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THE ENERGETICS OF *COLPIDIUM CAMPYLUM* STOKES, WITH A NOTE ON THE VERTICAL DISTRIBUTION OF CILIOPHORA IN THE MUD OF LOCH LEVEN, KINROSS

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Abstract

The energetics of the holotrich ciliate Colpidium campylum fed on the bacterium Moraxella sp. at 10°C, 15°C and 20°C were investigated.

The parameter used for ascertaining growth was the volume of protoplasm produced measured by means of a Coulter Counter with a mean cell volume converter attachment. Growth and consumption were measured in relation to food availability as indicated by the ratio of bacteria:protozoan. Mean cell volume variation and reproduction were also measured in relation to food availability and energy consumed.

Protozoan and bacterial material were harvested by centrifugation and freeze-dried for dried weight determinations and calorimetry studies. The energy content of Colpidium and its food source was determined with a Phillipson microbomb calorimeter.

Respiration was measured in a Warburg respirometer. Oxygen uptake in relation to population density and cell size was considered as well as the production of information concerning the heat lost during respiration for incorporation into energy budgets.

Energy budgets of two types were constructed: a 24-hour energy budget for an individual and the life-span or generation energy budget for an individual. Gross growth efficiencies, net growth efficiencies and assimilation efficiencies were considered in detail.

In addition to laboratory work the vertical distribution of Ciliophora in the mud of Loch Leven, Kinross, Scotland, was also considered; three sites, two shallow and one deep, being sampled with a core sampler over a 12 month period.

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GENERAL INTRODUCTION

General Protozoological Physiology

The science of Protozoology started with the observations of Antony van Leeuvenhook (1632-1723) using simple lenses which he ground himself. Since that time the study of these microscopic fauna has progressed to the sophisticated physiological and biochemical studies of today.

Free-living Protozoa lend themselves well to laboratory study by virtue of the ease with which most species can be cultured. The techniques of culturing have been refined with time and many investigations have been devoted to this aim (Hargitt and Fray 1917, Peters 1921, Parpart 1928, Glaser and Coria 1930, Phelps 1936, Hjelm 1970 and others). The controlled methods of today essentially involve two basic techniques;

i) the axenic culture, where the Protozoa are grown under highly sterile conditions in a nutrient medium, and

ii) the monoxenic culture where the Protozoa are grown with a single species of food organism.

The need for sterility in both methods is of the utmost importance, and present day methods of sterilisation allow a high degree of experimental control.

Early workers such as Woodruff and Baitsell (1911a, 1911b) were unaware of the importance of controlling the species and concentration of the good organism on which their cultures of Paramecium fed. While endeavouring to isolate the factors

responsible in bringing about rhythms in the reproductive activity of Paramecium in a medium of beef extract they failed to monitor or control the bacterial flora. They stated that temperature showed a certain amount of correlation with fluctuations in the fission rate, but in all probability the fluctuations they describe were the result of increased temperature producing an increase in the bacterial population in the nutrient medium they used, which naturally caused an increase in the reproductive activity of Paramecium.

Cutler and Crump (1924) were the first to become aware of the importance of food concentration in relation to reproduction in a ciliated protozoan. They found that where the ratio of bacteria per protozoan was less than 500:1 little or no reproduction occurred in 24 hours, but at a ratio of 1,024,000:1 the number of divisions became as great as 5.3.. Following this discovery many investigations were devoted to the effect of concentration of food or suitability of different species of food organism on the reproduction of Protozoa (Cleveland 1928, Luck, Sheets and Thomas 1931, Heatherington 1934, Johnson 1936, Burnbanck 1942, Rudinska 1951).

Harding (1937) as well as considering the effect of food concentration on division in Glaucoma pyriformis also considered the effect of food concentration on cell size. At low food to protozoan ratios he found that both the fission rate and cell size were dependent on food availability, but high food concentrations, although increasing cell size, had

no effect on the fission rate which maintained a maximum level. The investigation of Harding (1937) was probably the first in which the size of the ciliate was altered at will, between wide limits, with the rate of multiplication kept constant. Later studies on variation in cell size (Hamilton and Preslan 1969, Curds and Cockburn 1971) also relate cell size to food concentration, although Hamilton and Preslan (1970) consider the density of the ciliate community to be the major determining factor.

Energetics

In recent years the study of ecological energetics has gained momentum, involving both the field approach as well as the laboratory investigation. One of the first studies of this type was that of Lindeman (1942) on energy transfer in a natural community. Following this pioneering work energetics did not, as Engelman (1961) points out, spread to all types of natural communities but remained focussed on marine and fresh-water habitats. Further, investigations tended to be mainly restricted to the phyla Arthropoda and Mollusca, and many valuable contributions have emerged (Gere 1956, Golley 1960, Mukerji and LeRoux 1969a, 1969b, Lawton 1970, 1971, Hughes 1970, McNeill 1971, Smith 1972, Grahame 1973a, 1973b, and others).

As Slobodkin (1962) points out "The relation between energetics and the numerical properties of a population must

be in terms of energy budget analysis in which the population is considered as steady-state system through which potential energy passes". Energetics is based essentially on the physical laws relating to energy. Odum (1953) elucidates this point by discussing the first and second laws of thermodynamics in relation to energy flow in ecological systems. The first law of thermodynamics states that energy may be transferred from one type into another, but is never created or destroyed, and the second law states that no process involving an energy transformation will spontaneously occur unless there is a degradation of the energy from a concentrated form into a dispersed form; it is tautological that energy transformations cannot be 100% efficient.

The equation which expresses energy transformation in an organism was first used by Ivlev (1939, 1945) following Terroine:-

$$Q = Q' + Q_R + Q_T + Q_V + Q_W$$

where:- Q = energy consumed as food

Q'= energy converted to growth

Q_R= energy egested or not utilized

Q_T= energy of primary heat

Q_V= energy of external work

Q_W= energy of internal work

Ricker (1946) showed that Q_T, Q_V and Q_W could be combined and expressed as the heat lost during respiration; thus the equation is simplified to :-

$$\text{Input} = \text{Growth} + \text{Respiration} + \text{Egestion}$$

$$\text{Assimilation} = \text{Growth} + \text{Respiration}$$

Because we are considering energy, it follows that each component of the energy budget must be expressed as units of energy. Previously the calorie has been the unit of energy employed by workers in this field; however, in accordance with Standard International practice (see Kaye and Laby 1956) present day studies use the joule as a unit of energy. The calorie is defined as the energy required to raise 1 gram of water through $1^{o}C$ to $15^{o}C$; the joule on the other hand is the work done by a force of 1 newton acting through a distance of 1 metre.

From a consideration of the various components of an energy budget it is possible to ascertain the efficiency of growth and assimilation in an organism. The efficiency of an organism as a converter of energy is described by the net production or growth efficiency K_2, the gross production or growth efficiency K_1 and assimilation efficiency U-1, calculated thus (Prus 1972 based on Ivlev):-

$$K_1\% = \frac{P}{C} \times 100$$

$$K_2\% = \frac{P}{A} \times 100$$

$$U - 1 = \frac{A}{C}$$

where:- P = production or growth

A = assimilation

C = consumption or food ingested

The Energetics of Protozoa

Although many of the tools available to modern science allow the control and accurate measurement of parameters in physiological studies on microorganisms, the recent popularity of energetics studies has largely by-passed the Protozoa. As was pointed out earlier there is a vast quantity of information available on the energetics of arthropod and molluscan invertebrates, and yet there is little work of this kind on the Protozoa except for the work of Heal (1967a). Protozoa lend themselves well to laboratory studies. Most species are easily maintained in culture, as are their food organisms - bacteria, algae and other protozoan species, so that the comparative lack of interest shown in them by investigators of animal energetics is perhaps strange, particularly when one considers that Protozoa are often very abundant in aquatic environments and have been reported to constitute 93% of all the species of benthic animals in one brackish water study (Muus 1967).

The energy budget equation for a protozoan has been expressed by Heal (1967b) as :-

$$\text{Amount of food ingested} = \underbrace{\text{Amount of Protozoa produced} + \text{Amount lost in respiration}}_{\text{Amount assimilated}} + \text{Amount excreted}$$

In a protozoan energetics study, ingestion or consumption, growth and respiration are measured directly, but because of the impracticability of attempting to collect egested matter, the amount of energy egested and excreted is calculated from

the other parameters of the energy budget. Heal (1967b) makes the point that an energy budget of this type is readily obtained from laboratory populations, but there are dangers in extrapolating to the field.

Most studies on the energetics of Protozoa constitute only partial energy budgets. Such investigations are mainly concerned with studying the feeding rate and the associated growth rate, thus producing a measure of yield, or to use the terms applied in energetics, gross growth efficiency (Coleman 1964, Proper and Garver 1966, Curds and Cockburn 1968, 1971, Prus 1972). These workers have reported high gross growth efficiencies in ciliated protozoan species ranging from 40-78%, thus suggesting that the Protozoa are very efficient converters of energy, far more efficient than other invertebrates that derive their energy from the primary producers (Gere 1956, Richman 1958, Prus 1968a) where gross growth efficiencies reported are lower.

General Ecology of Protozoa

There have been many investigations into the abundance and distribution of protozoan species in fresh-water (Wang 1928, Moore 1939, Cole 1955, Webb 1961, Cairns 1965, 1966, Goulder 1971, and others) and in marine habitats (Fenchel and Jansson 1966, Fenchel 1967, Saifullah 1971 and others). An important fact which emerges from these studies, which were carried out on the continents of Europe and America, is that the Protozoan

species are cosmopolitan in their distribution. Species which occur in America are also found in Europe and display similar patterns of distribution.

Not only has the ecology of Protozoa been investigated in the wild, but those species which commonly occur in sewage treatment plants have also received scrutiny, for the obvious purpose of elucidating the role they may have in the purification of sewage. Several workers have shown that some ciliate and flagellate species possess the ability to flocculate bacteria (Harding 1943, Curds 1963). Curds, Cockburn and Bandyke (1968) have shown that the addition of ciliates to activated-sludge greatly improved the quality of the effluent. Later work by Curds and Fey (1969) indicated that ciliated Protozoa play a major role in the removal of Escherichia coli from sewage. A survey of the protozoan fauna of British percolating filters and activated-slude plants revealed that all the 52 percolating filters examined contained ciliates, and all but 3 of the 56 activated-sludge plants were found to have populations of ciliated Protozoa (Curds and Cockburn 1970). Further, activated-sludge plants which delivered high quality effluents harboured a wide variety of ciliated Protozoa in large numbers, while those plants which produced turbid low quality effluents, either lacked Protozoa, or contained only small numbers of a few species. Clearly, not only are Protozoa widely distributed in natural habitats, but also possess an important applied role in sewage treatment plants.

The Aims of the Present Study

The aim of the present investigation into the energetics of Colpidium campylum was to produce a series of detailed energy budgets over a range of temperature, relating energy uptake and utilization to energy availability. Care was taken to use a series of temperatures which Colpidium would encounter in its natural habitat. Loch Leven, Kinross was taken as an example of the natural environment of Colpidium. The water temperature rarely exceeds 20°C in this lake, so that in the present investigation 20°C was the highest experimental temperature used.

A subsidiary part of my research project was concerned with a study of ciliated Protozoa in their natural environment. The location was Loch Leven, Kinross and this part of the project formed part of the International Biological Programme on the loch.

CHAPTER 1 CULTURING

Prior to experimental work it was necessary to establish, in the laboratory, flourishing populations of a typical bacterial feeding holotrich ciliate and a suitable food organism. As a first step quantities of mud were collected from Loch Leven, Kinross, using a Jenkins core sampler (Jenkins and Mortimer 1938).

1.1 Protozoa

1.1.1 Materials and methods

The medium used in preliminary attempts to culture various ciliate species was that described by Sonneborn (1950) which consisted of 2.5g of powdered baked lettuce boiled in 1 litre of distilled water. After boiling the pH of the medium was adjusted to between 6.0-7.0 using calcium carbonate; it was then filtered, and autoclaved at 15lbs/sq.in. for 15 minutes. Aerobacter aerogenes, a bacterial food source, was then innoculated into the medium 24-28 hours before use. This bacterium had previously been maintained in this department by another research worker.

Freshly collected Loch Leven mud was examined in petri-dishes under a binocular microscope, individual ciliates were removed with a micro-pipette, and introduced into depressions in Butt slides. Each depression contained 0.5ml of the medium prepared as described above, innoculated with Aerobacter aero genes. The slides were then placed in covered petri-dishes and

incubated at room temperature (approximately 20°C).

Each depression was examined daily, and where division of the ciliates had occurred, the individuals were each re-isolated into fresh medium. Once a species had become established, that is dividing and feeding actively, stock hay-infusions cultures (Sonneborn 1950) were set up. These cultures consist of approximately 10g of chopped hay boiled for 10-15 minutes in 1ℓ of distilled water. A bacterial food source (Aerobacter aerogenes) was innoculated into the infusion 24 hours before the introduction of the Protozoa. Stock cultures of this type maintain themselves without attention for 4 months or longer. Such cultures were incubated at 24°C and 15°C.

1.1.2 Results

Of the twelve species which were isolated from the mud, four were successfully established in baked lettuce medium, these species were: Paramecium caudatum (Ehrenberg), Paramecium aurelia (Ehrenberg), Tetrahymena pyriformis (Ehrenberg) and Colpidium campylum (Stokes). These species are all typical bacterial-feeding Holotrich ciliates belonging to the order Hymenostomatida. All of these species were then maintained in stock hay infusions.

A decision had to be made as to which of these species would be used as the experimental organism in the construction of a ciliate energy bodget. The choice depended partly on the results of the culturing and investigation of a bacterial food source, but there was a bias towards Colpidium since it appears

to be the least worked on of the four species.

1.2 Bacteria

Mud from Loch Leven was brought to the laboratory in a previously sterilised jar. Smears of the mud were made aseptically on to 5% yeast agar plates. The plates were incubated at 24°C and 15°C for 48 hours. One of the resultant colonies was subcultured and tests to compare the suitability of this isolate with A. aerogenes were carried out. Colpidium was used for these tests because as has already been stated there was a preference towards using this species in the energetics studies. Such tests were necessary since some workers have shown that some bacteria are unacceptable or prove to be poor food sources to certain ciliates (Burbanck 1942, Curds and Vandyke 1966).

Four 250ml conical flasks each containing 50ml of baked lettuce medium were autoclaved. Two of the flasks were innoculated with A. aerogenes and two with the Loch Leven isolate. The flasks were incubated for 24 hours at 24°C. Protozoa were sterilized to remove bacteria from the cell surface according to the method of Parpart (1928). Briefly,the individual ciliates were transferred with sterile micropipettes through 5 washes of autoclaved lettuce medium in Butt slides, a 15 minute period was allowed between each transfer and the ciliates were left for 2 hours in the last change of medium.

The cultures were incubated at 20°C. Counts were commenced after 72 hours by pipetting 0.5ml, or less if necessary, from a well agitated culture into a depression slide and counting directly. The number of individuals produced from each individual was calculated as follows:-

$$k = \text{Log}_2 N \text{ per unit time}$$

$$= \frac{\text{Log}_{10} N}{\text{Log}_{10} 2} = \frac{\text{Log } N}{0.3010}$$

where:

k = fission constant in the equation $N = 2^{kt}$

N = number of animals after time t produced from a single animal.

1.2.1 Results

Figure 1 shows the number of generations produced by Colpidium feeding on A. aerogenes and the Loch Level isolate. The mean of the two cultures for each of the two food sources was plotted. The Loch Level isolate appeared slightly better than A. aerogenes, although the growth curves for both food sources show a close resemblance.

Plates of the isolate were sent to the Torry Laboratories at Aberdeen for a reliable identification. The isolate was found to be a species of the genus Moraxella, which is a non-motile, oxidase positive, gram negative rod.

1.2.2 Conclusions

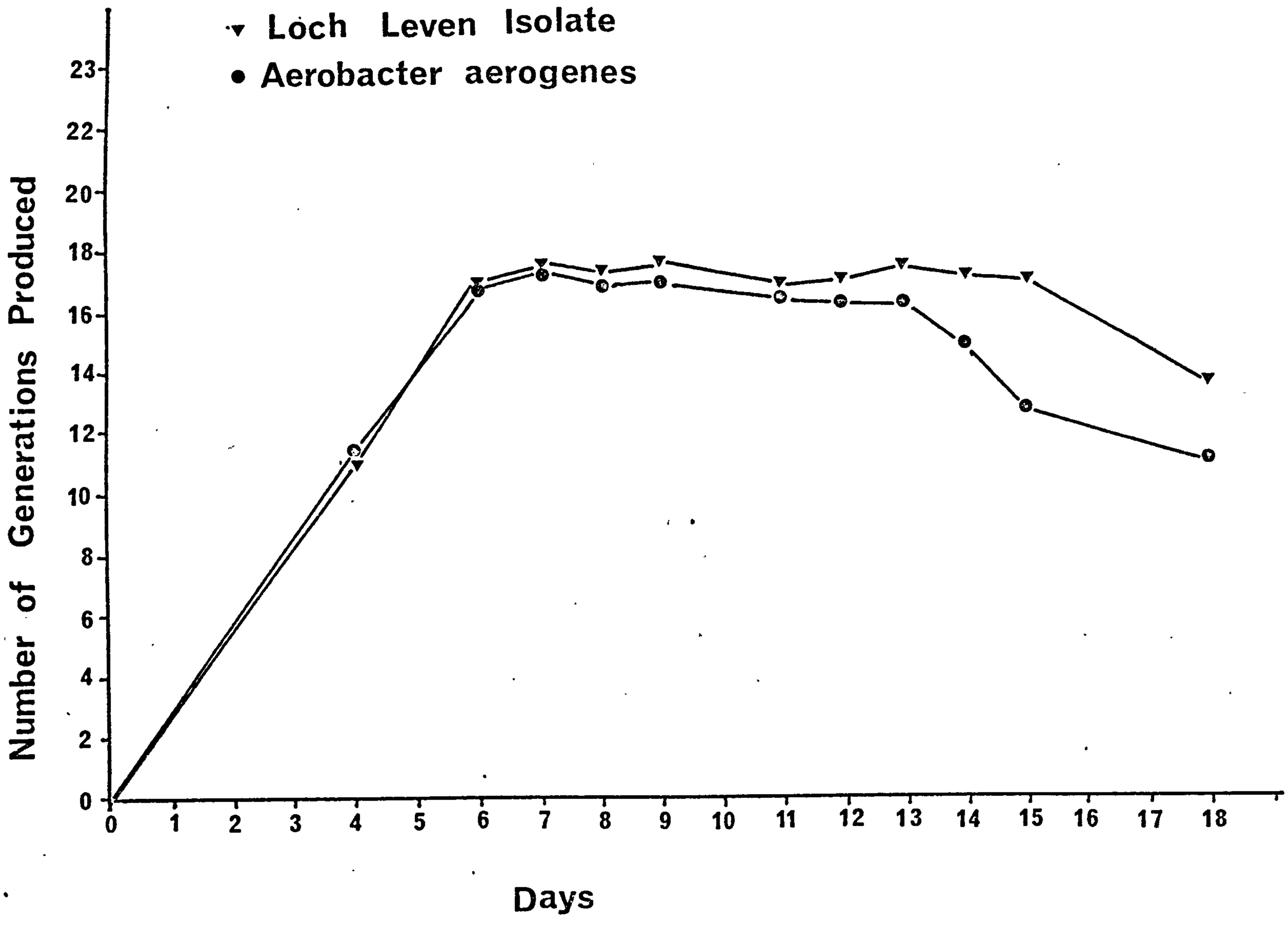
Since Colpidium appeared to feed satisfactorily on Moraxella this ciliate species was selected as the experimental

Figure 1

Comparison of the Loch Leven isolate with

Aerobacter aerogenes as a food source for *Colpidium*

as indicated by the number of generations produced


Loch Leven Isolate
Aerobacter aerogenes
Number of Generations Produced
0
2
4
6
8
10
12
14
16
18
20
22
23
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Days

organism for this energetics study. Both Colpidium and its food source have been routinely maintained in this laboratory during a research period of 24 months.

CHAPTER 2 CONSUMPTION AND GROWTH

2.1 Preliminary Growth Studies

Before detailed studies on food consumption and growth in Colpidium could be undertaken an important consideration had to be met, namely, which parameter would be used to measure growth.

Reproduction as a measure of growth has been used by many workers in the past including Cutler and Crump (1924), Luck, Sheets and Thomas (1930), Heatherington (1934) and Johnson (1936). More recently, reproduction as a parameter of growth has been utilized by Heal (1967a) and Curds and Cockburn (1968) in energetics studies. The accuracy of this method of estimating the quantity of protoplasm produced, i.e. growth, relies upon the size reached before each division remaining constant. This however, is not the case in Tetrahymena pyriformis (Curds and Cockburn 1971) and Uronema sp. (Hamilton and Preslan 1969), where considerable variation in the mean cell volume of the population was demonstrated and was apparently dependent on the number of bacteria, or other food organism, available to each protozoan.

Consequently, the volume of protoplasm produced may be a more accurate measure of growth in a species where size variation had been demonstrated. This idea gains weight from the findings of Kimball, Casperson, Svensson and Carlson (1959) from their work on Paramecium aurelia. They suggest that the growth rate and the division rate are capable of independent variation, and

further that size is an adaptive character. They also suggest, that the response of growth and division rates to various conditions has been subject to selection to bring about adaptive changes in size in those situations commonly met in the organism's natural habitat.

In view of this vital consideration, preliminary experiments on the growth of Colpidium were carried out to ascertain if size variation occurred in this species, and if so to what extent.

2.1.1 Materials and methods

Eight 500ml conical flasks, each containing 400ml of baked lettuce medium were prepared as described in section 1.1.1. Moraxella grown on yeast agar plates for 72 hours at 24°C were washed off the plates using sterile distilled water to form a bacterial suspension; 1ml of this suspension was added to each of the cultures which were then incubated at 24°C. After incubation for 24 hours the concentration of bacteria in the lettuce medium cultures was between 10-16 x 10^7/ml.

A colony of 20 individuals of Colpidium was added to each flask. Four of the cultures were incubated at 10°C and four at 20°C.

Counts and size determinations were commenced after 48 hours. Counting was carried out directly as described in section 1.2 during the experimental period of 28 days. Size measurements were made using a microscope and eye-piece graticule, ten individuals being measured from each culture.

The length and width were ascertained, and assuming the shape of Colpidium to be an ellipsoid the following equation was used to calculate the volume:-

$$V = \frac{d^2 \ell}{6}$$

where:- V = volume

d = diameter

ℓ = length

The mean of the ten determinations was taken to give a measure of the mean cell volume of the protozoan population in each culture flask.

Bacteria were counted using a standard bacterial counting chamber with Thoma markings (Hawksley, England). The bacteria in 100 squares were counted, the number per square was calculated and multiplied by a factor of 2×10^7 to give the number of bacteria per ml (Mackie and Macartney 1960). This procedure was repeated three times for each sample and the mean of the three determinations taken.

2.1.2 Results

Considerable variation in size occurred as shown in Figures 2-5. At 20°C the cultures had a shorter life than those incubated at 10°C, but the same basic pattern emerged at both temperatures. In all cases, cell volume was large at the beginning of the experiment when food was plentiful. At that time also the density of the ciliate population was low. As the food supply became depleted and the density of the Colpidium population increased, the mean cell volume fell. At 20°C the

Figure 2 a and b - Cultures 1 and 2

Mean cell volume variation in Colpidium in relation to food supply and population size at 10°C

Key:- mean cell volume ●

population density ■

bacterial population ▲

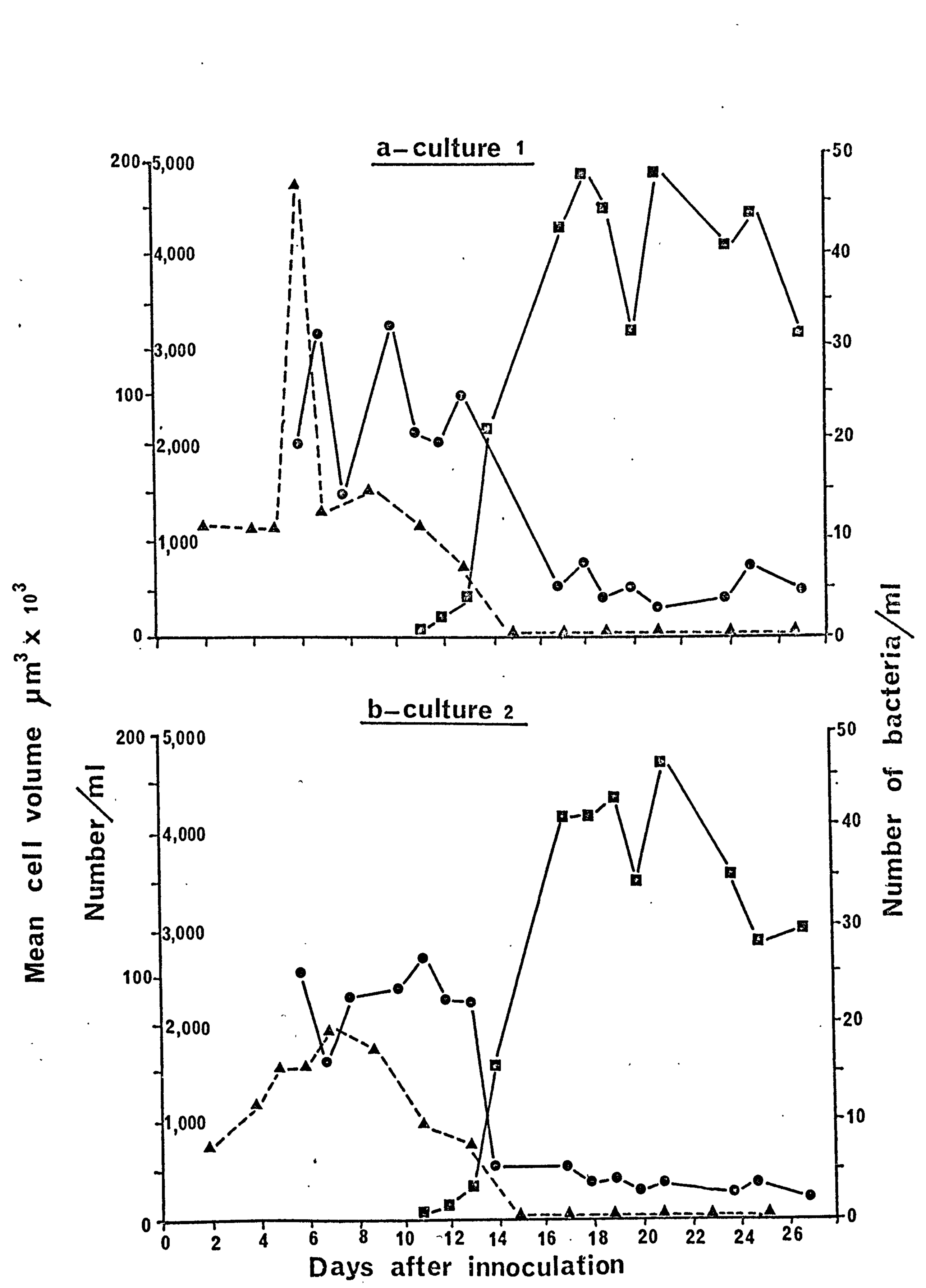

a–culture 1
200
100
0
5,000
4,000
3,000
2,000
1,000
50
40
30
20
10
0
Mean cell volume μm³ x 10³
Number/ml
Number of bacteria/ml
b–culture 2
200
100
0
5,000
4,000
3,000
2,000
1,000
50
40
30
20
10
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26
Days after innoculation

Figure 3 a and b - Cultures 3 and 4

Mean cell volume variation in Colpidium in relation to food supply and population size at 10°C

Key:- mean cell volume ●

population density ■

bacterial population ▲

a–culture 3

b–culture 4

Mean cell volume $\mu m^3 \times 10^3$

Number/ml

Number of bacteria/ml

Days after innoculation

0 2 4 6 8 10 12 14 16 18 20 22 24 26

0 100 1,000 2,000 3,000 4,000 200 5,000

0 10 20 30 40 50

Figure 4 a and b - Cultures 5 and 6

Mean cell volume variation in Colpidium in relation to food supply and population size at 20°C

Key:- mean cell volume ●

population density ■

bacterial population ▲

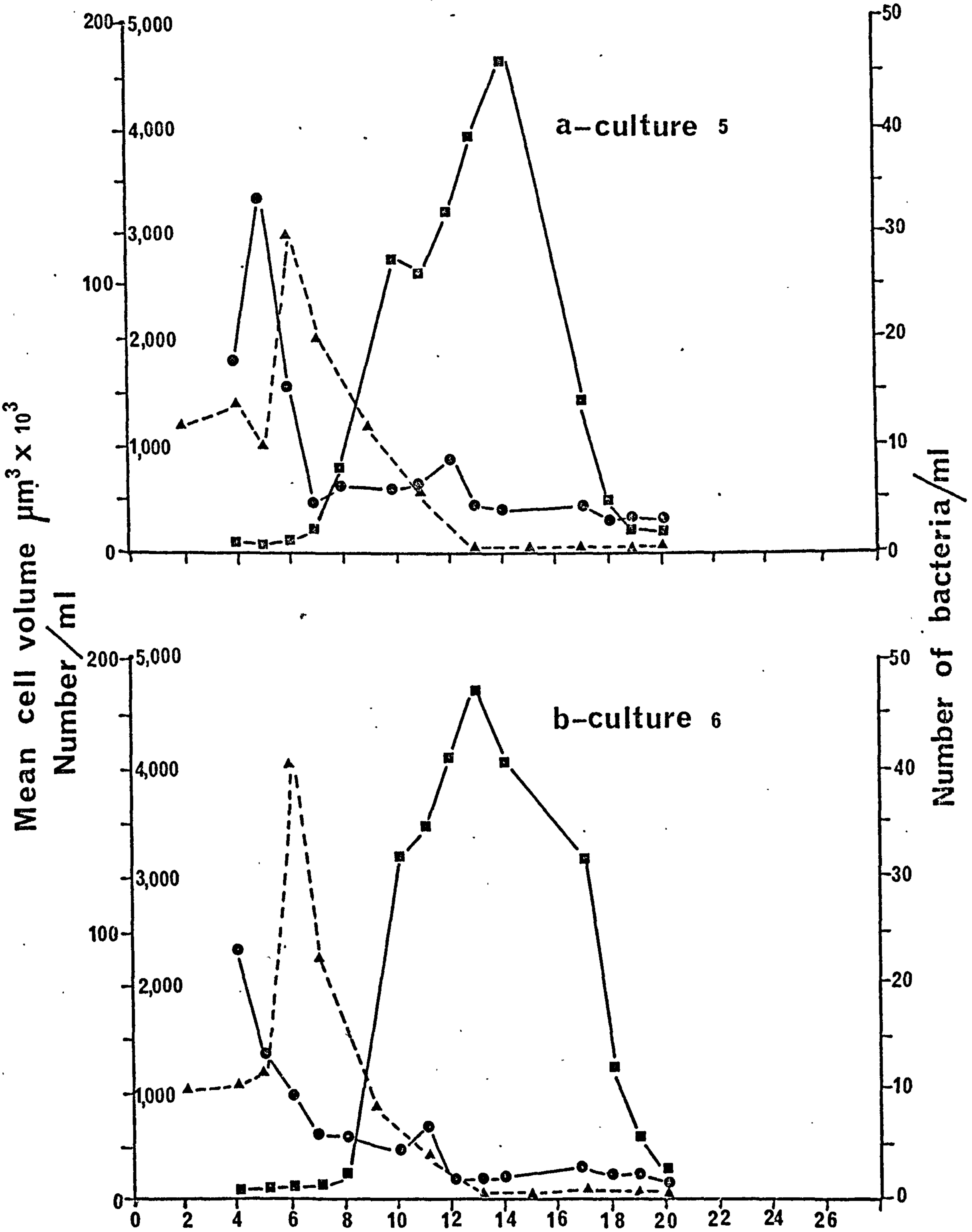

a–culture 5
b–culture 6
Mean cell volume $\mu m^3 \times 10^3$
Number/ml
Number of bacteria/ml
200
100
0
5,000
4,000
3,000
2,000
1,000
50
40
30
20
10
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26
Days after innoculation

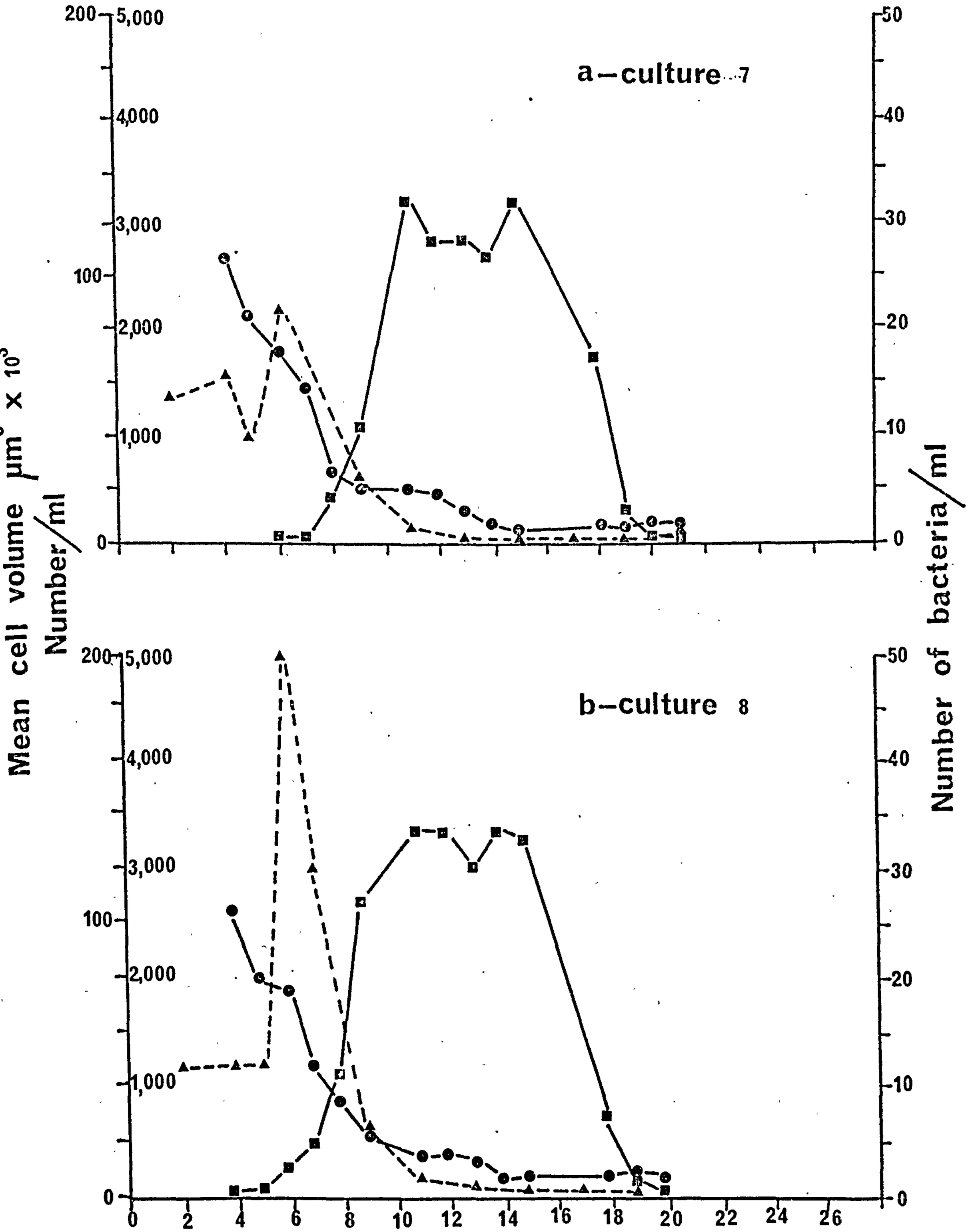
Figure 5 a and b - Cultures 7 and 8

Mean cell volume variation in Colpidium in relation to food supply and population size at 20°C

Key:- mean cell volume ●

population density ■

bacterial population ▲


a—culture 7
b—culture 8
Mean cell volume μm³ x 10³
Number of bacteria/ml
200
100
0
5,000
4,000
3,000
2,000
1,000
50
40
30
20
10
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26
Days after innoculation

cell size had fallen from a maximum size of 100-130 x $10^3 \mu m^3$ to less than 20 x $10^3 \mu m^3$ by the 14th day after innoculation. At 10^oC the mean cell volume fell from between 100-140 x $10^3 \mu m^3$ to a mean size of less than 30 x $10^3 \mu m^3$ by the 14th day after innoculation in cultures 2, 3 and 4 (Figures 2b and 3a and 3b) and the 17th day in culture 1 (Figure 2a).

At both 10^oC and 20^oC the maximum density of the Colpidium population coincided with the fall of the mean cell volume to the low values quoted above. At that time also the bacterial food supply had fallen to a very low level as the Figures show.

2.1.3 Discussion

Size variation is apparently dependent on the concentration of the food supply, as indicated by the downward trend of both the bacterial concentration and the mean cell volume. A similar pattern was obtained for Tetrahymena pyriformis by Curds and Cockburn (1971) who with Hamilton and Preslan (1969) concluded that food availability was a major factor in governing the size of ciliate cells.

The conclusion that the rate of growth and the rate of reproduction may be capable of independent variation has been drawn by Kimball et al.(1959). The results of the present study with Colpidium would indicate that growth and reproduction should be considered as two separate rates in an energetics study on ciliated protozoan, since clearly, the production of a dense population is not necessarily indicative of a large increase in protoplasm production or growth. The results show

that the ciliates merely divide at a smaller size when food supply becomes limited. There may be no real increase in the actual protoplasmic volume of the population but the total volume of protoplasm may simply be divided into a larger number of units.

Obviously, the effect of variables such as temperature and food concentration upon the rates of reproduction and growth need to be carefully controlled.

2.2 Growth and Food Consumption

When considering the method of measuring growth and reproduction in a protozoan species and relating these rates to the amount of food consumed, one important complicating factor arises and this is that the food source itself is capable of rapid division given favourable conditions. Dead bacteria appear to be unacceptable to Colpidium, and in any case do not really represent a direct reflection of the natural environment where this species probably feeds on living microorganisms. In consequence, experimental design must in some way allow for the division of the food organism.

