceolate, gradually narrowed to long slender acumen, margin usually plane; angular cells strongly incrassate, irregularly quadrate, middle angular cells mostly 17 - 23  $\mu$ m, cells in mid-leaf 54 - 102  $\mu$ m long. Capsule inclined, obloid to sub-cylindrical, curved or straight, 1.5 - 2.8 mm long, lid rostellate to rostrate, 0.5 - 0.9 mm long; spores 13 - 20  $\mu$ m. Fruit occasional to frequent, autumn n = 10. Green or pale green patches on rocks, walls, bark, logs, soil, etc., in sheltered or exposed habitats



especially where acidic, very common.

Var. resupinatum (Tayl.) Schimp.

Plants slender. Leaves  $\pm$  straight, usually directed upwards especially at stem and branch tips, 0.6 - 1.4 mm long, margin entire; angular cells  $\pm$  quadrate, thick-walled, hyaline, mid-leaf cells 50 - 85  $\mu$ m long. Capsule erect, sub-cylindrical, straight or slightly curved 1.5 - 2.3 mm long; lid 0.8 - 1.3 mm long with subulate beak; spores 15 - 23  $\mu$ m. Fruit frequent, autumn. Pale green patches on bark, logs, rocks and walls in slightly shaded habitat, frequent.

Var. lacunosum Brid.

Plants robust. Leaves  $\pm$  straight and imbricate to falcato-secunds 1.5 - 3.0 mm long, concave, ovate to ovate-oblong to oblong-lanceolate and tapering to channelled apex, margin recurved below, plane or inflexed above, sub-entire to denticulate towards apex; angular cells quadrate to rounded-quadrate, incrassate, cells in mid-leaf 30 - 100  $\mu$ m long. Capsule  $\pm$  erect, sub-cylindrical, straight or slightly curved; lid rostrate, seta very short. Fruit rare, autumn, winter. Dull green to glossy, yellowish-green to golden brown patches, on usually basic soil, rocks, wall tops, etc., common.

H. mammillatum (Brid.) Loeske

Dioecious. Plants very slender to medium-sized or filiform, stems procumbent or creeping, irregularly pinnately branched, branches procumbent to ascending, spreading to parallel with stem. Leaves falcato-secund, ovate to lanceolate, gradually tapering to channelled acumen, margin plane or slightly recurved below, sub-entire to denticulate; nerve very short and double or wanting; basal cells narrowly rhomboidal with rounded ends, incrassate, porose or not, angular cells  $\pm$  irregularly quadrate to quadrate, strongly incrassate,



cells in mid-leaf 30 - 65  $\mu$ m. Capsule erect or inclined, oblong-ellipsoid to sub-cylindrical, straight or slightly curved, 1.4 - 2.3 mm long; lid mamillate 0.3 - 0.5 mm long; outer peristome teeth longitudinally striate or finely papillose above; spores are variable in size 12 - 23  $\mu$ m. Fruit apparently common in larger forms, autumn  $\underline{n}$  = 10. Yellowish-green to green patches on bark, fallen logs, rocks and wall tops, shaded conditions, rarely on soil. Common.

Var. mammillatum

Plants slender to medium sized, stems procumbent or creeping, irregularly pinnately branched, branches procumbent to ascending, spreading to parallel with stem. Leaves falcato-secund, ovate to lanceolate, gradually tapering to channelled acumen, margin plane or slightly recurved below, sub-entire to denticulate, nerve very short and double or wanting; basal cells with rounded ends, incrassate, porose or not, angular cells  $\pm$  irregularly quadrate to quadrate, strongly incrassate, cells in mid-leaf 3.0 - 6.5  $\mu$ m. Capsule erect or inclined, straight or slightly curved, 1.4 - 2.2 mm long; lid mamillate 0.3 - 0.5 mm long; spores variable in size, 12 - 23  $\mu$ m. Fruit common, autumn.  $\underline{n}$  = 10. Green patches on bark, rocks and wall tops, shaded conditions. Common in western and northern Britain but rare elsewhere except in humid habitats.

Var. filiforme

Plants extremely slender, procumbent, stems long, distantly pinnate, with long  $\pm$  straight  $\pm$  parallel, almost filiform branches, forming very low patches. Leaves small, regular, falcato-secund usually denticulate; nerve short; angular cells  $\pm$  irregular, incrassate, cells in mid-leaf 28 - 44  $\mu$ m. Capsules small, inclined, sometimes  $\pm$  erect,

spores small, 11 - 16  $\mu$ m. Fruit rare.  $n = 10$ . Yellowish-green patches on trunks of trees, rarely on vertical rock. Common in woodland in western and northern Britain, occasional elsewhere.

H. jutlandicum Holmen & Warncke

Dioecious. Plants medium-sized, stem greenish, procumbent to ascending, densely and regularly pinnately; pseudoparaphyllia lanceolate-subulate to triangular with subulate apex. Leaves not crowded, slightly overlapping, falcato-secund to ovate, tapering to acuminate to filiform apex, margin plane, sub-entire to denticulate towards apex; nerve very short; basal cells narrowly rhomboidal, incrassate, angular cells incrassate, rounded-quadrate, cells in mid-leaf 40 - 70  $\mu$ m. Capsule inclined, obloid to shortly cylindrical, curved, 1.2 - 1.5 mm long, outer peristome teeth vertically striate or smooth above; lid rostrate; spores 12 - 15  $\mu$ m. Fruit rare, winter, spring. Pale green tufts or patches on heaths, moorland and montane habitats. Common in suitable habitats.

- 1 Nerve very distinct, 90-180  $\mu$ m long, angular cells 6-7.5  $\mu$ m long,  
pseudoparaphyllia 150-190  $\mu$ m wide at base H. vaucheri  
Nerve lacking or distinct, ( 0) 40-80 (-110)  $\mu$ m long, angular cells 10-16.5  $\mu$ m wide,  
pseudoparaphyllia 50-120  $\mu$ m wide at base 2
- 2 Shoots closely or densely pinnately branched, setae (-20) -30 (-45) mm long,  
plants very pale green H. jutlandicum  
Branching irregular to irregularly pinnate, setae ( 8) -17 (-25) mm long,  
plants pale to dark green, sometimes tinged with yellow or bronze 3
- 3 Leaves directed upwards at apex, capsule + straight H. cupressiforme var. resupinatum  
Leaves directed downwards at apex, capsule curved 4
- 4 Plant robust, stem ( 0.5) 0.7-1.3 (-1.8) mm wide,  
usually yellow or bronze-tinged H. cupressiforme var. lacunosum  
Plant slender to medium sized, stem ( 0.1) 0.2-0.5 (-0.6) mm wide,  
not yellow or bronze-tinged 5

5 Lid rostellate to rostrate, leaf cells (45) 68 (-102)  $\mu$ m long H. cupressiforme var. cupressiforme

Lid mammillate, leaf cells 28-75  $\mu$ m long 6

6 Leaves ( 1.3) 1.6-1.9 (-2.5) mm long, angular cells very incrassate with walls 0.6-2.2  $\mu$ m thick, usually on  $\pm$  horizontal substrata

H. mammillatum var. mammillatum

Leaves ( 0.8) 1.1-1.3 (-1.7) mm long, angular cells with thin walls  
0.1-0.5  $\mu$ m thick, usually on vertical substrata

H. mammillatum var. filiforme



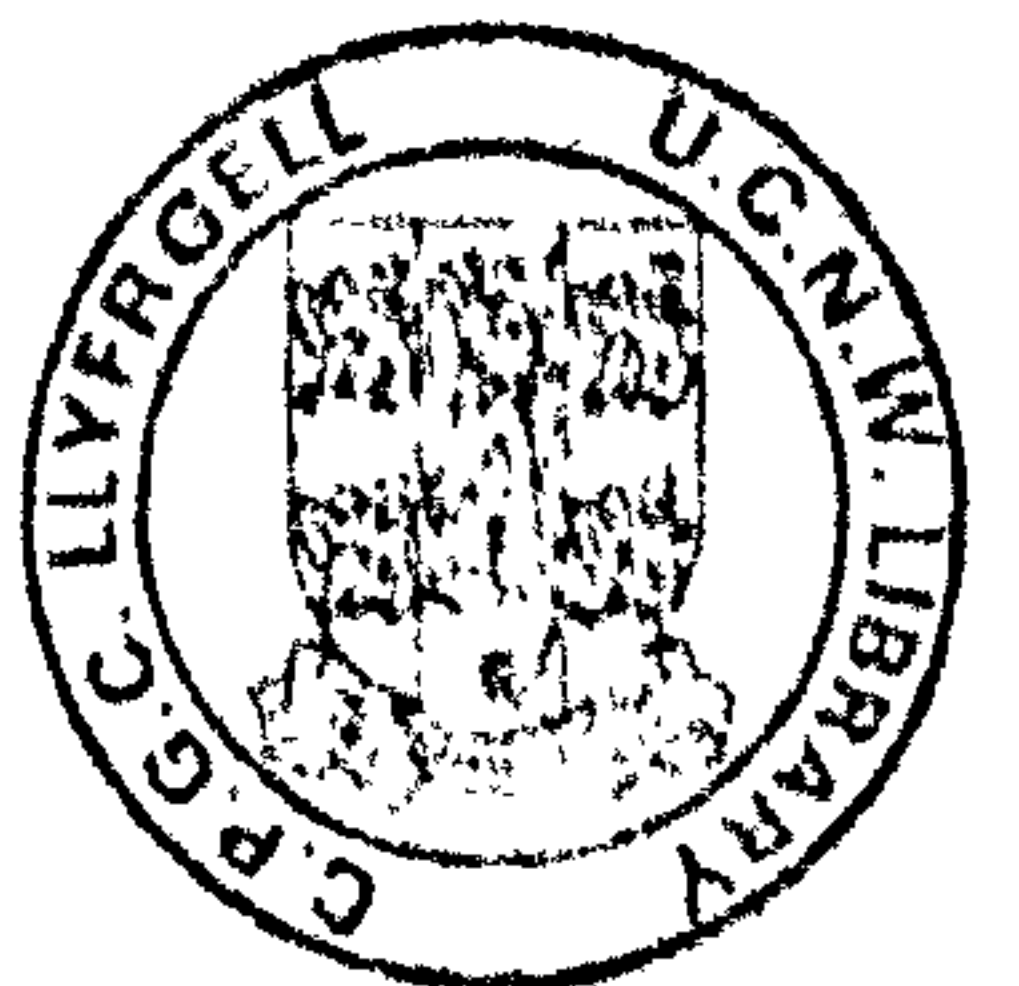
## CHAPTER 6

### Variation in *Atrichum undulatum*

#### 6.1 Introduction

The situation in *A. undulatum* where there are three cytotypes, one haploid, one diploid and the third triploid is of particular interest. They are, however, morphologically indistinguishable (Smith, 1978b). Cultivation experiments are needed to study the variation between and within the cytotypes and to ascertain whether or not they are morphologically indistinguishable. Similar work has been carried out by Newton (1968) in *Tortula muralis* with  $n = 26, 27$  and  $50, 52$ . No differences were found between the two haploid races or between the two diploids. Whilst some populations with  $n = 50, 52$  are distinct from haploids others were indistinguishable from the haploids. Although the natural chromosome races of Funariaceae are indistinguishable the artificial polyploids produced by Wettstein (1940) had larger cells than plants from which they were derived. Ramsay (1967) studied *Hypopterygium rotulatum* with  $n = 9, 18$  ca. 27, 36 found few differences between them. Smith and Newton (1968) in studies on *Funaria hygrometrica* (in which  $n = 28$  and  $56$ ), *Physcomitrium pyriforme* ( $n = 26$  and  $52$ ), *Pohlia nutans* ( $n = 22, 33$ ) and *A. undulatum* ( $n = 7, 14, 21$ ) could find no differences between cytotypes within these species.

As the three cytotypes in *A. undulatum* are apparently indistinguishable it is necessary to include as many characters as possible in this study to obtain satisfactory results.



## 6.2 Cytological Investigation

The Polytrichales is an order that is particularly well known cytologically. Some 240 species have been counted; of these 76% are haploid with  $\underline{n} = 7$ , 17% are diploid with  $\underline{n} = 14$  and 7% are of higher ploidy (Smith, 1978b). There are a few examples of chromosome numbers not based on  $\underline{x} = 7$ , but it is likely that they are erroneous and they should be discounted. There are a few examples in the genus Polytrichum of intraspecific polyploidy (e.g. Polytrichum alpinum var. brevifolium, P. commune, P. formosum, P. longisetum and P. piliferum. (Fritsch, 1972).

The biosystematic situation in A. undulatum is of a particular interest. Three cytotypes are known, one haploid, one diploid and the third triploid. Wilson (1911) reported a count of 16 - 17 for British material. Heitz (1926) reported the chromosome number of this species as "14 - 16" and in (1928) as "(20-)21(-22)". This is the earliest report of intraspecific polyploidy in mosses. Cytological variation appears to occur throughout the range of A. undulatum (see Table 7).

Haploid and diploid gametophytes have been reported from North America (Lowry, 1948, 1954; Steere, Anderson and Bryan, 1954; Bird, 1962; Khanna, 1967) and both occur in India (Chopra, 1959 (as A. subseriatum); Chopra and Bhandari, 1959). There have been reports of the three cytotypes from Japan (Tatuno, 1953, 1960; Kurita, 1938, 1950; Noguchi and Osada, 1960; Yano, 1957; Tatuno and Kise, 1970). There have been a number of reports of various cytotypes from different parts of Europe, the Ukraine (Lazarenko and Visotskya, 1964; Lazarenko, 1967; Visotskya, 1967; Fetisova and Visotskya, 1970; Lazarenko et al., 1971; Barzarina and Solonia, 1972; Lazarenko and Lesnyak, 1977), Austria (Bryan, 1973), Czechoslovakia (Wigh, 1972), Denmark (Holmen, 1958), Latvia (Visotskya and

Table 7. Showing the haploid, diploid and triploid counts reported in the literature for Atrichum undulatum.

<u>n</u>	Author	Source
14-16	Heitz (1926)	Europe
20	Heitz (1928)	Europe
16-17	Wilson (1911)	G. Britain
21	Lewis (1957)	G. Britain
14,21	Smith & Newton (1966)	G. Britain
7,21	Smith & Newton (1968)	G. Britain
21	Holmer (1958)	N. Europe
21	Bryan (1973)	Austria
21	Wigh & Strandhede (1971)	Swedish & Dutch
21	Wigh (1972)	Central & South Europe
14	Lowry (1948)	N. America
7,14	Lowry (1954)	N. America
7	Steere, Anderson & Bryan (1954)	N. America
7	Khanna (1967)	N. America
7	Bird (1962)	N. America
21	Kurita (1938)	Japan
7	Kurita (1950)	Japan
7,21	Tatuno (1953)	Japan
7,14,21	Tatuno and Kise (1970)	Japan
7	Yano (1957)	Japan
7,14,21	Noguchi & Osada (1960)	Japan
14	Tatuno (1960)	Japan

Table 7 continued:-

<u>n</u>	Author	Source	
14	Chopra & Bhandari	India	
7	Kumar & Anderson in Love (1968)	India	
21	Lazarenko & Visotskaya (1964)	Ukraine	U.S.S.R.
14,21	Lazarenko & Visotskaya (1965)	Ukraine	U.S.S.R.
21	Festisova & Visotskaya (1970)	Estonia	U.S.S.R.
21	Visotskaya (1967)	Ukraine	U.S.S.R.
14,21	Lazarenko (1967)		
21	Visotskaya (1970)	Caucasus	U.S.S.R.
7,14	Lazarenko & Lesnyak (1977)	Kazakhstan and Tadzhikistan	
7	Lazarenko (1967)		



Fetisova, 1969), Lithuania (Danilkiv and Visotskya, 1975) and Sweden (Wigh and Strandhede, 1971).

Counts reported from Britain are  $\underline{n} = 14$  (Smith and Newton, 1966, 1968),  $\underline{n} = 21$  (Lewis, 1957; Smith and Newton, 1966, 1968), and from Ireland  $\underline{n} = 7$  and 21 (Smith and Newton, 1968).

In a large scale study Lazarenko and Lesnyak (1977) examined 307 gatherings from European U.S.S.R., they found no haploids, 6 diploids with  $\underline{n} = 14$  and 301 were triploids with  $\underline{n} = 21$ . They concluded that in spite of morphological similarity in the chromosome races of A. undulatum they ought to be considered as cryptic sibling species. Smith and Newton (1968) also commented that they could find no morphological differences between different cytotypes.

Clearly A. undulatum has three chromosome races and it was studied to investigate the possibility of there being a correlation between chromosome number and geographical distribution and between chromosome number and morphology in the British Isles.

#### Materials and Methods

Methods used are similar to those for H. cupressiforme s.l. (Page 19). Material of A. undulatum was collected from different areas of Britain, allowed to grow in polythene bags in the laboratory at 10 - 22°C for at least two weeks. The new shoots that were produced were examined cytologically using aceto-orcein stain. About 0.5 cm of the shoot apex was fixed in acetic acid : absolute alcohol : chloroform (1:1:1) for 2-4 hours (Ramsay, 1969). Fixed material was stained in a saturated solution of aceto-orcein in 45% acetic acid for 12 hours at room temperature. After staining the apex was transferred to a drop of aceto-orcein on a slide. The shoot was macerated with a brass rod and a cover slip added and tapped with the point of a needle to spread the cells.

Pressure was applied to cover slips to spread the chromosomes.

### Results and Discussion

Chromosomes of gametophyte mitosis are illustrated in Fig. (44b, c, d). The karyotype is but described in terms of relation lengths (in  $\mu\text{m}$ ) as follows:

The haploid  $\underline{n} = 7$

Chromosome	1 (6.34 $\pm$ 0.11)
	2 (6.34 $\pm$ 0.10)
	3 (6.32 $\pm$ 0.90)
	4 (5.32 $\pm$ 0.12)
	5 (5.25 $\pm$ 0.11)
	6 (4.19 $\pm$ 0.10)
	7 (3.65 $\pm$ 0.13)

The diploid  $\underline{n} = 14$

Chromosome	1 (6.18 $\pm$ 0.60)
	2 (6.11 $\pm$ 0.22)
	3 (5.93 $\pm$ 0.80)
	4 (5.72 $\pm$ 0.06)
	5 (5.66 $\pm$ 0.15)
	6 (5.63 $\pm$ 0.11)
	7 (4.85 $\pm$ 0.16)
	8 (4.78 $\pm$ 0.18)
	9 (4.77 $\pm$ 0.11)
	10 (4.74 $\pm$ 0.05)
	11 (4.70 $\pm$ 0.15)
	12 (4.67 $\pm$ 0.14)
	13 (3.27 $\pm$ 0.08)
	14 (3.30 $\pm$ 0.12)

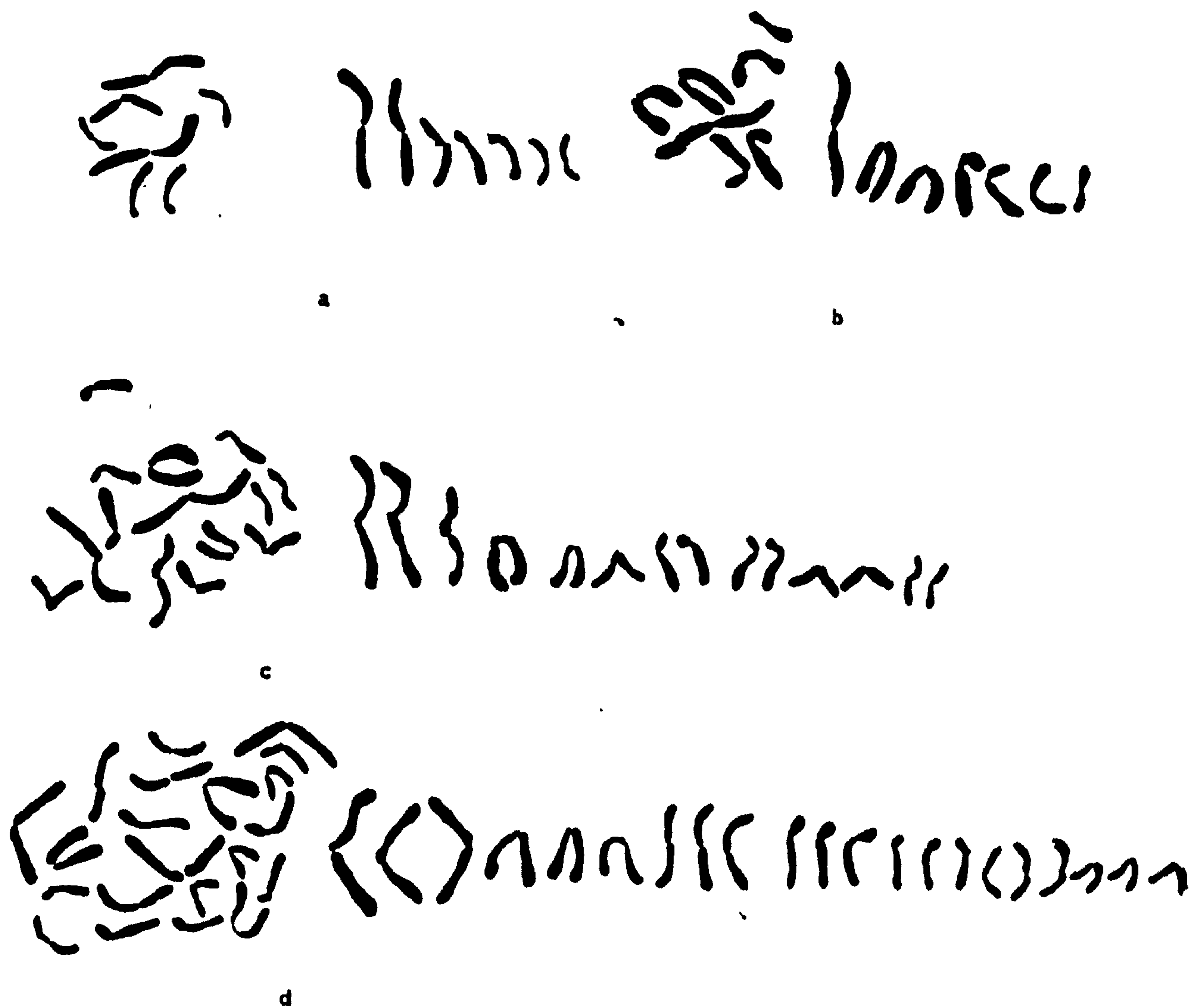


Fig. 44. Somatic chromosomes in the gamatophyte of Atrichum

(a) A. crispum ;

(b) haploid A. undulatum;

(c) diploid A. undulatum;

(d) triploid A. undulatum.

The triploid  $\underline{n} = 21$

Chromosome	1 (6.03 $\pm$ 0.11)
	2 (6.13 $\pm$ 0.15)
	3 (6.10 $\pm$ 0.10)
	4 (5.92 $\pm$ 0.05)
	5 (5.90 $\pm$ 0.10)
	6 (5.91 $\pm$ 0.13)
	7 (5.80 $\pm$ 0.12)
	8 (5.70 $\pm$ 0.21)
	9 (5.70 $\pm$ 0.15)
	10 (4.90 $\pm$ 0.04)
	11 (4.80 $\pm$ 0.14)
	12 (4.75 $\pm$ 0.12)
	13 (4.75 $\pm$ 0.15)
	14 (4.81 $\pm$ 0.13)
	15 (4.75 $\pm$ 0.12)
	16 (4.40 $\pm$ 0.09)
	17 (4.30 $\pm$ 0.05)
	18 (4.25 $\pm$ 0.12)
	19 (3.50 $\pm$ 0.14)
	20 (3.60 $\pm$ 0.06)
	21 (3.45 $\pm$ 0.18)

From Appendix 2 it would appear that the mean length of chromosomes of the haploid is significantly greater than that of the diploid and triploid which they do not differ significantly.

From the above data it would appear that the diploid has seven pairs of chromosome, each pair consisting of two chromosomes of almost equal length and similar centromere position. In the triploid the chromosomes appear to be in threes, each consisting of three chromosomes of almost



equal length and similar centromere position.

It seems to be that A. undulatum is a good example of an intraspecific polyploid series, with the following karyotype formulae

$$\underline{n} = 7: V + 2V + 2j + 2v$$

$$\underline{n} = 14: 2V + 4V + 4j + 4v$$

$$\underline{n} = 21: 3V + 6V + 6j + 6v$$

Smith (1978b) comments on using such formulae that the differences in shape and size implied by the formulae may be misleading as there is often a complete intergradation in size and position of centromeres between related species. Karyotype formulae may be used for rough comparisons. There is no significant variation in size and centromere position within cytotypes of populations of different origins (Fig. 44b, c, d).

Lowry (1954) suggested that reports of haploid and triploid races in A. undulatum (Figs. 45g, h, 46k, l) form the most extended natural intraspecific polyploid series known in mosses. Data that have been accumulated since indicate that A. undulatum is not exceptional. Naturally occurring intraspecific poliploidy with three or more cytotypes have been found in Amblystegium riparium ( $\underline{n} = 12, 24, 36$ ), Funaria hygrometrica ( $\underline{n} = 9, 18, 27, 36$ ) and Pohlia nutans ( $\underline{n} = 11, 22, 33$ ) and in the liverwort Dumortiera hirsuta ( $\underline{n} = 9, 18, 27$ ) (Smith, 1978b).

In British material of A. undulatum examined by Newton (1968) the meiotic bivalents are very large relative to SMCs., those of the haploid form being largest, and those of the triploid form smallest (see Fig. 45b, c, d).

Bryan (1973) reported a count of  $\underline{n} = 21$  from Austrian material where meiosis proceeds normally. The meiotic karyotype is similar to that of triploid British material (see Fig. 45e).

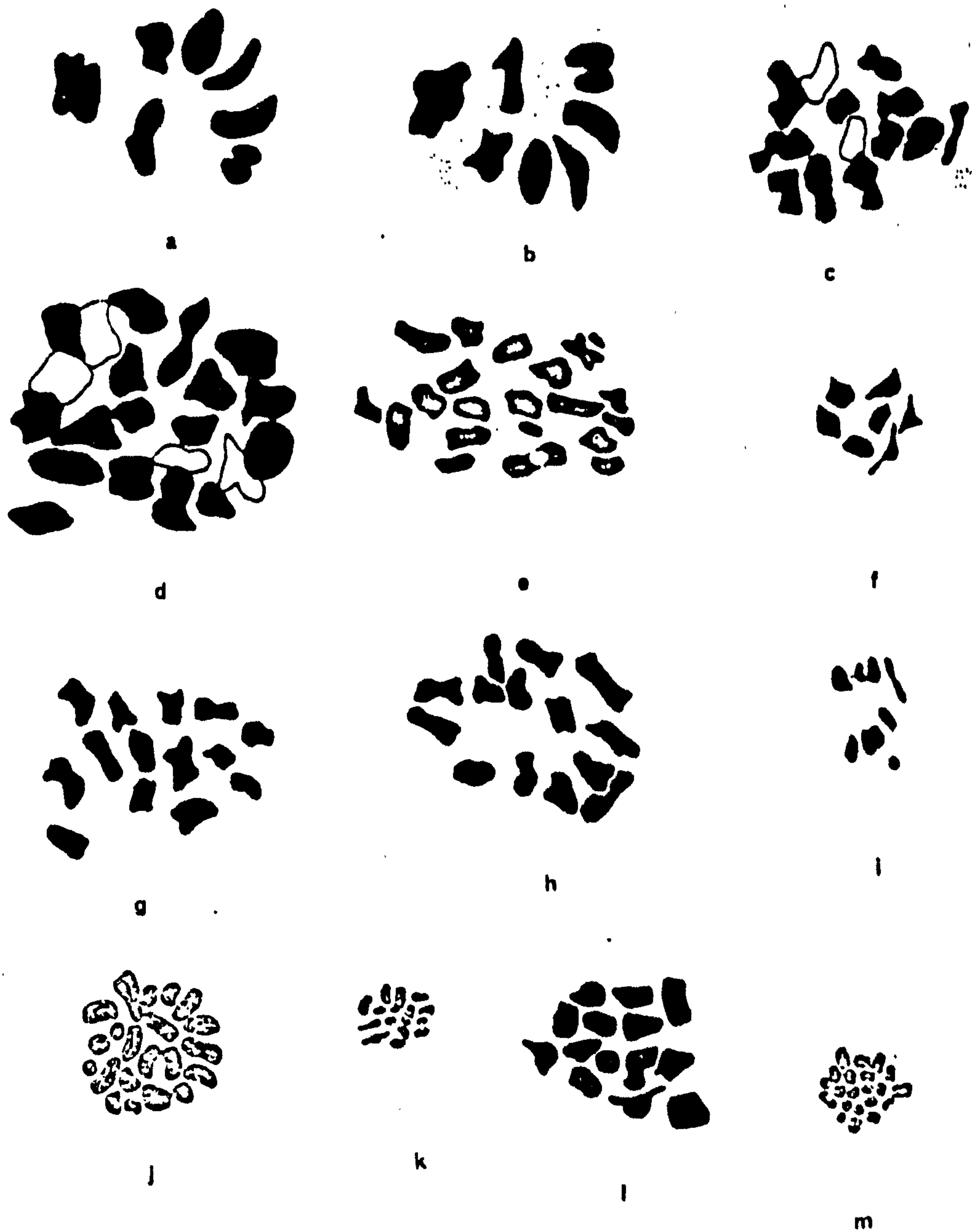


Fig. 45. Illustrations of meiotic chromosomes of A. undulatum by:

- |  |                                      |
|--|--------------------------------------|
| (a) Smith & Newton (1968);                     | (b-d) Newton (1968);                 |
| (e) Bryan (1972) ;                             | (f) Khanna (1967) ;                  |
| (g,h) Lowry (1954);                            | (i) Steere, Anderson & Bryan (1954); |
| (j) Lazarenko & Visotska (1964) ;              | (k) Lazarenko & Vysotskaya (1965);   |
| (l) Lazarenko, Visotskaya<br>& Lesnyak (1971); | (m) Visotska (1970).                 |

Russian material with  $\underline{n} = 7, 14$  and  $21$  has been reported. The diploid and triploid are relatively smaller than those produced from the British material. On the other hand the presence of the largest bivalent agrees with that of British material (see Figs. 45j-m). The haploid count  $\underline{n} = 7$  has been reported by Lazarenko (1967) and Visotstkya (1965).

In North America  $\underline{n} = 7$  and  $14$  have been reported. Lowry (1954) reported  $\underline{n} = 14$  and  $21$ . Meiotic bivalents in American material (see Figs. 45g, h) of diploid plants are similar to those in British plants. Steere, Anderson and Bryan (1954) reported  $\underline{n} = 7$  (Fig. 45i) and Khann (1967) also reported  $\underline{n} = 7$  from North America (Fig. 45f).

The mitotic karyotype has been studied by many authorities. Wigh and Strandhede (1971) reported  $\underline{n} = 7$  and  $21$  from Danish and Swedish material (Fig. 46i). He says "it is hardly possible to distinguish different size classes among the chromosomes though the longest one is about twice as long as the shortest chromosome". He also reported (1972)  $\underline{n} = 21$  from central and southern Europe (Fig. 46j). There is a difference in size range from British material (see Fig. 44b-d).

Kurita (1938) studied the gametophyte chromosomes of A. undulatum from Japan, he reported  $\underline{n} = 21$  (Fig. 46a). Tatuno (1960) reported  $\underline{n} = 7, 14$  and  $21$  (Fig. 46b, c, d). It would appear that there are some similarities with and differences from triploid British material (see Fig. 44d). Tatuno and Kise (1970) reported  $\underline{n} = 7, 14$  and  $21$  (Figs. 46e-h) according to the formulae:

$$n = 7: V(H, X \text{ or } Y) + 3V + 2J + m(h)$$

$$n = 14: V(H, X \text{ or } Y) + V(H, Y) + 6J + 4J + 2m(h)$$

$$n = 21: 2V(H, X) + V(H, Y) + 9V + 6J + 3m(h)$$



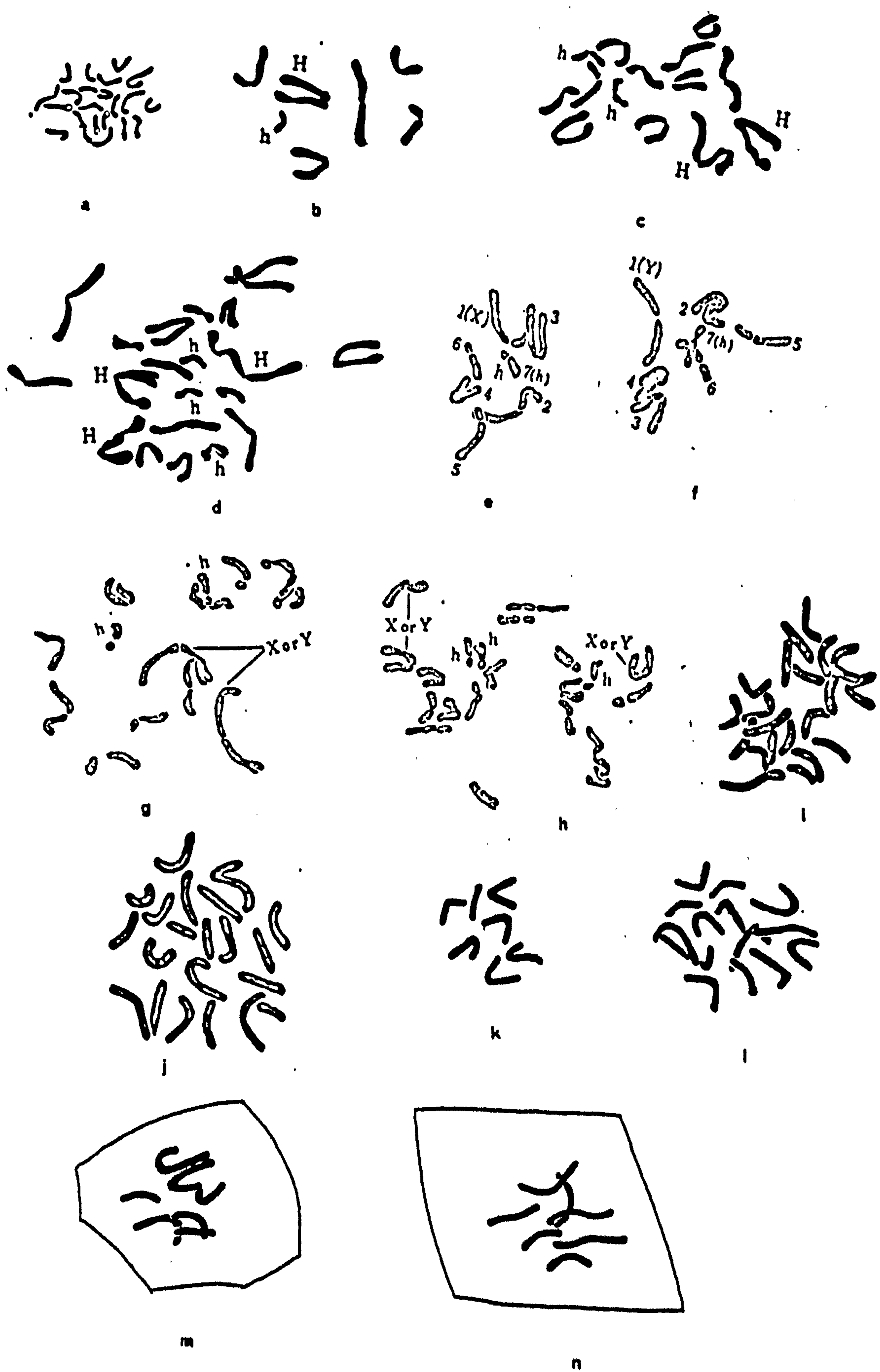


Fig. 46. Illustrations of mitotic chromosomes of Atrichum.

A. undulatum (a-l) and A. crispum (m,n) by:

(a) Kurita (1938);

(b-d) Tatuno (1960)

(e-h) Tatuno & Kise (1970);

(i) Wigh & Strandhede (1971)

(j) Wigh (1972);

(k-n) Lowry (1954)



The labelling of the largest and smallest chromosomes as heterochromatic should be discounted. Tatuno (1941) commenced the procedure of labelling these chromosomes as "H" and "h" and this has been dogmatically followed by Japanese authors ever since. This approach is highly unsatisfactory and may obscure the real situation (Smith, 1978b). Newton (in Smith, 1978b) says "in so far as quantities of heterochromatic are concerned the largest chromosome in Pellia epiphylla and P. neesiana undoubtedly equate with the H-chromosome of Tatuno (1941) but the smallest chromosome of the latter species consists of only about 26% heterochromatin compared with 35% in chromosome 5. In P. endiviifolia the largest and smallest chromosomes contain about 13% and 18% of their length as heterochromatin, only slightly more than in other chromosomes".

Further, the assumption that particular chromosomes are sex chromosomes is based on purely circumstantial evidence and in the absence of proof such chromosomes are best referred to as sex-associated chromosomes (Smith, 1978b). A more realistic representation of the karyotype formulae of Tatuno and Kaise (1970) would be:

$$n = 7 : v + 3v + 2J + j$$

$$n = 14 : 2V + 6V + 4J + 2j$$

$$n = 21 : 3V + 9V + 6J + 3j$$

Although it is likely that haploid plants are dioecious (R.E. Longton, pers. comm.), the diploid and triploid are likely to be monoecious and it is nonsense to designate any chromosomes as sex-associated in monoecious diploid or triploid plants. But, assuming that there really are distinguishable morphological differences, then this is of relevance as will be mentioned later.

A comparison of the karyotype formulae of Tatuno and Kise (1970) with the British material show that there are some similarities and some

differences. These may be summarized as follows:

British  $\underline{n} = 7: V + 2V + 2j + 2v$

Japanese  $\underline{n} = 7: V + 3V + 2J + j$

British  $\underline{n} = 14: 2V + 4V + 4j + 4v$

Japanese  $\underline{n} = 14: V + V + 6V + 4J + 2j$

British  $\underline{n} = 21: 3V + 6V + 6j + 6v$

Japanese  $\underline{n} = 21: 2V + V + 9V + 6J + 3j$

Lowry (1954) suggests that diploid A. undulatum arose from haploid material by apospory and that the triploid arose as the result of hybridity between the haploid and the diploid by apospory. That the diploid arose from the haploid and the triploid as the result of hybridity between the two is acceptable, but that apospory is involved is not. It seems more likely that chromosomes doubling results from diplospory (Smith, 1978b), in the first instance a diploid spore forming by diplospory in a diploid capsule. The triploid is likely to have arisen as the result of the failure of meiosis in a hybrid between a haploid and diploid. Diploid spores have been reported several times from naturally occurring plants. It therefore seems probable that doubling of the chromosome number results from diplospory rather than apospory.

Mehra and Khanna(1961) quote the work of Lowry on A. undulatum as an example of allopolyploidy. Smith (1978b) comments that this is a misinterpretation of the situation. This is a theoretical allopolyploidy but as the three genomes are similar the triploid plants are functionally autopolyploid .

The observations of Tatuno and Kise (1970) provide circumstantial evidence supporting the suggested mode of origin assuming that their "X" and "Y" chromosomes really are distinguishable. The diploid with "X" and "Y" would be derived by diplospory in a sporophyte resulting from the fusion of gametes from haploid male and female plants. The triploid, in this instance quoted would arise as the result of diplospory in a sporophyte resulting from the fusion of an "X+Y" diploid male gamete and an "X" haploid female gamete. This does, however, illustrate the absurdity of labelling chromosomes "X" and "Y" in polyploids since they clearly have no sex-determining function.

It has been suggested by Rose (1951b) and Nyholm (1971) that A. undulatum var. minus in a hybrid between A. undulatum and A. angustatum . In Britain A. undulatum var. minus often occurs outside the range of the former and it seems more likely that var. minus is a hybrid between different cytotypes of A. undulatum (Smith, 1978b).

It would appear from Fig. (44a) that A. crispum has seven chromosomes. The three largest are metacentrics, two being of almost equal size, then two subterminal and the remaining two metacentrics of almost equal size.

$$\underline{n} = 7 = 2V + V + 2j + 2v$$

These somatic chromosomes of the British material of A. crispum (Fig. 44a) appear not to differ from the north American material (Lowry, 1954) (see Fig. 46m, n).



## CHAPTER 7

### Morphological Variation of Cytotypes

#### 7.1 Materials and Methods

Several approaches were taken in the study of A. undulatum.

These were:-

1. Study of field and herbarium material
2. Cultivation experiments
3. Nuclear DNA content
4. The size of heterochromatin bodies
5. Isozymes
6. Distribution of A. undulatum cytotypes.

Methods used are similar to those described for H. cupressiforme s.l. on page ( 8 ). Each gathering was divided into two parts, one was cultured, the other preserved as an herbarium specimen. Living material was cultured at Pen-y-Ffridd Field Station, Bangor in an unheated glasshouse in a mist unit on peat-based potting compost in 25 cm flower pots. The pots were covered with polythene bags to prevent cross-contamination. Plants were unshaded and watered with tap water (pH 7.5). As all cultures were grown in a limited area (300 cm x 150 cm) at the same time, it could be assumed that growth was under uniform conditions. Three months growth was found to give satisfactory results for the purposes of this study. Samples from living material were taken, before and after culture under uniform conditions and preserved for later examination.

Moving means of five shoots, with at least twenty five samples, were found to give satisfactory results. Samples were dissected and the parts mounted in gum chloral.



## 7.2 Characters Selected for the Study of A. undulatum Cytotypes

### 1. Plant Morphology

a. Plant height: There is a considerable degree of over-lapping between the three cytotypes (see Table 8). The amount of variation in plant height under uniform conditions was lower than that in the wild, suggesting both genetic and environmental variation.

### 2. Leaf Morphology and Anatomy

#### a. Upper part of stem

(i) Leaf Length: The amount of variation in culture was lower than that in wild in both haploid and triploid. In the diploid there was a slight increase (see Table 8). There was also a considerable degree of overlap among the cytotypes. As with height, the haploid, diploid and triploid cytotypes seem to be genetically variable.

(ii) Leaf Width: There was a remarkable degree of overlapping among the cytotypes. The amount of variation decreased with culture in all cytotypes (see Table 8), suggesting that leaf width is affected by environmental factors.

(iii) Cell Width: Like other characters, cell width also showed a considerable degree of overlapping. There was a slight increase in the amount of variation in culture in the triploid. A decrease in haploids and diploids suggest a relatively low environmental and genetical variability.

#### b. Middle part of stem

(i) Leaf Length: There is a marked degree of overlapping between the cytotypes. There was a relatively slight decrease in the amount of variation in all plants (see Table 8) after culture.

(ii) Leaf Width: The cytotypes show considerable overlap. The amount of variation was almost the same before and after culture in the triploid

plants; there was a slight increase in the haploid and a decrease in the diploid after culture (see Table 8 ).

c. Acumen shape measured in degrees

This character showed a considerable degree of overlapping between the cytotypes. There was a decrease in the amount of variation in the triploid and an increase in the diploids in culture; this amount was almost the same in haploids.

d. Percentage of serration in the leaf

This character showed a considerable degree of variation between the cytotypes. The amount of variation was almost the same under both culture in haploid, with a decrease in both diploid and triploid after culture (see Table 8).

e. The angle between the tooth and leaf margin

There was a considerable degree of overlapping between the cytotypes. There was a marked increase in the amount of variation in culture in the triploid. An increase in the haploid and almost the same under both conditions in the diploid. This suggests that there is genetic variability in this character in the triploid.

f. Tooth length

There was considerable overlapping between the cytotypes. The amount of variation was almost the same in the haploid before and after culture; there was an increase in both diploid and triploid after culture (see Table 8).

g. Number of lamellae

The number of lamellae is 2-4, rarely 5 or 6. This character showed some degree of overlapping between the cytotypes. The amount of variation before and after culture in diploid plants was almost the same (see Table 8).

On the other hand there was a decrease after culture in the diploid and the haploid.

h. Lamellae height

The lamellae are 2-4, rarely 5, 6, 7 cells height. This character showed some degree of overlap between the cytotypes. There was a decrease in the amount of variation after culture in the cytotypes.

i. Angular cell wall thickness

Three categories were scored: thin, medium and thick. The cytotypes overlapped and were indistinguishable. Most plants had thin walls and retained this after culture, suggesting some degree of genetical stability.

j. Leaf margin

Three categories were scored: not detectable, distinct and very distinct. Most plants had distinct margins, almost all of these retained it after culture, suggesting some degree of genetical stability.

k. Nerve strength at apex

Three categories were scored: vanishing, sometimes detectable and distinct. All plants except a few had a vanishing nerve and retained this after culture.

l. Leaf size near the stem base

Three categories were scored: smaller than these above, equal and larger. All plants except some had smaller leaves at the stem base and 90% of these retained this character after culture.

m. Number of teeth

Three categories were scored: teeth single, teeth sometimes in pairs and teeth in pairs. Most plants have teeth in pairs, a few were in the second category.



### 3. Sporophyte

As sporophytes did not develop in culture, the following characters were studied from wild and herbarium material:

#### a. Seta Length

This was a variable character. The length of the seta is 1.4 - 3.1 cm and sometimes reaches 4.2 cm. All plants show an overlap and they were indistinguishable. Discrimination between the cytotypes using this character is not possible.

#### b. Seta Posture

Three categories were scored: inclined, intermediate and erect. All plants except 5% of the gatherings had erect setae, the 5% had intermediate. All plants overlap and are indistinguishable.

#### c. Capsule Length

The capsule length ranges from 3.4 - 6.2 mm. All plants overlap and were indistinguishable. Discrimination between the cytotypes using this character is not possible.

#### d. Number of peristome teeth

The number of teeth varies from 30 - 34. Most plants have 31 or 32 teeth. The number of peristome teeth of no taxonomic value.

#### e. Operculum Length

The length of the operculum in the cytotypes varied from 1.4 - 3.1 mm. The cytotypes overlap and are indistinguishable.

#### f. Beak Length

Three categories were scored smaller than the capsule, almost equal to the capsule length and larger than the capsule. All plants except a few had a beak of almost equal length to the capsule and were indistinguishable.



Table 8. Showing the Mean and Variability in Cytotypes of A. undulatum  
Before and After Culture.

CYTOTYPE	BEFORE CULTURE				AFTER CULTURE			
	$\bar{X}$	SD	SE	CV	$\bar{X}$	SD	SE	CV
n = 21								
1 Plant height	4.11	1.16	0.212	26.95	4.346	0.75	0.138	18.26
2 Leaf length	7.37	0.62	0.134	9.98	7.62	0.54	0.099	7.15
3 Leaf width	1.72	0.29	0.057	18.04	2.06	0.28	0.052	13.94
4 Cell width	22.90	4.06	0.589	14.11	25.03	4.34	0.79	17.35
5 Leaf length	6.35	0.56	0.113	9.74	6.76	0.49	0.090	7.35
6 Leaf Width	1.36	0.21	0.034	13.92	1.616	0.21	0.040	13.59
7 % of Serrate	79.00	11.66	1.91	13.23	76.33	9.73	1.77	12.75
8 Acumen angle	21.97	6.31	1.16	28.80	31.03	15.11	1.38	24.35
9 Teeth angle	46.83	11.24	2.50	29.20	36.76	1.55	2.90	43.29
10 Tooth length	6.613	1.42	0.268	22.211	6.07	1.31	0.283	25.53
11 Lamella in no.	3.40	1.08	0.217	35.05	3.70	1.21	0.24	35.60
12 Lamella cell in height	3.93	1.09	0.23	31.97	4.1	0.44	0.221	29.60
n = 14								
1 Plant height	4.84	1.45	0.172	17.75	4.914	0.67	1.034	13.40
2 Leaf length	7.20	0.76	0.168	11.68	7.87	0.43	2.57	12.66
3 Leaf width	1.69	0.36	0.065	19.36	1.668	0.28	0.045	13.70
4 Cell width	23.24	4.27	0.73	15.77	23.20	2.18	0.420	9.05
5 Leaf length	6.25	0.50	0.134	10.74	6.96	0.55	0.059	4.27
6 Leaf width	1.39	0.235	0.046	16.07	1.80	0.23	0.0346	9.62
7 % of Serrate	78.00	11.72	2.516	16.13	79.24	8.64	2.315	14.60
8 Acumen angle	21.12	7.98	1.165	27.58	27.60	8.93	1.917	34.73
9 Teeth angle	49.40	12.02	2.57	26.022	49.50	14.13	2.45	24.84
10 Tooth length	6.81	1.26	0.28	20.528	6.44	1.27	0.32	25.23
11 Lamella in no.	3.32	1.304	0.250	37.620	3.12	0.75	0.166	26.68
12 Lamella cell in height	3.92	1.34	0.215	27.475	4.16	0.81	0.179	21.59
n = 7								
1 Plant height	4.17	0.88	0.230	26.30	4.38	5.17	0.13	16.10
2 Leaf length	7.316	0.66	0.164	11.21	7.512	12.89	0.086	5.73
3 Leaf width	1.808	0.23	0.068	18.81	1.70	0.22	0.057	16.89
4 Cell width	22.48	3.09	0.840	18.70	25.52	2.10	0.436	8.55
5 Leaf length	6.27	0.55	0.118	9.47	6.668	0.29	0.110	8.26
6 Leaf width	1.40	0.16	0.038	13.55	1.56	0.17	0.0475	15.21
7 % of Serrate	7.68	12.13	2.49	16.26	83.20	11.57	1.729	16.40
8 Acumen angle	21.72	4.54	1.46	33.79	26.20	9.58	1.786	34.10
9 Teeth angle	46.40	8.58	2.37	25.56	46.80	12.27	2.82	30.12
10 Tooth length	6.728	1.20	0.278	20.721	6.20	1.62	0.25	20.54
11 Lamella in no.	3.28	1.19	0.227	34.67	3.36	0.83	0.15	22.53
12 Lamella cell in height	3.80	1.20	0.223	29.422	2.92	0.89	0.162	27.82

Character 1 in mm; for 2, 3, 5 & 6, 1 unit = 0.43 mm; 4, in um; for 10, 1 unit = 10 um.

g. Calyptra

Three categories were scored: calyptra without teeth, sometimes toothed and toothed. All plants except few had toothed calyptras, a few had the second category. This character of no systematic value.

h. Spores

This is a variable character with size ranging from 12 - 30 and the cytotypes were indistinguishable. This character is of no systematic value.

As no single character by itself is discriminatory, it was clearly necessary to see if all the characters taken in combination would separate these cytotypes from one another.

7.3 Variability Within Cytotypes of A. undulatum

1. Plant Height - Increased in culture in haploids, diploids and triploids (Figs. 47a - 49a). The variation in height in culture decreased in the three cytotypes (Table 8).
2. Leaf Length (in upper part of shoot): Leaf length was almost the same in both conditions accompanied by a decrease in leaf length variation in haploids. There was a slight increase in length, but the variation in length in culture is almost the same in diploids (Figs. 47 - 49b). Triploids increased their leaf length in culture and the variation decreased slightly (see Table 8).
3. Leaf Width (in upper part of shoot): In haploids there was a slight decrease in width, with a slight decrease in variation in culture. Diploids showed a slight decrease in width and also a slight decrease in variation in culture. There was a slight increase in width and a decrease in variation in triploids after culture (see Table 8, Figs. 47-49c).

Fig. 47. Histograms illustrating variation of haploid A. undulatum

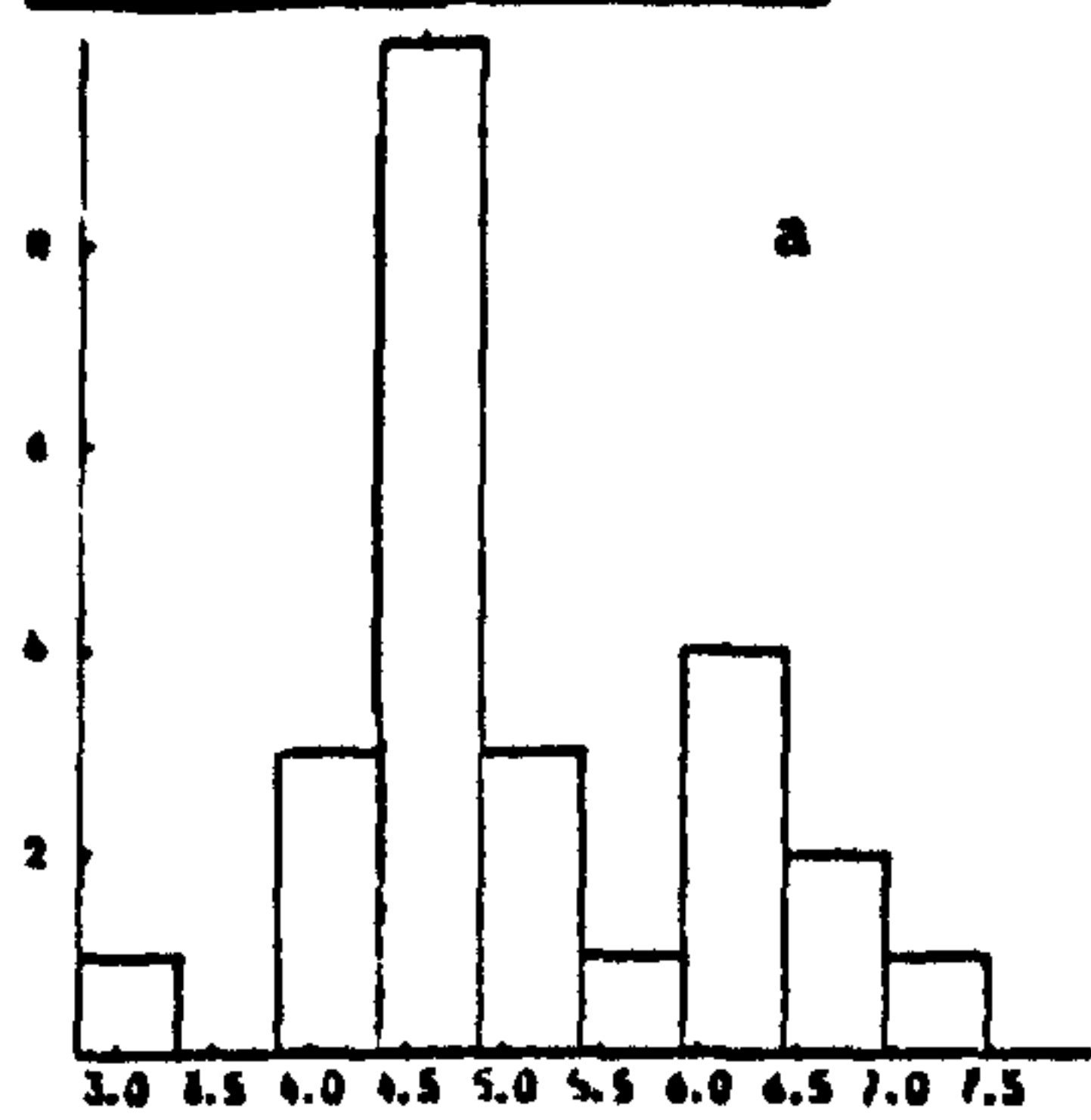
- |   |  |
|---|--|
| (a) plant height;                         | (b) leaf length (from upper part of stem); |
| (c) leaf width (from upper part of stem); | (d) cell width (from upper part of stem);  |
| (e) leaf length (from mid-stem);          | (f) leaf width (from mid-stem);            |
| (g) percentage of serration;              | (h) the angle of the acumen;               |
| (i) tooth angle;                          | (j) tooth long;                            |
| (k) number of lamellar cells;             | (l) height of lamellar cells.              |

Note that the Y-axis is not identical in all cases.

