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# ON THE CHEMICAL COMPOSITION OF SOME UNICELLULAR ALGAE

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**Abstract**—The gross chemical composition of the golden-brown algae, *Chrysochromulina kappa*, *Chrysochromulina polylepis*, *Prymnesium parvum*; the yellow-green alga, *Olisthodiscus* sp.; and the green alga *Pedinomonas minor*, are given in terms of amounts per average cell. The following constituents were assayed: cell volume, dry weight, protein, lipid, carbohydrate, deoxyribonucleic acid, ribonucleic acid, phospholipid, acid-soluble phosphorus, phosphoprotein phosphorus, and chlorophylls *a*, *b* and *c*. Two strains of *Prymnesium parvum* were assayed and gave similar results. When cultures of the Israeli strain of *P. parvum* grown in constant light were placed in darkness no very marked change in average cell composition other than a drop in carbohydrate content was produced, whereas when the alga had been previously grown with a regimen of 8 hr darkness per 24 hr there was a smaller drop in carbohydrate content on darkening (the initial carbohydrate content being only about half that of cells grown in constant illumination) and also a marked drop in lipid. This could be interpreted as indicating that carbohydrate is the first food reserve to be utilized, after which lipid is consumed. It appears probable that much of the carbohydrate in the cultures grown with light and dark periods is not available as reserve food. No indication of the nature of the fish toxin found on darkening cultures of *P. parvum*, grown in constant illumination, was obtained by studying changes in gross chemical composition.

## INTRODUCTION

WHILST there has been a considerable amount of work on various rather specialized aspects of the chemistry of a few unicellular algae, little has been done to account for the entire cell in chemical terms and very few per cell values have been determined. The majority of values have been given as percentages of dry or wet weight. The former values are not as generally useful as per cell values since the amounts of storage products, such as lipid and carbohydrate, vary markedly with the state of nutrition of the cell. Values on a wet weight basis are, in general, of less value in experiments carried out over a period of time unless any changes in the degree of hydration of the cells during the course of the experiment are given.

The fine structure of all of the organisms studied (except *Olisthodiscus* sp.) have been described: *Chrysochromulina kappa*[1,2]; *Chrysochromulina polylepis*[3]; *Pedinomonas minor*[4]; *Prymnesium parvum*[5,6].

The present work was undertaken as a preliminary to further biochemical studies on these organisms.

## RESULTS

The results of the analyses on *Chrysochromulina kappa*; *Chrysochromulina polylepis*; *Prymnesium parvum*; *Olisthodiscus* sp. (Plymouth No. 239) and *Pedinomonas minor* are shown

[1] M. PARKE, I. MANTON and B. CLARK, *J. Marine Biol. Assoc. United Kingdom*, **34**, 579 (1955).
[2] I. MANTON and G. F. LEEDALE, *J. Marine Biol. Assoc. United Kingdom*, **41**, 519 (1961).
[3] I. MANTON and M. PARKE, *J. Marine Biol. Assoc. United Kingdom*, **42**, 565 (1962).
[4] H. ETTL and I. MANTON, *Nova Hedwigia*, **8**, 421 (1964).
[5] I. MANTON and G. F. LEEDALE, *Arch. Mikrobiol.* **45**, 285 (1963).
[6] I. MANTON, *J. Roy. Microscop. Soc.* **83**, 317 (1964).