Two methods are available, firstly the method suggested by Heal (1967b) and used by him in 1967 in his quantitative feeding studies on a soil amoeba species (Heal 1967a). His method has an appealing simplicity. It involves placing a known number of bacteria, or any suitable food organism, and a known number of protozoa in a non-nutrient medium and measuring

the number of bacteria and protozoa at the end of a specified time. Because of the variables which may cause complications in such studies - e.g. the accumulation of metabolic waste products from both the food organism and the protozoa - the length of the experiment should be as short as possible.

The second method is that tried by Curds and Cockburn (1968) where bacteria were grown in known concentrations of sucrose, the concentration of which determines the yield of bacteria. When the bacteria reached the stationary phase of their growth curve, a known number of protozoa were added. This method assumes no bacterial division in the stationary phase, and since the experiment is carried out in a nutrient medium one must be certain that the protozoa are incapable of utilizing any part of the substrate which might still be available after the attainment of the bacterial stationary phase.

The method described by Heal (1967b) relying on a non-nutrient medium was selected for the current study, with counts or protozoa and bacteria plus the additional measurement of the volume of the protozoan populations.

2.2.1 Materials and methods

General

The experiments were set up as a series of bacteria:protozoan ratios ranging from 0.0-4.0 x 10^6 bacterial:protozoan. The ratio represents an index of food availability. Ten replicates were carried out at each food concentration within each series of experiments at $10^{o}C$, $15^{o}C$ and $20^{o}C$. The

experiments were carried out over a six-month period.

Autoclaved medical flats were used as experimental vessels. The non-nutrient medium used was autoclaved distilled water in which the bacteria appeared to survive in an active condition for the 24 hours over which the experiments were run. This was tested by plating bacteria after use in an experiment, and successful growth was obtained.

Controls of bacteria and protozoa alone were set up.

Protozoa

The Colpidia were grown in distilled water with bacteria as a food source for 48 hours at the temperature at which the experiment was to be carried out, in order that the animals should be fully acclimatized to the experimental temperature.

Protozoa were harvested by centrifugation at 1,000r.p.m. for 15 minutes in conical centrifuge tubes. A speed of 1,000 r.p.m. was chosen because at speeds above 1,500-2,000r.p.m. the contractile vacuoles became very enlarged and many of the animals burst. It was therefore deemed prudent to use a speed well below this critical level. After centrifugation the supernatant fluid was discarded and the centrifugate was re-suspended in sterile distilled water and recentrifuged at 1,000 r.p.m. for a further 15 minutes. The centrifugate was again resuspended in a small quantity of sterile distilled water. The density of Colpidium was then estimated by means of a Coulter Counter, the operation and use of which will be discussed later. The appropriate volume of the concentrate for addition to the

Bacteria

Moraxella was grown for 72 hours on 5% yeast agar plates at 24°C. The bacteria were washed off the plates with sterile distilled water. Counts of the number of bacteria/ml in the harvested concentrate were made using a bacterial counting chamber as outlined in section 2.1.1., and the quantity of the concentrate to be added to the experimental flasks was consequently calculated.

Having determined the number of bacteria and protozoa harvested, appropriate quantities of each were added to sterile distilled water (the experimental medium) to constitute the desired ratio of bacteria to each Colpidium. As far as possible the protozoan number per ml was always approximately 500, and the bacterial number was varied to render the different ratios in each series of experiments. The volume of the medium containing the protozoa and bacteria in each medical flat was always 50ml in each replicate.

Immediately after the flats had been set up, counts of protozoa and bacteria were made and protozoan mean cell volume determined. The experimental vessels were then incubated at either 10°C, 15°C or 20°C for 24 hours; at the end of this period the counts were repeated.

Bacteria were counted using the counting chamber method already outlined. Protozoa were sized and counted, as has been previously stated, by electronic means using a Coulter Counter.

Plate 1

The Coulter Counter

A = box containing electronic control and counting circuits

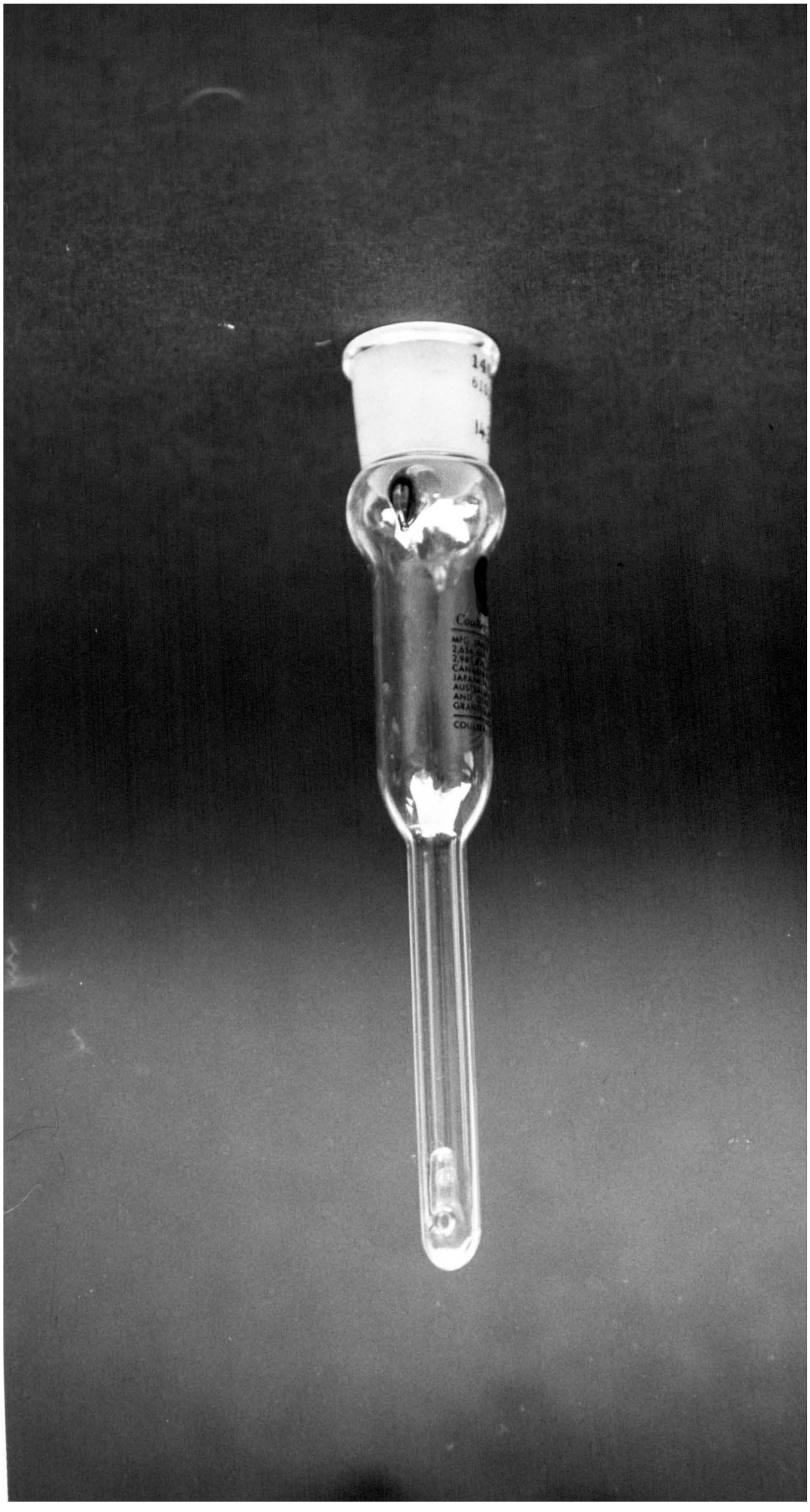
B = glassware - calibrated mercury manometer

C = mean cell volume converter attachment


A
B
C
COULTER COUNTER
MONITOR
THRESHOLD
APERTURE CURRENT
MEAN CELL VOLUME

Plate 2

Glass orifice tube



Coulter Counter

A model ZB Coulter Counter, with a mean cell volume converter attachment, was used for counting and sizing the Colpidium. The instrument is illustrated in Plate 1.

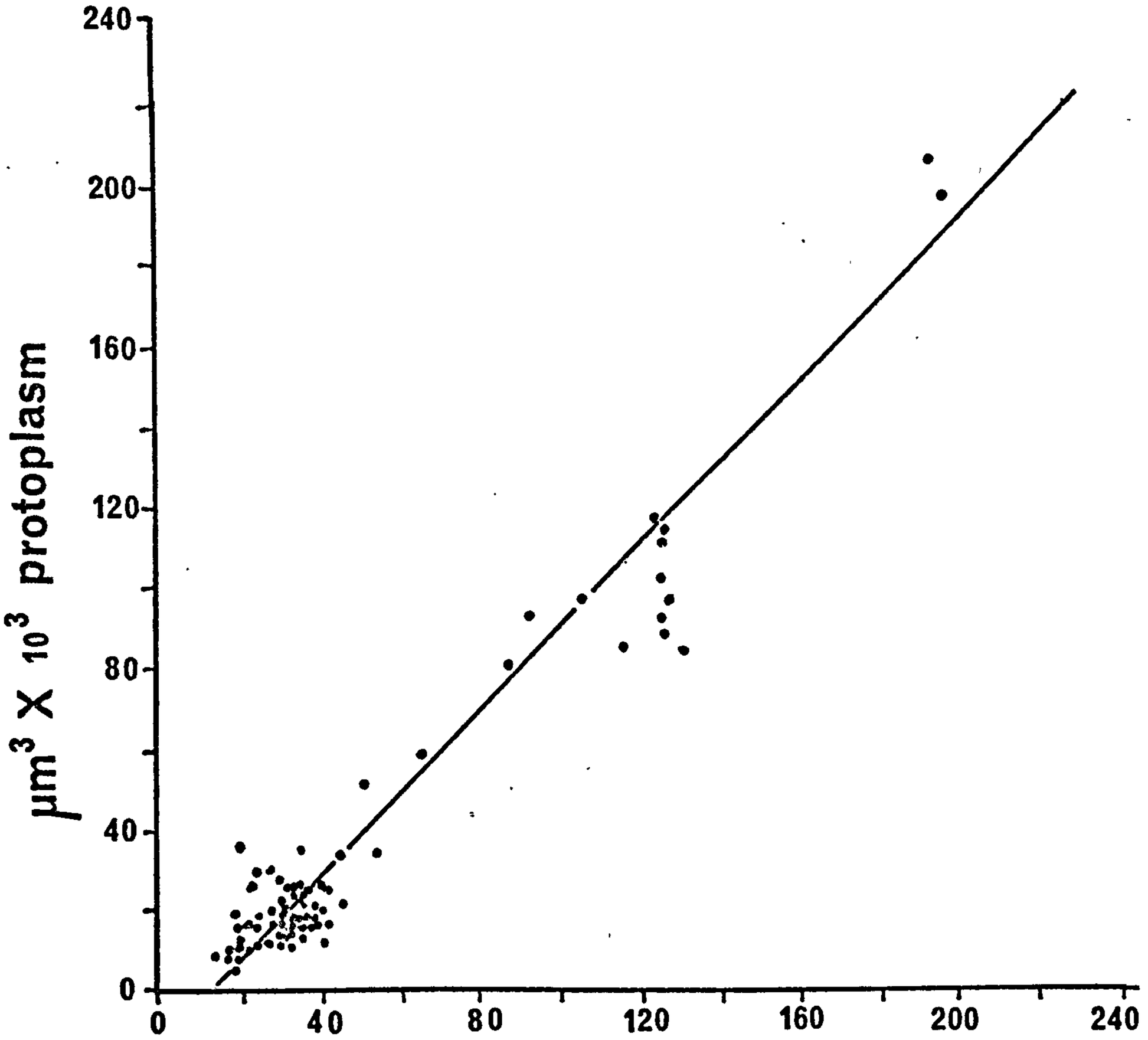
The principle of operation involves drawing in a known volume of sample through an orifice of known diameter in the wall of a glass tube, by means of a calibrated mercury manometer. For this work a glass tube with an orifice diameter of 140μm was used, as shown in Plate 2. The lower end of the orifice tube sits in a beaker of electrolyte in which the sample is suspended. There are two electrodes between which a current flows, one on the outside of the orifice tube, which like the orifice itself is submerged in the sample, and the other on the inside of the orifice tube. As an exactly measured 0.5ml sample is drawn through the orifice, each particle partially obscures the orifice and raises the resistance between the electrodes. The change in resistance is observed by the instrument as a voltage pulse. The pulse train is displayed on an oscilloscope and the pulses are counted electronically; the pulses are summated, and the total number of particles in the sample counted is displayed. The amplitude of the pulses is proportional to the particle volume, thus by integrating the amplitude of the pulses the mean cell volume converter provides the mean cell volume of the sample counted.

Calibration of the volume measurements is necessary. Standard sized particles (comprising various pollens, spores

Figure 6

The mean cell volume calibration curve for use with the mean cell volume converter attachment of the Coulter Counter

Mean Cell Volume Calibration Curve



and latex spheres) obtained from Coulter Electronics were used initially, and a calibration curve drawn from the results. A linear relationship existed between the volume of the standard particles and the value obtained from the machine. However, when the values obtained from the mean cell volume converter for Colpidium were read off the curve, the resultant volumes were inconsistent with the volumes determined for the same protozoan sample by microscopic measurements (see section 2.1.1). The conductivity of the measured particle affects the resistance change and hence the mean cell volume value (M.C.V.).

The conductivity of the standard particles obtained from Coulter Electronics differed from the conductivity of the Protozoa, necessitating calibration with the Protozoa themselves. This fact became apparent before the experiments in section 2.1 were carried out and therefore provided an opportunity for calibrating the instrument as well as an investigation of size variation. Thus the samples from these experiments were run through the machine and the results of the procedure correlated with the results obtained by the microscopic measurements outlined in section 2.1.1. The results are presented in Figure 6, again a linear relationship was obvious, and a linear regression was performed to determine the line of best fit (the smallest mean square error). The line of regression of y on x may be written (Weatherburn 1961):-

$$y = bx + a$$

where the slope $$b = \frac{\mu_{11}}{\sigma^2_x}$$

$$\text{the covariance } \mu_{11} = \frac{1}{N}\sum_{i=1}^{N}(x_i - \bar{x})(y_i - \bar{y})$$

$$= \mu_{11} - \bar{x} \cdot \bar{y}$$

where $$\mu_{11}^{1} = \frac{1}{N}\sum_{i=1}^{N} x_i y_i = \overline{xy}$$

$$\sigma_x^2 = \frac{1}{N}\sum_{i=1}^{N}(x_i - \bar{x})^2$$

$$= \frac{1}{N}\sum_{i=1}^{N}(x_i)^2 - \bar{x}^2$$

$$= \overline{x^2} - \bar{x}^2$$

where y = microscopically measured size

x = mean cell volume reading.

The electrolyte used in these studies was Fledon-Heig invertebrate saline, details of which are presented in the appendix. This solution had a resistance of 20kΩ when diluted 4:1 with the sample to be counted. Initially Peters physiological medium for ciliates (Peters 1921) was tried but proved to have too high a resistance and consequently too low a conductivity for use in this model Coulter Counter. In order to cut down the risk of volume changes due to the salt solution the samples were only mixed with the saline immediately before each count. Observations and measurements under a microscope show that for the first 3-4 minutes the volume of the animals showed no detectable change. After this period however, they became progressively smaller until death occurred.

Five replicate counts were made for each experimental reading; the mean of the counts and the mean of the mean cell volume values were taken.

Calculations.Modified after Heal (1967a)

$$V_t = (N_f \times \text{M.C.V.}) - (N_o \times \text{M.C.V.})$$

$$V_i = \frac{V_t}{N_o}$$

$$B_c = B_f - B_o$$

$$B_i = \frac{B_c}{N_o}$$

$$G = \frac{\text{Log } N_f - \text{Log } N_o}{\text{Log } 2}$$

where :- B_c = Bacteria consumed in 24 hours

B_i = Number of bacteria consumed by each individual present at T_o

B_f = Bacteria remaining after 24 hours

B_o = Bacteria present at T_o

G = Number of generations

N_f = Number of animals after 24 hours

N_o = Number of animals at T_o

T_o = Starting time of experiment

V_i = Volume of protoplasm produced by each individual at T_o

V_t = Total volume of protoplasm produced in 24 hours.

2.2.2 Results

Growth is expressed at μm^3 of protoplasm produced in 24 hours by each individual present at the beginning of the experiment (T_o), and consumption is expressed in terms of the number of bacteria eaten in 24 hours by each individual present at T_o. In the following chapters this data will be translated into terms of dried weight and energy, which will in turn allow it to be incorporated into an energy budget for Colpidium.

Figure 7

Growth as the volume of protoplasm produced by *Colpidium* at different bacteria:protozoan ratios at 10°C, 15°C and 20°C

(Each point represents the mean of 10 experiments)

GROWTH at 10^0C, 15^0C & 20^0C

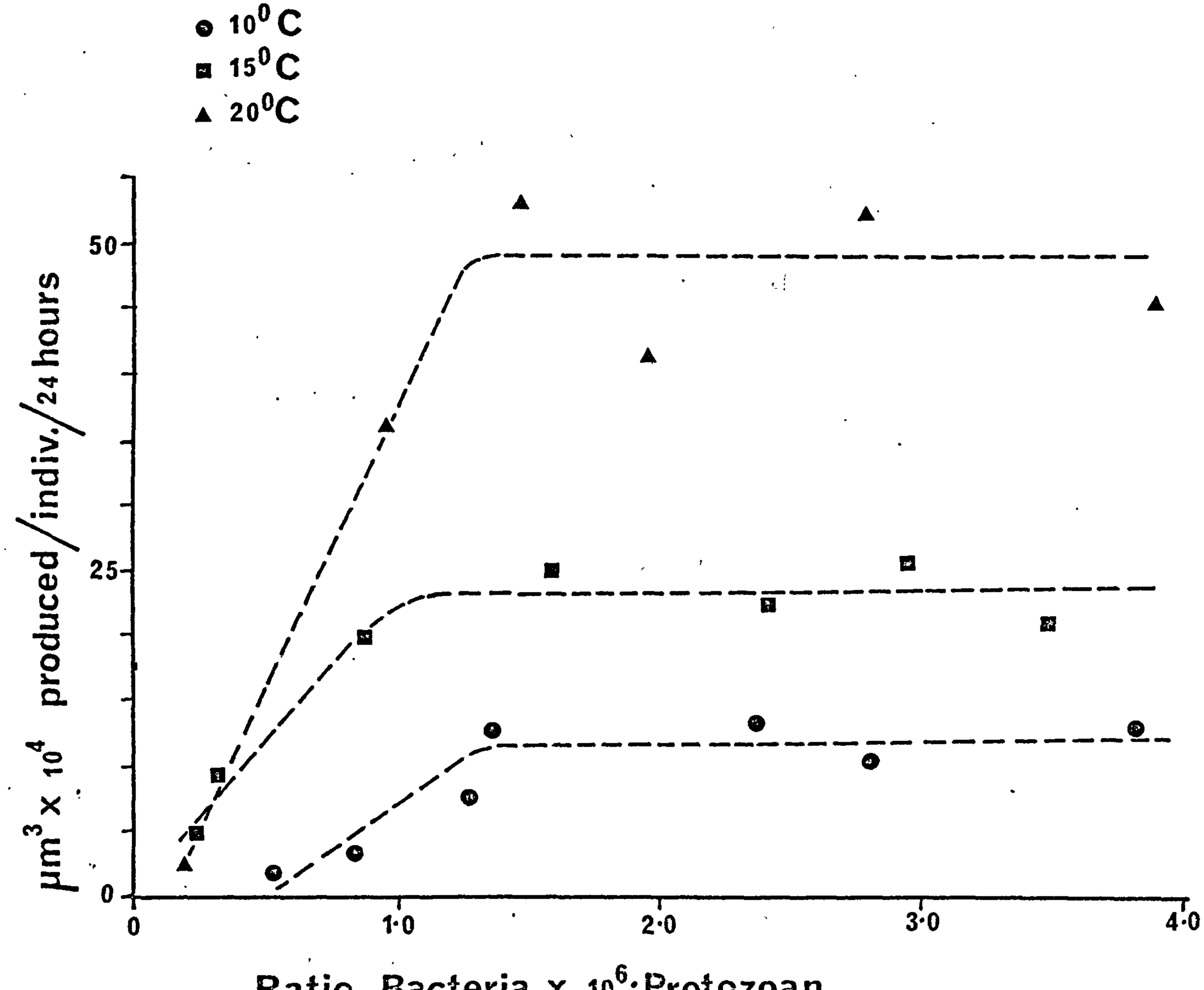
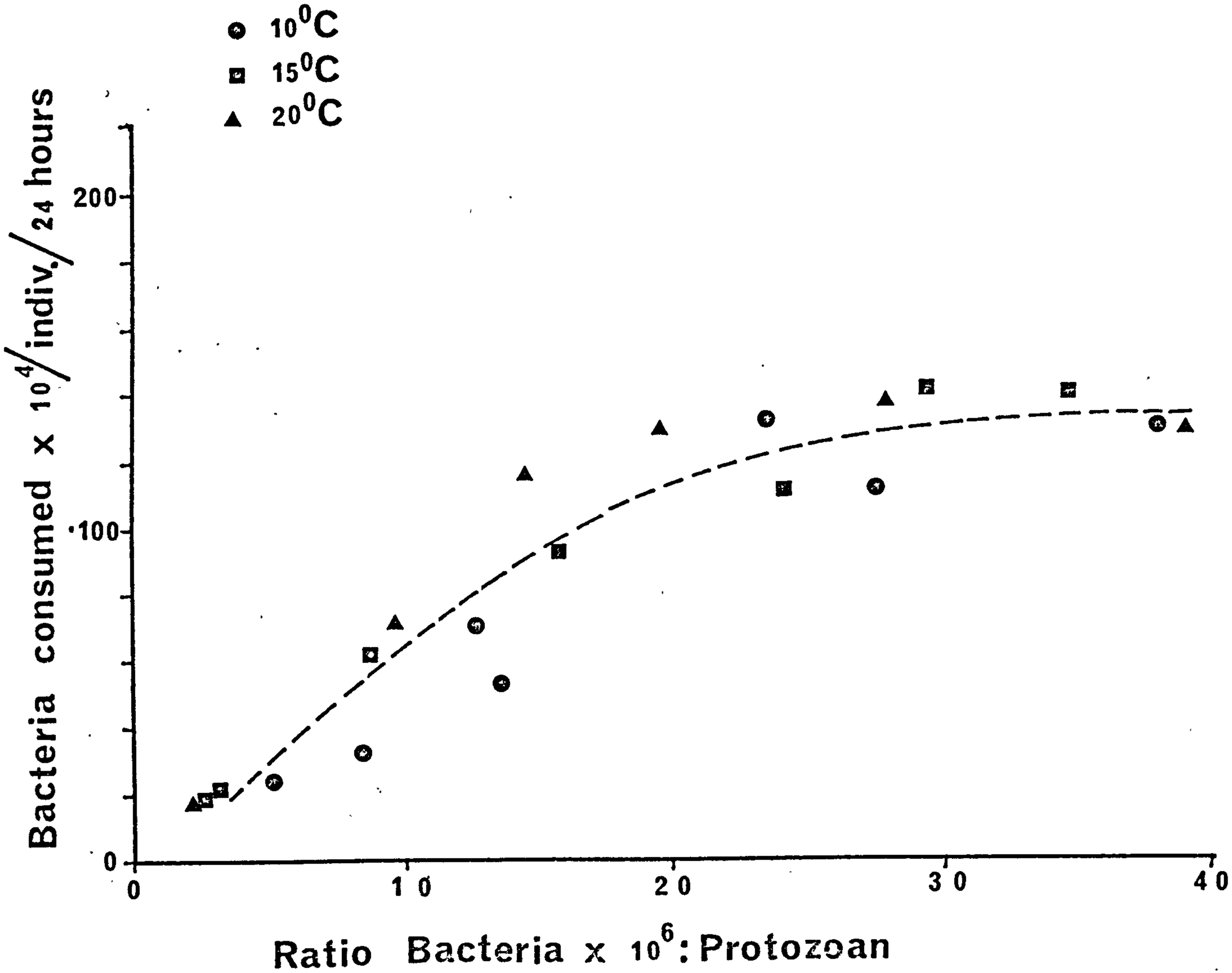


Figure 8

Consumption of bacteria by Colpidium at different bacteria:protozoan ratios at 10°C, 15°C and 20°C

(Each point represents the mean of 10 experiments)

CONSUMPTION at 10^0C, 15^0C & 20^0C



The effect of food concentration on growth

Figure 7 shows the pattern of growth obtained over the range of food concentration 0.0-4.0 x 10^6:1 at 10^oC, 15^oC and 20^oC. At each of the three temperatures investigated growth increased exponentially and then levelled out to a plateau. In all cases the maximum level of growth was attained near a food ratio of 1.0 x 10^6:1 bacteria per protozoan.

The effect of temperature on growth

As Figure 7 clearly shows temperature exerts a marked effect on the level of growth achieved over the range of food concentration considered.. At 10^oC the maximum level of growth was between 10-15μm^3 x 10^4 of protoplasm/individual/24 hours, at 15^oC the maximum lay between 20-25μm^3 x 10^4/individual/24 hours and at 20^oC between 40-45μm^3 x 10^4/individual/24 hours.

An increase of 5^oC within the temperature range studied appeared sufficient to raise the amount of protoplasm produced by 40-50%.

The effect of food concentration on consumption

The results are presented in Figure 8. Like growth, consumption increased exponentially with increased food concentration, but the levelling off effect was not apparent until a food concentration in the region of 2.5 x 10^6:1 bacteria per protozoan. Thus the number of bacteria consumed by each individual reached its maximum at a higher concentration than that at which growth attained a maximum. In other words consumption continued to increase despite the fact that growth

had attained its maximum level. Maximum consumption was in the region of 125 x 10^4 bacteria consumed/individual/24 hours.

The effect of temperature on consumption

Unlike growth, consumption was not greatly affected by temperature as Figure 8 shows. There was a slightly higher consumption at 20°C over that at 15°C and also for the consumption at 15°C over that at 10°C, but in general the curves obtained over the food concentration range considered followed each other very closely at all three temperatures.

The effect of consumption on growth

At 10°C and 15°C (see Figures 9 and 10) there was an initial rise in growth with increasing consumption, up to an intake of 50-80 x 10^4 bacteria/individual/24 hours, thereafter increased consumption did not produce an increase in growth, which maintained a plateau. Where consumption fell below 30 x 10^4 bacteria/individual/24 hours at 10°C there was in some cases a decrease in the weight of the Protozoa, suggesting that some endogenous cellular material was utilized to compensate for insufficient consumption.

At 20°C as shown in Figure 11, the pattern of growth which emerged in relation to the quantity of food consumed, differed from that obtained at 10°C and 15°C. Growth at 20°C increased with increased consumption. The indications are that growth began to level out where 120-130 x 10^4 bacteria were ingested/individual/24 hours.

The effect of temperature on the growth rate has already

Figure 9

Growth, in terms of protoplasm produced, as a product of consumption of bacteria at $10^{o}C$

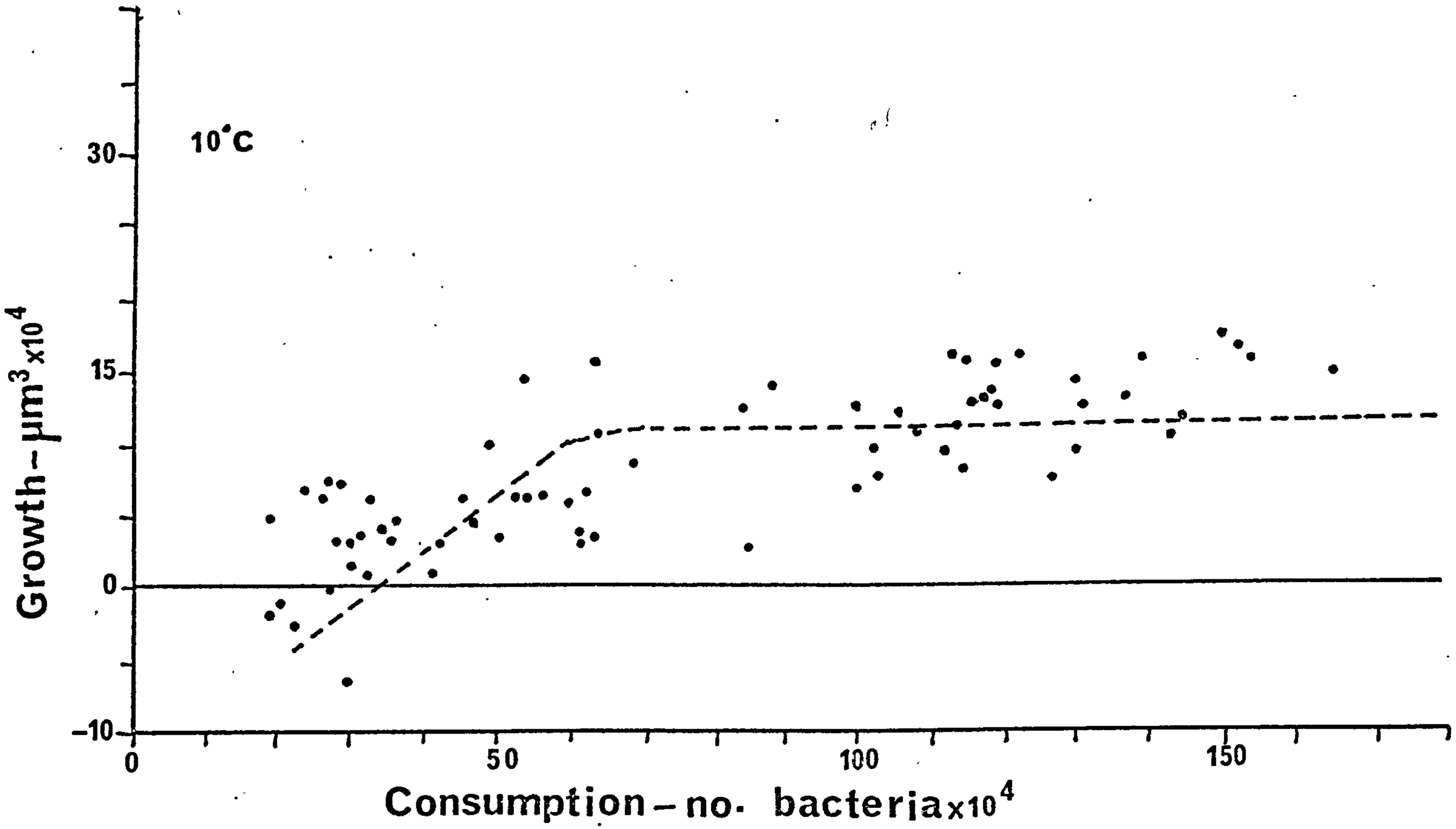

10°C
30
15
0
−10
0
50
100
150
Growth – µm³ x10⁴
Consumption – no. bacteria x10⁴

Figure 10

Growth, in terms of protoplasm produced, as a product of consumption of bacteria at $15^{o}C$

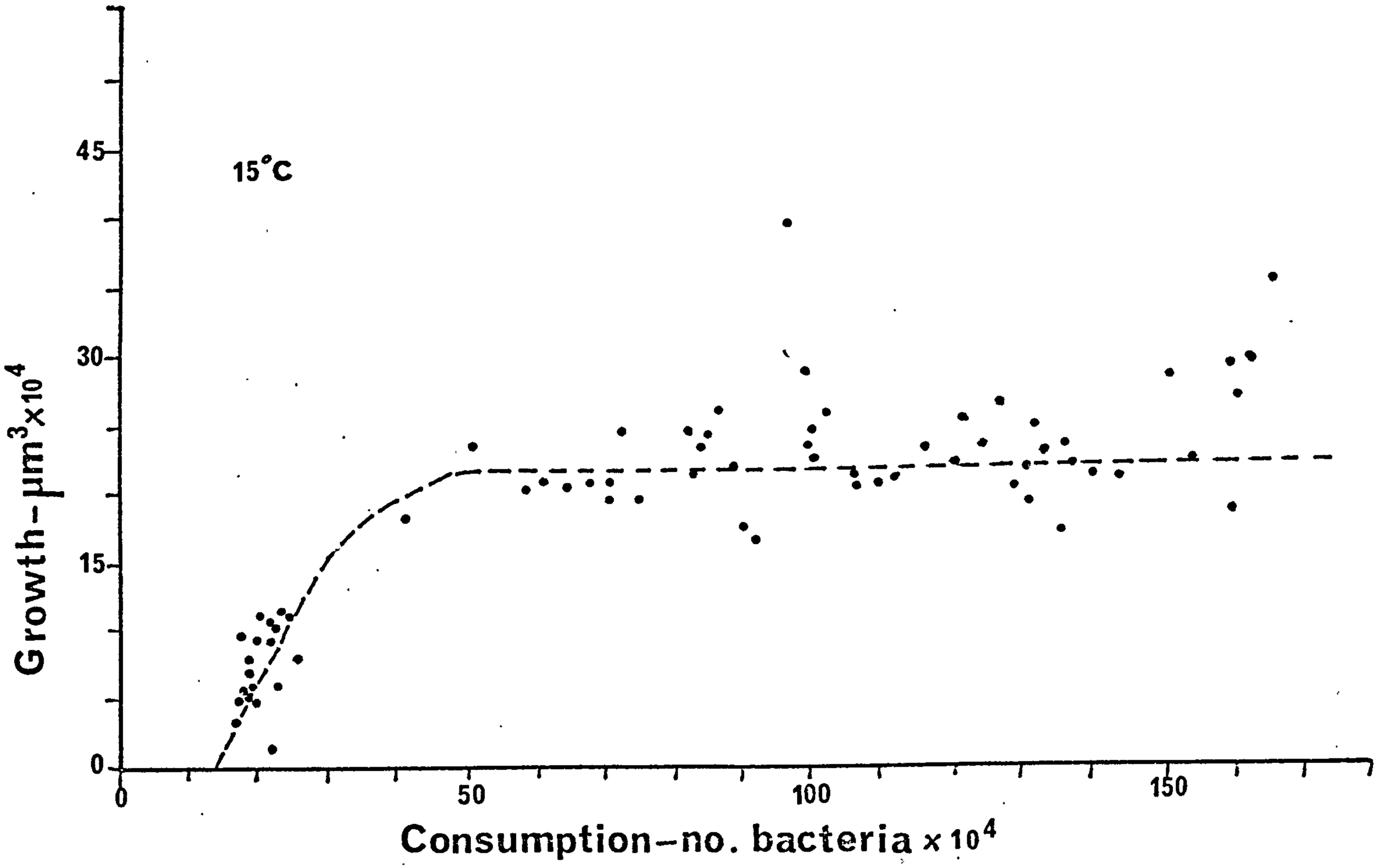
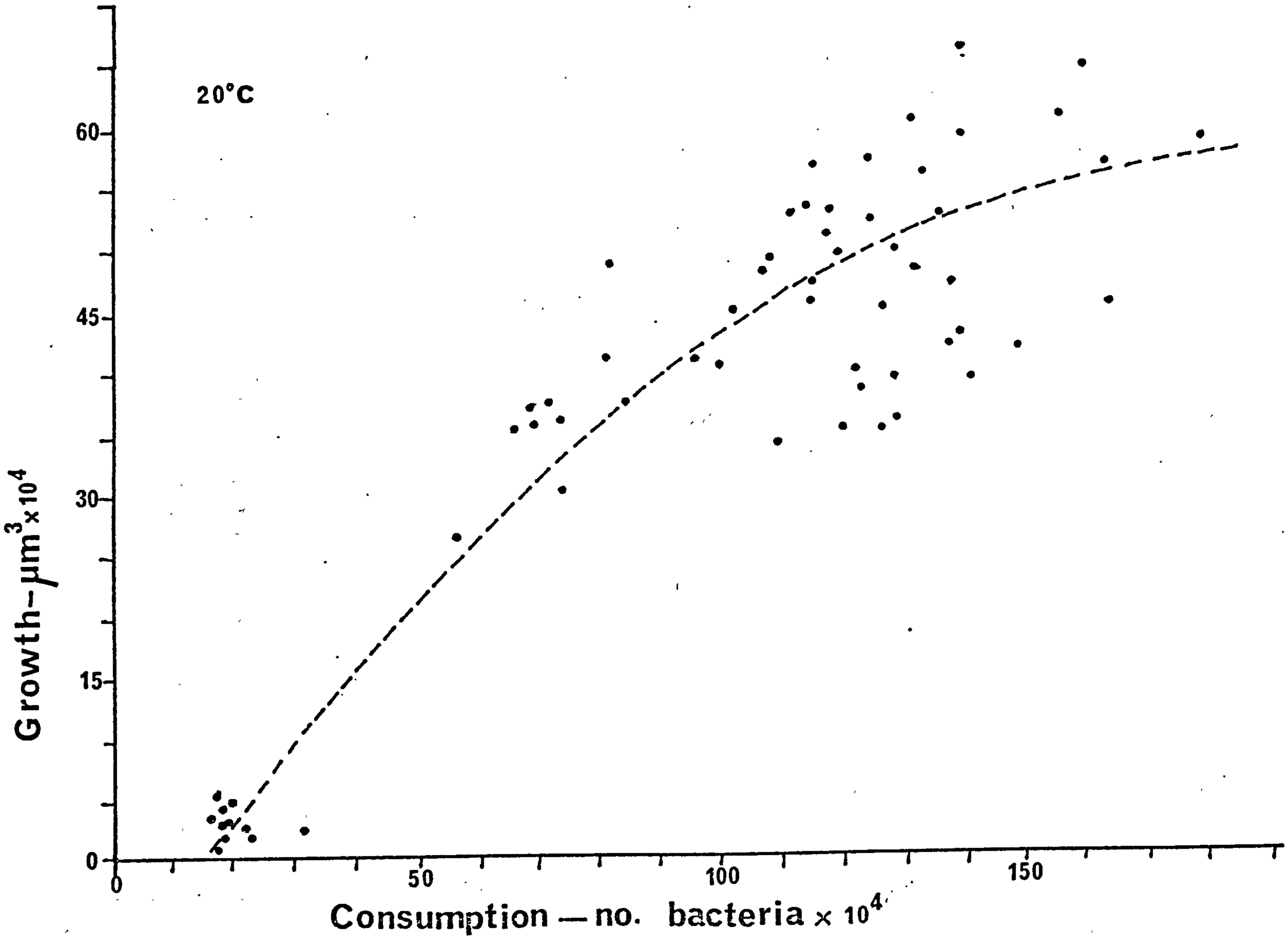

15°C
45
30
15
0
0
50
100
150
Growth-μm³×10⁴
Consumption-no. bacteria × 10⁴

Figure 11

Growth, in terms of protoplasm produced, as a product of consumption at $20^{o}C$


20°C
60
45
30
15
0
0
50
100
150
Growth-$\mu m^3 \times 10^4$
Consumption — no. bacteria $\times 10^4$

been described with reference to food concentration, which essentially dictates the quantity of food consumed.