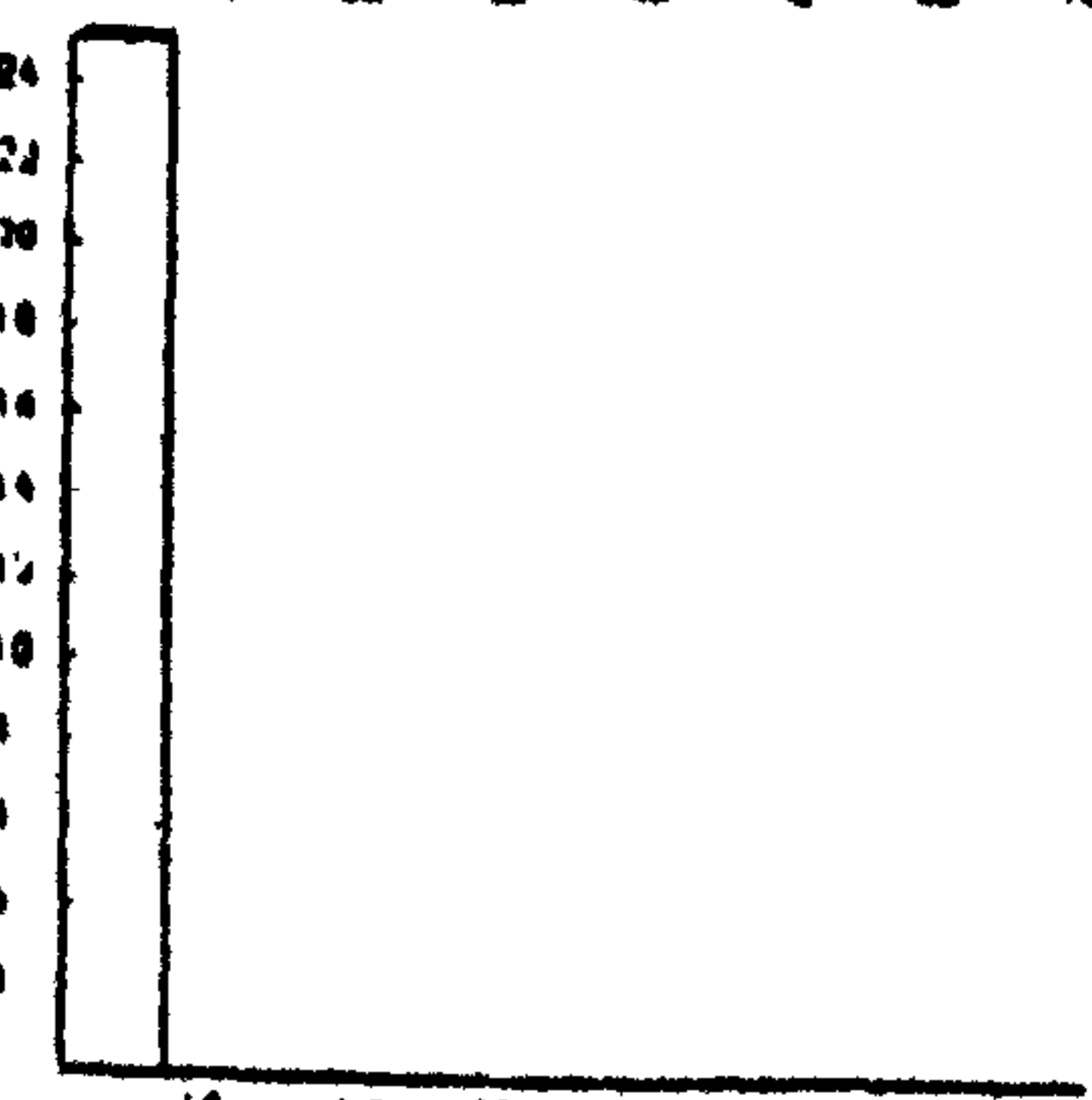
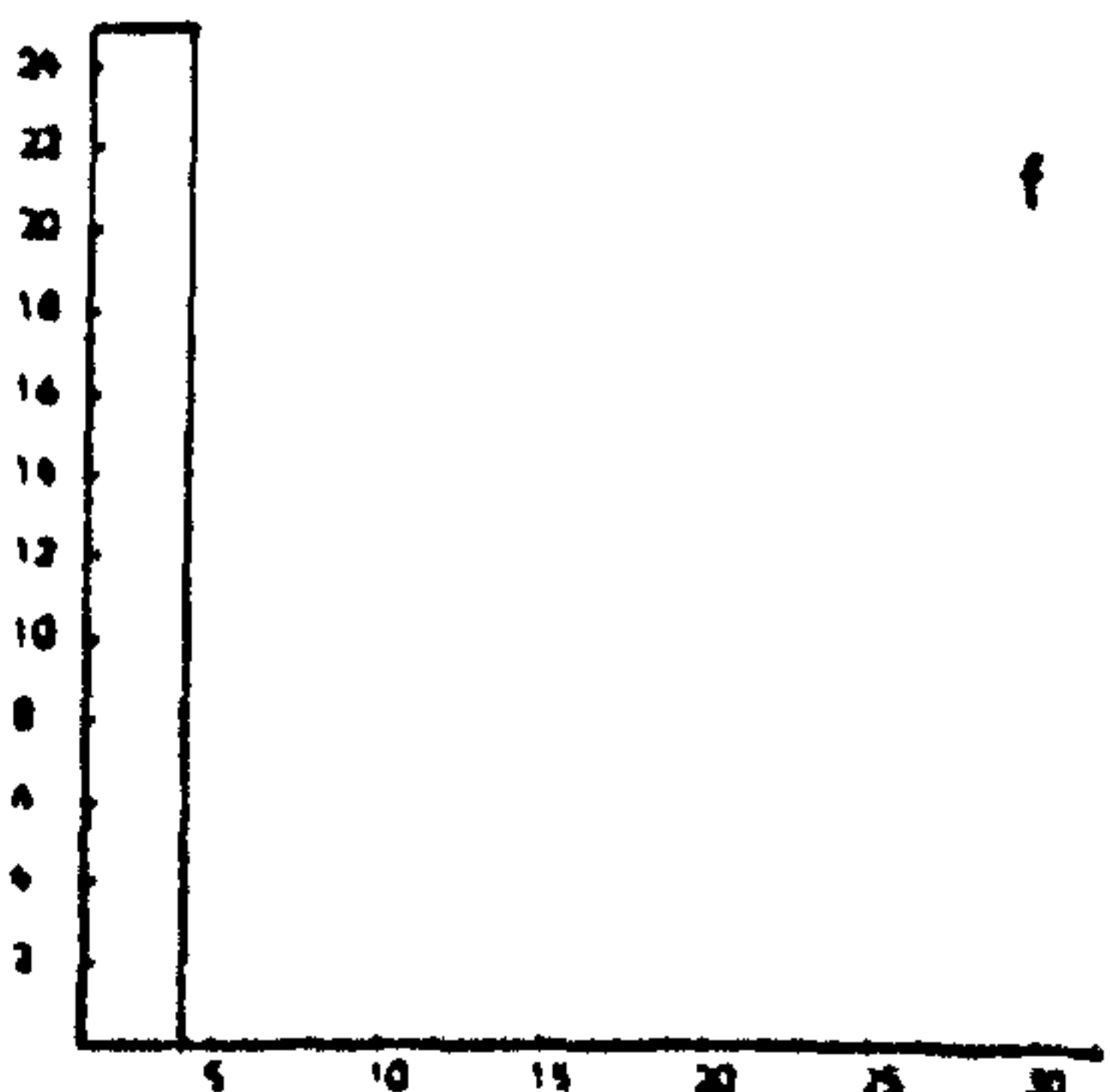
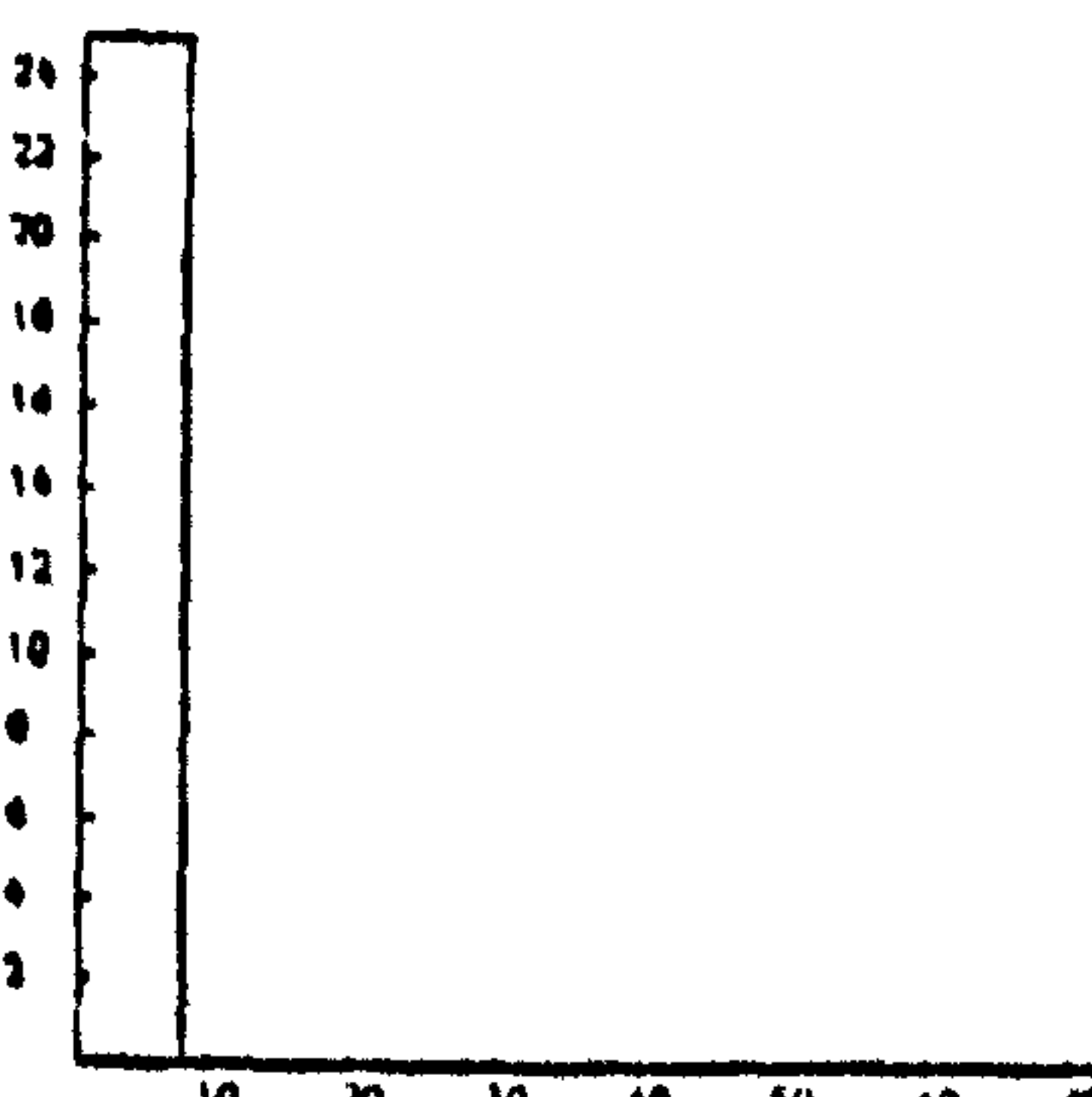
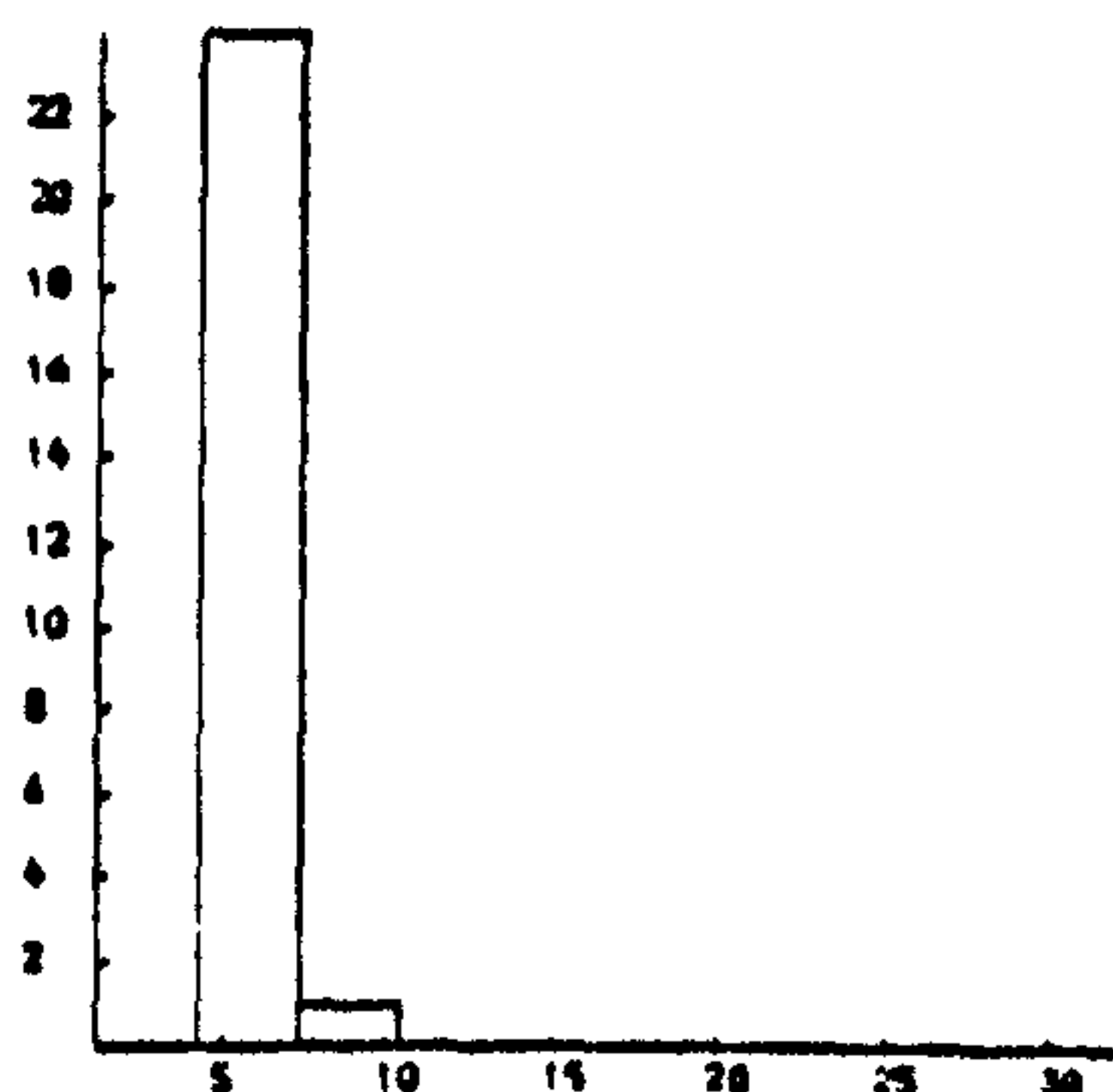
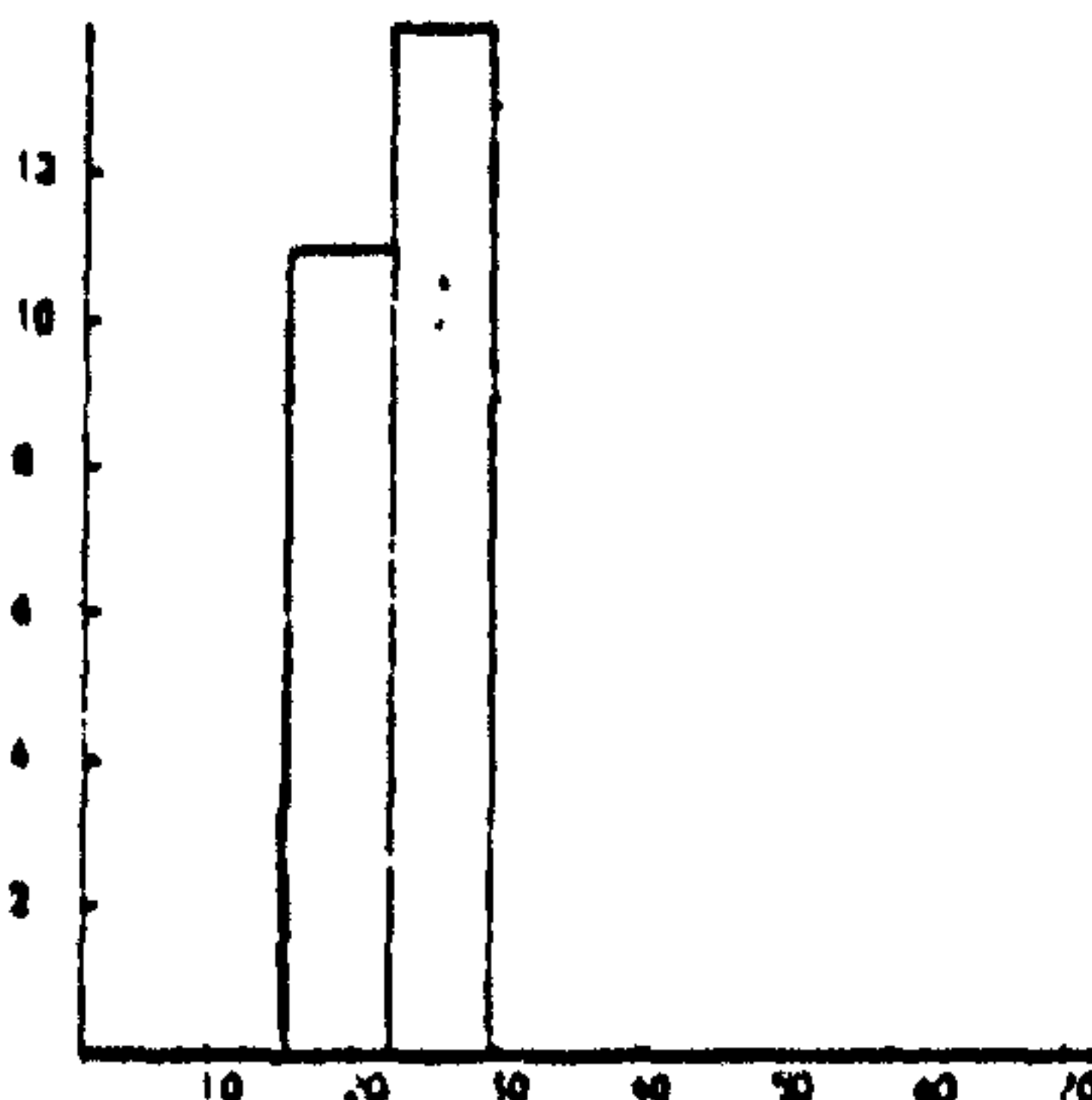
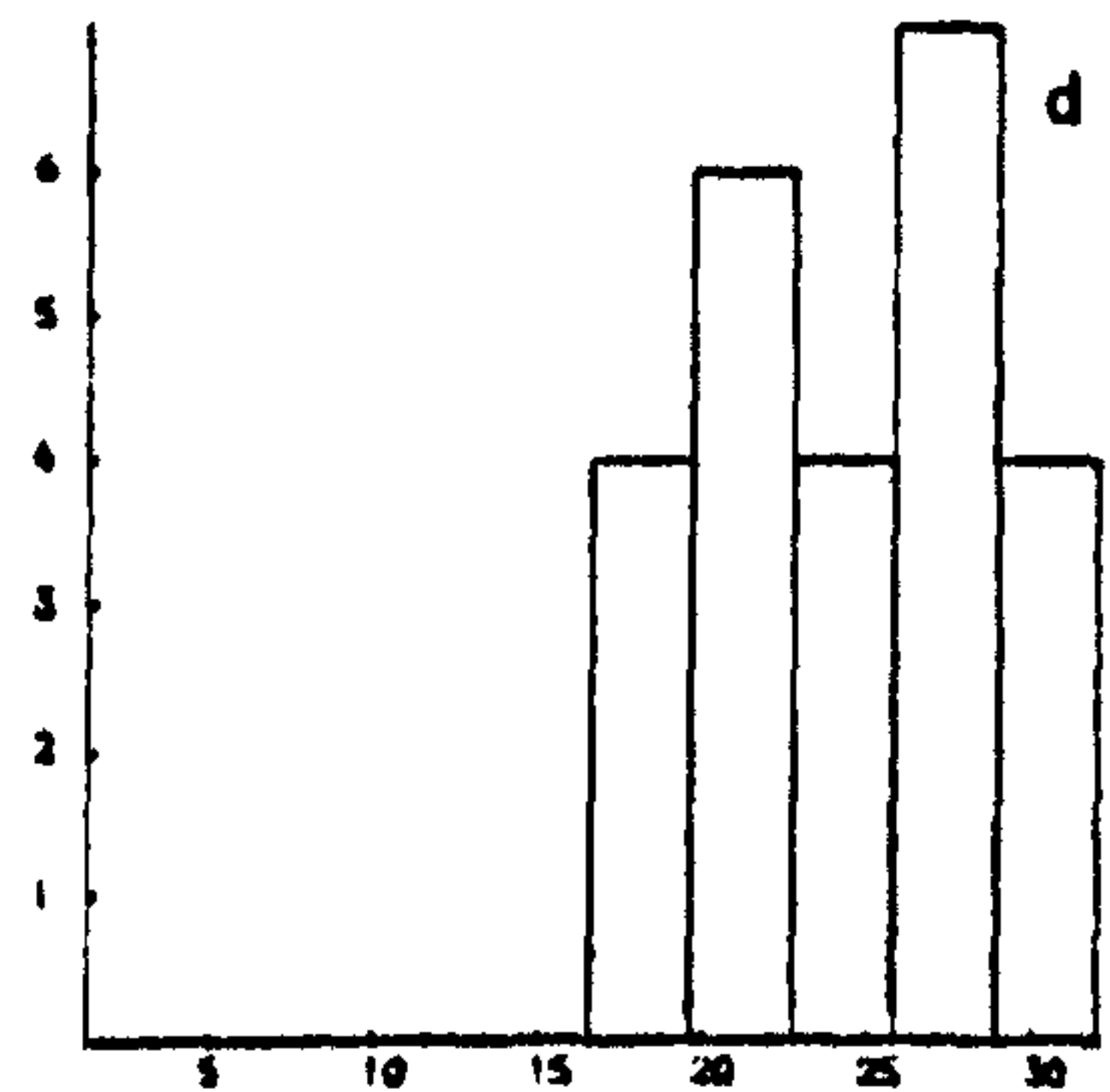
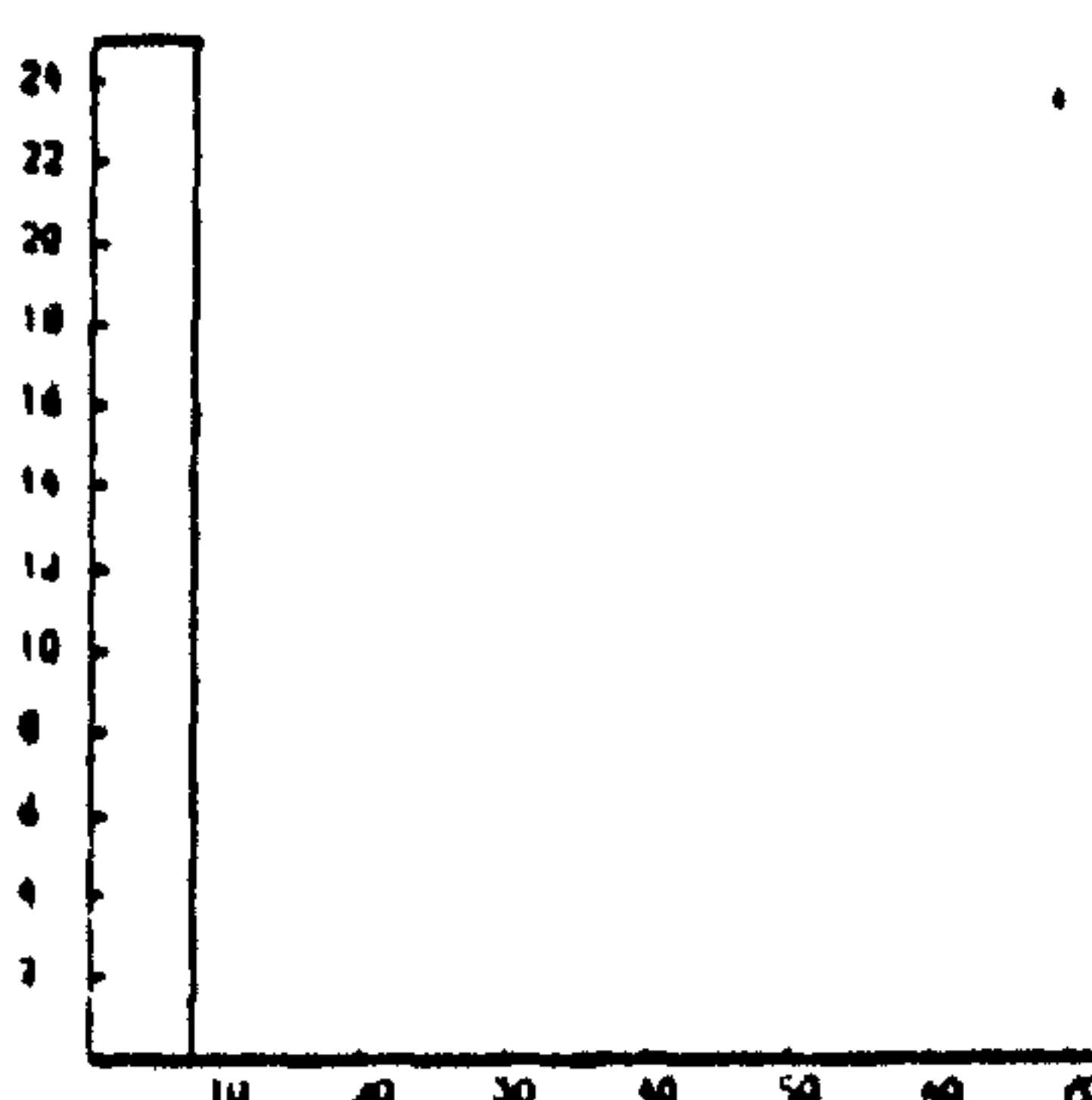
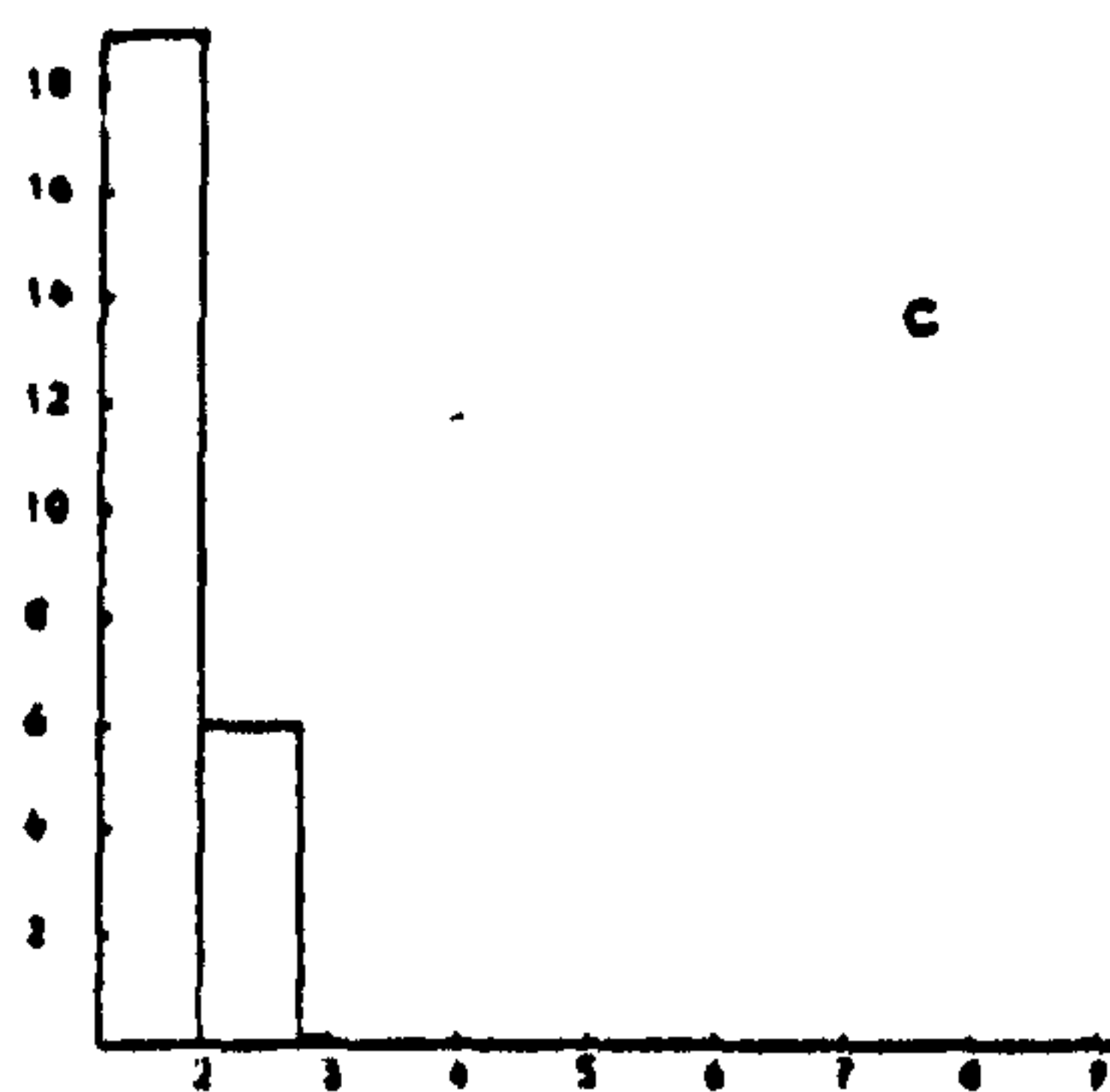
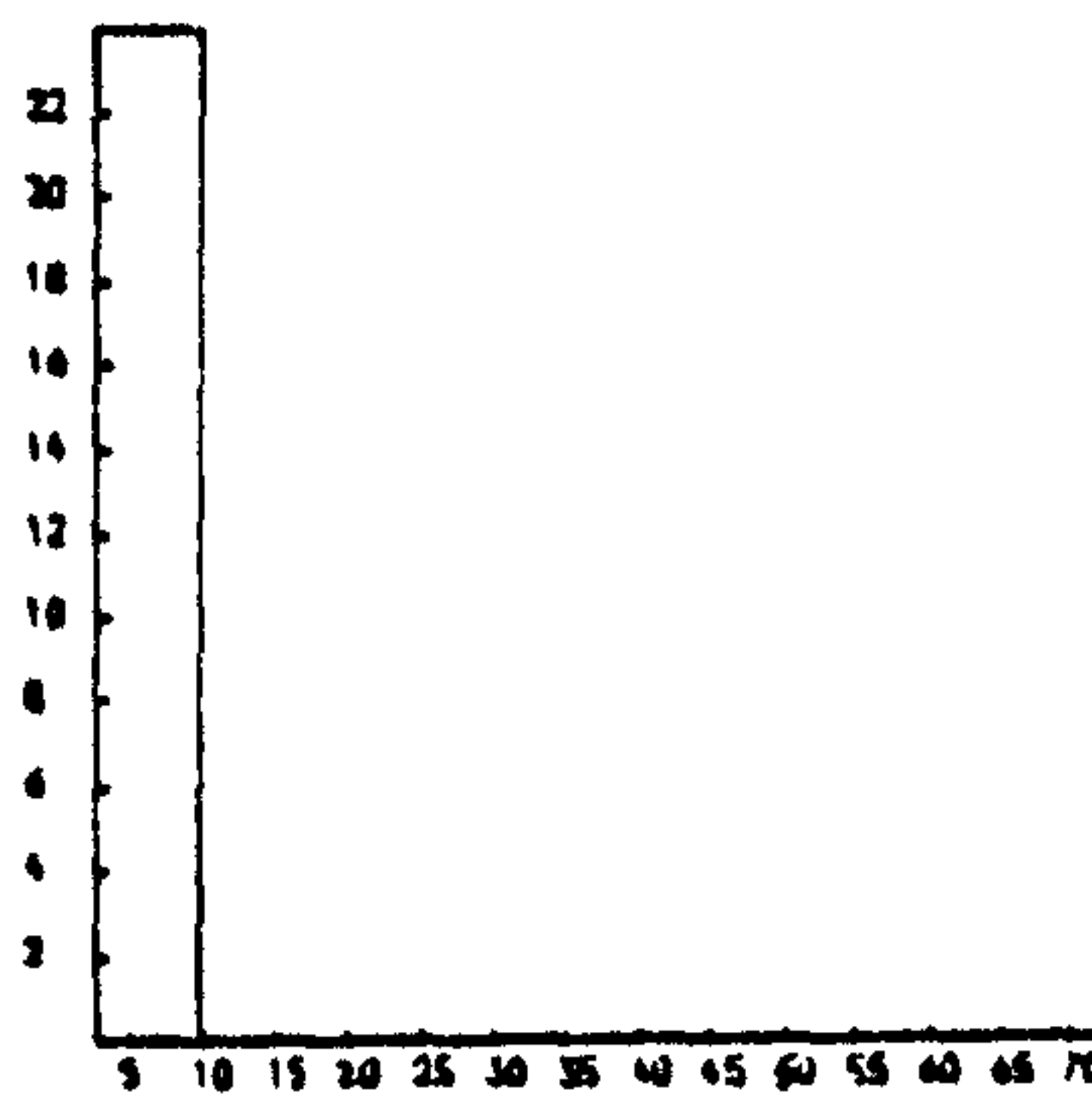
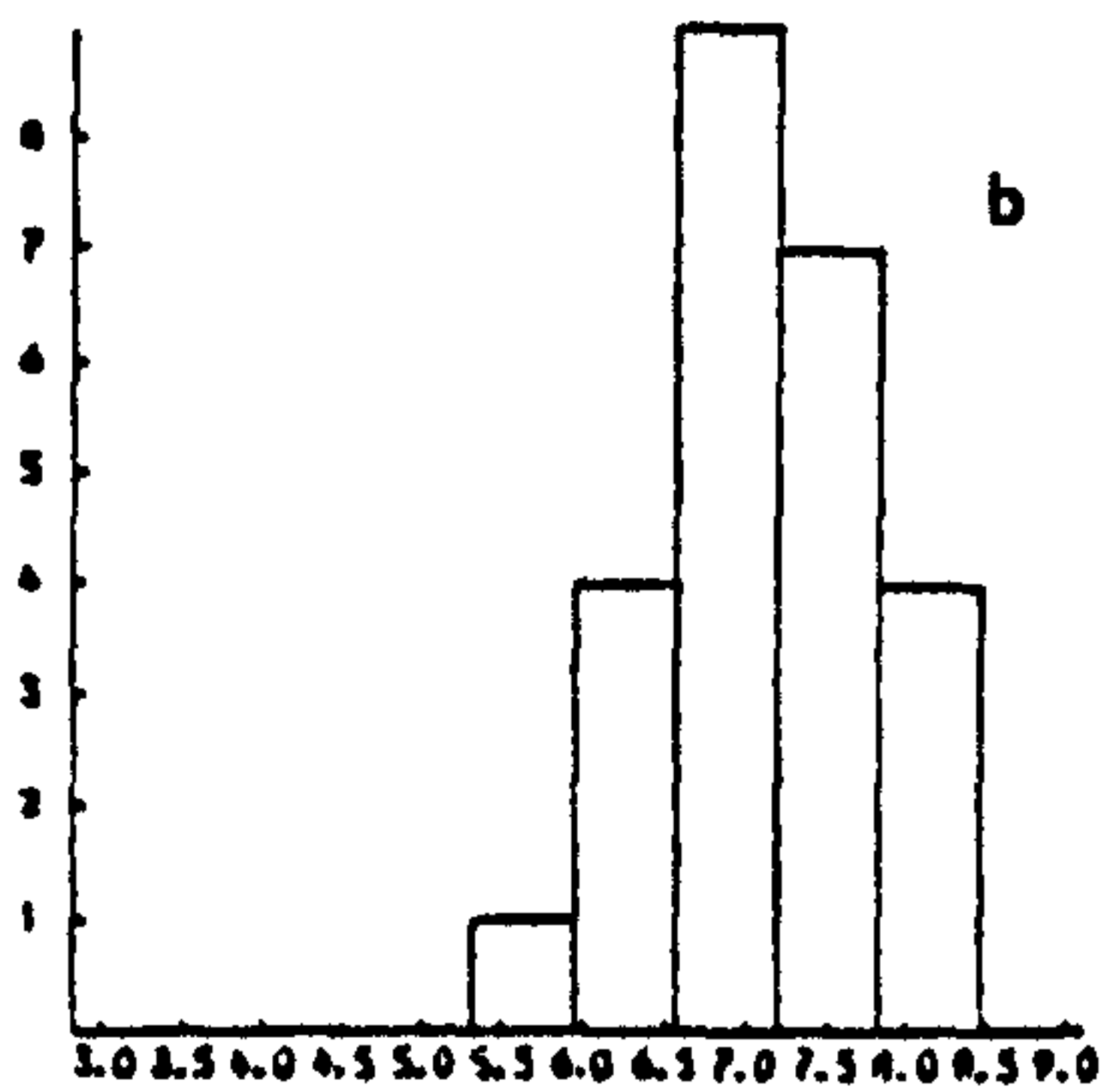
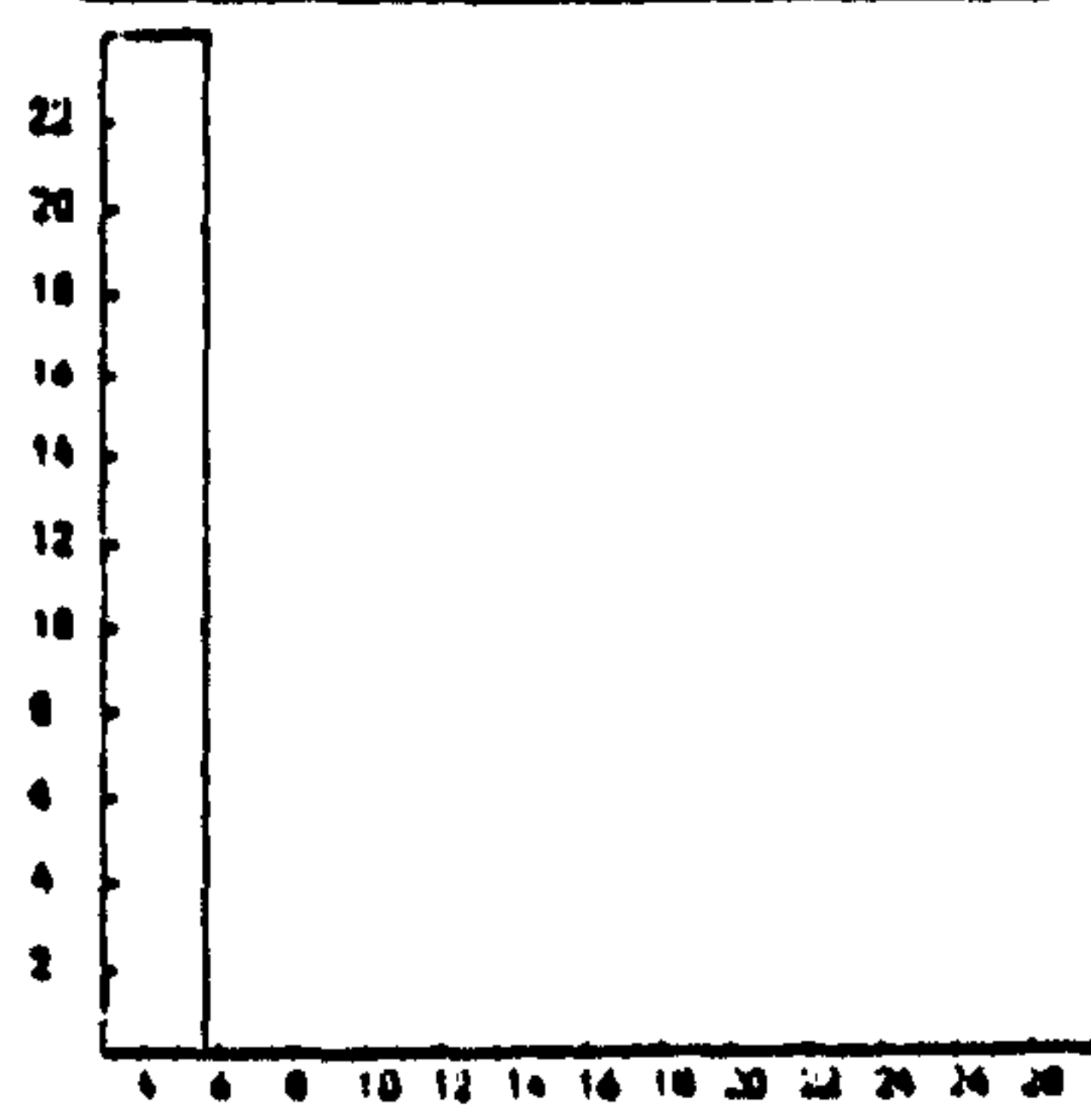
Character (a) in mm; for b, c, e & f, 1 unit = 0.43 mm; d, in  $\mu\text{m}$ ;  
for j, 1 unit = 10  $\mu\text{m}$ .



