THE NATURE OF ALGAL AND RELATED FLAGELLA

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In all ciliated or flagellated systems the basal body is the only cell constituent which is invariably found associated with the vibratile process. Rootlets and cytoplasmic fibres which occur in some systems appear to be entirely absent in others[1]. Thus it appears that the essential mechanism of flagellar movement must be contained in the flagellum together with its partner, the basal body. Such a conclusion is indicated also by the microdissection experiments of WORLEY[2], in which cilia of *Anodonta* intestinal cells still functioned even after the rootlets had been severed.

It also seems that the flagellum itself is by no means passive in its association with its partner. Thus BRADFIELD[3] has shown that the orientation of the basal body and the basal micron of the cilium does not vary appreciably during motion in the case of the lateral and latero-frontal cells of *Mytilus*, whilst GRAY[4], in a study of the movements executed by flagella and cilia, concluded that energy must be added along the length of the vibratile process.

The morphology of flagella and cilia has been investigated by many workers, and with the aid of the electron microscope a striking common feature has been revealed. Material from a large variety of sources has been examined, and almost without exception the vibratile process has been found to be constituted mainly of a bundle of eleven fibrils, nine of which form a ring round the other two. Important contributions in this field have come from MANTON and her co-workers[5], who have examined the spermatozoids of the Pteridophyta, Bryophyta, and green and brown algae; FAWCETT AND PORTER[1], who have examined ciliated epithelium from a number of animals including mammals; and BRADFIELD[3], who has investigated mainly spermatozoa.

One might expect therefore that an examination of the properties of this fibrillar bundle would give the clue to flagellar motion, and it is the purpose of this present paper to discuss the preparation and general nature of this material. In the systems used the flagellar sheath was very thin, and the matrix too, as judged from electron micrographs, did not appear to contribute any considerable quantity of material.

EXPERIMENTAL

Preparation of flagellar material

The systems investigated have been the flagellated single cells of the Chlorophyceae and the spermatozoa of the perch, trout and char. Most of the work has been carried out on *Polytoma uvella*.

Polytoma uvella. The organisms were grown on a sterilized medium of 0.2% hydrated sodium acetate, 0.2% "Difco" yeast extract, and 0.2% "Difco" bacto-tryptone in distilled water. After inoculation the flasks were set aside and the cells harvested by centrifugation as the logarithmic growth phase was coming to an end. This stage, which was easily detected from the appearance of the culture, was reached in between five and nine days, depending on the laboratory temperature. Seven days at 18°C was a usual incubation period.

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The harvested cells were washed by very careful suspension in distilled water followed by centrifugation. Never was the material washed more than twice since the osmotic shock tends to be lethal and continued washing was usually sufficient to kill most of the material. Such cells, which have been killed without fixation, detach their flagella practically spontaneously and the latter are lost in the wash water. Isotonic salt solutions and sucrose solutions were also tried as washing agents, but since the results were not greatly superior to those obtained with distilled water the practice was not continued.

After washing, the organisms were suspended in distilled water, a few drops of chloroform or toluene added, and the material subjected to about thirty seconds mild shaking by hand. Under these conditions the cells were rendered immotile and were presumably dead. Observations with the optical microscope showed that the flagella had become detached and were usually represented by a fluffy material. This indicated considerable disintegration. The cells were spun away and Fig. 1, which is an electron micrograph of the suspension which remained, shows the extensive splitting into the constituent fibrils which had taken place.