Controls

Controls of Protozoa without food were usually found to be dead at the end of 24 hours, on a few occasions some moribund specimens were encountered. The controls of bacteria only varied by an average of 5.5% over the experimental period, suggesting that little or no bacteria division occurred, in fact the majority of the variation was probably due to counting error. Analysis of counts shows that the values lie between ±2 S.E. of the mean.

The pH of experimental cultures varied between pH 6-7 during the course of the experiments. Protozoa are known to be tolerant of a wide pH range so that a variation of this order probably has no significant effect.

All the results from which the graphs were constructed are presented in the appendix.

2.2.3 Discussion

Very little work on the energetics of the Protozoa has been undertaken and consequently the literature does not offer a great deal for comparative purposes. In recent years the work of Curds and Cockburn (1968,1971) and Heal (1967a) has provided some quantitative data on the feeding and growth of Tetrahymena pyriformis and Acanthameoba sp. respectively.

Heal (1967a) noted that increased consumption did not increase reproduction, which was his parameter of growth. In the current study on Colpidium a similar pattern, namely that

increased consumption did not increase growth, emerged particularly at 10°C and 15°C. Curds and Cockburn (1968) were able to show that the relationship between the initial concentration of food and the yield of Protozoa was linear at low concentrations, but was inhibited at higher concentrations. This agrees with the results of the present investigation, where growth was linear at low food ratios, but levelled out as food availability increased at higher ratios. Colpoda steinii displayed a very similar pattern of growth, where the rate of growth was limited at higher food concentrations (Proper and Garver 1966). These authors suggested that the inhibition of growth at high food concentrations is caused either by the rate at which the individual cells can ingest bacteria, or by the rate at which the bacteria are metabolised. The latter suggestion is probably the case, as the current study showed that growth is not limited by the rate at which bacteria are ingested at higher food ratios, since consumption continued to rise even after maximum growth had been attained at 10°C and 15°C.Thus, the indications are that at temperatures between 10°C and 15°C, growth is limited by the rate at which the protozoan cell can metabolise the bacteria it ingests at higher food availabilities. The situation at 20°C is less clear; possibly at higher temperatures growth is limited by the rate at which bacteria can be ingested. At low levels of food availability, where growth and consumption are linear, the rate of growth is almost certainly limited by the rate at which the bacteria are ingested at all the temperatures considered.

It is interesting that at 10°C and 15°C consumption increased even after growth had reached its maximum level, since obviously more bacteria were ingested than were necessary to sustain maximum growth. There seems to be no mechanism to prevent this obvious inefficiency. Increased feeding with increased food concentration has also been demonstrated by Harding (1937) in Glaucoma pyriformis (syn. Tetrahymena pyriformis) at 25°C. Harding (1937) showed that the feeding rate never became independent of food concentration as it did in Colpidium since for every increase in concentration there was an increase in food vacuole formation. In Acanthamoeba sp. consumption was found to be linearly related to food availability at low food ratios, at higher ratios, however, the results became very variable and no obvious relationship between consumption and availability was apparent (Heal 1967a).

At 20°C the results of the study on Colpidium show a variation over the pattern which emerged at 10°C and 15°C. At these lower temperatures consumption continued to increase even after maximum growth rates had been reached; at 20°C however, the maxima for growth and consumption occur near the same food ratio. This would suggest that at higher temperature the maxima of growth and consumption become more closely related.

CHAPTER 3 MEAN CELL VOLUME

3.1 Introduction

The significance of variation in the mean cell volume of a ciliate population in relation to growth is apparent from the preceding chapter. In the light of the feeding and growth studies which have been discussed previously, it is of value to consider the effect of temperature and controlled food concentration on the cell size of Colpidium.

3.2 Materials and Methods

The results for the effect of food concentration and temperature on the mean cell volume of Colpidium were obtained from the detailed series of experiments described in Chapter 2, section 2.2 - Consumption and Growth.

Mean cell volumes were obtained by using the Coulter Counter mean cell volume attachment as described in section 2.2.1.

Bacterial dried weights were obtained by converting consumption values in terms of number of bacteria eaten from the preceding chapter into dried weight using a value of 0.79pg/individual bacterial cell, S.D. 0.30pg obtained as described in Chapter 4, section 4.2.

3.3 Results

At consumption levels below 400ng/24 hours/individual the mean cell volume decreased. Above this level of consumption the mean cell volume attains a maximum, as shown in Figures 12-

Figure 12

Mean cell volume changes in Colpidium as a product of the quantity of food consumed (dried weight of bacteria) at 10°C

Mean Cell Volume at 10 °C

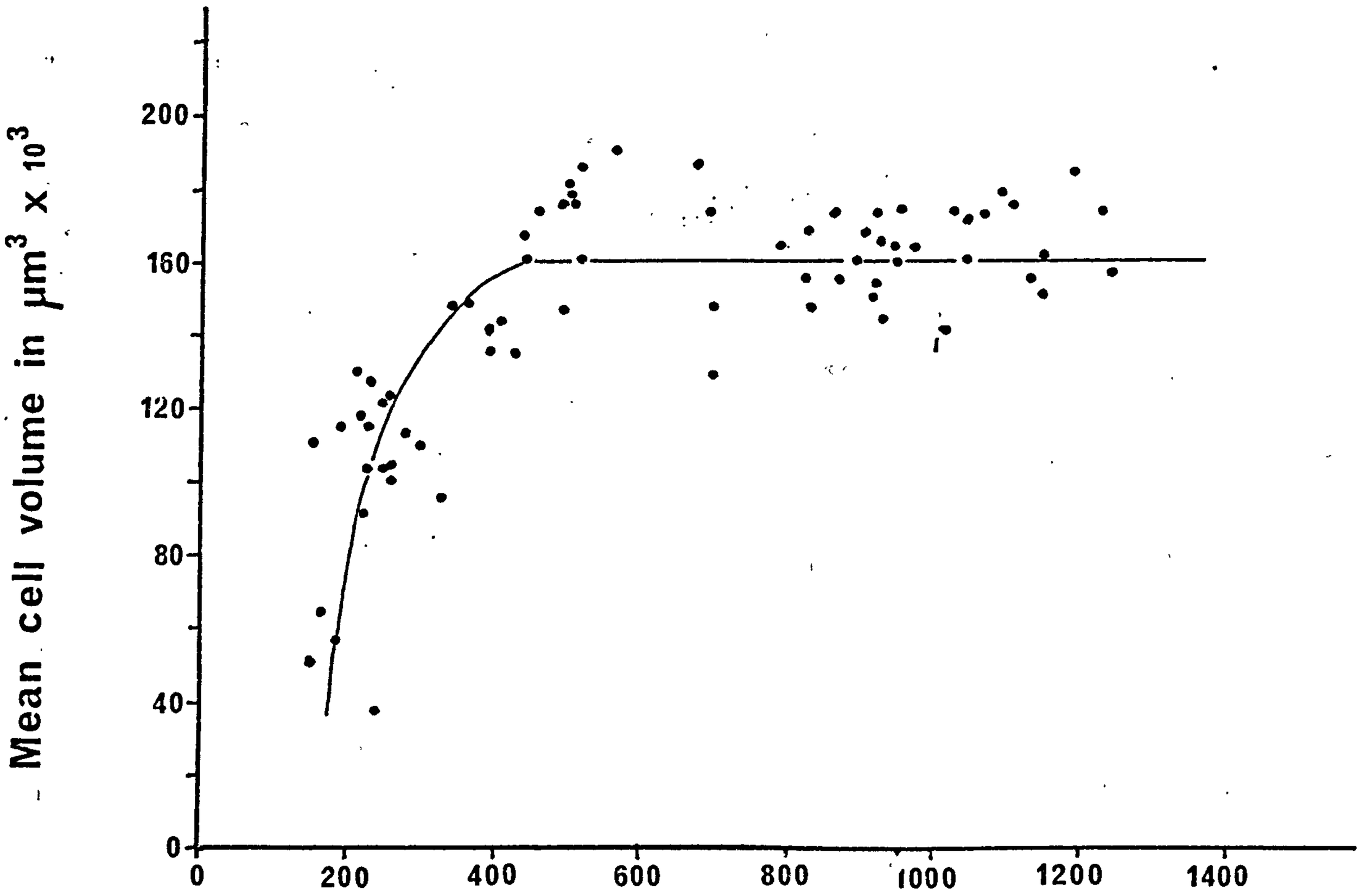


Figure 13

Mean cell volume changes in Colpidium as a product of the quantity of food consumed (dried weight of bacteria) at 15°C

Mean Cell Volume at 15 °C

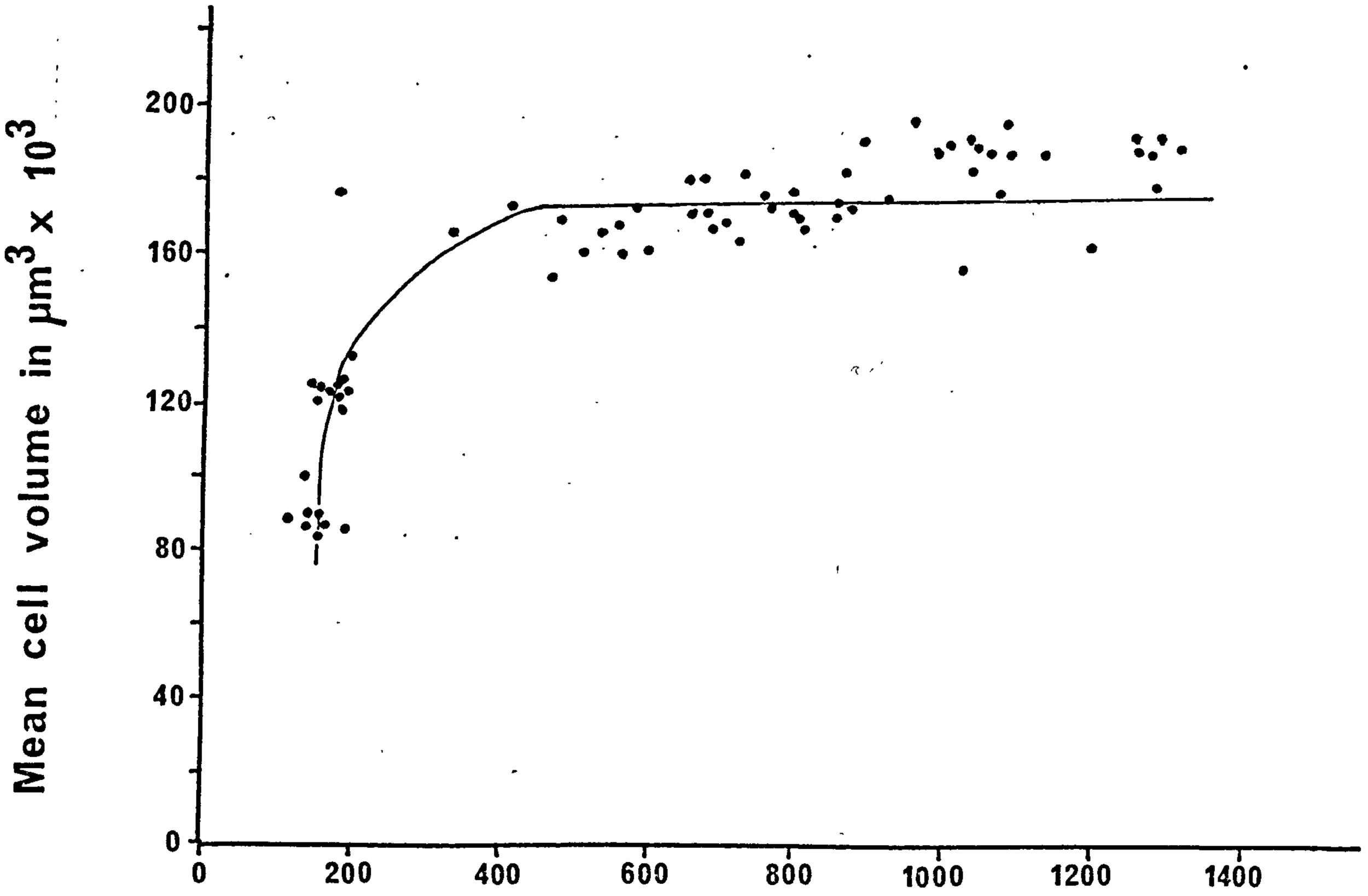
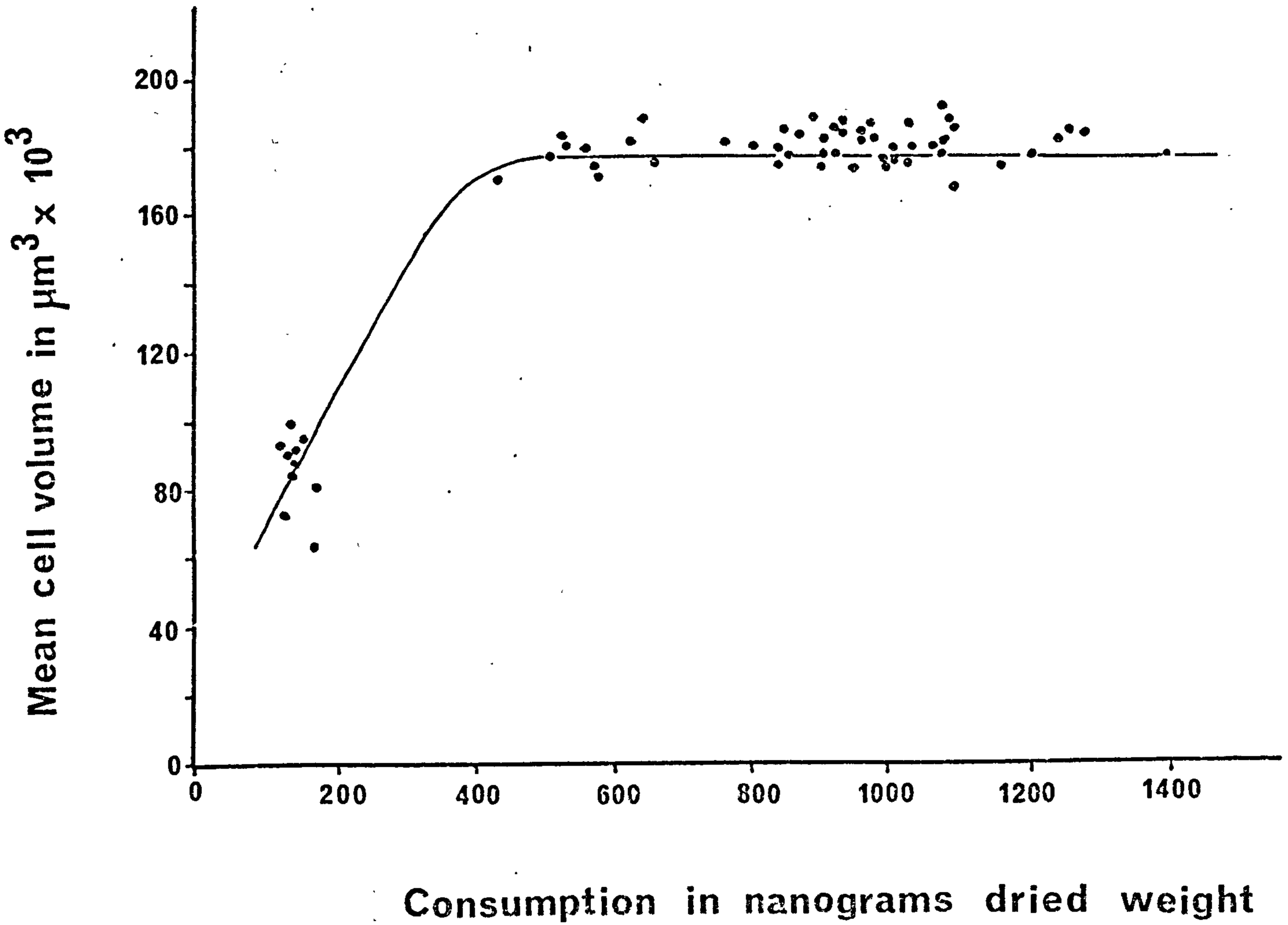


Figure 14

Mean cell volume changes in Colpidium as a product of the quantity of food consumed (dried weight of bacteria) at 20°C

Mean Cell Volume at $_{20}{}^{o}C$



14. The maximum mean cell volume appears slightly lower at 10°C, but in general the maximum limit does not differ greatly over the temperature range considered, and lies between 160-180$\mu m^3 x10^3$.

The minimum sizes recorded in these experiments range between 40-100μm^3 x 10^3. In Chapter 2, section 2.1 under conventional culture conditions values of less than 10μm^3 x 10^3 were recorded after the stationary phase. At this point the ratio of food organisms per protozoan had assumed a very low level and the animals were in a state of starvation.

3.4 Discussion

Decreased volume associated with decreased food concentration and presumably therefore decreased consumption have been demonstrated in Tetrahymena pyriformis by Harding (1937) and by Curds and Cockburn (1971). Decreased cell volume in an ageing culture associated with decreasing food concentration has also been shown to occur in Uromena species by Hamilton and Preslan (1968), maximum cell volumes were reached in the late exponential and stationary phases. Kimball, Caspersson, Carlson and Svensson (1959) demonstrated that a lower food concentrations division occurred at lower weights, that is smaller volume, in Paramecium aurelia.

The findings of the present research where various availabilities of food were presented to Colpidium would confirm, to some extent, the findings of the above authors, that decreased bacterial concentration and hence lower food consumption exert

an effect on cell size. Where the initial food concentration was high enough to allow a consumption above 400ng bacteria/ individual/24 hours (equivalent to 50 x 10^4 bacterial cells) mean cell volume remained fairly constant. Below this level of food intake mean cell volume decreased. This is contrary to the situation reported by Harding (1937), where growth rate in Tetrahymena was dependent on food supply below a concentration of 0.6 x 10^6 bacteria/cc, but above this range the cell size continued to increase while growth did not; a somewhat contradictory situation, since increased cell size should result in increased growth.

It has been suggested, at least for Protozoa grown under steady-state conditions (i.e. in a chemostat), that cell size is dependent on the density of protozoan cells (Hamilton and Preslan, 1970). An increase in population density caused an increase in mean cell volume in that population. A situation which is the opposite from that demonstrated for Colpidium in Chapter 2, section 2.1 under typical culturing conditions and from the findings of the present consideration of cell size variation. In Chapter 2, section 2.1 where the cultures were set up and allowed to age, increased density associated with depletion of food supply brought about a diminution in mean cell volume. In the present instance where the ciliates were studied for a 24 hours period in a range of food concentrations, mean cell volume reached a maximum associated with a particular level of food intake, below which is decreased. The initial density of the protozoan population was the same in each

experiment.

Thus within the limits of the present study it would appear that the mean cell volume of Colpidium is dependent very largely on the quantity of food consumed.

Twenty-four hours, the length of the experimental period, seemed sufficient to cause a reflection of the prevalent experimental conditions in the mean cell volume. At low bacterial to protozoan ratios, cells which had possessed a maximum volume prior to the experiment, had decreased in size to the levels shown in Figures 12-14 at food intakes below 400ng /individual/24 hours. A rapid response to changing conditions is indicated, which supports the findings of Kimball et al.(1959), who showed that starved cells of Paramecium regained a normal weight by the second division after the starvation period. Hamilton and Preslan (1969) showed that cells from an aged culture of Uronema when transferred to a fresh culture displayed some increase in cell volume even after the short period of two hours. It would seem, therefore, that ciliates are capable of responding rapidly to changes in their environmental conditions.

The mean cell volume of Colpidium displayed no significant variation over the temperature range ($10^{o}C$-$20^{o}C$) considered. Summers (1963) working with Tetrahymena pyriformis also showed no variation in cell size over the $10^{o}C$-$20^{o}C$ range; however, at $25^{o}C$, the optimum growth temperature for Tetrahymena, a marked decline in mean cell volume occurred, while at $30^{o}C$ the volume increased to the highest level found over the entire temperature range.

CHAPTER 4 DRIED WEIGHT, CALORIMETRY AND GROSS GROWTH EFFICIENCY

4.1 Introduction

In order to ascertain the level of growth efficiency or conversion efficiency (that is the proportion of food ingested which is converted into growth), in Colpidium over the range of food availabilities and temperatures considered in Chapter 2, section 2.2, and assimilation which will be considered in a later chapter, the calorific or energy content of both the ciliate and its bacterial food source had to be determined.

The present study appears to be one of the first in which the actual energy content of both bacteria and Protozoa have been used. Heal (1967a) used dried weights when estimating the conversion efficiency of Acanthameoba sp. fed on yeast cells of Saccharyomyces cerevisiae, as did Curds and Cockburn (1968) in Tetrahymena pyriformis fed on bacteria, also using carbon content in an axenic culture in a medium of proteose-peptone and yeast extract. Further studies by these workers (Curds and Cockburn 1971) involved using dried weights, but at this time they were aware of mean cell volume variations and their methods took account of this factor. Growth efficiencies in Colpoda steinii fed on bacteria have been investigated using dried weights by Proper and Garver (1966). Estimates of the yield of the Protozoa have been arrived at by means of labelled bacteria, Coleman (1964) used ^{14}C labelled bacteria in feeding experiments with the rumen ciliate Entodinium caudatum

In energy budgets the various components of the equation

are expressed in units of energy. If protozoan energy budgets are to have any comparative value with energy budgets on metazoan animals it is essential that similar units are adopted. In this work the unit of energy used will be the joule, in accordance with standard international practice.

4.2 Materials and Methods

4.2.1 Dried weights

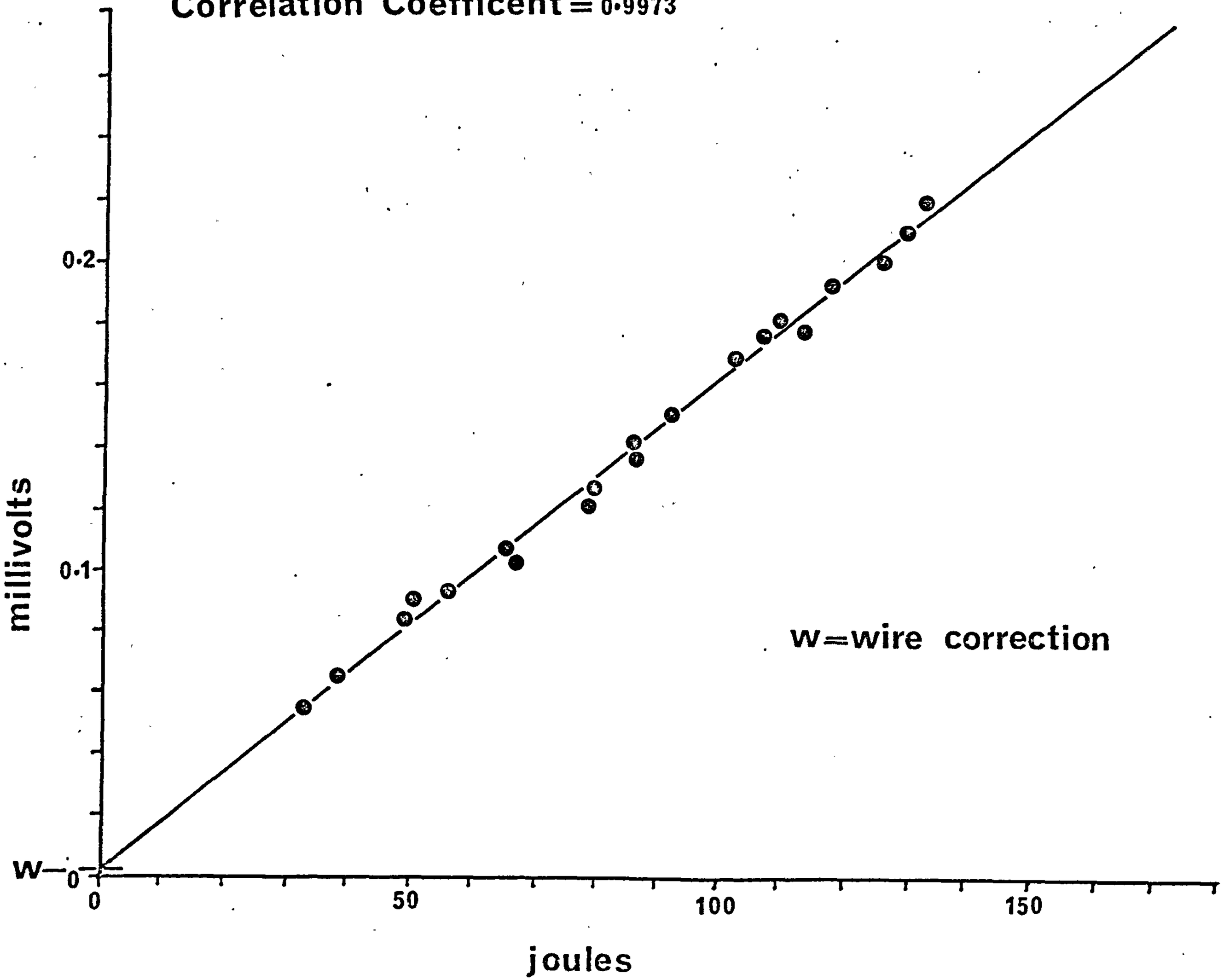
Cultures of Colpidium were grown for 48 hours at food concentrations above those which had been shown to be necessary for maximum growth (see Chapter 2, section 2.2), at 10°C, 15°C and 20°C. At the end of this period each culture was poured through a bolting silk sieve with a mesh size of approximately 2,500-10,00μm^2 to remove any flocculated bacteria. Samples of the sieved culture were then removed for counts of bacteria and Protozoa and the determination of protozoan mean cell volume. Cultures were centrifuged for 30 minutes at 1,000r.p.m.. The supernatant fluid was poured off and retained for counts of bacteria and Protozoa still suspended. The sediment was carefully collected and freeze-dried until a constant weight.

The methods used in counting of bacteria and the counting and sizing of Protozoa have already been fully outlined in Chapter 2, section 2.2.1. Bacteria were counted in order to apply a correction for the number of bacteria harvested with the Protozoa.

Bacteria were grown on 5% yeast agar plates for 72 hours.

Figure 15

The Benzoic Acid calibration curve


Correlation Coefficent = 0·9973
0·2
0·1
millivolts
W
0
0
50
100
150
joules
w=wire correction

The plates were washed with sterile distilled water and after the removal of samples for counting the bacterial "milk" was centrifuged for 15 minutes at 3,000r.p.m. The supernatant fluid was poured off and retained for the determination of the numbers still suspended. The sediment was collected and freeze-dried until a constant weight was achieved.

4.2.2 Calorimetry

A Phillipson (1964) microbomb calorimeter as manufactured by Gentry-Weigert of Aiken, Carolina was used for calorific determination. A Telsec chart recorder set on the 0.5mV range was attached to the output of the calorimeter. The apparatus was calibrated according to Phillipson (1964) and Prus (1968a). A thermo-chemical standard, benzoic acid ($C_6H_5.COOH$) yielding 26,455.0 joules/gm as determined by the National Physical Laboratories, was used for calibration. Twenty pellets ranging from 1-5mg were combusted and a line of regression was calculated (Figure 15) from the results using the equation in section 2.2.1. The relationship between the weight of benzoic acid (and hence the energy content) and the calorimeter output voltage was found to be linear over the range examined. The value between 0 and the intercept of the curve on the vertical axis represents the heat output from the platinum wire of the bomb, a value of 0.0031mV. The energy, E, stored in the bomb firing circuit can be calculated from the charging voltage, V, and the capacitance, C, expressed in farads, since

$$E = \tfrac{1}{2} C V^2 .$$

The capacitance is 3,900μF, and the charging voltage is 35V, hence E is 2.4joules, equivalent to 0.0038mV by using the benzoic acid calibration. The difference between the value and the measured wire correction may be attributed to external resistance wire losses.

It must be noted that in the past the units of energy used were calories, in this study, as has already been stated, the joule will be used (1 calorie = 4.184 joules).

4.3 Results

4.3.1 Dried weights

The dried weights for Colpidium grown at 10°C, 15°C and 20°C corrected for the bacteria harvested with the Protozoa are tabulated in Table 1. The variation between the weight of Protozoa grown at different temperatures was not great and a mean value was taken and used in all calculations.

Bacterial dried weights are tabulated in Table 2. Again a mean value was taken and used in all calculations.

4.3.2 Calorimetry

The yield of energy from the combustion of Colpidium samples is tabulated in Table 3. The mean value obtained was derived from 18 determinations, some of the dried samples were combined in order to provide pellets of sufficient size for combustion in the bomb calorimeter. A correlation coefficient (for equation see Chapter 5, section 5.2) of 0.9952 (P=<0.001) was obtained for the relationship of pellet weight and energy yield in joules.

TABLE 1

Dried Weights of Colpidium

Sample No.	Temperature	Dried weight in picograms
1	20°C	0.171
2		0.182
3		0.102
4		0.137
5		0.115
6		0.296
7		0.207
8	15°C	0.131
9		0.290
10		0.132
11		0.211
12		0.132
13		0.211
14	10°C	0.108
15		0.111
16		0.259
17		0.054
18		0.092
19		0.236
20		0.241
	MEAN	0.170 S.D. 0.070

TABLE 2

Dried Weights of Moraxella (Bacterial food source)

Sample No.	Weight/indiv picograms
1	0.9
2	0.4
3	0.4
4	0.5
5	1.1
6	1.2
7	0.9
8	0.8
9	0.9
MEAN	0.79
S.D.	0.30

TABLE 3

Calorific Determinations on Colpidium

Sample No.	Joules/mg	% Ash
1	22.419	2.04
2	19.643	1.79
3	19.084	1.52
4	19.009	0.83
5	19.906	3.32
6	18.584	0.89
7	21.116	1.60
8	20.822	4.70
9	20.946	3.38
10	20.000	4.12
11	17.911	
12	19.719	1.41
13	19.872	2.25
14	22.973	1.36
15	17.392	1.45
16	20.176	0.88
17	20.909	0.91
18	22.223	1.12
MEAN	20.1519	1.975
S.D.	1.5002	1.193

The energy contained in the bacterial food source Moraxella as determined from the combustion of 30 pellets is tabulated in Table 4. A correlation coefficient of 0.9905 (P=<0.001) was obtained for the dependence of energy content in joules on the weight of the pellet.

The ash content of both Colpidium and the bacteria, based on the residue remaining on the platinum pan after combustion, and therefore only an approximation, was 1.9% for Colpidium and 12.9% for the bacteria.

4.3.3 Growth efficiencies

The results for growth and consumption in Chapter 2, section 2.2 were first converted to dried weight and then to joules using the results previously outlined. The percentage of the food consumed which was converted to growth was then calculated. The final results for all experiments on growth and consumption may be found in the appendix.

Since growth displays a levelling off at higher consumption levels, as shown in Figures 16-18, it follows that the highest conversion will occur where growth reaches its maximum, but will fall thereafter because consumption continues to rise in spite of maximum growth having been attained. In other words, at higher levels of consumption a smaller proportion of the food ingested is converted to protoplasm. Consequently there would appear to be an optimum growth efficiency based on the quantity of food eaten and this in turn is determined by the concentration of the available food. This relationship is clearly illustrated in Figures 16-18.

TABLE 4

Calorific Determinations on Moraxella

Sample No.	Joules/mg	% Ash
1	20.639	18.4
2	20.063	16.8
3	20.641	12.1
4	19.954	17.2
5	20.000	17.6
6	20.000	16.1
7	20.827	16.4
8	20.283	14.2
9	20.824	12.3
10	20.544	10.6
11	20.565	11.1
12	20.103	10.3
13	20.492	16.4
14	20.000	12.4
15	20.779	11.7
16	19.507	13.6
17	21.341	9.2
18	20.227	11.4
19	20.366	12.6
20	20.833	11.5
21	20.976	14.6
22	20.761	13.1
23	20.989	8.8
24	20.597	7.9
25	20.671	9.7
26	19.858	13.0
27	19.529	10.6
28	20.582	11.9
29	19.517	16.1
30	19.912	12.2
MEAN	20.3627	12.99
S.D.	0.4653	2.82

Figure 16

Growth, consumption and gross growth efficiency at 20°C

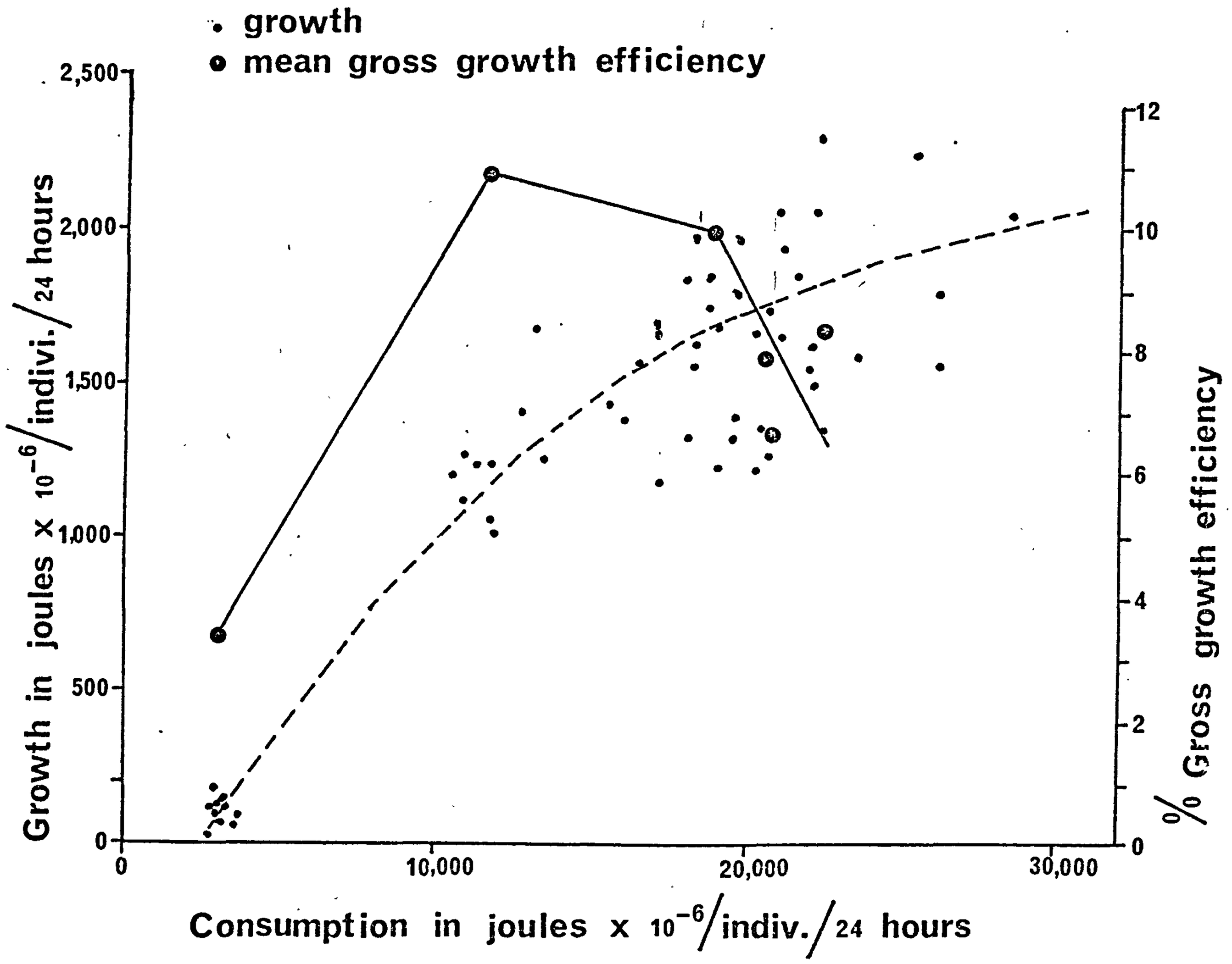

growth
mean gross growth efficiency
2,500
2,000
1,500
1,000
500
0
Growth in joules x 10⁻⁶/indivi./24 hours
12
10
8
6
4
2
0
% Gross growth efficiency
0
10,000
20,000
30,000
Consumption in joules x 10⁻⁶/indiv./24 hours