TABLE 1. THE GROSS CHEMICAL COMPOSITION OF SOME ALGAE EXPRESSED IN AMOUNTS PER AVERAGE CELL

| Assay | *Chryso-chromulina kappa* | *Chryso-chromulina polylepis* | *Olistho-discus* sp. | *Pedinomonas minor* | | *Prymnesium parvum* (Israeli strain) | *Prymnesium parvum* (Israeli strain)* | *Prymnesium parvum* (Droop strain) | *Prymnesium parvum* (Droop strain)* |
|---|---|---|---|---|---|---|---|---|---|
| Time of growth (days) | 67 | 32 | 42 | 20 | 27 | 12 | 12 | 11 | 13 |
| Culture cell concentration ($\times 10^{-6}$ ml) | 0·82 | 0·23 | 0·92 | 8·98 | 8·55 | 3·20 | 2·71 | 2·13 | 2·49 |
| Cell volume ($\times 10^{-10}$ ml) | 2·04 | 7·35 | 19·0 | 0·80 | - | 3·27 | 3·30 | 5·61 | 4·84 |
| Dry weight (pg) | 30·7 | 101·6 | 248·0 | 11·19 | 12·15 | 43·3 | 56·5 | 61·4 | 59·5 |
| Dry weight as % wet weight | 15·0 | 13·8 | 13·1 | 14·0 | — | 13·2 | 17·1 | 10·9 | 12·3 |
| Protein (pg) | 9·6 | 30·5 | 103·4 | 4·6 | 2·94 | 19·8 | 16·6 | 18·8 | 16·8 |
| Lipid (pg) | 10·0 | 48·4 | 34·4 | 2·36 | 1·81 | 11·32 | 12·21 | 23·3 | 22·7 |
| Lipid-free dry weight (pg) | — | 61·2 | 210·0 | 8·71 | 10·47 | 32·22 | 42·61 | 41·8 | 37·0 |
| Carbohydrate (pg) (as glucose) | -- | - | 12·0 | 3·10 | 6·90 | --- | 13·9 | - - | 19·35 |
| Total nucleic acid (pg) | 0·435 | 1·04 | 6·31 | 0·839 | 0·526 | 0·618 | 1·367 | 1·136 | 1·287 |
| DNA (pg) | 0·129 | 0·315 | 1·948 | 0·095 | 0·104 | 0·273 | 0·309 | 0·317 | 0·290 |
| RNA (pg) | 0·306 | 0·725 | 4·36 | 0·744 | 0·422 | 0·345 | 1·058 | 0·819 | 0·997 |
| "RNA" (orcinol) (pg) | 0·60 | 3·2 | 5·84 | 0·905 | 1·726 | — | -- | 2·145 | - |
| Phospholipid (pg) | 0·75 | 2·44 | 7·71 | 0·51 | 0·39 | 0·489 | 0·860 | 0·60 | 0·94 |
| Acid soluble phosphorus (pg) | 0·057 | 0·156 | 1·71 | 0·014 | 0·028 | 0·131 | 0·156 | 0·221 | 0·227 |
| Phosphoprotein phosphorus (pg) | 0·018 | 0·034 | 0·23 | 0·016 | 0·009 | 0·010 | 0·011 | 0·013 | 0·004 |
| Total phosphorus (pg) | 0·149 | 0·390 | 2·88 | 0·133 | 0·105 | 0·223 | 0·338 | 0·371 | 0·397 |
| Chlorophyll *a* (pg) | | | 2·58 | 0·278 | 0·185 | 0·729† | 0·846 | - | |
| Chlorophyll *b* (pg) | | - - | nil | 0·133 | 0·125 | nil | nil | | - |
| Chlorophyll *c* (pg) | | | 0·81 | | - | | 0·403 | - | |
| Chlorophyll $c_1$‡ (pg) | | | 0·62 | | | - | - - | | - |

* Grown with constant illumination. The other *P. parvum* cultures were grown with alternating light and dark periods.

† A 30-day culture, grown with light and dark periods, of cell concentration $5·6 \times 10^{6}$ ml, gave 1·10, nil, 0·47, and 0·48 pg per average cell for chlorophyll *a*, *b*, *c* and $c_1$‡ respectively.