BEFORE CULTURE



AFTER CULTURE





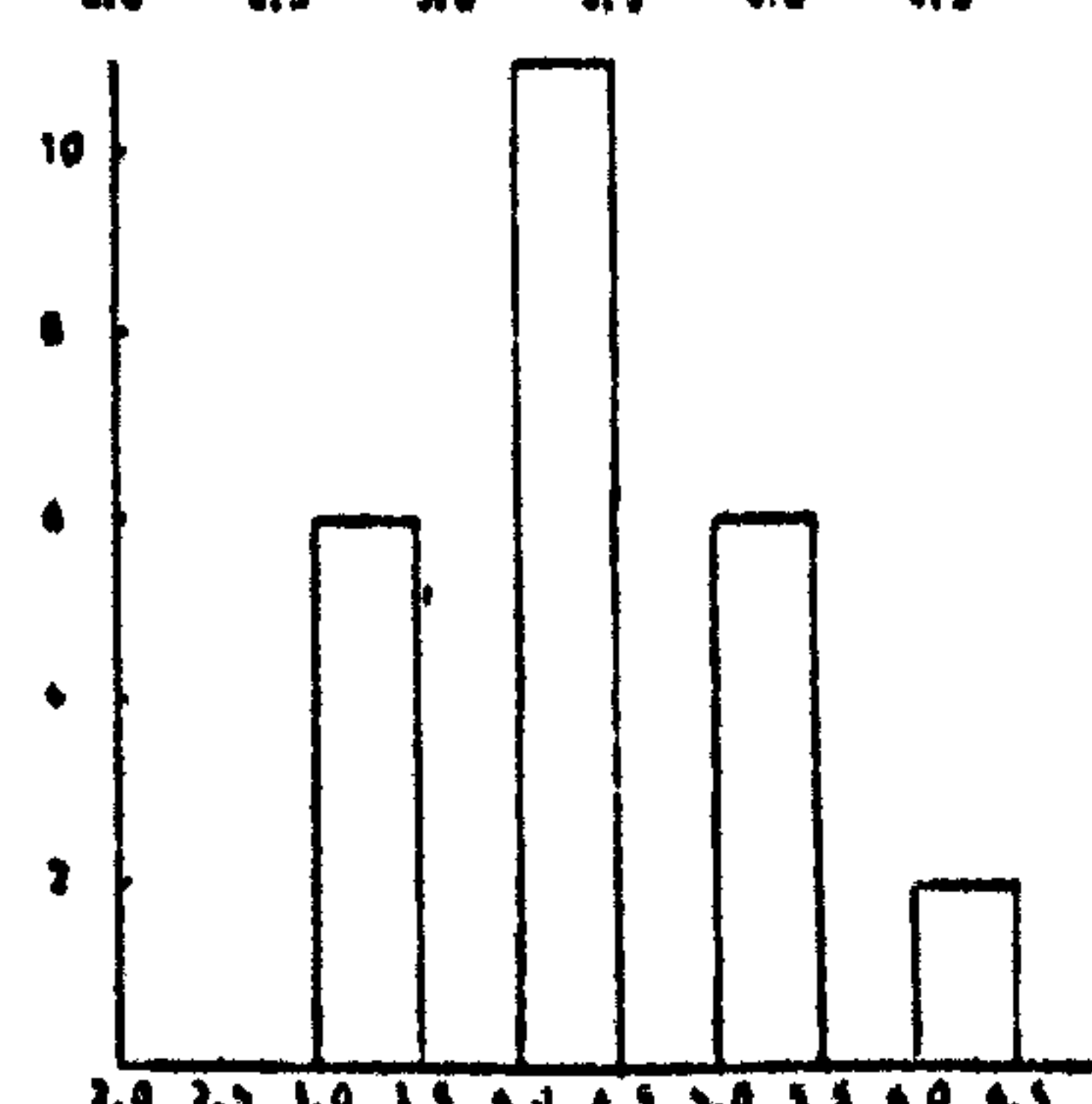
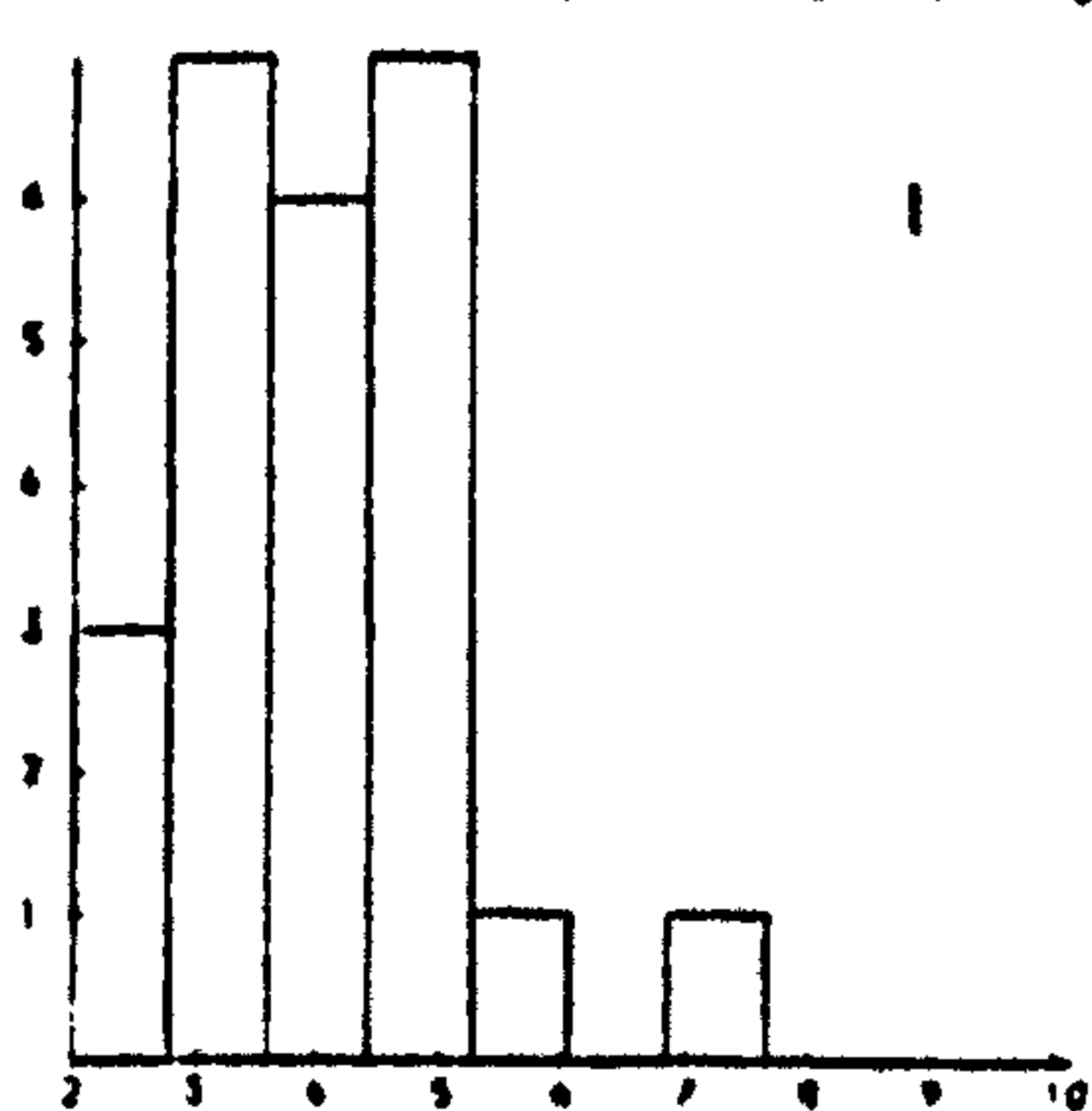
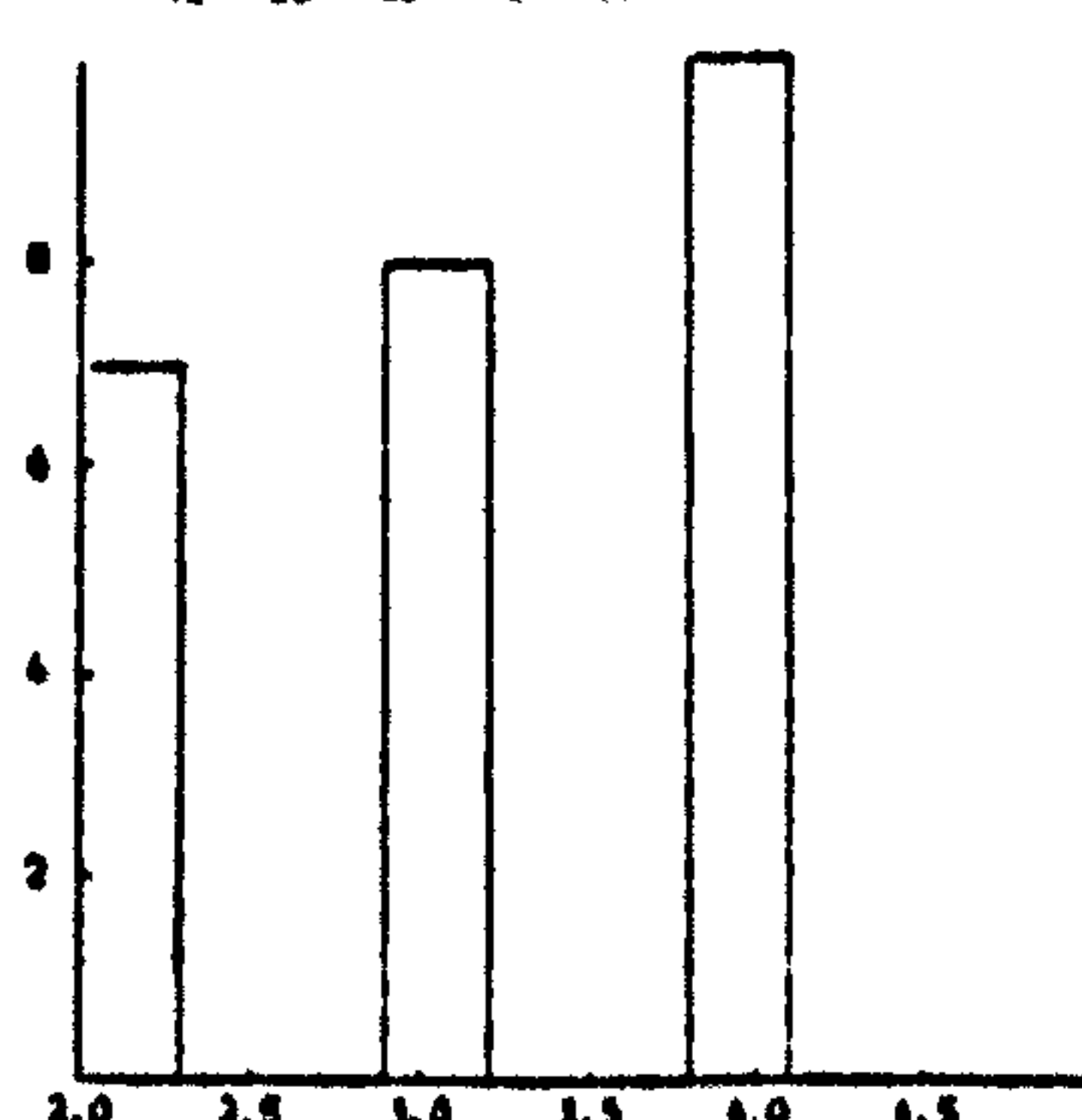
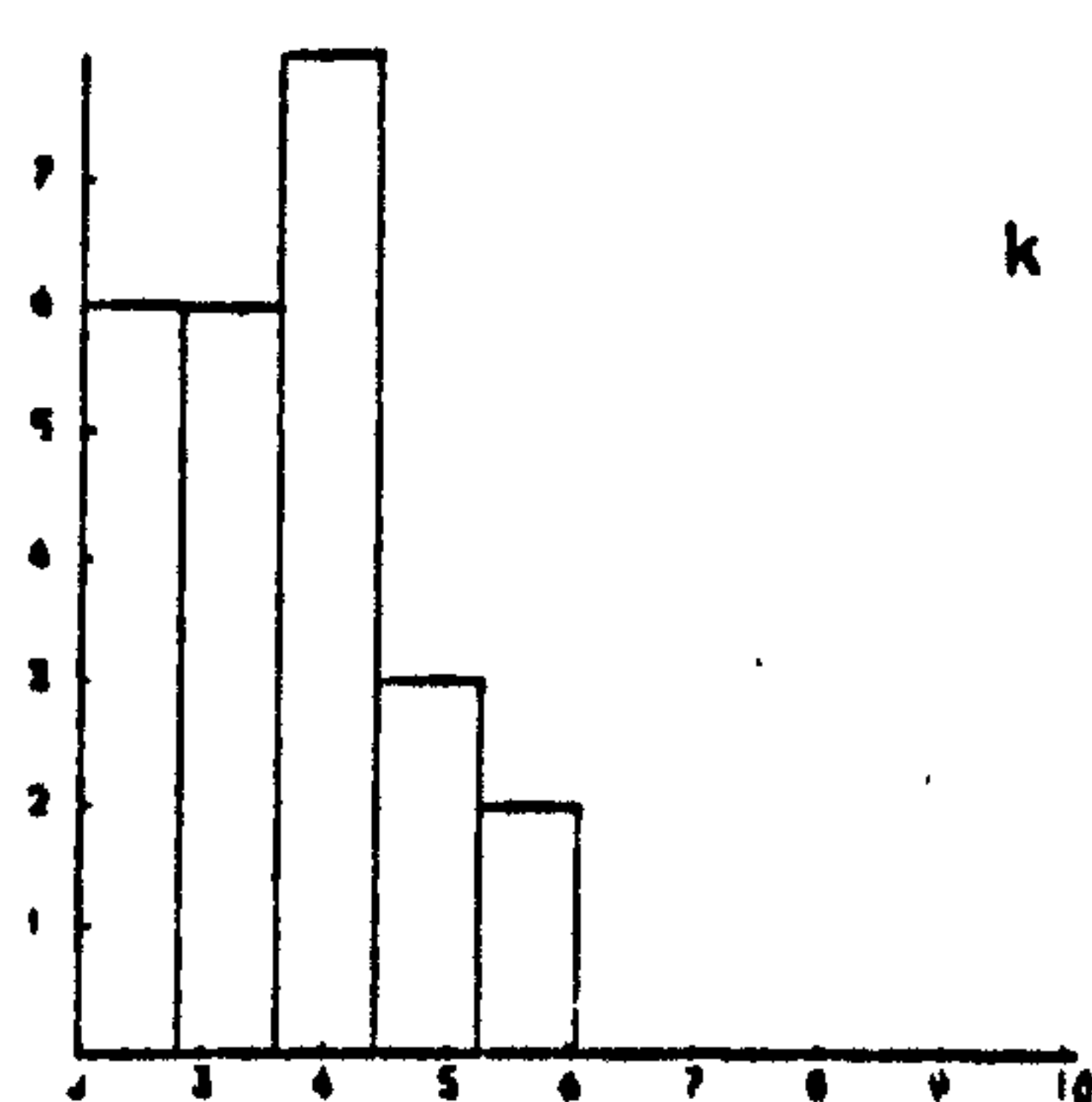
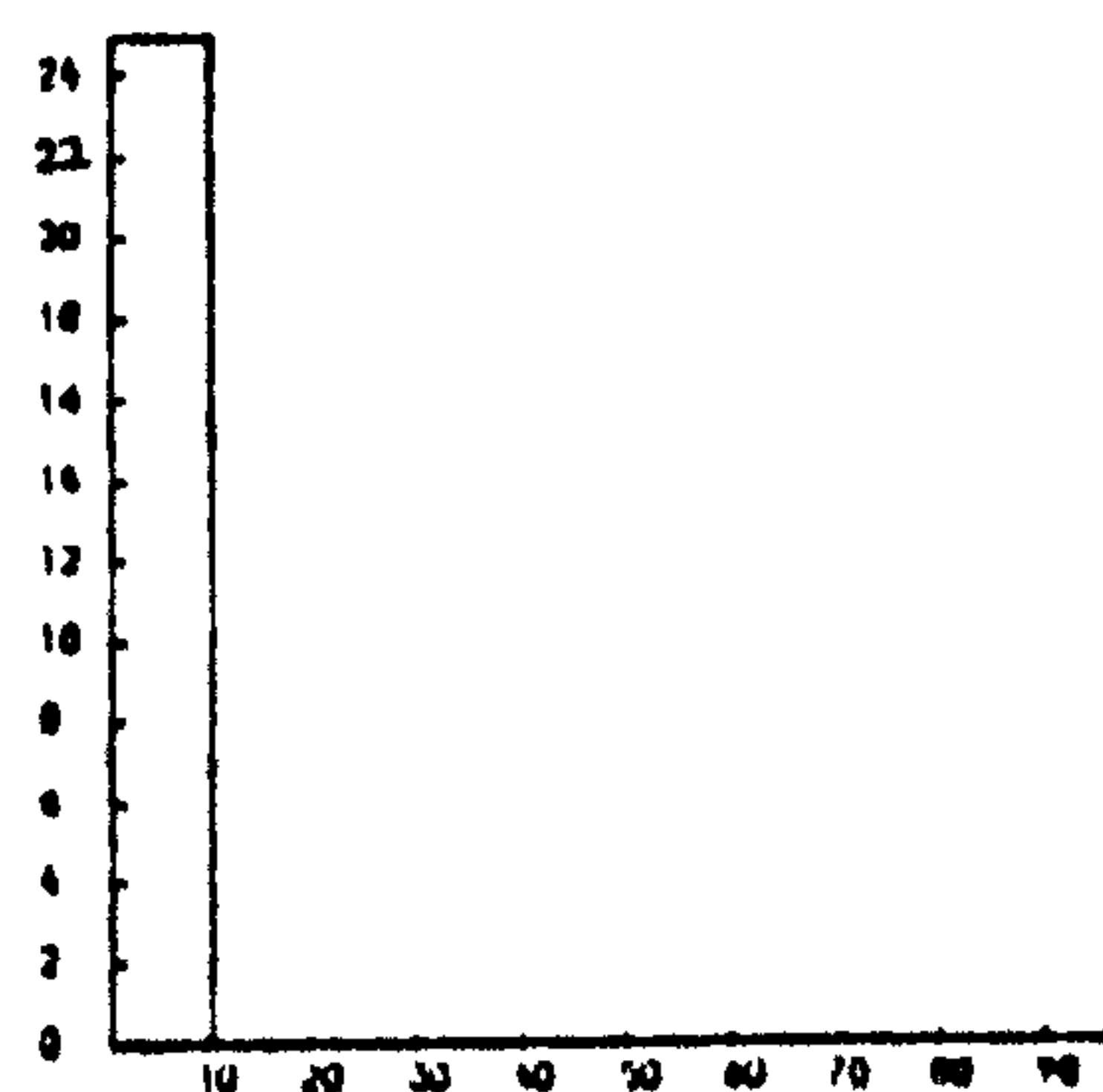
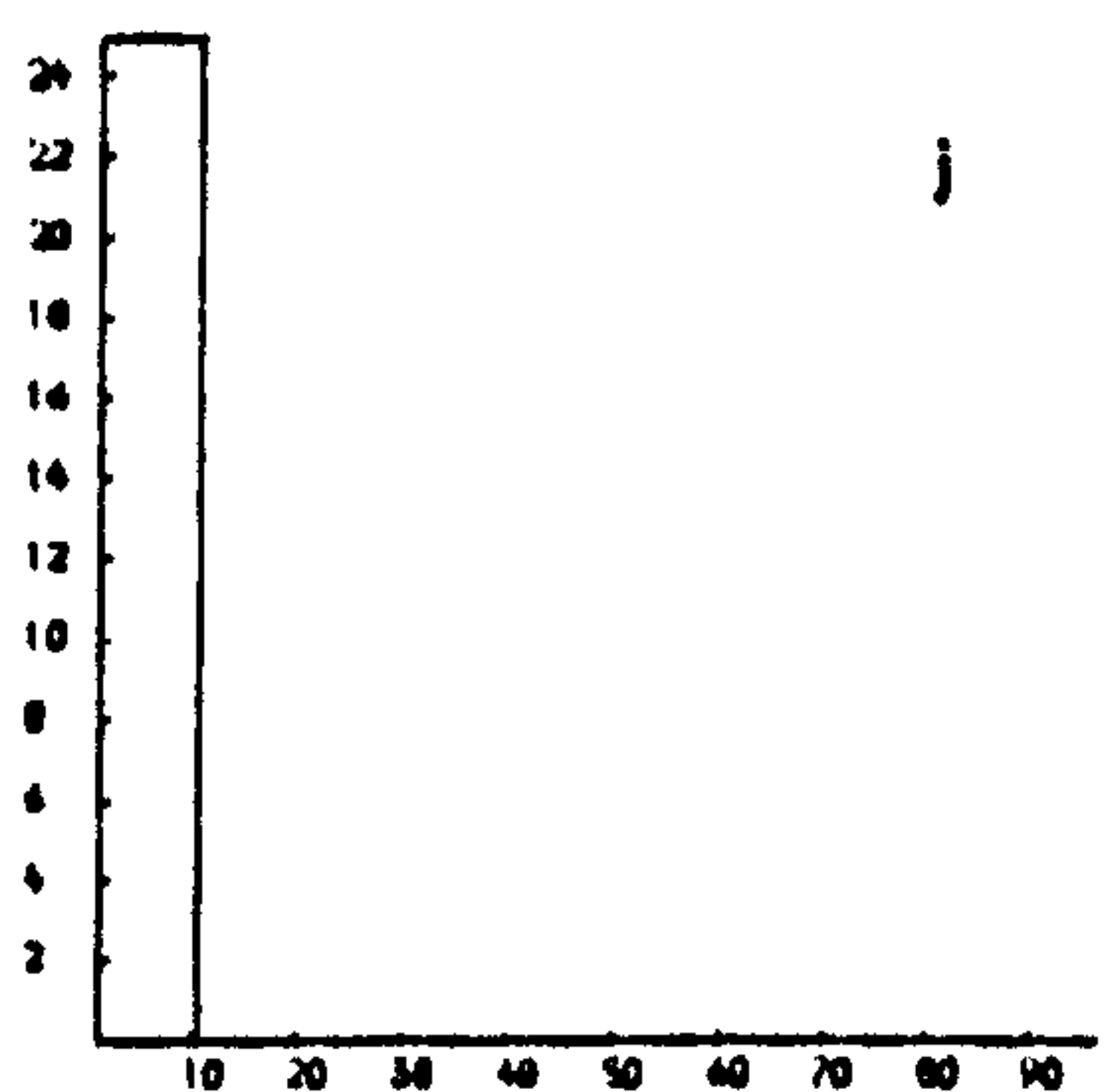
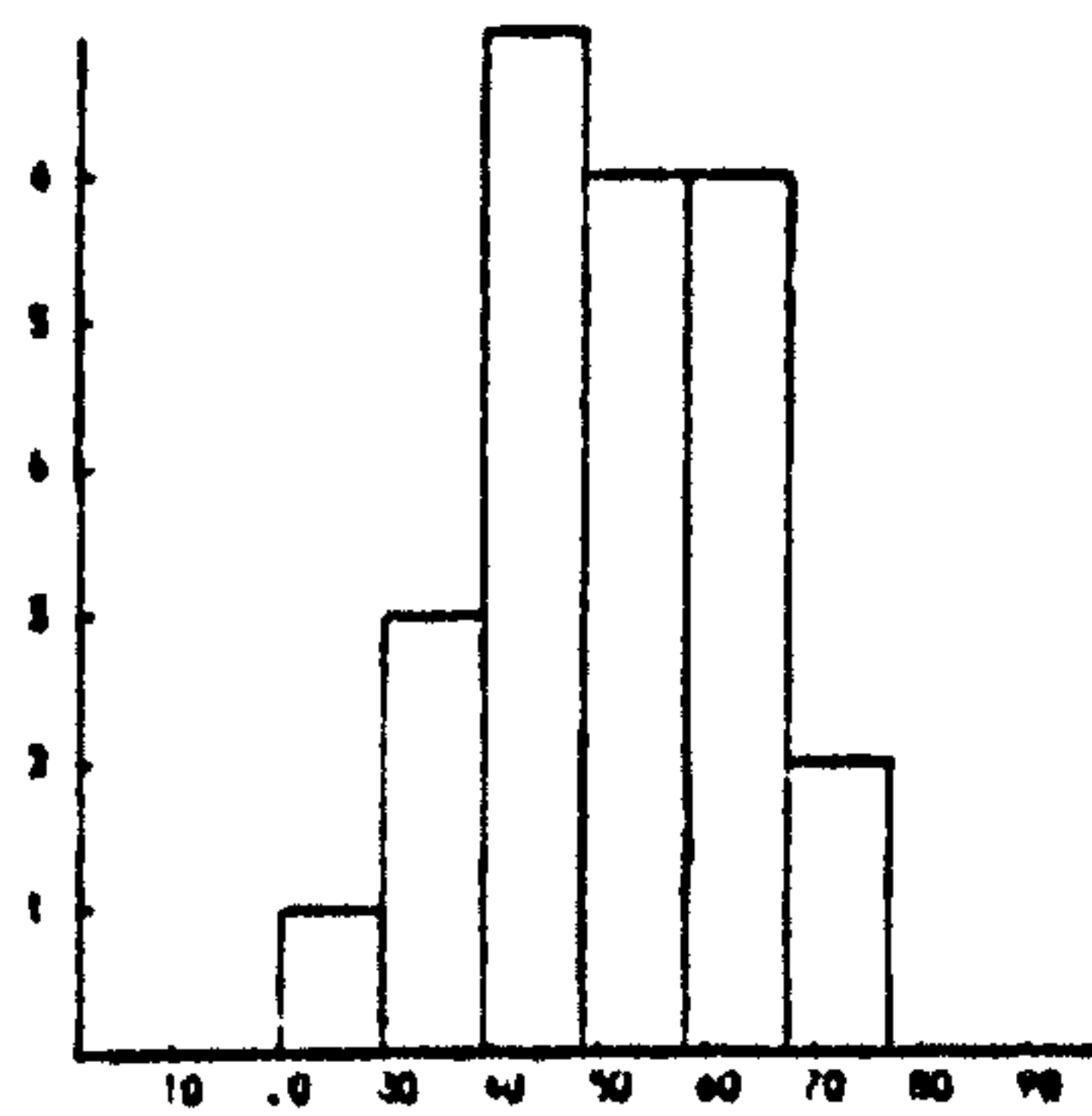
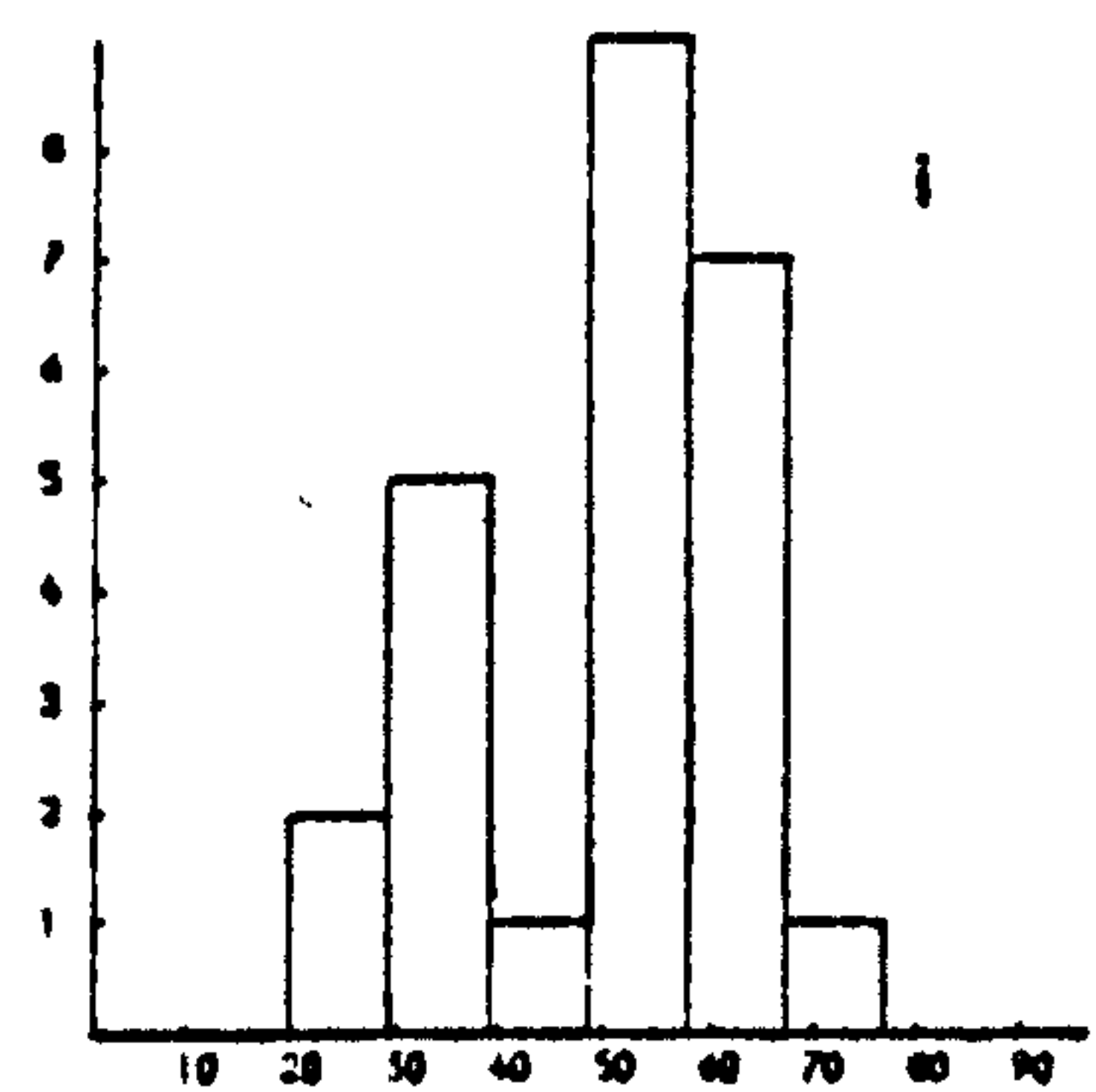
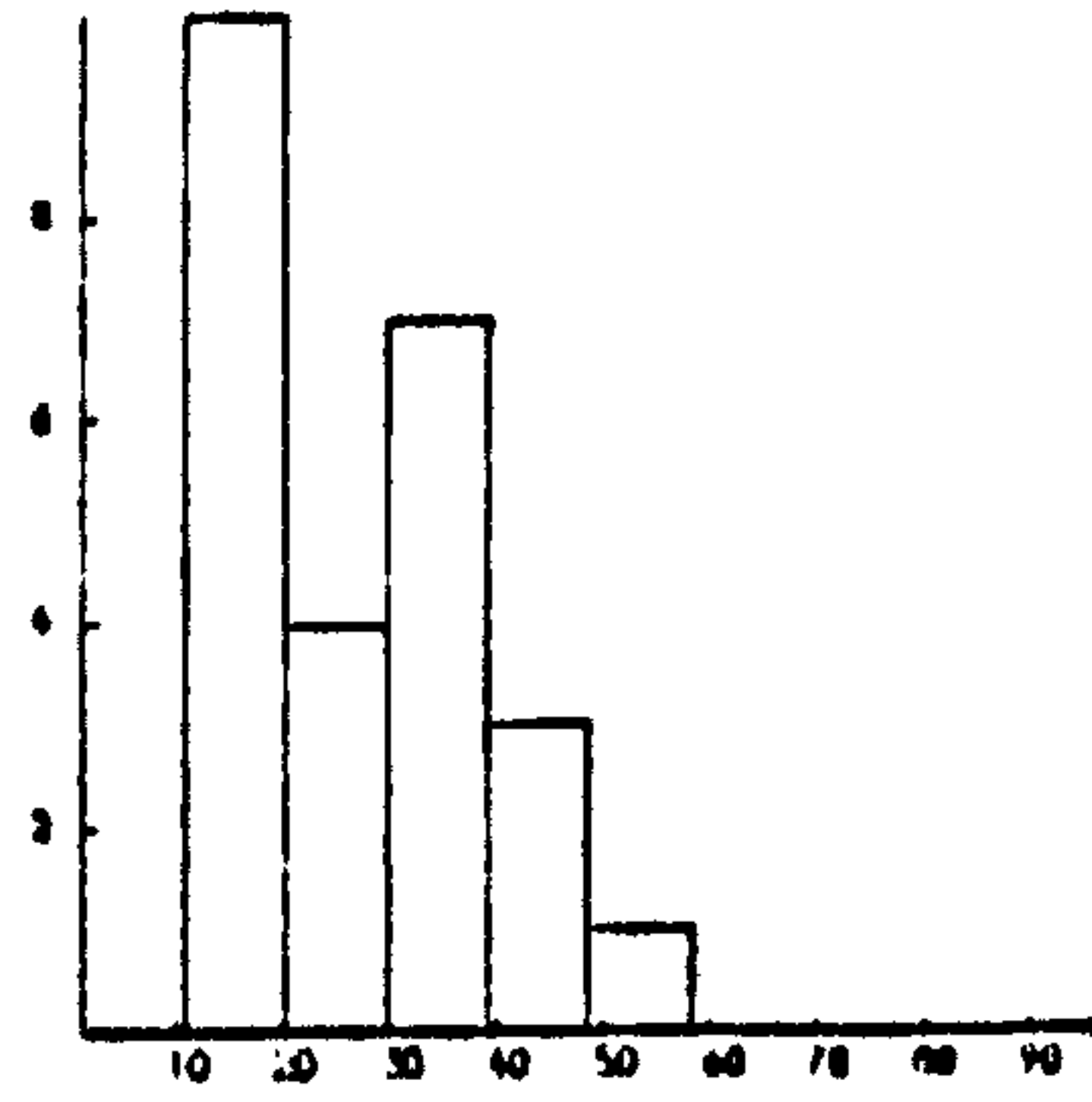
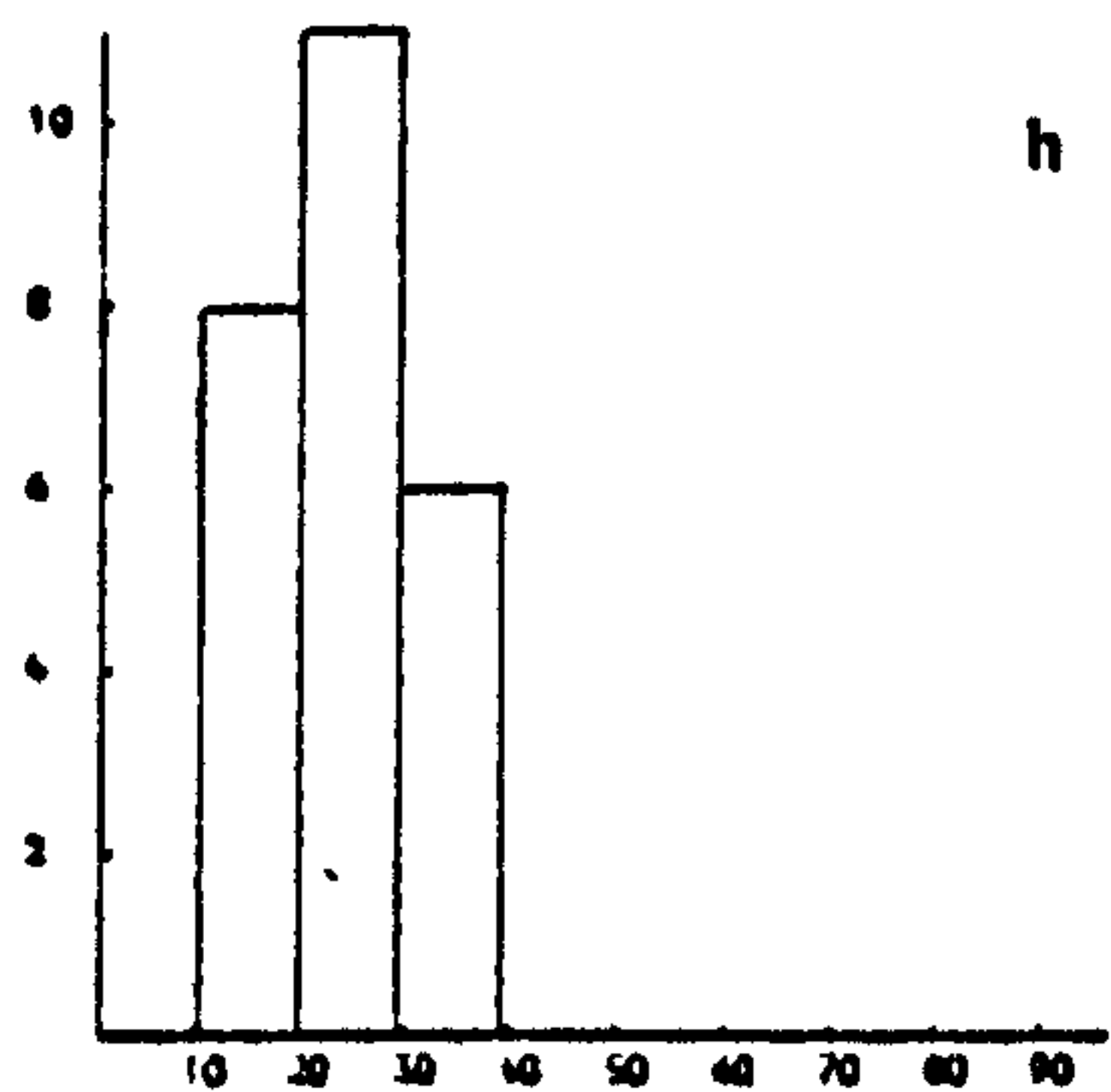
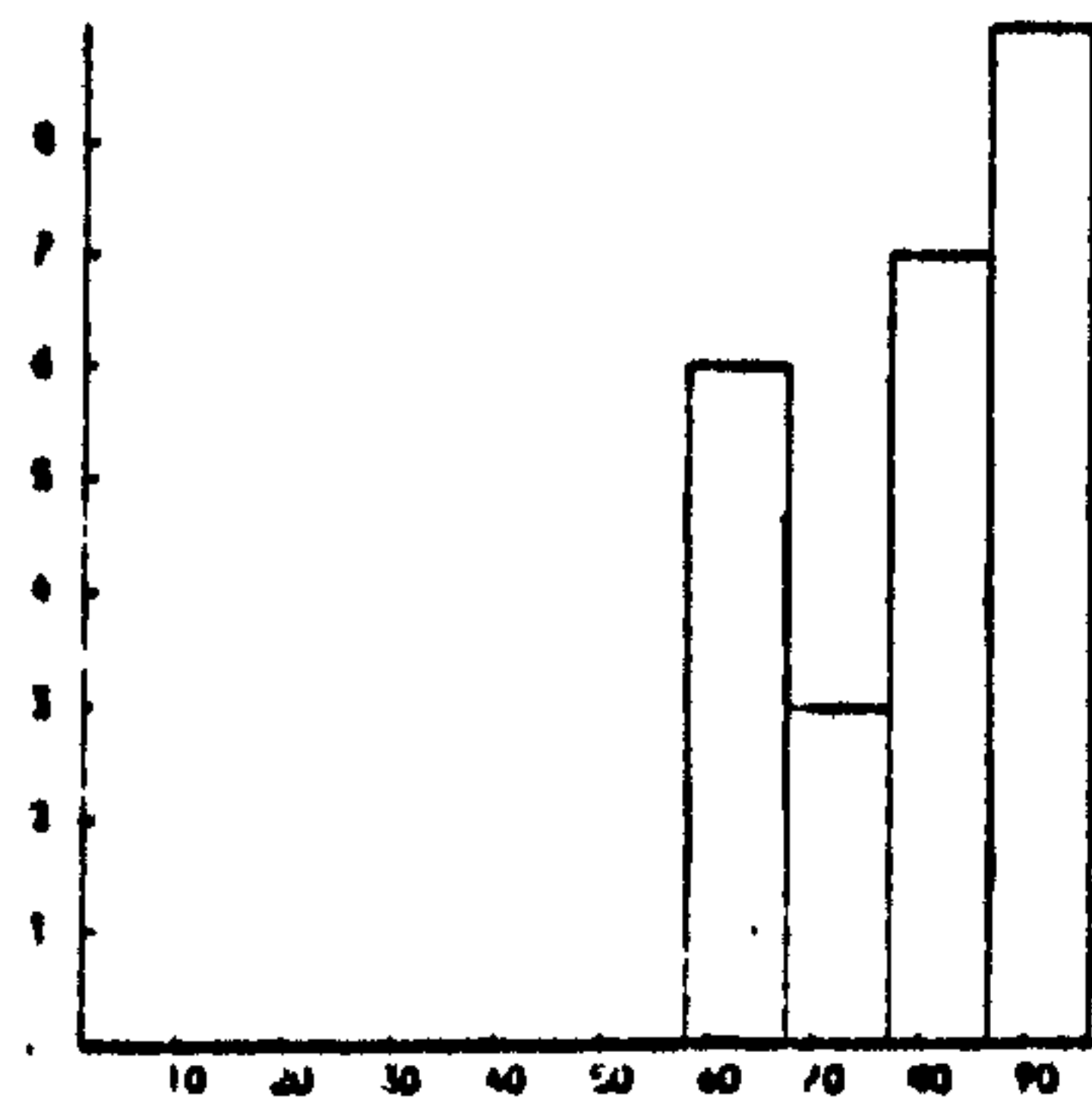
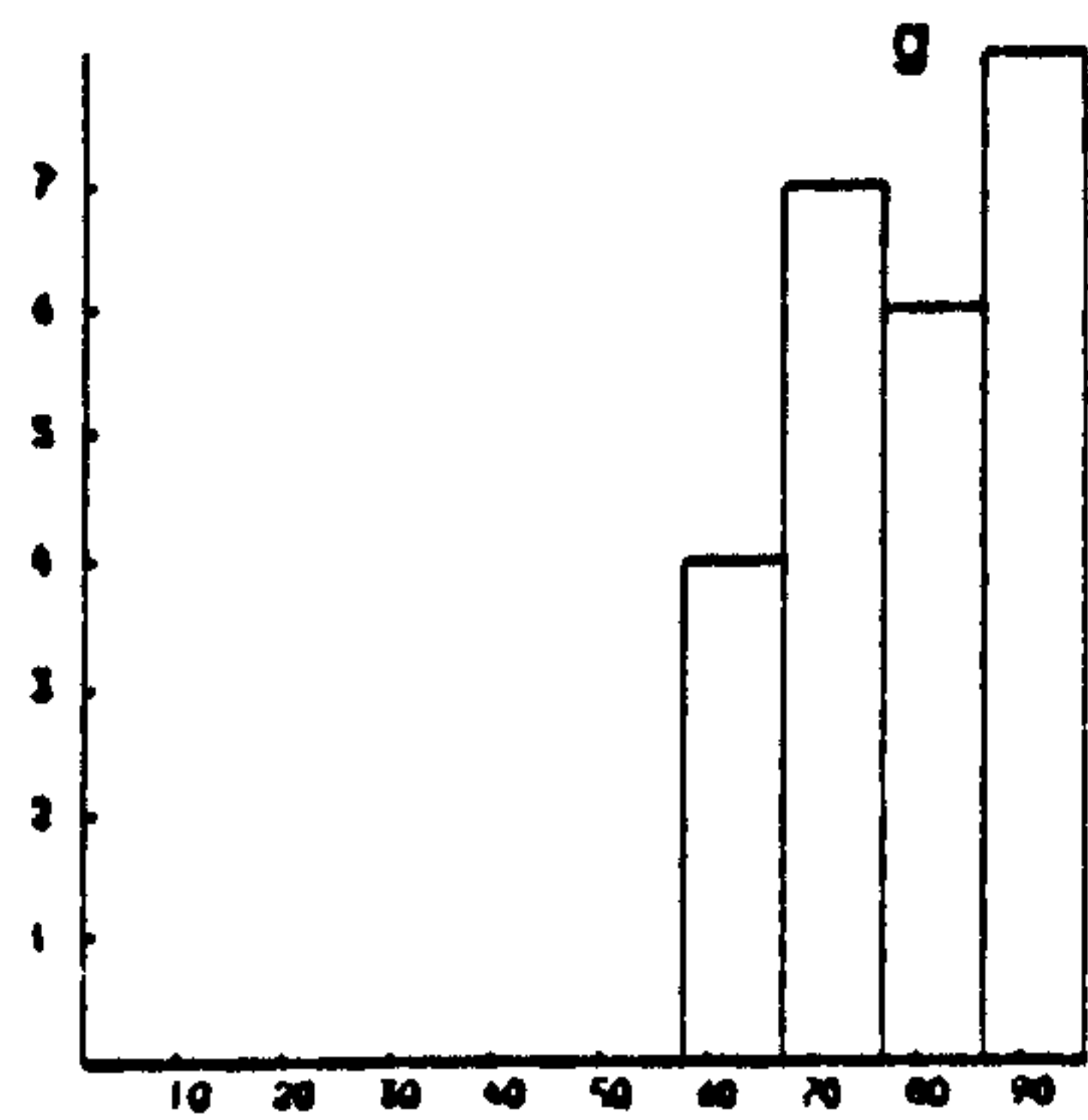


Fig. 48. Histograms illustrating variation of diploid A. undulatum

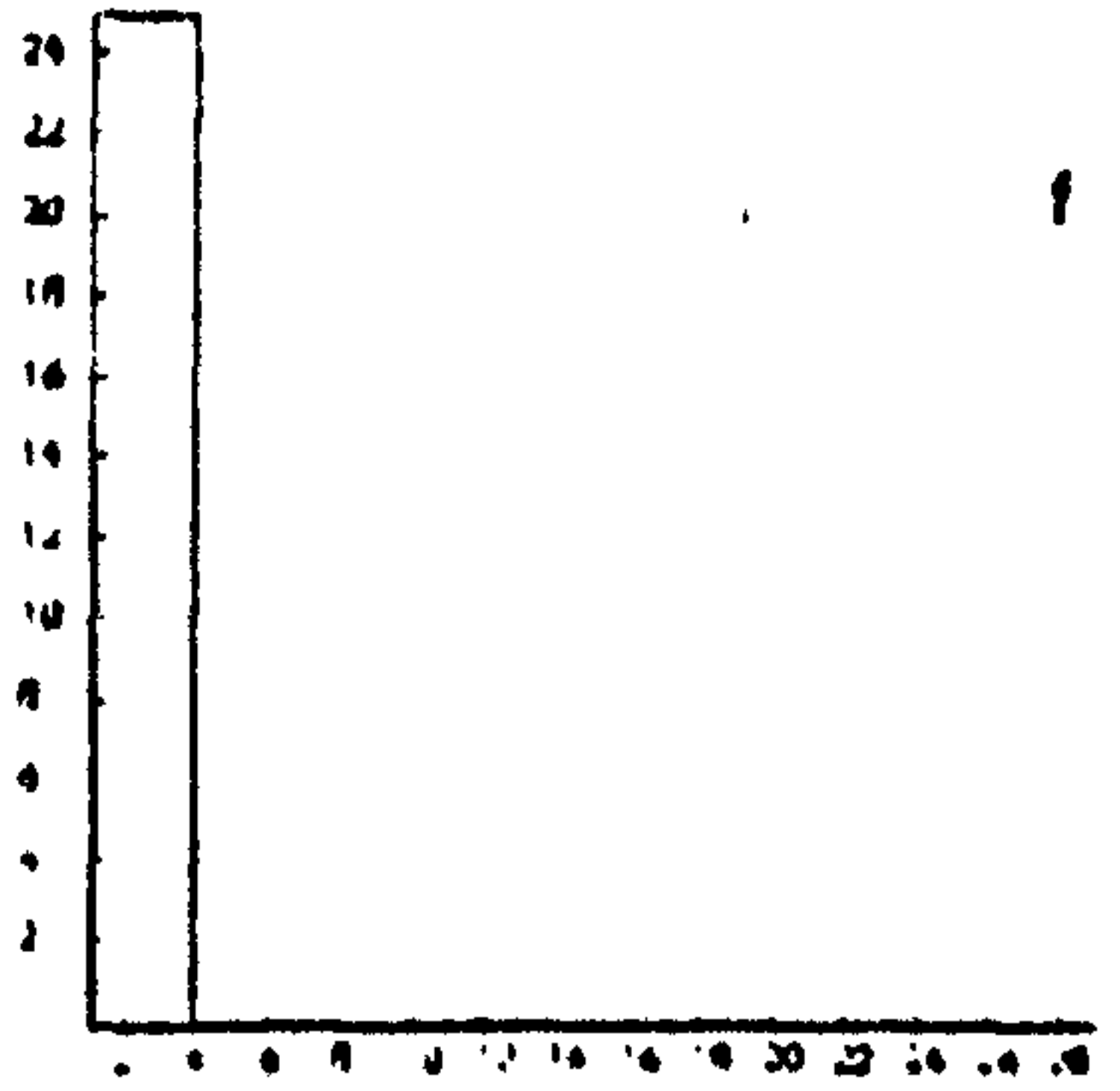
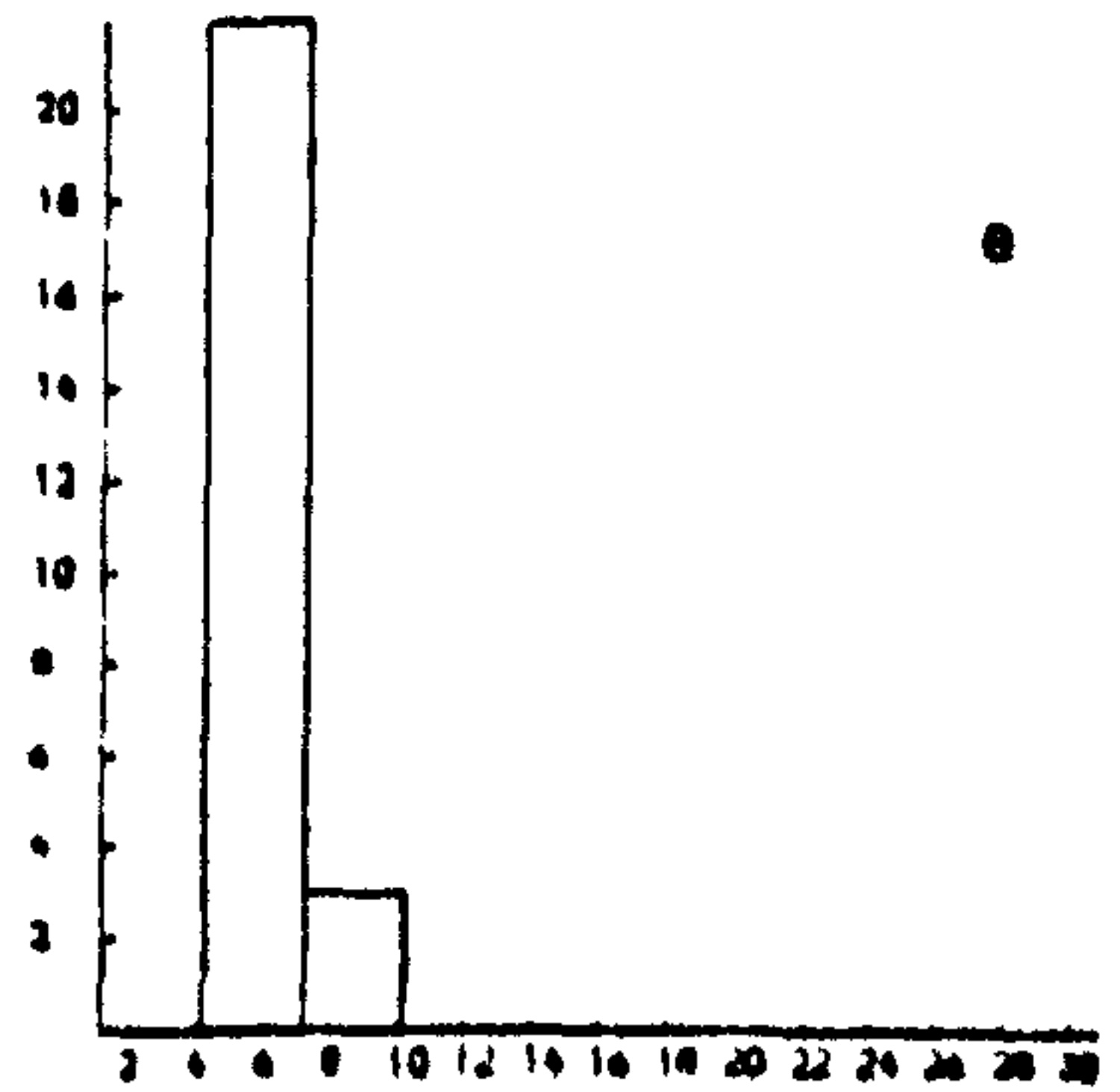
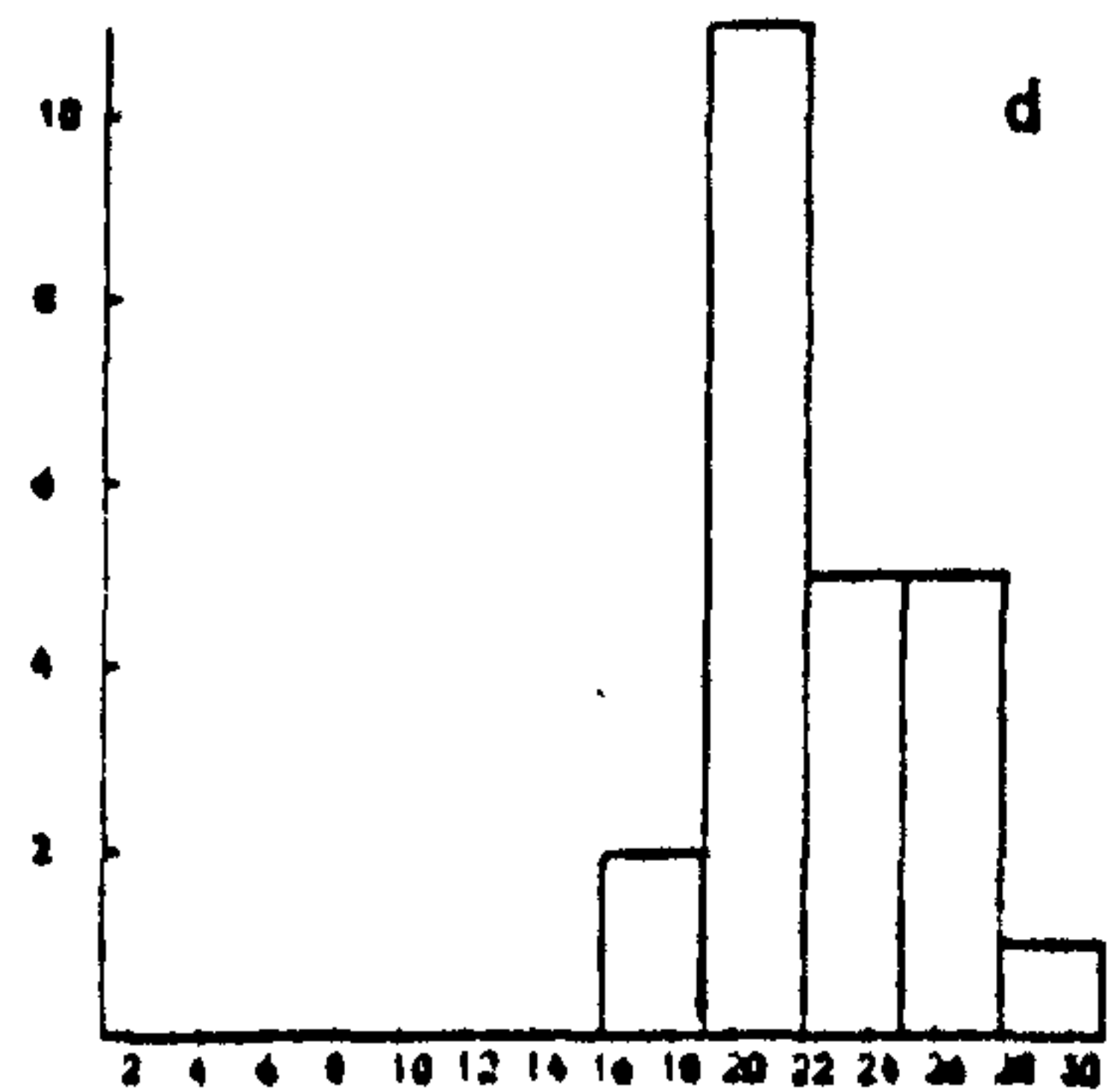
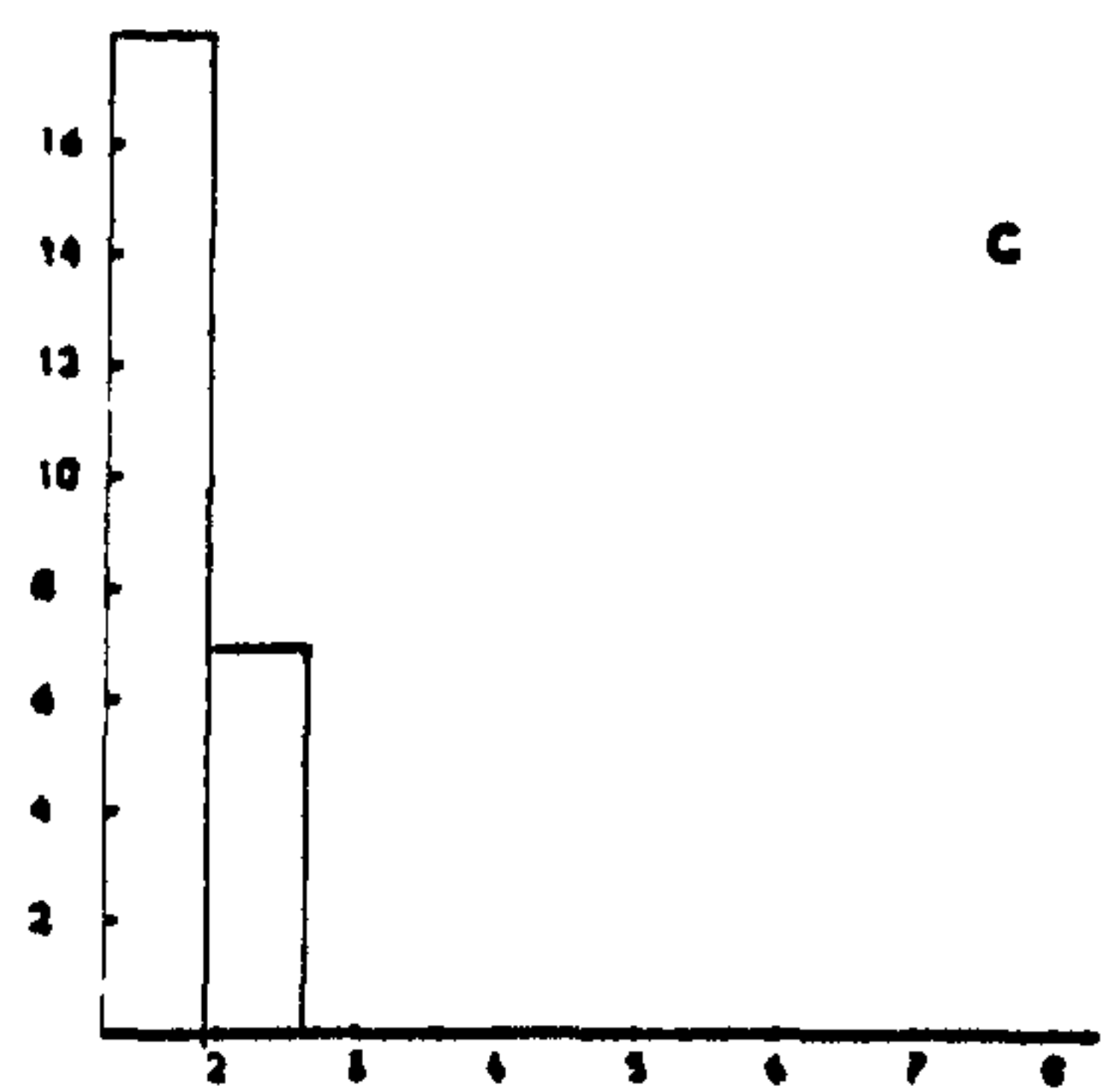
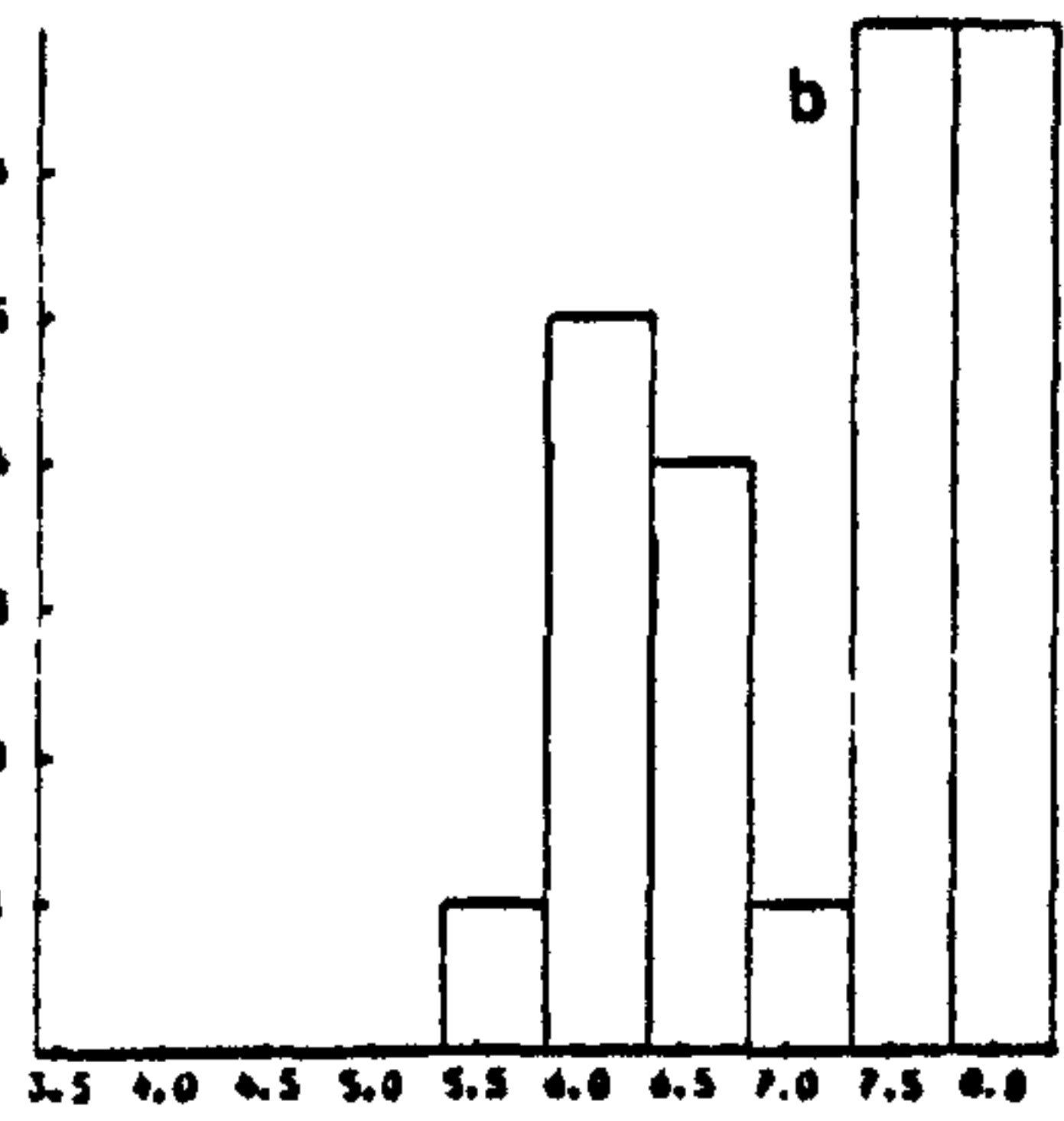
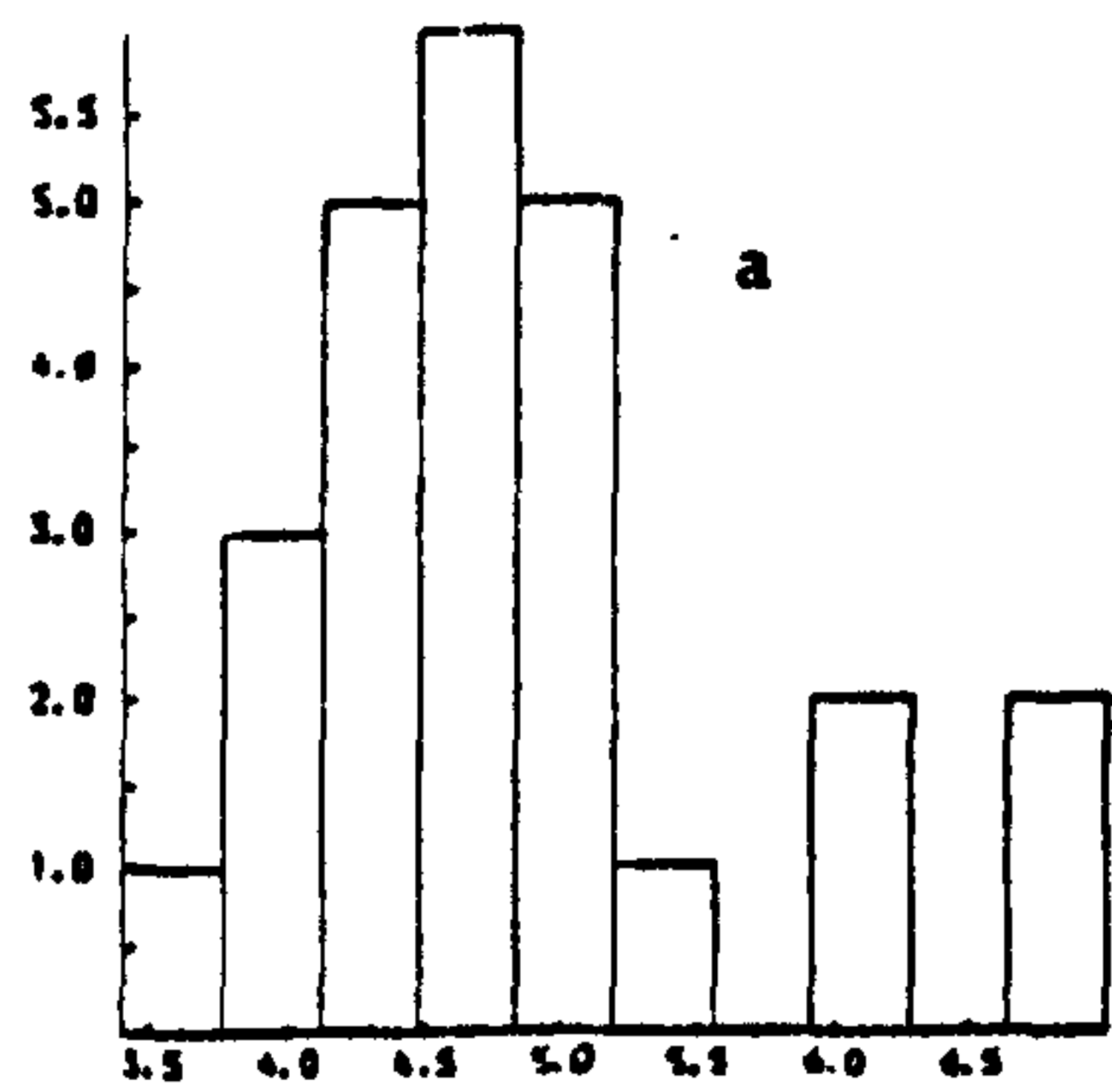
- |  |  |
|--|--|
| (a) plant height ;                         | (b) leaf length (from upper part of stem); |
| (c) leaf width (from upper part of stem) ; | (d) cell width (from upper part of stem);  |
| (e) leaf length (from mid-stem);           | (f) leaf width (from mid-stem);            |
| (g) percentage of serration ;              | (h) the angle of the acumen ;              |
| (i) tooth angle ;                          | (j) tooth long ;                           |
| (k) number of lamellar cells ;             | (l) height of lamellar cells .             |