Figure 17

Growth, consumption and gross growth efficiency at 15°C

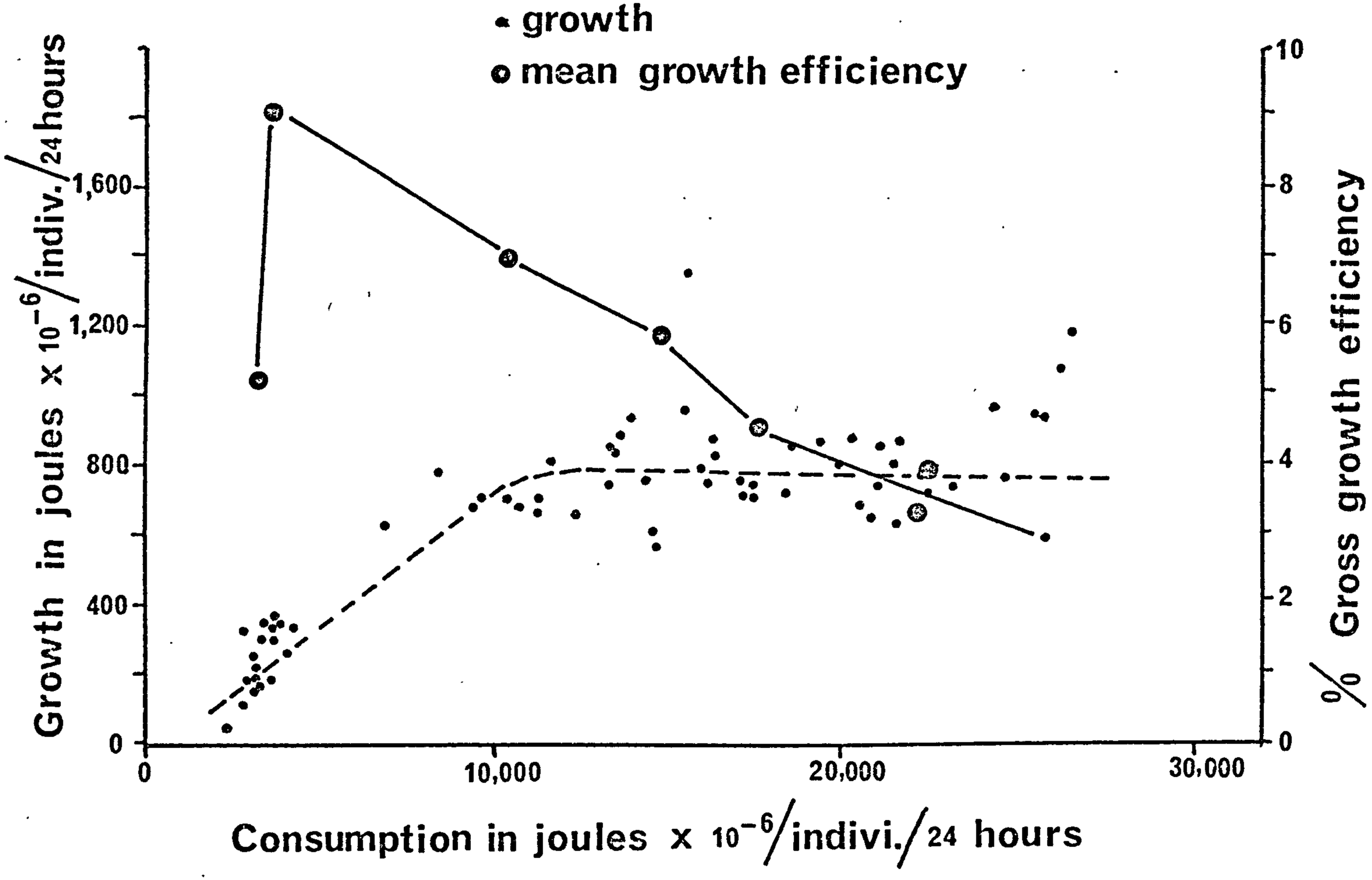
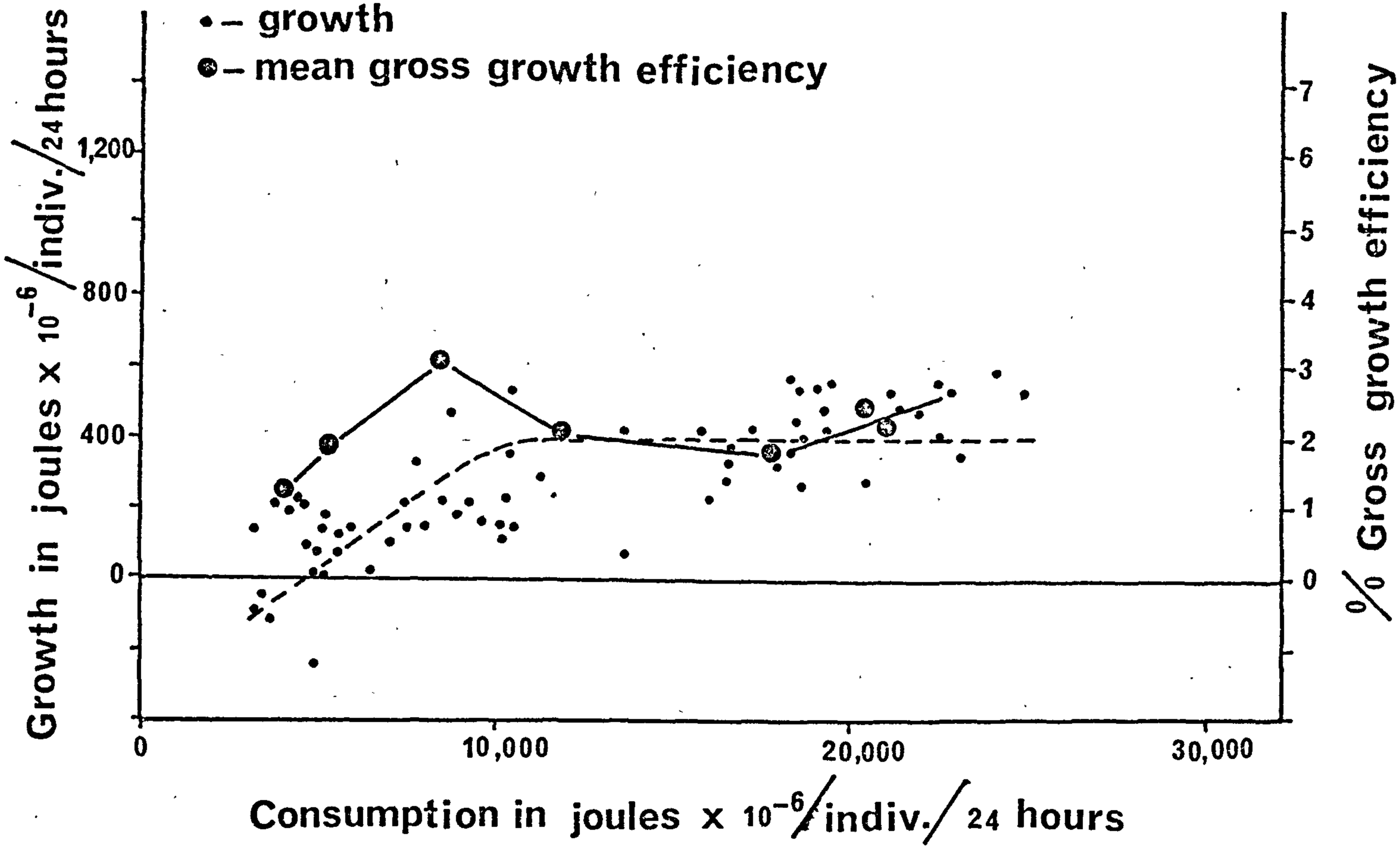

growth
mean growth efficiency
Growth in joules x 10⁻⁶/indiv./24 hours
0
400
800
1,200
1,600
0
10,000
20,000
30,000
Consumption in joules x 10⁻⁶/indivi./24 hours
% Gross growth efficiency
0
2
4
6
8
10

Figure 18

Growth, consumption and gross growth efficiency at $10^{o}C$


• – growth
⊗ – mean gross growth efficiency
Growth in joules x 10⁻⁶/indiv./24 hours
1,200
800
400
0
% Gross growth efficiency
7
6
5
4
3
2
1
0
0
10,000
20,000
30,000
Consumption in joules x 10⁻⁶/indiv./24 hours

The efficiency of conversion varied with temperature as one would expect, with increased growth efficiency dependent on increased temperature. The maximum at 20°C was in the region of 11%, at 15°C a maximum of 9% was achieved and at 10°C maximum growth efficiency was approximately 3%.

4.4 Discussion

The energy content of Colpidium lies close to the values obtained from the combustion of other invertebrates by other workers. Prus (1968b) found adult female Tribolium castaneum to contain 6.5329 calories/mg, equivalent to 27.33J/mg, Lawton (1971) showed the damselfly Pyrrhosoma nymphula to possess a calorific content of 5.124-5.292 calories/mg during its life-cycle, which represents 21.44-22.14J/mg. Higher values were shown in Podisus maculiventris, an hemipteran, (Mukerji and LeRoux 1969a), when adult males contained 8.613-5.747 calories/mg and females 8.688-5.485 calories/mg, these values convert to 36.03-24.09J/mg and 36.35-22.95J/mg respectively. All these values are from energetics studies an arthropods, a group that has received much attention in this field.

The dried weights obtained in this study are somewhat higher than one would expect for a protozoan representing around 17% of the wet weight assuming a specific gravity of 1. Percentage dried weights for arthropods which have an exo-skeleton range from 15-30% (Gere 1956, Klekowski, Prus and Zyromska-Rudska 1967, Prus 1968a, 1972, Lawton 1971). Comparable information from the literature on Protozoa is not

easily available, largely because authors do not express dried weight as a percentage of wet weight, or because cell dimensions are not always given with the dried weight for a single animal. Using the figure for the dried weight of an individual Tetrahymena cell stated by Curds and Cockburn (1968), and using the cell volumes recorded by them in a later paper (Curds and Cockburn 1971) approximate dried weights of around 5% of the wet weight are obtained. From the data given by Proper and Garver (1966) for the ciliate Colpoda steinii of an average cell volume 10,000μm^3 and an average dried weight of 1.2×10^{-6}mg per cell one can obtain a figure which represents 12% of the wet weight, again assuming a specific gravity of 1. This figure is not a great deal different from that obtained for Colpidium.

Variation in the percentage dried weights of Protozoa is probably a result of the methods employed. The procedure used by Proper and Garver (1966) involved comparing the dried cell suspension of a known concentration with the supernatant dried weight, a method in some ways similar to that used with Colpidium. Curds and Cockburn (1968) obtained their dried weight value by filtering thoroughly washed suspensions of the organism through pre-washed oxoid membrane filters. The filters were dried and weighed. Washing the organisms was a precaution against the collection of bacteria with the Protozoa. Proper and Garver (1966) make no mention of precautions against the inclusion of bacteria in their protozoan dried weight value.

The growth efficiencies of up to 11% found for Colpidium are low when compared with those reported for Protozoa by other workers. Efficiencies ranging from 37%-78% have been found in various ciliate species and one sarcodine species (Coleman 1964, Proper and Garver 1966, Heal 1967a, Curds and Cockburn 1968,1971). Even allowing for the higher experimental temperatures used by these workers the growth efficiencies found in Colpidium are still low by comparison.

The low values may be due to the method used for measuring growth in this study. Coleman (1964) who fed ^{14}C labelled bacteria to the rumen ciliate Entodinium caudatum, found that 50% of the labelled bacterial carbon was retained by the Protozoa. Proper and Garver (1966) used mass cultures of Colpoda steinii and after drying the recovered Protozoa obtained an extremely high yield of 78%; 0.78g of Protozoa were produced from 1g of bacteria. Heal (1967a) multiplied the results of his feeding and growth experiments by the dried weights of the food organism and Acanthanoeba sp. to give a growth efficiency of 37%. Curds and Cockburn (1968) used mean dried weights for Tetrahymena pyriformis and its bacterial food source and by application to their feeding and growth studies obtained a yield of 50%. Thus a number of techniques have been applied over a range of temperature by various authors and the range of growth efficiency which has been demonstrated within the Protozoa is apparently considerable.

The growth efficiencies which have been found in protozoan species by some of the authors quoted above are high when

compared with other invertebrates. Prus (1968b) quotes growth efficiencies of 13-23% for Tribolium, and 5% for Asellus aquaticus (Prus 1972). Klekowski et al.(1967) give a figure of 30% production efficiency for Tribolium. The damselfly Pyrrhosoma nymphula displays growth efficiencies ranging from 15%-60% during its life-cycle (Lawton 1971). Gere (1956) found conversion efficiencies as low as 0%-7% for litter feeding Diplopoda and Isopoda. McNeill (1971) showed that the heteropteran Leptopterna dolabrata had growth efficiencies ranging from 15.6%-16.8%. Thus, the growth efficiencies obtained in Colpidium although lower than those which have been demonstrated in other protozoan species, are within the range reported for many other invertebrate animals. Colpidium's growth efficiency also compares favourably with that of some bacterial species of 13%-34% as demonstrated by Rahn (1940).

CHAPTER 5 REPRODUCTION

5.1 Introduction

Reproduction by binary fission does not strictly speaking constitute part of the energy budget equation for a ciliate. There are no special reproductive products and consequently no energy is expended in the synthesis of such products, however, energy is expended in bringing about the actual fissionary process which will be measured as part of the heat lost during respiration. The normal process of binary fission in Colpidium results in the formation of two daughter cells, each individual produced is merely half of the assimilated energy of the parent cell. Thus sexual reproduction is a direct product of growth.

It is, however, of interest to consider the effect of temperature and food availability on the rate of reproduction in Colpidium, especially for comparison with the many workers who have used reproduction (i.e. the number of animals produced) as opposed to direct measurements on the volume of protoplasm produced, as a parameter in growth studies (Cutler and Crump 1924, Heatherington 1934, Curds and Vandyke 1966, Heal 1967a, Curds and Cockburn 1968, Winet and Jahn 1971).

5.2 Materials and Methods

The results for reproduction were obtained during the experiments on consumption and growth, the procedure of which is fully outlined in Chapter 2, section 2.2.1. The equation used in the calculation of the number of generations produced

is given in Chapter 1, section 1.1.2.

In order to draw a comparison between the efficacy of the two parameters which have been used for measuring growth, that is the exact volume of protoplasm produced as opposed to the number of individuals produced, the correlation coefficients of protoplasm production as a result of consumption and reproduction as a result of consumption, at 20°C, 15°C and 10°C, were calculated using the following equation :-

$$r = \frac{\sum[(x-\bar{x})\ (y-\bar{y})]}{\sqrt{\sum[(x-\bar{x})^2]\sum[(y-\bar{y})^2]}}$$

where

r = correlation coefficient

x = protoplasm produced and the number of generations produced in 24 hours by each individual present at the beginning of the experiment

y = consumption by each individual present at the beginning of the experiment

5.3 Results

The number of generations produced in 24 hours as a function of the quantity of bacteria consumed, in joules, is shown in Figures 19-21. The results at 10°C and 15°C (Figures 19 and 20) show considerable scatter, but there is a general trend towards the production of higher numbers of generations, and consequently a shorter generation time up to 2,000-10,000 J $x10^{-6}$/individual/ 24 hours respectively. At 20°C, a shown on Figure 21, an upward trend related to energy intake is well marked, and the levelling off effect seen at 10°C and 15°C is less obvious.

Figure 19

Reproduction as a product of the energy consumed at $10^{o}C$

REPRODUCTION at 10^0C

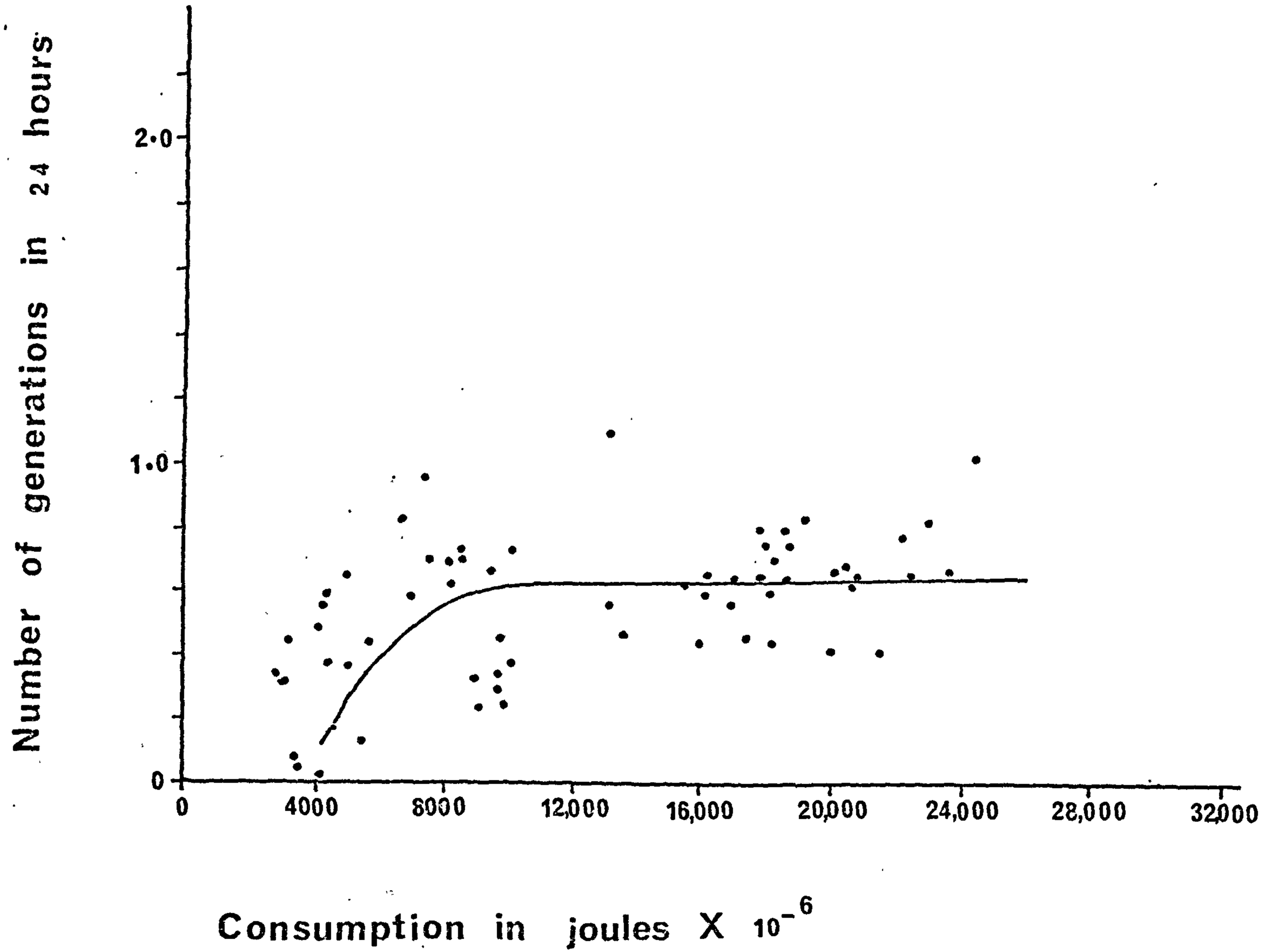


Figure 20

Reproduction as a product of the energy consumed at $15^{o}C$

REPRODUCTION at 15^0C

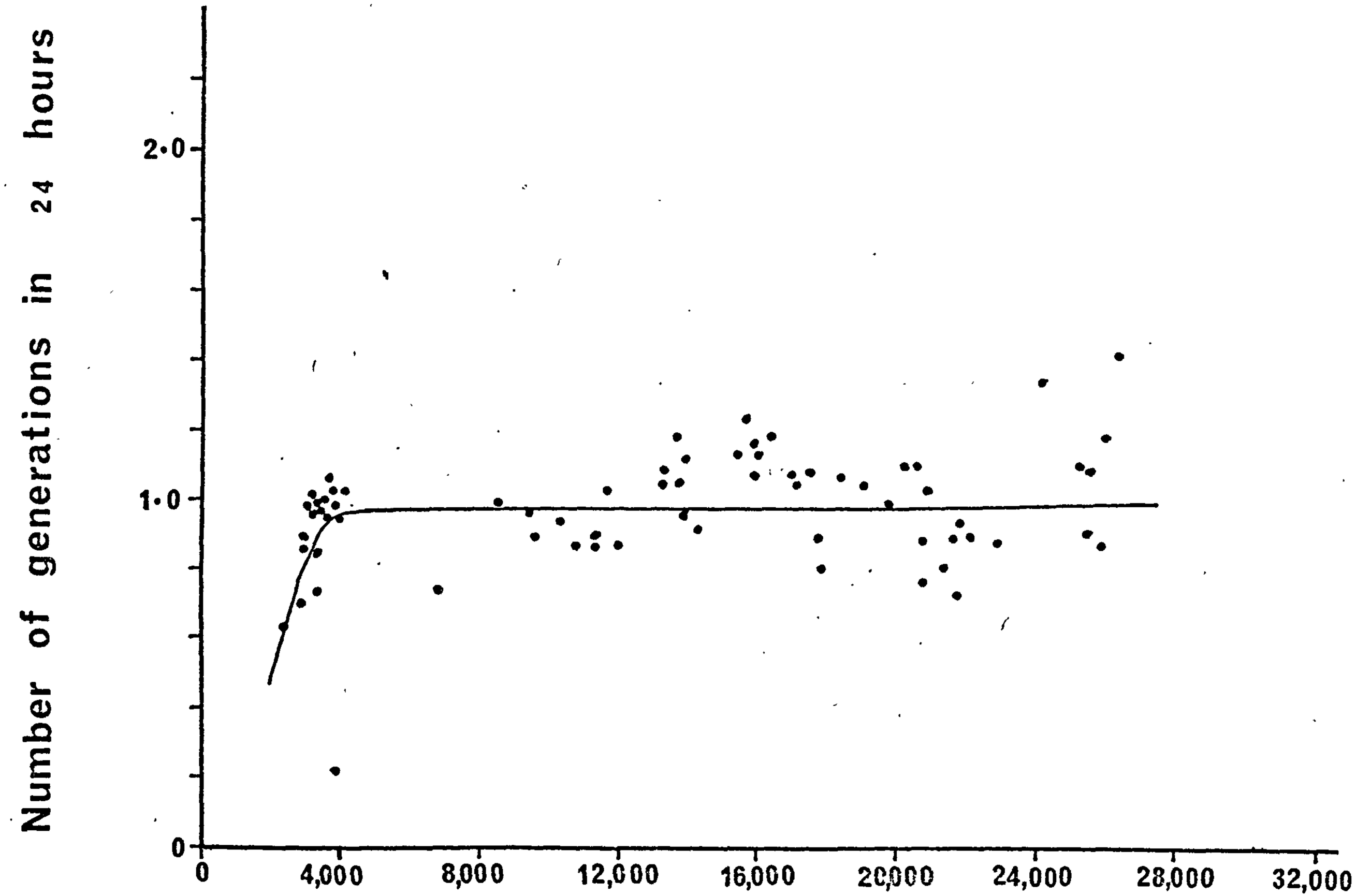
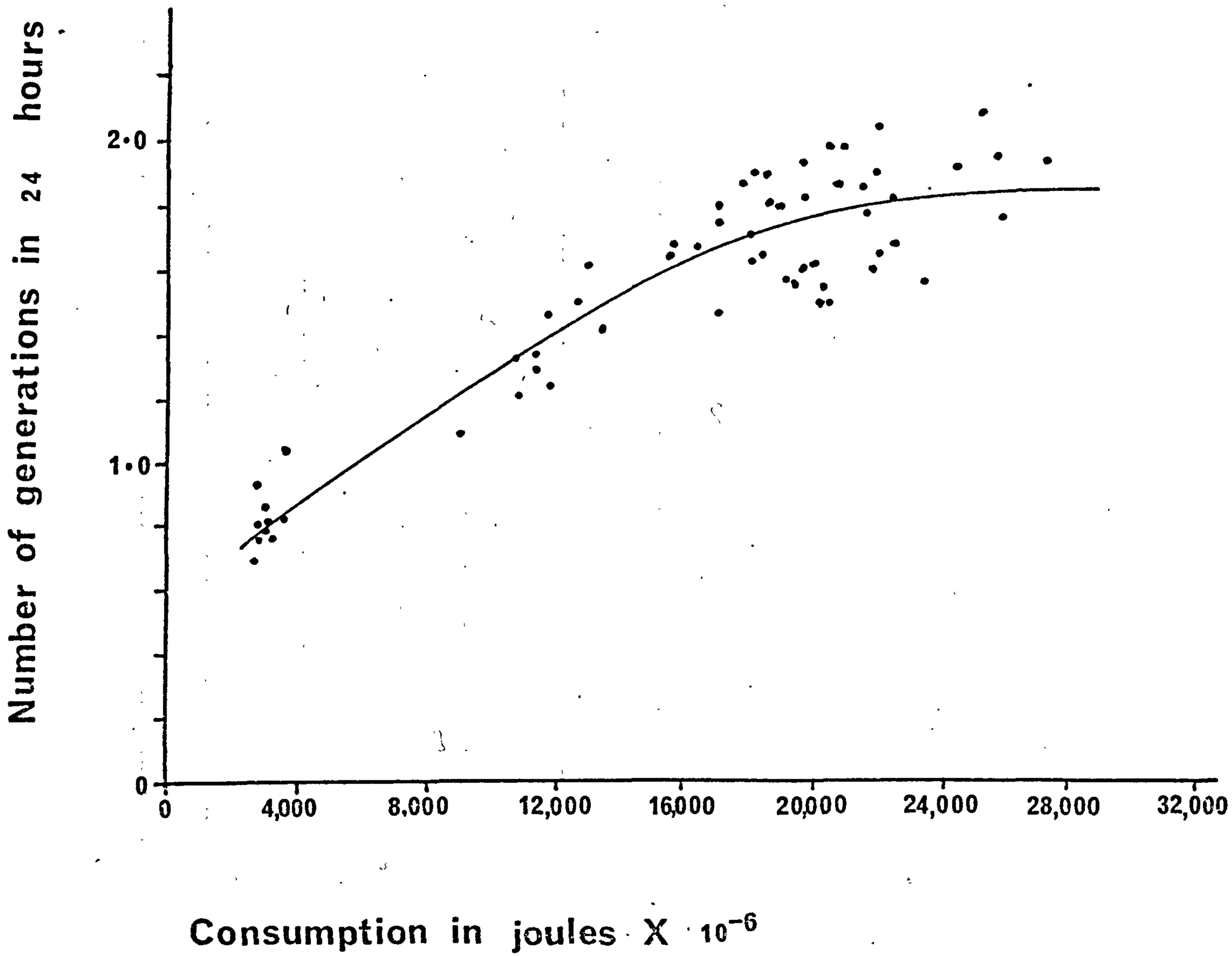


Figure 21

Reproduction as a product of the energy consumed at $20^{o}C$

REPRODUCTION at 20 °C



At 10°C the indications are that no reproduction occurred below a consumption of 2,000-6,000 J x 10^{-6}/individual/24 hours. At 15°C and 20°C the exact level of consumption at which reproduction ceased is not clear, but would appear to lie below 4,000 J x 10^{-6}/individual/24 hours.

Temperature was also found to exert an influence, in addition to the quantity of food eaten. The generation time decreased with increased temperature. Table 5 shows the number of generations produced at various food:Protozoa ratios at the three temperatures studied.

TABLE 5

Ratio Bacteria:Protozoa	20°C No.Gens.	S.D.	15°C No.Gens.	S.D.	10°C No.Gens.	S.D.
0.0-0.5 x 10^6:1	0.08	0.10	0.87 0.90	0.14 0.24	0.36	0.22
0.5-1.0 x 10^6:1	1.32	0.14	1.01	0.10		
1.0-2.0 x 10^6:1	1.81 1.62	0.10 1.17	1.08	0.14	0.73 0.39	0.10 0.28
2.0-3.0 x 10^6:1	1.78	0.17	1.07 1.00	0.10 0.14	0.70 0.54	0.14 0.10
3.0-4.0 x 10^6:1	1.71	0.14	0.88	0.10	0.67	0.14

The value of r (correlation coefficient) for reproduction and consumption , and the volume of protoplasm produced and consumption are shown in Table 6.

TABLE 6

x and y	Temp.	r	No.pairs
No.Generations and Consumption	20°C	0.92	60
	15°C	0.35	70
	10°C	0.54	70
Volume of Protoplasm and Consumption	20°C	0.89	60
	15°C	0.80	70
	10°C	0.80	70

All values of r were significant to a value of <0.001.

5.4 Discussion

The relationship between bacterial concentration and reproduction was demonstrated as early as 1924 by Cutler and Crump working with Colpidium colpoda. They found that the number of divisions increased with increased food:protozoan ratios. The relationship was also demonstrated in a somewhat cruder manner by Johnson (1936) who, with experiments at two bacterial levels, one five times greater than the other, found higher reproductive rates at the higher bacterial density. A similar phenomenon has been found in the carnivorous protozoan Woodrufia metabolica by Salt (1967), where the density of the carnivore decreased with the number of paramecia eaten.

The results of the present study on Colpidium confirm the findings of the authors cited above. At higher levels of consumption, however, there is a levelling off in the number of generations produced. Cutler and Crump (1924) did not find a levelling off effect, but the highest ratio of bacteria:

protozoan they used was 1,024,000:1; in the present work the ratios extended over the range 0.0-4.0 x 10^6:1. Harding (1937) working on Glaucoma pyriformis found that fission rate was dependent on bacterial concentration at low food:protozoan ratios but remained constant at higher bacterial concentrations. Phelps (1936) working with the same species in axenic cultures demonstrated a levelling off in the rate of reproduction at higher nutrient concentrations as was shown for Colpidium in the present study and Glaucoma by Harding (1937) in monexnic cultures of different bacterial concentrations. In the Suctoria where reproduction involves the production of motile dispersal forms by means of budding, a different situation prevails. The Suctoria appear to have an optimum feeding level below and above which embryo production decreases, as demonstrated in Tokophrya infusorium (Rudzinska 1959).

The influence of temperature upon the reproduction of Protozoa has been documented by several workers (Woodruff and Baitsell 1911a, 1911b, Johnson 1936). As one would expect increased temperature is reflected in a decrease in the length of the generation time. Woodruff and Baitsell (1911a), while trying to isolate the factors responsible for fluctuations in populations of Paramecium spp., noted that higher temperatures raised the level of reproduction, although the fluctuations persisted. In all probability the fluctuations they described were brought about by variation in the density of the bacterial flora, a variable they did not control or monitor.

Although the mechanism controlling reproduction is considered to change more slowly in response to changing conditions than the growth controlling mechanism (Kimball et al.1959, Hamilton and Preslan 1969), I found that reproduction had responded to various feed availabilities within 24 hours. At low ratios and hence low consumptions, the number of generations produced in 24 hours decreased. The rapidity of response to change of temperature was not investigated, since all the animals in experiments described in Chapter 2, section 2.2 had been previously acclimatized to the experimental temperatures.

As was pointed out in the introduction (5.1) it was considered necessary to evaluate the effectiveness of measuring the volume of protoplasm produced directly instead of counting the number of animals produced and duly multiplying this figure by a dried weight value obtained from drying cultures of unknown mean cell volume (Heal 1967a, Curds and Cockburn 1968). The correlation coefficients of growth by volume as a result of consumption versus the number of generations produced as a result of consumption, as shown in Table 6, are all significant to a value of 0.001. It is however obvious that volume as a product of consumption shows a closer and consistent correlation ($r=0.8-0.89$) over the temperature range $10^{o}C-20^{o}C$ than the number of generations produced as a result of consumption, which although showing a close correlation at $20^{o}C$ ($r=0.92$), are less closely correlated at $10^{o}C$ and $15^{o}C$ ($r=0.35-0.51$). Nevertheless, since all the values of r were significant to a

probability level of <0.001, indicating that they were very highly significant, it would appear, at least from this test, that both methods are satisfactory.

CHAPTER 6 RESPIRATION

6.1 Introduction

There have been many investigations into the respiratory exchange of the Protozoa. The first of these studies was that of Vernon (1895) on a radiolarian. Thereafter work of this kind was carried out on species from various protozoan groups.

Within the Ciliata, a few species have received much attention, notably species of the genus Paramecium (Lund 1918a, b and c, Necheles 1924, Leichsenring 1925, Kalmus 1928, Howland and Bernstein 1931, Pace and Kimura 1944, Pringle and Stewart 1961, Stewart 1966). The methods employed by these workers for measuring oxygen consumption included the Warburg apparatus, the Kalmus respirometer, and Winkler technique and the cartesian diver.

The effect of various external factors upon the respiration of ciliate species has been widely considered. Such factors include the effect of temperature (Wachendorff 1912, Leichsenring 1925, Pace and Lyman 1947, Sarojini and Nagabhushanam 1966), population density (Specht 1935, Pace and Kimura 1944, Pace and Kimura 1947), the hydrogen ion concentration (Hall 1941) and the effect of cyanide upon the oxygen uptake (Lund 1918, Pitts 1932, Lwoff 1934, Hall 1941).

The respiratory metabolism of Colpidium campylum has received some attention (Hall 1938, 1941). Hall's (1941) work on the effect of cyanide on the oxygen uptake of Colpidium dispelled the previously-held idea that the oxidation process

in ciliates was insensitive to cyanide. Their insensitivity to cyanide implied that oxidation in ciliates differed essentially from that of other aerobic cells. Earlier workers (Pitts 1932, Lwoff 1934) reported an initial inhibition followed by a recovery. However, they had failed to recognise the reversibility of the cyanide effect, and the tendency for HCN to distil over into the KOH inset of the respirometer flask.

The present work was carried out primarily to obtain an estimate of the heat liberated during respiration in order that an energy budget for Colpidium could be constructed. Ideally, in a study of this kind the heat lost during respiration should be measured directly in a microcalorimeter, since the indirect method involving respirometry assumes a totally aerobic respiration. Unfortunately such devices, although in existence, are as yet not quite developed enough to cope with the problems afforded by the Protozoa. Consequently at present one must be content with measuring the energy liberated during respiration by an indirect means.

In addition to the aim previously outlined the study was also intended to provide information on the effect of temperature, population density and cell size on the oxygen consumption of Colpidium campylum. Respiratory quotients were also considered.

6.2 Materials and Methods

6.2.1 Measurement of oxygen consumed

Oxygen consumption was measured using Warburg constant volume respirometers (Warburg 1926). The respirometer consisted of a flask which is attached by ground glass stopper to a manometer. Each flask has a central well for the reception of the alkali used to absorb the carbon dioxide liberated during respiration. The manometer is filled with Krebs solution (Krebs 1951), giving a reading equivalent to 10,000mm at N.T.P. Each respirometer was calibrated according to Dixon (1952) and Umbreit, Burris and Stauffer (1949). The quantity of oxygen consumed is obtained by multiplying the flask constant (k) for a given respirometer by the change in the reading of the manometric fluid of that respirometer, thus:-

$$x = h\,k$$

where x = the amount of gas absorbed

h = the corresponding manometric reading

The Thermobarometer - As one end of the manometer tube is open to the atmosphere the Warburg apparatus is sensitive to slight changes in barometric pressure. In order to correct for the effect of pressure changes a respirometer containing only a small quantity of distilled water is placed in the water bath with the experimental respirometers. The thermobarometer is read when the other respirometers are read and its reading is subtracted from the experimental readings.

6.2.2 Measurement of respiratory quotients (R.Q.)

Respiratory quotients were obtained by using the direct method of Warburg (Dixon, 1952, Umbreit et al.1949). This method involves the use of two respirometers each containing the same number of animals. In the first flask the CO_2 evolved is absorbed by alkali in the normal way, and the reading of this apparatus gives the amount of oxygen consumed. In the second respirometer the alkali is omitted, and the reading obtained is the result of both O_2 consumed and CO_2 evolved. In order to calculate the quantity of CO_2 evolved the following equation is used:-

$$x\ CO_2 = \left(h - \frac{x\ O_2}{k\ O_2} \right) k\ CO_2$$

xO_2 is obtained from the first respirometer, kO_2 and kCO_2 are the constants of the second respirometer and are derived as described by Dixon (1952) and Umbreit et al.(1949), h is the manometric reading.

The respiratory quotient is obtained thus:-

$$R.Q. = \frac{CO_2 \text{ produced}}{O_2 \text{ consumed}}$$

6.2.3 Procedure

Protozoa were grown in sterile distilled water with varying concentrations of bacteria for 48 hours prior to respirometry experiments at a temperature which corresponds to the experimental temperature.

The Colpidium were centrifuged for 10 minutes at 1,000r.p.m., the supernatant fluid was discarded and the sediment re-suspended

in sterile distilled water and recentrifuged at the same speed for a further 10 minutes. The supernatant fluid was again discarded and the sediment re-suspended in sterile distilled water. This procedure was carried out in order to remove as many of the bacteria as possible from the Protozoa.

The central well of each flask was filled with a roll of filter paper (Whatmans starch free No.40) which extended at least 5mm above the lip of the well (Dixon 1952). 0.4ml of 2N NaOH was pipetted into the centre well of each flask and 4ml of Colpidium suspension was pipetted into the main part of each flask. The number of Colpidia per ml and their mean cell volume were determined using a Coulter Counter as described in section 2.2.1.

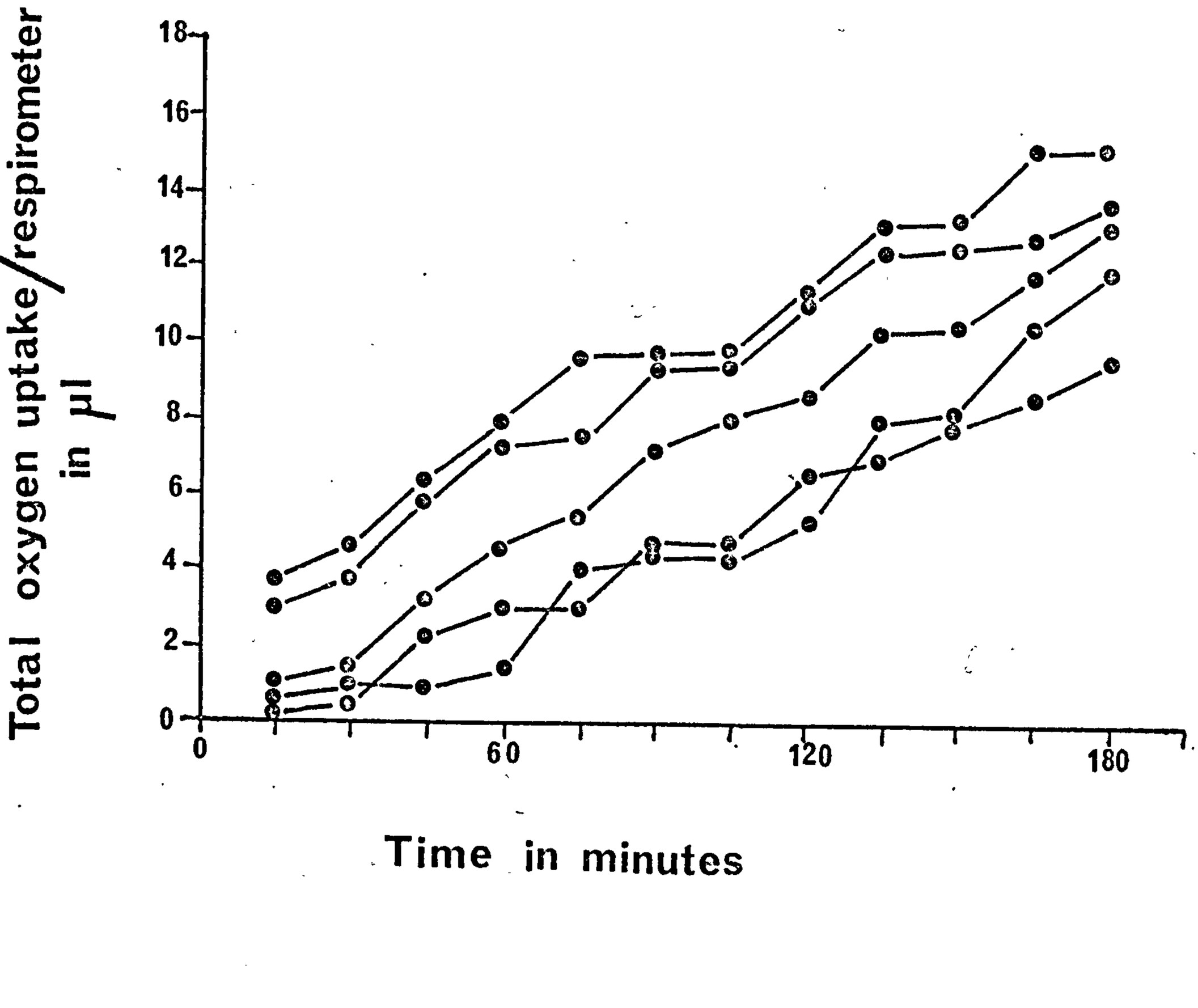
The number of animals in each flask was varied throughout the series of experiments since it has been demonstrated by Pace and Kimura (1944) and Pace and Lyman (1947) that oxygen uptake per individual increases with decreasing density. Oxygen uptake by animals of different sizes was also considered.