‡ Determined by the method of Parsons.[29]

TABLE 2. THE GROSS CHEMICAL COMPOSITION OF SOME ALGAE EXPRESSED AS PERCENTAGES OF CELL DRY WEIGHT

| Assay | *Chryso-chromulina kappa* | *Chryso-chromulina polylepis* | *Olistho-discus* sp. | *Pedinomonas minor* | | *Prymnesium parvum* (Israeli strain) | *Prymnesium parvum* (Israeli strain)* | *Prymnesium parvum* (Droop strain) | *Prymnesium parvum* (Droop strain)* |
|---|---|---|---|---|---|---|---|---|---|
| Relative cell volume† | 2·55 | 9·19 | 23·75 | 1·00 | — | 4·09 | 4·12 | 7·01 | 6·05 |
| Protein | 31·3 | 30·0 | 41·7 | 41·1 | 24·2 | 45·7 | 29·4 | 30·6 | 28·2 |
| *Lipid* | 32·6 | 47·6 | 13·9 | 21·1 | 14·9 | 26·1 | 21·6 | 37·9 | 38·2 |
| Lipid-free dry weight | — | 60·2 | 84·7 | 77·8 | 86·2 | 74·4 | 75·4 | 68·1 ± 2·1 | 62·2 |
| Carbohydrate | — | — | 4·8 | 27·7 | 56·8 | — | 24·6 | — | 32·5 ± 1·1 |
| Total nucleic acid | 1·42 | 1·02 | 2·54 | 7·50 | 4·33 | 1·43 | 2·42 | 1·85 | 2·16 |
| DNA | 0·42 | 0·31 | 0·78 | 0·85 | 0·86 | 0·63 | 0·55 | 0·52 | 0·49 |
| RNA | 1·00 | 0·71 | 1·76 | 6·65 | 3·47 | 0·80 | 1·87 | 1·33 | 1·67 |
| Phospholipid | 2·44 | 2·4 ± 0·077 | 3·1 | 4·6 | 3·2 | 1·1 | 1·5 | 1·0 | 1·6 |
| Acid soluble phosphorus | 0·19 ± 0·012 | 0·15 | 0·69 | 0·13 | 0·23 | 0·30 | 0·28 | 0·36 | 0·38 |
| Phosphoprotein phosphorus | 0·06 ± 0·014 | 0·033 ± 0·004 | 0·09 | 0·14 ± 0·005 | 0·07 | 0·023 | 0·02 | 0·02 | 0·007 |
| Total phosphorus | 0·49 ± 0·03 | 0·38 | 1·16 | 1·19 | 0·86 | 0·51 | 0·60 | 0·60 | 0·67 |
| Chlorophyll *a* | — | — | 1·04 | 2·5 | 1·5 | 1·7 | 1·5 | — | — |
| Chlorophyll *b* | — | — | nil | 1·2 | 1·0 | *nil* | *nil* | — | — |
| Chlorophyll *c* | — | — | 0·33 | — | — | — | 0·7 | — | — |
| Chlorophyll *c*‡ | — | — | 0·25 | — | — | — | — | — | — |

* Grown in constant illumination. The other *P. parvum* cultures were grown with both light and dark periods.
† Cell volume relative to the smallest organism *Pedinomonas minor*.
‡ Determined by the method of Parsons.[29]
— Indicates not determined.

in Table 1, expressed as amounts per average cell; and as percentages of cell dry weight in Table 2. The percentage of total dry mass accounted for in terms of known materials is given in Table 3. All the cultures were harvested towards the end of the logarithmic phase of growth

TABLE 3. THE PERCENTAGE OF THE TOTAL DRY MASS OF ALGAL CELLS ACCOUNTED FOR IN TERMS OF KNOWN SUBSTANCES

| Algae | Per cent total dry mass accounted for | Time of growth (days) |
|---|---|---|
| *Chrysochromulina kappa* | 65·5* | 67 |
| *Chrysochromulina polylepis* | 78·8* | 32 |
| *Olisthodiscus sp.* | 63·6 | 42 |
| *Pedinomonas minor* | 97·5 | 20 |
| *Pedinomonas minor* | 100·4 | 27 |
| *Prymnesium parvum* (Israeli strain) | 73·5* | 12 |
| *Prymnesium parvum*† (Israeli strain) | 78·3 | 12 |
| *Prymnesium parvum* (Droop strain) | 70·8* | 11 |
| *Prymnesium parvum*† (Droop strain) | 101·5 | 13 |

* Not including carbohydrate, which was not assayed in these cases.
† Grown in continuous light.

