any granules pass into the canal. When the opening is wide and the flagella point outward, there is a steady flow into the periphery in anesthetized as well as in unanesthetized animals. When the entrance is wide and the flagella point into the pouch, the flow is into the stomach. The entrance into and the exit from the gonadal cavities show very similar conditions (Figs. 5 and 6).

The stimuli affecting the muscles are not known. However, 1 to 2 hours after feeding, when the stomach is full of digestive material, two or three exits are usually wide open, and flow out of the stomach is almost continuous. At this time, the entrance into the gonads is usually open, but the exit is sometimes open and sometimes closed. An hour or two later when the stomach is nearly empty, it is common to see digestive matter circulating within the gonadal cavity, with the "sphincters" on both sides of the cavity closed. The net effect of sphincter control on the gastric side appears to be such that coarse material such as the exoskeletons of brine shrimp never get into the circulation at all. Large cellular clumps are broken up and almost squeezed into the canals by the muscular action of the stomach. On the peripheral side, the marginal canal receives much less granular material than the gonads do, and it begins to show a distinct increase in granules long after the gonadal cavities become filled with them.

Observations and measurements on the living system and various supportive findings give rise to the following conclusions.

1) The gastrovascular system of *Phialidium* is truly circulatory in that material is circulated in it, but the system has a complex and highly variable pattern, partly because it is subdivided functionally into compartments and partly because the possibility for reversal of flow exists in any part of the system.

2) The essential and main driving force is delivered by the action of long flagella (minimal length 55 μ). This is demonstrated by the fact that flow continues in anesthetized animals and that it occurs in the canals of isolated immobile pieces of animals in the absence of gastric or umbrellar musculature (*6*). The flow is always in the direction of the flagella. Where the flagella are looped or disordered, the currents are irregular and display whirlpools. In addition, the motion pictures show that peripheral granules commonly overtake central ones, an indication that the currents are fastest at the periphery.

3) Muscular action plays a twofold role. Slow and seemingly erratic contractions in the walls of the gastric pouches create pressure and suction at their exits into the canals. The positive or negative pressure waves produced are usually weaker than the pressure continually generated by the flagella. Occasionally, however, they are strong enough to stop and reverse the flow, and when this happens the flagella flip, reversing their net effect. Unless muscular action interfers with the flow, the flagella remain in their orientation and the currents, once set in motion, continue. Muscle also appears to widen or narrow the lumen in three well-defined locations, the exit from the gastric pouches and the proximal and distal ends of the gonads. The rhythmic contractions of the umbrellar musculature, previously thought to play the major role in driving the circulation, appear to have little influence on it. They may cause very brief local interruptions of flow through bending and clamping the canals, and they may help in preventing large clumps of debris from clogging the passages. The functional sphincters described still do not fully account for the flexibility of the system. In addition to them, the "whirlpools" arising anywhere in the canals in regions of fluctuating pressure will serve as temporary blocks to linear flow in either direction.

4) The system is under low pressure. With the dimensions of the system (Fig. 1) and the speed of flow one can, by assuming the viscosity of the circulating fluid to be that of sea water, estimate the pressure by applying Poiseuille's law. At a speed of 100 μ/sec, the pressure gradient must be of the order of 0.12 mm-Hg.

Gray (*7*) has pointed out that the movement of fluids in tubes by means of flagella is reasonably effective only if the diameter of the tube is not much more than twice the length of the flagella. In the radial canals of *Phialidium*, the flagella are twice as long as the tubes are wide, and they are very effective in driving the contents in either direction. Flagellar action in the wider compartments is bound to be less effective in promoting linear flow, but it creates circular currents well suited to the relatively slow digestive processes occurring in the gastric and gonadal cavities. In most animals, the function of cilia is taken over in circulatory systems by muscular action (*8*). Even in hydromedusae the inherent inability of cilia to change their direction actively must be overcome by muscle. It is the combination of flagellar and muscular motive forces which makes this system unusual, if not unique.