Note that the Y-axis is not identical in all cases

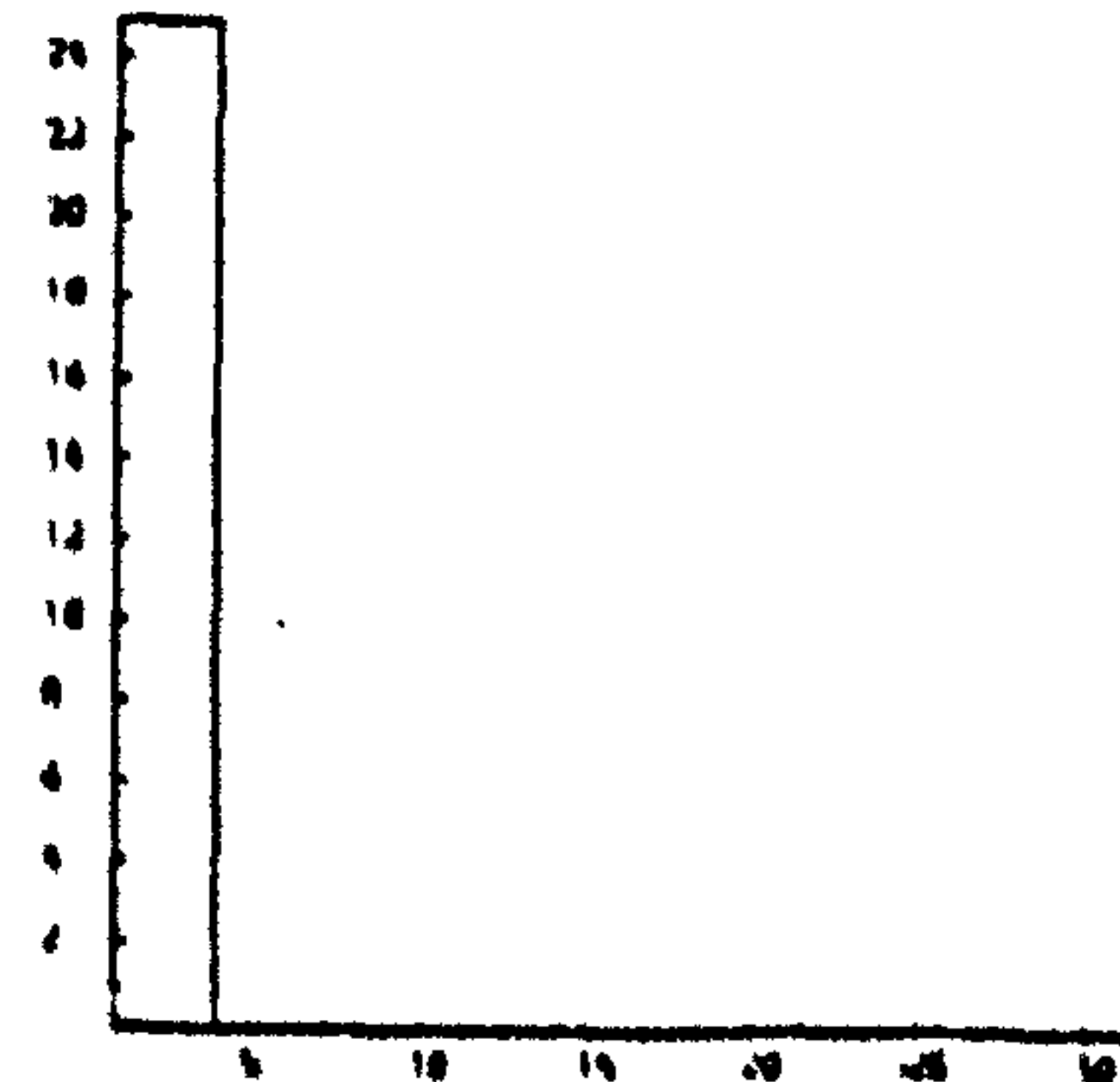
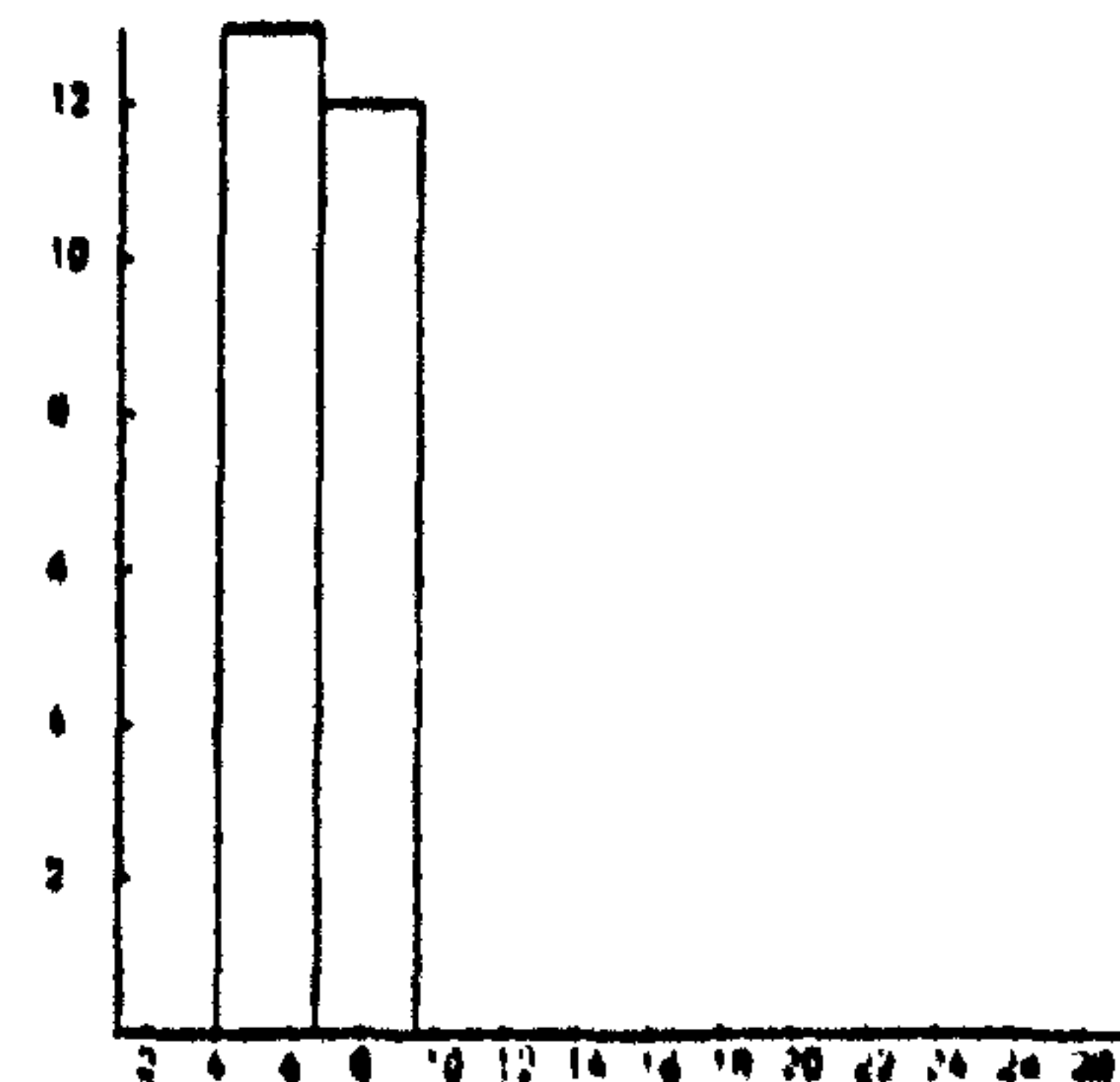
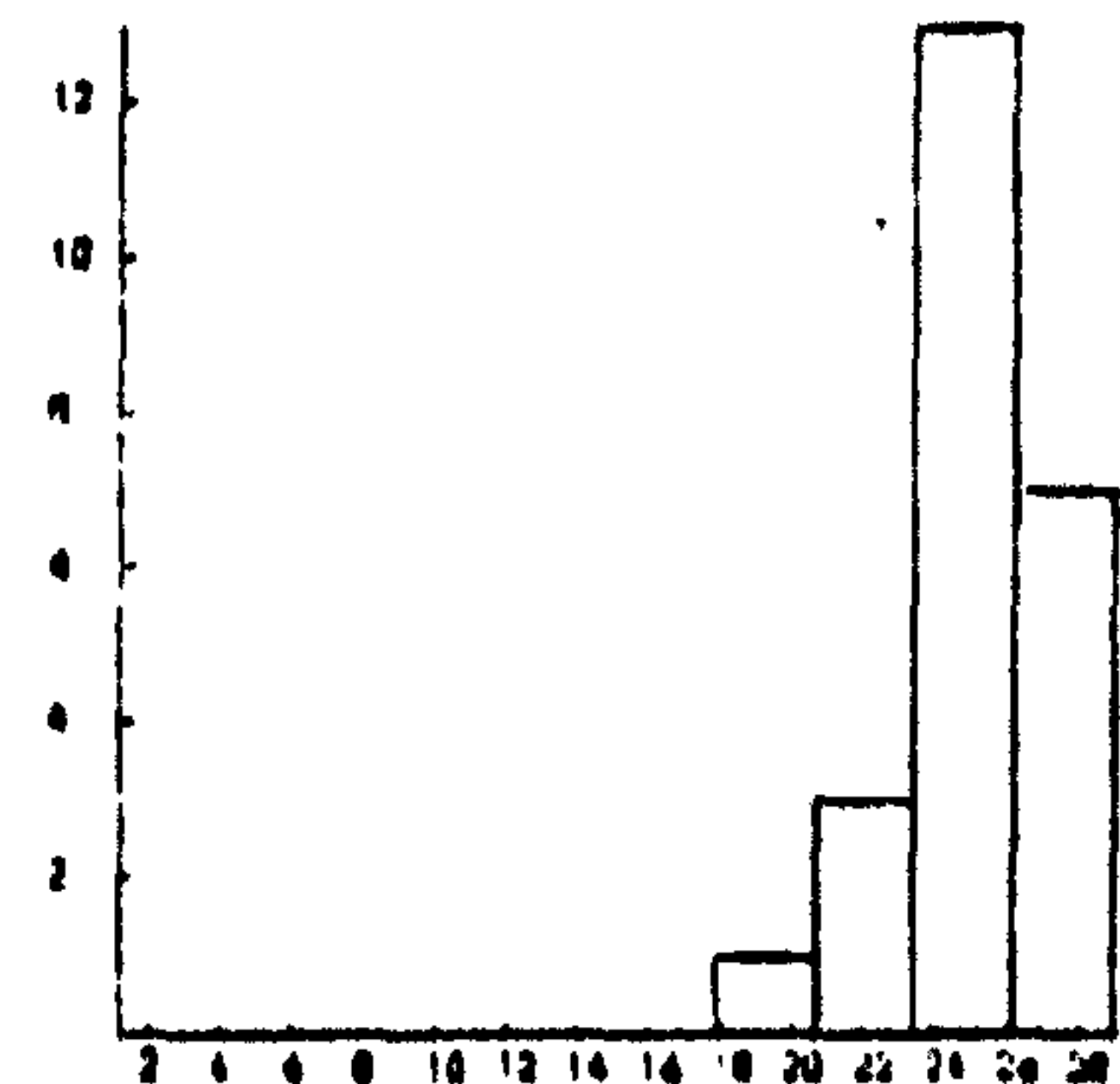
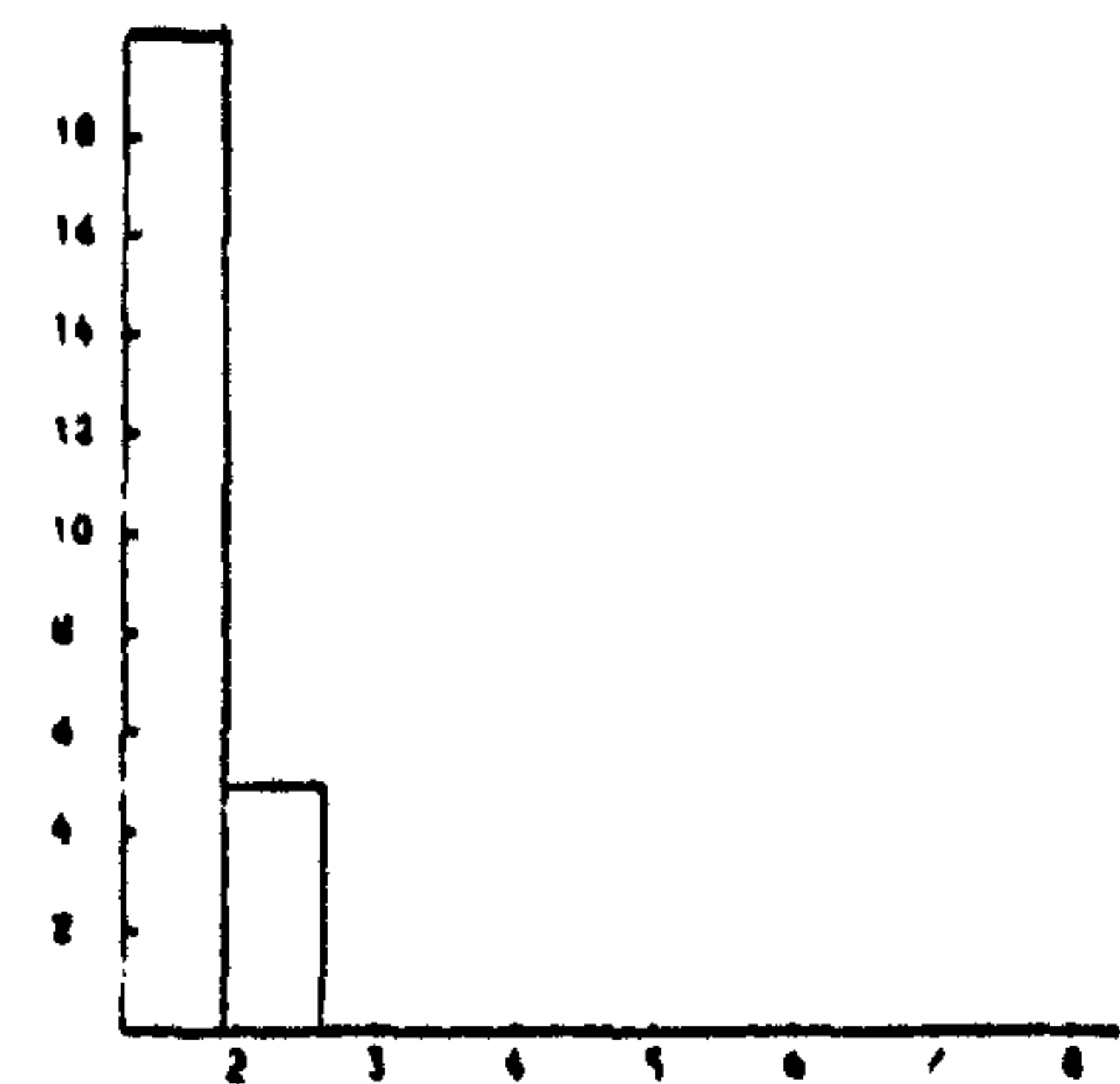
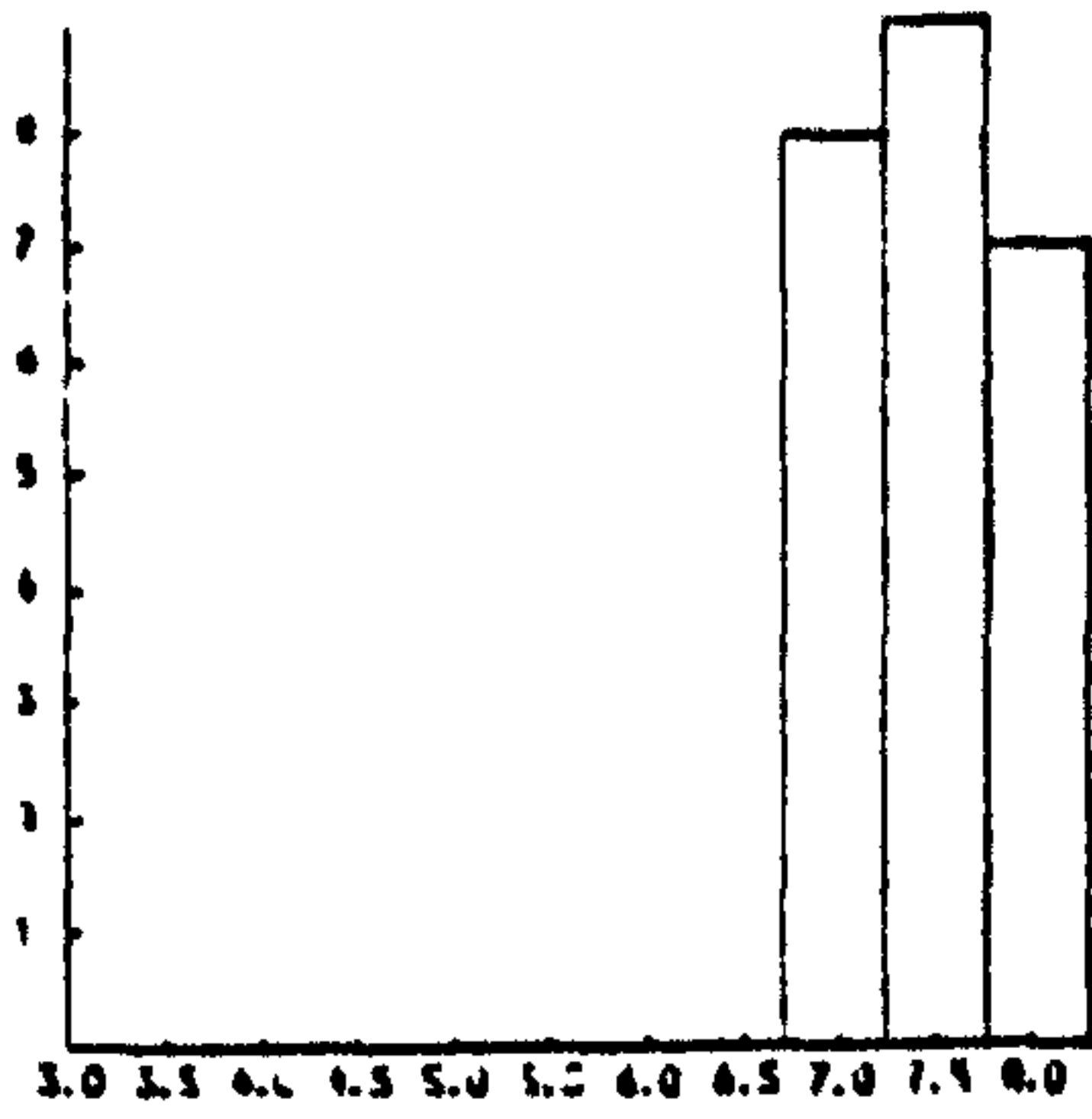
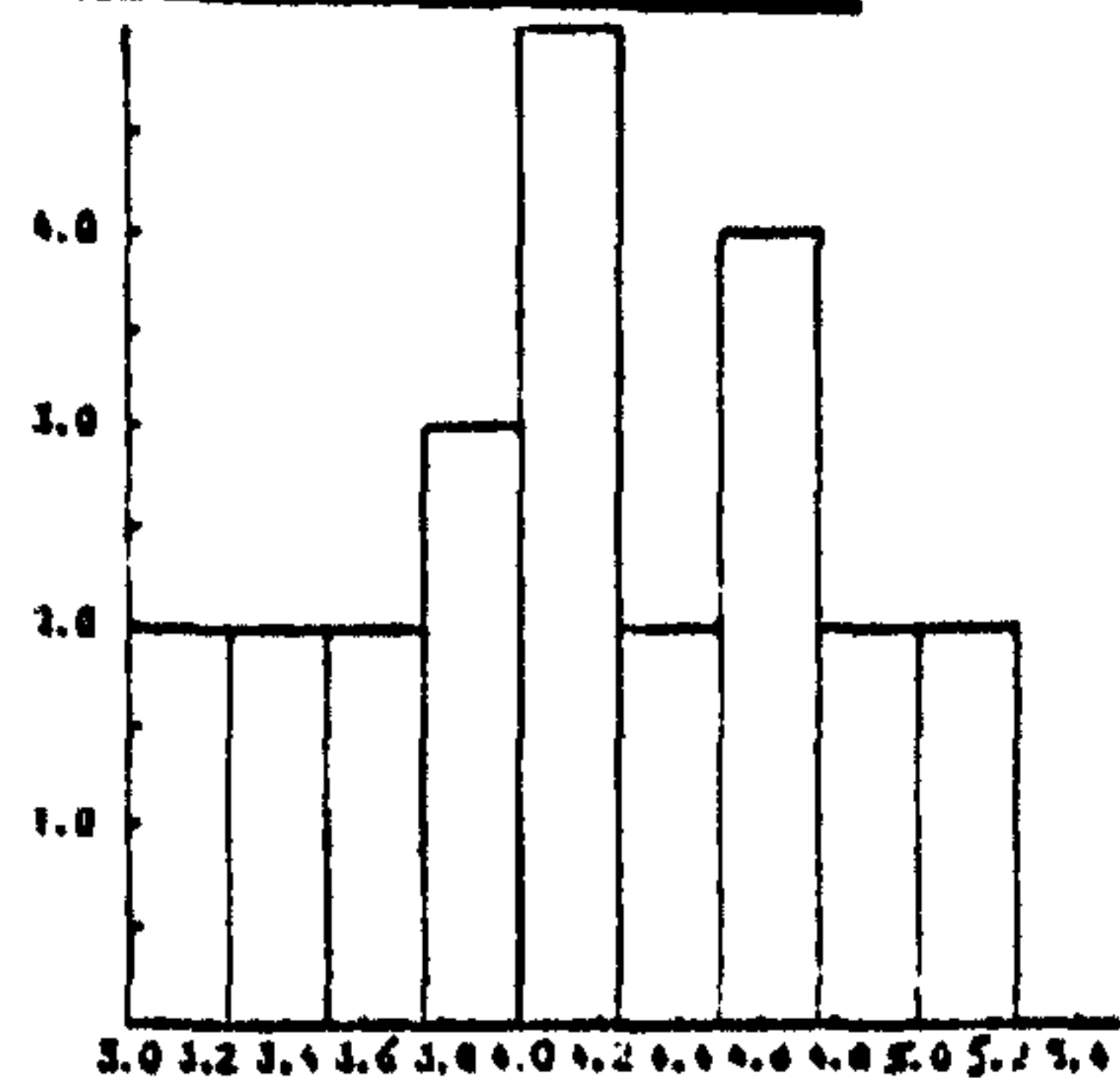
Character (a) in mm; for b, c, e & f, 1 unit = 0.43 mm; d, l in  $\mu\text{m}$  ;

for j, 1 unit = 10  $\mu\text{m}$ .

# BEFORE CULTURE



# AFTER CULTURE



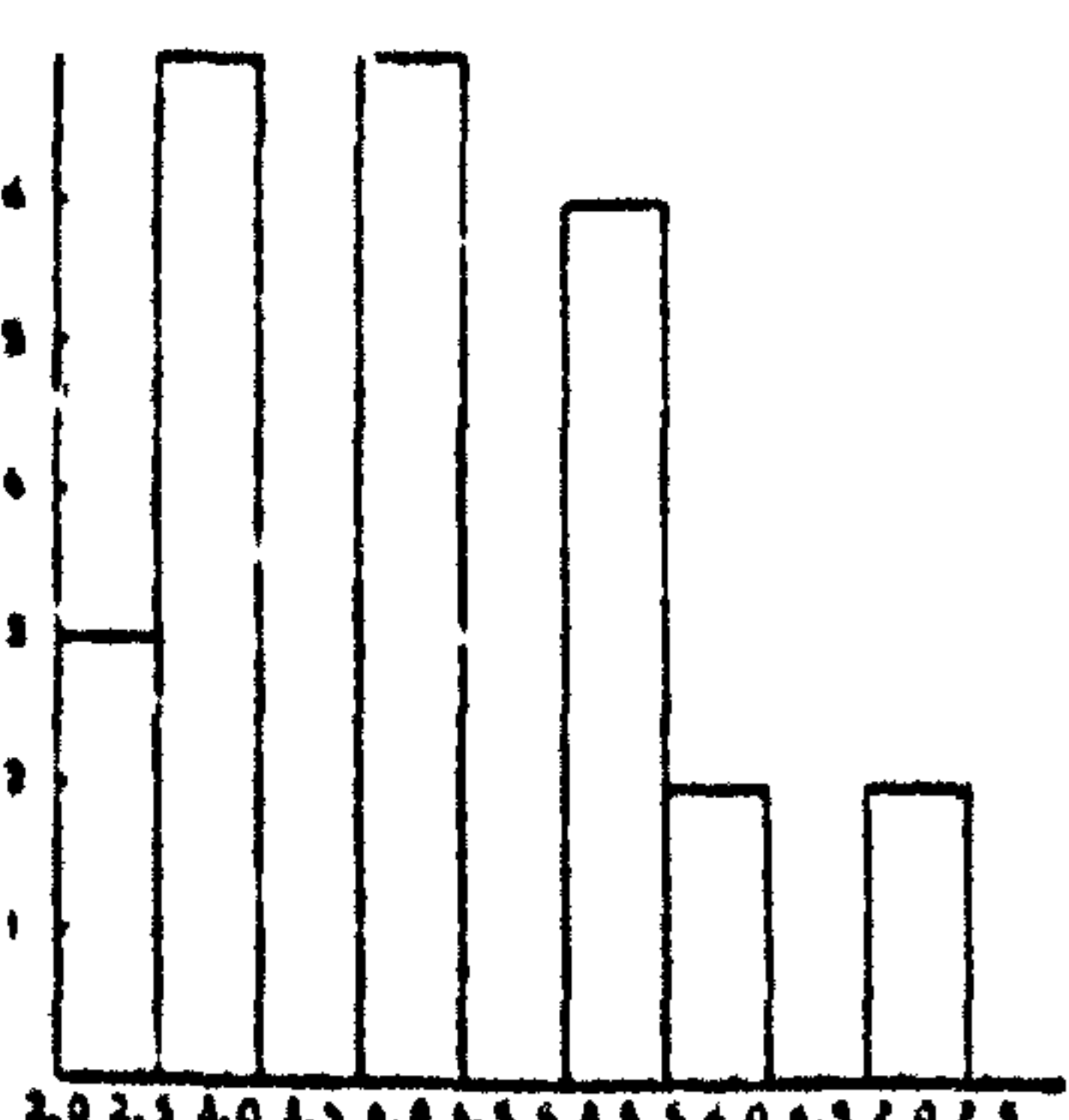
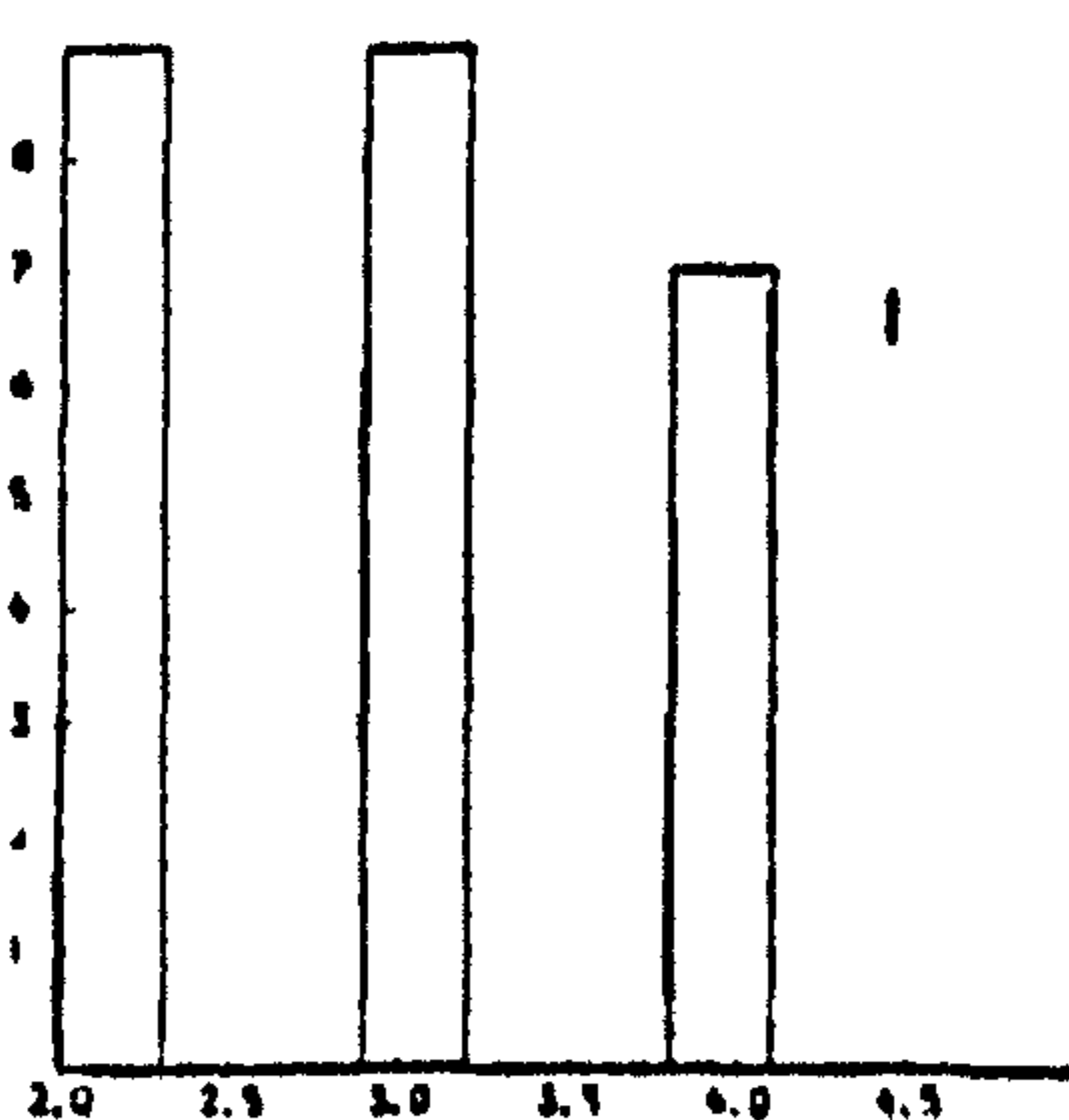
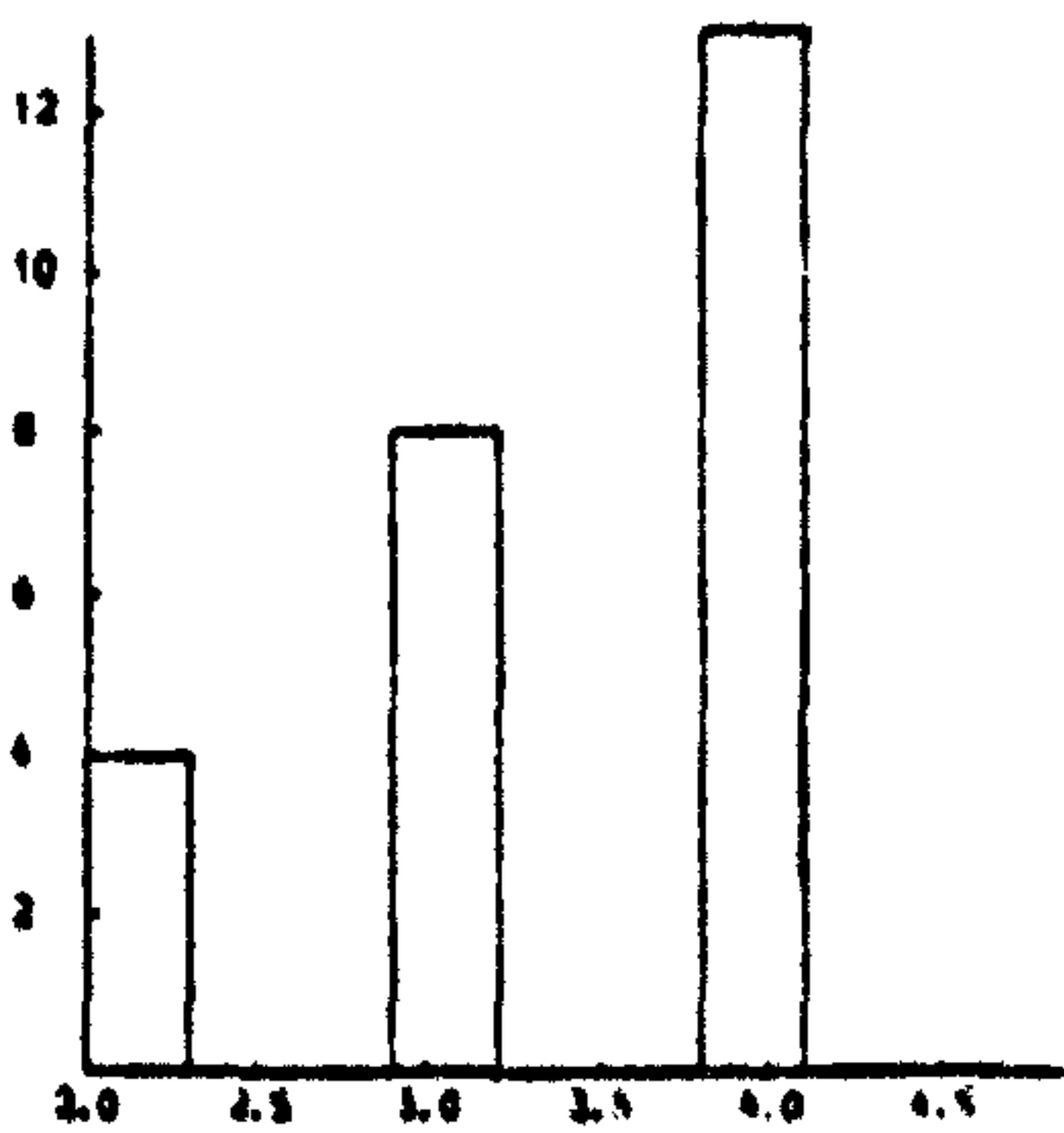
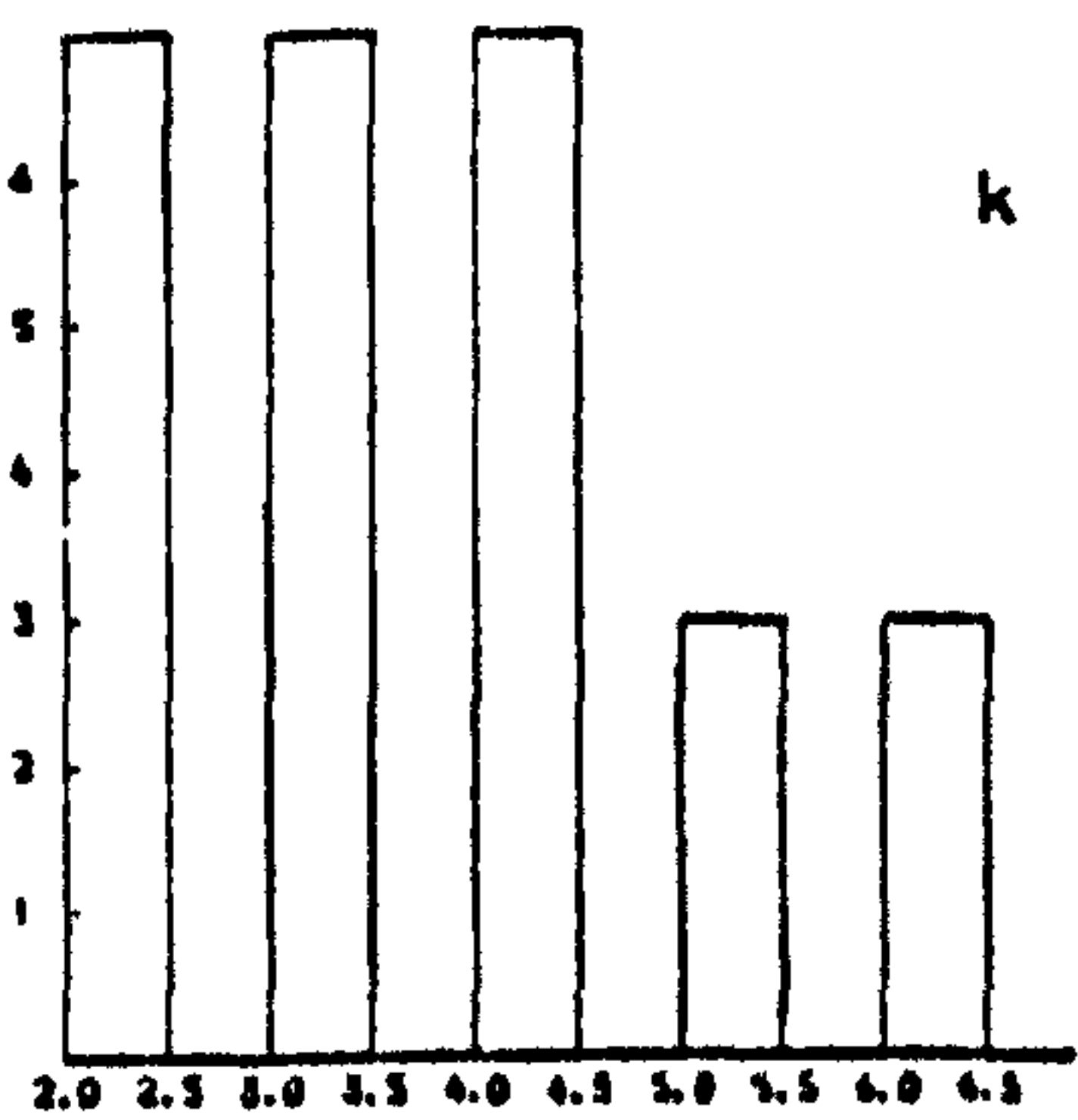
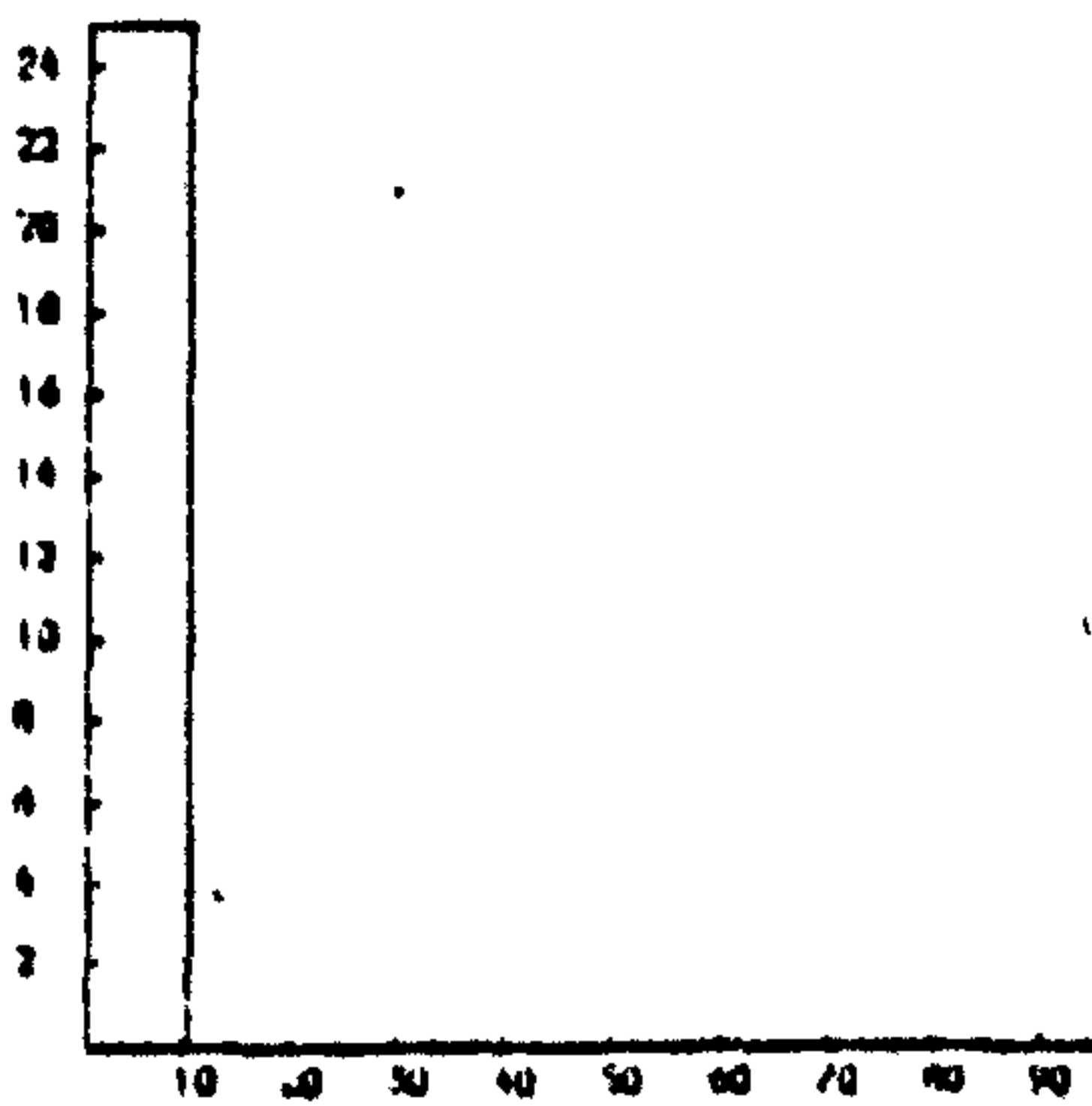
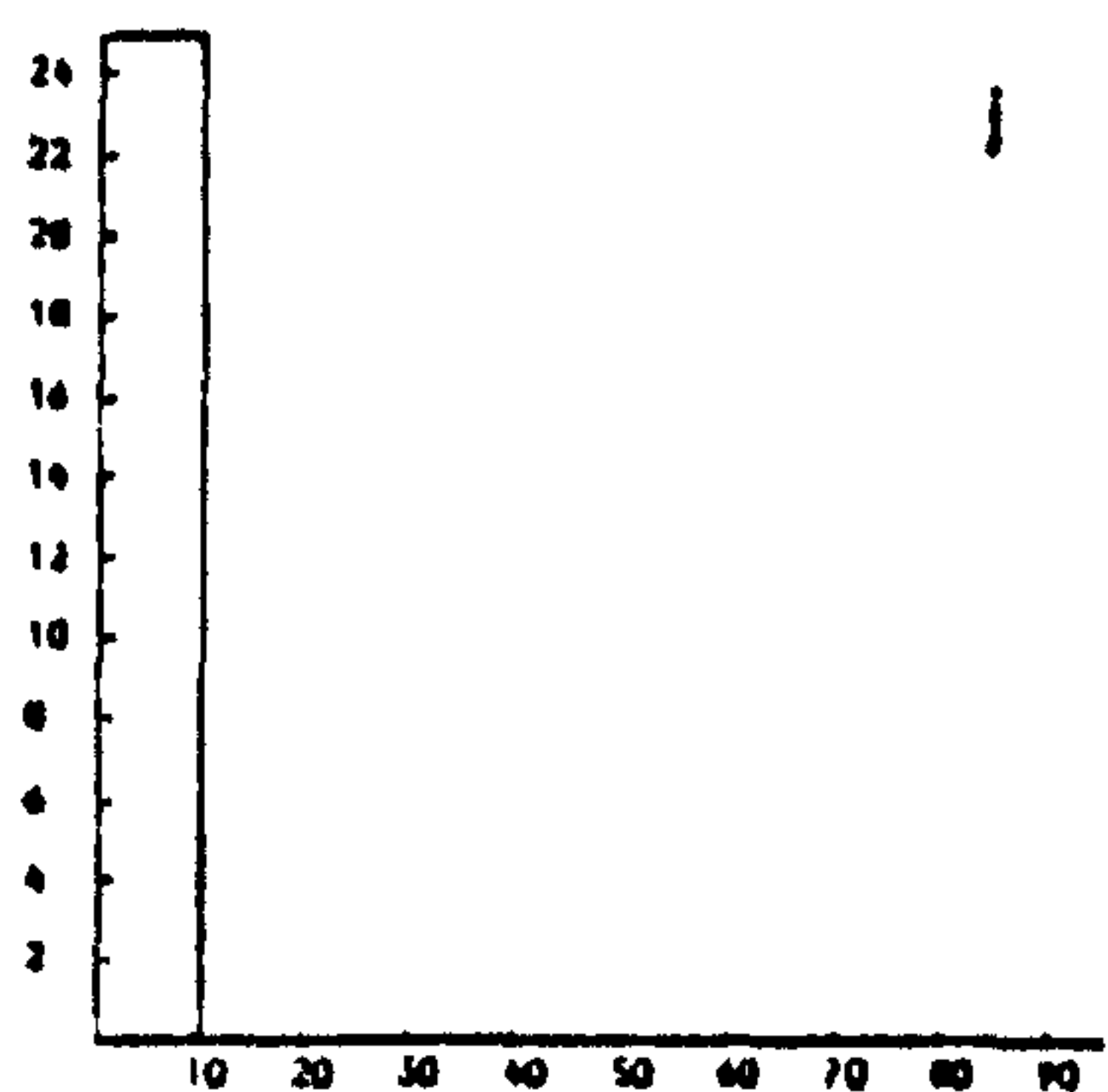
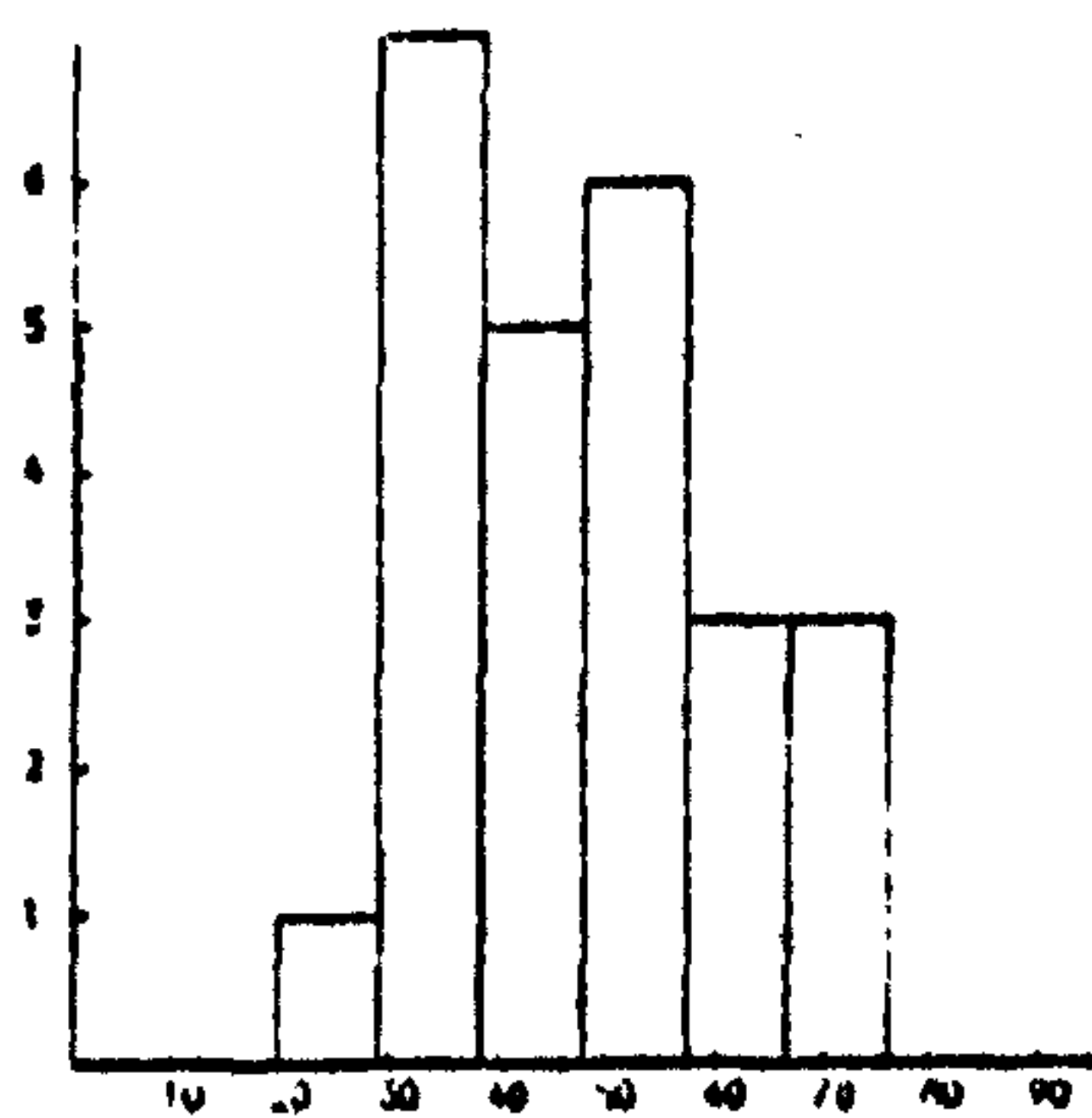
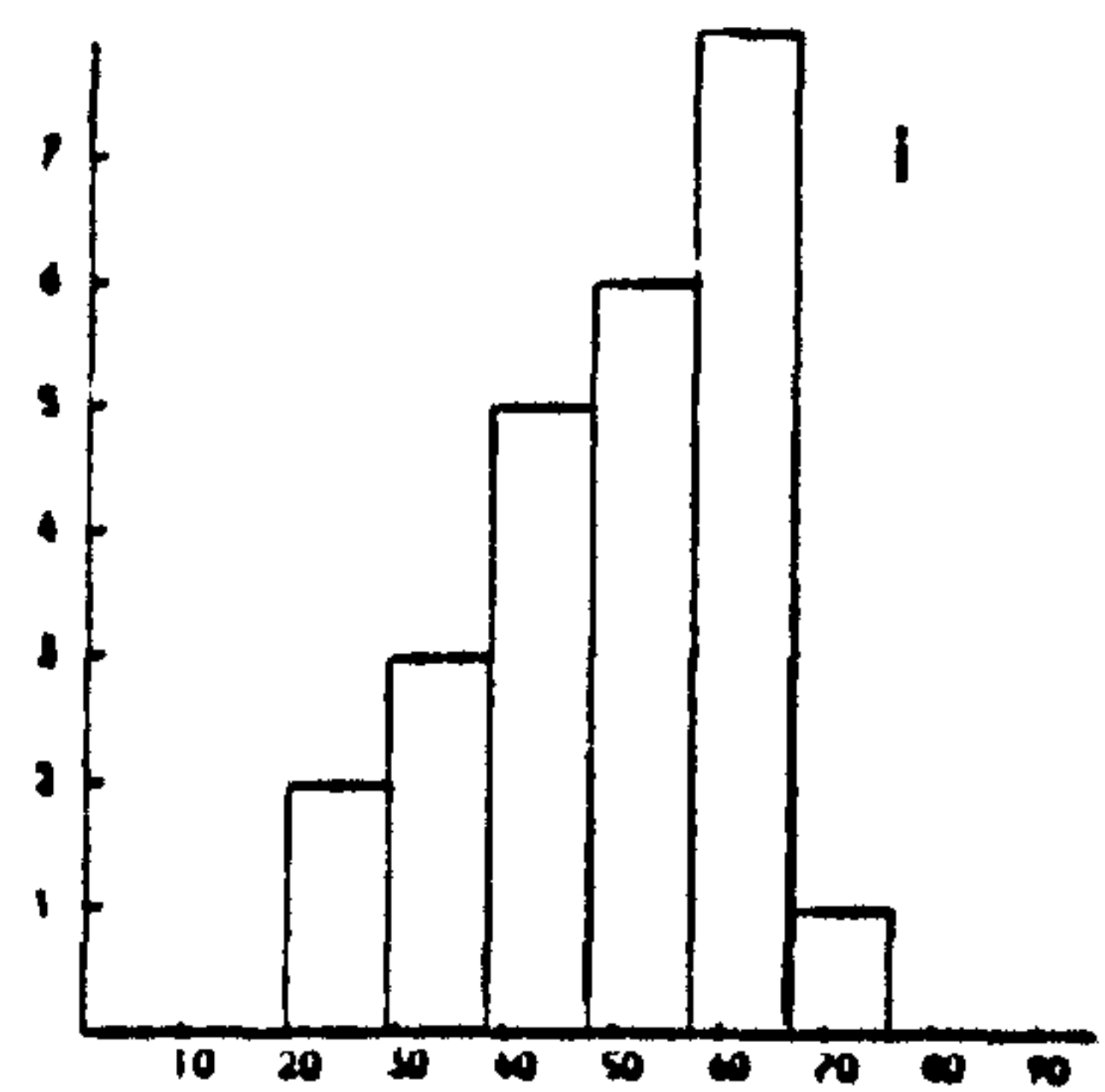
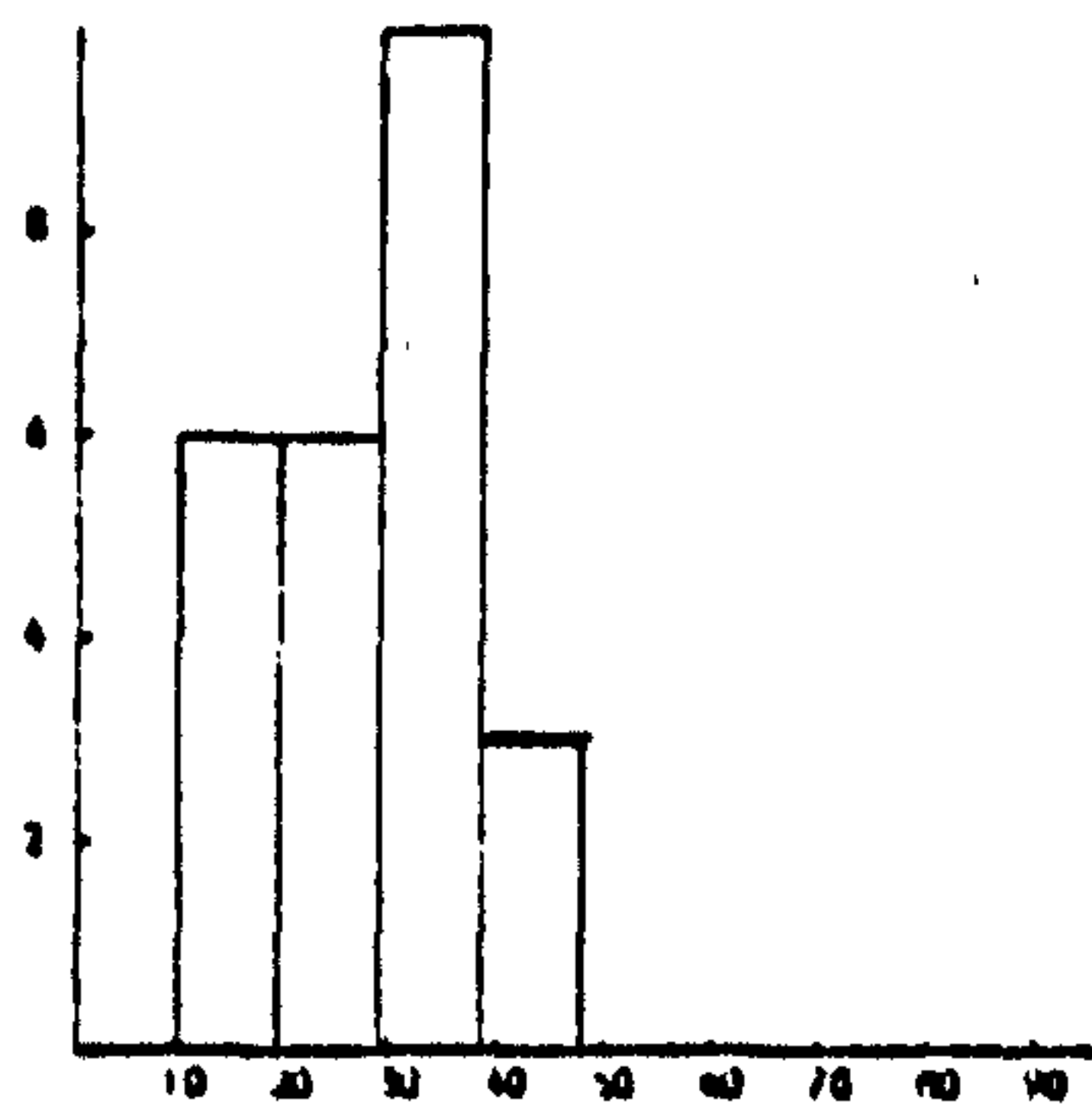
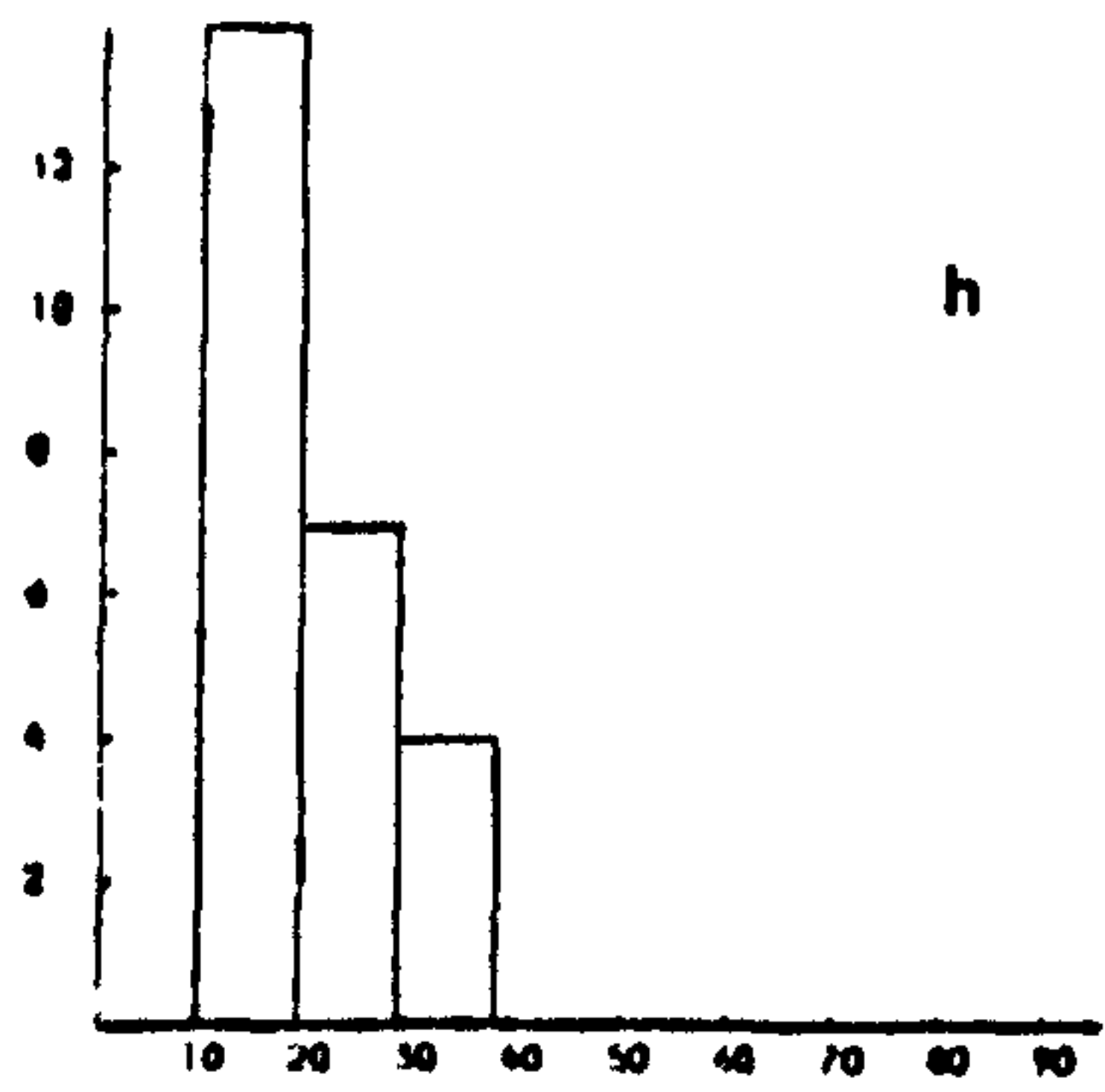
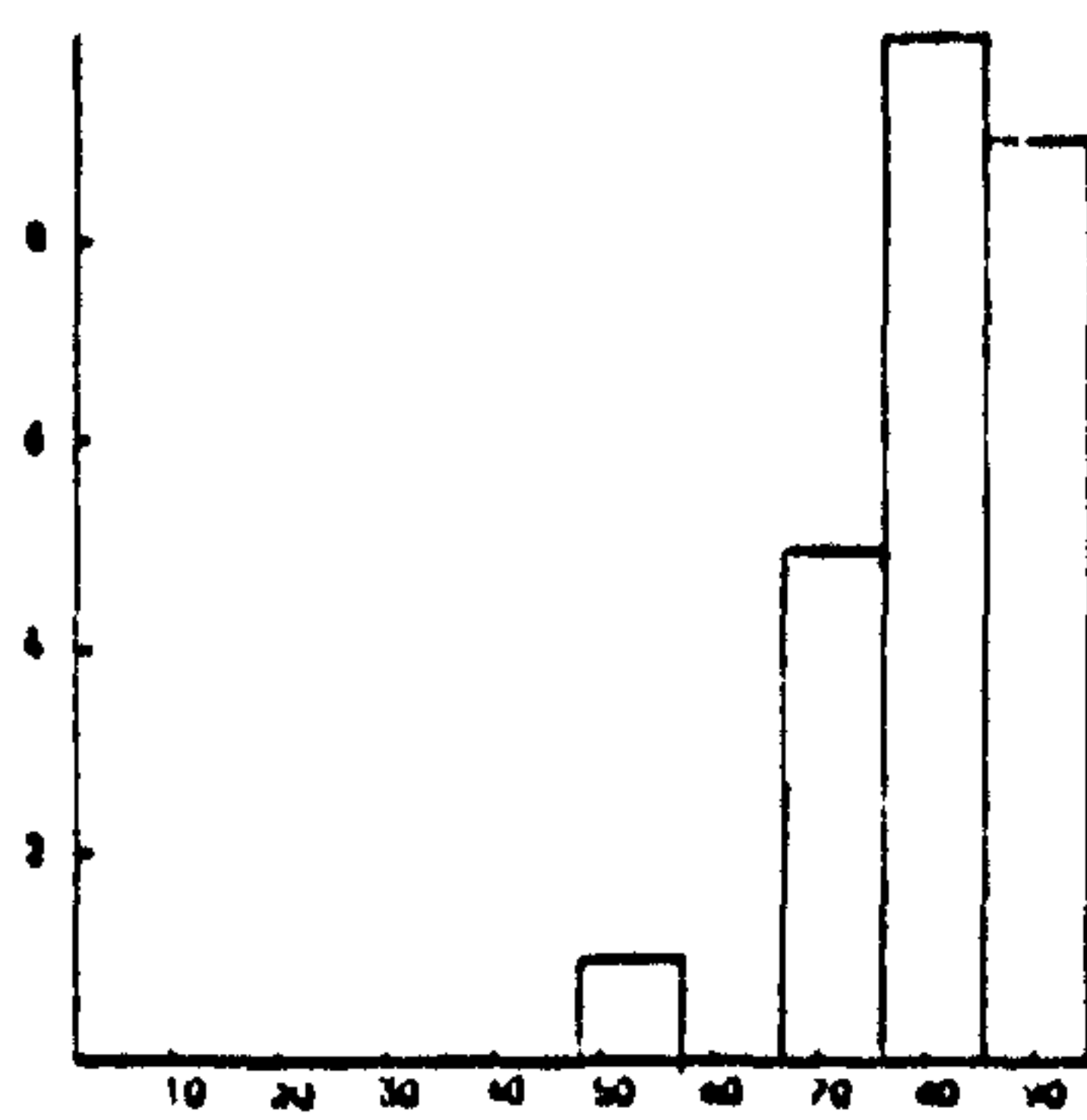
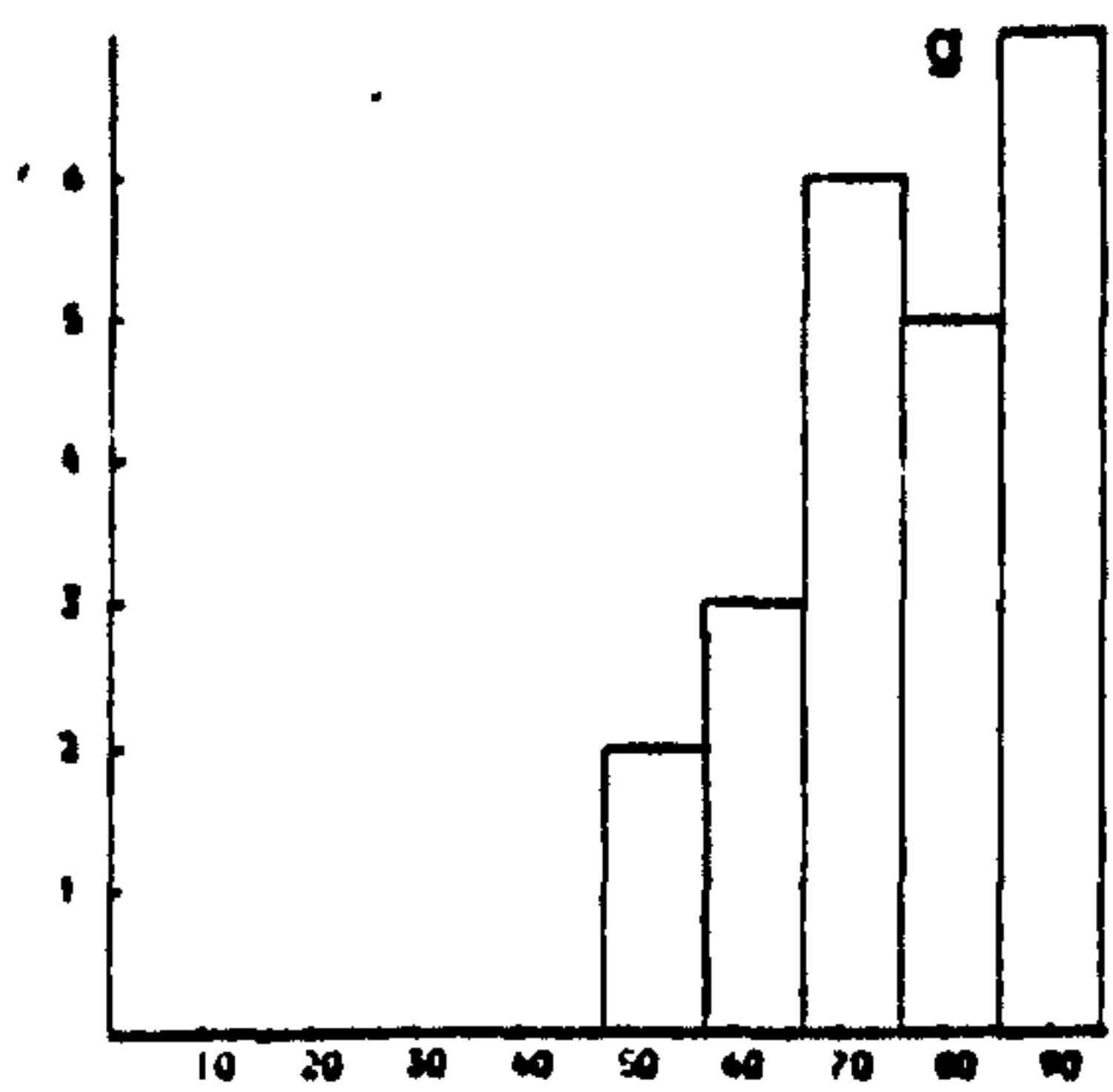