(except for the Chrysochromulinae in which the phase of growth was not known; and the 27-day culture of *Pedinomonas* which was harvested at the end of the stationary phase).

In single determinations, the mean time taken to double the cell population at about day seven of growth (grown in constant light or with light and dark periods), was found to be 2·3 days for the Droop strain, and 2·9 days for the Israeli strain, of *Prymnesium parvum*. No evidence of synchronization of cell division was obtained in this organism in the cultures grown with light and dark periods.

The 27-day culture of *Pedinomonas* gave unusual results on harvesting. A fair proportion of the cells were of only slightly greater density than the culture medium and thus could not be tightly packed on centrifugation. There was also a tightly packed lower deposit. Microscopic examination indicated that the less dense material consisted of intact non-motile cells and that virtually no cellular debris was present, as would be expected if the culture was degenerating. Electron microscopic examination of thin sections of embeddedcells indicated that most of the cells from this culture had large osmiophilic extraplastidic globules of about 1 $\mu$ diameter. These globules were not apparent in the younger cultures. As far as could be judged from the electronmicrographs the amount of plastidic storage material was similar in cells from the young and the older cultures.

The time course of colour production in the anthrone reaction for carbohydrate, using cells in the logarithmic phase of growth, for *Prymnesium*, *Pedinomonas* and *Olisthodiscus* resembled those found by Yemm and Willis[7] for D-glucose, D-galactose and D-mannose.

[7] E. W. YEMM and A. J. WILLIS. *Biochem. J.* 57, 508 (1954).

As Reich and Parnas[8] had shown that darkening of a culture of *Prymnesium parvum* (Israeli strain) grown with constant illumination, led to the development of ichthyotoxic activity in the culture, it was felt worthwhile to determine whether any difference in gross chemical composition of this organism could be detected after darkening a similar light-grown culture for seven hours. Two such experiments yielded similar results. There was a mean increase in cell concentration of 8 per cent during the dark period. The major change was a drop of about 15 per cent in cell dry weight, whilst there was only a 3 per cent fall in mean cell volume. The former was largely due to a 17 per cent drop in lipid-free dry weight, there being little change in protein or lipid. There was a mean drop in carbohydrate content of 37 per cent, probably due to utilization of food reserves. Little change was found in the other components assayed.

Darkening of *P. parvum* cultures grown with light and dark periods produced slightly different results. There were drops in average cell volume, 15 per cent; dry weight, 10 per cent; lipid, 29 per cent; and carbohydrate, 10 per cent. The cell concentration increased by 5 per cent. The utilization of lipid as well as carbohydrate probably reflects inadequate carbohydrate reserves. The carbohydrate concentration in these cells was about half that of cells grown in continuous light and presumably little was available as reserve food. Lipid concentration was similar in both types of culture.

## DISCUSSION

The results show that these organisms, which have volumes varying over 24-fold, have a relatively similar gross chemical composition. This finding agrees with that of Parsons *et al.*[9] who found similar results for marine phytoplankton. Protein ranges from 24–46 per cent, lipid 14–48 per cent, and carbohydrate 5–57 per cent of cell dry weight. Lipid and carbohydrate will obviously be very variable, depending upon the state of nutrition of the cells (e.g. as seen in *Pedinomonas*). The ranges for the minor constituents are: nucleic acid, 1–7 per cent; DNA, 0·31–0·86 per cent; RNA, 0·7–6·65 per cent; phospholipid, 1·0–4·6 per cent; acid soluble phosphorus, 0·13–0·69 per cent; phosphoprotein phosphorus, 0·02–0·14 per cent; total phosphorus, 0·49–1·19 per cent of dry weight. It is particularly noticeable that the DNA per average cell is relatively constant in the same organism, and that it is the same in the two strains of *P. parvum*. This presumably reflects a constant amount of DNA per non-dividing cell and either a constant proportion of dividing cells in the cultures at any one time or a very low proportion of cells with a twofold amount of DNA. These results would enable one to determine the number of cells in a sample by means of DNA rather than by cell counting.