The respirometers were placed in a constant temperature water-bath and allowed to equilibrate for 30 minutes with the flasks open to the atmosphere. The apparatus carrying the respirometers was shaken at 125 cycles per minute with a horizontal movement of 5cm.

The shaking speed is critical, as the rate of shaking must be sufficient to allow the rate of oxygen uptake to be independent of the rate of oxygen diffusion into the medium

Figure 22

Typical pattern of oxygen uptake in Colpidium


Total oxygen uptake/respirometer in µl
0
2
4
6
8
10
12
14
16
18
0
60
120
180
Time in minutes

containing the animals. At low shaking speeds oxygen uptake is dependent on the rate of oxygen diffusion into the medium (Dixon and Elliot 1930). A speed of 120 cycles/minute has been shown by Hall (1938) to render oxygen consumption independent of the diffusion equilibrium between the gas space and the fluid bathing the cells.

After the 30 minute equilibration period the taps were closed and the readings were commenced. Readings were taken at 15 minute intervals over a 3 hour period.

The pH of the cell suspension was between 5.5-6.5. The optimum pH for respiration in Colpidium campylum lies around pH 5.5 (Hall 1941).

6.3 Results

6.3.1 Oxygen consumption

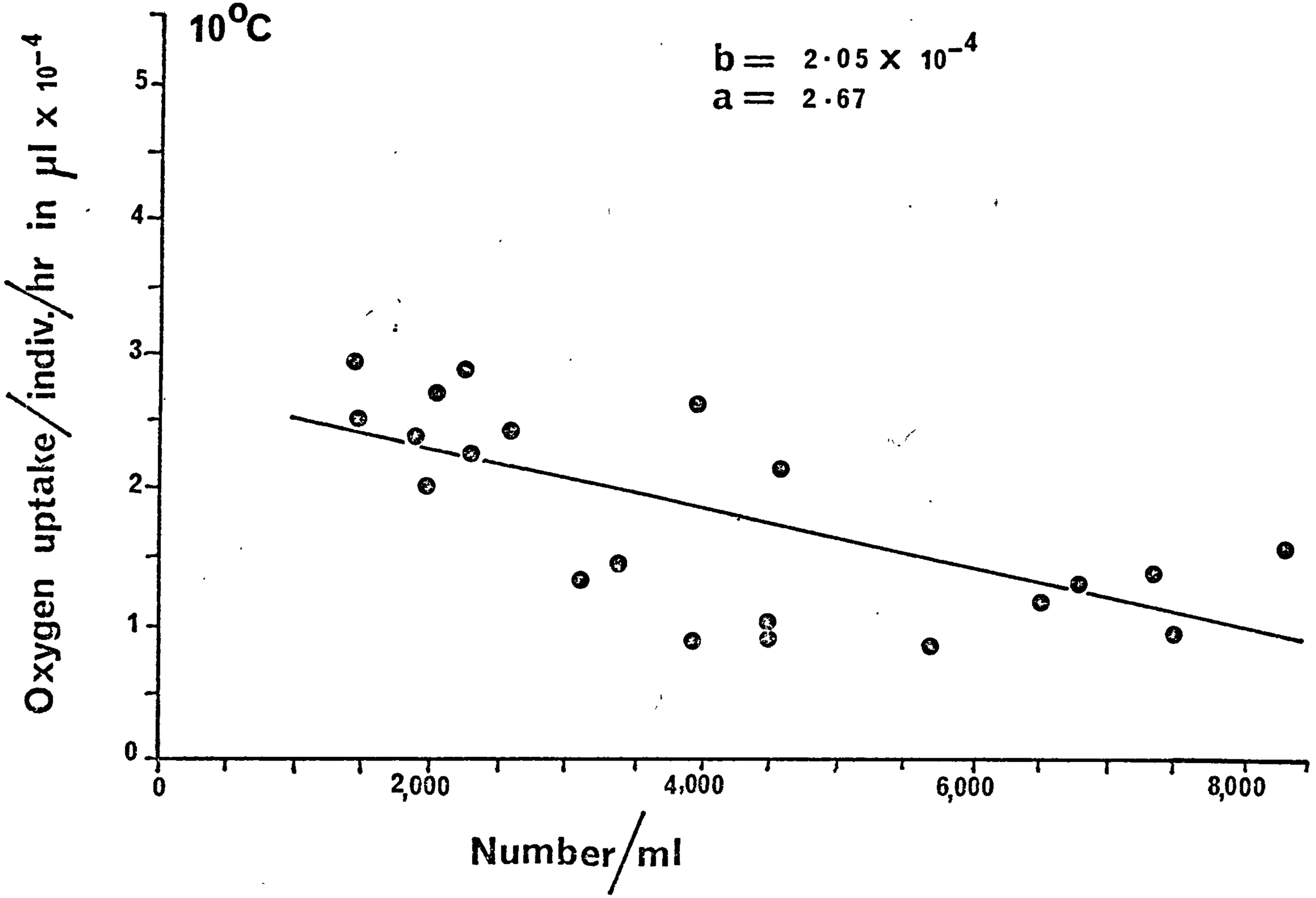
The typical pattern of the rate of oxygen uptake in Colpidium is shown in Figure 22. Because some variability in the rate was encountered, the mean oxygen uptake/hour for each experiment was calculated and used in all further analysis.

A clear relationship between the density of Colpidia and their individual oxygen consumption emerged. This is shown in Figure 23-25. Each point represents the mean of the results obtained from 7 replicate experiments. Linear regressions were performed and the correlation coefficients (r) calculated; all were significant to a level of 0.001. Increased density causes a significant decrease in the oxygen uptake of the individual cells.

Figure 23

Oxygen uptake per individual Colpidium as influenced by the population density at $10^{o}C$

$r = 0.700$ ($P = <0.001$)



$b = 2 \cdot 05 \times 10^{-4}$

$a = 2 \cdot 67$

Figure 24

Oxygen uptake per individual Colpidium as influenced by the population density at 15°C

$r = 0.797$ ($P = <0.001$)

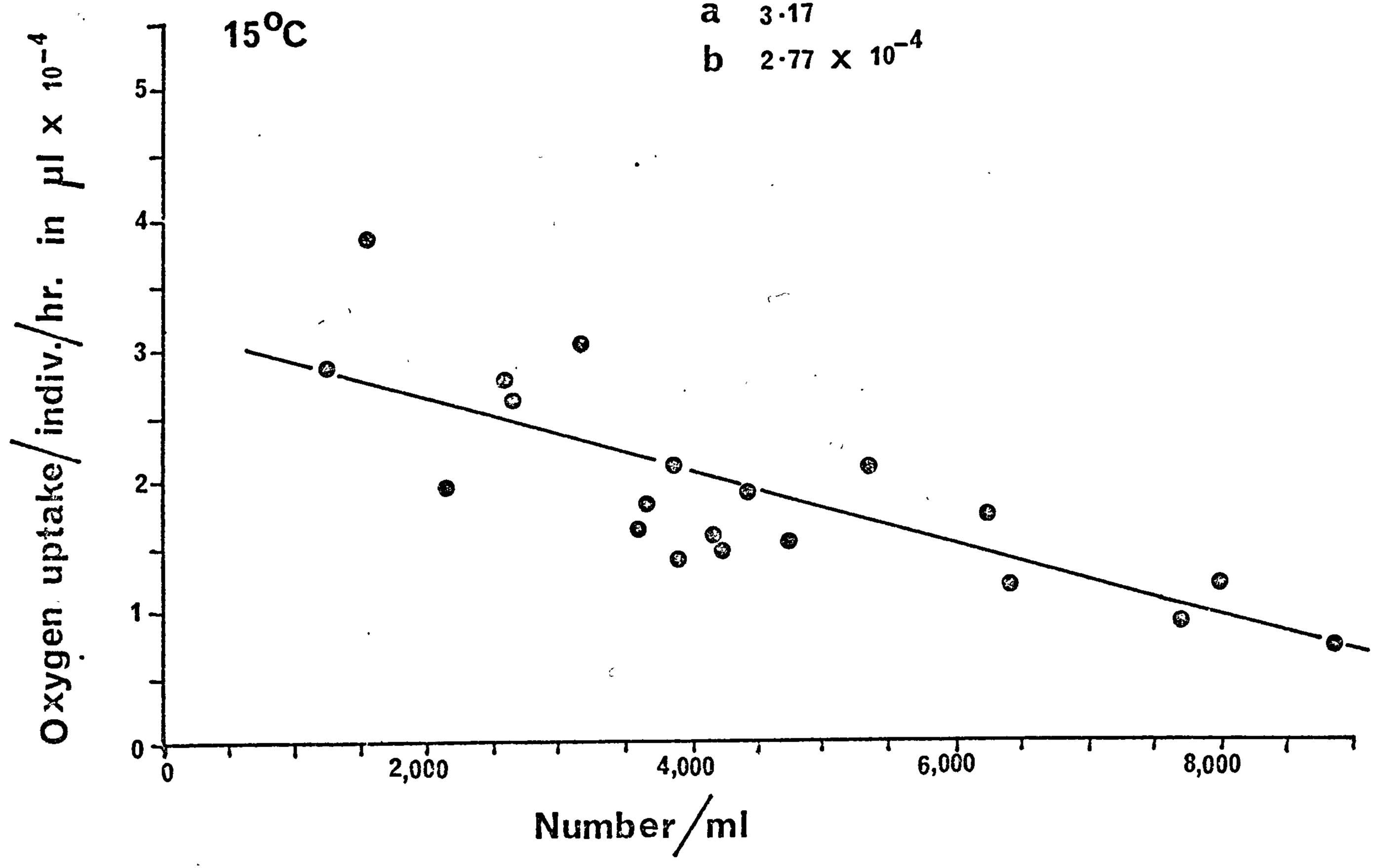
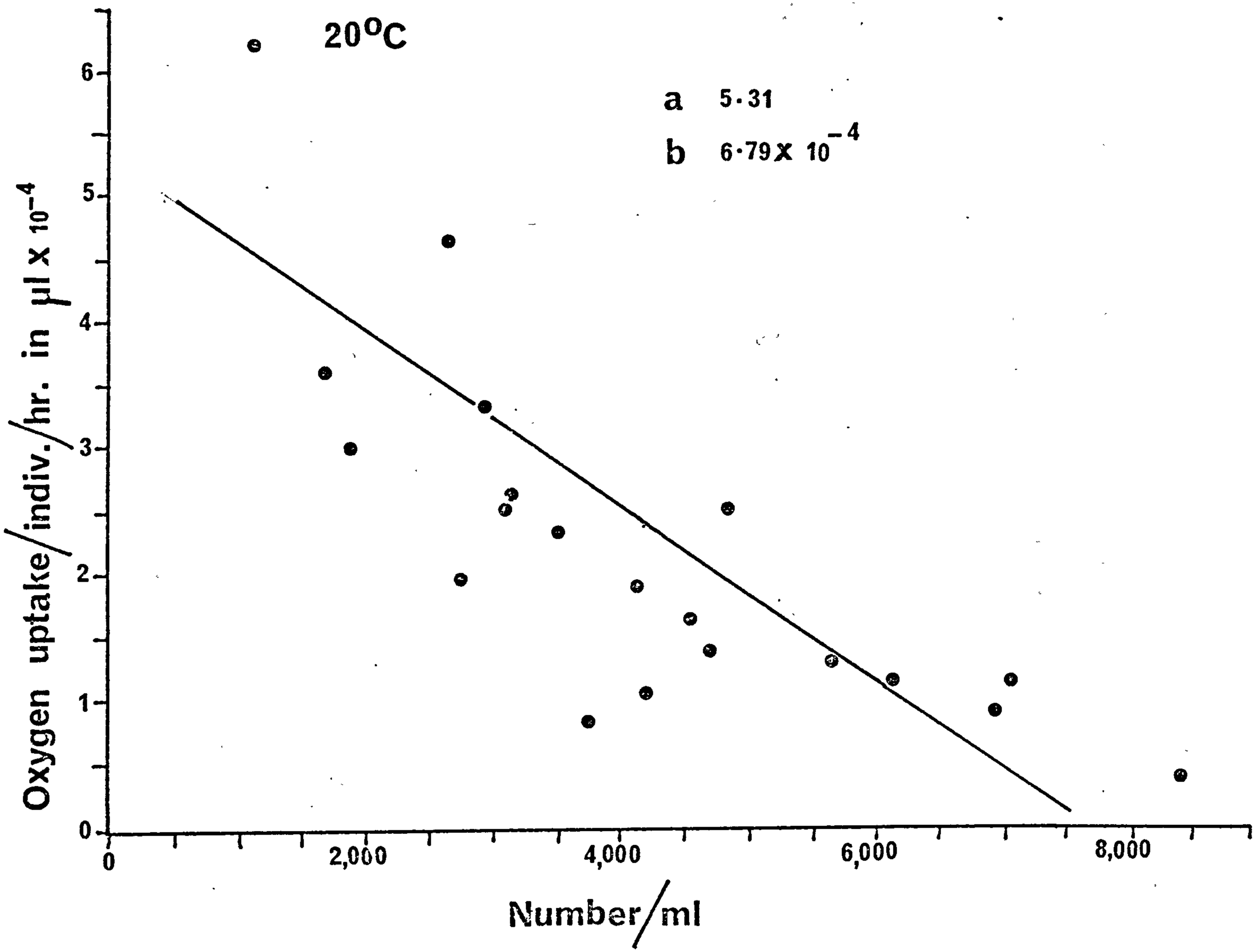

15°C
a 3·17
b 2·77 x 10⁻⁴
Oxygen uptake/indiv./hr. in µl x 10⁻⁴
0
1
2
3
4
5
0
2,000
4,000
6,000
8,000
Number/ml

Figure 25

Oxygen uptake per individual Colpidium as influenced by the population density at 20°C

r = 0.759 (P = <0.001)


20°C
a 5·31
b 6·79 X 10⁻⁴
Oxygen uptake/indiv./hr. in µl X 10⁻⁴
0
1
2
3
4
5
6
0
2,000
4,000
6,000
8,000
Number/ml

A relationship between the size of Colpidium and their oxygen uptake was also apparent as Figures 26-28 indicate. Again linear regressions and correlation coefficients were calculated. At 10°C and 15°C (Figures 25 and 26) the values for r were highly significant (P=<0.01). At 20°C (Figure 27), however, the scatter was considerable and the correlation coefficient was not significant. Increased cell mass results in the increased uptake of oxygen. In order to obtain animals of different mean cell volumes, it was necessary to grow Colpidium in different concentrations of food. Some of the levels used were of necessity much lower than those necessary to achieve maximum growth. Thus the ciliates were of varied nutritional status.

If the rate of oxygen uptake per μm^3 of cell substance is plotted against cell volume, as in Figures 29-31, the rate of oxygen uptake per unit volume of cell substance can be seen to decrease with increasing cell volume. The relationship is a product of the surface to volume ratio of the cells. Thus a large Colpidium consuming more oxygen than a smaller cell, consumes less oxygen per unit volume of protoplasm.

The data from which the figures were compiled is tabulated in the appendix.

The effect of temperature upon the respiration rate of Colpidium showed that a decrease in temperature produced a decrease in the volume of oxygen consumed, see Table 7.

Figure 26

Oxygen uptake per individual Colpidium as influenced by cell size at 10°C

$r = 0.509$ ($P = 0.01$)

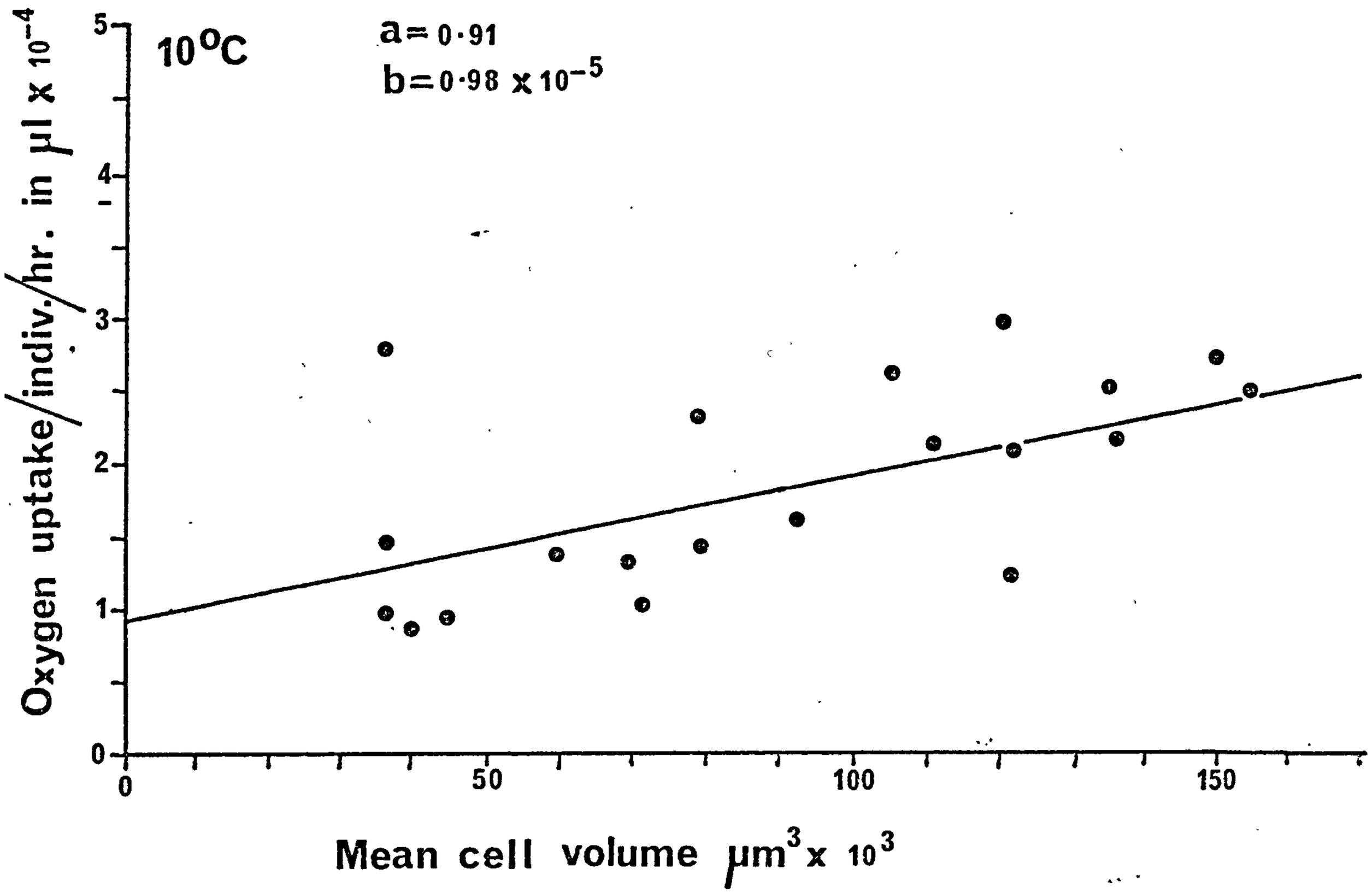

10°C
a= 0·91
b= 0·98 x 10⁻⁵
Oxygen uptake/indiv./hr. in µl x 10⁻⁴
0
1
2
3
4
5
0
50
100
150
Mean cell volume µm³ x 10³

Figure 27

Oxygen uptake per individual Colpidium as influenced by cell size at 15°C

r = 0.804 (P = <0.001)

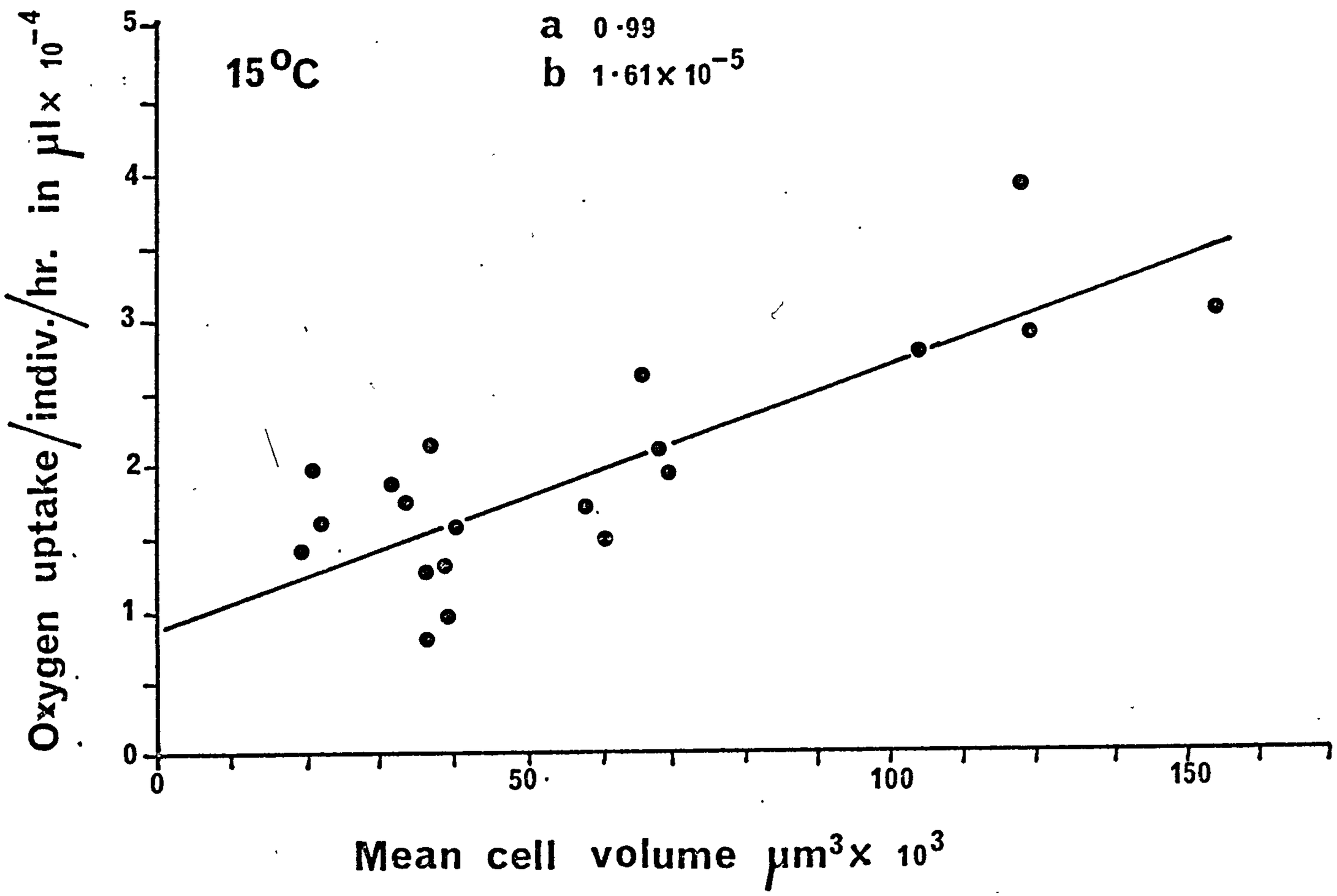

15°C
a 0·99
b 1·61 x 10⁻⁵
Oxygen uptake/indiv./hr. in µl x 10⁻⁴
0
1
2
3
4
5
0
50
100
150
Mean cell volume µm³ x 10³

Figure 28

Oxygen uptake per individual Colpidium as influenced by cell size at 20°C

$r = 0.325$ ($P = <0.1$)

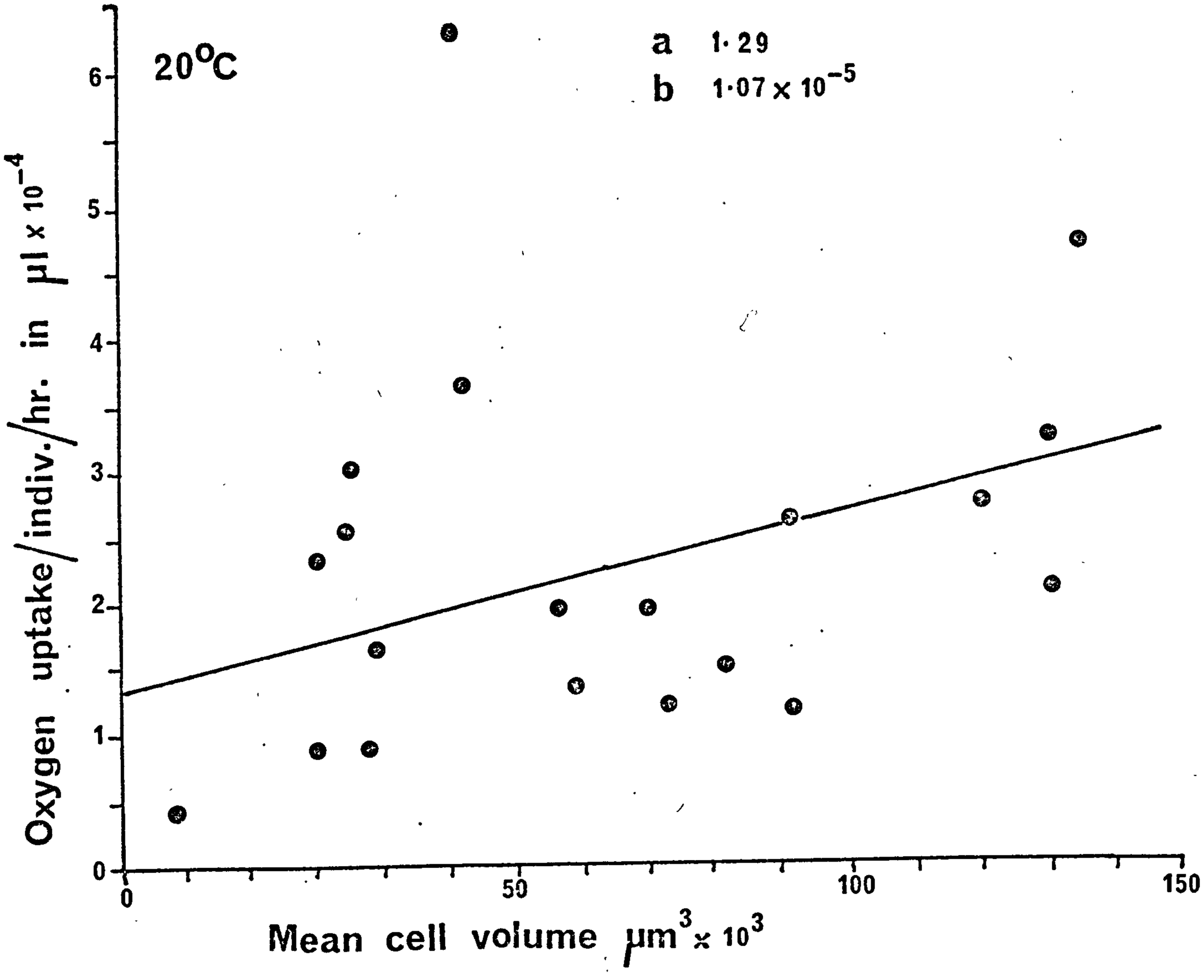

20°C
a 1·29
b 1·07 x 10⁻⁵
Oxygen uptake/indiv./hr. in µl x 10⁻⁴
0
1
2
3
4
5
6
0
50
100
150
Mean cell volume µm³ x 10³

Figure 29

Oxygen uptake per μm^3 of protoplasm per hour in relation to mean cell volume at $10^{o}C$

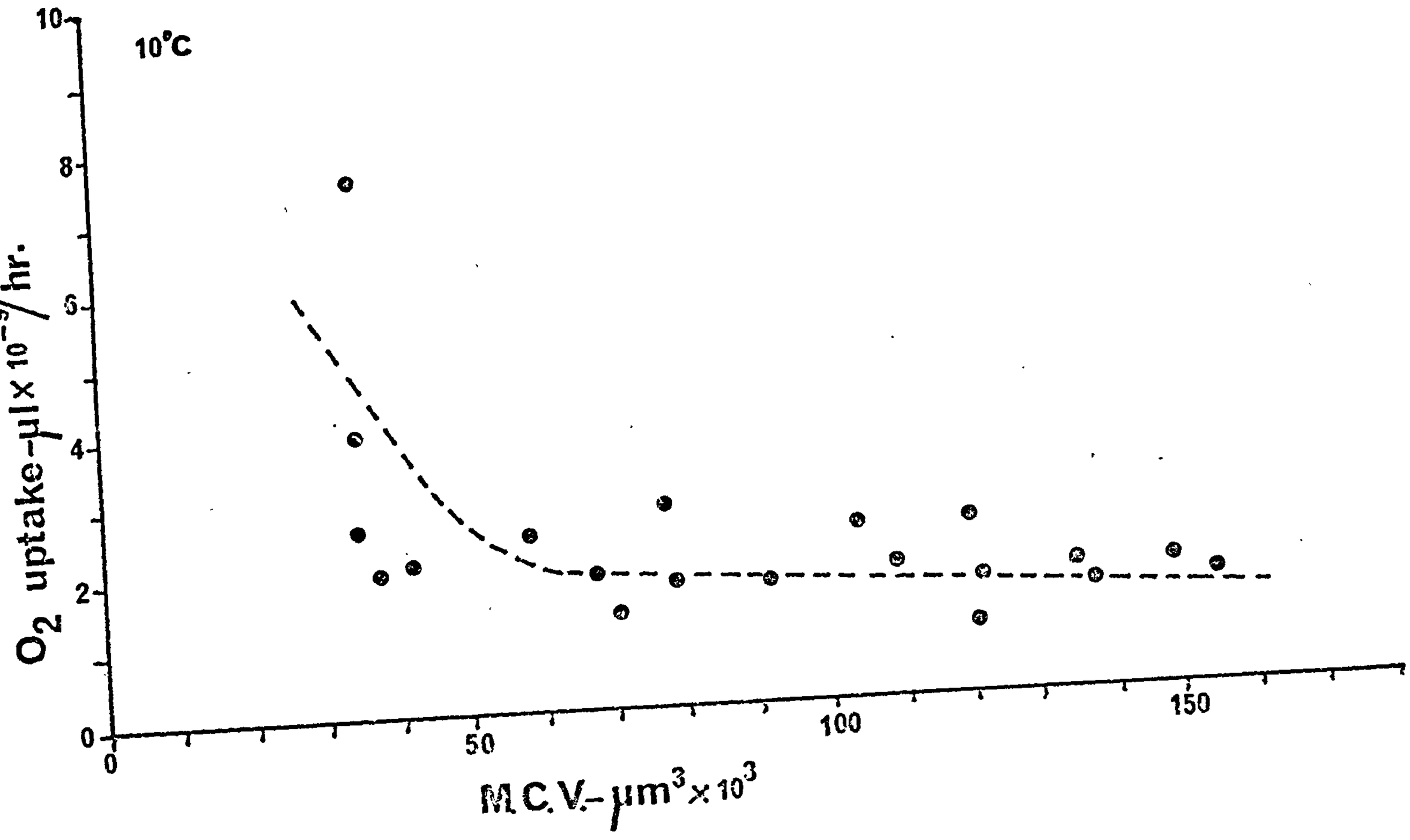

10°C
O2 uptake-µl x 10-9/hr.
M.C.V.-µm3 x 10³
0
2
4
6
8
10
50
100
150

Figure 30

Oxygen uptake per μm^3 of protoplasm per hour in relation to mean cell volume at $15^{o}C$

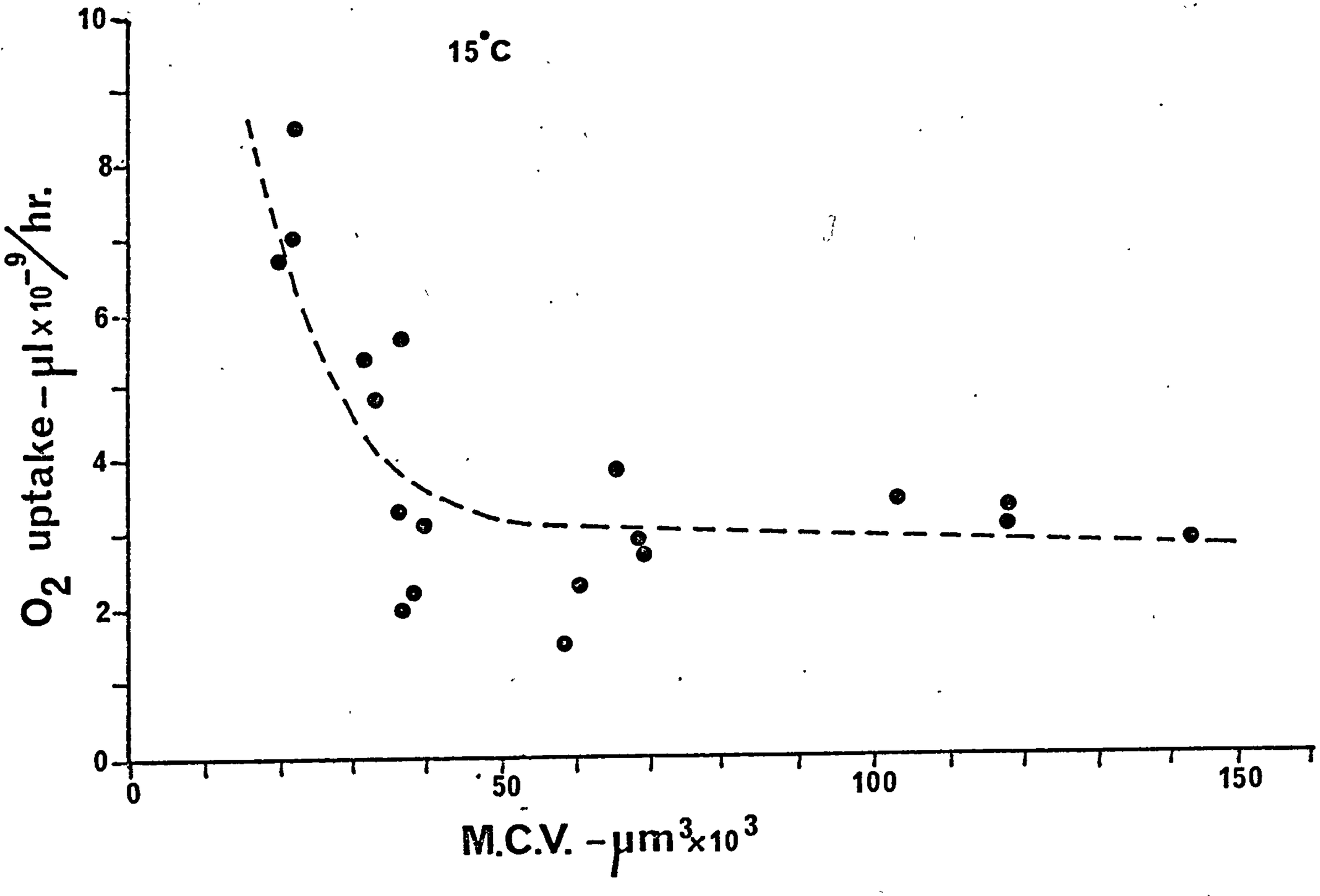

15°C
10
8
6
4
2
0
0
50
100
150
O_2 uptake – $\mu l \times 10^{-9}$/hr.
M.C.V. – $\mu m^3 \times 10^3$

Figure 31

Oxygen uptake per μm^3 of protoplasm per hour in relation to mean cell volume at $20^{\circ}C$

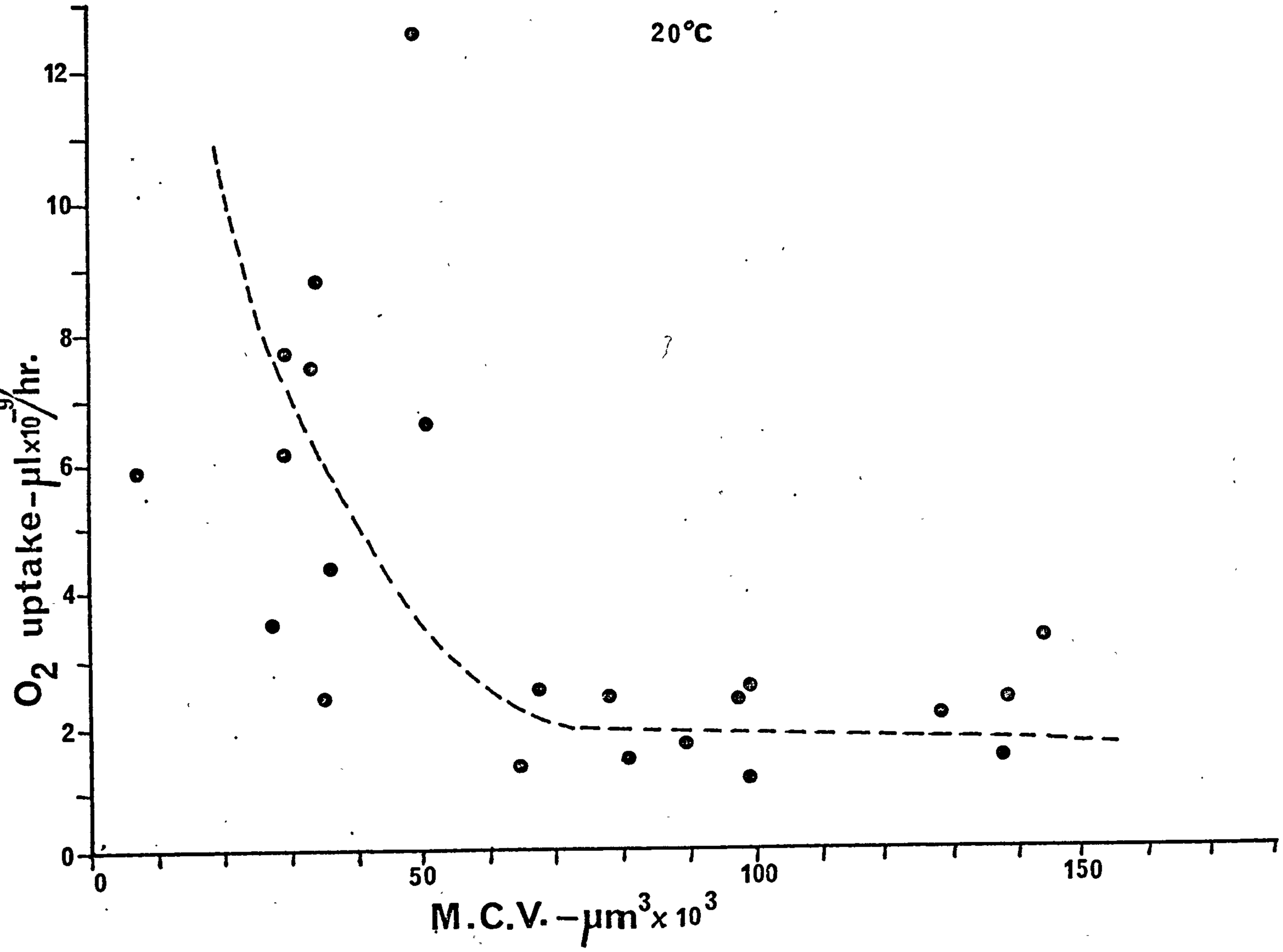

20°C
12
10
8
6
4
2
0
O_2 uptake-$\mu l \times 10^{-9}$/hr.
0
50
100
150
M.C.V. $-\mu m^3 \times 10^3$

TABLE 7

Temp.	mean O_2/indiv/hr	S.D.	$O_2/\mu m^3/hr$	S.D.
20°C	2.4×10^{-4}	1.41	4.01×10^{-9}	3.01
15°C	1.93 "	0.77	3.94 "	1.89
10°C	1.79 "	0.69	2.21 "	1.49

6.3.2 Respiratory quotients

Twenty determinations of R.Q. were carried out at 10°C, 15°C and 20°C. All the animals used were grown at food concentrations above those which have been shown to be necessary for maximum growth (see Chapter 2, section 2.2.2). The length of the experiment in each case was 4 hours plus 30 minutes prior equilibration time. In all cases the number of animals per ml was around 5,000. The results are tabulated in Table 8.