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Adenosine Triphosphate Usage by Flagella

Abstract. *Comparison of beat frequencies with rates of dephosphorylation of adenosine triphosphate by glycerinated sea urchin spermatozoa as functions of adenosine triphosphate concentration suggests that each molecule of the flagellar adenosine triphosphatase, dynein, dephosphorylates one adenosine triphosphate molecule during each beat cycle.*

Dynein, the adenosine triphosphatase isolated from the cilia of *Tetrahymena pyriformis* (*1*), has a molecular weight of approximately 600,000 and a specific activity of 1.3 to 3.5 micromoles of phosphorus per milligram of protein per minute at 20°C, depending on the degree of depolymerization and the conditions of cation activation (*2*). This enzyme, which is believed to be re-

sponsible for the ATP-generated movements of cilia and flagella, will therefore dephosphorylate 13 to 35 molecules of ATP (adenosine triphosphate) per second per enzyme molecule (*2*). This number is significant, because it is of the same order of magnitude as the beat frequencies of cilia and flagella (*3–5*). The passage of a bend along a cilium or flagellum may therefore be associated with the use of one ATP molecule by each enzyme molecule distributed along the appropriate half of the cilium. Such a model would be appropriate to the generation of flagellar bending by the propagation of abrupt transitions between bent and unbent states, as recently suggested (*5, 6*), and some of its kinetic properties at the molecular level have already been considered (*7*).

This model predicts that the rate at which a flagellum uses ATP for movement should be proportional to its beat frequency. A test of this prediction is complicated by the difficulty of measuring the use of ATP by flagella under conditions of normal movement and by the possibility that ATP will be used for other activities besides movement, for example, for membrane transport, perhaps by the adenosine triphosphatase which Gibbons found associated with the membrane fraction of *Tetrahymena* cilia (*8*).

By the use of glycerinated flagella, movement and adenosine triphosphatase activity can both be studied under similar conditions, but it is not certain that the enzyme activity is completely coupled to movement under these conditions. Studies with glycerinated flagella have indicated that the beat frequency increases with ATP concentrations from 10^{-3} to $10^{-4}M$ (*9*), but estimates of the Michaelis-Menten constant (K_m) for the adenosine triphosphatase activity of glycerinated flagella (*10*) or purified dynein (*2*) have given values of the order of $10^{-5}M$, indicating that the enzyme activity is relatively independent of ATP concentration above $10^{-4}M$. A reexamination of this problem under more favorable conditions has now given a different result, which is more consistent with the model discussed above.

Spermatozoa of a sea urchin, *Lytechinus pictus*, have been used for these experiments because of the high degree to which the movements of their flagella can be reactivated by ATP after extraction with solutions containing 50 percent glycerol (*11*). Glycerinated spermatozoa were prepared by mixing concentrated spermatozoa with an equal volume of sea water, and then adding the mixture to four volumes of a 62.5 percent (by volume) solution of glycerol containing 0.25*M* KCl, 0.01*M* $MgCl_2$ and 0.01*M* of *p*H 7.8 tris-thioglycolate buffer (*10*) at −10°C. The preparations were stored at −10° to −20°C and used within a few hours after they were mixed.

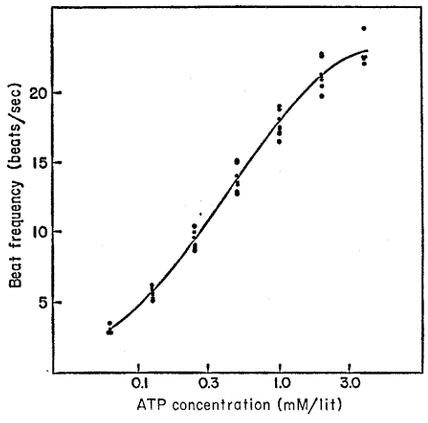

Fig. 1. Effect of ATP concentration on the beat frequency of glycerinated sea urchin spermatozoa. Each point represents the average of measurements of 20 spermatozoa. The curve was obtained by fitting a straight line to the points when the reciprocal of the beat frequency was plotted against the reciprocal of ATP concentration.