The DNA values obtained are of the order of magnitude which might be expected from their cell sizes. The observed parallelism between DNA content and cell size has been noted before and it has been suggested that this is due to a requirement for a number of replicates of the genetic unit to provide enough metabolic units in the cytoplasm (assuming that the genetic units that control the formation of these units operate at a relatively slow rate).[10]

The proportion of RNA is much greater in *Pedinomonas* than in the other organisms studied. Electron-microscopically this organism shows a higher relative concentration of ribosomes than the other organisms and the RNA results are probably mainly a reflection of this.

[8] K. Reich and I. Parnas, *J. Protozool.* **9**, 38 (1962).
[9] T. R. Parsons, K. Stephens and J. D. H. Strickland, *J. Fisheries Research Board Can.* **18**, 1001 (1961).
[10] G. Brawerman and H. S. Shapiro. In *Comparative Biochemistry* (Edited by M. Florkin and H. S. Mason). Vol. 4, p. 107. Academic Press, New York (1962).

*Olisthodiscus* shows a relatively higher concentration of acid-soluble phosphorus than the other organisms. This alga has a much lower lipid and carbohydrate content than the other organisms and only 64 per cent of its total dry weight has been accounted for in terms of known substances. The organism was found to have an ash content of 10·4 per cent of the dried weight. Thus about 25 per cent of the dry weight of the cell is unidentified. It is possible that the higher acid-soluble phosphorus reflects, at least in part, an unidentified storage product.

The dry mass of the cells studied can be accounted for reasonably well (excepting *Olisthodiscus*) in terms of defined materials. Values lower than 100 per cent can be expected in the marine and brackish organisms as no estimation of inorganic salt content was made. These chrysophycean organisms are also covered with layers of scales which are of a very resistant, but unknown nature (J. C. Green, personal communication). It is probable that these were undetermined in assay.

Also of interest are the chlorophyll values per average cell. *P. parvum* has two plastids (on average) per cell and *Pedinomonas* has one. The figures are thus the per plastid values in the case of *Pedinomonas*, and twice this in the case of *Prymnesium*.

The results of the darkening experiments with cultures of *P. parvum* grown with constant illumination indicate that either the extracellular fish toxin released is of extremely high potency (as there is little change in cell composition, particularly in protein and lipid concentration, on darkening) and perhaps released from stored toxin or toxin precursor,[11] or that there is very rapid synthesis and release of toxin on darkening: or both. If, as has been suggested, the toxin is of protein nature[12] one might expect an increase of ribosomes (and RNA) on darkening in the latter cases. This is not found. Some of the properties of the toxin are not consistent with it being a protein and Shilo and Rosenberger[13] have suggested that it may be a lipid.

The changes on darkening a culture of *P. parvum* grown with dark periods can be interpreted as indicating that much of the carbohydrate in these organisms is not available as a food reserve, and, taken in conjunction with the darkened light-grown culture experiments, that carbohydrate is the reserve foodstuff to be first utilized, followed by lipid. Interpretation of the results of darkening experiments is rendered difficult by the continued division of the organism during the dark period. The results, which are per *average* cell, to some extent reflect a redistribution of cellular material among a greater number of organisms. One must assume that synthesis is considerably reduced during the period of darkening, although *P. parvum* shows some heterotrophic characters.[14]