The R.Qs lie between 0.7 and 0.8 with a slight increase in R.Q. with decreasing temperature. The R.Q. for the oxidation of fat is 0.71 and for protein 0.80, while that for carbohydrate is 1.0 (Petrusewicz and Macfadyen 1970). Thus the R.Qs obtained for Colpidium are intermediate between those characteristic of the oxidation of fats and proteins.

6.4 Discussion

Considerable variation is encountered in the oxygen uptake of a number of protozoan species as demonstrated by various authors (see Table 9). For the purposes of comparison with the present study and between the results of these authors, the values given in Table 9 have been converted to $\mu\ell \times 10^{-4}$ O_2

TABLE 8

RESPIRATORY QUOTIENTS IN COLPIDIUM CAMPYLUM AT 10°C, 15°C and 20°C

Temp. 10°C	15°C	20°C
0.908	0.962	0.762
0.907	0.871	0.941
1.030	0.343	0.840
0.745	0.983	0.753
0.750	0.921	0.617
0.688	0.690	0.497
0.989	0.413	0.612
0.709	0.892	0.724
0.860	0.931	0.502
0.876	0.700	0.820
0.949	0.913	0.959
1.037	0.921	0.849
0.755	0.481	0.908
0.688	0.499	0.704
0.704	0.631	0.696
0.377	0.654	0.453
0.834	0.400	0.840
0.562	0.868	0.298
0.606	0.752	0.814
0.880	0.841	0.840
0.7961	0.7333	0.7287
S.E. 0.0037	0.0148	0.0037

consumed/10^6 cells/hour, or $\mu\ell$ O_2 consumed/10^3 cells/hour. Even within one species there is a wide variability in the reported respiration rates, as Table 9 clearly shows.

Obviously there are a great many variables in studies of this kind, and these may in part account for the wide differences in respiration rate reported within one species. The type of respirometer used and the temperature control of the water-bath are important. The critical rate at which the respirometers are shaken has already been discussed (see section 6.2.3). Bacteria are known to have high metabolic rates, and in many cases, particularly where bacteria have been used as a protozoan food source, the precaution of removing the bateria from the Protozoa was not undertaken. The importance of this measure is emphasised if one considers the work of Cook and Haldane (1931) who demonstrated an oxygen up-take of 17 5$\mu\ell$ O_2/hour/mg bacterial nitrogen in B. coli communis in the absence of any oxidisable substrate. Hall (1938) using the figures of Cook and Haldane (1931) estimated an oxygen uptake of 40$\mu\ell$/hour/mg dried weight of bacterial suspension in the presence of an oxidisable substrate. By way of comparison he quotes a consumption of 35$\mu\ell$ O_2/hour/mg dried weight for the ciliate Glaucoma pyriformis (syn. Tetrahymena pyriformis). The density and cell size of the protozoan population being respired are also factors which may contribute to the degree of oxygen consumption by single cells.

TABLE 9

RESPIRATION RATES IN OTHER SPECIES AS DEMONSTRATED BY OTHER WORKERS

Authors	Species	O_2/indiv/hr x$10^{-4}\mu\ell$	Respirometer used	Temp. °C
Laybourn (this work)	Colpidium campylum	1.79-2.43	Warburg	10-20
Hall (1938)	"	1.12	"	19.8
Emerson (1929)	Blepharisima	30-70	"	20
Pringle and Stewart (1961)	Paramecium aurelia	12.27-55.06	Cartesian diver	26
Stewart (1966)	"	7.85	"	26
Pace and Kimura (1944)	P.caudatum	21.1-97.0	Barcroft-Warburg	15-35 15-35
Kalmus (1928)	"	5.2	Kalmus	21
Howland and Berstein(1931)	"	3.3-4.9	"	20
Sarojini and Nagabushanam (1966)	Spirostomum ambiguum	2.91	Warburg	25
Specht (1935)	"	25.0	"	25
Pace and Lyman (1947)	Tetrahymena geleii	1.9-3.7	Barcroft-Warburg	25
Sarojini and Nagabushanam (1967)	Chilophrya labiata	0.12-0.34	Warburg	24-25
"	Strobilidium gyrans	0.96-1.04	"	24-25
"	Blepharisima perisicum	0.28-0.32	"	24-25
"	Loxodes vorax	0.12-0.18	"	24-25
"	Dileptus granulosus	0.76-0.98	"	24-25
"	Coleps hirtus	1.15-1.52	"	24-25
"	P.aurelia	1.35-1.52	"	24-25
"	P.calkinsii	1.58-1.96	"	24-25
"	P.multinucleatum	1.29-1.69	"	24-25
"	P.caudatum	1.93-2.82	"	24-25
"	Stentor sp.	0.41-0.43	"	24-25
"	Euplotes aediculata	0.96-1.08	"	24-25

The rate of oxygen uptake by each protozoan in the present study at 20°C is twice that quoted by Hall (1938) for Colpidium campylum. However, when drawing the comparison one must bear in mind that his work involved animals grown axenically, while the Colpidium campylum used in this study were from a monoxenic culture.

The effect of the density of animals per unit volume of medium and the rate of oxygen uptake by each individual has been noted by a number of workers (Specht 1935, Pace and Kimura 1944, Pace and Lyman 1947). Pace and Kimura (1944) while studying the effect of temperature on the respiration of Paramecium aurelia and Paramecium caudatum, noted that the rate of oxygen uptake per individual is greater when fewer animals are used. The density of the population was considered in more detail by Pace and Lyman (1947) who showed that oxygen consumption per animals was inversely proportional to population density in Tetrahymena geleii. Specht (1935), however, showed that at above 1,000 Spirostomum ambiguum cells/ml the rate of oxygen uptake was constant, wherease below a density of 1,000 animals /ml the rate of uptake increased until a maximum was reached. It must be noted that Specht (1935) used the Warburg apparatus shaken at a speed of only 60 cycles per minute, a speed below that necessary to render oxygen consumption independent of the diffusion equilibrium between the gas phase and the medium.

The results obtained in this study would indicate a close relationship between the population density and oxygen uptake

per protozoan. The causative factors may include the build up of metabolic waste products, and decreased locomotory activity due to crowding.

The relationship between body size and respiration rate in Metazoa sp. is well documented (e.g. Itô 1964, Lawton 1971, Smith 1972). The larger the animal of a particular species the greater its oxygen uptake. Since such a pattern exists in the higher animals it is worthwhile considering if such a relationship is encountered in the Protozoa.

A decrease in oxygen consumption associated with decreased weight, and hence decreased size, has been demonstrated in the amoeba Chaos chaos during starvation experiments (Holter and Zeuthen 1948). The amoeba, which is a carnivorous species, usually died after one month of starvation. The problem is whether the decrease in oxygen uptake is entirely the result of decreased volume, or whether it is the result of a changed metabolism due to starvation. This consideration is relevant to the present study on Colpidium, where animals of varied nutritional status had to be used in order to obtain results for a wide range of cell size. Leichsenring (1925) reports decreased oxygen uptake related to starvation in Paramecium caudatum, but she attributed the effect entirely to deprivation of food and makes no mention of decreased cell size.

Several attempts to relate oxygen uptake to volume and surface area have been tried on an interspecific basis. Sarojini and Nagabhushanam (1967), while investigating the

respiration of 13 species of ciliates, showed no trends or correlations in the relationships of respiratory rates to volume, surface area or surface:volume ratios. They concluded that oxygen uptake is a product of locomotory habits and food getting habits rather than the size of the species. A comparison of oxygen uptake in Paramecium aurelia and Paramecium caudatum by Pace and Kimura (1944) lead them to conclude that the rate of oxygen uptake is not related to surface area but to volume.

One would expect the respiration rate of a protozoan to vary with the temperature of its environment. A decrease in oxygen uptake with decreased temperature has been demonstrated by a number of authors (Wachendorff 1912, Leichsenring 1925, Pace and Kimura 1944, Pace and Lyman 1947, Sarojini and Nagabhushanam 1966). An optimum temperature for respiration has been shown for Tetrahymena geleii (Pace and Lyman 1947). Oxygen uptake decreased below and above 25°C. A similar effect was observed in Spirostomum ambiguum, with oxygen uptake following the same pattern as in Tetrahymena geleii (Sarojini and Nagabhushanam 1966).

The magnitude of the change in respiration rate with reference to temperature has been quoted as four times greater at 17°C than at 7°C in Colpidium colpoda, a decrease of 75% with a drop of 10°C (Wachendorff 1912). Leichsenring (1925) demonstrated a decrease of 34% between 20° and 10°C, and a decrease of 30% between 20°C and 15°C in Paramecium caudatum. The results of Pace and Kimura (1944) for the same species

corresponded to a decrease of approximately 16% between 20°C and 15°C. In Tetrahymena geleii a decrease of 74% in oxygen uptake occurs between 20°C and 10°C (Pace and Lymen 1947). A decrease in the mean respiration rate/individual of 27% occurred between 20°C and 10°C in the present investigation on Colpidium campylum.

If one accepts that an optimum temperature exists for respiration in a given protozoan species, then obviously the decrease in the rate of oxygen consumption between any given temperatures will depend very largely on the optimum temperature for that species. Another point which is all too often ignored, is any possible effect produced by not using animals previously acclimatized to the experimental temperature. In most of the examples cited with reference to temperature and oxygen uptake, the protozoa were grown at one temperature only, yet their respiration rates were investigated at a series of temperatures. In many cases mention of the temperature used for growth was omitted.

The respiratory quotients of a number of ciliate species have been studied. In Paramecium aurelia R.Q. ranged from 0.65-0.99 and increased with temperature; a similar trend occurred in Paramecium caudatum, where R.Qs between 0.73-0.90 occurred (Pace and Kimura 1944). These authors suggested that Paramecium utilized more carbohydrate at higher temperatures. The situation in Colpidium differs, in that R.Q. showed a slight downward trend with increased temperature, although the mean

R.Q. values for Colpidium lie within the range reported for Paramecium. Other figures quoted in the literature are an R.Q. of near 1.0 for Blepharisma spp. at 20°C (Emerson 1929) and 0.820 for Spirostomum ambiguum at 25°C (Sarojini and Nagabhushanam 1966).

CHAPTER 7 ENERGY BUDGETS

7.1 Introduction

Despite an increase in energetics studies, both in the field and the laboratory, the Protozoa are a group which have been largely ignored. Studies which constitute partial energy budgets for protozoan species exist (Coleman 1964, Proper and Garver 1966, Curds and Cockburn 1968, 1971). All these studies dealt with feeding and growth and the results were not expressed in terms of energy. Heal (1967a) calculated a complete energy budget for Acanthamoeba sp. in terms of the percentage of consumption used for growth and respiration (assimilation) and the percentage of the ingested food which was egested.

In an energetics study on a micro-organism the energy budgets constructed are related to variables such as food concentration and temperature, since the former factor alters the quantity of food that a protozoan consumes (see section 2.2.3 Chapter 2) and both factors influence the way in which the ingested energy is utilized by the cell. The equation used in the construction of the energy budgets is as follows:-

$$C = P + R + E + U$$

$$A = P + R$$

$$E + U = C - A$$

where :- A = assimilation

C = energy consumed as food

E = egested energy

P = growth

R = heat lost during respiration

U = nitrogenous excretion

As was pointed out in the general introduction, egested and excreted energy are calculated and not measured directly.

Protozoan energy budgets will obviously differ from the energy budgets which have been constructed for higher invertebrates, with reference to time. Other energy budgets relate to a season, or the life-cycle of a species. The life of an individual protozoan may be only a matter of hours before the total cell mass divides to become two individuals, under normal asexual reproductive conditions. In addition, whereas in other invertebrates the adults produce offspring and then die so that the biomass of the dead animals has been lost from the population, in the Protozoa reproduction does not involve the production of a corpse, since the cell mass of the parent is passed on complete to the following generation.

Daily energy budgets are sometimes calculated for invertebrate species (Prus 1972, Klekowski 1970). Again the Protozoa provide a problem, in that when one is calculating such a budget one must bear in mind the fact that what is one individual at the beginning of 24 hours may be several individuals at the end of 24 hours.

With these considerations in mind two types of energy budget were constructed for Colpidium. Firstly a 24-hour energy budget, and secondly a life-cycle energy budget, were calculated from the experimental investigations which have preceded this

chapter. The value and implications of these two types of energy budget will be discussed.

7.2 Methods

7.2.1 General

Two types of energy budget were constructed for Colpidium using the energy budget equation previously outlined. The 24-hour energy budget corresponds to the instantaneous energy budget described by Prus (1968b) and the energy budget for the life-span of the ciliate corresponds to the cumulative energy budget described by Prus (1968b).

7.2.2 Calculation of 24-hour energy budget for an individual

This budget is based on the energy intake and expenditure of an individual present in the initial population in a 24-hour period. Consumption C and growth P were obtained from the experiments detailed in Chapter 2 section 2.2 and converted into joules by the use of the data obtained from the studies outlined in Chapter 4.

The heat lost during respiration R was calculated by taking the average oxygen uptake per μm^3 of protoplasm per hour at each temperature (see Chapter 6, Table 7) and multiplying this figure by the mean cell volume value of the animals in the consumption and growth experiments described in Chapter 2 section 2.2, thus :-

$$O_2 \text{ consumed/indiv./hr} = \left[O_2/\mu m^3/hr \times M.C.V\right] \times 24$$

The value of the oxygen consumed/individual/24 hours thus obtained was converted to units of energy by using the oxy-caloric coefficient of 4.85 calories/ml O_2, where the substrate of respiration is a mixture of organic substances (Winberg et al. 1971). This mean oxycaloric coefficient was used because although the mean R.Qs obtained in Chapter 6, Table 8 were between 0.7-0.8, the R.Qs. from which the mean values were derived showed considerable variation as Table 8 shows. In accordance with standard international practice calories were converted to joules (1 calorie = 4.184 joules). Throughout the energy budgets in this study the μJ or $Jx10^{-6}$ was used.

Excretion and egestion E+U were calculated, as has already been stated, and represents that part of the ingested energy which is not assimilated.

7.2.3 Calculation of an energy budget for a generation of Colpidium

This type of energy budget shows energy uptake and utilization during the life-span of an individual Colpidium. The length of time over which a generation extended was calculated from the results for reproduction stated in Chapter 5, section 5.3. By calculation consumption C and growth P were derived from the 24-hour energy budget as was respiration R. Egestion and excretion E+U were calculated as before.

7.3 Results

7.3.1 24-hour energy budget

The 24-hour energy budget is calculated on the energy intake and utilization of one of the individuals present in the initial population at the beginning of a 24 hour period. It does not take reproduction into consideration; thus the individual may divide into two individuals within the 24 hours, but the two daughter cells are still regarded as one individual in this energy budget. It is essentially an energy budget indicating the potential of one protozoan in a 24-hour period.

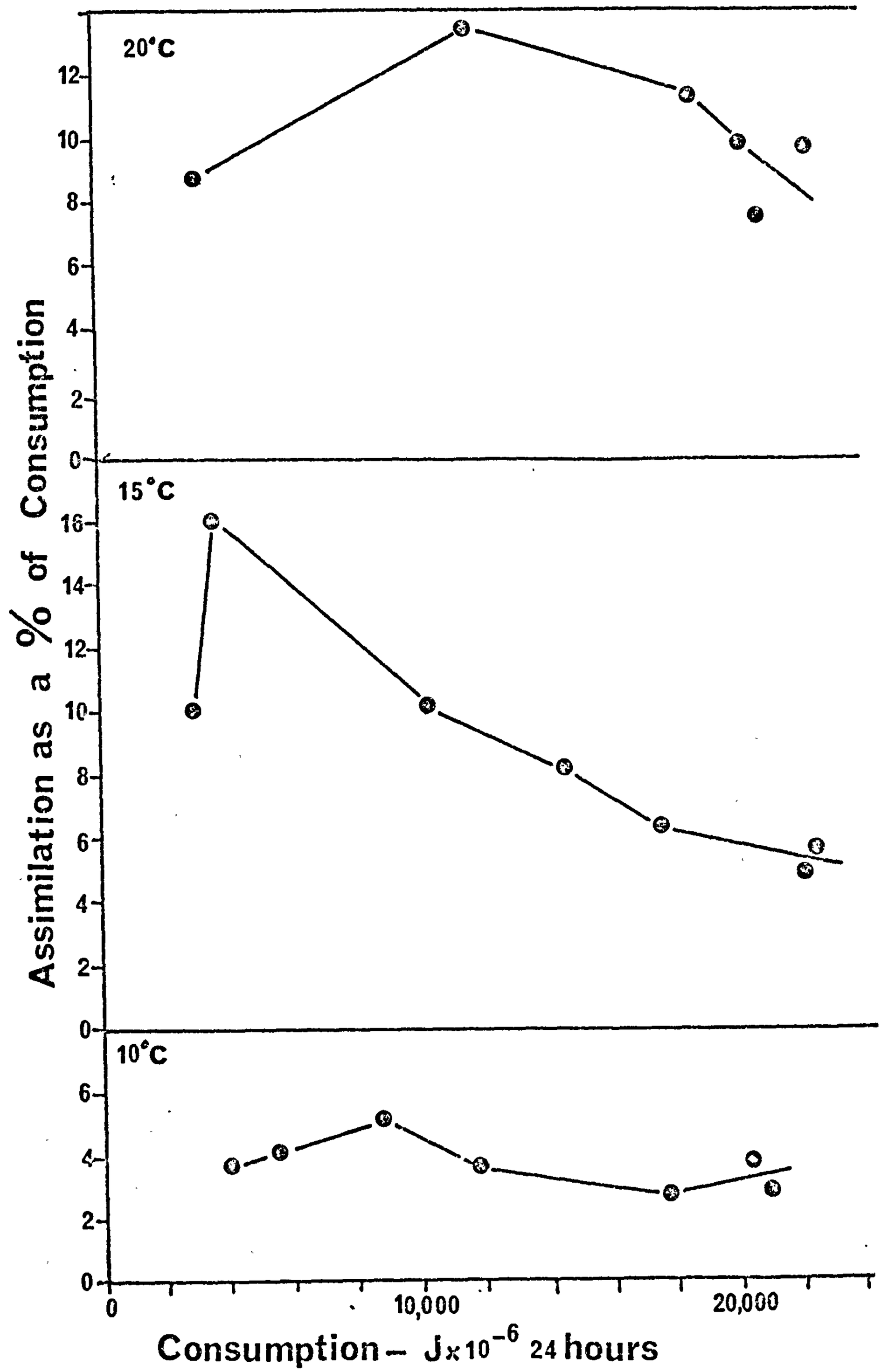
(i) Consumption C

This component of the energy budget has already been discussed at length in Chapter 2, section 2.2. The quantity of food ingested in terms of energy at different food concentrations is shown in Tables 10, 11 and 12. Briefly reiterating the points already discussed in Chapter 2, consumption showed only a slight variation with increased temperature, but increased in relation to increased food concentration until a maximum was attained at a food: protozoan ratio of 2.5×10^6:1.

The amount of energy needed to be consumed to maintain the cell without growth appears to lie between 4,000-5,000 $\times 10^{-6}$J/individual/24 hours at 10°C, and below 2,000-3,000 $\times 10^{-6}$J/individual/24 hours at 15°C and 20°C (see Chapter 4, Figures 16-18).

Figure 32

Assimilation as a percentage of consumption at different levels of energy consumption at 10°C, 15°C and 20°C.


20°C
12
10
8
6
4
2
0
15°C
16
14
12
10
8
6
4
2
0
10°C
6
4
2
0
0
10,000
20,000
Assimilation as a % of Consumption
Consumption – J x 10⁻⁶ 24 hours

(ii) Assimilation A=P+R

The heat lost during respiration R and growth P are shown as individual components and jointly as assimilation in Tables 10, 11 and 12.

Respiration showed an initial increase with increased consumption (consumption being essentially a product of the initial food concentration), levelling off as ingestion increased. This is due to variation in the cell mass over the range of consumption studied, see Chapter 3, section 3.2. At low levels of food intake mean cell volume is small, increasing to a maximum with increased consumption. Consequently the heat lost during respiration followed the same trend, since in Chapter 6, section 6.2.1 it was shown that oxygen consumption increased with increased cell mass.

Growth and growth efficiency have been discussed at length in Chapters 2 and 4 respectively. Growth in Colpidium has been shown to increase with increased consumption until a maximum was reached, and in addition, the level of growth achieved was shown to be related to temperature, increasing with higher temperature within the range studied.

Assimilation as a percentage of consumption or assimilation efficiency (U-1 as described in the general introduction) is illustrated in Figure 32 and tabulated in Tables 10, 11 and 12. As the Figure shows, assimilation is overall higher at higher temperatures, although one of the values obtained

TABLE 10 A 24 HOUR ENERGY BUDGET AT DIFFERENT FOOD CONCENTRATIONS 20°C

Approx Init.Food Index	Consmpn. $(C)_{-6}$ Jx10	Growth $(P)_{-6}$ Jx10	Respn $(R)_{-6}$ Jx10	Egestn. Excretn. $(E+U)_{-6}$ Jx10	Assimln. $(A)_{-6}$ Jx10	Growth as % of Cons.	Respn. as % of Cons.	Egestn. Excretn. as % of Consmpn.	Assimln. as % of Consmpn.	Respn. as % of Assimln.	Growth as % of Assimln.
$0.2x10^6$:1	3040	101	168	2771	269	3.3	5.5	91.2	8.8	62.5	37.5
$1.0x10^6$:1	11618	1243	347	10028	1590	10.6	2.9	86.5	13.5	16.3	83.7
$1.5x10^6$:1	18839	1801	356	16682	2157	9.5	1.8	88.7	11.3	16.5	83.5
$2.0x10^6$:1	20904	1272	344	19288	1616	6.0	1.6	92.4	7.6	21.3	78.7
$3.0x10^6$:1	22234	1823	347	20615	1619	8.2	1.5	90.3	9.7	21.4	78.6
$4.0x10^6$:1	20365	1581	338	18446	1919	7.7	1.7	90.6	9.4	17.6	82.4

each result represents the mean of 10 replicate experiments

TABLE 11 A 24 HOUR ENERGY BUDGET AT DIFFERENT FOOD CONCENTRATIONS 15°C

Approx InitFood Index	Consmpn. $(C)_{-6}$ Jx10	Growth $(P)_{-6}$ Jx10	Respn. $(R)_{-6}$ Jx10	Egestn. Excretn. $(E+U)_{-6}$ Jx10	Assimln. $(A)_{-6}$ Jx10	Growth as % of Cons.	Respn. as % of Cons.	Egestn. Excretn. as % of Consmpn.	Assimln. as % of Consmpn.	Respn. as % of Assimln.	Growth as % of Assimln
$0.25x10^6$:1	3062	167	157	2736	324	5.4	5.1	89.5	10.5	48.5	51.5
$0.4x10^6$:1	3525	319	246	2960	565	9.0	6.9	84.1	15.9	43.5	56.5
$1.0x10^6$:1	10217	708	332	9087	1130	6.9	3.2	89.9	10.1	29.4	70.6
$1.5x10^6$:1	14683	863	336	13484	1199	5.8	2.3	91.9	8.1	28.0	72.0
$2.5x10^6$:1	17684	782	320	16582	1102	4.4	1.8	93.8	6.2	29.0	71.0
$3.0x10^6$:1	22435	874	361	21200	1235	3.9	1.6	94.6	5.5	29.2	70.8
$3.5x10^6$:1	22236	741	363	21132	1104	3.3	1.6	95.1	4.9	32.1	67.9

each result represents the mean of ten replicate experiments

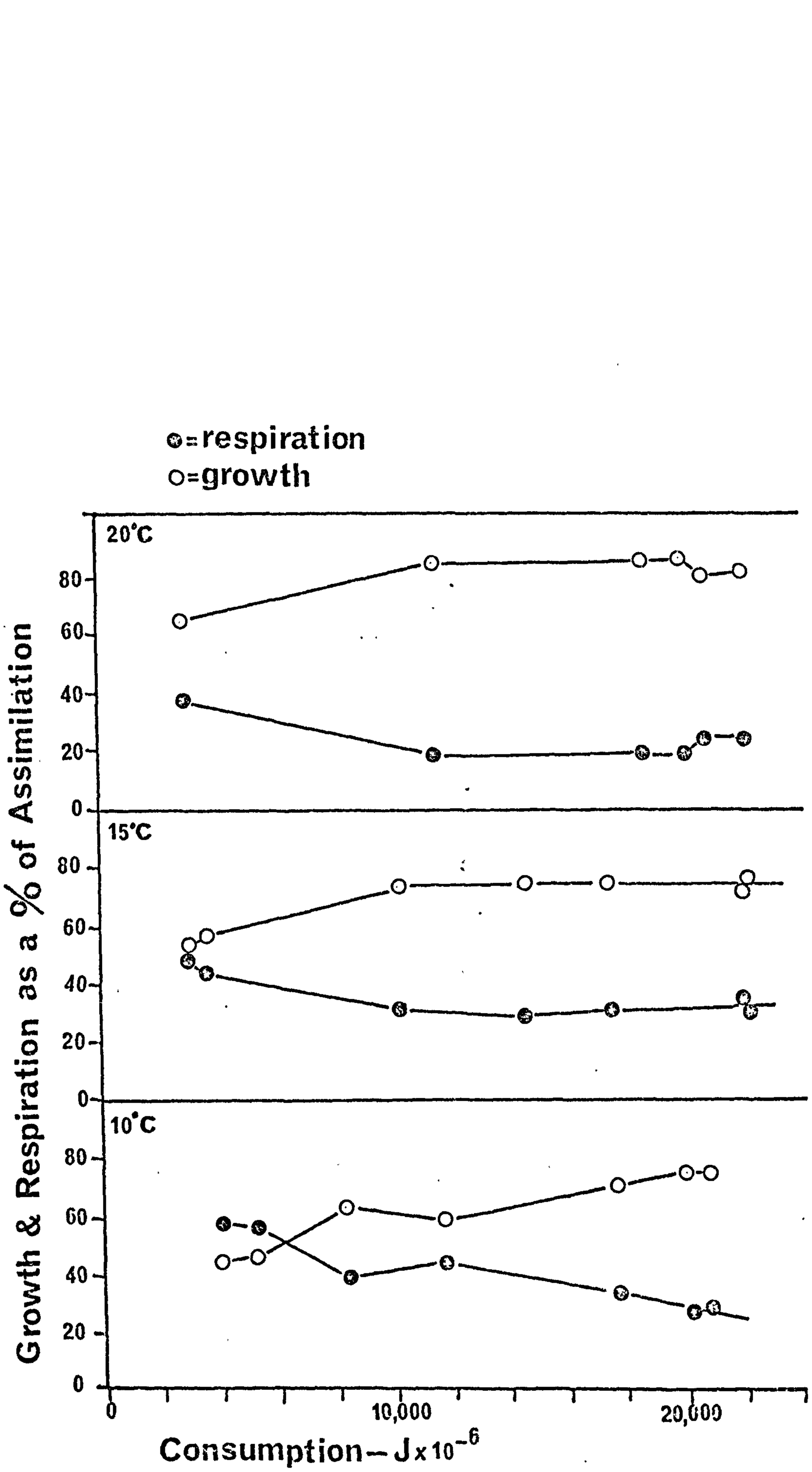
TABLE 12 A 24 HOUR ENERGY BUDGET AT DIFFERENT FOOD CONCENTRATIONS 10°C

Approx Init.Food Index	Consmpn. (C) $Jx10^{-6}$	Growth (P) $Jx10^{-6}$	Respn. (R) $Jx10^{-6}$	Egestn. Excretn. (E+U) $Jx10^{-6}$	Assimln. (A) $Jx10^{-6}$	Growth as % of Cons.	Respn. as % of Cons.	Egestn. Excretn. as % of Consmpn.	Assimln. as % of Consmpn.	Respn. as % of Assimln.	Growth as % of Assimln.
$0.5x10^6$:1	4021	72	92	3857	164	1.7	2.2	96.1	3.9	56.1	32.9
$0.9x10^6$:1	5261	95	118	5048	213	1.8	2.2	96.0	4.0	55.4	44.6
$1.5x10^6$:1	8407	262	176	7968	444	3.1	2.0	94.9	5.1	39.6	60.4
$2.0x10^6$:1	11969	253	184	11532	487	2.1	1.5	96.4	3.6	42.1	57.9
$2.5x10^6$:1	20961	464	164	20333	628	2.2	0.7	97.1	2.9	26.1	73.9
$3.0x10^6$:1	17894	342	154	17398	496	1.9	0.9	97.2	2.8	31.0	69.0
$4.0x10^6$:1	20350	487	183	19680	670	2.9	0.9	96.2	3.8	27.3	72.7

each result represents the mean of ten replicate experiments

Figure 33

Net growth efficiency and the heat lost during respiration at different levels of energy consumption at 10°C, 15°C and 20°C


●=respiration
○=growth
20°C
15°C
10°C
80
60
40
20
0
Growth & Respiration as a % of Assimilation
0
10,000
20,000
Consumption – J x 10⁻⁶

at 15°C, that of 15.9% at a low consumption level, was higher than any of the values found at 20°C. As both the Tables and Figure 32 show assimilation efficiency varied with food consumption as did growth efficiency (see Chapter 4, section 4.3). In the case of both assimilation efficiency and growth efficiency the pattern over the range of food intake obtained in this study was similar. At 20°C and 15°C assimilation efficiency showed a marked maximum at lower food intake decreasing as food intake increased. At 10°C the maximum was less well marked.

If one considers the manner in which the assimilated energy is used at each temperature, a clear pattern emerges as shown in Figure 33. The proportion of the assimilated energy used for growth (K_2 net growth efficiency - see general introduction) increases with increased temperature, yet the proportion of the energy lost as heat during respiration decreases. In other words as temperature decreases, more of the assimilated energy is respired and less is incorporated as growth.

(iii) Egestion and Excretion E+U = C-(P+R)

Egestion and excretion are two components of a protozoan energy budget which are calculated from consumption and assimilation as outlined in section 7.1. Obviously, since assimilation increased with temperature and consumption remained fairly constant over the temperature range considered, it follows that egestion and excretion decreased

TABLE 13

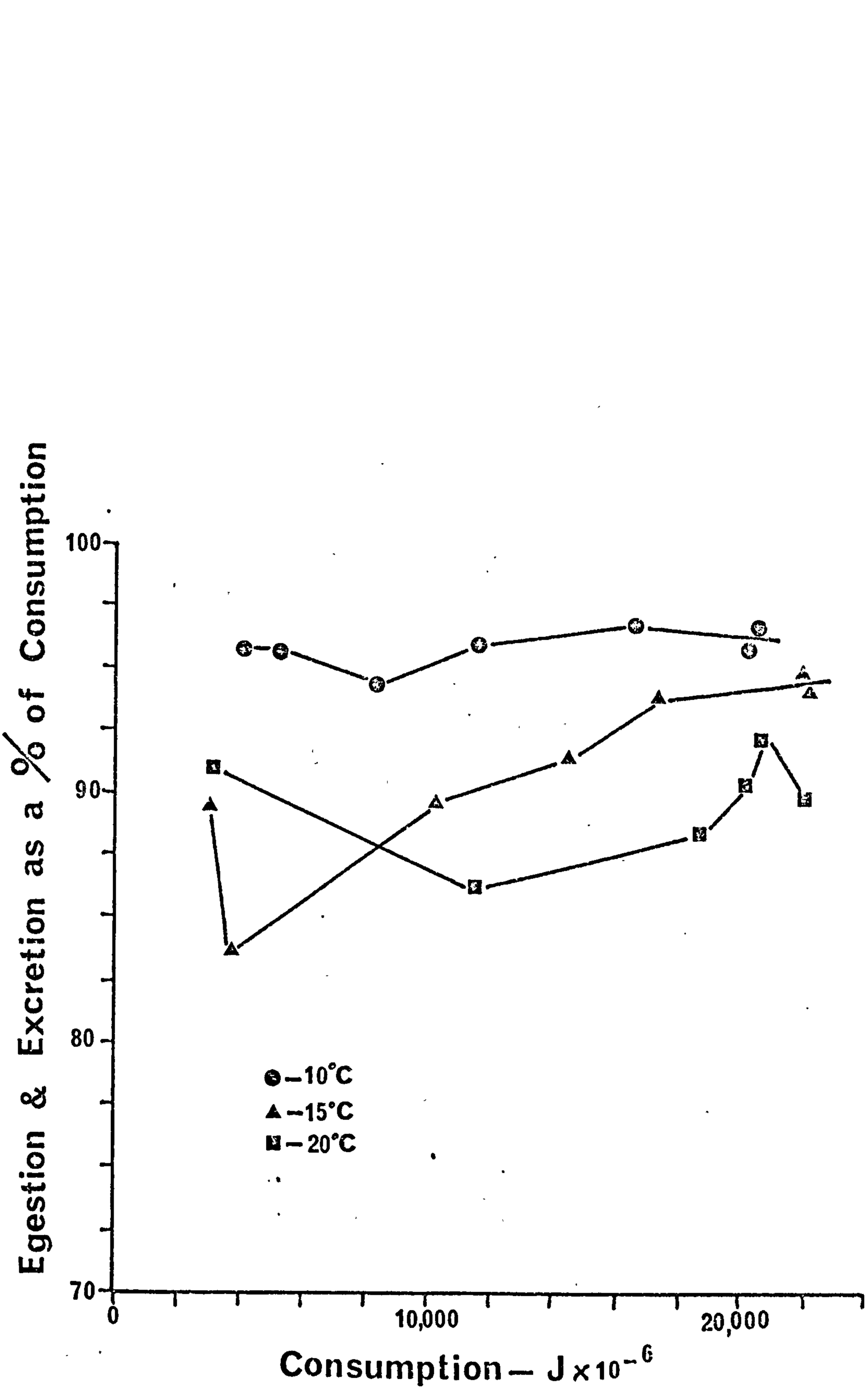
MEAN MAXIMUM ENERGY BUDGET FOR AN INDIVIDUAL IN 24 HOURS AT 10°C, 15°C and 20°C

Temp. °C	Consmpn. (C) $Jx10^{-6}$	Growth (P) $Jx10^{-6}$	Respn. (R) $Jx10^{-6}$	Egestn. Excretn. (F+U) $Jx10^{-6}$	Assimln. (A) $Jx10^{-6}$	Growth Efficy. %	Assimln. Efficy. %	Net Growth Efficy. %
20	21167	1558	343	19266	1901	7.4	8.9	81.9
15	20785	765	348	19672	1113	3.7	5.4	69.9
10	19735	431	167	19137	598	2.1	3.0	72.1

Each of the figures quoted is based on the mean of 30 replicate experiments

Figure 34

Egestion and excretion as a percentage of consumption at different levels of energy consumption at 10°C, 15°C and 20°C


Egestion & Excretion as a % of Consumption
100
90
80
70
0
10,000
20,000
10°C
15°C
20°C
Consumption — J x 10⁻⁶

with higher temperature. This point is illustrated in Figure 34 and tabulated in Tables 10, 11 and 12.

(iv) Complete energy budget

Thus the various components of the energy budget for Colpidium show variation in relation to the quantity of food consumed. A mean energy budget for each temperature can be compiled from data obtained for a food ratio of above 2.5×10^6:1 beyond which consumption became independent of food concentration. In such energy budgets each component is at its maximum level, see Table 13. It must be noted that although consumption, growth, respiration and egestion are at their maxima in this mean energy budget, the animals is not operating at its most efficient, because as has already been pointed out in Chapter 4, section 4.3, and under the heading of assimilation in the present section, the highest growth and assimilation efficiencies occur at food concentrations below the level necessary for maximum consumption but at the point where growth reached its maximum.

7.3.2 The generation energy budget

The generation energy budget expresses the consumption and utilization of energy during the life-span of an individual Colpidium, from the point where it becomes an independent cell, by binary fission from a parent cell, to the point where it undergoes binary fission to produce two daughter cells.