Fig. 49. Histograms illustrating variation of triploid A. undulatum

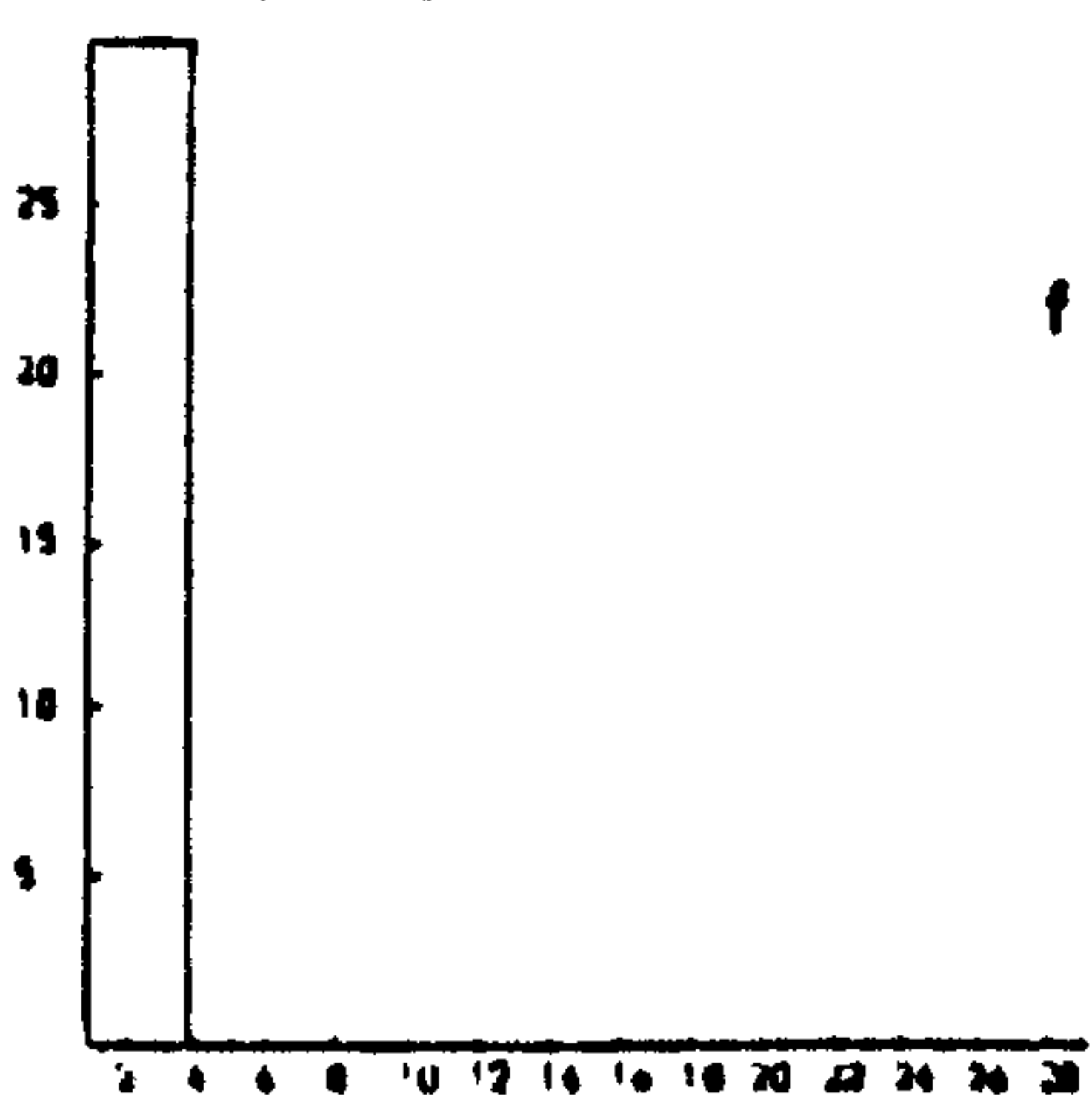
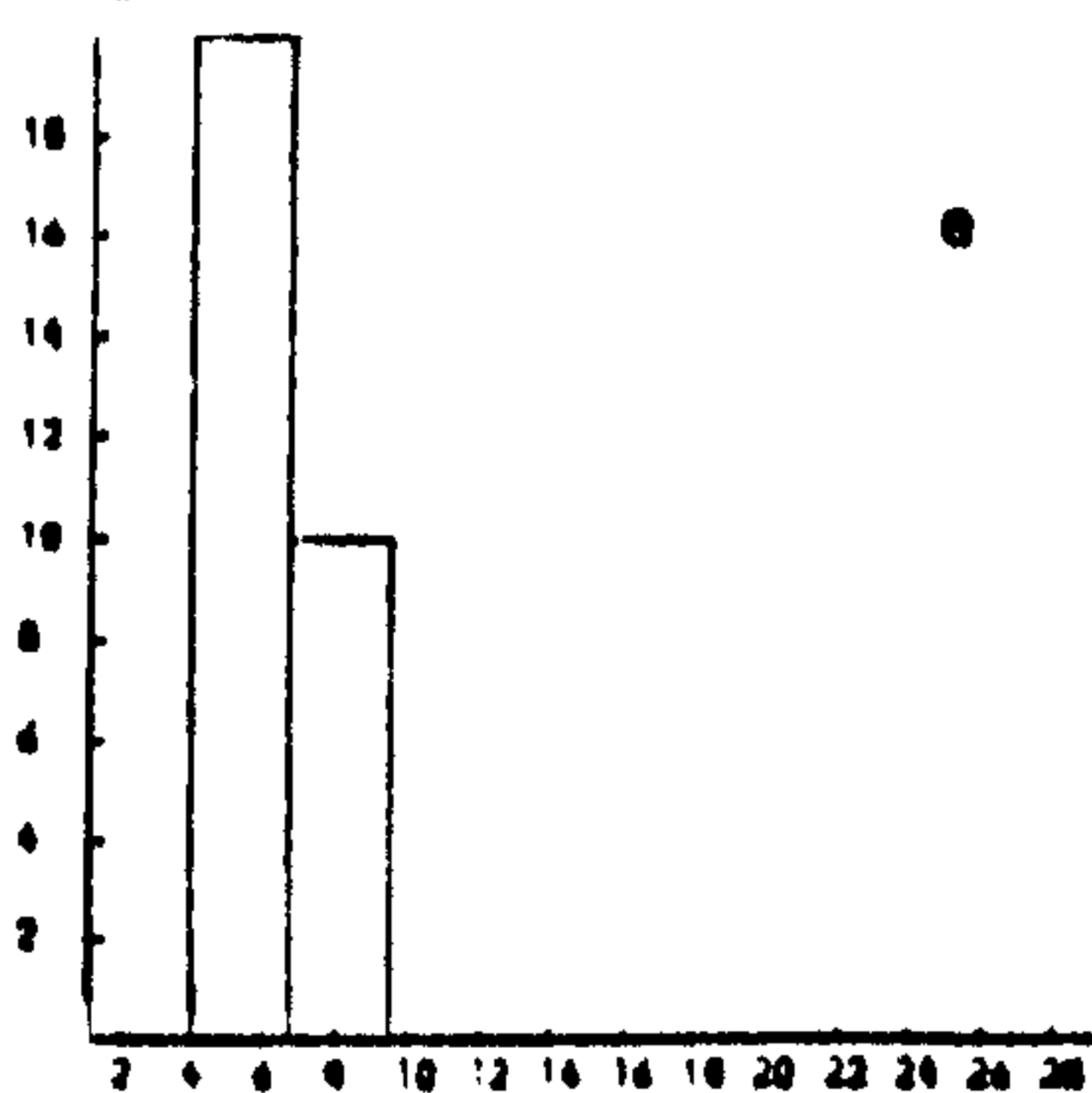
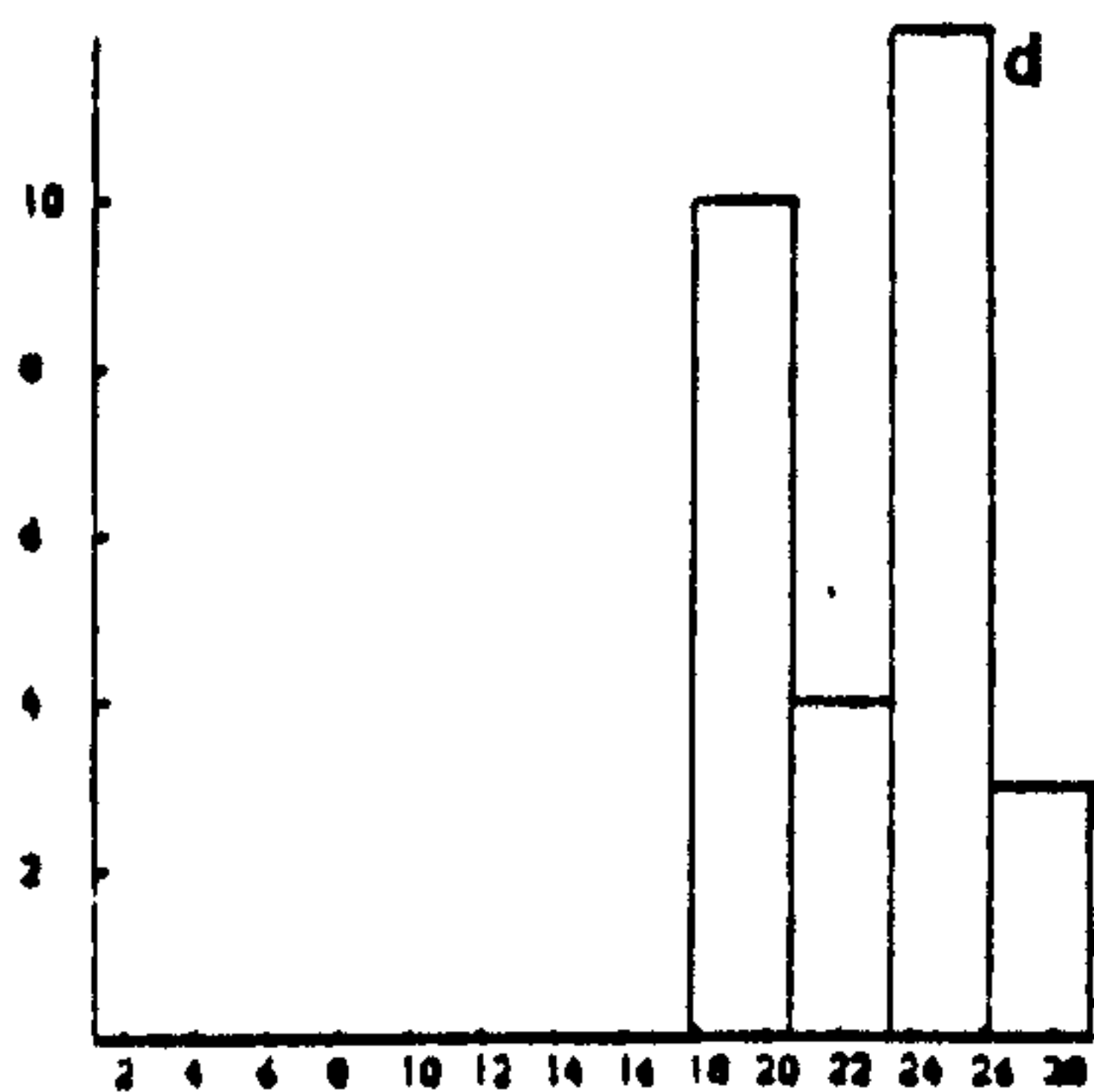
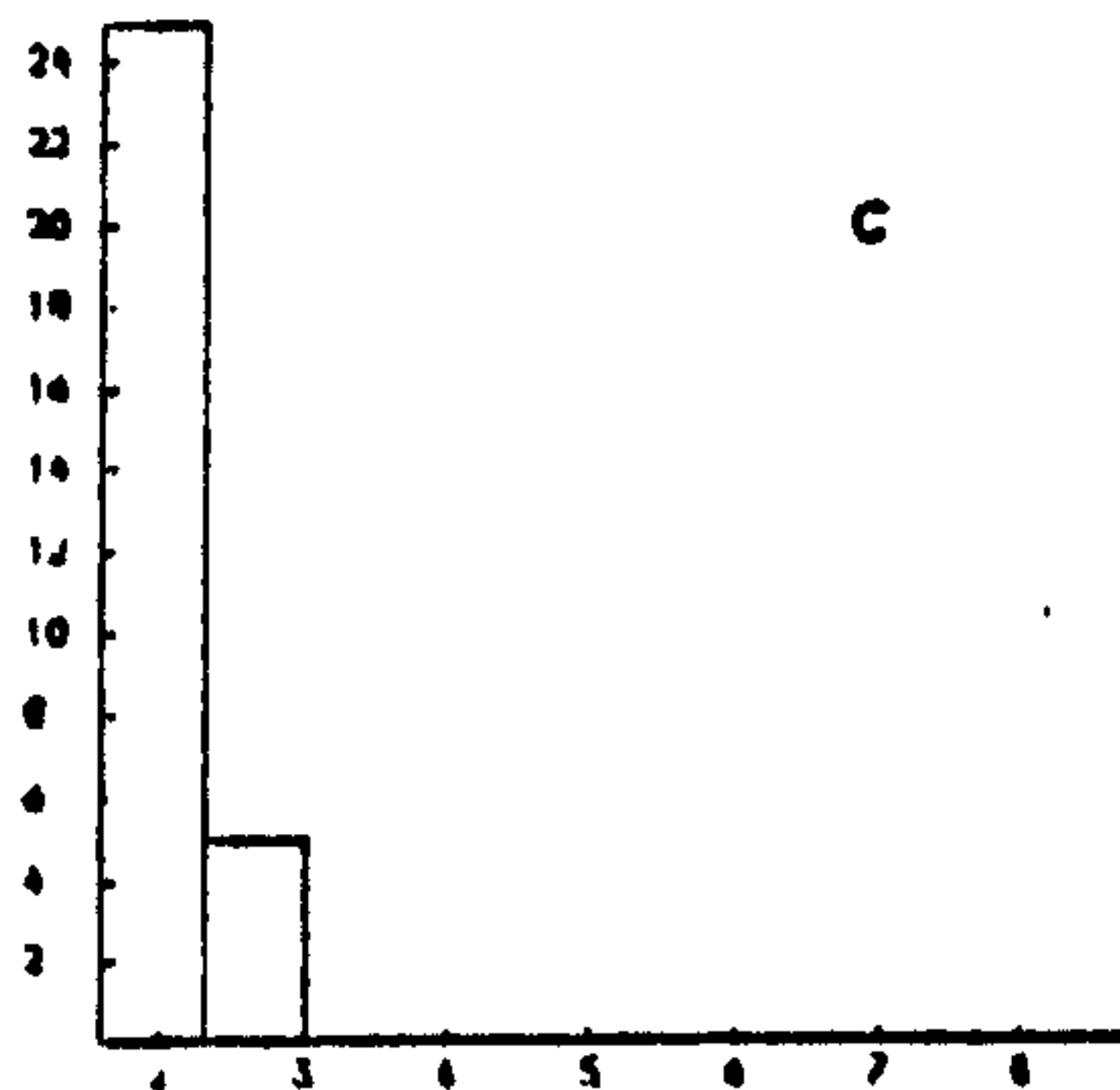
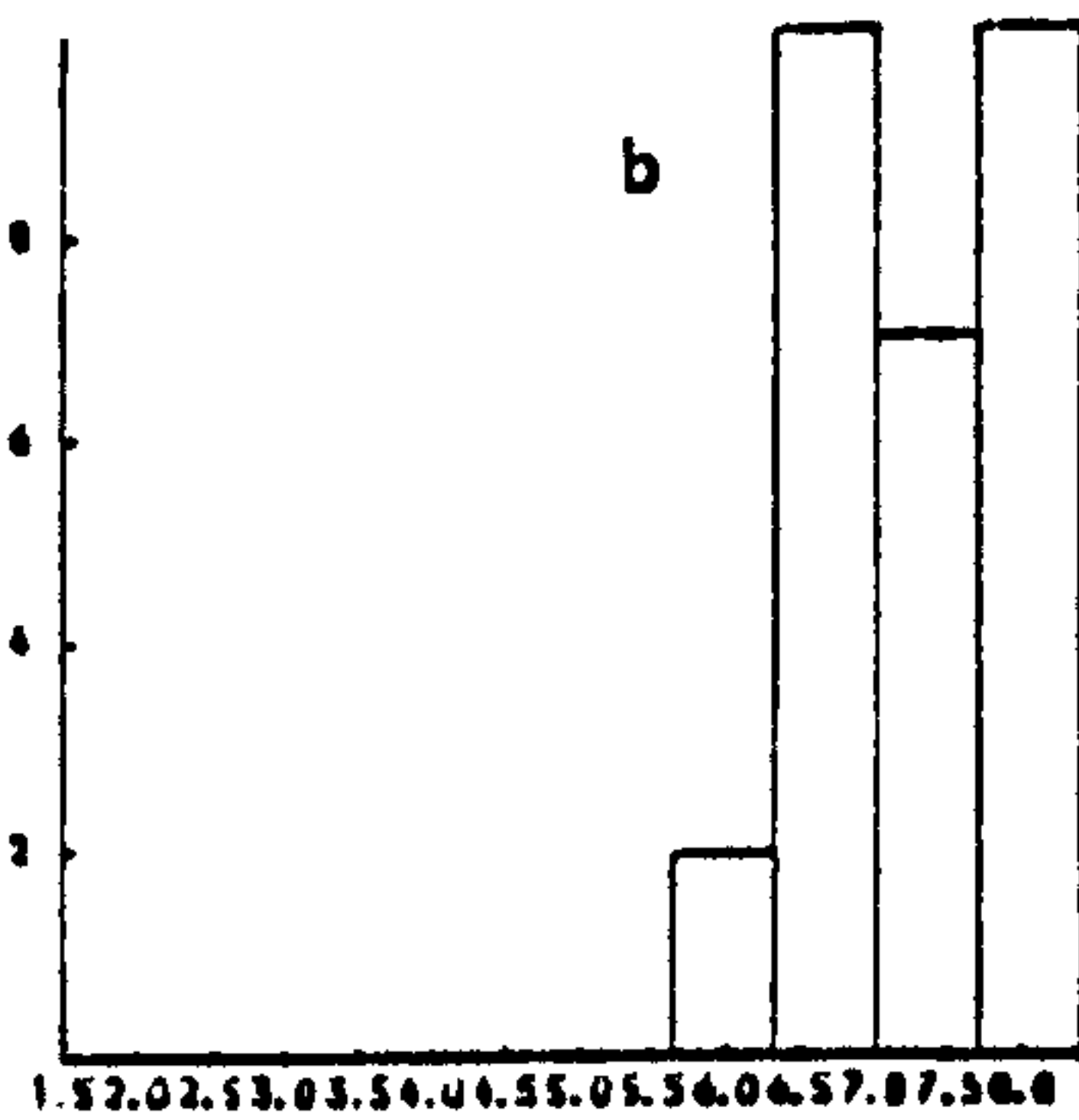
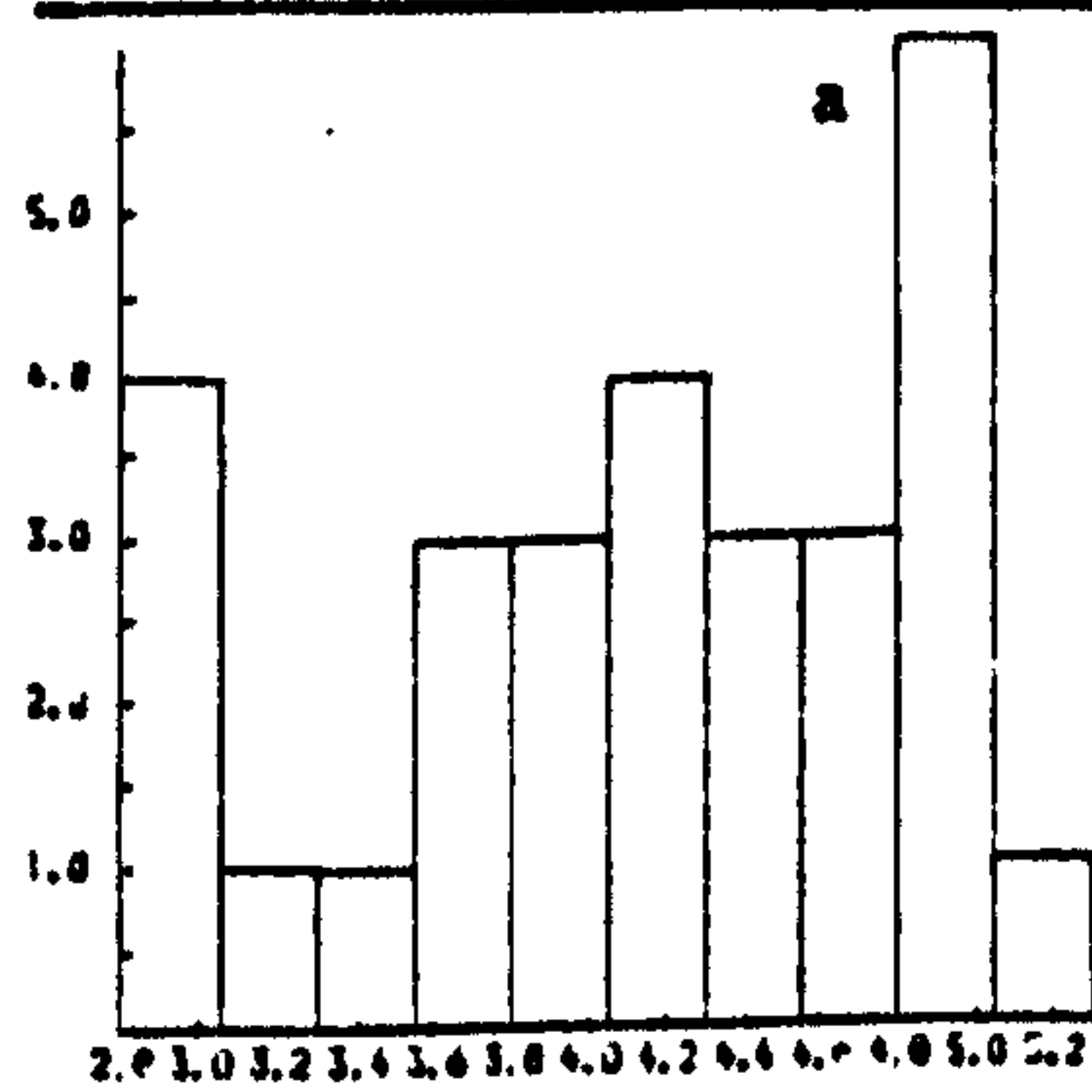
- |   |   |
|---|---|
| (a) plant height;                         | (b) leaf length (from upper part of stem) |
| (c) leaf width (from upper part of stem); | (d) cell width (from upper part of stem); |
| (e) leaf length (from mid-stem) ;         | (f) leaf width (from mid-stem) ;          |
| (g) percentage of serration;              | (h) the angle of the acumen ;             |
| (i) tooth angle;                          | (j) tooth long;                           |
| (k) number of lamellar cells ;            | (l) height of lamellar cells .            |

Note that the Y-axis is not identical in all cases.

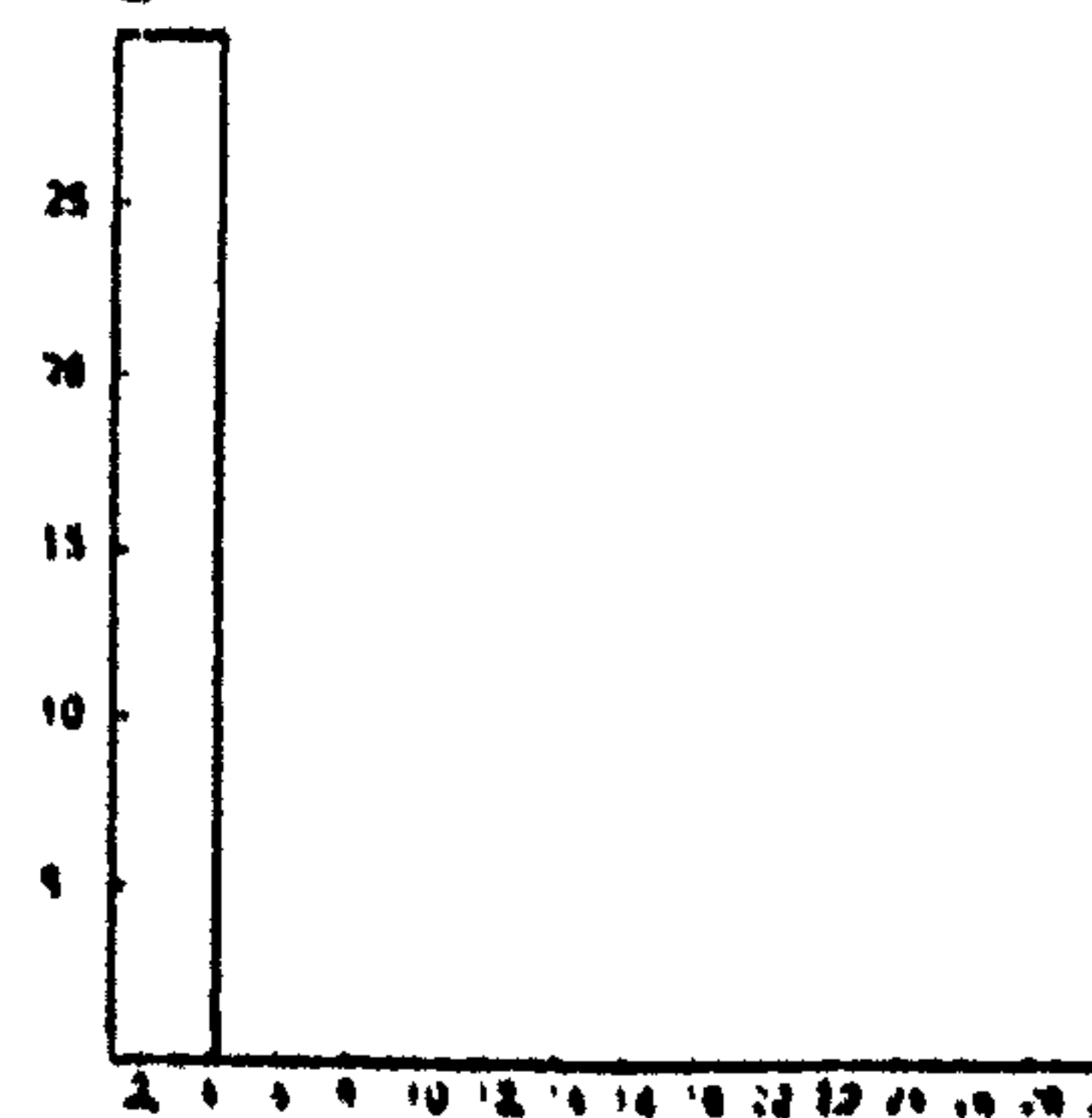
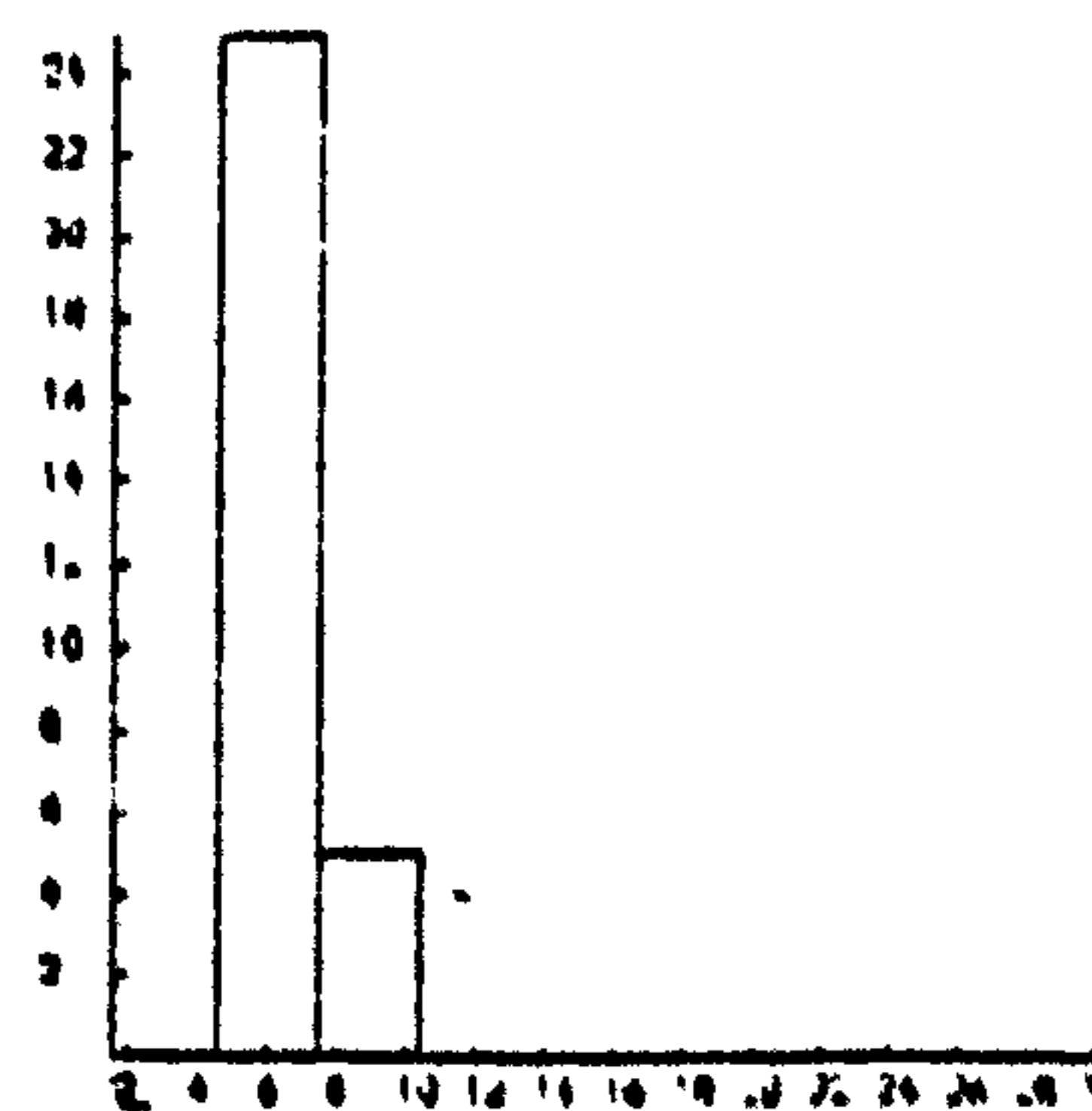
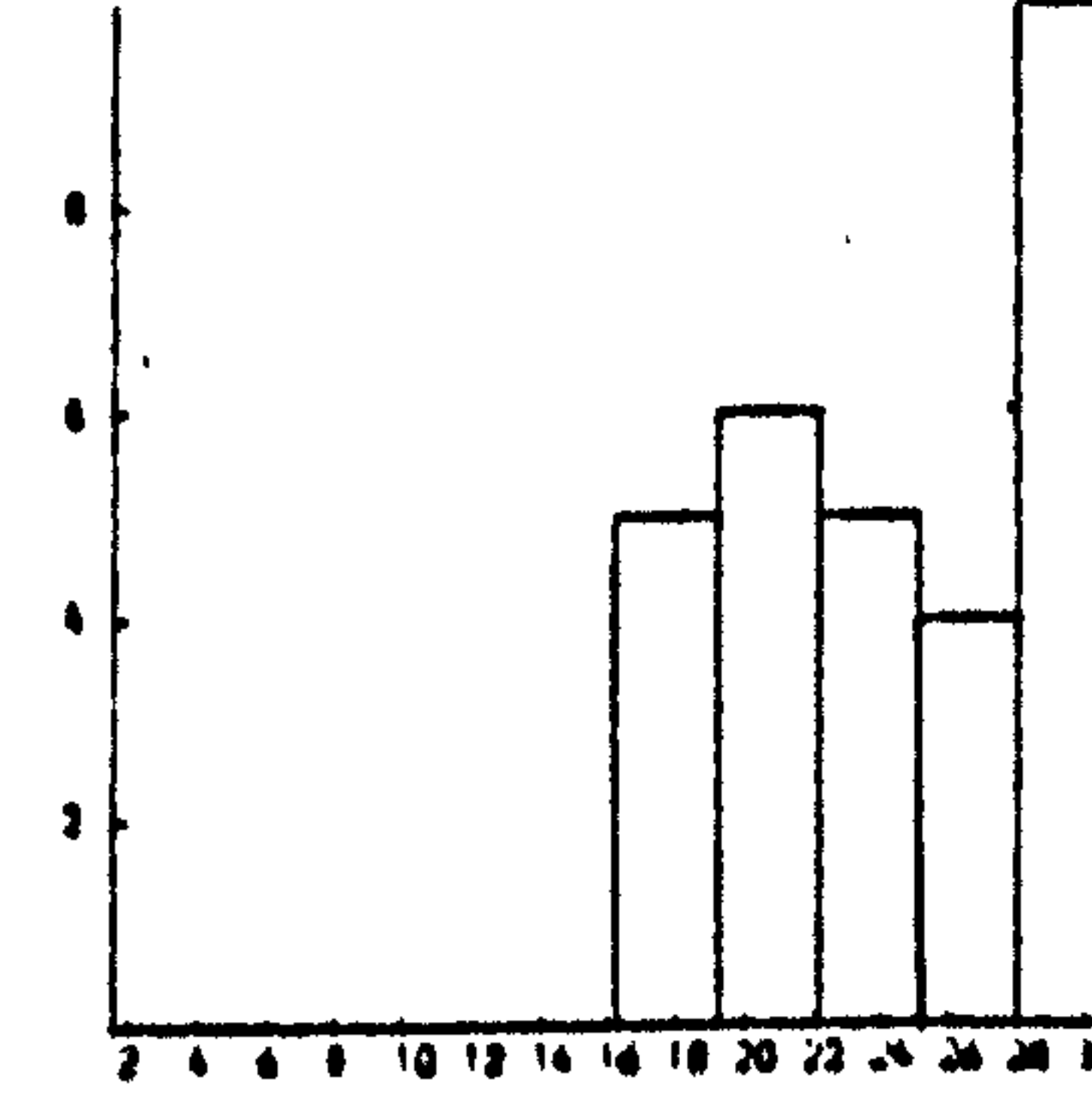
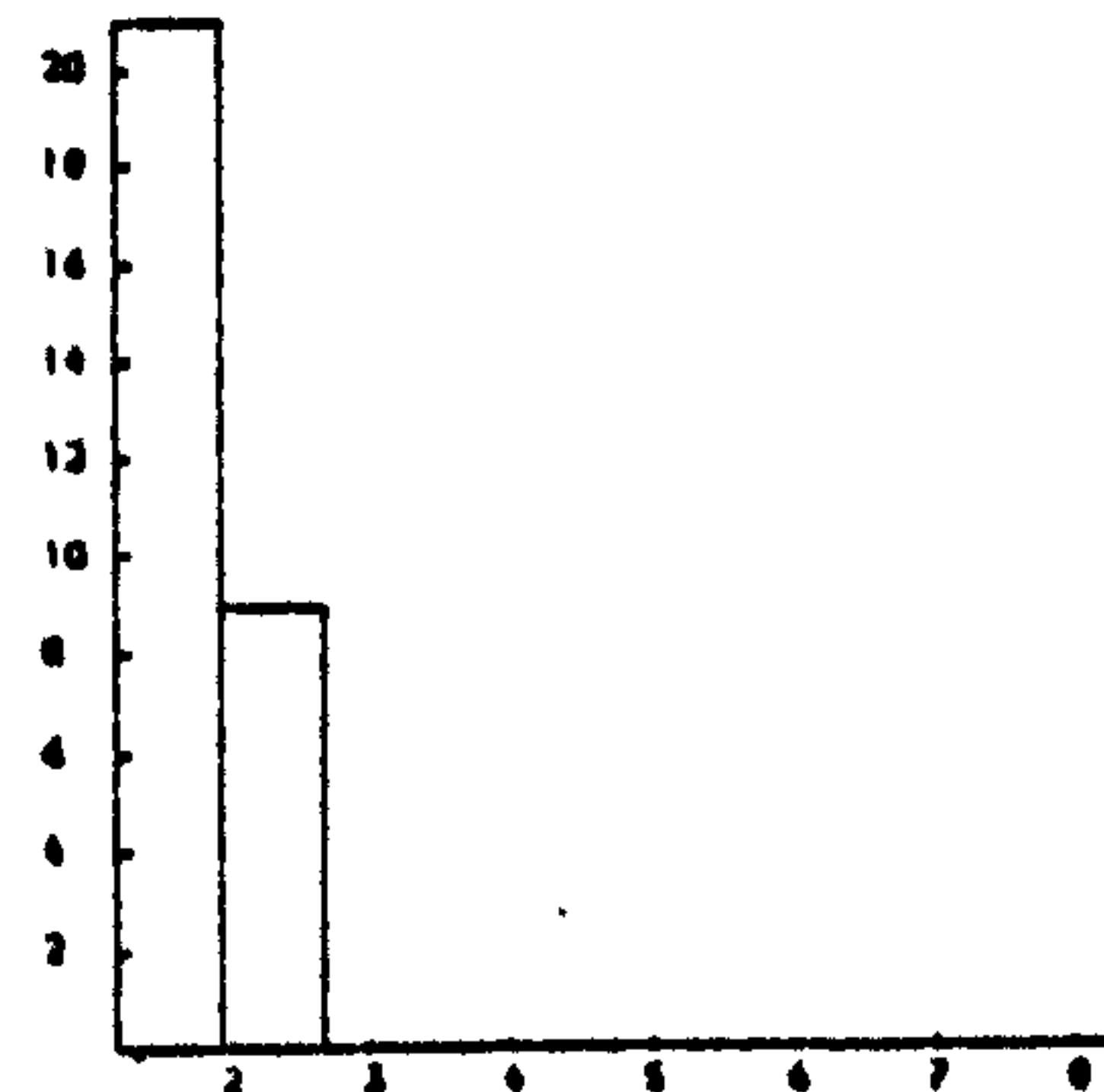
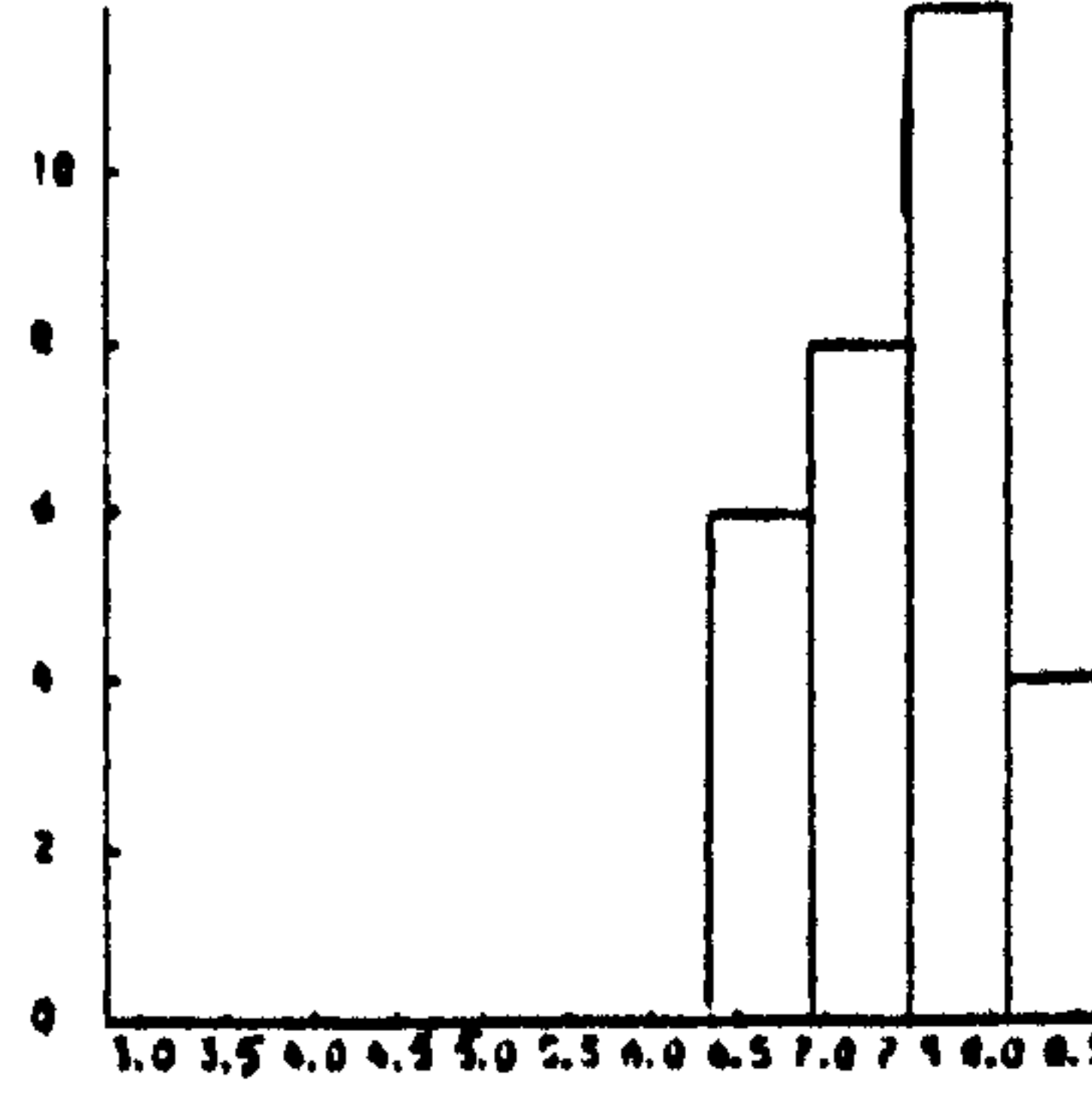
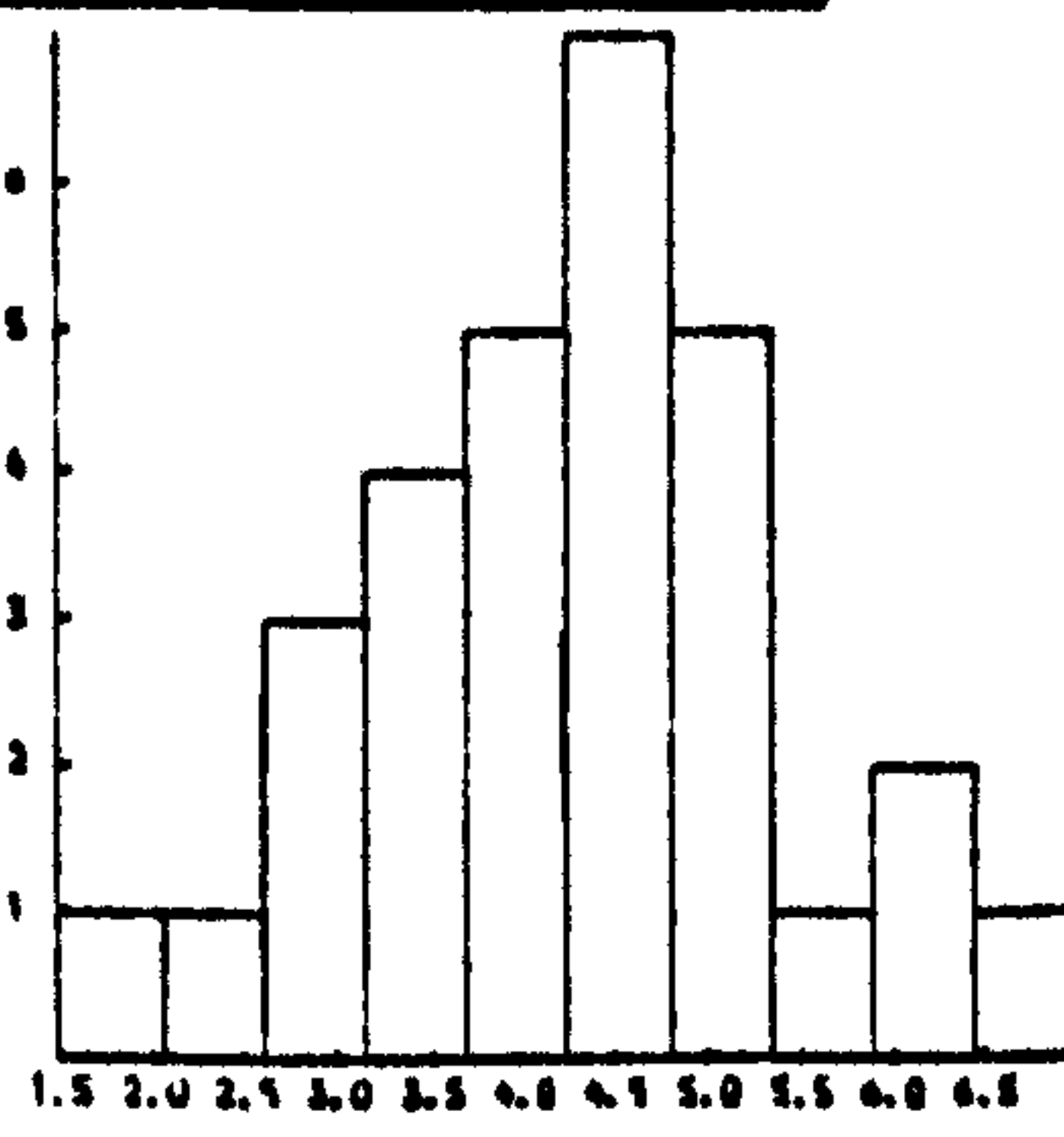
Character (a) in mm; for b, c, e & f, 1 unit = 0.43 mm; d, in  $\mu\text{m}$ ;