A comparison of the results with those of other workers shows a higher proportion of lipid and pigment in the *Chrysophyceae* and *Chlorophyceae* than those found by Parsons *et al.*[9] and a lower proportion of phosphorus. Protein contents appear to be somewhat lower than their values. The protein, lipid and carbohydrate values for *Pedinomonas* are very similar to those found by Ketchum and Redfield[15] for a number of *Chlorophyceae*. These authors found that cells grown in a nitrogen deficient medium had a low protein content and a raised carbohydrate content. This observation may well explain the results from the 27-day culture of *Pedinomonas*. It appears probable that the greater proportion of carbohydrate in the older culture is due to carbohydrate-containing extraplastidic globules. These have a different

[11] R. F. ROSENBERGER, Unpublished data (cited in Ref. [13]) (1960).
[12] M. SHILO (Shelubsky), M. ASCHNER and M. SHILO (Goldsweig) *Bull. Res. Council Israel* **2**, 446 (1953).
[13] M. SHILO and R. F. ROSENBERGER, *Ann. N. Y. Acad. Sci.* **90**, 866 (1960).
[14] J. J. A. MCLAUGHLIN, *J. Protozool.* **5**, 75 (1958).
[15] B. H. KETCHUM and A. C. REDFIELD, *J. Cell. Comp. Physiol.* **33**, 281 (1949).

electronmicroscopic appearance to the plastidic storage material. The DNA and volume per average cell are of the same order as those found by Tamiya and his co-workers[16] for *Chlorella ellipsoidea*. Similarly the dry weight, phosphorus, nitrogen, carbohydrate, lipid and protein values of *Pedinomonas* resemble those obtained by Hase *et al.*[17] for cells of *Chlorella ellipsoidea* of similar size to *Pedinomonas*.

Little information is available about algae related to the other organisms; Gibbs[18] has reported chlorophyll *a* values per cell for *Ochromonas danica* which were about half those here obtained with *Prymnesium parvum*.

Bidwell[19] has reported that mannitol is the main alcohol soluble product of photosynthesis in *Olisthodiscus* sp. and that the main insoluble carbohydrate product gave rise to glucose on hydrolysis. The results of the anthrone reaction agree with these findings.

The findings here presented add a little to the meagre literature on the chemical composition of these types of organism. However, it is obvious that many more species need to be examined so as to give a fuller picture. This type of investigation may prove useful as an aid to the taxonomy of the algae, provided that growth conditions are well defined, and also as a guide to their nutritive value.

## EXPERIMENTAL SECTION

### *Cultures*

*Chrysochromulina kappa* (Plymouth K); *Chrysochromulina polylepis* (Plymouth No. 136) and *Olisthodiscus* sp.* (Plymouth No. 239) were provided as unialgal bacteria-containing cultures by Dr. Mary Parke of the Plymouth Laboratory. They were grown in Erdschrieber culture media at 14° with a regimen of 16 hr of light (200 lumen/ft$^2$) and 8 hr of darkness per day.

*Pedinomonas minor* (Strain 17B) had been provided as a bacteria-containing unialgal culture by Dr. Hans Ettl and was later rendered axenic by a dilution technique. It was grown under the conditions described by Ricketts[21] at 14°.

Axenic *Prymnesium parvum* Carter (Israeli strain) was grown at 24° as described by Ricketts[22] and also under similar conditions but with a regimen of constant light (200 lumen/ft$^2$). Dr. M. R. Droop of the Marine Station, Millport, also kindly provided a sample of his strain of *Prymnesium parvum* (axenic) which was grown in a similar manner to the Israeli strain.

### *Harvesting*

The cultures were pooled, well mixed, and samples taken for cell counts as described later. (K and 136 cultures were in 1·5 l. volumes, the remainder in 100 ml volumes.) The cultures were then centrifuged at 1000 *g* for 10 min and the supernatants decanted. The deposit, in the case of the *Chrysochromulinae*, was resuspended quantitatively in 10 ml sea-water and recentrifuged at 1000 *g* /15 min. The volume of deposit was noted and the supernatant removed and

* This alga has been classified in the Order *Heterochloridales* in the Class Xanthophyceae, but its systematic position is uncertain (see Parke and Dixon[20]).