The energy budgets for a generation span of Colpidium as

TABLE 14

ENERGY BUDGET FOR ONE GENERATION OF COLPIDIUM AT DIFFERENT FOOD CONCENTRATIONS 20°C

Approx. Initial Food Ratio	Av.Gen. Time hours	Av. M.C.V. $\mu m^3 x10^3$	Consmpn. $(C)_{-6}$ $Jx10^{-6}$	Growth $(P)_{-6}$ $Jx10^{-6}$	Respn. $(R)_{-6}$ $Jx10^{-6}$	Egestn. Excretn. $Jx10^{-6}$	Assimln $(A)_{-6}$ $Jx10^{-6}$
0.2×10^6:1	27.0	86	4321	114	189	4018	303
1.0×10^6:1	19.6	177	9520	1022	283	8215	1305
1.5×10^6:1	12.8	182	10050	961	190	8899	1151
2.0×10^6:1	14.8	176	12600	785	212	11603	997
3.0×10^6:1	13.4	177	12410	1018	198	11194	1216
4.0×10^6:1	13.4	181	11370	883	188	10299	1071

each result is based on the mean of 10 replicate experiments

TABLE 15

ENERGY BUDGET FOR ONE GENERATION OF COLPIDIUM AT DIFFERENT FOOD CONCENTRATIONS $15^{o}C$

Approx. Initial Food Ratio	Av.Gen. Time hours	Av. M.C.V. $\mu m^3 x10^3$	Consmpn. (C) $Jx10^{-6}$	Growth (P) $Jx10^{-6}$	Respn. (R) $Jx10^{-6}$	Egestn. Excretn $Jx10^{-6}$	Assimln. (A) $Jx10^{-6}$
$0.25x\ 10^6:1$	30.7	82	3916	213	200	3503	413
$0.4\ x\ 10^6:1$	28.2	128	4142	374	289	3479	663
$1.0\ x\ 10^6:1$	26.4	164	11238	774	365	10099	1139
$1.5\ x\ 10^6:1$	22.0	175	13459	791	308	12360	1099
$2.5\ x\ 10^6:1$	22.0	168	16210	761	293	15156	1054
$3.0\ x\ 10^6:1$	24.4	188	22808	888	367	21553	1255
$3.5\ x\ 10^6:1$	26.9	186	24922	830	407	23685	1237

each result is based on the mean of 10 replicate experiments

TABLE 16

ENERGY BUDGET FOR ONE GENERATION OF COLPIDIUM AT DIFFERENT FOOD CONCENTRATIONS $10^{\circ}C$

Approx. Initial Food Ratio	Av.Gen. Time hours	Av. M.C.V. $\mu m^3 x10^3$	Consumpn. (C) $Jx10^{-6}$	Growth (P) $Jx10^{-6}$	Respn. (R) $Jx10^{-6}$	Egestn. Excretn. $Jx10^{-6}$	Assimln. (A) $Jx10^{-6}$
0.5×10^6:1	67.4	86	11292	202	263	10827	465
0.9×10^6:1	400.0	110	86051	1583	1960	82508	3543
1.5×10^6:1	38.2	149	11489	358	280	10851	638
2.0×10^6:1	63.1	173	31468	665	484	30319	1149
2.5×10^6:1	33.3	164	29083	643	227	28213	870
3.0×10^6:1	37.5	153	27959	534	240	27185	774
4.0×10^6:1	35.8	171	30355	726	273	29356	999

each result is based on the mean of 10 replicate experiments

related to the initial food concentration are shown in Tables 14, 15 and 16. The food concentration (and consequently consumption) has been shown to exert a marked effect on the length of the generation time (see Chapter 5, section 5.3). Below an ingestion of approximately 10,000 x 10^{-6}J/individual/24 hours the generation time increased. Above this level of consumption the generation time remained fairly constant.

With reference to the Tables 14, 15 and 16, it will be seen that in all cases, with the exception of one unusual result at 10^{o}C at a food concentration of 0.9 x 10^{6}:1, assimilation increased with a decrease in the length of the generation time. Associated with the increase in assimilation there was also an increase in the mean cell volume. Mean cell volume in Colpidium has been shown to be influenced by consumption (see Chapter 3, section 3.3); below an intake of 400 nanograms dried weight of bacteria/individual/24 hours (equivalent to 8145J x 10^{-6}) mean cell volume decreased; above this level of consumption mean cell volume maintained a plateau.

Thus increasing assimilation associated with increased consumption, which is largely determined by the concentration of the available food, was related to a decrease in the generation time and an increase in the mean cell volume. This point is illustrated graphically in Figures 35-37. At 20^{o}C and 15^{o}C the relationship of assimilation, mean cell volume and length of the generation time as correlated with consumption is clearly apparent from Figures 35 and 36; at 10^{o}C, see Figure 37,

Figure 35

The relationship of assimilation, generation time and mean cell volume over a range of energy consumption at 20°C

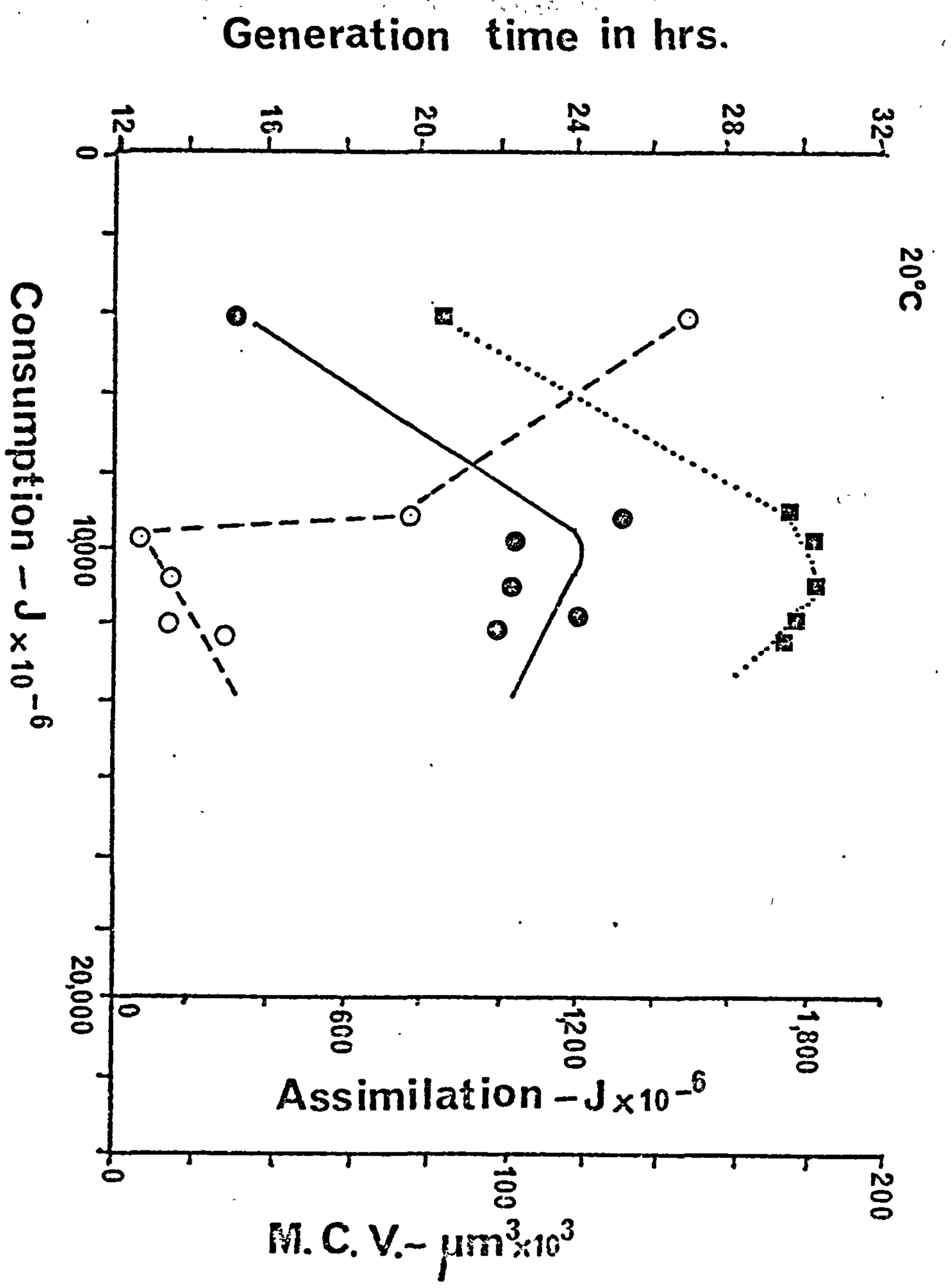

Generation time in hrs.
12
16
20
24
28
32
0
10000
20,000
Consumption – J ×10⁻⁶
20°C
■ M C V
● Assimilation
○ Generation time
0
600
1,200
1,800
Assimilation – J ×10⁻⁶
0
100
200
M. C. V.– μm³×10³

Figure 36

The relationship of assimilation, generation time and mean cell volume over a range of energy consumption at 15°C

Key:- mean cell volume ▪

assimilation ●

generation time o

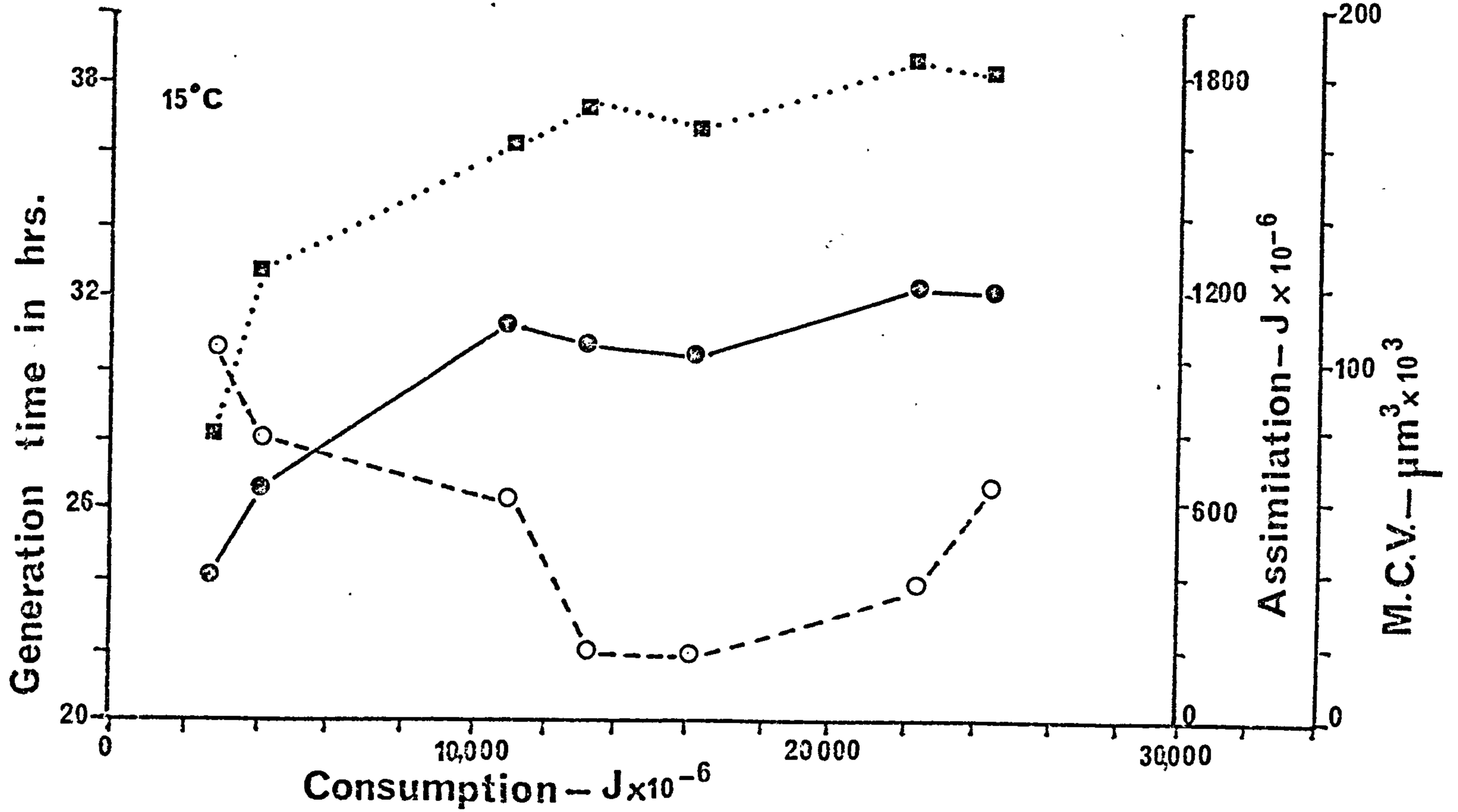

15°C
38
32
26
20
0
10,000
20000
30,000
Consumption – J x10⁻⁶
Generation time in hrs.
1800
1200
600
0
200
100
0
Assimilation – J x 10⁻⁶
M.C.V. – μm³ x 10³

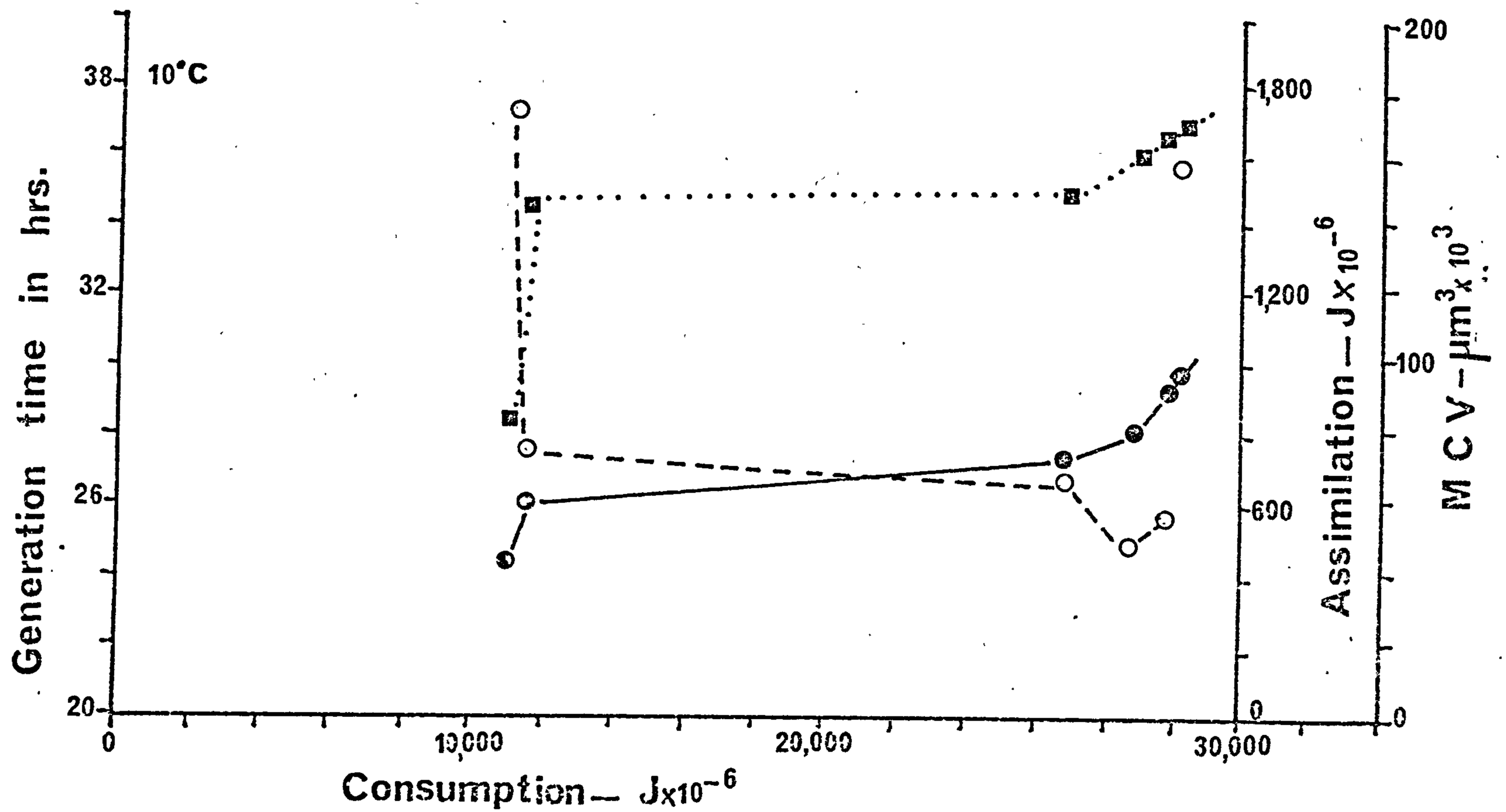
Figure 37

The relationship of assimilation, generation time and mean cell volume over a range of energy consumption at 10°C

Key:- mean cell volume ■

assimilation ●

generation time ○


Generation time in hrs.
38
32
26
20
10°C
0
10,000
20,000
30,000
Consumption— J x 10⁻⁶
1,800
1,200
600
0
Assimilation — J x 10⁻⁶
200
100
0
M C V - μm³ x 10³

however, the pattern is less well illustrated. In fact, the generation energy budget at 10°C (see Table 16) does show considerable variability, especially at the lower levels of consumption. At low levels of assimilation, where the length of the generation time increased, the animals apparently divided at a smaller size at the three temperatures considered, as indicated by the reduction in the mean cell volume.

From Tables 14, 15 and 16 and Figures 35-37 a comparison of the consumption during a generation by an individual shows that less food is ingested at higher temperatures. This is largely because the length of the generation is decreased by increased temperature. In terms of energy flow, this means that less energy is expended by one generation in the production of the following generation as temperature increases, at least within the temperature range considered in this study.

Obviously the influence of temperature on the way in which the assimilated energy is utilized by Colpidium, as outlined previously under the section dealing with the 24 hour energy budget, also applied to the generation energy budget.

7.4 Discussion

Within a study of this type designed to provide energy budgets for an asexual continuously dividing microorganism, there are obviously a great many variables which will influence each component of the budget. In particular, the adaptability

of protozoan species with reference to size change in response to changing cultural conditions has been demonstrated by Hamilton and Preslan (1969) and Curds and Cockburn (1971) as well as in the present study. The effect of food concentration on growth and consumption is clearly important, as the present investigation has shown, and this important variable imposes a considerable influence on the ingestion and utilization of energy in Colpidium.

In studies of bacteria a linear relationship between the glucose concentration in the growth medium and the dried weight of Aerobacter aerogenes produced has been found to exist (Ligeri et al. 1964). At higher concentrations of glucose a lower yield was found and similar results were found in other substrates. Bacteria and Protozoa have in common their ability to undergo rapid division, and Colpidium like Aerobacter is influenced by the concentration of its energy source. In Colpidium the linear relationship between growth and food concentration exists only up to a concentration 1.0×10^6:1 bacteria per protozoan, thereafter a plateau was attained. From the results of Ligeri et al. (1964) it would seem that a linear relationship exists between the energy source and the yield of bacteria up to an optimum concentration, thereafter declining.

Consumption of energy has been shown to be limited by food concentration in metazoan invertebrates. Mukerji and LeRoux

(1969b) working with the hemipteran Podisus maculiventris fed on live larvae of Galleria mellonella, found that nymphs showed increased consumption as food supply was increased. They were able to demonstrate a linear relationship between daily food consumption and increased weight. Hydra pseudoligaetis showed increased consumption with increased food supply (Schroeder 1969); growth efficiency increased in relation to food intake to a maximum level. A differing situation appears to exist for Daphnia pulex fed on Chlamydomonas cells as demonstrated by Richman (1958). Consumption was shown to be independent of the food concentration but was dependent on the size of the Daphnia. Growth in Daphnia decreased with decreased food concentration. In Colpidium the amount of the ingested energy transformed into growth was not linearly related to consumed energy over the entire food availability range as has been pointed out. Consumption in Colpidium was dependent on food concentration up to a food ratio of 2.5×10^6:1 bacteria per protozoan. At higher ratios consumption was independent of food supply.

A variable which may influence the energy budget for a given species is the type of food source. The amphipod Hyalella azteca showed variable assimilation efficiencies associated with different food types (Hargrave 1970). With bacteria an assimilation efficiency of 60-82% was obtained, 75% for diatoms and values as low as 5-15% for blue-green algae

Additionally, the ingestion rate of sediment was related to the assimilation of microflora added to the sediment. Thus, sediment mixed with blue-green algae was not ingested as rapidly as sediment which contained diatoms. Lawton (1970) also demonstrated variation in assimilation efficiency with different food sources in the larval damselfly Pyrrhosoma nymphula, as did Carefoot (1967) in the growth rates of Aplysia punctata fed on eight species of algae. Although in the present study on Colpidium the energy budgets constructed are based on one food source only (a food which was apparently highly acceptable to Colpidium, see Chapter 1, section 1.2.2) a number of workers have demonstrated variable reproduction with different bacterial flora in the same ciliate species (Luck, Sheets and Thomas 1931, Burbanck 1942, Curds and Vandyke 1966). It seems probable that different bacterial food sources would produce variation in the energy budget for a ciliate.

The assimilation efficiencies obtained for Colpidium are low in comparison to that of 58% calculated for Acanthamoeba sp. by Heal (1967a) at 25°C. However, the assimilation efficiencies demonstrated in Colpidium lie close to some of those reported for other primary feeders. The results of Gere (1956) indicate that litter feeding Diplopoda and Isopoda have assimilation efficiencies in the range of 4-21%. Richman (1958) showed Daphnia pulex preadults to assimilate 6.6-23.88% of their ingested energy, and adults to assimilate 14.22-31.72% of

consumed energy. The level of assimilation depended on the number of Chlamydononas cells ingested. Other authors report higher assimilation efficiencies in primary feeders. The litter feeder Tracheoniscus rathbei utilizes an average of 33% of its ingested energy (White 1968), defoliating insects in a hazel coppice 35.5-39.6% (Smith 1972) and Littorina littorea approximately 87% (Grahame 1973).

The assimilation efficiencies reported in the literature for carnivorous feeding species are all higher than those which have been shown to occur in most primary feeders. The damselfly Pyrrhosoma nymphula has an assimilation efficiency between 81.2-90.6% (Lawton 1970), the opisthobranch Navanax inermis an assimilation efficiency of 62% (Paine 1965) and the phalangid Mitopus moris 44-74% (Phillipson 1960). Detrital feeders range in assimilation efficiency from 30.3% for Asellus aquaticus (Purs 1972) to 45-61% for Scrobicularia plana (Hughes 1970). In general carnivorous feeders appear to assimilate more of the energy they consume than do detrital and herbivorous feeders.

Welch (1968), by a review of the literature, has shown that carnivorous animals which have high assimilation efficiencies have low net growth efficiencies, while herbivorous and detrital feeding animals, although assimilating a smaller proportion of their ingested food, have higher net growth efficiencies. Thus carnivores lose more of their assimilated energy during respiration than to herbivores and detrivores. Colpidium fits into the pattern described by Welch (1968), in that, except at

low food concentrations, where respiration accounted for the largest proportion of assimilated energy, net growth efficiencies ranged from 60.4-73.9% at 10°C, 67.9-72.0% at 15°C and at 20°C 78.7%-83.7%. As the level of energy consumed fell towards the maintenance level, that in the energy needed to maintain the existing volume of cell material without growth, the percentage of assimilation that the energy of respiration constituted increased while the net growth efficiency decreased.

Temperature exerted a marked effect on the energetics of Colpidium as the results have indicated. Not only was the level of assimilation increased by temperature, but the net growth efficiency also showed a variation with temperature. In general as temperature increased from 10°C to 20°C, the amount of energy which Colpidium incorporated as growth increased; conversely as temperature decreased from 20°C to 10°C more energy was used in respiration. In this respect Colpidium appears to differ from some higher invertebrates; Lawton (1970) for example, found that assimilation efficiency showed little difference at 4°C and 10°C in Pyrrhosoma nymphula. White (1968) found that Tracheoniscus rathbei showed increased consumption and excretion at higher temperatures. Again this situation differs from that which prevails in Colpidium, where consumption was not greatly affected by temperature, and as a result the increased assimilation associated with higher temperature resulted in a decrease in excretion and egestion with increased temperature.

An energy budget based on the length of the life of an individual Colpidium, or a single generation, provides a clearer insight into the energy intake and utilization of a protozoan. Here the effect of temperature and food availability on the energetics of Colpidium takes on another dimension. These two factors determine the length of the generation time, so that three interrelated variables - temperature, food availability and time, determine the amount of energy consumed, assimilated and egested in the life-span of an individual. Since a ciliate is basically in continuous reproduction, i.e. there are no non-reproductive larval stages as there are in higher invertebrates, the energy which is assimilated is essentially directed towards the immediate aim of producing two daughter cells.

From the budgets outlined in Tables 14-16 it is apparent that reproductive efficiency, in terms of the energy which is ingested and consequently removed from the environment, increases with increased temperature, so that with a given concentration of food a larger population of individuals can be produced from that food as temperature rises. This has obvious ecological implications, in that the more efficient the production from the primary producers by the ciliates, the greater will be the biomass of ciliates available for predation by the carnivores, which include other species of Protozoa as well as Metazoa.

Another point which emerges from the generation energy

budgets and which has been touched on in previous chapters (Chapter 2, section 2.1, Chapter 3, Chapter 5) is the adaptive character of Colpidium at low food availabilities. Instead of ceasing reproduction when the source of the available energy is very low and assimilation is decreased, some mechanism causes division to occur at a smaller size. This phenomenon has a great ecological advantage since the chances of an individual of the species finding another suitable food source, and thus perpetuating the species, is enhanced by an increase in numbers.

CHAPTER 8 FIELD SAMPLING

8.1 Introduction

Protozoa possess a cosmopolitan distribution, a useful characteristic, since it allows the standard works on identification to have a worldwide applicability. In freshwater benthic environments Protozoa often occur in large numbers and are represented by an enormous number of species, and yet their place in the ecological system is not clearly understood. They presumably form a food source for many small metazoans. Within the protozoan community there is a complex structure, with bacterial and detrital feeders preyed upon by a successive heirachy of carnivorous forms (Picken 1937), forming the pyrimidal system described by Elton (1927). This concept has been supported by Faure-Fremiet (1950) and by Webb (1956) from her extensive study of the protozoan communities of a brackish environment.

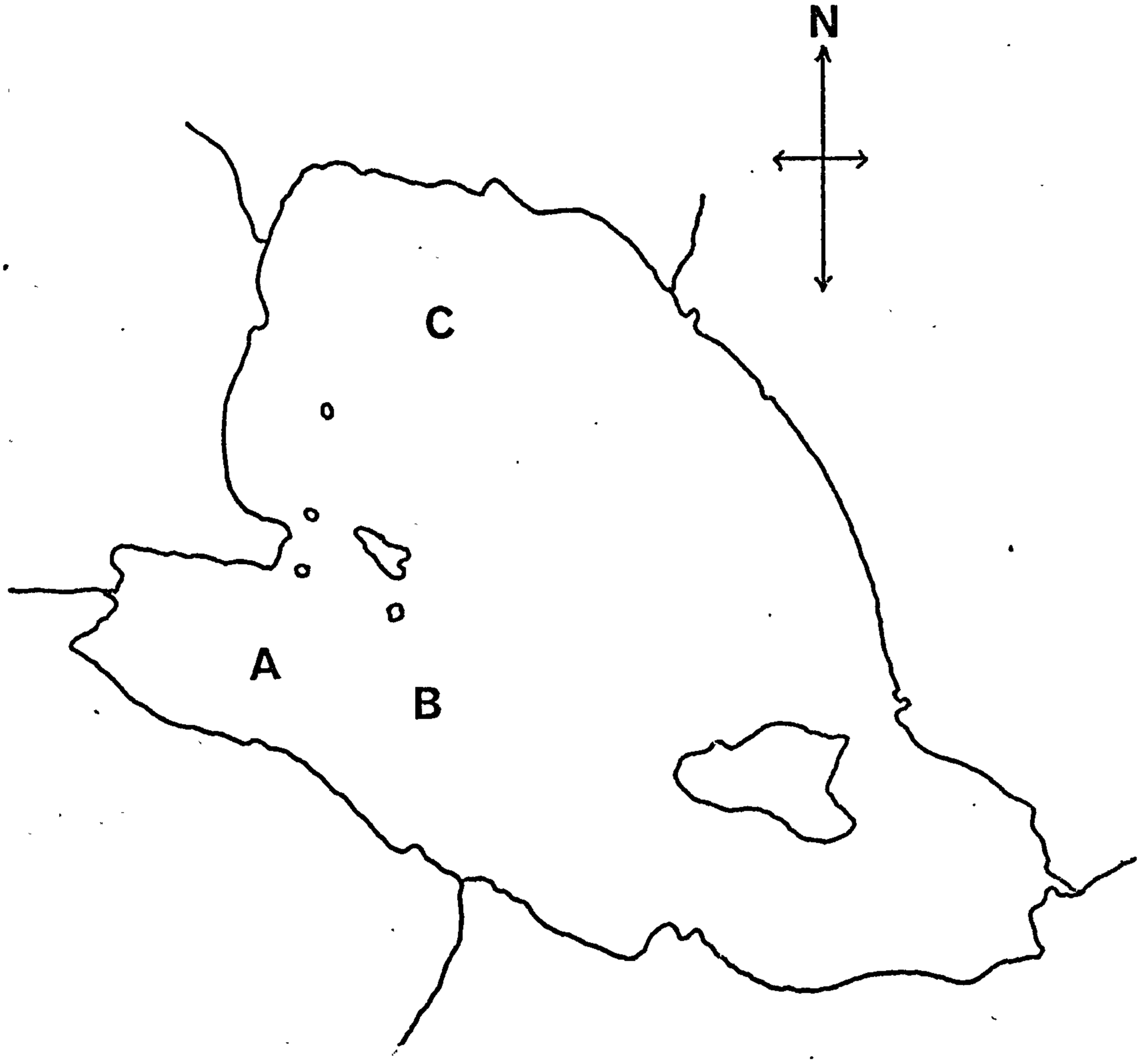
The present field sampling programme on the communities of Ciliophoroa in the soft sediments of Loch Leven, Kinross, was initiated in order to provide some information on the numbers, vertical distribution and species composition of ciliates in this important fresh-water body. Loch Leven is a nature reserve, and a study of it has formed part of the International Biological Programme, (PF-section). The present work on the Ciliophora is a small part of a large project on the loch.

Initially the aim of correlating the extensive laboratory studies on the energetics of a bacterial feeding ciliate -

Figure 38

A map of Loch Leven, Kinross, to show the position of the sampling sites

LOCH LEVEN



Colpidium campylum, to the natural communities of bacterial feeders in the loch was entertained. As the work progressed, however, it became very clear, that without an intimate knowledge of the fluctuations in the bacterial flora of Loch Level, and the various other factors implicated in controlling the distribution and abundance of Protozoa, such an aim, apart from estimating ciliate biomass, could not be achieved.

Protozoa have been shown to occur to varying depth in muddy sediments (Moore 1939, Cole 1955, Goulder 1971), it was therefore necessary to use a core sampler instead of merely sampling the mud surface as some workers have done (Webb 1961, Cairns 1965, 1966, Patrick, Cairns and Roback 1967).

8.2 Materials and Methods

8.2.1 General

Loch Leven is a large but relatively shallow (surface area 13.3 sq.km., average depth 3.9m) eutrophic lake, situated in Kinross, Scotland. The loch is exposed to wind action and the waters are well mixed throughout the year. As a result the loch does not undergo thermal stratification, except under rate anticyclonic conditions, and then only for a matter of a few days (Smith 1974).

8.2.2 Sampling sites

The benthic mud was sampled at three sites, see Figure 38. Sites A and B were situated in the southwest of the loch, at a depth of 3 and 4 metres respectively, and were sampled monthly from April 1972 until March 1973. Site C was situated at a

depth of 23 metres in the North Deeps and was visited monthly from October 1972 until March 1973.

8.2.3 Sampling device

A simple core sampler modified from the design of Moore and Neill (1930) was used. The sampling device is shown in Plate 3. The sampler consists of a graduated perspex tube with a detachable water pressure operated valve situated at its top end, and a brass nozzle at the opposite end.

After a core of mud had been obtained and hauled to the surface, the bottom of the corer was closed by the insertion of a rubber bung. The valve was unscrewed and removed and the top end of the tube sealed by another rubber bung. The cores were stored upright in a specially constructed carrying box until the return to the shore laboratory where the cores were divided up. Samples obtained with this type of core sampler were apparently undisturbed with the overlying flocculent layer intact.

The cores were divided by means of the plunger illustrated in Plate 3. Firstly, the water overlying the mud was siphoned off with a rubber hose. Then the plunger was pushed down the tube until it made contact with the mud surface, excess water escaping up the hollow tube of the plunger. The upper end of the plunger was then closed with a clip, the bung in the bottom of the corer tube removed and the mud core pushed out, allowing appropriate lengths to be chopped off into containers.

The core was split into 2cm deep samples. The extreme flocculence of the upper part of the core would not permit accurate division into thinner layers.

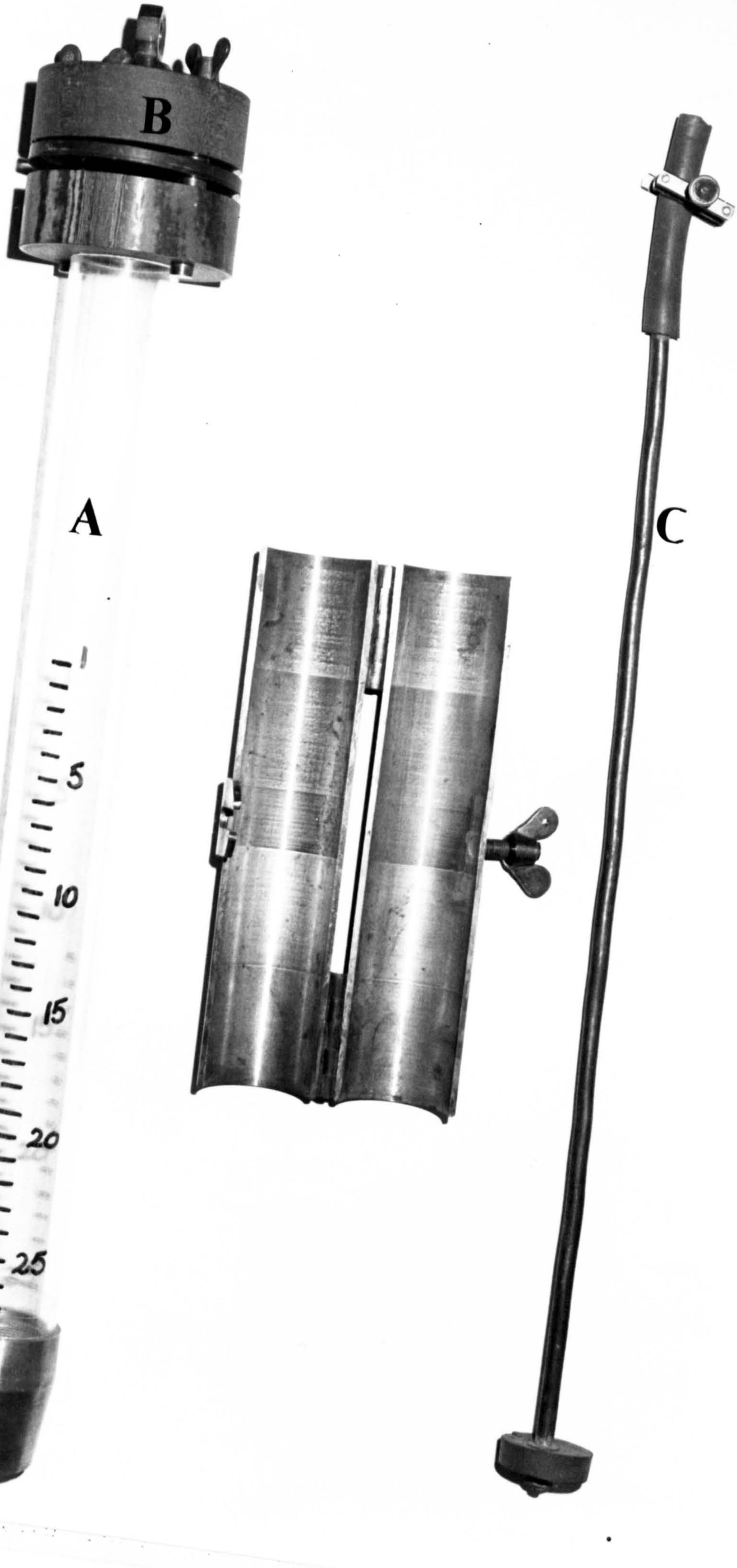
Plate 3

The core sampling device

A = graduated (cm) perspex tube

B = water pressure operated valve

C = plunger


B
A
C
5
10
15
20
25

8.2.4 Examination of samples

1ml samples of mud were removed from a thoroughly mixed layer sample, and diluted with 2ml of distilled water. The suspension was spotted out onto microscope slides and examined at a magnification of x100, higher magnifications were used for identification when necessary. All the individuals in a 1ml mud sample were counted and identified where possible. Two 1ml samples from each layer sample were examined and the mean of the numbers found taken.

8.3 Results

The vertical distribution and relative abundance of the Ciliophora in the Loch Level mud are shown in Fibure 39. Most of the ciliates were encountered in the top 2cm, occasionally small numbers were found in the 2-4cm layer and on four occasions single specimens were found in the 4-6cm layer. Ciliates were never found below 6cm.