Observations on beat frequency were carried out by diluting small amounts of glycerinated sperm suspension with solutions containing 0.25*M* KCl, 0.004*M* $MgSO_4$, 0.1*M* urea, 0.001*M* potassium thioglycolate, 0.002*M* tris buffer, 3 percent polyvinylpyrrolidinone and variable amounts of ATP. A molecular weight of 625 was assumed for the hydrated disodium ATP used to prepare solutions at various ATP concentrations. The *p*H of the solutions was adjusted to 7.8 at 16°C, and all observations were carried out at 16°C on a water-cooled microscope stage. Normally, spermatozoa swimming with asymmetrical but otherwise nearly normal wave patterns were selected for stroboscopic measurement (*4*) of their beat frequencies. However, at the lowest ATP concentrations, some spermatozoa loosely attached to the slide or cover glass were measured, because of the small numbers of freely swimming spermatozoa in these preparations. Photographs of the type of movement obtained under these conditions have been shown (*11*).

In agreement with results from sea urchin spermatozoa and other glycerinated flagellar systems (*9*), the beat frequency increases with increasing ATP concentration (Fig. 1). The results resemble a typical Michaelis-Menten enzyme-substrate interaction, and when the reciprocal of the beat frequency is plotted against the reciprocal of the ATP concentration, the points yield a straight line from which a value for K_m of 0.43 m*M* and a maximum beat frequency of 25.6 beats per second can be determined. The fact that the maximum frequency is slightly less than the frequency (28.5 beats per second) of normal spermatozoa under these conditions of temperature and viscosity (*11*) may be an indication that the conditions used for reactivation do not optimally simulate the normal internal environment of the flagellum.

The rate of dephosphorylation of ATP by glycerinated spermatozoa was measured at 16°C and *p*H 7.8 with a recording *p*H-stat (E. H. Sargent), in an open reaction vessel with a layer of mineral oil floating on top of the solution to prevent uptake of atmospheric carbon dioxide. Spermatozoa were suspended in 15 ml of a solution identical to that used for the observations on beat frequency, except that the tris buffer was omitted and in most experiments the thioglycolate was replaced by 1 m*M* dithiothreitol, which increased the *p*H stability of the solutions. The reaction was started by addition of 50 μl of glycerinated sperm suspension, and it was followed for 5 to 10 minutes. In most cases, the rate of the reaction was nearly constant over this period. The system was calibrated by adding known amounts of KH_2PO_4 and recording the steps obtained in reaction rate records. Sperm densities of different preparations were compared on the basis of turbidity measurements, by diluting 20 μl of glycerinated sperm suspension with 1.0 ml of distilled water, adding 3.0 ml of cold 10 percent trichloroacetic acid, and measuring the optical density at 540 mμ. These measurements were then related to actual sperm densities obtained by microscope counts of more than 6000 spermatozoa; a counting chamber 0.1 mm deep and an eyepiece micrometer grid were used.

The rate of dephosphorylation of

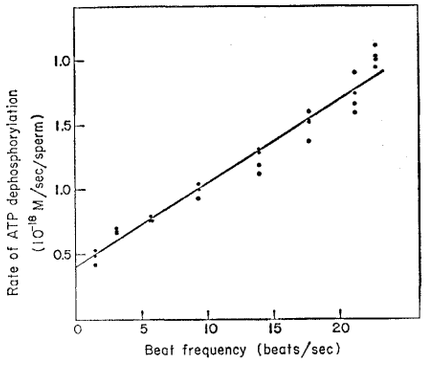

Fig. 2. Dephosphorylation of ATP by sperm suspensions at various beat frequencies obtained by varying the ATP concentration. Each point represents a single measurement of the rate of ATP dephosphorylation. The line was obtained by the method of least squares, and has a slope of 0.065×10^{-18} mole per beat per sperm.

ATP under these conditions does not vary with ATP concentration in the manner expected for a single enzyme obeying Michaelis-Menten kinetics. Most likely, these sperm preparations contain several active adenosine triphosphatases, and other methods will be needed to individually characterize them. In Fig. 2, the results from experiments with four sperm preparations are presented by plotting the rate of ATP dephosphorylation against the average beat frequency observed at the same ATP concentration. This mode of presentation suggests that a portion of the adenosine triphosphatase activity may be independent of the beat frequency, but that the major portion may be proportional to the beat frequency, with approximately 0.65×10^{-19} mole of ATP dephosphorylated per beat by each spermatozoon.