It was originally hoped to isolate the flagella in a fairly whole condition by high-speed centrifugation, but such a procedure resulted only in a minute preparation of that fraction which was not quite so badly disintegrated. Attempts were made to obtain intact material from cells fixed by formalin. Once fixation had taken place considerable violence was needed to detach the flagella and usually a percentage of cell debris was formed in the process. In any case, since fixed material would have been of very limited use, the method was not continued. Eventually the disintegrated material was obtained from the suspension by reducing the pH to about 5.0 with 1% acetic acid. Under these conditions precipitation occurred. The supernatant had a negligible nitrogen content and an electron microscopic examination showed that little observable material was left behind. It is, however, appreciated that a small quantity of such substances as fats, carbohydrates, and compounds of small molecular weight probably remained in the supernatant. An electron micrograph of a flagellar precipitate is shown in Fig. 2. If the material was for chemical analysis, two volumes of alcohol were also added at this stage and the precipitate spun away, washed, and freeze-dried. The yield was 25 to 40 mg from about ten litres of culture. If further purification was required the addition of alcohol was omitted and the precipitate dissolved at pH 7 and then reprecipitated. Fig. 3 shows an electron micrograph of a dissolved precipitate. Complete disintegration has taken place and to all intents and purposes a true solution of the flagellar material has been obtained.

Chlorogonium elongatum (forma) and Polytomella caeca. These organisms were grown and harvested in the same way as *Polytoma*. On the addition of distilled water or on quite mild shaking, however, a fair proportion of the cells were ruptured and the preparation was spoiled by the presence of debris. Some information on the tyrosine and tryptophan contents was obtained from material purified by reprecipitation, but the material was not considered suitable for general use. In the case of *Polytoma* no corresponding cell breakdown was ever observable either with the optical or with the electron microscope, this important aspect being checked for every preparation.

Spermatozoa from perch and brown trout. The milt was expressed from the ripe fish and diluted with distilled water. Four careful washings were considered sufficient to remove the seminal fluid, and continued washing, here as with *Polytoma*, has a harmful effect. A suspension of the spermatozoa was then shaken vigorously by hand for about two minutes. The flagella which became detached in the process were obtained as previously described. Preparations were carried through on the same day as the milt was expressed from the fish.

Spermatozoa from char. Although the char used in these experiments were quite ripe, only a little milt was obtainable at any one time by the usual process of squeezing along the line of the vas deferens. The fish were therefore killed and the testes dissected out, washed, and minced. By squeezing the mince through muslin the sperm were expressed and collected in distilled water. Microscopic examination showed that little solid matter was present in the suspension apart from sperm cells and flagella. Unfortunately the latter had already become detached from the heads and this occurred no matter how carefully the processing was carried out. After centrifuging the cells away the flagella were obtained by precipitation in the usual manner. Since the starting material was not pure milt and it was not possible to wash the sperm before detaching the flagella, the final preparation was not thought pure enough for general use, but some useful information was obtained from it.

The chemical nature of the flagellar precipitate

Ash and nitrogen content. Ash contents were all less than 3% and usually about 1%. Nitrogen contents by the micro-Kjeldahl procedure were 14.8 to 15.2%.

General protein nature. Paper chromatography of an acid hydrolysate of *Polytoma* flagella indicated the presence of the three basic amino acids and the two acidic amino