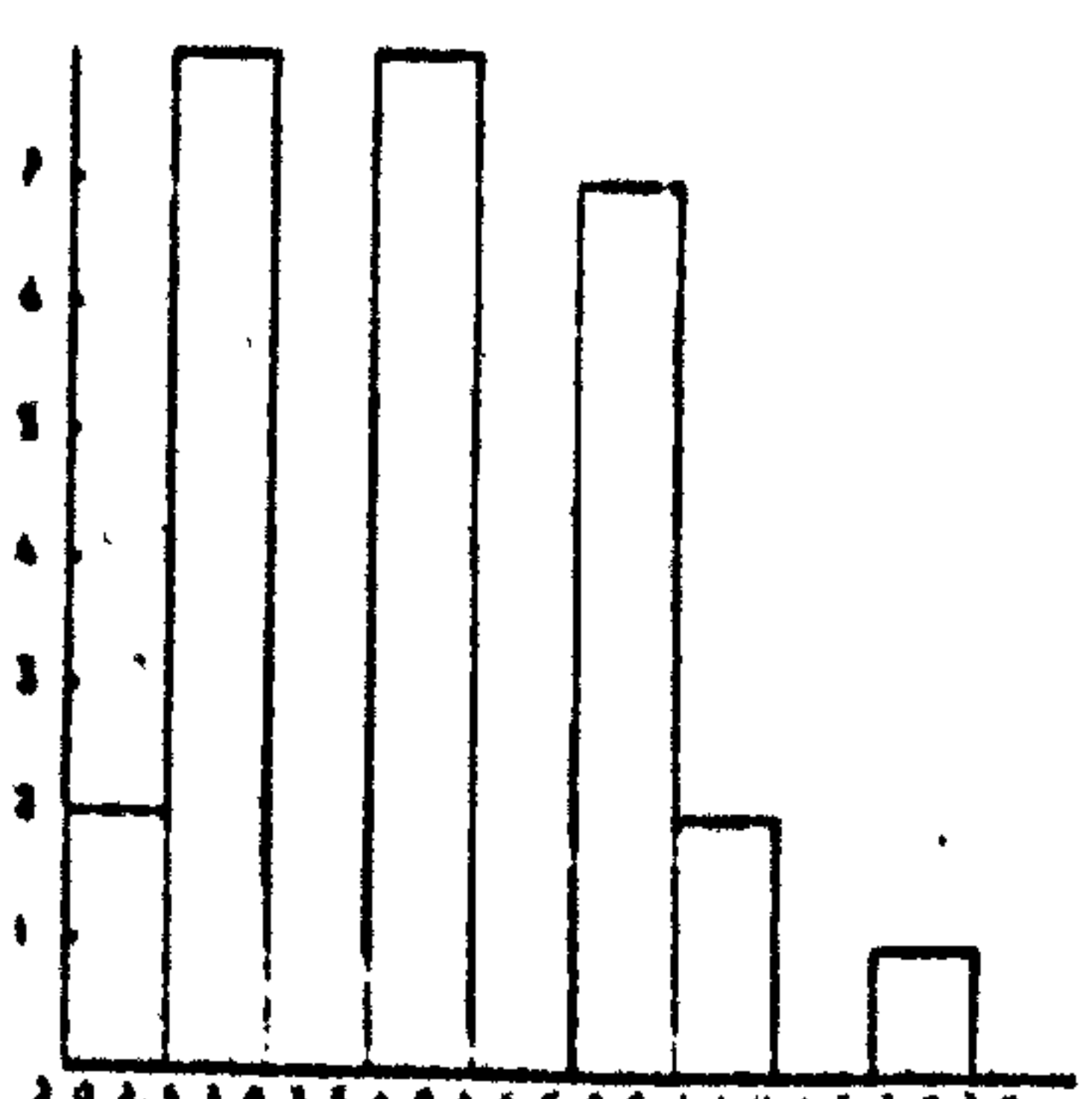
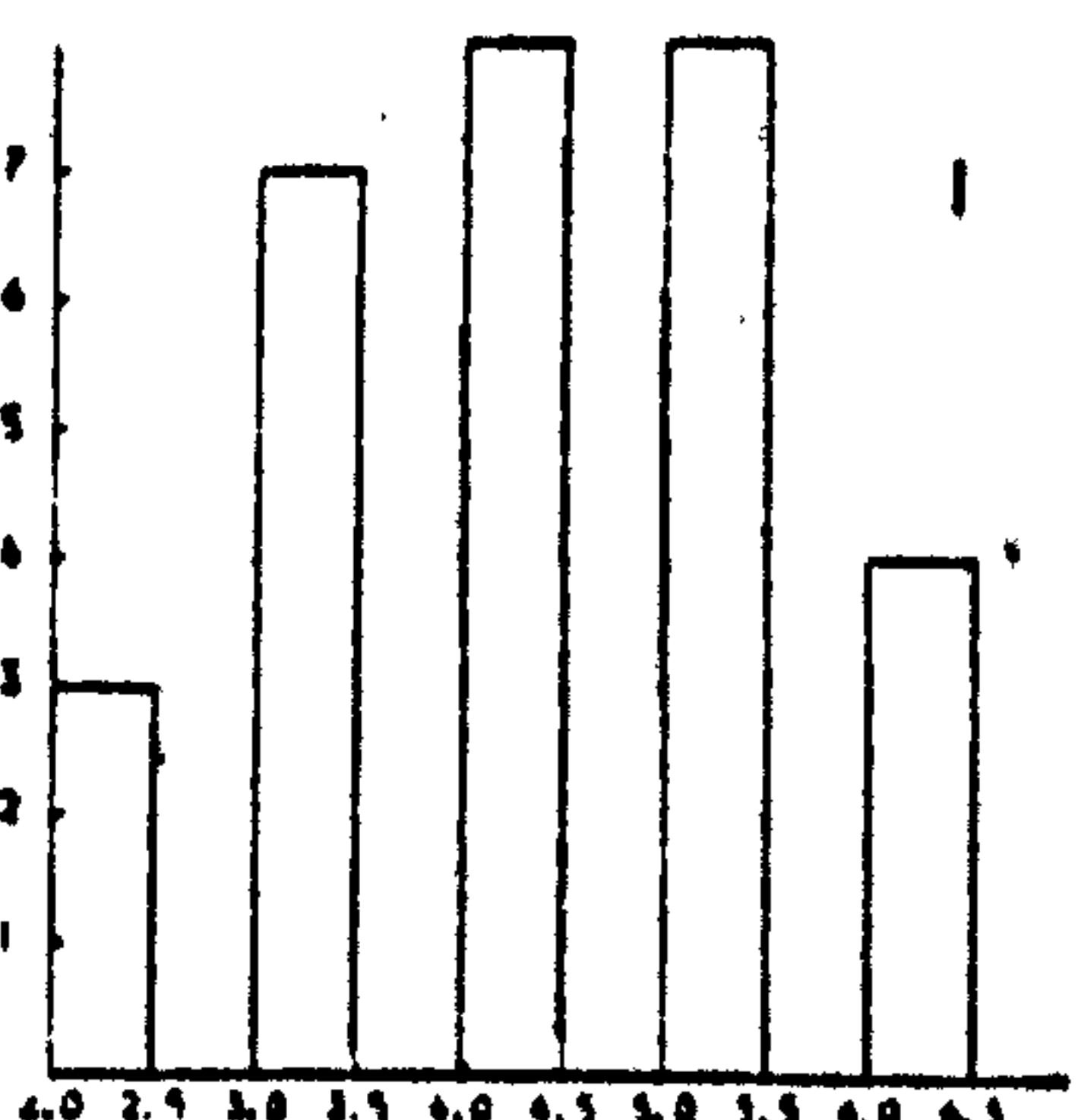
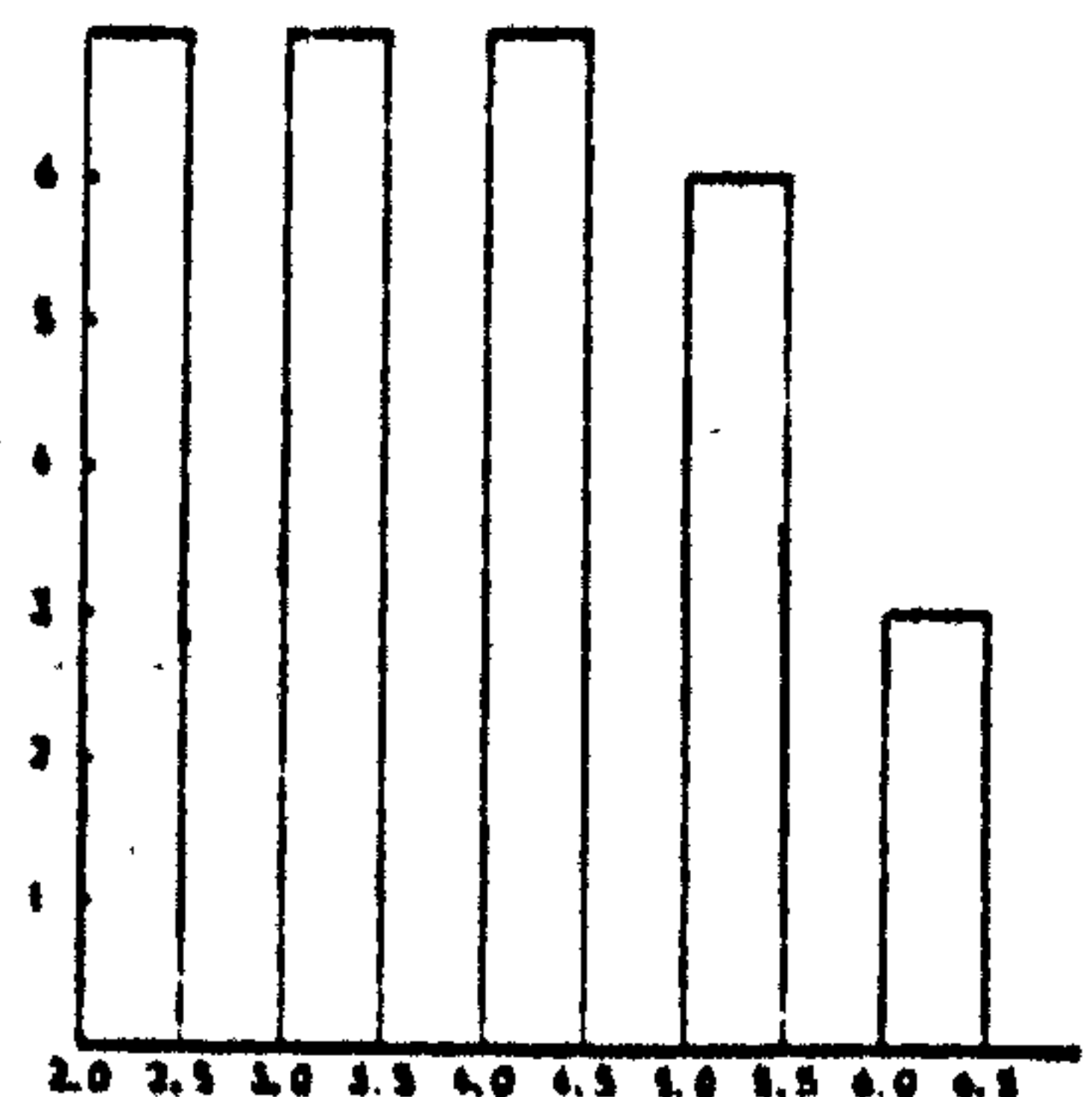
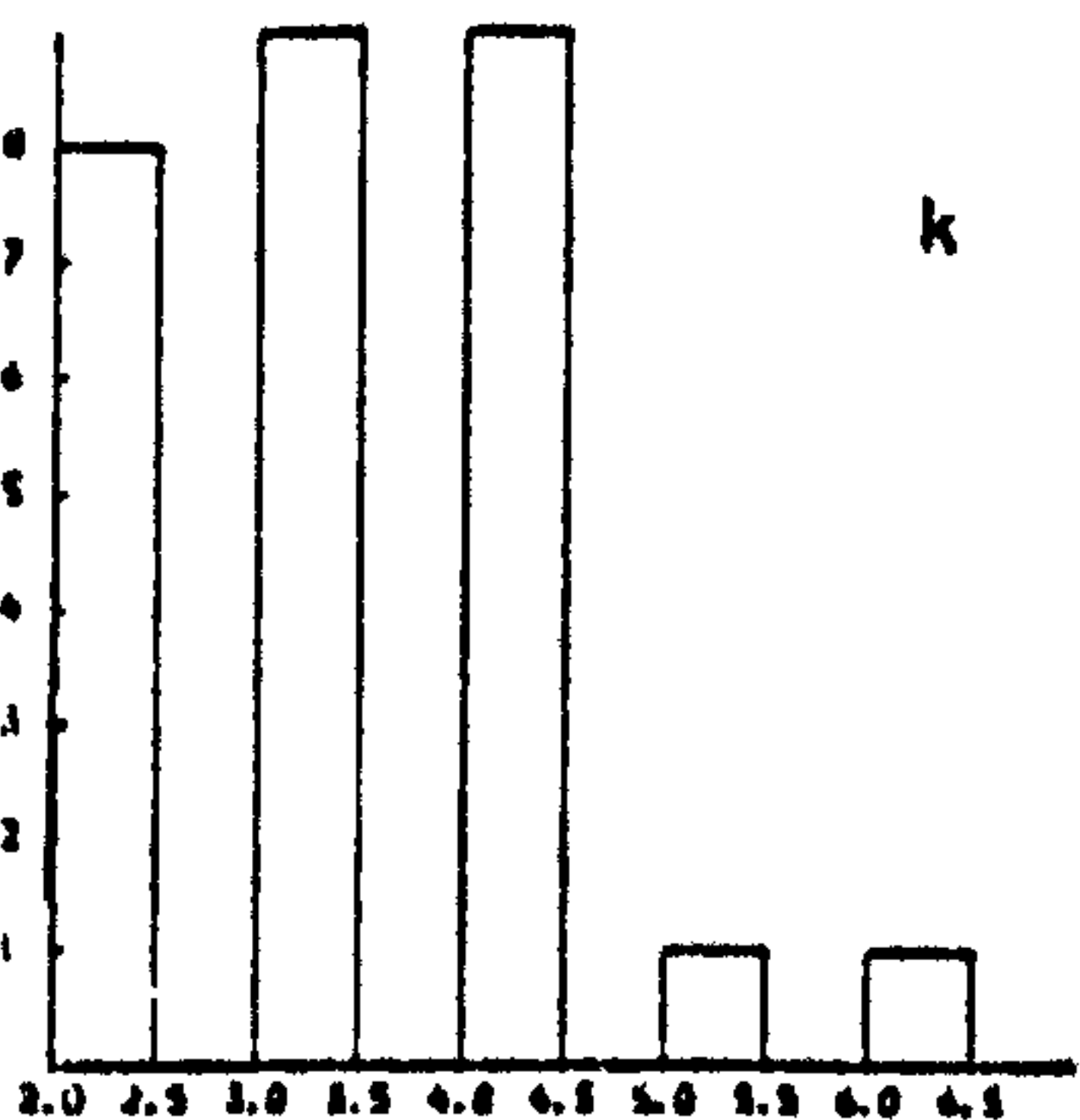
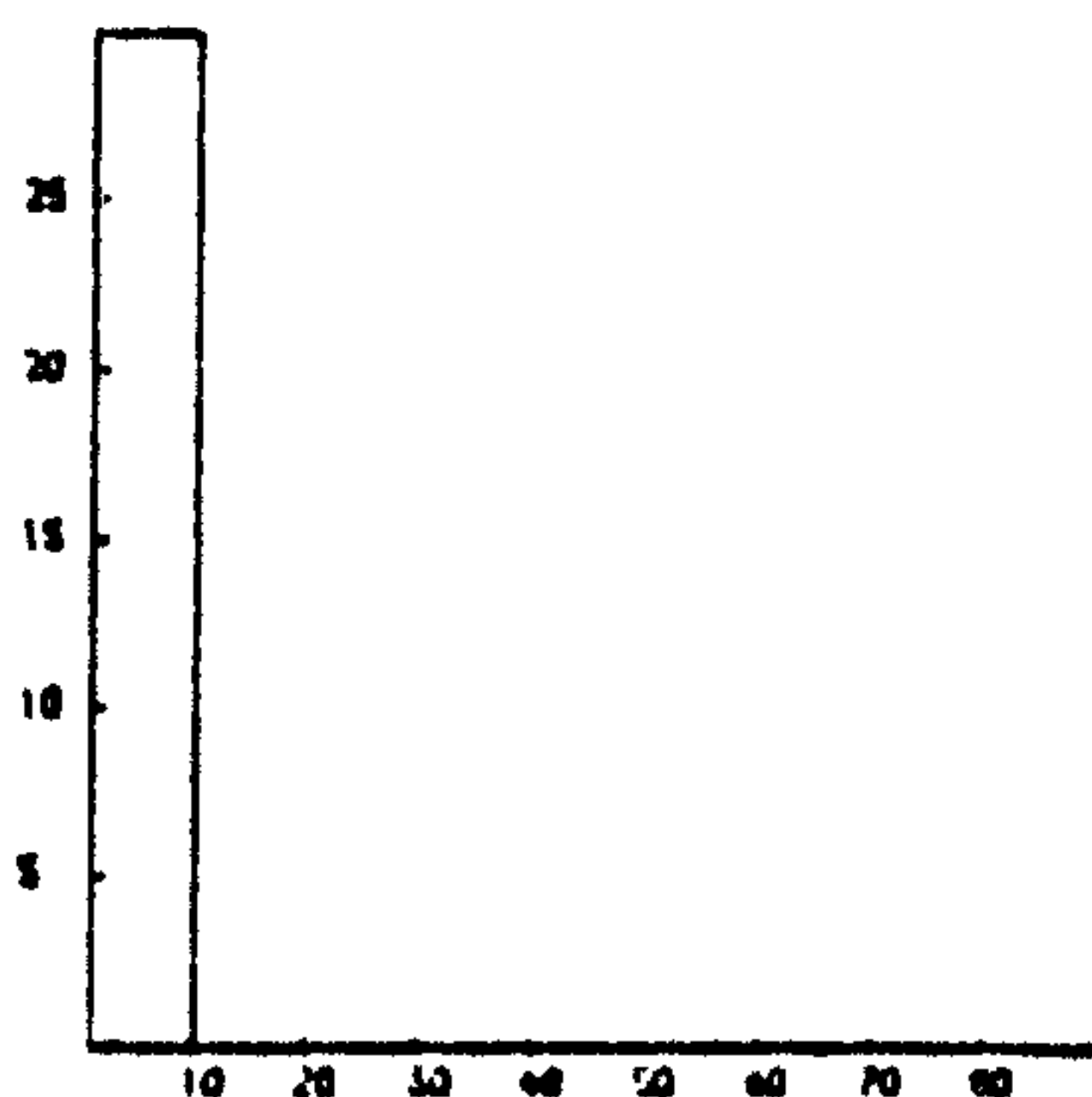
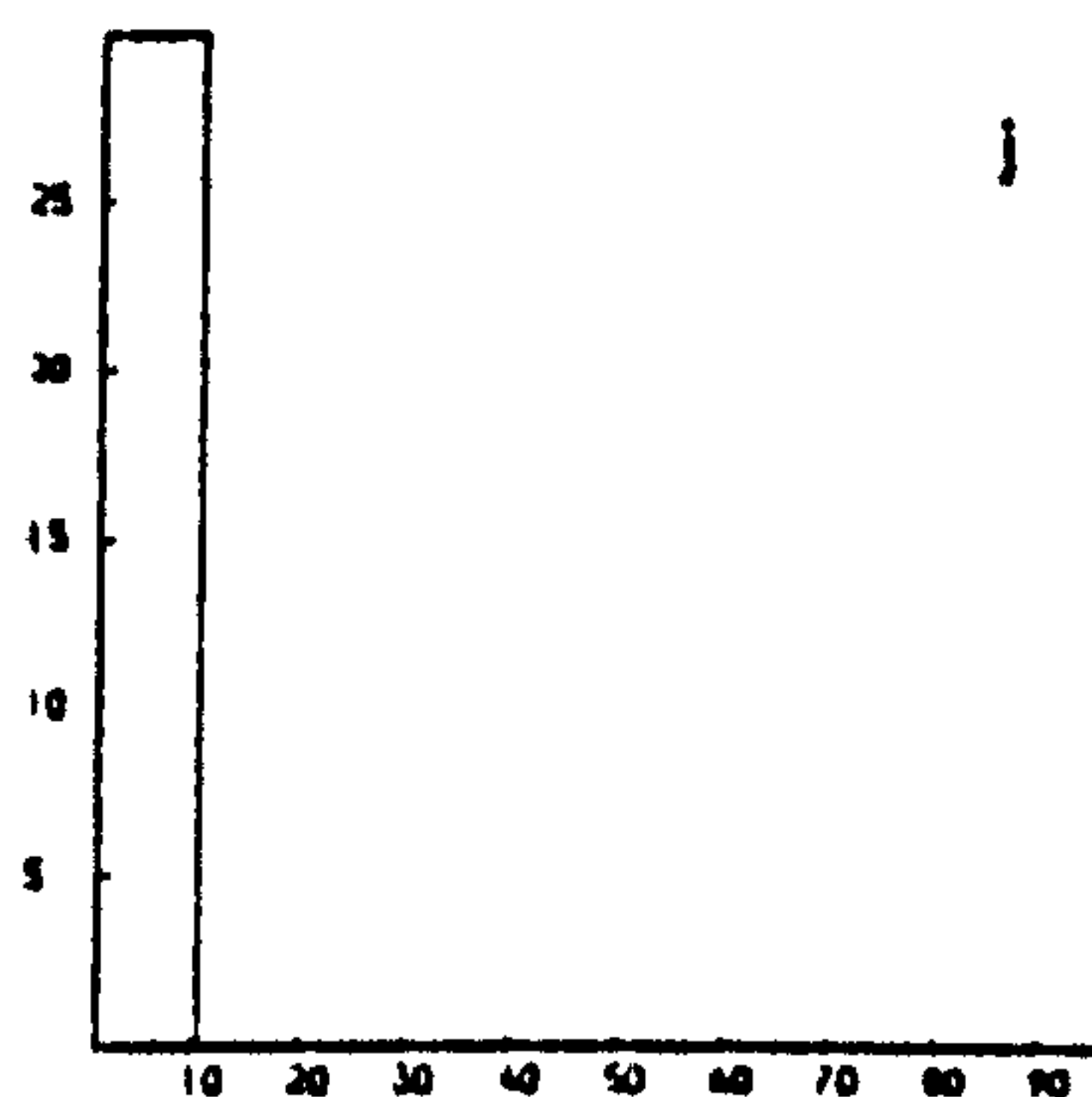
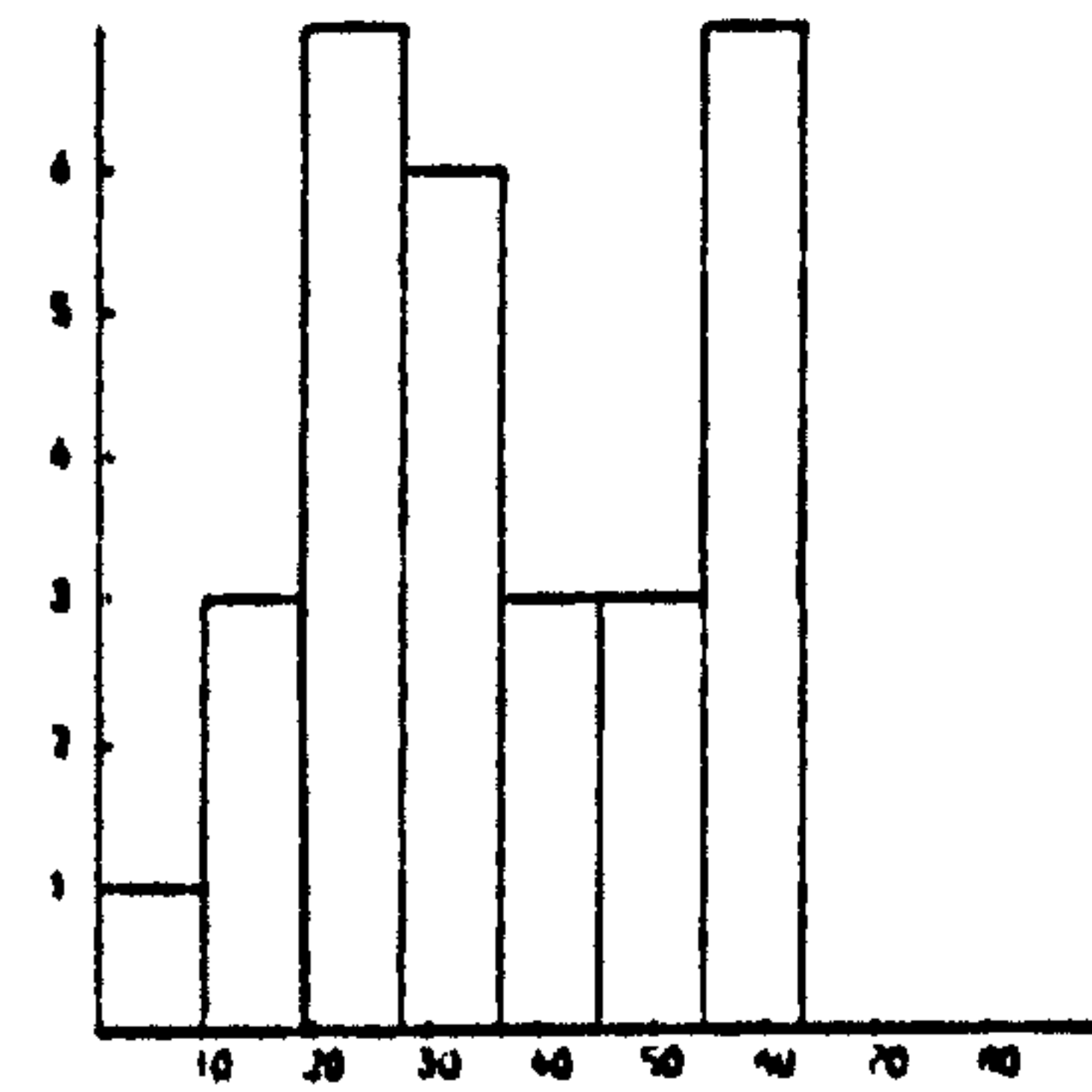
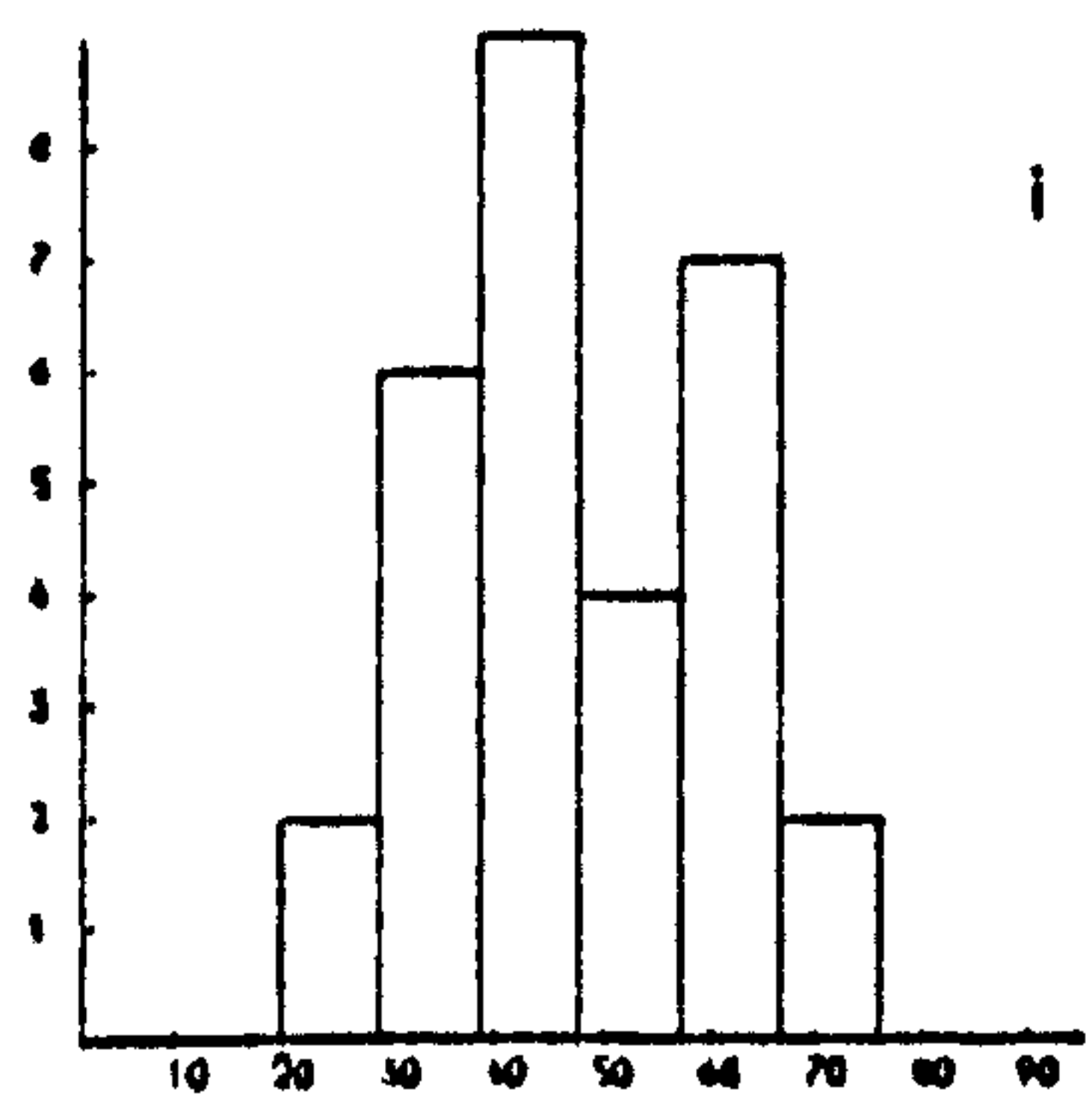
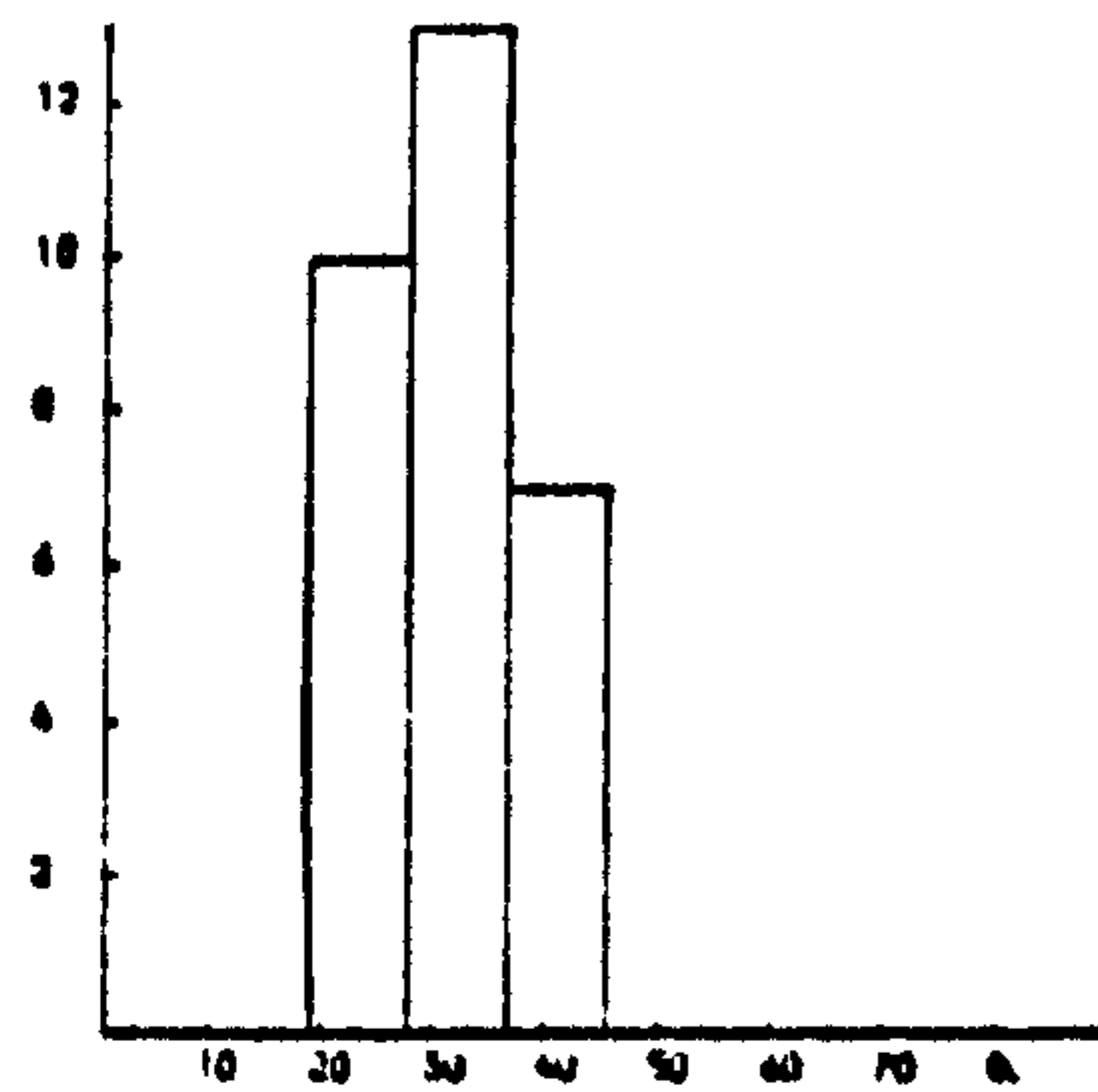
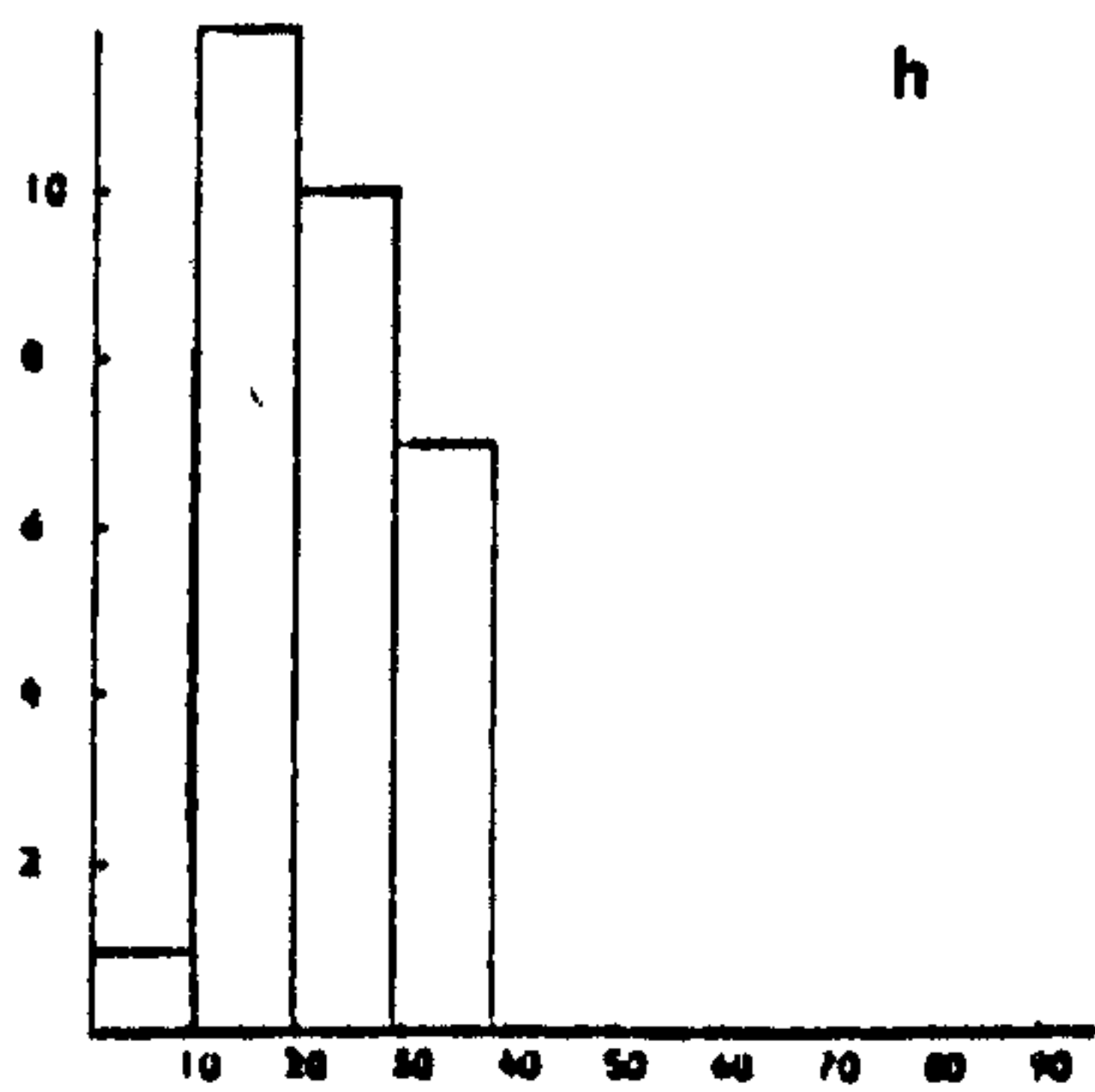
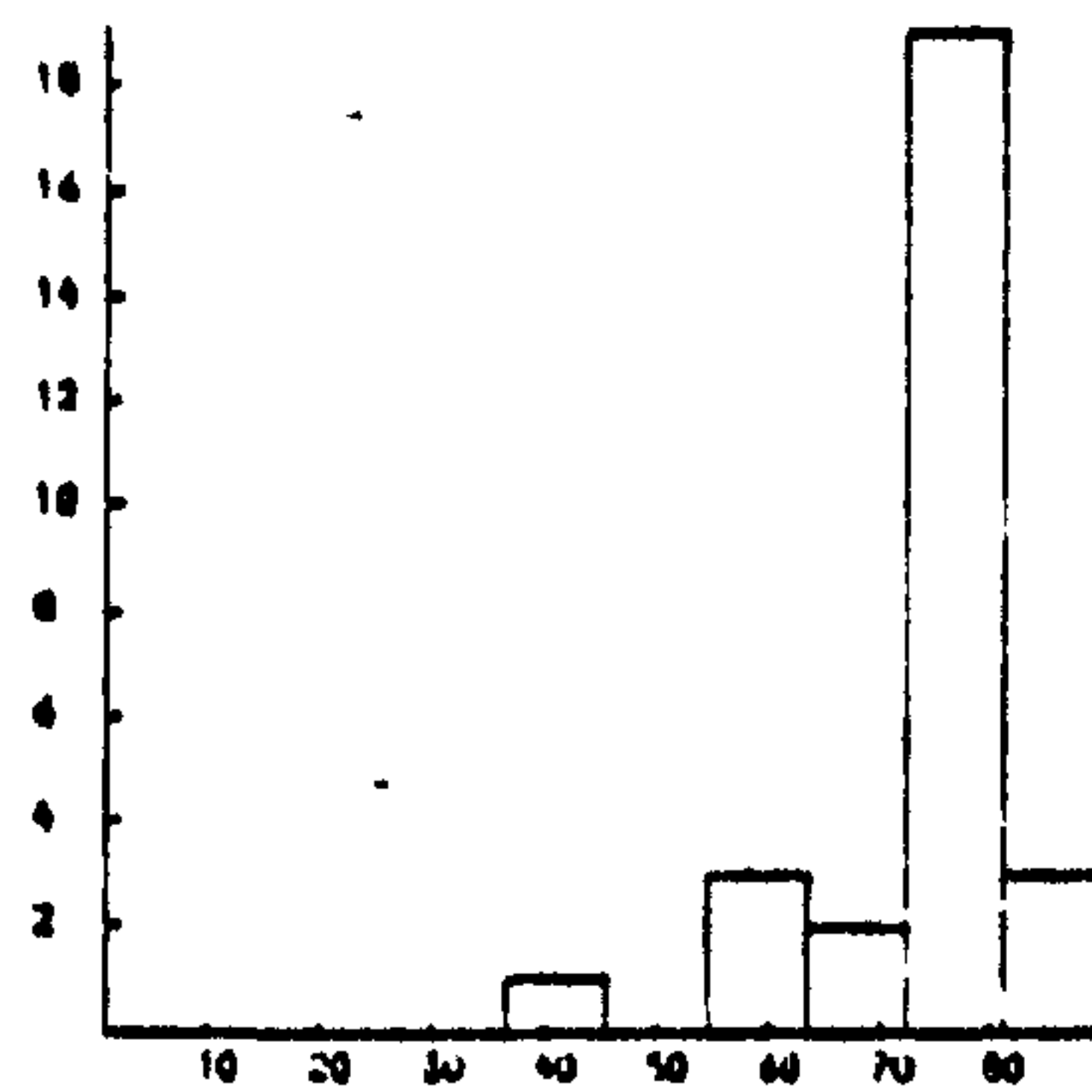
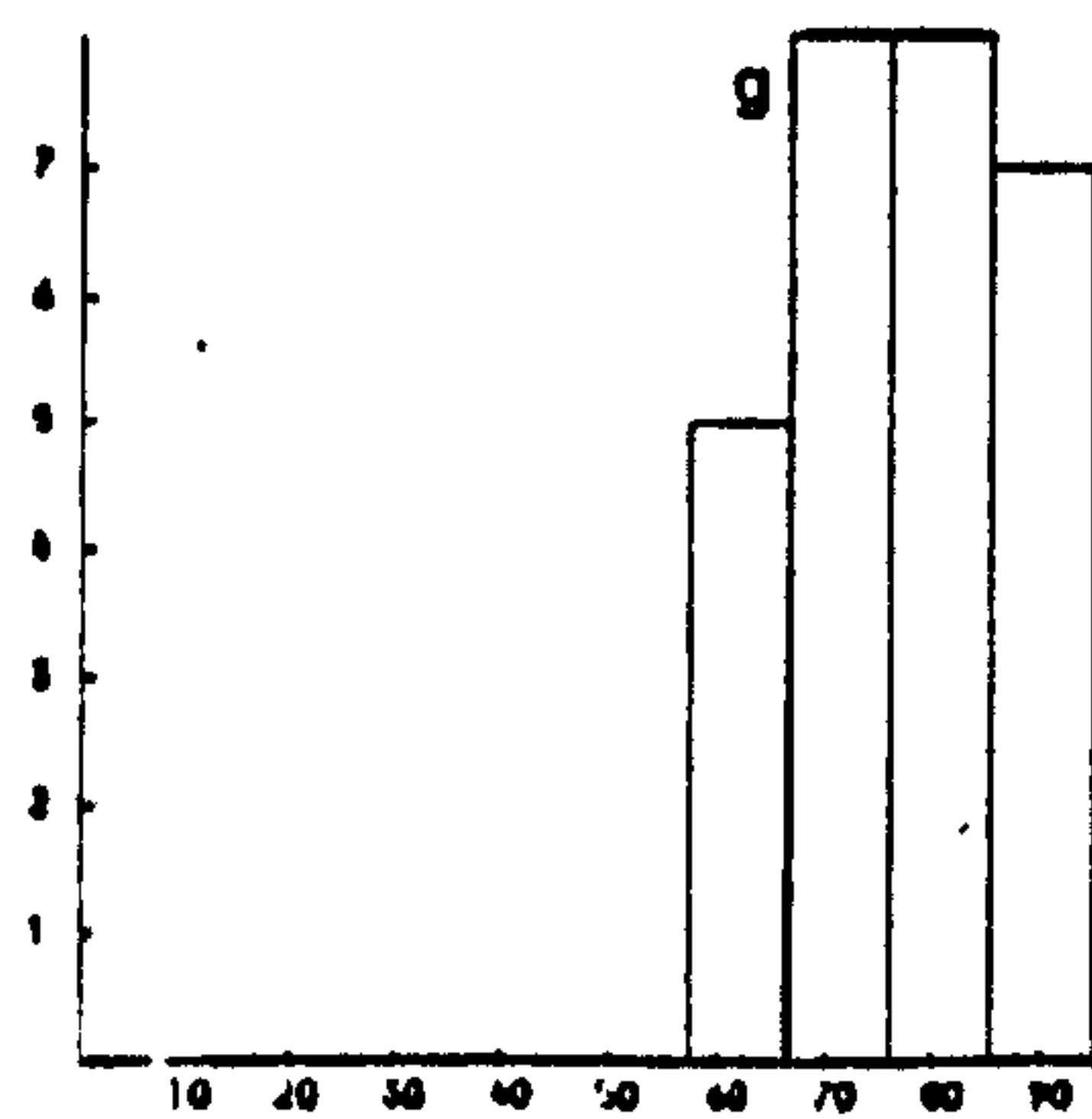
for j, 1 unit = 10  $\mu\text{m}$ .

# BEFORE CULTURE



# AFTER CULTURE





4. Cell Width (in upper part of shoot): There was an increase in cell width, while the variation in width decreased after culture in haploids. In diploids cell width is almost the same under both conditions and a decrease in variation is detectable. In contrast triploids increased in cell width and the variation also increased in culture (Table 8, Figs. 47d- 49d).

5. Leaf Length (from middle part of shoot): The leaf length in both cultures was almost the same, and so was the amount of variation in haploids after culture. There was a slight increase in length and decreases in variation in diploids and triploids after culture (Table 8, Figs. 47e- 49e), suggesting that this character is slightly influenced by environmental factors and the variation is partly genetic in nature.

6. Leaf Width (from middle part of shoot): There was a slight increase in width and a slight increase in variation in haploids after culture. An increase in width and a decrease in variation is shown in diploids. There was a slight increase in width, but the variation in width is almost the same under both culture in triploids (see Table 8, Figs. 47f- 49f), suggesting that this character is slightly influenced by environmental factors.

7. Percentage of Teeth: There was an increase in the percentage of teeth in culture, but the variation is almost the same in haploids. A similar increase in diploids was found, but there was a slight decrease in variation. There was a decrease in triploids but the variation is almost the same under both conditions (Table 8, Figs. 47g- 49g), suggesting some environmental effects.

8. Acumen Angle: This angle increased in culture in the three cytotypes (Figs. 47h- 49h), but the variation was increased in diploids, decreased in triploid and almost the same in haploids (Table 8), suggesting some



environmental influence.

9. Tooth Angle: This angle is almost the same in culture in haploids and diploids, while it increased in triploids (Figs. 47i - 49i). The amount of variation in culture increased in both haploids and triploids. In contrast this slightly decreased in diploids after culture (see Table 8). This character is influenced by some environmental factors.

10. Tooth Length: Tooth length slightly decreased in culture in the three cytotypes (see Figs. 47j - 49j). The variation slightly increased in diploids and haploids while it is almost the same in haploids before and after culture (see Table 8), suggesting low environmental effects.

11. Number of Lamella Cells: The lamella cells were almost the same in number in both conditions and the variation decreased in culture in haploids and diploids. In contrast the number of lamella cells and the variation in number are almost the same in triploids under both cultures (see Table 8, Figs. 47 - 49k), suggesting that this character is slightly influenced by environmental factors.

12. Height of Lamellae Cells: There was a decrease in the number of cells in haploids after culture, but the variation was almost the same. There are slight increases in both diploids and triploids after culture, but the variation decreased and almost the same in the two cytotypes respectively (see Table 8, Figs. 47i - 49i), suggesting that this character was little affected by the environment.

#### 7.4 Numerical Approach

-The same MIXORD technique as described on page ( 30 ) for H. cupressiforme s.l. was used as follows:-

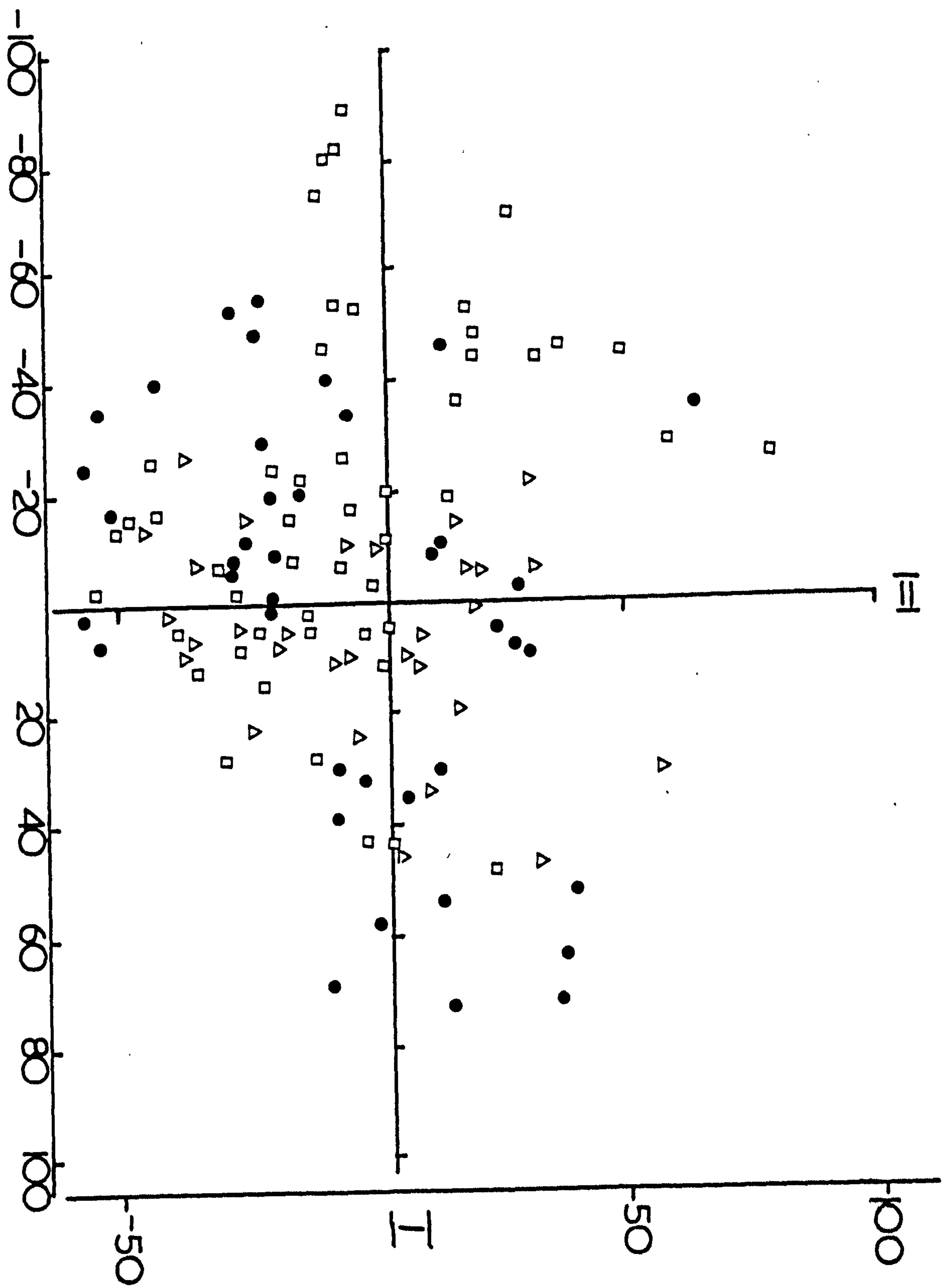


Fig. 50. Ordination of morphological characters for A. undulatum herbarium and field material. Key to symbols:

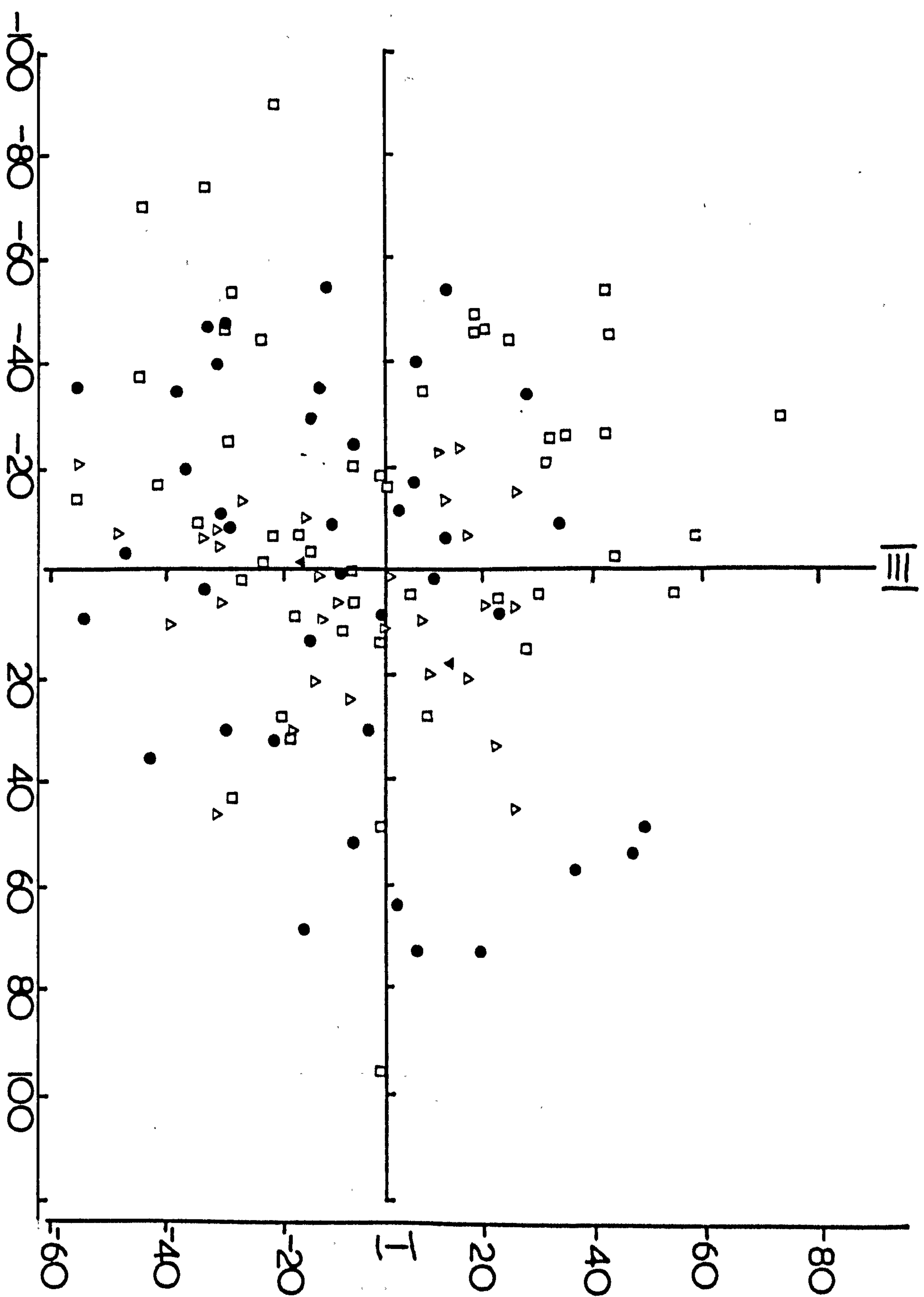
- ( Δ ) Specimens referred to haploid A. undulatum
- ( ● ) Specimens referred to diploid A. undulatum
- ( □ ) Specimens referred to triploid A. undulatum

Note that axis I plotted against II.

Fig. 51. Ordination of morphological characters for A. undulatum  
herbarium and field material. Key to symbols:

- (  $\Delta$  )     Specimens referred to haploid A. undulatum
- (  $\bullet$  )     Specimens referred to diploid A. undulatum
- (  $\square$  )     Specimens referred to triploid A. undulatum

Note that axis I plotted against III.





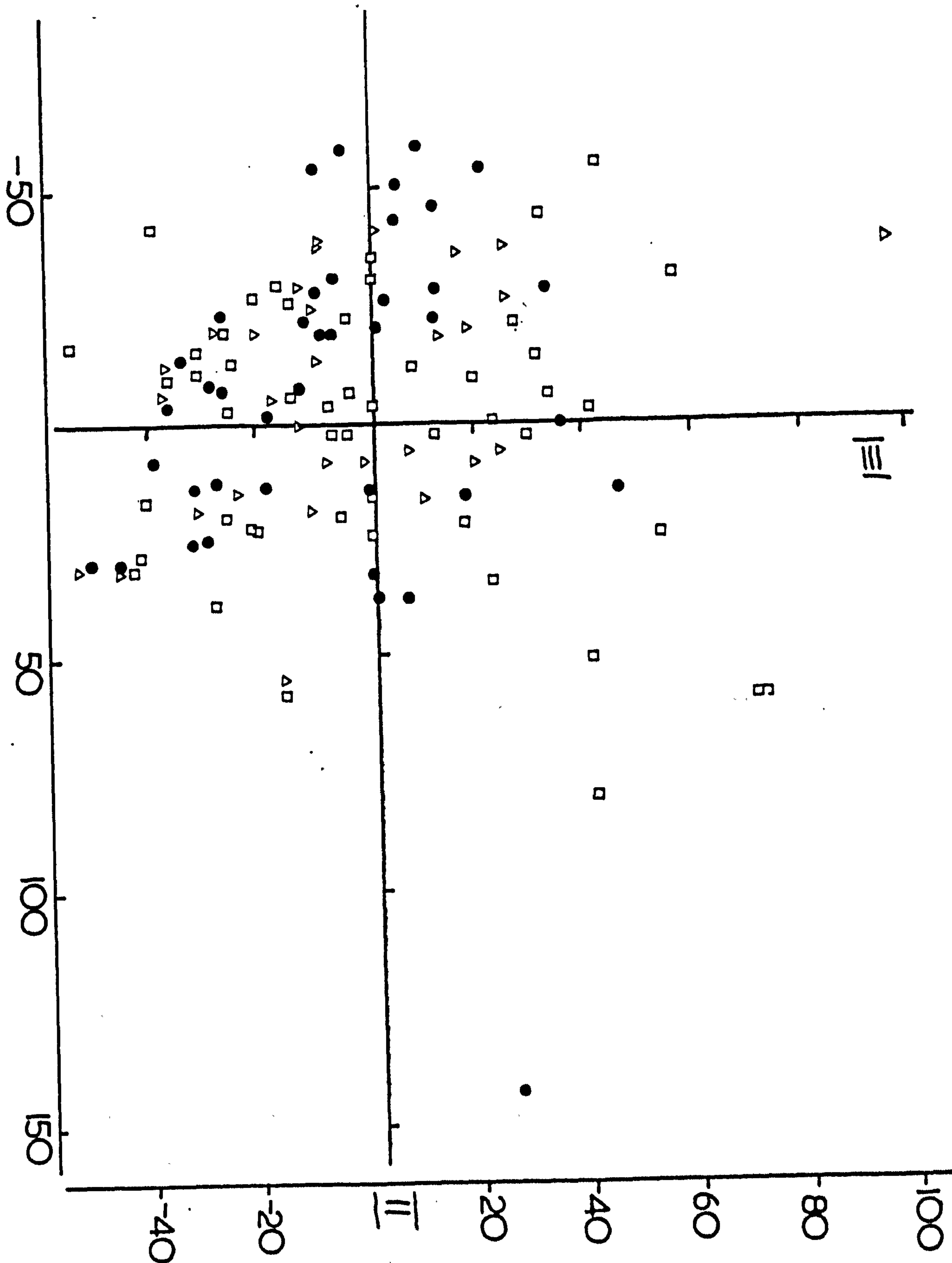


Fig. 52. Ordination of morphological characters for A. undulatum herbarium and field material. Key to symbols:

- (  $\Delta$  ) Specimens referred to haploid A. undulatum
- (  $\bullet$  ) Specimens referred to diploid A. undulatum
- (  $\square$  ) Specimens referred to triploid A. undulatum

Note that axis II plotted against III.

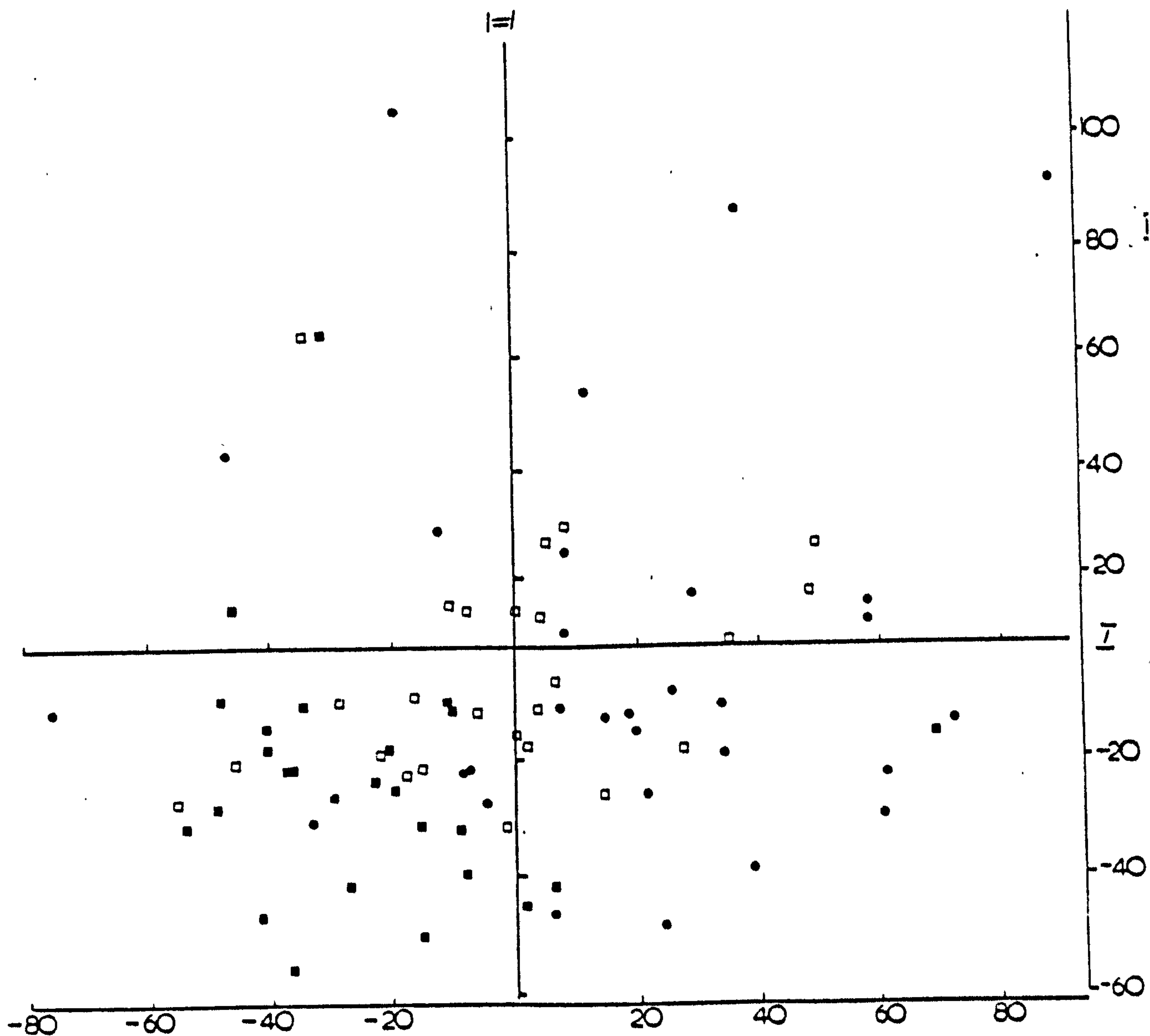


Fig. 53. Ordination of morphological characters for A. undulatum wild material before culture. Key to symbols:

- ( ■ ) Specimens referred to haploid A. undulatum
- ( □ ) Specimens referred to diploid A. undulatum
- ( ● ) Specimens referred to triploid A. undulatum

Note that axis I plotted against II.

