this limit of detectability. An experimental determination of precision on samples of simulated DNA, having a bromine content equivalent to about 1 percent replacement, indicates an error of approximately $\pm 1$ percent. This error is the relative standard deviation of the determinations, for one-minute integration, of fractional bromine content of eight separate 35-$\mu$g samples taken from the same preparation of simulated DNA with added bromine.

The weight of the sample need not be accurately determined, since the fraction by weight of bromine in DNA, $P_{Br}$, comes directly out of the experimental determination of ratios and the ratios are independent of the mass of the sample. Assays have been carried out on samples as small as 10 $\mu$g, although with this size of sample there is a loss of precision compared to 35-$\mu$g samples.

The technique is also applicable to the analysis of iodine, and it can be used to determine the degree of substitution of iodinated as well as brominated purine and pyrimidine analogs. Because of the higher ionization energy of the K shell of the iodine atom and the higher energy of the I-K$\alpha$ photon, two basic changes in the instrument are required to obtain an equivalent order of magnitude of detectability for iodine. The x-ray tube must have the capacity to operate at a voltage of at least 100 kv, and the proportional counter must be filled with a gas having a higher absorption for I-K$\alpha$ than the argon has. The use of a krypton gas at 1 atm should be adequate. The K$\alpha$ lines of bromine and iodine may be resolved easily since they are sufficiently separated. This indicates the feasibility of a simultaneous analysis for iodine and bromine with a single proportional counter (*6*).

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25 October 1963

## Zoospores in *Scenedesmus obliquus*

Abstract. *In basal medium without a nitrogen source a 3-day-old culture of* Scenedesmus obliquus *produced zoospores, which were biflagellated, had a parietal chloroplast, lacked a pyrenoid, and were apparently not walled. Identical results were produced with new clones. A reconsideration of the relationships and classification of this organism is now essential.*

*Scenedesmus*, which might be the most widely distributed fresh-water algal genus (*1*), has been studied extensively both in nature and in the laboratory. The genus was monographed twice during the last 50 years (*2*) with the taxonomic disposition of many isolates based on prolonged observation of laboratory cultures. Reproduction is said to occur solely by the production of nonmotile spores which become arranged in a definite pattern within the parent cell before release as a colony.

Beginning with Beijerinck's work with *S. acutus*, in which only unicells were formed in basal medium (*3*), there have been many investigations dealing with pleomorphism (*4*). Stages of *Scenedesmus* resembling *Dactylococcus, Chlorella, Oocystis*, and *Ankistrodesmus* are common in culture.

When *Scenedesmus obliquus* (Fig. 1) (*5*) was grown in basal medium (*6*) from which the ammonium nitrate had been withheld, a few zoospores were observed (Fig. 2). To eliminate the possibility of a mixed culture, we established new clones. Upon subsequent starvation zoospores appeared in six clonal cultures (*7*). No motility or any evidence of foreign organisms were noted in control flasks of basal medium. Aseptic procedures were used throughout.

Growth in basal medium, from which the ferric chloride, magnesium sulfate, or the potassium phosphates had been withheld, did not stimulate motility. With ammonium nitrate starvation, zoospores were evident after 3 days in continuous fluorescent illumination (4400 lu/m$^2$) and motility terminated after 36 hours. Although there were not usually great numbers of zoospores, and the effects of starvation were apparent, a parietal chloroplast, two flagella of equal length, and an occasional stigma were observed. Pyrenoids were always present in vegetative cells, but were not seen in the zoospores. Apparently, they do not possess a wall; elongate, fusiform and spherical types were observed. When zoospores in a hanging drop became quiescent they assumed a spherical shape and lost their flagella, but in the absence of a nitrogen source there was no further development. Techniques for transferring zoospores to a basal medium, and at the same time retaining their viability, will have to be developed.

With the presence of flagellated cells, induced by conditions which stimulate sexuality in some species of *Chlamydomonas* (*8*), the existence of a sexual phase in *Scenedesmus* is now a possibility.