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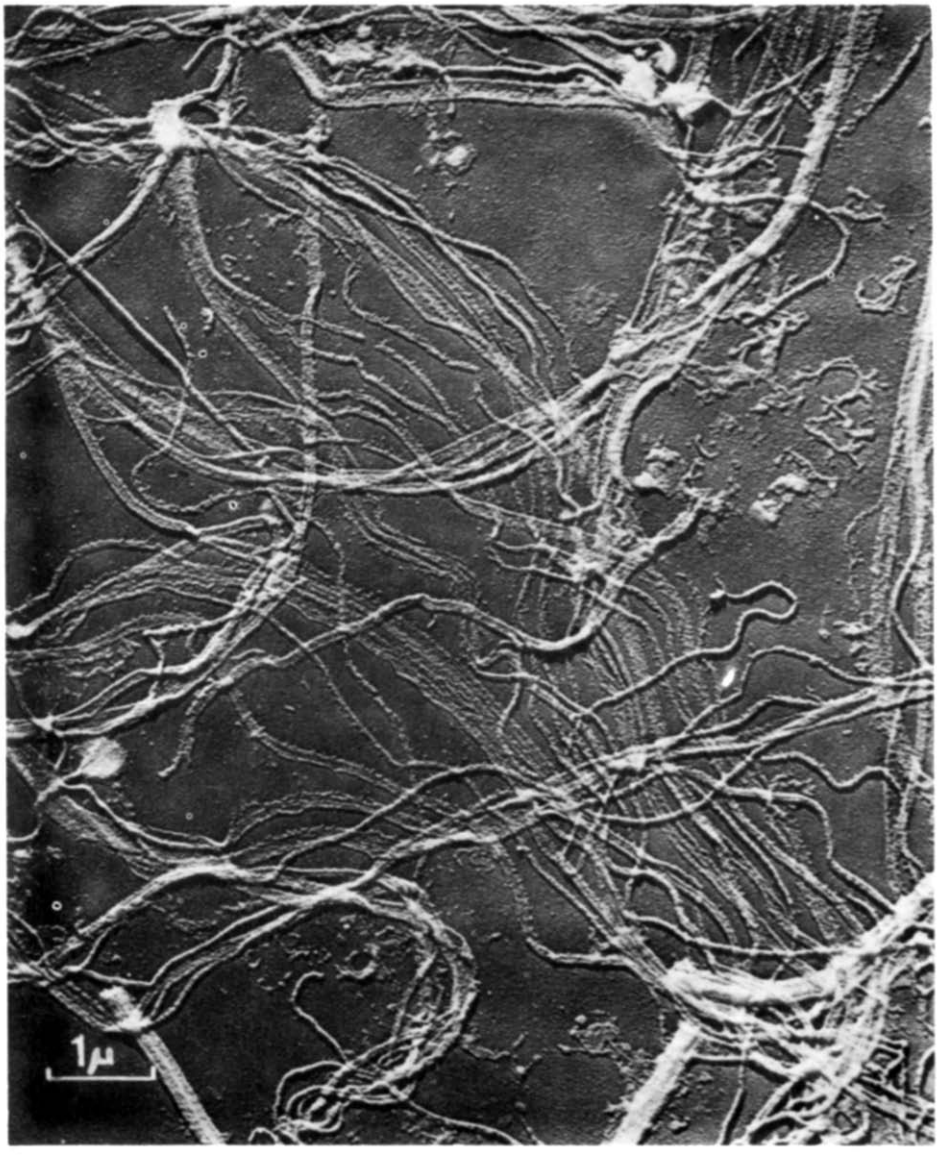

FIG. 1. Electron micrograph of a suspension of disintegrating *Polytoma* flagella.

acids, cystine, serine, glycine, threonine, alanine, tyrosine, valine, phenylalanine, the leucines, and proline.

The infra-red absorption, kindly measured by Dr. K. D. PARKER, showed the material to be proteinaceous, and an examination of the 1650 cm^{-1} region provided the additional information that it existed substantially in the α-form. No β-component was detected. X-ray powder photographs of material dried down from a solution in the same manner as used in the infra-red investigation showed only two diffuse rings at about 4.65 A and 9.8 A, but such a protein pattern is not specific enough to indicate with any certainty the intramolecular configuration.

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Fig. 2. Electron micrograph of a *Polytoma* flagellar precipitate.

Carbohydrate content. This was determined on *Polytoma* flagella by the anthrone method as described by TREVELYAN AND HARRISON[6]. Nine different preparations gave values, in terms of glucose units, ranging from 0.6% to 6.2%. A small and variable amount of carbohydrate may thus be said to be present in material prepared by precipitation.