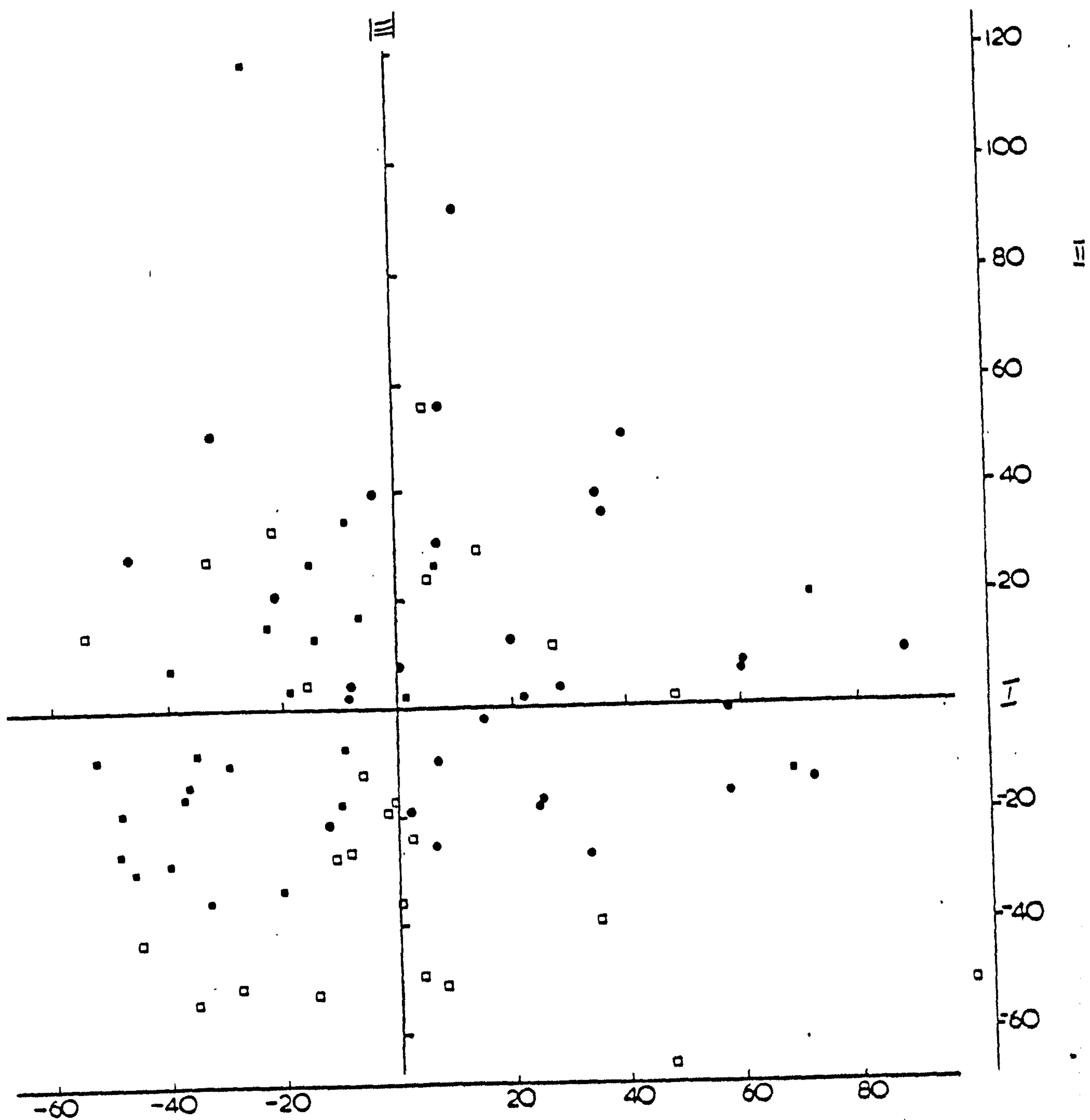


Fig. 54. Ordination of morphological characters for A. undulatum wild material before culture. Key to symbols:

- ( ■ ) Specimens referred to haploid A. undulatum
- ( □ ) Specimens referred to diploid A. undulatum
- ( ● ) Specimens referred to triploid A. undulatum

Note that axis I plotted against III.

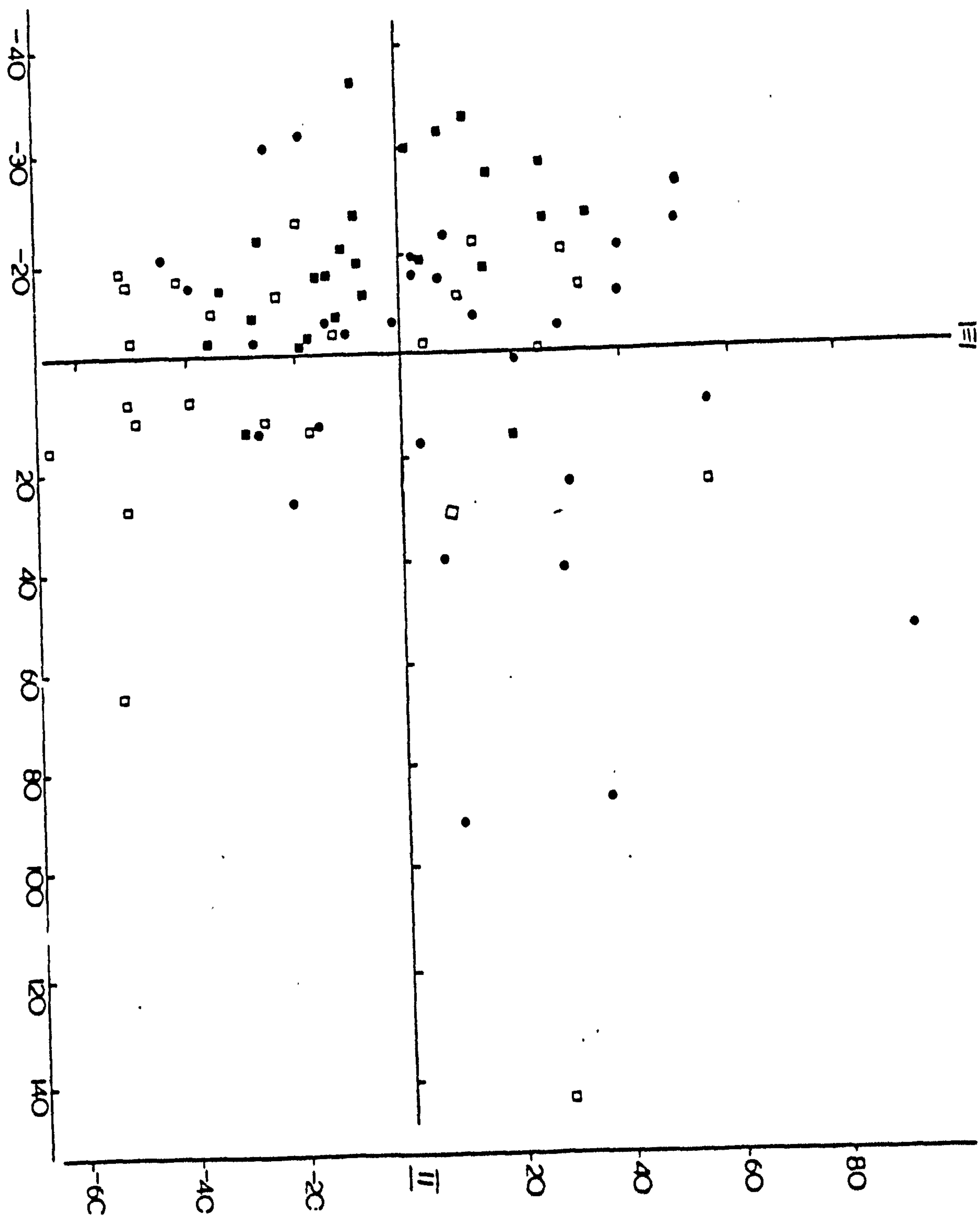


Fig. 55. Ordination of morphological characters for A. undulatum wild material before culture. Key to symbols:

- ( ■ ) Specimens referred to haploid A. undulatum
- ( □ ) Specimens referred to diploid A. undulatum
- ( ● ) Specimens referred to triploid A. undulatum

Note that axis II plotted against III.



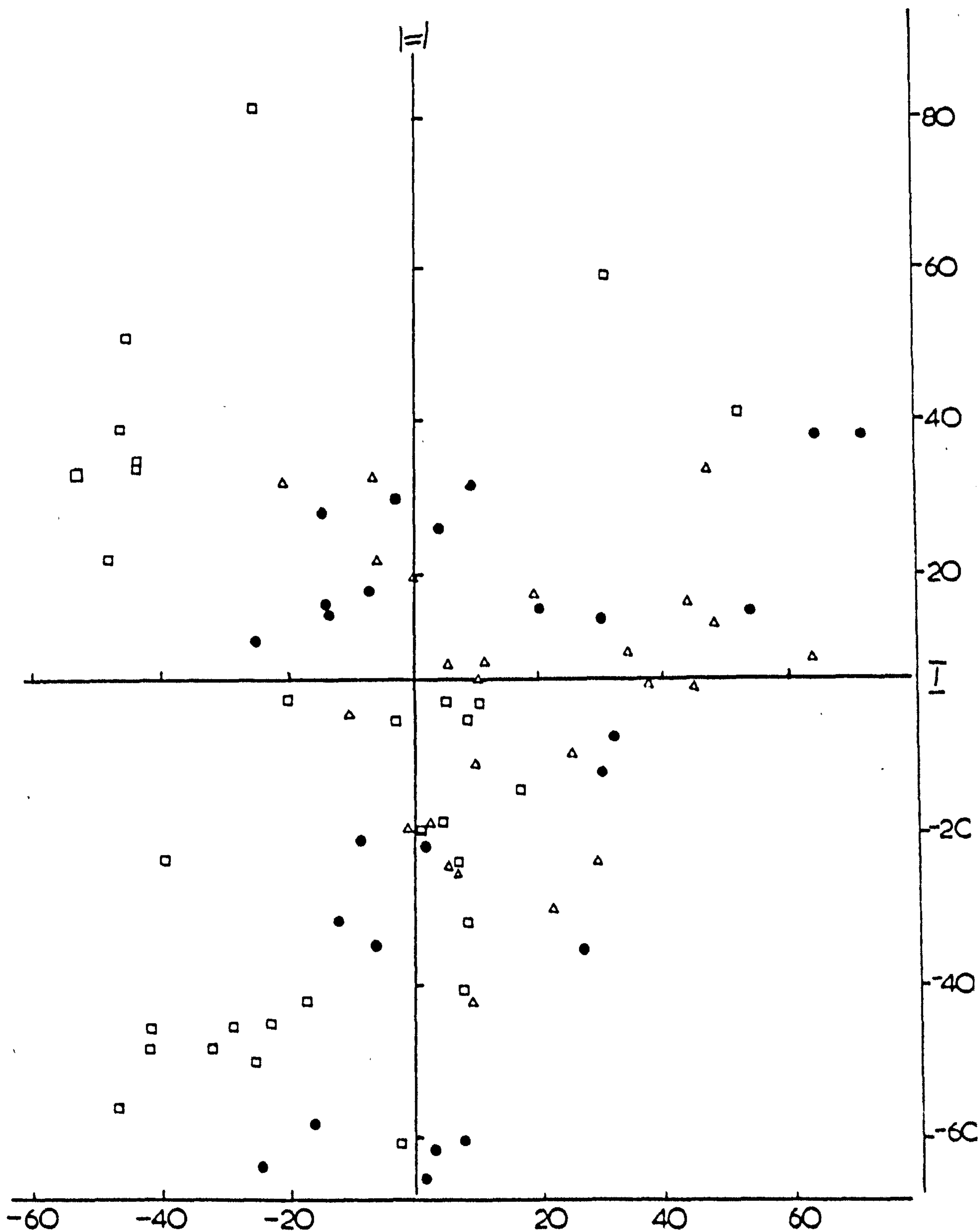


Fig. 56. Ordination of morphological characters for A. undulatum after culture. Key to symbols:

- ( Δ ) Specimens referred to haploid A. undulatum
- ( ● ) Specimens referred to diploid A. undulatum
- ( □ ) Specimens referred to triploid A. undulatum

Note that axis I plotted against II.

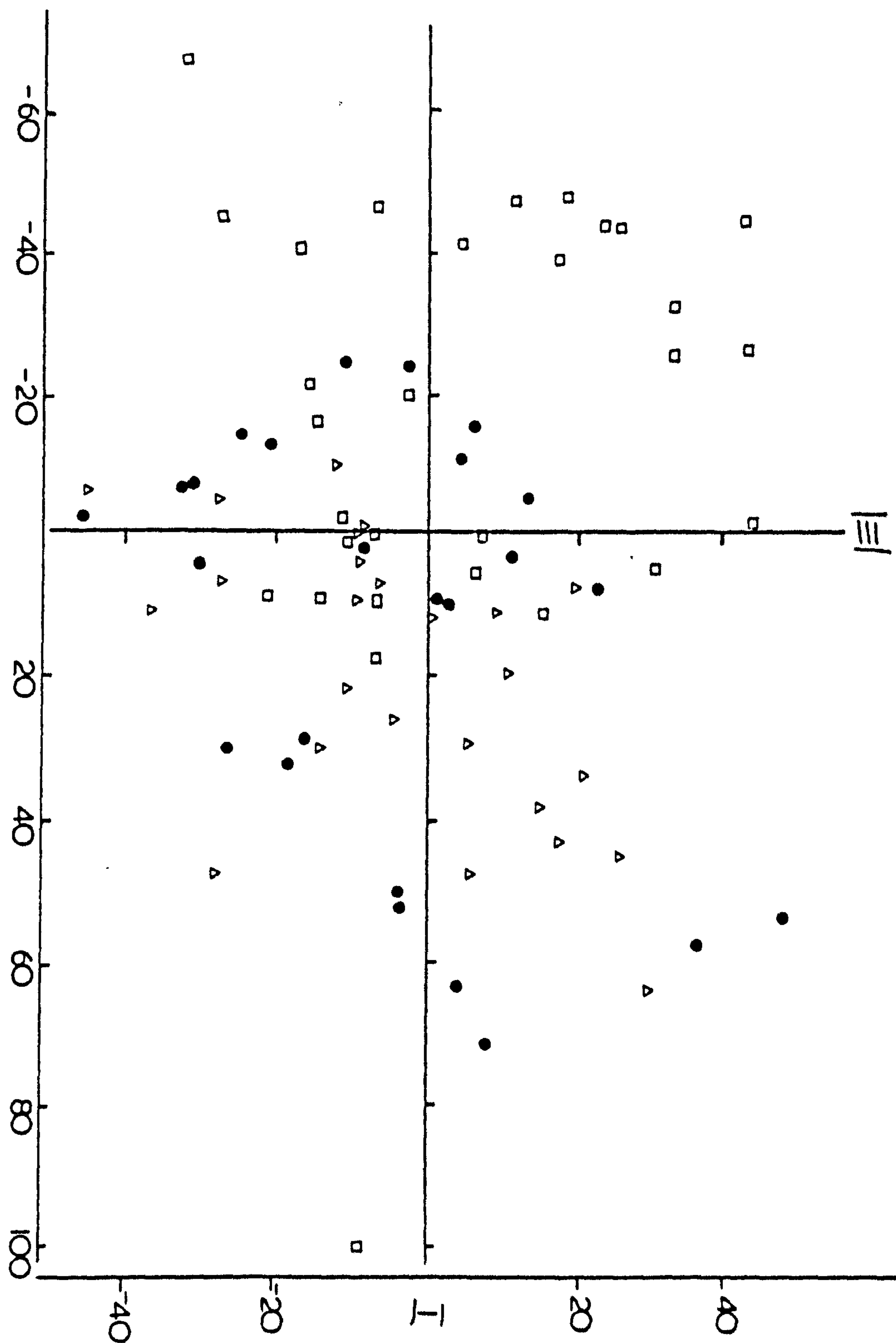


Fig. 57. Ordination of morphological characters for A. undulatum after culture. Key to symbols:

- (  $\Delta$  ) Specimens referred to haploid A. undulatum
- (  $\bullet$  ) Specimens referred to diploid A. undulatum
- (  $\square$  ) Specimens referred to triploid A. undulatum

Note that axis I plotted against III.

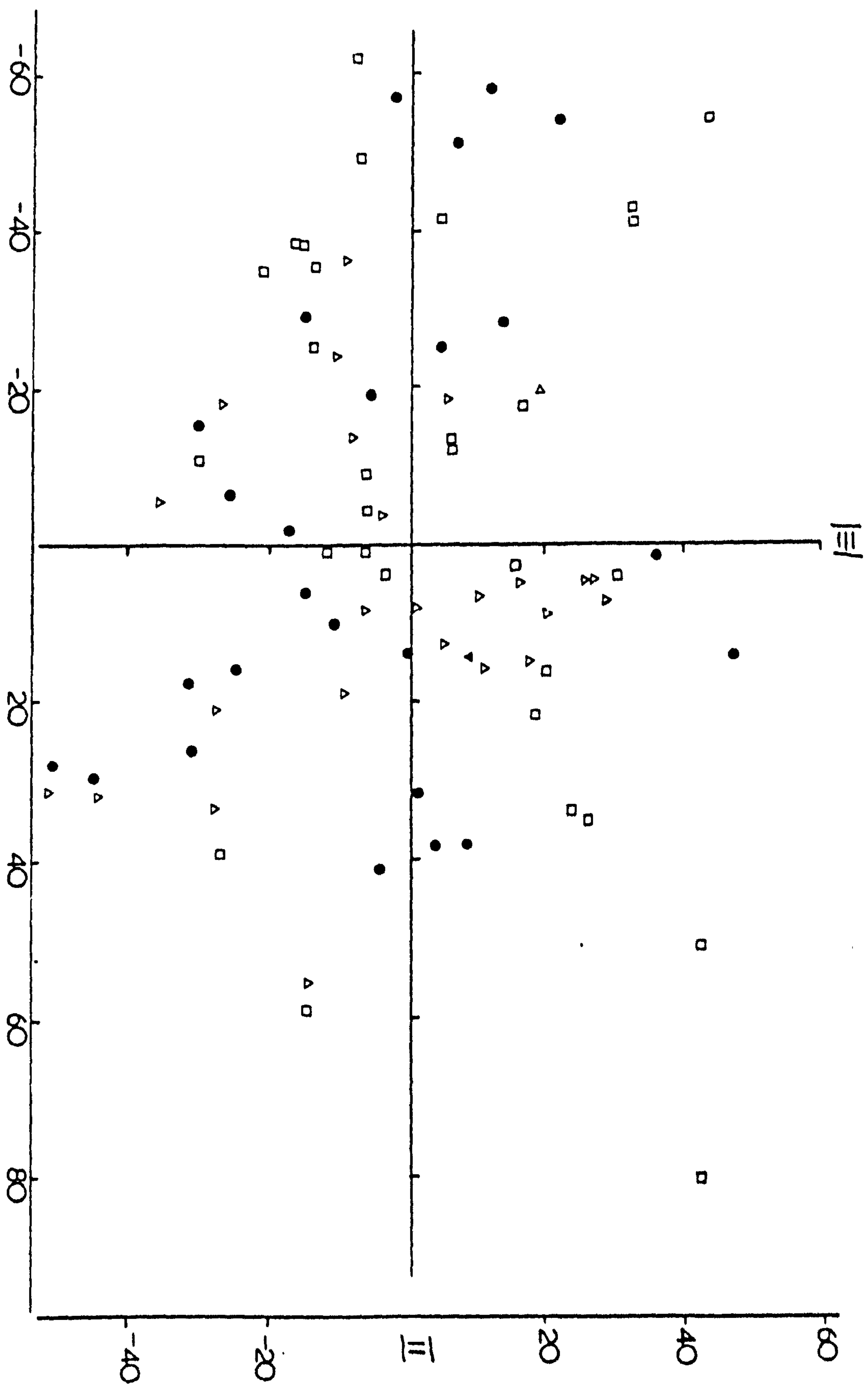


Fig. 58. Ordination of morphological characters for A. undulatum

after culture. Key to symbols:

- (  $\Delta$  ) Specimens referred to haploid A. undulatum
- (  $\bullet$  ) Specimens referred to diploid A. undulatum
- (  $\square$  ) Specimens referred to triploid A. undulatum

Note that axis II plotted against III.

1. To herbarium and wild material
2. To wild material
3. To wild material after culture

From Fig. (50 - 55) it can be seen that the three cytotypes overlap and are indistinguishable as might be expected from the discussion of individual characters above.

After culture the three cytotypes showed a similar intergradation in gametophyte characters (see Fig. 56 - 58).

As no differences were detectable between haploid, diploid and triploid races, these cytotypes should be treated as a single taxonomic species containing three cytotypes. This conclusion is not in line with that of Lazarenko and Lesnyak (1977) who say "these should be treated as cryptic sibling species", but is with that of Smith (1978h) who says "... best to regard A. undulatum as a single species containing three cytotypes".

It is of interest that in A. undulatum there is no tendency for diploids and triploids to be larger than haploids. In Tortula muralis (Newton, 1968) diploids tend to be larger although they intergrade completely with haploids. In the case of Tortula muralis it was suggested that the new diploids were larger but that there was selection for smaller plants on successive generations. This would be in line with observations of Wettstein (1940) who noted a diminution in size in successive generations in artificial polyploids of Funariaceae. That diploids in T. muralis are often larger than haploid gametophytes (Newton, 1968) suggests that some are of recent origin whilst those diploids that are of similar size to the haploids have been selected for over a number of generations. The large diploids correspond to the plant referred to as T. muralis var. rupestris. As an indication of the



relative frequency of what might be new polyploids compared with haploids and old polyploids may be obtained from Duncan (1926). Duncan records T. muralis var. muralis which contains the latter categories of plants from 112 English and 40 Irish vice-counties whilst var. rupestris is recorded from 63 and 9 vice-counties respectively. That there is not the slightest indication that polyploids are larger than haploids in A. undulatum suggests that either new polyploids are of similar size to parental haploids or that new polyploids, if larger, are of very rare occurrence and were not encountered during the present investigation.

Rose (1951b) and Nyholm (1971) suggest that A. undulatum var. minus is a hybrid between A. undulatum and A. angustatum. Smith (1978b) commented that in var. minus the sporophyte is stunted and the spores abnormal and apparently sterile and this is indicative of a hybrid nature. The distribution of A. angustatum in Britain precludes it being one of the parents of A. undulatum var. minus often occurs outside the range of the former and it seems likely that var. minus is a hybrid between different cytotypes.

Against the idea that var. minus is a hybrid between different cytotypes are the observations of Dr. M.E. Newton (pers. comm.) who examined capsules in mixed populations of diploid and triploid plants. The sporophytes were all normal var. undulatum sporophytes and, whilst there were some irregularities at anaphase-1 of meiosis there were always 14 or 21 bivalents. If hybridisation had occurred between diploid and triploid gametophytes then at least some capsules with 14 bivalents and 7 univalents would have been found. Further, in mature sporophytes, the spores were normal; var. minus sporophytes contain spores which are very variable in size and with many aborted.

Lowry (1954) suggests that triploid A. undulatum was derived by

apospory from the hybrid haploid X diploid A. undulatum. The suggestion that there is a hybrid between haploid and diploid A. undulatum is acceptable, but that by apospory is involved is not. Smith (1978b) commented that it seems more likely that in such a hybrid meiosis is irregular and that occasional spores with a balanced complement of 21 chromosomes occur. Diploid spores have been reported from naturally occurring plants. It therefore seems probable that doubling of the chromosome number results from diplospory rather than apospory. Higher plants frequently produce diploid microspores (Stebbins, 1971) - the situation in mosses is not different from higher plants (Smith, 1978b).

## CHAPTER 8

### Variation in non-morphological characters

#### 8.1 Isozymes in three cytotypes of *A. undulatum*

Electrophoresis of xanthin dehydrogenase (XDH) system was carried out on a horizontal slab of poly acrylamide gel. The method is described in page 38 .

Only one (XDH) isozyme phenotype was found (see Fig. 59) in the three cytotypes of *A. undulatum*. This consists of two bands one small and the other larger. The smaller band was white while the other was dark blue. These three cytotypes of *A. undulatum* have the same number, position, shape and colour of bands, thus indicating a close genetical relationship between them, supporting the suggestion that these plants form an autopolyploid series and are best treated as a single species with three cytotypes.

#### 8.2 DNA content of nuclei

##### Introduction

Measurement of the relative DNA content of nuclei from gametophytes from different populations may provide evidence of different ploidy levels of these populations and may provide an efficient mean of differentiating populations where karyotype analysis is difficult or impracticable. The DNA content of nuclei of gametophytes may provide a useful taxonomic criterion and may provide evidence of evolution of one taxon from another. Table 9 and Fig. 60 show the absolute DNA contents of nuclei from gametophytes of four bryophytes in relation to the contents of nuclei of green algae and annual or ephemeral higher plants. DNA contents of the four bryophytes were estimated from nuclear volumes. These estimated DNA contents per nucleus are relatively low but overlap with the minimum values for vascular plants. The estimated DNA per chromosome is in the lower range for land

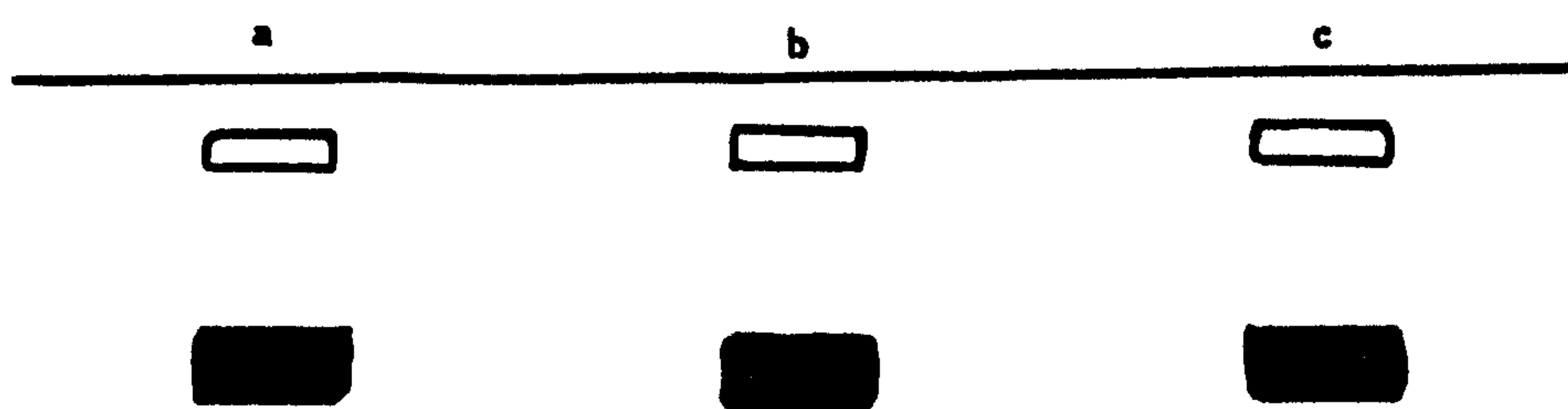


Fig. 59. The isozyme bands of Xanthine Dehydrogenase (XDH) system for three cytotypes of A. undulatum.

(a) haploid ;

(b) diploid ;

(c) triploid.



Table 9. The absolute DNA contents of nuclei from gametophytes of four bryophytes and from nuclei of green algae and higher plants (Sparrow et al. 1972).

Organism	Chromosome no. (n)	Ref.	NV ( $\mu^3$ )	Ref.	ICV ( $\mu^3$ )	Ref.	DNA/cell (haploid)	Ref.	DNA/chromosome
<b>Bryophyta (mosses and liverworts)</b>									
<i>Marchantia polymorpha</i> (gametophyte)	9	13	13	13	1.4	13	$9.0 \times 10^8$	128	$1.0 \times 10^8$
							$1.6 \times 10^9$	132	$1.8 \times 10^8$
<i>Mnium</i> sp. (gametophyte, shoot apex)	ca.7	119	59	62	8.4	62	$7.8 \times 10^9$	132	$1.1 \times 10^9$
<i>Riccia</i> sp. (gametophyte, cells near apical notch)	8	62	64	62	8.0	62	$8.6 \times 10^9$	132	$1.1 \times 10^9$
<i>Sphagnum</i> sp. (gametophyte, central meristem of shoot apex)	19	131	20	62	1.1	62	$2.6 \times 10^9$	132	$1.4 \times 10^8$
<b>Psilopsida</b>									
<i>Psilorum nudum</i> (shoot apex, apical cell derivatives)	104	62	3844	62	18.5	62	$2.6 \times 10^{11}$	132	$2.5 \times 10^9$
<i>Imbricaria</i> sp. (shoot apex, apical cell derivatives)	208	47	9366	62	22.5	62	$6.3 \times 10^{11}$	132	$3.0 \times 10^9$
<b>Lycopsidea (club mosses)</b>									
<i>Isotria engelmannii</i> (shoot apex)	11	131	498	62	22.6	62	$3.3 \times 10^{10}$	132	$3.0 \times 10^9$
<i>Lycopodium lucidulum</i> (shoot apex)	132	47	296	62	1.1	62	$2.0 \times 10^{10}$	132	$1.5 \times 10^8$
<i>Selaginella kraussiana</i> var. brownii (shoot apex)	10	119	77	62	3.8	62	$5.1 \times 10^9$	132	$5.1 \times 10^8$
<b>Sphenopsida (horsetails)</b>									
<i>Equisetum arvense</i> (nonapical cells of shoot apex)	108	47	1124	62	3.2	62	$7.5 \times 10^{10}$	132	$6.9 \times 10^8$
<i>E. hyemale</i> (nonapical cells of shoot apex)	108	47	1060	62	4.9	62	$7.1 \times 10^{10}$	132	$6.6 \times 10^8$
<b>Pteropsida (ferns)</b>									
<i>Asplenium nidus</i> <sup>†</sup>	144	47	280	62	1.9	62	$3.7 \times 10^{10}$	132	$2.6 \times 10^8$
<i>Cystopteris fragilis</i> <sup>†</sup>	84	47	447	62	5.3	62	$6.0 \times 10^{10}$	132	$7.1 \times 10^8$
<i>Onoclea sensibilis</i> <sup>†</sup>	37	62	842	62	22.8	62	$1.1 \times 10^{11}$	132	$3.0 \times 10^9$
<i>Ophioglossum petiolatum</i> (nonapical root cells)	ca.310	47	4124	62	4.0	62	$2.8 \times 10^{11}$	132	$5.3 \times 10^8$
<i>Osmunda cinnamomea</i> <sup>†</sup>	22	47	724	62	32.9	62	$9.6 \times 10^{10}$	132	$4.4 \times 10^9$
<i>Pteridium aquilinum</i> <sup>†</sup>	32	49	316	62	6.1	62	$4.6 \times 10^{10}$	132	$8.5 \times 10^8$
<i>Pteris longifolia</i> <sup>†</sup>	58	62	451	62	7.8	62	$6.0 \times 10^{10}$	132	$1.0 \times 10^9$
<i>Rumohra adiantiformis</i> <sup>†</sup>	41	47	335	62	8.2	62	$6.5 \times 10^{10}$	132	$1.1 \times 10^9$
<i>Salvinia</i> sp. (apical cells)	27	62	176	62	3.2	62	$1.2 \times 10^{10}$	132	$4.4 \times 10^8$

<sup>†</sup> Gametophyte, 3-cell stage.



plants. The lowest DNA content of the four bryophytes falls below, but the highest value exceed, the upper limit found in green algae. It is claimed that nuclear volumes or DNA contents of a range of higher plant species are correlated with complexity (Sparrow et al. 1972). There is no evidence that the DNA content in the four bryophytes is correlated with complexity as it is within the green algae.

The estimation of nucleic acid contents by chemical means requires large amounts of material and is relatively time consuming. The DNA contents of single nuclei can be estimated by measuring the density of Feulgen staining. The light absorbed by the stained nucleus is proportional to the DNA content. A more sensitive measure can be made using a fluorochrome which stains DNA in a quantitative manner. Such a flurochrome is DAPI. Ohher methods depend upon the relationship between nuclear volume and DNA content. These relationships show that nuclear volume and interphase chromosome volume are directly proportional to DNA content per cell and per chromosome, repsectively. Therefore, when the nuclear volume of meristematic cells is known an estimate of DNA content can be made (Sparrow et al. 1972). This method appears to be inadequate especially when the interphase chromosome volume is obtained by dividing the average of the interphase nucleus by the somatic chromosome number and represents the volume occupied by an average chromosome at interphase, neglecting other nuclear components such as the nucleolus or volume changes during replication of chromosomes. Clearly the accuracy of measurements with very small nuclei is questionable. To assess the DNA contents in nuclei, Feulgen stain was used in the past, but there are many difficulties, for example excess or too little stain effects the results as does the influence of the background. To obtain satisfactory results the fluorochrome stain was applied to the three cytotypes of A. undulatum.



The fluorochrome 4,6-diamidino-2-phenylindole DAPI has been shown to bind specifically to DNA. The DAPI/DNA complex fluoresces with an intensity of about 15-20 times that of DAPI alone. The fluorescence of the DAPI/DNA complex has been used as a quantitative estimate of the DNA (Brunk et al. 1979, Lin et al. 1977). Background fluorescence can be reduced to a negligible amount. DAPI unlike some other stains, such as chromomycin A<sub>3</sub>, does not display rapid quenching. This allows scanning of preparations under the Ultra Violet (U.V.) microscope and accurate focussing on the nucleus with little loss of intensity. The simplicity of the staining procedure coupled with the brightness of the DAPI/DNA complex provides a convenient technique for cell cycle studies and comparative estimate of DNA contents of nuclei.

#### Materials and Methods

Four gametophyte cells from each of twenty five samples of each cytotype were measured. Plant material was fixed in 5% glutaraldehyde EM in Tris buffer pH 7 for 5 minutes. The excess fixative was then removed and replaced with the fluorochrome DAPI.

The stain was made up as a stock solution of 1000 ng/ml in Tris buffer pH 7, 100 mM NaCl and 10 mM EDTA. All reagents were started in the dark at 3°C. The fixed tissue was squashed directly on a clean slide. Staining with DAPI appears almost instantaneously. Stained material was observed under a Leitz Werzlar microscope fitted with an incident U.V. light source and photomultiplier coupled to a pen recorder. Filters used were BG3, UG1, giving a final wave length of 350 nm. The DAPI/DNA complex fluoresces at 450 nm. All nuclear measurements were made from nuclei in cells of young gametophytes near the shoot apex.

As the variation is proportional to the mean value in the first peak (G<sub>1</sub>) of the three cytotypes, transformation of data by means of log<sub>10</sub> was



suggested to overcome this proportional variation. A one way analysis of variance was carried out. Tukey's test was suggested to be suitable for this sort of data (see Appendix 3).

### Results and Discussion

Bimodality was found in the three cytotypes of A. undulatum (Fig. 61 a,b,c) indicating that some cells had increased DNA contents as they were measured in S, G<sub>2</sub> or M phases of cell cycle. In the haploid strains the basal level (mean value of cells in G<sub>1</sub>) is estimated to be 60 units. The second peak of the distribution would seem to correspond to an expected G<sub>2</sub> mean of 130. The G<sub>1</sub> level in the diploid cytotype is estimated to be 110 and again the G<sub>2</sub> mean is approximately twice this value. The triploid peaks indicate a mean higher than that of the diploid viz 160 for G<sub>1</sub> and approximately twice this value for the G<sub>2</sub> nuclei

In the haploid strain the mean value of the first peak is estimated to be  $55.5 \pm 4.2$  units. The first peak of the diploid cytotype is estimated to be  $90.80 \pm 3.95$  units. The mean value of the first peak of the triploid cytotype is estimated to be  $134.06 \pm 4.89$  units. These results show that the mean relative DNA contents increase from the haploid to diploid to triploid as would be expected from karyotype. Differences between diploids and haploids, triploids and haploids and between triploids and diploids were compared using Tukey's test. The Log<sub>2</sub>, Log<sub>3</sub> and Log<sub>1.5</sub> values are outside the 95% confidence intervals. Therefore the increase in the DNA content is not proportional to increase in chromosome number.

From these results, it would appear that there are differences in DNA content between the three cytotypes of A. undulatum. Polyploidy plays a role in the elevation of DNA, but poliploidy is only one of other forces forming the basis of the variability. Stebbins (1950) pointed out some examples where certain temperate genera have larger chromosomes and

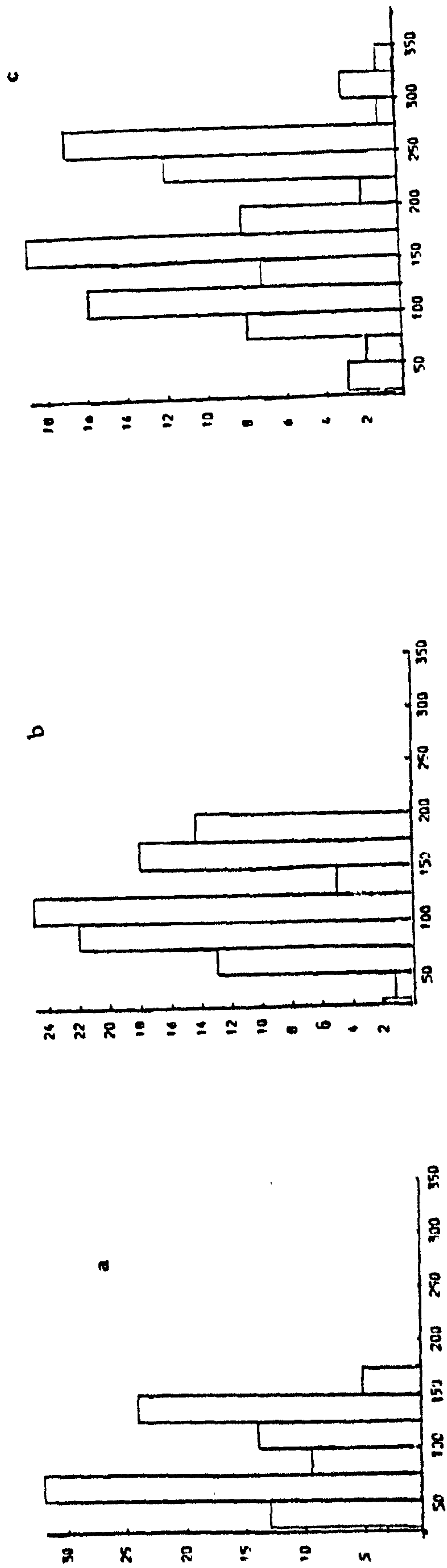


Fig. 61. Histograms of the amount of DNA in A. undulatum

(a) haploid;

(b) diploid;

(c) triploid.

nuclei than tropical ones. There are significant differences in chromosome size and DNA content in closely related diploid species (Stebbins, 1950). These examples suggest the existence of selective forces influencing the amount of DNA per nucleus and per chromosome (Sparrow, 1972).

Clearly, polyploidy has played a main role in generating the three cytotypes of A. undulatum but there is some evidence from the present study that loss or gain of DNA has also been involved since the polyploids were formed. Variation in chromosome size is known in tropical and temperate numbers of the same genera. Significant differences in chromosome size or DNA content is known to occur in some closely related species of higher plants (Stebbins, 1950)

### 8.3 Heterochromatin bodies in A. undulatum

#### Introduction

Heterochromatin was first recognized by Heitz in 1928. He found that certain parts of Pellia chromosomes were more densely staining than most during prophase of mitosis and remained condensed during telophase and the ensuing interphase. The presence of heterochromatin in other bryophytes has been reported by many authorities (e.g. Lorbeer, 1934; Inoue, 1967; Segawa, 1971; Newton, 1977a,b). Heterochromatin bodies have been used to identify different levels of polyploidy (Anderson, 1964; Berrie, 1957; Wigh, 1975). Anderson (1964) says: "In so-called polyploid species Heitz found more than one heterochromosome and consequently more than one heteropycnotic body in the interphase nuclei of these polyploids. Heitz concluded, however, that a species is polyploid if it has more than one heterochromosome". In Brachythecium rutabulum, B. rivulare complex Wigh (1975) found that one large heteropycnotic body indicated that a species was haploid and two large bodies that it was diploid. Further, he



found one body occurs in dioecious species and two in autoecious species. Some authorities discuss these bodies in relation to sex chromosomes in bryophytes (Tatuno, 1960; Mehra and Khana, 1961; Berrie, 1963; Khana, 1971). Kurita (1938) reported more heterochromatin in the large X- than in the Y-chromosome of two species of Pogonatum. Anderson (1963) reported more heterochromatin in female nuclei of Anomodon species than in male nuclei. Unequal amount of heterochromatin in plant nuclei has been reported by Jackimsky (1935) who found that Pellia epiphylla ( $n = 18$ ) had in its complement a pair of chromosomes which differed only in their distribution of heterochromatin. Diploid and triploid forms of Dumortiera hirsuta with  $n = 18$  and 27, were found to have two or three times the number of interphase heterochromatin bodies than the haploid form (Newton, 1977b). Newton (1971) found that the size of heterochromatin bodies in interphase nuclei offered a means of distinguishing between male and female plants of Plagiomnium undulatum in most instances.

The amount of DNA or its reactivity to Feulgen stain is reduced by low temperature (Lacour et al., 1956) . In contrast, Newton (1971) found in studying the size of heterochromatin bodies in the male and female Plagiomnium undulatum that temperature had no affect on heterochromatin body size.

Tatuno (1941) found a remarkable uniformity in the presence of a large and/or a small heterochromatin body in liverworts, and suggested calling large and small heterochromatic chromosomes, H- and h- chromosomes respectively, each of which is universally present in the haploid gametophytic complement of liverworts. The extension of these terms H and h to heterochromatic moss chromosomes was suggested by Yano (1957) but moss heterochromatin is not confined to these two chromosome, and there are difficulties associated with their designation as H or h-chromosomes (Berrie, 1963). Attempts by



Newton (1977a) to identify H- and h-chromosomes in British hepatics proved inconclusive indicating the undesirability of their stereotype recognition in liverworts. The quantities of heterochromatin associated with the longest chromosome in Pellia epiphylla and Pneesiana undoubtedly equate with H-chromosome of Tatuno (1941), but the smallest chromosome of the latter species consists of only about 26% of heterochromatin compared with 35% in chromosome 5. In P. endiviifolia the largest and smallest chromosomes contain 13% and 18% of their length as heterochromatin, only slightly more than in the other chromosomes (Newton, 1977a). On the basis of this evidence there seem no grounds for designating the largest and smallest chromosomes H and h respectively and Newton (1977a) proposed the abandonment of these terms.

Clearly, with three cytotypes, it would be of interest to investigate the heterochromatin content of interphase nuclei of Atrichum undulatum and to see if temperature had any influence on the size of heterochromatin bodies. The possibility that temperature might effect the size of heterochromatin bodies of interphase A. undulatum was therefore considered, but observations indicate that temperature has no effect on the size of heterochromatin bodies of interphase A. undulatum.

#### Materials and Methods

Twenty five haploid, diploid and triploid samples were chosen for this study from various localities in England, Wales and Scotland. Material was kept at laboratory temperature from one to six months, further material was cultured in an unheated mist unit in a glasshouse at Pen-y-Ffridd Field Station and a number of samples were kept at (5°C to -5°C) in refrigerators in the School of Plant Biology, Bangor.

Table 10. Showing the size and the mean value of heterochromatin bodies in haploid, diploid and triploid Atrichum undulatum.

Cytotype	Size of heterochromatin bodies in $\text{mm}^2$	Mean
Haploid	2.88 - 4.80	$3.70 \pm 1.29$
Diploid	2.90 - 4.81	$3.94 \pm 1.15$
Triploid	3 - 5.80	$4.44 \pm 1.16$

Table 11. Showing the d value of heterochromatin bodies in the three cytotypes of Atrichum undulatum

Haploid and Diploid	Diploid and Triploid	Haploid and Triploid
-0.552	-0.55	-0.915

Cytological preparation technique was similar to that used for Hypnum cupressiforme and Atrichum undulatum chromosomes (Pages 19, 65). Drawings of heterochromatin bodies were made using a camera lucida and the size of drawings calculated in square millimetres.

#### Results and discussions

The number of nuclei needed to give a suitable sample for each population was determined by calculating the moving means for haploid, diploid and triploid specimens. A sample size of 25 nuclei gave satisfactory results. Table 10 showing the size and the mean value of heterochromatin bodies in the three cytotypes.

From Table 11 it can be seen that the mean size of triploid A. undulatum is not significantly larger ( $d = -0.915$ ;  $P > 0.05$ ) than the mean size of the haploid. The mean size of triploid is not significantly larger ( $d = 0.55$ ;  $P > 0.05$ ) than the mean size of the diploid and the mean size of diploid plants is also not significantly larger ( $d = -0.552$ ;  $P > 0.05$ ) than the mean size of haploid plants. Clearly the size of heterochromatin bodies in interphase nuclei A. undulatum cannot be used to distinguish between haploid, diploid and triploid plants in most instances.



## CHAPTER 9

### Distribution and Conclusion

#### 9.1 Distribution of *A. undulatum* cytotypes

*A. undulatum* is a very common plant, (Dixon 1924, Rose 1951 a, Watson, 1968, Smith, 1978a). It occurs in a variety of habitats, growing as yellowish-green to green patches or scattered plants on loose soil in woods, on banks, heaths, in fields. An investigation of the distribution of three cytotypes of *A. undulatum* in Britain was carried out. Data for this study were compiled from field material collected from different localities. Material was determined by Dr. A.J.E. Smith. Field gatherings were allowed to grow in polythene bags for at least three weeks to enable them to produce young shoots. These were then examined cytologically to determine the chromosome number, using the method described on page . Four hundred and seventy two samples were counted from different localities and geographical areas. For details see Appendix 4.

Data were analysed using Chi squared ( $\chi^2$ ). Whenever there are two methods of cross-classification, each of which is made up by several more or less qualitative subdivisions, the data can be arranged in the form of a contingency table.  $\chi^2$  is calculated as a measure of deviation between observed and expected. The formula used is:

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

where the summation symbol  $\Sigma$  indicates summation over all the compartments. The number of degrees of freedom involved is the product of one less than the number of rows and one less than the number of columns. Probability of a deviation as large as that observed being due to chance is obtained from tables of Chi-squared.



The British Isles were divided into four major geographical areas. The national grid reference was used to divide mainland Britain into three areas: North (N), South-east (SE) and South-west (SW). Ireland was taken as a separate area (I). Five contingency tables were examined to show the most possible associations between chromosome number and geographical distribution of A. undulatum.

### Results

There is a significant association between ploidy and geographical distribution, where chi-squared is highly significant at  $P < 0.001$  (see Tables 12, 13, 16).

### Discussion

These results disagree with the Null hypothesis which assumes that there is no association between numbers of different ploidies in different areas. If this hypothesis is true then the deviation between observed and expected values is due to chance, a situation which is clearly not true. Inspection of these tables also shows that this highly significant association is almost mainly due to the distribution of the haploid and partly to the triploid cytotype. In flowering plants it is suggested (Stebbins, 1971) that the proportion of polyploids is lowest in warm temperature or sub-tropical regions and increases towards both the tropics and the arctic. In flowering plants there is a close correlation between ployploidy and effective vegetative reproduction in colder regions (Smith, 1978b). Steere (1954) pointed out that there is not this complication in mosses. The results in Fig. 62 and Table 17 show that haploid A. undulatum is more common in southern areas of Britain which are relatively warmer than in the north which is considered to be relatively colder. This agrees with the hypothesis mentioned by Stebbins (1971).

Table 12. Contingency table relating the four geographical areas and haploid, diploid & triploid A. undulatum (observations (O); expectations (E); and value of  $\chi^2$  corresponding to probability with 6 degrees of freedom

Cytotypes	Geographical areas				Total
	I	N	S.E	S.W	
Haploid	O = 0 E = 2.11 P <0.05	O = 3 E = 15.25 P <0.001	O = 21 E = 15.46 P <0.05	O = 26 E = 17.16 P <0.01	50
Diploid	O = 7 E = 6.61 P >0.05	O = 44 E = 47.59 P >0.05	O = 47 E = 48.25 P >0.05	O = 58 E = 53.54 P >0.05	156
Triploid	O = 13 E = 11.27 P >0.05	O = 97 E = 81.15 P <0.05	O = 78 E = 82.27 P >0.05	O = 78 E = 91.29 P <0.05	266
Total	20	144	146	162	472

$$\chi^2 = 24.64$$

$$P < 0.001$$

Table 13. Contingency table relating south and north of Britain and haploid, diploid & triploid A. undulatum, values of  $\chi^2$  corresponding to probability with 2 degrees of freedom

Cytotypes	Geographical areas		
	S	N	Total
Haploid	O = 47 E = 34.07 P <0.05	O = 3 E = 15.92 P <0.01	50
Diploid	O = 105 E = 101.53 P >0.05	O = 44 E = 47.46 P >0.05	149
TriPloid	O = 156 E = 172.39 P >0.05	O = 97 E = 80.60 P <0.05	253
Total	308	144	452

$$\chi^2 = 20.634$$

$$P < 0.001$$

Table 14. Contingency table relating south east and south west plus Ireland and haploid, diploid and triploid A. undulatum, values of  $\chi^2$  corresponding to probability with 2 degrees of freedom

Cytotypes	Geographical areas		
	SE	SW & I.	Total
Haploid	O = 21 E = 20.92 P >0.05	O = 26 E = 26.07 P >0.05	47
Diploid	O = 47 E = 49.85 P >0.05	O = 65 E = 62.14 P >0.05	112
Triploid	O = 78 E = 75.22 P >0.05	O = 91 E = 93.77 P >0.05	169
Total	146	182	328

$$\chi^2 = 0.4794$$

$$P > 0.05$$



Table 15. Contingency table relating the four geographical areas and diploid, triploid A. undulatum, values of  $\chi^2$  corresponding to probability with 3 degrees of freedom

Cytotypes	Geographical areas				Total
	N	SE	SW	I	
Diploid	O = 44	O = 47	O = 58	O = 7	156
	E = 52.12	E = 46.2	E = 50.27	E = 7.39	
	P > 0.05	P > 0.05	P > 0.05	P > 0.05	
Triploid	O = 97	O = 78	O = 78	O = 13	266
	E = 88.87	E = 78.79	E = 85.72	E = 12.6	
	P > 0.05	P > 0.05	P > 0.05	P > 0.05	
Total	141	125	136	20	422

$$\chi^2 = 3.917$$

$$P > 0.05$$

Table 16. Contingency table relating the four geographical areas with haploid and diploid plus triploid A. undulatum, values of  $\chi^2$  corresponding probability with 3 degrees of freedom

Cytotypes	Geographical areas				
	N	SE	SW	I	Total
Haploid	O = 0	O = 3	O = 21	O = 26	50
	E = 2.11	E = 15.25	E = 15.46	E = 17.16	
	P >0.05	P <0.01	P >0.05	P <0.01	
Diploid and triploid	O = 20	O = 141	O = 125	O = 136	422
	E = 17.88	E = 128.7	E = 130.53	E = 144.83	
	P >0.05	P >0.05	P >0.05	P >0.05	
Total	20	144	146	162	472

$$\chi^2 = 20.65$$

$$P < 0.001$$

Table 17. Percentages of haploid, diploid and triploid cytotypes of  
A.undulatum in four geographical areas of the British Isles.

Taxon	North	South east	South west	Ireland	TOTAL
Haploid	2.08%	14.38%	16.05%	0%	10.59%
Diploid	30.55%	32.19%	35.80%	35%	33.05%
Triploid	67.36%	53.42%	48.15%	65%	56.36%

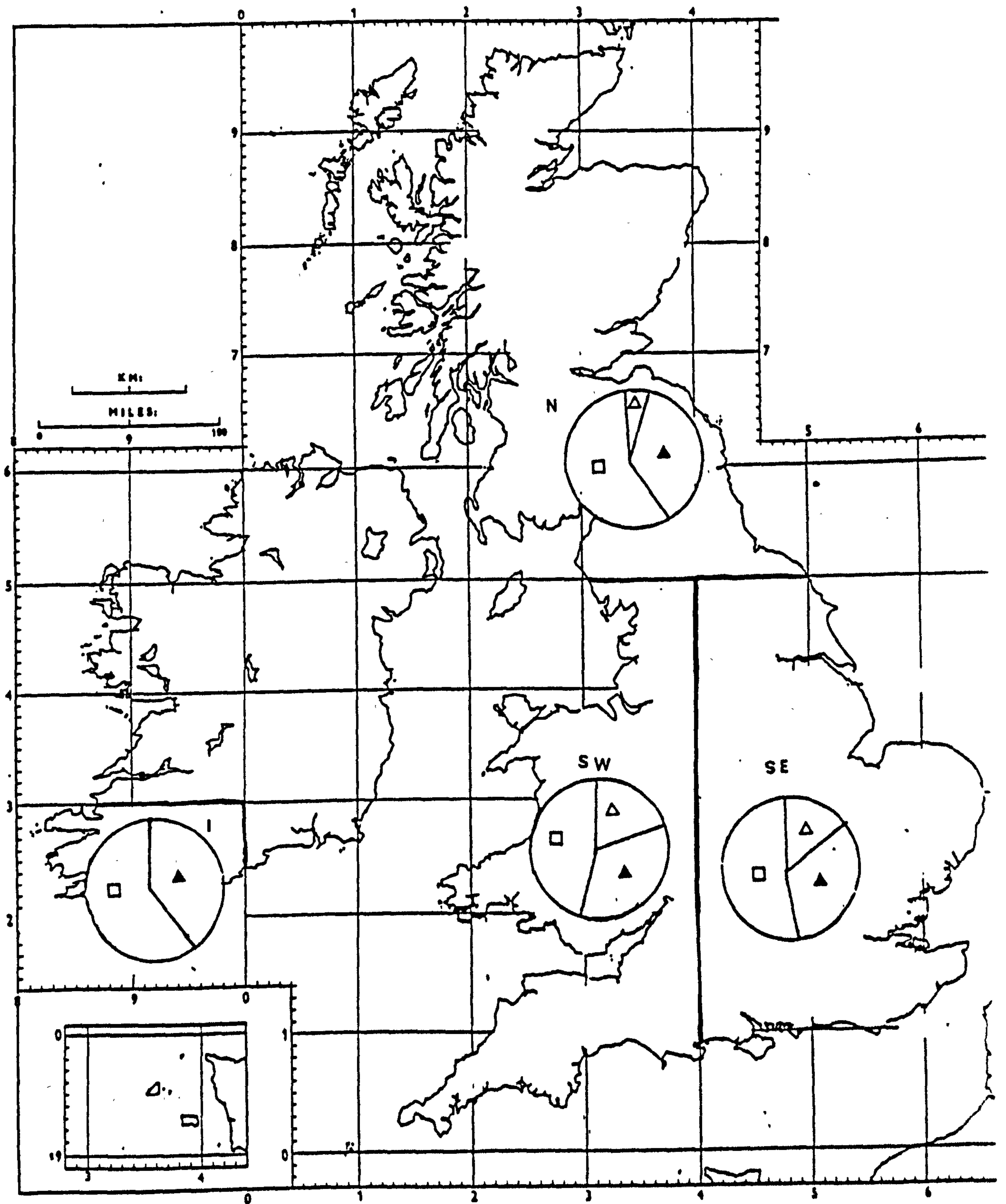


Fig. 62. The proportional distribution of haploid, diploid and triploid A. undulatum in the four geographical areas of the British Isles. Haploid ( Δ ), Diploid ( ▲ ), and Triploid ( □ ).



In another example Lazarenko and Lesnjak (1977) found no haploid A. undulatum in western U.S.S.R. with continental climate with much colder winters than Britain, thus extrapolating the situation in Britain.

## 9.2 Conclusion

In Atrichum undulatum there are three cytotypes haploid, diploid and triploid which are indistinguishable morphologically. Morphological variation is both genetical and environmental. Cultivation experiments suggest that plant height is genetically variable and that leaf length is also genetically variable. The angle between teeth and leaf margin shows genetic variability. There is some degree of genetical stability in angular cell wall thickness. Sporophyte characters seta length, seta posture, capsule length, number of peristome teeth, operculum length, calyptra and spore are variable and the three cytotypes overlap completely. From this investigation it is clear that there is no correlation between morphology and cytotype, but that there is a relationship between cytotype and geographical distribution. Inspection of contingency tables shows that this is significant and due to the distribution of the haploid.