16 H. Tamiya, Y. Morimura, M. Yokota and R. Kunieda, *Plant Cell Physiol.* **2**, 383 (1961).
17 E. Hase, Y. Morimura and H. Tamiya, *Arch. Biochem. Biophys.* **69**, 149 (1957).
18 S. P. Gibbs, *J. Cell Biol.* **15**, 343 (1962).
19 R. G. S. Bidwell, *Can. J. Bot.* **35**, 945 (1957)
20 M. Parke and P. S. Dixon, *J. Marine Biol. Assoc. United Kingdom*, **44**, 499 (1964).
21 T. R. Ricketts, *Phytochem.* **4**, 725 (1965).
22 T. R. Ricketts, *J. Roy. Microscop. Soc.* **83**, 459 (1964).

combined with the initial supernatant. This procedure produced an algal deposit which was estimated to contain not more than 5 per cent by volume of bacteria.

The *Olisthodiscus* culture used contained bacteria which were almost all adhering to the culture flask. By means of careful decantation (which resulted in a few clumps of bacteria in the supernatant) and filtration through Whatman No. 54 filter paper it was possible to rid the culture of practically all its bacteria as judged microscopically. The cells were still motile after this treatment, although the cell concentration was reduced by about 50 per cent. It was not possible to determine whether there had been a selection of smaller cells using this procedure. This cell suspension and the pooled *Pedinomonas* and *Prymnesium* cultures were harvested by centrifugation at 1000 *g* / 10 min, the supernatants decanted and pooled, and the deposit resuspended in about 10 ml of supernatant and quantitatively transferred to a graduated centrifuge tube. The cells were then packed to constant volume at 1000 *g*. The packed cell volume was noted and the supernatant combined with the initial supernatant. Cell counts were done on all the pooled supernatants. Knowing the volume and cell concentration of the culture used and the cell counts of the combined supernatants it was possible to calculate the number of cells present in the packed deposit. It was found impossible to resuspend the packed deposit uniformly in order to carry out accurate cell counts for direct determination of the number of cells in the deposits.

Cell counts were carried out by mixing two volumes of well mixed culture with one volume of 25% (v/v) aqueous formalin. A Fuchs-Rosenthal haemocytometer was then flooded and the volume containing in the region of a thousand cells noted. Two entirely separate assays were carried out on each culture or supernatant.

In all cases the packed cell deposit was rapidly cooled in an ice bath after removal of the final supernatant and then resuspended to a known volume with distilled water. In most cases it was impossible to remove completely the culture medium supernatant without loss of cells from the deposit. The resuspended deposit therefore contained about 0·1 ml of culture medium. It was calculated that this would make negligible difference, at the dilutions used, to the phosphate and nitrogen assays later carried out. Chlorophyll assays were immediately carried out on the resuspended deposit and the remainder of the suspension stored at - 20 until required for assay.

*Assays*

The cell volume was calculated by dividing the packed cell volume by the cell number, the assumption being made that the cells were sufficiently flexible to pack completely and exclude culture medium. An approximate cell wet weight was calculated by assuming a density of one for the packed cells.

Dry weights were determined by taking a known aliquot of the aqueous cell suspension and drying at 100 to constant weight.

Lipid was determined gravimetrically, utilizing the dried deposit mentioned above. This was extracted successively with 3 × 15 ml alcohol:ether 3:1 (v/v) and 2 × 15 ml acetone, centrifuging between each extraction, and decanting the supernatants. The final deposit was extracted with 5 ml boiling chloroform, cooled, and 10 ml alcohol: ether 3:1 (v/v) added to allow sedimentation by centrifugation. The supernatants (containing the total lipids) were all pooled, evaporated to dryness, dessicated, and weighed. The lipid-free deposit was also dried to constant weight.