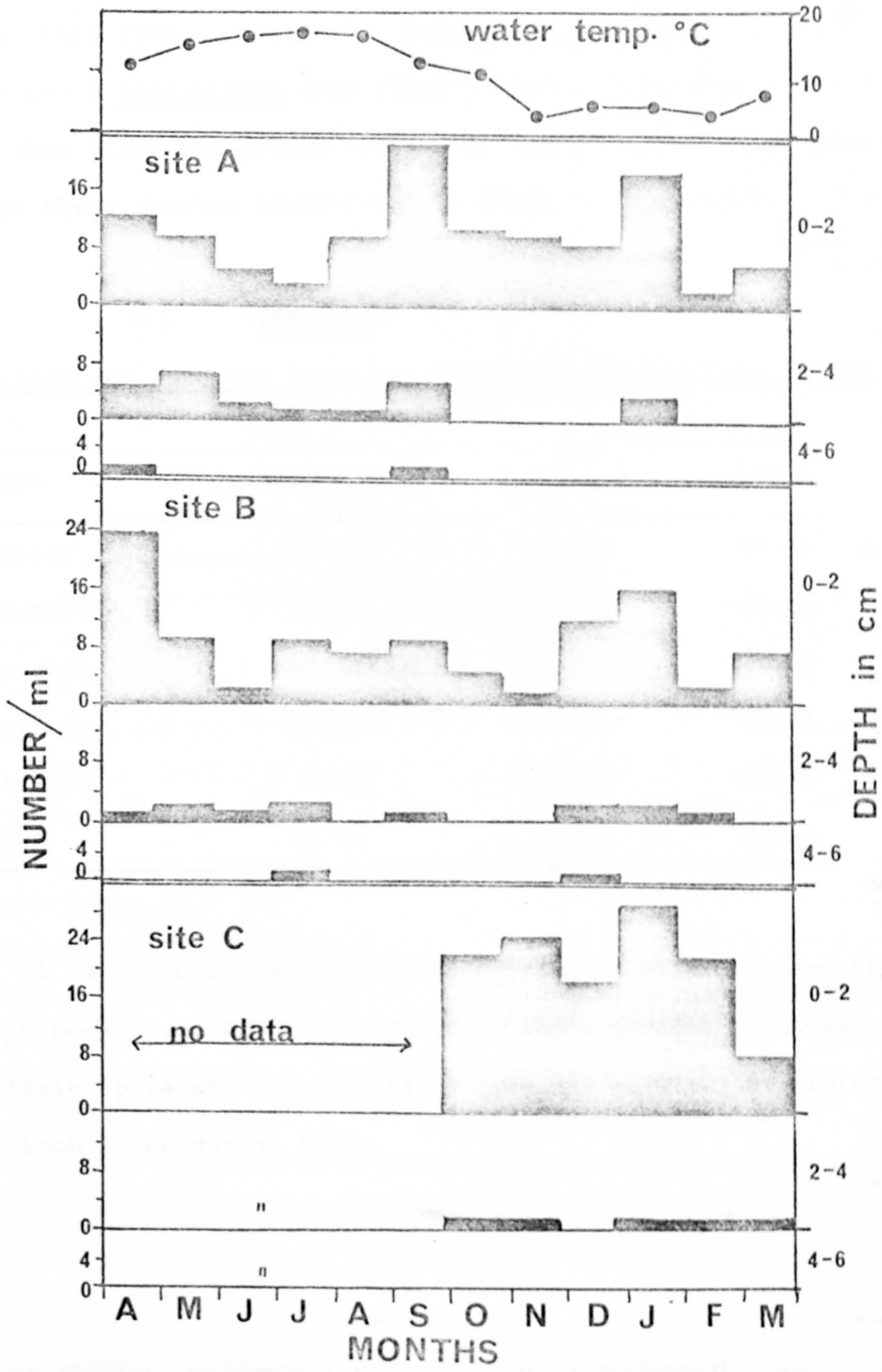
The numbers found were low; the maximum number found on any one occasion was $64/cm^2$ and the minimum number $2/cm^2$. Consistently higher numbers were recorded at the deeper site C than at sites A and B.

Temperature seemed to have no effect on either the size or the vertical distribution of the ciliate communities which both fluctuated through the period of sampling as the histograms show.

The species composition of the communities varied widely throughout the twelve month period at sites A and B and no

Figure 39

The vertical distribution of Ciliophora in the mud of Loch Leven, Kinross


water temp. °C
20
10
0
site A
16
8
0
8
0
4
0
2-4
4-6
site B
24
16
8
0
8
0
4
0
2-4
4-6
site C
24
16
8
0
8
0
4
0
no data
n
n
2-4
4-6
0-2
0-2
0-2
NUMBER/ml
DEPTH in cm
A M J J A S O N D J F M
MONTHS

consistent pattern emerged, see Table 18. At site C, however, a few species dominated the community during the six months that this site was visited. Frontonia leucas and species of the genus Spirostomum were found in relatively large numbers at this site, the proportion of the total community, at site C, that these species constituted is shown in Table 17.

TABLE 17

Spirostomum spp. and Frontonia leucas as a percentage of the community at site C

Month	Spirostomum spp.	F. leucas	Others
October	34.0%	4.7%	61.3%
November	34.0%	34.6%	31.4%
December	45.7%	31.4%	22.9%
January	20.3%	16.9%	62.8%
February	0.0%	11.8%	88.2%
March	37.0%	25.0%	38.0%

C

A total of 59 species of Cilophora have been recorded from Loch Leven, see Table 18, including those species recorded by Guthrie (personal communication) from her work on the Protozoa of Loch Leven during 1967.

TABLE 18

CILIOPHORA - LOCH LEVEN SPECIES AND MONTHLY OCCURENCE

(Nomenclature according to Kahl 1930-35)

Recorded by J.E.M. Laybourn except:-

* recorded by Dr. M.G. Guthrie

** recorded by Dr. M.G. Guthrie and J.E.M. Laybourn

	Months when recorded											
Class Ciliata	A	M	J	J	A	S	O	N	D	J	F	M
Subclass Holotricha												
Chilodonella cucullus Muller	-	-	-	-	-	-	*	-	-	*	-	-
Chilophyrya labiata Edmondson	-	-	-	*	-	*	-	-	-	-	-	-
Coleps Elongatus Ehrenberg*	-	-	-	-	-	-	-	-	-	-	-	-
Coleps hirtus Muller**	*	*	*	-	-	-	*	*	-	*	-	-
Coleps odospicus Noland*	-	-	-	-	-	-	-	-	-	-	-	-
Chlamydodon sp.*	-	-	-	-	-	-	-	-	-	-	-	-
Colpidium campylum Stokes	-	-	-	-	*	*	*	-	*	-	*	*
Colpidium colpeda Ehrenberg	*	-	-	-	-	*	*	*	*	*	-	-
Cohnilembus fusiformis Cohn	-	-	-	-	-	*	-	*	-	-	-	-
Dichilum cuneiforme Schewiakoff	-	*	-	*	-	-	*	-	*	*	*	-
Dileptus anser Muller**	*	*	*	*	-	*	-	-	-	-	-	-
Didinium sp.	-	-	-	-	*	*	-	-	-	-	-	-
Frontonia leucas Ehrenberg	-	-	-	*	-	*	*	*	*	*	*	*
Hemiophyrs procena Ponard*	-	-	-	-	-	-	-	-	-	-	-	-
Holophrya simplex Schewiakoff	*	*	-	*	*	*	-	*	-	*	-	-
Homalozoon vermiculae Stokes*	-	-	-	-	-	-	-	-	-	-	-	-
Lacrymaria olor Muller**	*	*	*	*	*	*	*	-	-	-	-	-
Lacrymaria pupula Muller**	-	-	-	-	-	-	-	-	-	-	-	-
Lembadion bulliuam Perty*	-	-	-	-	-	-	-	-	-	-	-	-
Litonotus sp.	*	-	-	-	-	*	*	*	*	-	-	-
Loxodes magnus Stokes**	-	-	-	-	*	-	-	-	-	-	-	*
Loxodes vorax Stokes**	-	-	-	*	-	*	*	*	*	*	-	*
Loxophyllum helus Stokes*	-	-	-	-	-	-	-	-	-	-	-	-

	A	M	J	J	A	S	O	N	D	J	F	M
Loxophyllum setigerum Quennerstedt*	-	-	-	-	-	-	-	-	-	-	-	-
Mesodinium sp.*	-	-	-	-	-	-	-	-	-	-	-	-
Monochilum frontatun Schewiakoff	*	*	-	*	*	*	*	-	*	-	*	*
Nassula aurea Ehrenberg	-	*	*	-	*	-	*	-	*	*	-	-
Paramecium aurelia Ehrenberg	-	*	-	-	-	*	-	-	-	-	*	-
Paramecium bursaria Ehrenberg	*	-	-	-	*	-	-	-	-	-	-	-
Paramecium caudatum Ehrenberg	-	-	-	-	-	-	*	-	*	*	-	-
Paramecium trichium Stokes	-	-	-	-	-	-	*	-	-	*	-	-
Tetrahymena sp.	*	-	*	*	-	*	*	*	-	-	-	-
Urocentrum turbo Muller**	*	-	-	-	-	*	-	-	-	-	-	-
Uronema sp.	-	-	-	-	-	*	-	-	-	*	-	-
Urotricha farcta Stokes	*	-	-	-	-	*	-	-	-	-	-	-
Subclass Spirotricha												
Aspidisca lynceus Ehrenberg**	-	-	-	-	-	*	-	-	-	-	-	-
Blepharisima sp.	-	-	-	*	*	*	-	*	*	-	-	-
Colpoda cucullus Muller	*	-	-	*	*	*	-	*	*	-	*	*
Euplotes patella Muller	-	-	-	*	*	*	*	-	*	-	*	*
Euplotes sp.	-	-	-	-	-	*	-	*	*	*	*	-
Gonostomum strenuum Engelmann	-	-	-	-	-	*	*	-	-	*	-	-
Halteria grandinella Muller**	*	*	*	*	*	*	-	*	-	*	*	-
Oxytricha steigera Stokes	*	-	-	-	-	-	-	-	-	-	-	-
Spirostomum filium Ehrenberg	-	*	-	-	*	-	-	*	-	-	-	-
Spirostomum loxodes Stokes	-	*	-	*	-	-	-	-	-	-	-	-
Spirostomum minus Roux**	-	-	*	*	*	*	*	*	*	*	-	*
Spirostomum teres Claparede and Lachmann	-	*	-	-	-	-	*	-	*	-	*	*
Stentor coeruleus Ehrenberg	-	-	-	-	-	-	-	-	-	-	-	-
Stentor polymorphous Muller**	*	*	*	-	-	-	-	-	-	-	-	-
Stichotricha sp.*	-	-	-	-	-	-	-	-	-	-	-	-
Strombidium sp.*	-	-	-	-	-	-	-	-	-	-	-	-
Strombilidium sp.*	-	-	-	-	-	-	-	-	-	-	-	-
Stylonchyia putrina Stokes	*	*	*	-	*	*	*	-	*	*	-	-
Urostyla trichogaster Stokes	-	-	-	-	-	*	-	-	*	-	-	-
Tachysoma sp.*	-	-	-	-	-	-	-	-	-	-	-	-

	A	M	J	J	A	S	O	N	D	J	F	M
Subclass Petritricha												
Vorticella sp.	-	-	-	-	-	-	*	-	*	-	-	-
Vorticella convallaria Linnaeus*	-	-	-	-	-	-	-	-	-	-	-	-
Class Suctoria												
Podophyrya sp.	*	*	*	*	-	*	-	-	-	-	-	*
Tokophyra sp.	-	*	-	*	-	-	*	-	-	-	-	-

8.4 Discussion

The depth to which ciliates were found in this study agrees closely with the vertical distribution in freshwater sediments reported by Moore (1939) and Cole (1955), both of whom found no ciliates below 5cm. Goulder (1971) was unable to find any ciliates below 4cm. In all cases the majority of the Ciliophora were in the surface mud layer with a decrease in density downwards.

It is not possible, without detailed experimental evidence, to isolate the factors which determine the abundance and distribution of Protozoa. Undoubtedly, food supply is an important factor as Noland (1924) suggests, but physical and chemical factors, such as sediment texture and oxygen availability also exert an influence (Goulder 1971, Bryant and Laybourn 1974).

A fine sediment, such as mud, where the interstices are small, will not only hamper locomotion, but will not permit the diffusion of oxygen to depth in the mud. A coarse sediment, with large interstices will have the opposite effect, and Protozoa have been found to a depth of 20cm in a sandy marine sediment by Fenchel and Jansson (1966). Most protozoan food sources (Bacteria, algae and other Protozoa) are probably most abundant in the surface layer of the sediment, and this of course would also contribute to the larger number of ciliates in this stratum.

With only three sampling sites it is not possible to make

any detailed generalizations about the communities of Ciliophora in Loch Leven. Certainly, ciliates were more abundant at site C, a deep water site, when compared with sites A and B which were both shallow. Although no quantitative work was carried out on protozoan food, algae, which may constitute a food source to many ciliate species including Frontonia leucas and Spirostomum spp. (Sandon 1932), appeared to be more abundant at site C than at sites A and B. This fact might contribute to the high incidence of these ciliate species in the North Deeps. The North Deeps, which is a kettle hole in the loch bed, possibly provides a more stable environment than the shallow sites, which are probably subject to considerable mixing and are hence less stable. The density of the ciliate communities found in this study is low when compared with those reported by other workers from freshwater sediments in two North American and one British lakes (Moore 1939, Cole 1955, Goulder 1971). The reasons are not clear.

No apparent seasonal pattern in either numbers or species composition occurred in Loch Leven. Seasonal fluctuations related to temperature have been found in planktonic protozoan communities by Saifullah (1971) in a marine situation and Wang (1928) in a freshwater pond. Many of the species reported by Wang (1928) were also identified in the mud of Loch Leven. Fenchel (1967) found maximum benthic ciliate numbers in the summer months in a marine environment, although he reported large active populations throughout the year.

Variation in the species composition brought about by thermal stratification in Esthwaite Water have been noted by Webb (1961), and by Moore (1939) in Douglas Lake, Michigan. Webb (1961) showed that as the water of the hypolmnion became depleted of oxygen the protozoan community contained species capable of withstanding low oxygen concentrations, (e.g. Coleps spp., Spirostomum spp., Stentor spp., and Urocentrum turbo). When the water below the thermocline became completely depleted of oxygen, only species capable of withstanding anoxic conditions remained (e.g. Plagiopyla nasuta, Metopus spp.). The absence of a marked thermal stratification in Loch Leven may account for a lack of any detectable seasonal variation in the species composition.

Peaks in ciliate numbers in bottom sediments associated with peaks in Daphnia production in the plankton have been reported by Grabacka (1971). In Polish fingerling ponds this author showed that ciliate density increased after the maximum production of Daphnia spp., and suggested that the development of bacterial growth on the dead bodies of these crustaceans might be responsible. The ciliate communities in my study showed no such relationship with peaks which occurred in the zooplankton crustaceans of Loch Leven as demonstrated by Johnson and Walker (1974).

From the dried weight and calorific values obtained during laboratory studies (Chapter 4, section 4.3) it was possible, using the approximate dimensions quoted by Kahl (1930-35) and

Kudo (1971), and calculating volumes, to estimate the mean monthly biomass of Ciliophora in the mud of Loch Leven. The figure obtained represents an average monthly biomass of 1.009kJ/m^2. Thus, Protozoa do not appear to contribute a great deal towards the total animal biomass; a fact also noted by Muus (1967) in a study of a brackish environment in Danish waters, where although the Ciliophora constituted 93% of the total number of benthic animals, they formed only 0.4% of the total animal biomass. In Loch Leven the mean monthly biomass of ciliates (representing an annual production of 12.108kJ/m^2) is extremely low when compared to the annual chironmid larval production which represents 587.5kJ/m^2 (Charles, East, Gray and Murray 1974).

GENERAL DISCUSSION

Cutler and Crump (1924) were probably the first workers to recognise the importance of food concentration in controlling reproduction, and most later studies attempted to control and monitor this extremely important variable in physiological investigations of growth, reproduction and feeding on Protozoa. It has also been shown that ciliates may be selective in their choice of food, and that not all bacteria provide a suitable energy source. Some species are toxic to ciliates, others slightly toxic, while others will only support poor growth; favourable bacterial food sources have been shown to support good growth in comparison (Curds and Vandyke 1966). Thus the species of food organism and its concentration represent two variables in feeding, growth and reproduction studies on ciliated Protozoa. Both of these variables were given consideration in this study.

Feeding and growth in Colpidium (see Chapter 2, section 2.2.3) are clearly influenced by food concentration and temperature. Below an initial food concentration of 1.0×10^6 bacteria per protozoan, growth was dependent on food concentration, but above this level the growth rate was independent of the available food. Temperature determined the maximum level of growth attained, growth increasing with increased temperature. Consumption was also dependent on food concentration, but in this case up to a ratio of 2.5×10^6:1 (higher than for growth), above which it became independent of food availability. Temperature did not

greatly influence the maximum level of ingestion. Both Heal (1967a) and Curds and Cockburn (1968) found growth to be linearly related to food concentration at low food-to-protozoan ratios. Curds and Cockburn (1968) and Proper and Garver (1966) also found that growth was limited at higher food concentrations; the present findings on Colpidium are very similar to the pattern demonstrated by these workers.

The consumption in Colpidium differed from the findings of Harding (1937) who showed that feeding never became independent of food concentration in Glaucoma pyriformis . However, Heal (1967a) found consumption to have a linear relationship with food concentration at low food:protozoan ratios. When food was abundant his results were very variable and no obvious relationship between consumption and availability was apparent.

Since the parameter selected for measuring growth was the volume of cellular material produced multiplied by a dried weight factor, as done in similar studies (Heal 1967a, Curds and Cockburn 1968), detailed information on the variations in the mean cell volume of populations grown in a range of food concentrations at 10°C, 15°C and 20°C also emerged (see Chapter 3). Consumption, which as previously stated was dependent on food concentration up to a ratio of 2.5×10^6:1, had a distinct effect on the mean cell volume of the population. Where less than 400 nanograms dried weight of bacteria were consumed by each Colpidium in the initial population during 24 hours, mean cell volume maintained a maximum level. Temperature had little

effect on mean cell volume. From the results of the present study the quantity of food consumed appeared to be the major factor influencing the cell size maintained by Colpidium. This conclusion is in agreement with Curds and Cockburn (1971) and Hamilton and Preslan (1969), but contrary to the suggestion of Hamilton and Preslan (1970) from a study of steady-state populations of Uronema sp. where the density of the protozoan population was said to be the governing factor.

Detailed information on reproduction in relation to food supply and temperature was also obtained from the long series of experiments on growth and feeding (see Chapter 2, section 2.2). The reproductive rate, as indicated by the number of generations produced in 24 hours, displayed a variation with temperature and energy consumed (see Chapter 5). Temperature had the overall effect of reducing the reproductive rate as temperature fell; the influence of temperature on the rate of reproduction has been documented by other workers (Woodruff and Baitsell 1911a, 1911b, Heal 1967a). Where less than 2,000-10,000J x 10^{-6} were consumed per individual in 24 hours at $10^{o}C$ and $15^{o}C$, the generation time increased; that is the number of generations produced in 24 hours decreased. Above this level of energy intake, however, reproduction was independent of consumption. Much the same pattern has been shown to prevail in Glaucoma pyriformis Harding 1937), where at low food concentrations reproduction was found to have a linear relationship with food availability, but at higher bacterial

concentrations the rate of reproduction became independent of food concentration. At 20°C the rate of reproduction in Colpidium increased with consumption, with some indication of a levelling-off where between 20,000 - 30,000 J x 10^{-6} were consumed by each individual in the initial population in 24 hours.

Returning to the question of mean cell volume, where a decrease occurred below an intake of 400 nanograms dried weight of bacteria/individual/24 hours (equivalent to 8145 x 10^{-6}J), it is evident that Colpidium not only increases the time span of a generation at low food concentrations, but also divides at a smaller size.

Respiration studies in the Protozoa are many and varied. The results obtained for individual species by different workers often display great variability, the possible reasons for which are discussed in Chapter 6, section 6.4. The population density of Colpidium was found to exert a marked effect on oxygen uptake, a phenomenon also noted by other workers (Specht 1935, Pace and Kimura 1944, Pace and Lyman 1947). Variation in oxygen uptake with population density obviously represents yet another variable in an energetics study on a protozoan species, along with good concentration and food species.

A clear relationship between body weight or size and oxygen uptake has been demonstrated in many metazoan species (e.g. Lawton 1971, Smith 1972). A similar relationship was found to exist in Colpidium at 10°C and 15°C but at 20°C there was considerable scatter and no clear relationship could be

proved. Since the mean cell volume of Colpidium has been shown to decrease below certain consumption levels, it follows that the heat lost during respiration as indicated by the oxygen uptake will also decrease, and this in turn will affect the degree of assimilation. Above the food intake level where mean cell volume remains constant irrespective of consumption the heat lost during respiration does not vary to any great extent.

The various components of the energy budget equation having been determined directly by experiment, except for egestion and excretion which were calculated, two types of energy budgets were constructed (see Chapter 7). The twenty-four-hour energy budget was intended to indicate the potential of an individual in 24 hours, in terms of consumption and assimilation, and takes no account of the fact that the energy assimilated may have been divided between several individuals by binary fission within the 24 hours. The second type of energy budget which was constructed, was based on the time span of a generation, or the life of one individual under a set of specified conditions. Obviously, the length of the generation varies with consumption (see Chapter 5, section 5.3) and the amount of food consumed is determined by the food concentration.

In the case of both types of energy budget the energy consumed and utulized was related to food concentration at each temperature. As has already been pointed out food concentration has been shown to be an important variable in growth and feeding,

both in this study and in investigations by other workers (Cutler and Crump 1924, Luck, Sheets and Thomas 1931, Harding 1937), therefore a consideration of energy intake and use by a protozoan must correlate directly with the concentration of the available energy to have any meaningful value.

The gross growth efficiencies wiich emerged from the study on Colpidium (up to 11%) were low in comparison to the growth efficiencies reported in other protozoan species of 37-78% (Coleman 1964, Proper and Garver 1966, Heal 1967a, Curds and Cockburn 1968, 1971), but were comparable with those found in other primary feeding invertebrates where gross growth efficiencies of less than 10% were recorded (Gere 1956, Prus 1972). Assimilation efficiencies in Colpidium were also lower than those reported in Acanthamoeba by Heal (1967a). The efficiency of assimilation in Colpidium are again comparable with those reported in other invertebrates at the same trophic level. Richman (1958) reported assimilation efficiencies of 6.6-23.88% for pre-adult Daphnia pulex and 14.22-31.72% for adults and Gere (1956) found litter feeding Diplopoda and Isopoda to have assimilation efficiencies ranging from 4-21%. An important factor which emerged from the present study was that both growth efficiency and assimilation efficiency were found to vary with the concentration of the energy source offered to Colpidium. This phenomenon was, as has already been pointed out (Chapter 2, section 2.2.3; Chapter 7, section 7.4) a result of the fact that growth and consumption became independent of food concentration at different bacteria: protozoan ratios. Growth

reached its maximum at a lower concentration than consumption, so that both maximum growth and assimilation efficiency lie near the point where the growth rate became independent of food concentration. Above this point more energy was ingested than was necessary to sustain maximum growth and therefore the proportion of the ingested energy which was converted to growth and thus incorporated in assimilated energy decreased.

The part of the assimilated energy lost as heat during respiration became independent of food consumption at the same point as mean cell volume, since as was pointed out earlier, oxygen uptake was found to be related to size. Mean cell volume remained more or less constant above an intake of 400 nanograms dried weight of bacteria/individual/24 hours (equivalent to $8145J \times 10^{-6}$), so consequently did respiration. It follows that most of the variation in assimilation efficiency over the food concentration range considered in this study was due to variation in growth efficiency rather than to variations in the respiration rate.

Colpidium as an animal feeding on a primary producer fits into the general scheme of assimilation and growth efficiencies in aquatic consumers as outlined by Welch (1968). Welch showed that in general herbivores and detrital feeders, although assimilating a smaller proportion of their ingested energy, have higher net growth efficiencies than carnivorous species. Colpidium has high net growth efficiencies, except where the quantity of energy consumed fell to a low level, when the heat

lost during respiration accounted for the greater part of the assimilated energy.

Field studies on the species composition, seasonal distribution and vertical distribution of Protozoa in aquatic habitats are many (Wang 1928, Moore 1939, Cole 1955, Webb 1956, 1961, Fenchel and Jansson 1966, Fenchel 1967, Saifullah 1971, Goulder 1971). A point which emerges from these studies is the diversity and adaptability of protozoan species. The field sampling programme on the Ciliophora of the soft sediments of Loch Leven in the present project was initially intended to relate to the laboratory investigations on the energetics of a bacterial feeding ciliate. It became very apparent, however, that such an aim was not feasible without a clear picture of the abundance and fluctuations of the bacterial food organisms in the sediments of Loch Leven. Further the variables affecting the energetics of Colpidium to which attention has been repeatedly drawn in this section, added further complications.

Nevertheless, the work did provide some information on the general ecology of Ciliophora in the soft sediments of Loch Leven, in particular details of vertical distribution and species composition The Ciliophora of Loch Leven displayed no detectable seasonal variation in numbers of species composition. The lack of variation in the species composition of the ciliate community of Loch Leven is probably attributable to the absence of any prolonged thermal stratification in this water body, since Webb (1961) and Moore (1939) found a seasonal pattern in

species composition related to thermal stratification. The pattern of vertical distribution found in the present study was typical of ciliates in soft fresh-water sediments (Moore 1939, Cole 1955, Goulder 1971).

The mean monthly biomass calculated for Ciliophora in Loch Leven represents 1.009kJ/m^2 (equivalent to an annual production of 12.108kJ/m^2), a figure which is very low when compared with the annual chironomid larval production in Loch Leven which represents 578.5kJ/m^2 (Charles, East, Gray and Murray 1974). Indeed, the fact that Protozoa constitute a small proportion of the total animal biomass has been noted by Muus (1967), even when they represent a large percentage of the total animal population in terms of numbers.

Accepting Picken's (1937) theory of the structure of protozoan communities, as supported by Faure-Fremiet (1950) and Webb (1956), where detrital and herbivorous feeders are preyed upon by a successive heirachy of carnivorous forms, the energetics of protozoan communities has a wide scope for investigation. The flow of energy through the pyramid of the communities of these unicellular animals would be of exological interest, notwithstanding the value it might have in relation to higher invertebrates. Not only do other bacterial feeders require investigation, but algal and omnivorous species need detailed examination. It would be interesting to know whether carnivorous Protozoa resemble bacterial feeding species in their energetics, or if they are more analagous to other invertebrate carnivores.

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A P P E N D I X

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CONSUMPTION AND GROWTH AT 20°C

Exp.No.	Ratio	Consumption/indiv/24 hrs		Growth/indiv/24 hrs	
		No.bacteria x10^4	Joules x10^{-6}	Protoplasm μm^3 x 10^4	Joules x 10^{-6}
1	1:977,142	80.0	12867	41.2	1410
2	"	71.2	11444	37.4	1282
3	"	81.9	13155	49.0	1678
4	"	68.0	10942	37.0	1267
5	"	73.8	11867	36.0	1235
6	"	56.2	9041	26.9	921
7	"	68.0	10942	32.6	1115
8	"	73.6	11838	30.7	1052
9	"	65.7	10560	35.3	1207
10	"	84.1	13528	36.8	1259
11	1:204,296	19.3	3097	4.2	144
12	"	22.0	3540	2.2	76
13	"	18.7	3014	3.3	112
14	"	22.1	3556	1.9	63
15	"	17.7	2851	5.1	175
16	"	18.8	3020	3.6	124
17	"	17.1	2742	0.7	225
18	"	18.6	2981	2.9	102
19	"	18.4	2961	2.1	72
20	"	16.4	2642	3.3	113
21	1:2,812,000	114.3	18384	97.5	1964
22	"	126.4	20330	77.6	1562
23	"	177.9	28614	100.8	2030
24	"	147.9	23788	72.4	1458
25	"	138.1	22212	101.9	2054
26	"	114.2	18367	76.6	1542
27	"	114.7	18448	80.5	1621
28	"	158.9	25558	111.0	2239
29	"	135.6	21810	91.4	1841
30	"	154.4	24834	95.3	1920
31	1:3,933,786	128.4	20652	50.8	1740
32	"	99.6	16023	40.7	1393
33	"	132.0	21281	95.8	1931
34	"	127.7	20539	67.9	1357
35	"	97.3	15656	70.9	1430
36	"	124.1	19960	89.0	1794
37	"	118.8	19108	83.7	1686
38	"	136.9	22019	71.5	1440
39	"	162.8	26185	77.1	1553
40	"	138.5	22276	73.8	1487
41	1:1,492,268	107.5	17290	48.2	1651
42	"	117.5	18899	51.3	1757
43	"	139.1	22373	67.2	2302
44	"	123.6	19880	57.5	1969
45	"	117.1	18834	53.8	1841

46	"	112.5	18094	53.8	1841
47	"	130.6	21006	60.3	2063
48	"	102.4	16470	45.8	1569
49	"	113.4	18239	53.2	1821
50	"	107.6	17306	49.6	1699
141	1:1,990,836	140.4	22585	39.4	1348
142	"	137.5	22116	47.0	1609
143	"	162.7	26166	57.0	1609
144	"	120.4	19372	35.7	1222
145	"	121.9	19618	40.7	1394
146	"	126.9	20411	35.7	1222
147	"	108.0	17372	34.8	1190
148	"	122.1	19643	38.2	1308
149	"	128.5	20660	36.6	1253
150	"	131.2	21101	48.4	1657

CONSUMPTION AND GROWTH AT 15°C

Exp.No.	Ratio	Consumption/indiv/24hrs		Growth/indiv/24hrs	
		No. bacteria x 10^4	Joules x 10^{-6}	Protoplasm μm^3 x 10^4	Joules x 10^{-6}
171	1:256,011	22.5	2392	1.0	34
172	"	17.0	2742	3.4	116
173	"	19.3	3099	6.8	232
174	"	20.4	3274	4.9	167
175	"	19.1	3077	5.3	181
176	"	25.2	4054	7.9	270
178	"	17.9	2891	4.5	154
179	"	19.2	3093	4.7	161
180	"	18.3	2938	5.4	184
181	1:323,092	25.2	4053	17.3	348
182	"	23.4	3763	9.5	191
183	"	18.3	2943	16.5	332
184	"	20.4	3281	15.6	315
185	"	20.7	33329	18.0	363
186	"	22.3	3586	15.3	308
187	"	22.9	3683	17.9	356
188	"	23.7	3812	17.5	352
189	"	19.2	3088	12.4	250
190	"	23.1	3715	18.5	373
191	1:1,604,811	91.2	14675	28.1	567
192	"	88.4	14212	38.1	767
193	"	102.4	16475	44.1	887
194	"	100.0	16084	39.6	796
195	"	96.5	15513	67.8	1366
196	"	84.5	13592	40.7	820
197	"	82.8	13316	42.3	852
198	"	98.1	15783	49.1	989
199	"	85.4	13750	42.1	848
200	"	83.5	13428	36.8	741
201	1:2,471,136	129.7	20866	34.5	694
202	"	107.0	17219	37.0	745
203	"	151.9	24445	48.3	972
204	"	90.4	14532	29.9	603
205	"	107.7	17320	35.3	711
206	"	109.8	17662	38.5	775
207	"	116.1	18675	39.7	800
208	"	100.0	16084	38.3	771
209	"	100.4	16145	41.2	829
210	"	86.4	13892	45.7	920
211	1:889,094	64.4	10358	20.6	705
212	"	75.0	12263	19.1	653
213	"	52.7	8476	23.3	797
214	"	70.1	11275	20.9	714
215	"	70.5	11339	19.3	662
216	"	58.8	9457	20.0	685
217	"	60.0	9650	20.8	713

218	"	42.3	6803	18.6	637
219	"	67.7	10889	20.1	686
220	"	72.5	11661	24.2	829
231	1:2,912,088	137.5	22116	22.3	763
232	"	126.9	20411	26.3	899
233	"	112.3	18059	21.6	740
234	"	131.6	21162	24.9	854
235	"	163.2	26229	29.2	1000
236	"	160.7	25847	27.2	931
237	"	124.8	20073	23.9	817
238	"	133.8	21520	23.7	811
239	"	165.1	26552	35.0	1199
240	"	139.1	22378	21.3	728
241	1:3,530,000	135.5	21787	30.2	607
242	"	120.8	19422	43 2	870
243	"	154.2	24800	38.2	769
244	"	130.5	20990	32.9	662
245	"	136.9	21992	39.8	801
246	"	109.6	17635	35.0	105
247	"	144.0	23167	35.8	720
248	"	159.9	25720	46.6	940
249	"	130.8	21031	21.9	749
250	"	160.5	25817	17.1	586

CONSUMPTION AND GROWTH AT 10°C

Exp.No.	Ratio	Consumption/indiv/24hrs		Growth/indiv/24hrs	
		No.bacteria x10^4	Joules x10^{-6}	Protoplasm μm^3x10^4	Joules x10^{-6}
81	1:554,084	19.1	3072	-3.6	-74
82	"	29.4	4728	-11.9	-239
83	"	22.4	3602	-5.1	-102
84	"	23.2	3731	10.5	212
85	"	28.3	4551	5.1	102
86	"	28.4	4567	6.7	229
87	"	19.1	3072	4.9	167
88	"	27.6	4439	6.4	219
89	"	31.7	5098	4.8	164
90	"	20.8	3345	-1.6	-54
91	1:885,714	35.1	5645	4.6	92
92	"	32.0	5147	0.2	3
93	"	30.9	4970	1.7	23
94	"	34.2	5500	6.6	133
95	"	41.6	6691	1.7	34
96	"	27.4	4407	-0.4	-7
97	"	30.5	4905	4.8	96
98	"	26.4	4246	10.7	215
99	"	32.2	5179	9.4	188
100	"	38.6	5919	7.7	154
101	1:2,418,886	143.1	23023	10.2	350
102	"	128.0	20594	14.1	484
103	"	118.6	19071	13.9	478
104	"	153.3	24694	15.7	537
105	"	130.7	21027	9 9	339
106	"	154.9	24922	16.2	554
107	"	119.0	19143	15.9	544
108	"	108.9	17527	10.7	355
109	"	115.2	18535	15.9	547
110	"	131.0	21075	12.8	438
111	1:1,385,659	45.2	7266	10.4	210
112	"	63.9	10284	26.6	535
113	"	54.7	8794	24.2	487
114	"	54.9	8839	10.3	206
115	"	60.4	9711	9.5	191
116	"	48.8	7855	16.8	339
117	"	43.6	7006	5.4	109
118	"	47.1	7572	7.9	161
119	"	53.2	8458	6.4	219
120	"	50.9	8196	4.8	164
121	1:2,827,486	116.9	8794	12.1	415
122	"	103.1	16575	7.9	270
123	"	103.8	16689	11.2	382
124	"	115.5	18580	8.3	282
125	"	112.1	18037	9.7	332

126	"	119.9	19288	12.5	427
127	"	114.7	18452	11.3	388
128	"	99.2	15955	12.5	428
129	"	100.0	16084	6.8	234
130	"	127.4	20491	7.7	263
131	1:3,876,740	150.2	24158	17.3	593
132	"	145.7	23434	12.1	414
133	"	136.7	21987	13.3	455
134	"	115.4	18561	12.9	442
135	"	107.8	17338	12.6	431
136	"	114.0	18334	16.4	561
137	"	103.4	16631	9.8	334
138	"	165.2	21074	15.7	538
139	"	121.8	19590	16.3	558
140	"	139.2	22389	16.0	548
151	1:1,302,777	88.5	21347	14.1	483
152	"	62.8	10101	6.8	232
153	"	69.9	11243	8.8	301
154	"	57.0	9168	6.3	215
155	"	64.3	10336	10.7	366
156	"	84.4	13575	12.5	428
157	"	62.5	10062	4.2	143
158	"	86.0	13671	2.7	92
159	"	62.5	10052	3.8	130
160	"	63.1	10149	4.0	137

RESPIRATION DATA AT 10°C

No/ml	M.C.V. $\mu m^3 x 10^3$	O_2/indiv/hr $\mu\ell \times 10^{-4}$	$O_2/\mu m^3/hr$ $\mu\ell \times 10^{-9}$
1540	105.0	2.56	2.43
1966	122.0	2.01	1.64
2608	135.0	2.43	1.80
1962	154.0	2.40	1.54
4598	137.0	2.08	1.50
4515	71.0	1.00	1.39
8306	92.0	1.54	1.66
3412	35.5	1.41	3.97
6838	68.0	1.27	1.86
1938	120.0	2.91	2.40
5276	110.0	2.09	1.89
7336	59.0	1.37	2.45
4566	35.5	0.93	2.58
7534	43.0	0.91	2.11
2306	78.0	2.23	2.85
6500	121.0	1.19	0.96
4023	149.0	2.62	1.75
5765	38.0	0.81	2.11
3102	79.0	1.37	1.72
2053	36.0	2.75	7.62

each figure represents the mean of 7 experiments

RESPIRATION DATA AT 15°C

No/ml	M.C.V. $\mu m^3 \times 10^3$	O_2/indiv/hr $\mu\ell \times 10^{-4}$	$O_2/\mu m^3/hr$ $\mu\ell \times 10^{-9}$
3970	37.0	2.13	5.75
3644	59.0	1.66	2.80
4268	61.5	1.48	2.40
7846	39.0	0.91	2.33
6450	39.5	1.27	3.21
4798	40.5	1.54	3.80
4480	70.0	1.94	2.76
3918	20.5	1.41	6.86
4192	22.2	1.57	7.06
1312	119.0	2.89	3.41
2206	22.0	1.91	8.67
5416	69.0	2.10	3.06
2640	104.5	2.72	3.59
1584	119.0	3.89	3.32
3202	144.5	3.00	3.09
8030	37.0	1.23	3.32
2690	66.0	2.61	3.93
8918	37.0	0.77	2.08
3716	32.0	1.84	5.47
6316	34.0	1.72	4.97

each figure represents the mean of 7 experiments

RESPIRATION DATA AT 20°C

No/ml	M.C.V. $\mu m^3 \times 10^3$	O_2/indiv/hr $\mu\ell \times 10^{-4}$	$O_2/\mu m^3/hr$ $\mu\ell \times 10^{-9}$
3096	34.0	2.54	7.48
3572	30.5	2.35	7.68
4870	101.5	2.58	2.54
4194	66.0	1.95	1.38
4330	101.0	1.14	1.12
3166	130.0	2.68	2.04
2844	140.0	2.03	1.43
4768	91.0	1.47	1.60
8514	7.5	0.43	5.83
4654	37.5	1.63	4.36
3870	37.0	0.87	2.36
1896	35.0	3.05	8.72
3076	140.5	3.34	2.35
2734	145.5	4.70	3.21
1248	50.0	6.27	12.53
1694	52.0	3.63	6.51
5742	69.0	1.34	2.49
7182	83.0	1.18	1.40
7074	29.0	0.86	3.40
6292	79.0	1.91	2.35

each figure represents the mean of 7 experiments

FLEDON-HEIG INVERTEBRATE SALINE

in 1 litre distilled water

NaCl	7.0g
KCl	0.3g
$CaCl_2$	0.1g
$NaHCO_3$	1.5g
$MgSO_4$	0.3g