Some time ago, Fritsch (*9*) suggested that a motile stage might be found eventually in members of the family Coelastraceae, in which *Scenedesmus* is placed. Zoospores and gametes are known in the colonial forms of the related Hydrodictyaceae. Furthermore,

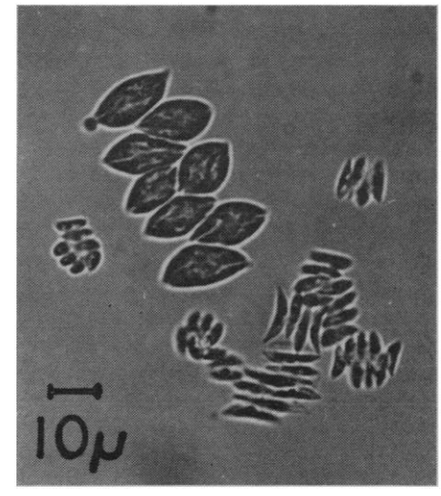


Fig. 1. *Scenedesmus obliquus* colonies.

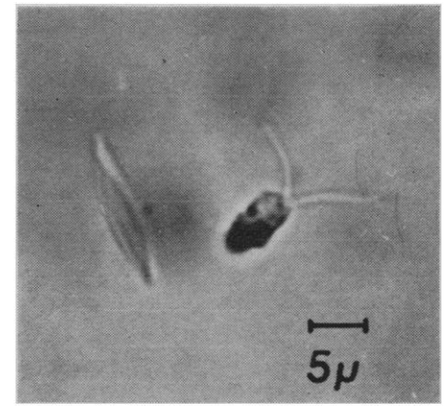


Fig. 2. A zoospore from an ammonium nitrate starved culture of *S. obliquus*. A wall from a mother cell is seen at the left. Material killed with $I_2KI$.

Fritsch questioned the feasibility of maintaining two families of colonial or coenobial forms. Discovery of zoospores in *Scenedesmus* makes a reconsideration of these families necessary (*10*).

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2 October 1963

## Feeding Response in *Aedes aegypti*: Stimulation by Adenosine Triphosphate

Abstract. *Taste receptors which evoke ingestion of blood in the mosquito,* Aedes aegypti *L., are stimulated by adenosine tetraphosphate, adenosine triphosphate, and adenosine monophosphate, in decreasing order. No other nucleotide is effective. Certain chelators can partially simulate the effect of nucleotides. The feeding response is elicited only at an osmotic pressure close to that of blood, and requires the presence of sodium ions.*

Behavioral studies on mosquitoes indicate that they possess contact chemoreceptors which react to water, sugars, salts and blood (*1*). One can assume that, as in the blowfly, there exist specifically sensitive neurons: some specifically sensitive to sugars, others to salts (*2*) and still others to water (*3*). Unlike the blowfly, which does not ordinarily feed on blood, the mosquito also possesses taste receptors that are stimulated by blood and probably are located on the dorsal wall of the buccal cavity. These chemoreceptors are specifically stimulated by adenosine phosphates of the blood cells in the diet (*4*). In the experiments reported here, our aim was to elucidate the specific molecular mechanism of stimulation of these taste receptors by adenine nucleotides.

In each experiment, about 100 or more female mosquitoes, of the species *Aedes aegypti* L., were used. They were held in cylinders covered with Silver-light membranes (*5*) through which they could probe and suck blood (*6*) or any other solution containing the stimulants. Unless starved, mosquitoes do not usually suck water or sugar solutions through a membrane. All the solutions offered were at 38°C, and contained a small amount of red dye (Safranin O) so that the percentage of the mosquitoes which fed on each solution through the membrane could be determined by the presence of the dye in the midgut. The nucleotides, at concentrations from $10^{-2}M$ to $10^{-8}M$, were offered in $0.15M$ solutions of NaCl adjusted to *p*H 7 by NaOH.

The percentage of the mosquitoes which fed on each solution was used as an index of the stimulatory effect. Among the adenine nucleotides tested, the effectiveness increased in the following order: adenine-5-monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), and adenosine tetraphosphate (A-tetra-P). The mono- and tri-phosphates of inosine (IMP, ITP), guanosine (GMP, GTP), and cytidine (CMP, CTP) were ineffective. The results indicate that the presence of the adenine moiety is a prerequisite for the effect. However, the number of the phosphate groups is also of importance (Table 1).