The results in Fig. 2 are too widely scattered to provide any firm evidence for a tight coupling between movement and ATP dephosphorylation. Such coupling seems unlikely in these preparations, since only a fraction of the spermatozoa in the reaction mixture are swimming actively, and variations in this fraction do not appear to be correlated with the rate of dephosphorylation. Evidence is also lacking that it is actually the dynein component that is responsible for the ATP dephosphorylation which is proportional to the beat frequency. These problems require further study. However, if the sole source of energy for flagellar movement is the indicated dephosphorylation of 0.65×10^{-19} mole of ATP per beat, a sea urchin spermatozoon which normally beats at 30 beats per second at 16°C will use approximately 2×10^{-18} mole of ATP per second. Since the work that it performs against the external viscous resistance appears to be about 3×10^{-7} erg per second (*5*), it must convert the free energy of ATP to external work at a rate equivalent to nearly 4 kcal per mole of ATP. This compares favorably with a value of 5.9 kcal per mole of creatine phosphate estimated for the work output of muscle (*12*).

The dynein molecules obtained from *Tetrahymena* cilia form chains with a spacing in vitro of 140 Å (*1*), while estimates of their spacing in vivo of 160 to 200 Å have been obtained (*13*). If sea urchin spermatozoa have a dynein with a configuration and localization similar to that of *Tetrahymena* cilia, a chain of dynein molecules with 140- to 200-Å spacing extending the length of a sperm flagellum, 42 μ (*5*), will contain 2 to 3×10^3 dynein molecules; and 18 chains, corresponding to one for each arm of each outer doublet fiber in the ciliary cross-section (*8*), will contain 3.6 to 5.4 $\times 10^4$ molecules, or 0.6 to 0.9×10^{-19} mole of dynein. Although a more direct and precise estimate of the number of dynein molecules in a sea urchine spermatozoon would be desirable, this estimate is close enough to the value of 0.65×10^{-19} mole per beat obtained for the rate of ATP dephosphorylation to support the suggestion that each dynein molecule uses one ATP molecule during each beat cycle.

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Histones in the Wild-Type and the Anucleolate Mutant of Xenopus laevis

Abstract. *A comparison of anucleolate mutant and wild-type tadpoles of* Xenopus laevis *reveals striking differences in histones. In the mutant, synthesis of histone fractions I and IIb is virtually absent and IIa is reduced, while fractions III and IV are significantly increased.*

Several studies have shown histones to be inhibitors in vitro of DNA-directed RNA synthesis (*1*). Moreover differential extraction of histone from its native association with DNA has resulted in the increased priming ability of the DNA (*2*). If we are to determine the role of histones in gene control, it would be desirable to relate the findings of these in vitro studies to known developmental alterations of gene readout, and to known repressed states of the nucleus. Condensed, or hetero, chromatin represents such a repressed state. Brown and his co-workers have shown that developmentally condensed chromatin in mealy bugs is genetically inactive (*3*) by virtue of repressed RNA synthesis (*4*). We have described histone differences in condensed and diffuse chromatin for this organism (*5*). The work of other laboratories also suggests a relationship between structural alteration of chromatin, genetic inactivity, and histone differences (*6*).

In the present study we have attempted to look at qualitative differences in histones associated with another nuclear alteration, the absence of nucleoli and nucleolar organizers in the anucleolate mutant of the African clawed toad, *Xenopus laevis*. This mutant (the homozygous recessive) lacks nucleoli, and is devoid of ribosomal RNA synthesis (*7*), but it manages to live to the swimming tadpole stage (*8*).

Sexually mature toads, phenotypically normal but heterozygous for the anucleolate condition (having one nucleous, rather than two), were injected with gonadotropin and mated. The resulting eggs were allowed to develop in dechlorinated tap water until they reached Nieuwkoop-Faber stage 28.

Approximately 1000 tadpoles were placed in 40 to 60 ml of water with 1 mc (10 to 36 mc/mmole) of sodium C^{14}-carbonate, 0.5 mg of penicillin per milliliter, and phenol red as *p*H indicator (*p*H 8). The tadpoles were