Lowry (1954) suggested that  $n = 21$  A. undulatum was derived by apospory from the hybrid  $n = 7$  A. undulatum x  $n = 14$  A. undulatum. It seems more likely that in such a hybrid meiosis is irregular and that occasional spores with a balanced complement of 21 chromosomes occur. Diploid spores have been reported several times from naturally occurring plants. It therefore seems more likely that in such a hybrid meiosis is irregular and that occasional spores with a balanced complement of 21 chromosomes occur. Polyploidy resulting from factors of meiosis - diplospory - has been suggested by Smith (1978b). It therefore seems probable that doubling of the chromosome number results from diplospory rather than apospory,

the latter only occurring under controlled laboratory conditions (Smith 1978b). Mehra and Khanna (1961) quote the work of Lowry (1954) on A. undulatum as an example of allopolyploidy. Smith (1978b) comments that this is a misinterpretation of the situation. Lowry suggests that  $n = 14$  A. undulatum is derived from  $n = 7$  A. undulatum by apospory and that these two gave rise to  $n = 21$  plants by hybridity followed by apospory. This is a theoretical allopolyploid but as the three genome are similar the  $n = 21$  plants are functionally autopolyploid.

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## APPENDIX 1

### Analysis of Carotenoid Pigments

#### A. Procedure for Extraction of Carotenoids (adapted from Davies, 1965)

1. The plant material was pounded for 1-2 min. with sand and a small quantity of acetone using a pestle and mortar. The extract was filtered under reduced pressure and the process repeated 2-3 times.
2. The extract was concentrated under reduced pressure and diluted with an equal volume of diethyl ether, then water, until 2 layers were formed.
3. The lower aqueous phase was run off and re-extracted with ether to remove any remaining lipid-soluble material and then discarded.
4. The ether solutions were bulked and washed with water to remove traces of acetone, then distilled to a small volume under reduced pressure and dried by standing over anhydrous sodium sulphate for about 1 hour.
5. The sodium sulphate was removed by filtering and the extract was evaporated to dryness on a hot water-bath in a stream of nitrogen.

#### B. Saponification Procedure

This step was necessary in order to remove unwanted lipid material.

1. Sufficient absolute alcohol was added to dissolve completely the dry extract, then 60% (W/V) aqueous potassium hydroxide was added, 1 ml to every 10 ml of ethanolic solution.
2. The alkaline mixture was left in the dark at room temperature under nitrogen for about 12 hours.

3. The alkaline solution was then diluted with 3 volumes of water and freshly distilled diethyl ether was added (1 volume to 3 of the alkaline water (ethanol phase)). The mixture was shaken firmly in a separating funnel and allowed to stand until 2 phases appeared, the carotenoids being in the upper (ether) phase. The lower phase was discarded.
4. The extraction with ether was repeated twice and the three extracts bulked.
5. The ether solution was washed free of alkali by shaking with its own volume of water. The washing was repeated several times, the water being discarded on each occasion.
6. The ether extract was dried by shaking with powdered anhydrous sodium sulphate (5-10 g per 100 ml of extract) and left to stand for 1-2 hours.
7. The sodium sulphate was filtered off and washed with fresh dry ether. The ether solution was concentrated by distillation in the dark under reduced pressure and finally blown to dryness with a stream of nitrogen on a hot water-bath.
8. The residue was stored under nitrogen at 0°C until ready for use.

#### C. Thin-Layer Chromatography

The stored extract was re-dissolved in 1-2 ml of fresh, dry ether and loaded on plates coated with Silica Gel GF 254. Effective separation of the carotenoid compounds was achieved using both 20% Ethyl acetate in Methylene chloride and 15% Methanol in Benzene as developing systems. Some spots had a spontaneous colouration; others only appeared after spraying with a saturated solution of antimony trichloride in chloroform

and heating at  $110^{\circ}\text{C}$  for 15-20 min. (Davies et al., 1963). The colours after treatment with the staining reagent are shown on the chromatograms (Figs. 30, 31).

APPENDIX 2

Comparison of Chromosome Length of haploid, diploid and triploid Atrichum undulatum using Tuckey's interval estimate

Tukey's interval estimate was used to calculate the 95% confidence intervals for the difference between mean value of pairs of cytotypes. The statistic  $q'$  was calculated by:

$$q' = q_{v,r} \times \sqrt{\frac{MSE}{2} \left( \frac{1}{n_1} + \frac{1}{n_2} \right)}$$

Where  $q$  was derived from tables for  $v$  = number of groups (i.e. cytotypes) and  $r$  = number of degree of freedom of error terms in the analysis of variance table. MSE is the mean square error from the analysis of variance.  $n_1, n_2$  are the sample size of the two groups. 95% confidence intervals were then calculated as:

$$(\bar{X}_i - \bar{X}_j) - q' \leq \mu_i - \mu_j \leq q' + (\bar{X}_i - \bar{X}_j)$$

Where  $\bar{X}_i, \bar{X}_j$  are the mean values for the two groups.

$\mu_i - \mu_j$  is the expected difference between cytotype means.

Chromosome number	Chromosome Length ( $\bar{X}$ ) in $\mu m$		
	Haploid	Diploid	Triploid
1	6.40	6.14	6.14
2	6.34	5.82	5.9
3	6.32	5.65	5.71
4	5.32	4.81	4.88
5	5.25	4.60	4.80
6	4.19	4.68	4.32
7	3.65	3.19	3.52
Total	5.353	4.984	5.038



<u>Source</u>	<u>S.S.</u>	<u>DF</u>	<u>MSE</u>	<u>F</u>	<u>PROB.</u>
1. Chromosome number	19.10	6	3.00	66.92	0.000
2. Cytotypes	18.01	2	0.28	6.18	0.0143
3. Error	0.55	12	0.04		
4. Total	0.54	20			

As  $P < 0.05$ , then the differences between groups (ploidy levels or cytotypes) is significant. The chromosomes in the three cytotypes are not equal in length, and significant differences have been found between them. The expected difference between the cytotype means was calculated to estimate which cytotype is responsible for this significant difference in length.

1. Between haploid and diploid cytotypes

$$(\bar{X}_1 - \bar{X}_2) - q' \leq \mu_1 - \mu_2 \leq q' + (\bar{X}_1 - \bar{X}_2)$$

$$0.369 - 0.285 \leq \mu_1 - \mu_2 \leq 0.285 + 0.369$$

$$0.084 \leq \mu_1 - \mu_2 \leq 6.654$$

Assuming that the chromosome length in the haploid and diploid cytotypes are equal, then  $\mu_1 - \mu_2 = 0$ , but as 0 is not inside the 95% confidence intervals, therefore the chromosome length in these cytotypes are not equal in length and the difference in length is significant.

2. Between diploid and triploid cytotype

$$(\bar{X}_3 - \bar{X}_2) - q' \leq \mu_3 - \mu_2 \leq q' + (\bar{X}_3 - \bar{X}_2)$$

$$0.054 - 0.285 \leq \mu_3 - \mu_2 \leq 0.285 + 0.054$$

$$-0.231 \leq \mu_3 - \mu_2 \leq 0.339$$

Assuming that the chromosome length in the diploid and triploid cytotypes are equal, then  $U_3 - U_2 = 0$ , which is inside the 95% confidence intervals.

### 3. Between haploid and triploid cytotype

$$(\bar{X}_1 - \bar{X}_3) - q' \leq \mu_1 - \mu_3 \leq q' + (\bar{X}_1 - \bar{X}_3)$$

$$0.315 - 0.285 \leq \mu_1 - \mu_3 \leq 0.285 + 0.315$$

$$0.03 \leq \mu_1 - \mu_3 \leq 0.600$$

As  $\mu_1 - \mu_3 \neq 0$ , then there is a significant difference in chromosome length between the two cytotypes.

### Conclusion

As the expected difference in means (i.e.  $\mu_i - \mu_j = 0$ ) between triploid and diploid cytotypes, which is inside the 95% confidence intervals, therefore the total chromosome length is not significantly different in these cytotypes. In the haploid and triploid and haploid and diploid cytotypes the difference in means is outside the 95% confidence intervals. Therefore there are significant differences between each group of cytotypes. It would appear that the chromosomes in the haploid cytotype are responsible for the significant difference found. The diploid and triploid chromosome lengths are similar, but both are different from the haploid chromosomes. The total chromosome length of the triploid is  $3/2$  times that of the diploid, but the total length in the diploid and triploid are not twice and three times that in the haploid

APPENDIX 3

Comparison of DNA Content of Three cytotypes of Atrichum undulatum

Using Tukey's Interval Estimate

Tukey's interval estimate was used to calculate the 95% confidence intervals for the differences between means of pairs of groups of samples. The statistic  $q$  was calculated by:

$$q' = q_{v,r} \cdot \sqrt{\frac{MSE}{2} \left( \frac{1}{n_1} + \frac{1}{n_2} \right)}$$

where  $q_{v,r}$  was derived from tables for  $v$  = number of groups (i.e. cytotypes) and  $r$  = number of degrees of freedom of error term in the analysis of variance.

MSE is Mean Square Error from the Analysis of Variance.

$n_1, n_2$  are sample sizes of the two groups.

95% confidence intervals were then calculated as:-

$$(\bar{x}_i - \bar{x}_j) - q' \leq (\mu_i - \mu_j) \leq q' + (\bar{x}_i - \bar{x}_j)$$

where  $\bar{x}_1, \bar{x}_2$  were the means of  $\log_{10}$  transform data for the two groups.

$(\mu_2 - \mu_1)$  was the expected difference between gatherings means.

The DNA content in haploid, diploid and triploid A. undulatum was assumed to be in the ratio of 1:2:3:. A  $\log_{10}$  transformation was applied to the data to give it a normal distribution and equal variance for the three cytotypes as follows:-

<u>Cytotype</u>	<u>Size (n)</u>	<u>Mean <math>\bar{x}</math></u>	<u>St. Dev.</u>
Haploid	45	1.78	0.117747
Diploid	58	1.80	0.112555
Triploid	65	2.10	0.125273



<u>Source</u>	<u>Sum of SQ.</u>	<u>D.F.</u>	<u>M.S.E</u>	<u>F</u>
Between	4.110306	2	2.055	146.00
Within	2.322456	165	0.01408	

Values of  $(\mu_2 - \mu_1)$  under the above assumption was taken as follows:-

1. Between diploid and haploid cytotypes  $\log_{10} 2 = 0.30102$

$$(\bar{x}_2 - \bar{x}_1) - q' \leq (\mu_2 - \mu_1) \leq q' + (\bar{x}_2 - \bar{x}_1)$$

$$0.02 - 0.0555 \leq (\mu_2 - \mu_1) \leq 0.0555 + 0.02$$

$$- 0.0355 \leq (\mu_2 - \mu_1) \leq 0.0755$$

2. Between triploid and haploid cytotypes  $\log_{10} 3 = 0.47712$

$$(\bar{x}_3 - \bar{x}_1) - q' \leq (\mu_3 - \mu_1) \leq q' + (\bar{x}_3 - \bar{x}_1)$$

$$0.34 - 0.054 \leq (\mu_3 - \mu_1) \leq 0.054 + 0.34$$

$$0.286 \leq (\mu_3 - \mu_1) \leq 0.394$$

3. Between triploid and diploid cytotypes  $\log_{10} 1.5 = 0.17609$

$$(\bar{x}_3 - \bar{x}_2) - q' \leq (\mu_3 - \mu_2) \leq q' + (\bar{x}_3 - \bar{x}_2)$$

$$0.3 - 0.0504 \leq (\mu_3 - \mu_2) \leq 0.0504 + 0.3$$

$$0.2496 \leq (\mu_3 - \mu_2) \leq 0.3504$$

As none of the three confidence intervals contain the expected values of  $\log_{10} 2$ ,  $\log_{10} 3$  and  $\log_{10} 1.5$  the assumption that the ratio of DNA content in haploid:diploid:triploid is 1:2:3 is therefore rejected by the data.



# APPENDIX 4

## Localities and Chromosome Numbers of *Atrichum undulatum* used in this study

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
6 miles, east of Longtown Cumbria	14	70	NY/468707	May 80	D.E. Ellis
Bacton Wood, Norfolk	14	27	TG/317312	9.11.80	M.J. Selwyn
Dowdswell Wood, near Cheltenham	7	33	SO/9920	24.8.79	J.A. Appleyard
4 miles south-south west of Kelso Roxburghshire	14	80	NT/696280	Aug. 80	D.E. Ellis
Bush Estate, Penicuik, Midlothian	14	83	NT/245633	Aug. 80	P.J. Lishtowlers
By River Dee, nr.Dent	14	65	SD/7087	2.9.79	F.E. Branson
Llangathen, Camarthenshire	21	44	SN/579214	26.11.79	Ray Woods
3/4 mile west of Ecclefechan Dunfresshire	21	72	NY/184747	Jan. 81	D.E. Ellis
Hunwick churchyard, Durham	14	66	NZ/192326	21.11.79	G.G. Graham
Hunwick churchyard, Durham	14	66	NZ/192326	12.11.79	G.G. Graham
Earth among roots?	14	65	NJ/1868	27.2.80	Roland Ruchter
Fownhope, Hereford	21	36	SO/5934	17.5.80	P.J. Port

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
Streamside, valley field Callander, Perthshire	21	87	NN/587097	22.4.80	D.E. Ellis
Clearing in lodgepole pine wood Wykeham Forest, Scarborough	7	62	SE/950874	May. 80	D.E. Ellis
North of Langholm - Castle over forest	21	72	NY/221971	June 80	D.E. Ellis
Riverside broadleaf wood south of Newcastleton	21	80	NY/475854	June 80	D.E. Ellis
Glendinning rigg, Canonbie Cumbria	21	70	NY/440759	May 80	D.E. Ellis
Cove Wood, Kirkpatrick Fleming Dumfriesshire	21	72	NY/265705	May 80	D.E. Ellis
St. Blasius Church, Shanklin	7	10	SZ/578805	June 80	L. Snow
Kielder Village, Northumberland	21	67	NY/638897	May 80	D.E. Ellis
Cove Wood, Kirkpatrick Fleming, Dumfriesshire	21	72	NY/258708	May 80	D.E. Ellis
Outskirts of Kielder village Northumberland	21	67	NY/627934	24.4.80	D.E. Ellis
12 miles south of Hawick, Roxburghshire	21	80	NY/469970	May 80	D.E. Ellis
3½ miles north-north south of Langholm Dumfriesshire	21	72	NY/351900	June 80	D.E. Ellis

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
4½ miles north-east Langholm, Dumfrieshire	21	72	NY/414894	13.4.80	D.E. Ellis
Outskirts of Kielder village	21	67	NY/630934	24.4.80	D.E. Ellis
5 miles north-east of Langholm, Dumfrieshire	21	72	NY/418896	13.4.80	D.E. Ellis
3½ miles north-north west of Langholm, Dumfrieshire	21	72	NY/336894	June 80	D.E. Ellis
3½ miles north-north west of Langholm, Dumfrieshire	21	72	NY/332897	June 80	D.E. Ellis
10 miles west-north west of Bellingham Northumberland	14	67	NY/679859	8.4.80	D.E. Ellis
5 miles north-east of Newcastleton Roxburghshire	14	80	NY/554920	6.4.80	D.E. Ellis
3 miles north east of Newcastleton	14	67	NY/690898	18.4.80	D.E. Ellis
3½ miles north-north west of Langholm	21	72	NY/349902	June 80	D.E. Ellis
Earth bank	21	95	NJ/3460	30.3.80	R. Richter
Near Eardisley, Herefords	14	36	SO/3150	5.5.80	P.J. Port
Rhayader (Claewen Dam)	14	43	SN/8763	26.4.80	P.J. Port



LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
Hinton Hill, Dyrham	14	34	ST/742763	9.4.80	G.W. Garlick
By stream in woodland	7	3	SS/8106	24.4.80	J. Appleyard
Woodland near stream	14	4	SS/8714	22.4.80	J. Appleyard
Pagets Wood near Fowahope, Herefords	21	36	SO/5934	17.5.80	P.J. Port
Walters Copse, Newtown, I. of Wight	7	10	SZ/430908	June 80	L. Snow
Soil-covered stump	21	3	SS/8503	26.4.80	J. Appleyard
Earth bank in beech wood	21	95	NJ/1662	3.4.80	R. Richter
Mattersley Hill farm, Mattersley, N. Notts.	7	56	SK/6889	29.8.80	P.A. Henley
Mattersley Hill farm, Mattersley, N. Notts.	14	56	SK/6889	29.8.80	P.A. Henley
Mattersley Hill farm, Mattersley, N. Notts.	7	56	SK/6889	29.8.80	P.A. Henley
Mattersley Hill farm, Mattersley, N. Notts.	21	56	SK/6889	29.8.80	P.A. Henley
Wooded banks of Afon Vernwy Dolanog, Mongomery	7	47	SJ/0712	Sept. 80	M.E. Newton
Gampshott Wood, near Horsley, Surrey	21	17	TQ/24	6.9.80	J.C. Gardiner
Biggs Grove, near Kimpton, Herts.	7	20	TL/199193	1.9.80	Bloom
Hall Lays Wood, near Knebworth, Herts.	21	20	TL/208206	27.8.80	Bloom
Wath Wood, St. Pauls Waldon	21	20	TL/186221	Aug. 80	Bloom



LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
Near Battle, E. Sussex	21	14	TQ/71	18.9.80	C.C. Townsend
Churchyard, Kings Wood, Surrey	21	17	TQ/25	28.8.80	J.C. Gardiner
Aber Valley	21	49	SH/67	9.7.80	S. Abd.
Aber Valley	21	49	SH/67	9.7.80	S. Abd.
	14	27	TG/157166	23.9.80	M.J. Selwyn
Sellers Wood, Bulwell, Nottingham	21	56	SK/5345	30.8.80	P.A. Henley
Ebbor Wood, N. Somerset	21	6	ST/522485	21.9.80	Vilma McAdam
Ebbor Wood, N. Somerset	21	6	ST/522485	21.9.80	Vilma McAdam
Ranmore Common, near Dorking, Surrey	21	17	TQ/15	27.9.80	J.C. Gardiner
Nant Melyn N.N.R. near Randumyn Carmarthenshire	7	44	SN/728467	Sept. 80	Ray Woods
Hayne Barton	7	4	SS/3702	10.10.80	J. Appleyard
Monkleigh	21	4	SS/4620	10.10.80	J. Appleyard
Ashridge Court	21	4	SS/6603	10.10.80	J. Appleyard
New Bridge Wood	14	4	SS/5628	10.10.80	J. Appleyard
Dippermill	7	4	SS/4406	10.10.80	J. Appleyard
Booton Common	21	27	TQ/112229	12.10.80	M. Selwyn

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
Yorkshire	21	62	SE/98	20.6.80	W.D. Foster
Yorkshire	7	62	SE/98	20.6.80	W.D. Foster
10 miles north-west of Langholm, Dumfriesshire	21	72	NY/256945	July 80	D.E. Ellis
6 miles north-west of Gretna, Dumfriesshire	21	72	NY/262742	July 80	D.E. Ellis
3 miles east-north east of Langholm, Dumfriesshire	21	72	NY/404867	Aug. 80	D.E. Ellis
1/2 mile east of Earlston, Berwickshire	21	81	NT/585389	Aug. 80	D.E. Ellis
Woods south-west Stapleford	21	58	SK/85	4.7.80	W.D. Foster
12 miles north-west of Langholm	21	72	NY/265965	July 80	D.E. Ellis
6 miles north Langholm, Dumfriesshire	21	72	NY/391956	July 80	D.E. Ellis
3/4 miles east of Earlston, Berwickshire	21	81	NT/587389	Aug. 80	D.E. Ellis
4 miles west of Tedburgh, Roxburghshire	21	80	NT/604195	Sept. 80	D.E. Ellis
9 miles north-west of Langholm, Dumfriesshire	14	72	NY/265950	Sept. 80	D.E. Ellis
9 miles north-west of Langholm, Dumfriesshire	14	72	NY/267952	Sept. 80	D.E. Ellis
1 mile north west of Langholm, Dumfriesshire	14	72	NY/353852	Sept. 80	D.E. Ellis

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
5 miles south-south east of Newcastleton, Roxburghshire	14	80	NY/520808	Sept. 80	D.E. Ellis
5 miles south-south east of Newcastleton, Roxburghshire	14	80	NY/521811	Sept. 80	D.E. Ellis
1 mile north-west of Langholm, Dumfrieshire	14	72	NY/353853	Sept. 80	D.E. Ellis
10 miles east-south east of Moffat, Dumfrieshire	21	72	NY/229930	Sept. 80	D.E. Ellis
10 miles east of Moffat, Dumfrieshire	21	72	NY/231927	Sept. 80	D.E. Ellis
9 miles north-west of Langholm, Dumfrieshire	21	72	NY/271955	Sept. 80	D.E. Ellis
Abberley Hill, Worcester	21	37	SO/761666	12.10.80	R. Fisk
7 miles north of Langholm, Dumfrieshire	21	72	NY/391956	July 80	D.E. Ellis
Bishops Wood, Worcester	14	37	SO/836684	25.10.80	R. Frisk
Abberley Hill, Worcester	21	37	SO/761667	12.10.80	R. Fisk
Townhurst Wood near Abinger, Surrey	21	17	TQ/14	14.9.80	J.C. Gardiner
Dunley Hill near Effingham, Surrey	14	17	TQ/15 SW	27.9.80	J.C. Gardiner
Norton, Presteign, Powys	14	43	SO/298680	12.10.80	P.J. Port

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
Hebblethwaite Gill, nr. Sedburgh	21	70	SD/692933	Oct. 80	P. Henley
Dovecote Gill, near Sedburgh	14	70	SD/693918	Oct. 80	P. Henley
Hebblethwaite Gill, W. Sedburgh	21	70	SD/692933	Oct. 80	P. Henley
Near Vestgate, Co Durham	14	66	NY/906387	31.7.80	Vilma McAdam
Near Westgate, Co Durham	14	66	NY/906387	31.7.80	Vilma McAdam
Gallows Wood, Barnet, South Humberside	21	54	TF/38643	July 80	J.M. Cressey
Near Barkway, Royston	21	20	TL/395353	16.7.80	Bloom
North of Mountfield, E. Sussex	7	14	TQ/2330	18.9.80	C.C. Townsend
Near Tarnhouse Tarn, north-east of Lupton	21	69	SO/5683	18.9.80	A.C. Crundwell
Near Capel, East Kent	21	15	TQ/634445	1.9.80	Trudy Side
Near Capel, East Kent	14	15	TQ/634445	1.9.80	Trudy Side
Near Capel, East Kent	7	15	TQ/634445	1.9.80	Trudy Side
Lon-y-Bryn, Bangor	14	49	SH/5771	Oct. 80	W.S. Lacey
Aber Valley, Coedydd, Gwynedd	14	49	SH/67	Oct. 80	S. Abd.
Aber Valley, Coedydd, Gwynedd	14	49	SH/67	9.7.80	S. Abd.
Near Breachwood Green	7	20	TL/156217	2.8.80	G. Bloom



LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
Lyncombe Wood	21	27	TG/157166	23.9.80	M.J. Selwyn
	7	5	ST/4219	22.10.80	J. Appleyard
	14	27	TG/157166	23.9.80	M.J. Selwyn
	14	27	TG/157166	23.9.80	M.J. Selwyn
Ayst St. Lawrence	14	20	TL/185173	18.8.80	G. Bloom
Near Wheathampstead	21	20	TL/167125	22.7.80	G. Bloom
Near Wheathampstead	14	20	TL/174164	24.7.80	G. Bloom
Milton, Bridge of Cally near Blairgowrie, Perthshire	21	89	NO/137570	Sept. 79	D.E. Ellis
3 miles east of Canonbie, Dumfriesshire	14	70	NY/223259	23.9.79	D.E. Ellis
Newcastleton, Roxburghshire	21	80	NY/45846	Sept. 79	D.E. Ellis
Blairgowrie, Perthshire	14	80	NO/177461	Sept. 79	D.E. Ellis
2 miles north of Selkirk, Selkirkshire	14	79	NT/468322	Sept. 79	D.E. Ellis
Abernethy, Perthshire	21	88	NO/188140	Sept. 79	D.E. Ellis
Near Blairgowrie, Perthshire	21	89	NO/055629	Sept. 79	D.E. Ellis
8 miles south-south east of Hawick, Roxburghshire	21	80	NT/566037	Sept. 79	D.E. Ellis

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
North side of Loch Tunnel, Perthshire	21	88	NN/803599	Sept. 79	D.E. Ellis
½ mile north of Langholm, Dumfriesshire	21	72	NY/367854	Sept. 79	D.E. Ellis
3 miles east of Langholm, Dumfriesshire	21	72	NY/412856	Sept. 79	D.E. Ellis
Near Blairgowrie, Perthshire	14	89	NO/052629	Sept. 79	D.E. Ellis
Blairgowrie, Perthshire	21	89	NO/1781460	Sept. 79	D.E. Ellis
10 miles south of Hawick, Roxburghshire	14	80	NY/537989	Sept. 79	D.E. Ellis
½ mile south west of south end of Bassenthwaite lake, Cumbria	14	70	NY/223259	17.9.79	D.E. Ellis
8 miles south-south east of Jedburgh	14	80	NT/709092	27.9.79	D.E. Ellis
3 miles east of Canonbie, Dumfriesshire	21	72	NY/430770	23.9.79	D.E. Ellis
Cum-Tal-Drum	21	44	SN/766268	Sept. 80	R.A. Woods
Abernethy, Perthshire	21	88	NO/186155	Sept. 80	D.E. Ellis
Newcastleton	14	80	NT/730083	8.9.79	D.E. Ellis
10 miles south-east of Newcastleton	21	80	NT/738088	8.9.79	D.E. Ellis

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
5 miles south-east of Newcastleton	14	80	NY/525814	8.9.79	D.E. Ellis
Newcastleton	21	80	NY/482878	8.9.79	D.E. Ellis
10 miles south-east of Newcastleton	21	80	NT/736084	8.9.79	D.E. Ellis
11 miles south-east of Hawick	21	80	NT/618022	Sept. 79	D.E. Ellis
5 miles south-east of Newcastleton	21	80	NY/524813	8.9.79	D.E. Ellis
10 miles south-east of Jedburgh Roxburghshire	21	80	NT/749092	Sept. 79	D.E. Ellis
11 miles south-east of Hawick	21	80	NT/622024	Sept. 79	D.E. Ellis
Between Bath and Marchfield, Somerset	14	6	ST/786799	June 79	J. Appleyard
Between Bath and Marchfield, Somerset	21	6	ST/786209	June 79	J. Appleyard
South-west of Glen Docherty, east of Kinlochewe	21	105	NH/0460	31.3.79	J.A. Paton
North-south side of Allt Mor, east of Kishorn River, Ross	21	105	NG/8442	30.3.79	J.A. Paton
Coulin Lodge, south-west of Kinlochewe, Ross	21	105	- NH/0056	28.3.79	J.A. Paton
North side, Kinlochewe, Ross	21	105	NH/0263	27.3.79	J.A. Paton
Llydsinan, Newbridge-on-Wye, Brecknock	14	42	SO/008586	July 79	R.G. Woods
Allt Rhyd y Croes, N.N.R. Carmarthenshire	21	44	SN/768479	July 79	R.G. Woods

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
Mattersley Hill farm, Mattersley, N. Notts.	21	56	SK/6889	29.8.80	P.A. Henley
Mattersley Hill farm, Mattersley N. Notts.	21	56	SK/6889	29.8.80	P.A. Henley
Aber Valley	21	49	SH/67	9.7.80	S. Abd.
Near Capel, East Kent	7	15	TQ/634445	1.9.80	Trudy Side
Near Capel, East Kent	14	15	TQ/634445	1.9.80	Trudy Side
Allt Rhyd y Croes, N.N.R. Carmarthenshire	7	44	SN/768479	July 79	R.G. Woods
Allt Rhyd y Croes, N.N.R. Carmarthenshire	7	44	SN/768479	July 79	R.G. Woods
Near Ightham, Kent	14	15	TQ/575530	July 79	A.G. Side
Ebbor Gorge, Somerset	7	6	ST/5248	7.4.79	J. Appleyard
Stocking Wood, Detling	21	15	TQ/8052	6.8.79	A.G. Side
Above Boarley, Kent	14	15	TQ/7660	6.8.79	A.G. Side
Above Boarley, Kent	7	15	TQ/7660	6.8.79	A.G. Side
Above Boarley, Kent	21	15	TQ/7660	6.8.79	A.G. Side
Stocking Wood, Detling, Kent	21	15	TQ/8059	6.8.79	A.G. Side
Ebbor Gorge, Somerset	14	6	ST/5248	7.8.79	J. Appleyard



LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
Priory Wood, near Bilsington, Kent	21	15	TR/0335	8.8.79	A.G. Side
South side Loch of Lowne, Dunkeld Perthshire	14	89	NO/043433	Sept. 80	D.E. Ellis
Brecon Beacons	14	42	SO/02	12.2.80	R.A. Woods
8 miles south-south east of Fedborough, Roxburghshire	21	80	NT/715095	20.9.79	D.E. Ellis
$\frac{1}{2}$ mile north of Langholm, Dumfriesshire	21	72	NY/366853	Sept. 79	D.E. Ellis
2 miles south of Langholm	14	72	NY/375823	Sept. 79	D.E. Ellis
1 mile north of Newcastleton, Roxburghshire	21	80	NY/491895	Sept. 79	D.E. Ellis
3 miles south-east of Hexham	14	67	NY/963614	19.2.80	D.E. Ellis
1 mile west of Newcastleton, Roxburghshire	7	80	NY/471876	17.2.80	D.E. Ellis
4 miles east of Brampton, Cumberland	14	70	NY/587627	19.2.80	D.E. Ellis
1 mile west of Stocksfield, near Newcastle, Northumberland	21	67	NZ/043608	18.2.80	D.E. Ellis
1 mile west of Stocksfield, near Newcastle, Northumberland	21	67	NZ/043607	18.2.80	D.E. Ellis

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
Withington Woods	21	33	SP/0314	7.10.79	J. Appleyard
Littleworth Common, near Esher, Surrey	21	17	TQ/147650	22.2.80	J.C. Gardiner
Stronochollins	21	101	NR/850799	Feb. 80	P. Highboulus
Guzedale forest near Lake Windermere	14	69	SD/340963	29.9.79	J.C. Gardiner
Styal Woods, Cheshire	21	58	SJ/828831	25.8.79	A.R. Outen
Styal Woods, Cheshire	7	58	SJ/828831	25.8.79	A.R. Outen
Styal Woods, Cheshire	14	58	SJ/828831	25.8.79	A.R. Outen
Lathkill Dale, Derbyshire	21	57	SK/211657	Aug. 79	Ray Woods
Holywell Dingle, Eardisley, Herefords	7	36	SO/312513	22.8.79	P.J. Port
Sharpley Plantation north-west of Murton, Co-Durham	21	66	NZ/374492	Aug. 79	Robin Stevenson
Pentraeth, Anglesey	21	52	SH/520792	11.12.79	C. Jones
Aron Gorge, Bristol	14	34	ST/563735	12.9.79	P. Martin
Yeld Wood, Kington, Herefords	21	36	SO/281569	2.11.79	P.J. Port
Bradnor Wood, Kington, Herefords	14	36	SO/275575	3.11.79	P.J. Port
Holywell Dingle near Eardisley, Herefords	21	36	SO/312513	28.11.79	P.J. Port

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
Hurstmonaux Castle, Sussex	21	14	TQ/645104	30.8.79	P.J. Port
Trewithen, East of Probus	7	2	SW/9147	21.9.79	J.A. Paton
North-east of Blisland, east Conwell	7	2	SX/1074	22.9.79	J.A. Paton
South-east of Wenford bridge, east Conwell	14	2	SX/0974	22.9.79	J.A. Paton
Allt Rhyd-y-groes stream, near Rhaeder-mwyn, Carmarthenshire	21	44	SN/770475	16.10.79	Ray Woods
Craig y Rhaider, near Cilycwm	7	44	SN/755437	15.2.80	Ray Woods
Bracken Gill, Dent	21	65	SD/7087	1.9.79	J.E. Branson
Victoria College, Jersey	21	113	90/42	Oct. 79	E.H. Dufeu
70 feet above R. Wharfe	21	64	SE/068560	Oct. 79	R.M. Henson
70 feet above R. Wharfe	14	64	SE/068560	Oct. 79	R.M. Henson
Vallee des Vaux near Helier, Jersey	21	113	90/42	Sept. 79	E.H. Dufeu
20 feet above R. Wharfe	21	64	SE/074558	Oct. 79	R. Henson
Bradnor Wood, Kington, Herefords	7	36	SO/279575	3.11.79	P.J. Port
Cholesbury, Bucks	14	24	TQ/932065	3.12.79	P.J. Port
Yeld Wood, Kington, Herefords	21	36	SO/279569	2.11.79	P.J. Port
Stonor, Oxforas	14	23	SU/741885	2.12.79	P.J. Port

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School Wood, Aymestry, Herefords	14	36	SO/432654	10.11.79	P.J. Port
South of Wellington, Shropshire	7	40	SJ/6409	8.9.79	M.E. Newton
South of Wellington, Shropshire	14	40	SJ/6409	8.9.79	M.E. Newton
South of Wellington, Shropshire	21	40	SJ/6409	8.9.79	M.E. Newton
Stronochulin Garden	21	101	NR/850798	Feb. 80	P. Highboulus
Whitehaven	14	70	NX/982175	24.2.80	C. Haworth
South-west of Much Wenlock, Shropshire	21	40	SO/5797	9.9.79	M.E. Newton
South-west of Much Wenlock, Shropshire	21	40	SO/5797	9.9.79	M.E. Newton
South-west of Much Wenlock, Shropshire	21	40	SO/5797	9.9.79	M.E. Newton
West of Cornbank Estate, Penicvik, Midlothian	21	83	NT/219603	Sept. 79	B.G. Bell
West of Cornbank Estate, Penicvik, Midlothian	21	83	NT/219603	Sept. 79	B.G. Bell
Flitwick Plantation, Flitwick, Beds.	14	30	TL/008343	Oct. 79	A.R. Outen
Claremount Garden, near Esher	14	17	TQ/128632	25.8.79	J.C. Gardiner
Broadmoor, near Wolton, Surrey	21	17	TQ/132450	16.9.79	J.C. Gardiner



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Borth, Cardigan	21	46	SN/608883	27.2.80	S. Agnew
Borth, Cardigan	14	46	SN/608883	27.2.80	S. Agnew
Bird Ringing Station, Nature Reserve, Knarborough	21	64	SE/3557	7.10.79	J.E. Branson
Coaley Wood, Coaley, Dursley	14	34	SO/794006	April 80	G.W. Gartick
Shrubbery, Parkhill, Arbroath	21	90	NO/646454	1.10.79	U.K. Duncan
Clowance, south of Cambourne	21	1	SW/637348	20.10.79	J.A. Paton
Queen's Drive, Cheadle Hulme, Cheshire	7	58	SJ/872869	27.8.79	A.R. Outen
Queen's Drive, Cheadle Hulme, Cheshire	21	58	SJ/872869	27.8.79	A.R. Outen
Aldbury Common, Herts.	21	20	SP/974119	29.8.79	A.R. Outen
Aldbury Common, Herts.	14	20	SP/974119	29.8.79	A.R. Outen
Hedge bottom	21	74	NX/276543	March 80	R.M. Henson
Near headwater of R. Blackwater, Farnham	7	17	SU/849498	28.2.80	J.C. Gardiner
St. Helen's Wood, Hastings, East Sussex	14	14	TQ/816121	23.2.80	M. McFarlane
Bookham Commons	14	17	TQ/563132	11.11.79	O. French

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Bookham Commons	21	17	TQ/563123	27.10.79	O. French
Hyarn Wood near Malmesbury	21	7	ST/9087	2.9.79	J. Appleyard
Queens Wood	21	8	SO/9725	March 80	J. Appleyard
New Plantation, Westerleigh	21	34	ST/688805	March 80	G. Garlick
New plantation, Westerleigh	14	34	ST/688805	March 80	G. Garlick
Port Marquet	21	113	90/42	Aug. 79	E.H. Dufeu
West of Todmorden	21	59	SD/875283	March 79	R.M. Henson
Mores Wood, near Coxtie Green, Brentwood Essex	21	18	TQ/562966	28.4.79	J.C. Gardiner
Near Ottershaw Church, Brachleshaw, Beds.	14	17	TQ/017634	13.2.80	J.C. Gardiner
Weald Country Park, Pilgrims Hatch, Brentwood, Essex	21	18	TQ/578952	28.4.79	J.C. Gardiner
Blannan Llandybie	21	44	SN/600141	Feb. 80	Ray Woods
Near Top College, Bangor	7	49	SH/579724	29.10.79	C. Jones
Risby, South of Beverley	21	61	TA/012352	23.9.79	E.R.B. Little
Risby, south of Beverley	21	61	TA/012352	23.9.79	E.R.B. Little
Risby, south of Beverley	14	61	TA/012352	23.9.79	E.R.B. Little

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
Risby, south of Beverley	21	61	TA/012352	23.9.79	E.R.B. Little
Whippendell Wood, Herts.	21	20	TQ/073977	23.9.79	A.R. Outen
Wavenden Heath	21	24	SP/936341	16.9.79	A.R. Outen
Wavenden Heath	14	24	SP/936341	16.9.79	A.R. Outen
Flitwick, Beds.	21	30	TL/0335	22.8.79	A.R. Outen
Bookham Commons, Area I	21	17	TQ/563132	3.11.79	O. French
	7	33	SP/0006	13.9.79	J. Appleyard
Wain Wood, Herts.	21	20	TL/181255	26.9.79	A.R. Outen
Wrest Park, Beds.	21	30	TL/094348	3.11.79	A.R. Outen
Hertford Heath, Herts.	14	20	TL/352109	15.9.79	A.R. Outen
Wavenden Heath	14	24	SP/935340	16.9.79	A.R. Outen
Flitwick plantation, Flitwick, Beds.	14	30	TL/009342	Sept. 79	A.R. Outen
Baysbrown Wood, Great Langdale	21	69	NY/318045	17.2.80	J.H.C. Fenton
Briar's Wood, north-west Dorset	21	9	ST586091	Sept. 79	M.E. Newton
Knebworth, Herts.	21	20	TL/249208	23.9.79	A.R. Outen
St. Helen's Wood Rd., Hastings, East Sussex	21	14	TQ/814121	19.2.80	M. McFarlane

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
Plashes Wood, near Standon	21	20	TL/378207	March 80	G. Bloom
Plashes Wood, near Standon	14	20	TL/383207	March 80	G. Bloom
Plashes Wood, near Standon	14	20	TL/383207	March 80	G. Bloom
Plashes Wood, near Standon	7	20	TL/383207	March 80	G. Bloom
Near Cole Green, near Welwyn Garden City	7	20	TL/272115	March 80	G. Bloom
Bookham Commons, Area A	21	17	TQ/125568	14.10.79	O. French
Astonbury, near Aston	21	20	TL/276214	20.2.80	G. Bloom
Bookham Commons, Area J	14	17	TQ/564134	27.10.79	O. French
From garden of 17 Austen Rd., Jordan Hill, Glasgow	21	77	NS/56	Oct. 79	A.M.G. Stirling
Penton Linns, 3 miles east of Canonbie, Dumfriesshire	21	72	NY/431776	Oct. 79	D.E. Ellis
North-west corner of Cannocks Wood, near Norton Green	21	20	TL/225228	March 80	G. Bloom
Bigby, Lincolnshire	21	54	TA/045085	Sept. 79	M. Gressey
Harles Wood	14	33	SP/0529	4.10.79	J. Appleyard
Guiting Wood	21	33	SP/0725	4.10.79	J. Appleyard
	21	33	SP/2316	6.10.79	J. Appleyard
Bledington	14	33	SP/2325	3.10.79	J. Appleyard



LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
By R. Windrush near Bourton-o-the- Water	21	33	SP/2116	6.10.79	J. Appleyard
	7	33	SP/155205	Oct. 79	J. Appleyard
Near Uckfield, East Sussex	21	14	TQ/449187	10.9.79	Vilma McAdam
Kincardineshire	21	91	NO/7604	9.8.79	Vilma McAdam
Near Hailsham, East Sussex	21	14	TQ/087569	12.9.79	Vilma McAdam
Near Hailsham, East Sussex	14	14	TQ/087569	12.9.79	Vilma McAdam
Kincardineshire	7	91	NO/762804	16.8.79	Vilma McAdam
Kincardineshire	14	91	NO/826854	16.8.79	Vilma McAdam
Kincardineshire	21	91	NO/879993	16.8.79	Vilma McAdam
Kincardineshire	21	91	NO/89	16.8.79	Vilma McAdam
Near Hailsham, East Sussex	21	14	TQ/087569	12.9.79	Vilma McAdam
Near Hailsham, East Sussex	7	14	TQ/087569	12.9.79	Vilma McAdam
Near R. banks	21	92	NO/39	Sept. 79	Vilma McAdam
Near Mill of Glenbeirie	21	91	NO/762804	16.8.79	Vilma McAdam
Green Grove, near Weston	14	20	TL/262314	Oct. 79	A.R. Outen
Old Brathay, Ambleside	21	69	NY/367032	18.2.80	J. Fenton

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
Godshill, I. of Wight	21	10	SZ/531813	17.2.80	L. Snow
Stockings Wood, Detling, Kent	14	15	TQ/8059	6.8.79	A. G. Kenneth
Stockings Wood, Detling, Kent	21	15	TQ/8059	6.8.79	A.G. Kenneth
Above Boarley, Kent	14	15	TQ/7660	6.8.79	A.G. Kenneth
Above Boarley, Kent	14	15	TQ/7660	6.8.79	A.G. Kenneth
Above Boarley, Kent	21	15	TQ/7660	6.8.79	A.G. Kenneth
St. John's Wood near Waterford	14	20	TL/325154	March 80	G. Bloom
Ranmore Common	21	17	TQ/505140	21.11.79	O. French
Bookham Commons, Area Q	21	17	TQ/558124	11.11.79	O. French
Probus, Cornwall	7	1	SW/84	Oct. 79	J.A. Paton
2 miles up Glen Callater, south of Braemar	14	92	NO/18	17.2.80	S. Agnew
East Row Beck, near East Barnly, N. Yorks.	21	62	NZ/822113	6.9.79	E.R.B. Little
East Row Beck, near East Barnly, N. Yorks.	21	62	NZ/822113	6.9.79	E.R.B. Little
Bookham Commons	21	17	TQ/125565	29.9.79	O. French
Westonbirt Arboretum, near Tetbury	7	34	ST/844899	6.10.79	P. Martin

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
Avon Gorge, Bristol	21	34	ST/563736	29.10.79	P. Martin
Wolton, Surrey	14	17	TQ/126472	16.9.79	J. Gardiner
Briar's Wood, north-west Dorset	21	9	ST/586090	Sept. 79	M.E. Newton
Plashis Wood near Standon	21	20	TL/383207	March 80	G. Bloom
Ashridge Park, Herts.	21	20	SP/990120	29.8.79	A.R. Outen
Ashridge Park, Herts.	14	20	SP/990120	29.8.79	A.R. Outen
Pentraeth, Anglesey	21	52	SH/520793	11.12.79	C. Jones
University Grounds, Glasgow	14	77	NS/567667	13.2.80	A.C. Crundwell
Marston Thrift, Beds.	21	30	SP/972415	1.11.79	A.R. Outen
Priory Wood near Bilsington, Kent	21	15	TR/0335	8.8.79	A.G. Side
Old Brathey, Ambleside	21	69	NY/367034	18.2.80	J. Fenton
Himley near Wolverhampton	7	39	SO/894906	16.2.80	J. Ashton
	21	5	ST/22	Oct. 79	J. Appleyard
Parkhill, near Arbroath, Argus	21	90	NO/651452	26.9.79	U.K. Duncan
Bury-me-Wick, Ztalybridge, Cheshire	14	58	SJ/976968	Oct. 79	M.E. Newton
West of Amfield Reservoir, Hollingwood, Cheshire	14	58	SK/0097	Oct. 79	M.E. Newton

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
Tickencote Laund, near Stamford	21	55	TF/0207	23.2.80	P. Jackson
Plushes Wood near Standon	21	20	TL/383207	17.9.79	G. Bloom
Bagmore Wood, north Dorset	21	9	ST/790311	Sept. 79	M.E. Newton
Lower Canfoed Wood near Ewhurst	14	17	TQ/082394	Nov. 79	O. French
Yetminster, north-west Dorset	14	9	ST/593111	Oct.79	M.E. Newton
Pentraeth, Anglesey	21	52	SH/520793	30.10.79	C. Jones
Kingshoe Wood, Beds.	14	30	TL/002342	28.10.79	A.R. Outen
Near Harrogate	7	64	SC/279539	March 80	R.M. Henson
	21	7	SU/6797	30.8.79	J. Appleyard
	14	7	ST/8686	30.8.79	J. Appleyard
	21	7	ST/8686	30.8.79	J. Appleyard
	21	7	ST/8086	30.8.79	J. Appleyard
	21	33	SU/0797	30.8.79	J. Appleyard
Bookham Commons	7	17	TQ/127563	14.10.79	O. French
Bookham Commons	14	17	TQ/564120	11.11.79	O. French
Crowhurst, Hastings, East Sussex	21	14	TQ/756126	23.2.80	M. McFarlane
Crowhurst, Hastings	21	14	TQ/756126	23.2.80	M. McFarlane



LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
	14	95	NJ/1863	26.2.80	R. Richter
Bush Estate, Penicuik, Midlothian	21	83	NT/245633	Aug. 80	P.J. Lishtowlers
How Wood, Wastwater Lake (70)	21	80	NY/143038	Sept. 80	
By Path in wood by Gouganebarra Lake, W. Cork	21	H3	10/0867	29.9.79	Smith
By path in wood by Gouganebarra Lake, W. Cork	21	H3	10/0867	29.9.79	Smith
Gouganebarra Lake	21	H3	10/0867	29.8.79	Smith
Gouganebarra Lake	14	H3	10/0867	29.8.79	Smith
Sheep's Head, S.W. of Bantry W. Cork	14	H3	00/7334	30.8.79	Smith
Sheep's Head, S.W. of Bantry W. Cork	14	H3	00/7334	30.8.79	Smith
Glengariff River, Glengariff, Co. Cork	21	H3	00/9257	26.8.79	Smith
Glengariff River, Glengariff, Co. Cork	21	H3	00/9257	26.8.79	Smith
Inchintaglin Bridge near Adrigole, W. Cork	21	H3	00/8151	31.8.79	Smith
Inchintaglin Bridge near Adrigole, W. Cork	21	H3	00/8151	31.8.79	Smith
Near Coomhola Bridge, W. Cork	21	H3	00/9955	27.8.79	Smith
Near Coomhola Bridge, W. Cork	21	H3	00/9955	27.8.79	Smith

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
By Roughty River, S. Kerry	14	H1	10/002670	30.8.79	G. Bloom
By Glengariff River, Glengariff, W. Cork	21	H3	00/9257	26.8.79	Smith
By Glengariff River, Glengariff, W. Cork	21	H3	00/9257	26.8.79	Smith
By Gonganebarra Lake, W. Cork	21	H3	10/0867	29.9.79	Smith
By Roughty River, S. Kerry	14	H1	00/9672	29.8.79	G. Bloom
Gerahies, S.W. of Bantry, W. Cork	21	H3	00/9145	30.8.79	Smith
By Gonganebarra Lake, W. Cork	14	H3	10/0868	29.8.79	Smith
By Glenlough, W. Cork	14	H3	00/8353	Sept. 79	J.A. Paton
Newbridge-on-Wye, Brecknock	21	42	TV/0058	10.7.79	R.G. Woods
Fedw Deg, Caernarfonshire	14	49	SH/796537	5.7.79	S. Abd.
Fedw Deg, Caernarfonshire	21	49	SH/797537	5.7.79	S. Abd.
Itaford Fawr	14	49	SH/723401	10.7.79	S. Abd.
Itaford Fawr	14	49	SH/724405	10.7.79	S. Abd.
Itaford Fawr	21	49	SH/726405	10.7.79	S. Abd.
Itaford Fawr	21	49	SH/728404	10.7.79	S. Abd.
Itaford Fawr	21	49	SH/726406	10.7.79	S. Abd.
Bryn Mawr	7	49	SH/645405	12.7.79	S. Abd.

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
Coed Caefali	14	49	SH/636405	12.7.79	S. Abd.
Coed Caefali	14	49	SH/636404	12.7.79	S. Abd.
Beaumaris, Anglesey	21	49	SH/597767	12.7.79	S. Abd.
Beaumaris, Anglesey	14	49	SH/595766	12.7.79	S. Abd.
Beaumaris, Anglesey	21	49	SH/595765	12.7.79	S. Abd.
Beaumaris, Anglesey	14	49	SH/599765	12.7.79	S. Abd.
Beaumaris, Anglesey	21	49	SH/581761	12.7.79	S. Abd.
Beaumaris, Anglesey	21	49	SH/582762	12.7.79	S. Abd.
Beaumaris, Anglesey	14	49	SH/583761	12.7.79	S. Abd.
Beaumaris, Anglesey	21	49	SH/581763	12.7.79	S. Abd.
Aber, Gwynedd, Caernarfonshire	21	49	SH/662718	30.7.79	S. Abd.
Aber, Gwynedd, Caernarfonshire	21	49	SH/663716	30.7.79	S. Abd.
Aber, Gwynedd, Caernarfonshire	14	49	SH/662716	30.7.79	S. Abd.
Aber, Gwynedd, Caernarfonshire	14	49	SH/661716	30.7.79	S. Abd.
Aber, Gwynedd, Caernarfonshire	14	49	SH/664719	3.8.79	S. Abd.
Aber, Gwynedd, Caernarfonshire	21	49	SH/666717	3.8.79	S. Abd.
Aber, Gwynedd, Caernarfonshire	14	49	SH/665715	3.8.79	S. Abd.

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
Aber, Gwynedd, Caernarfonshire	14	49	SH/665725	3.8.79	S. Abd.
Aber, Gwynedd, Caernarfonshire	14	49	SH/664725	3.8.79	S. Abd.
Aber, Gwynedd, Caernarfonshire	21	49	SH/665714	3.8.79	S. Abd.
Aber, Gwynedd, Caernarfonshire	21	49	SH/665722	3.8.79	S. Abd.
Aber, Gwynedd, Caernarfonshire	21	49	SH/665721	3.8.79	S. Abd.
Aber, Gwynedd, Caernarfonshire	14	49	SH/663724	3.8.79	S. Abd.
Ihiwlas, Anglesey	21	49	SH/535785	30.8.79	S. Abd.
Ihiwlas, Anglesey	21	49	SH/538788	30.8.79	S. Abd.
Ihiwlas, Anglesey	14	49	SH/536788	30.8.79	S. Abd.
Ihiwlas, Anglesey	21	49	SH/535786	30.8.79	S. Abd.
Ihiwlas, Anglesey	14	49	SH/534785	30.8.79	S. Abd.
Ihiwlas, Anglesey	14	49	SH/535789	30.8.79	S. Abd.
Ihiwlas, Anglesey	21	49	SH/536786	30.8.79	S. Abd.
Ihiwlas, Anglesey	14	49	SH/536787	30.8.79	S. Abd.
Tyn-y-wynydd, Anglesey	21	49	SH/547787	3.9.79	S. Abd.
Tyn-y-wynydd, Anglesey	14	49	SH/546786	3.9.79	S. Abd.
Tyn-y-wynydd, Anglesey	21	49	SH/546787	3.9.79	S. Abd.



LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
Tyn-y-mynydd, Anglesey	14	49	SH/547788	3.9.79	S. Abd.
Tyn-y-mynydd, Anglesey	21	49	SH/548788	3.9.79	S. Abd.
Tyn-y-mynydd, Anglesey	21	49	SH/546788	3.9.79	S. Abd.
Tyn-y-mynydd, Anglesey	21	49	SH/558797	1.10.79	S. Abd.
Tyn-y-mynydd, Anglesey	14	49	SH/559798	1.10.79	S. Abd.
Tyn-y-mynydd, Anglesey	14	49	SH/557798	1.10.79	S. Abd.
Tyn-y-mynydd, Anglesey	14	49	SH/557797	1.10.79	S. Abd.
Tyn-y-mynydd, Anglesey	21	49	SH/558798	1.10.79	S. Abd.
Tyn-y-mynydd, Anglesey	14	49	SH/558799	1.10.79	S. Abd.
3½ miles north-north west of Langholm, Dumfriesshire	21	72	NY/346897	March 81	D.E. Ellis
13 miles north-west of Langholm, Dumfriesshire	21	72	NY/207918	3.12.80	D.E. Ellis
½ mile east of Ecclefechan, Dumfriesshire	14	72	NY/186745	Jan. 81	D.E. Ellis
¾ miles west of Ecclefechan, Dumfriesshire	21	72	NY/182750	Jan. 81	D.E. Ellis
2½ miles west of Ecclefechan, Dumfriesshire	14	72	NY/155751	Jan. 81	D.E. Ellis
½ mile west of Ecclefechan, Dumfriesshire.	21	72	NY/186746	Jan. 81	D.E. Ellis

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4 miles north-west of Langholm, Dumfrieshire	14	72	NY/345901	Feb. 81	D.E. Ellis
3 miles north-north west of Langholm, Dumfrieshire	21	72	NY/334889	Jan. 81	D.E. Ellis
5 miles north of Rockerbie, Dumfrieshire	21	72	NY/134888	Jan. 81	D.E. Ellis
3/4 mile west of Rockerbie, Dumfrieshire	14	72	NY/184747	Jan. 81	D.E. Ellis
5 miles north of Rockerbie, Dumfrieshire	21	72	NY/125901	Jan. 81	D.E. Ellis
Kellet, Lancs.	7	60	SD/534697	12.11.80	M.J. Wigginton
	21	20	TL/189223	28.1.81	G. Bloom
	21	20	TL/223143	2.2.81	G. Bloom
	21	20	TL/193230	28.1.81	G. Bloom
	14	20	TL/248 83	26.1.81	G. Bloom
Near Preston	7	20	TL/181255	6.2.81	G. Bloom
Near Preston	14	20	TL/191239	28.1.81	G. Bloom

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Near Warton, Lancs.	21	60	SD/496733	12.11.80	M.J. Wigginton
Halton, Lancs.	14	60	SD/510646	12.11.80	M.J. Wigginton
Tackley, Oxon.	21	23	SP/466215	20.12.80	M.J. Wigginton
Near Warton, Lancs.	14	60	SD/498731	12.11.80	M.J. Wigginton
Warton, Oxon.	14	23	SP/434279	21.12.80	M.J. Wigginton
Wombourne, Staffordshire	14	39	SO/865935	Jan. 81	J. Ashton
5 miles south Lockerbie, Dumfrieshire	21	72	NY/130901	Jan. 81	D.E. Ellis
9 miles near south-east Lockerbie, Dumfrieshire	21	72	NY/207948	Feb. 81	D.E. Ellis
9 miles south-east Lockerbie, Dumfrieshire	21	72	NY/207949	Feb. 81	D.E. Ellis
2 miles south-west of Newcastleton, Roxburghshire	21	80	NY/454861	7.12.80	D.E. Ellis
4 miles north-west of Langholm, Dumfrieshire	21	72	NY/327891	March 81	D.E. Ellis
11 miles north-west of Langholm, Dumfrieshire	7	72	NY/252920	8.12.80	D.E. Ellis
3½ miles north-east of Langholm, Dumfrieshire	21	72	NY/403885	Feb. 81	D.E. Ellis
½ mile south-west of Newcastleton, Roxburghshire	14	80	NY/477872	Feb. 81	D.E. Ellis

LOCALITY	CHROMOSOME NO.	V.C.	GRID REFERENCE	DATE	COLLECTOR
13 miles north-west of Langholm, Dumfrieshire	14	72	NY/207922	3.12.80	D.E. Ellis
3½ miles north-east of Langholm, Dumfrieshire	14	72	NY/402885	Feb. 81	D.E. Ellis
5 miles north Lockerbie, Dumfrieshire	7	72	NY/125897	Jan. 81	D.E. Ellis
5 miles north Lockerbie, Dumfrieshire	21	72	NY/120897	Jan. 81	D.E. Ellis
5 miles north Lockerbie, Dumfrieshire	21	72	NY/117892	Jan. 81	D.E. Ellis
4 miles north-east of Newcastleton, Roxburghshire	21	80	NY/543923	March 81	D.E. Ellis
5 miles north Lockerbie, Dumfrieshire	21	72	NY/124896	Jan. 81	D.E. Ellis
Kellet, Lancs.	14	60	SD/314308	12.11.80	M.J. Wigginton
Bacton Wood	21	60	SD/317312	9.11.80	M.J. Wigginton
Warrington, Lancs.	21	60	SD/497732	12.11.80	M.J. Wigginton
Halton, Lancs.	21	60	SD/510645	12.11.80	M.J. Wigginton
Oxford clay	21	58	ST/7443	8.1.81	J. Appleyard
Near Church Stretton Shrop.	14	40	SO/448939	Nov. 80	R. Wood
	21	42	SN/954219	Nov. 80	R. Wood
Near Newbridge-on-Wye	21	43	SO/010566	Nov. 80	R. Wood