Initially these gravimetric assays were carried out in duplicate, but as the total harvested packed cell deposit generally had a volume of only about 1 ml later assays were carried out

singly. The volumes of cell suspension used were such that the dry weight of the aliquot was about 50 mg.

Duplicate aliquots of the cell suspension were fractionated by the method of Schneider[23] into acid-soluble, phospholipid, nucleic acid and protein fractions. These fractions were each analysed for organic phosphorus content. Aliquots of the fractions were evaporated to dryness, together with blank and standard phosphate tubes. To each was added 0·25 ml M.A.R. sulphuric acid. The contents were then digested by heating, with the aid of 100 vol hydrogen peroxide (not more than 0·25 ml), added dropwise, and cooling somewhat before each addition. The tubes were finally cooled and 2 ml of water saturated with $SO_2$ added. They were then heated at 100° for 15 min, cooled, neutralized to thymol-phthalein with 5 N NaOH, and made up to a known volume. Phosphorus was then determined by the method of Fiske and Subbarow.[24] This method of assay was evolved because it was found initially that hydrogen peroxide interfered with the phosphate assay, even after prolonged boiling of the digest. Total phosphorus was calculated by summation of the phosphorus in the various fractions. Phospholipid was calculated by assuming a conversion factor of phospholipid phosphorus $\times 25$.

Initially the deoxyribonucleic acid and ribonucleic acid of the nucleic acid extract were determined as described in Lathe and Ricketts.[25] It was found that the Dische reaction had too low a sensitivity to give accurate results for the amounts of material available. Deoxyribonucleic acid was therefore determined by the method of Ceriotti[26] using the same standards as before[25]. In some organisms the orcinol reaction for ribonucleic acid assay gave fallacious results, the calculated ribonucleic acid phosphorus being greater than the total nucleic acid phosphorus. Absorption curves of the colour produced in the orcinol reaction in these circumstances were identical to that produced by ribonucleic acid. This indicated that the trichloroacetic acid hydrolysis of the cells, used in preparation of the nucleic acid extract, had released bound ribose-like sugars into solution. The results of the orcinol assays are nevertheless given in the Results section (expressed as "RNA"), corrected for the colour produced by deoxyribonucleic acid. No estimation of polyphosphate content was made in the nucleic acid phosphorus assays and therefore these values and those of the RNA may be somewhat inaccurate. Other information indicates that any errors due to polyphosphate are unlikely to be, at the most, more than 20 per cent. Ribonucleic acid phosphorus content was taken as the difference between the nucleic acid phosphorus and deoxyribonucleic acid phosphorus.

Nitrogen was determined by distillation in a Markham's apparatus following micro-Kjeldahl digestion. Protein was calculated from this by assuming a conversion factor of $N \times 6{\cdot}25$, after correcting for the nitrogen contents of the nucleic acids which were assumed to be 15 per cent. This nucleic acid correction was about 5 per cent in all the organisms studied except *Pedinomonas*, in which it was about 15 per cent. Carbohydrate was determined by the anthrone reaction using the method of Trevelyan and Harrison.[27] The method was calibrated using glucose standards and the results are expressed as glucose.

Chlorophylls were determined by the method of Parsons and Strickland,[28] and chlorophyll

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*c*, additionally, by the method of Parsons.[29] When no values are given for chlorophyll *c* the method of estimation was that of MacKinney.[30] Carotenoids were not assayed as no suitable simple method was available.[25]

The total percentage of the dry weight accounted for in terms of known substances was taken as the sum of percentage dry weights of lipid, protein, carbohydrate, nucleic acid and acid soluble phosphorus.

All assays (except the gravimetric ones) were carried out in duplicate. For clarity, the deviation from the mean value is not given but is less than ± 3 per cent unless given in Table 2. The values refer to the average cell composition.

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