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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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.

dendritic membrane. Among these ions, Ca^{++} was considered as the most likely one to be affected by ATP, since there are numerous data establishing its importance in determining membrane permeability (*8*). Therefore, we studied the action of various compounds which could affect membrane-bound Ca^{++}, such as chelating agents [ethylenediaminetetraacetic acid (EDTA), dipicolinic acid (DPA)], or sodium oxalate, which forms an insoluble salt with Ca^{++}. A slight stimulatory effect was obtained with EDTA and DPA (Table 1) but sodium oxalate was not effective.

Our results suggest that the depolarization of the dendritic membrane depends on the removal of Ca^{++} ions from their sites of binding on the membrane. Adenosine triphosphate could act at this stage because of its chelating properties. In about one quarter of the mosquito population, Ca^{++} removal is sufficient to depolarize the nerve membrane, as shown by the results with the nonelectrolyte solutions. In the bulk of the population, however, the influx of Na^{+}, made possible by the removal of Ca^{++}, is necessary to lower the potential and to depolarize the membrane. It could be assumed that the nucleotide is bound through the adenine moiety to a specific receptor site on the surface of the membrane. The geometry of the ATP molecule may be such that the terminal phosphate groups of the bound nucleotide are brought in juxtaposition to a second site on the surface where the Ca^{++} ion is located. This may explain why ATP is superior to other polyphosphates as a stimulant and, despite its lower formation constant with Ca^{++} ions, is also superior to stronger chelating agents such as EDTA (*9*).

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Ribosomes and Ribonucleic Acids in Three Morphological States of Neurospora

Abstract. *Dormant ascospores contain the same ribosomal population as growing hyphae and resting conidia. This conclusion is based on analyses of sedimentation coefficients and of nucleotide composition of ribosomes, ribosomal RNA, and soluble RNA. Thus dormancy is not characterized by the absence of the important parts of the protein-synthesizing apparatus.*

Changes in the physiological state are accompanied by variations in the ribosomal population, affecting class distribution or physicochemical properties, of bacterial (*1*), yeast (*2*), and plant and animal cells (*3*); variations also occur in the nucleotide composition of ribonucleic acids (RNA) of protozoan cells (*4*). We have, therefore, compared the properties of the ribosomes and RNA present in three morphological states of the fungus *Neurospora crassa*.

Ribosomes present in extracts of both resting conidia and growing hyphae from *N. crassa* have similar physicochemical properties (*5*). These include sedimentation coefficients of ribosomes and ribosomal RNA (rRNA) and nucleotide composition of rRNA. Also, the nucleotide composition of total RNA and soluble RNA (sRNA) from conidia and hyphae is the same. Perhaps these results could have been predicted since conidia might be considered merely as segments of hyphae (*6*). They can be regarded as the product of a simple morphological change, are of asexual origin, and can germinate readily in nutrient medium without an activating treatment.

In contrast, ascospores of *N. crassa* have a sexual origin and are the result of a relatively complex mycelial differentiation. They require heat or chemical activation, and then only water and oxygen, in order to germinate. Nonactivated *Neurospora* ascospores can be stored for years in the presence of water without loss of viability (*7*). In addition, their respiratory activity is at a minimum, and it has been suggested that they do not contain all the enzymes present in hyphae (*7*). Ascospores are thus dormant and, in this respect, analogous to bacterial endospores. Endospores of *Bacillus subtilis* contain only two of the four classes of ribosomes present in the vegetative cells (*8*); the same study also suggested that rRNA is not the major portion of the total spore RNA.

Ascospores were obtained from the mating of wild-type strains (*9*) on Westergaard and Mitchell's synthetic medium (*10*). They were harvested and freed of contaminating conidia and hyphal fragments by flotation and sedimentation (*11*). The population and dry weight of ascospore suspensions were measured turbidimetrically after standardization by microscopical counting and dry-weight determination. The average dry weight of an ascospore was found to be 1.8×10^{-6} mg, of which 5 percent was RNA and 11 percent protein. Breakage of ascospores was effected by a modified French cell (*12*) at 23,000 pounds per square inch. Fractionation of extracts, RNA purification and determination of nucleotide composition were performed by methods described elsewhere (*5*). However, polyvinylsulfate was used routinely during RNA preparation to inhibit enzymatic degradation (*13*). The RNA

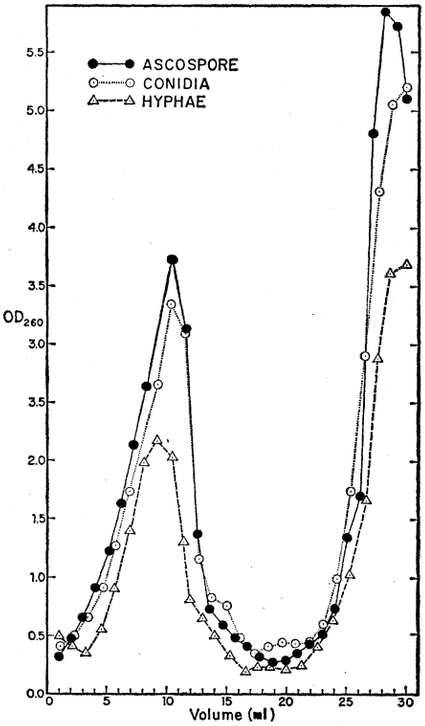

Fig. 1. Density-gradient centrifugation of extracts from ascospores, conidia and hyphae. Each extract was layered directly on a linear gradient of sucrose (20 to 3 percent) in 0.01*M* tris-HCl buffer (*p*H 7.4) containing 10 μmole/ml of $MgCl_2$. The extracts were centrifuged for 255 minutes at 25,000 rev/min in an SW25 head of a Spinco model L preparative ultracentrifuge. Fractions were collected and their optical density at 260 mμ was measured.