Hosoi (*4*) found that an osmotic pressure isotonic to blood was essential to obtain stimulation by AMP. We investigated the role of osmotic pressure and of various ions by offering the mosquitoes AMP or ATP in increasing concentrations of NaCl or other salts. The lowest concentration of NaCl which gave maximum results (more than 70 percent feeding) was $0.15M$. When the saline was replaced by an isotonic solution of a nonelectrolyte, never more than 25 percent feeding was observed. With distilled water (containing AMP) there was practically no feeding. With NaCl at $0.037M$, the percentage of mosquitoes feeding (Table 1) could not be further increased by adjusting the tonicity with lactose. Thus, it seems that in addition to the need for osmotic pressure, $Na^+$ is specifically required for the response to be at an optimum. When $Na^+$ was replaced by $K^+$, $Ca^{++}$ or $Mg^{++}$, the percentage of mosquitoes feeding was low, and when these ions were added to AMP in NaCl, a strong inhibitive effect was exerted (Table 2).

The dependence of the system on $Na^+$, and the inhibition of $K^+$, recalls the now well-known Hodgkin-Huxley-Katz theory of excitation which ascribes depolarization to a sudden increase in the $Na^+$ conductance of the membrane, followed by increase in $K^+$ conductance. It was, therefore, suspected that ATP, known as a good chelator, like any other polyphosphate (*7*), acts upon divalent ions of the

Table 1. The feeding response of mosquitoes exposed to nucleotides and calcium-binding agents. All compounds were offered in $0.15M$ solutions of NaCl. For abbreviations see text.

| Compound | No. exposed | Percent feeding ($10^{-2}M$) | No. exposed | Percent feeding ($10^{-3}M$) |
|---|---|---|---|---|
| ADP | 537 | 76 ±1.1* | 574 | 17 ±0.9 |
| AMP | 580 | 70 ±1.6 | 392 | 31 ±1.6 |
| ATP | 631 | 85 ±0.7 | 1037 | 58 ±0.8 |
| A-tetra-P | 322 | 85 ±1.6 | 504 | 67 ±1.6 |
| IMP | 134 | 1 ±0.0 | | |
| ITP | 165 | 1 ±0.0 | 268 | 0 ±0.0 |
| GMP | 155 | 2 ±0.0 | | |
| GTP | 195 | 2 ±0.0 | 209 | 1 ±0.0 |
| CMP | 94 | 0 ±0.0 | 75 | 1 ±0.0 |
| CTP | 192 | 0 ±0.0 | 217 | 0 ±0.0 |
| EDTA | 612 | 20 ±0.8 | 479 | 3 ±0.3 |
| DPA | 562 | 11 ±0.9 | 294 | 3 ±0.5 |
| Na-Oxalate | 170 | 0 ±0.0 | 90 | 1 ±0.0 |

* ± Standard error of the mean.

Table 2. Effect of various ions and nonelectrolytes, in solutions containing $10^{-2}M$ adenosine monophosphate, on the feeding response of mosquitoes.

| Composition of medium | No. exposed * | Percent feeding |
|---|---|---|
| Distilled water | 273 | 2 ± 0.4* |
| $0.037M$ NaCl | 373 | 28 ±2.6 |
| $.075M$ NaCl | 333 | 41 ±1.45 |
| $.15M$ NaCl | 537 | 76 ±1.1 |
| $.30M$ NaCl | 429 | 71 ±2.7 |
| $.15M$ KCl | 363 | 15 ±1.1 |
| $.10M$ $CaCl_2$ | 305 | 6 ±0.8 |
| $.10M$ $MgCl_2$ | 299 | 10 ±1.1 |
| $.15M$ NaCl + $0.15M$ KCl | 259 | 18 ±2.5 |
| $.15M$ NaCl + $0.10M$ $CaCl_2$ | 298 | 6 ±1.1 |
| $.15M$ NaCl + $0.10M$ $MgCl_3$ | 341 | 6 ±0.9 |
| $.30M$ glucose | 288 | 25 ±1.5 |
| $.30M$ sucrose | 476 | 22 ±0.8 |
| $.30M$ sucrose (without AMP) | 379 | 1 ±0.0 |
| $.30M$ lactose | 393 | 21 ±1.1 |
| $.037M$ NaCl + $0.225M$ lactose (isotonic) | 380 | 27 ±1.6 |

* ± Standard error of the mean.