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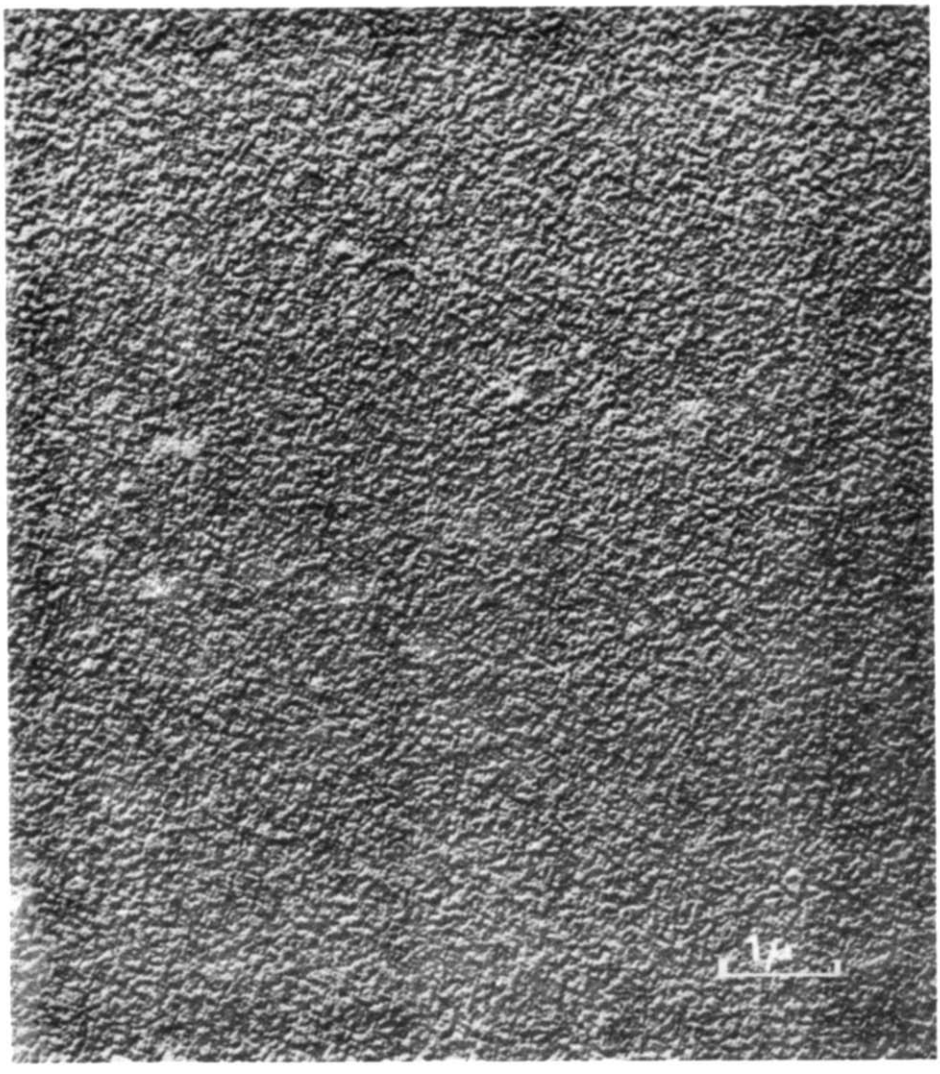

Fig. 3. Electron micrograph of a *Polytoma* flagellar precipitate after redissolving.

Hexosamine content. This analysis, again made only on *Polytoma* material, was carried out by a modified MORGAN AND ELSON procedure[7]. Five preparations gave results from 0.2% to 0.5%, from which it is reasonable to infer that the amount of hexosamine present is negligible.

Phosphorus analysis and the distribution of phosphorus. Phosphorus determinations were carried out on material obtained from *Polytoma*. After oxidation of the flagella with boiling 60% perchloric acid, the phosphate formed was estimated by the GOMORI method[8]. Four different preparations had phosphorus contents of 0.77%, 0.82%, 0.72% and 0.63%. The addition of alcohol in the final steps of the preparation was found to be essential in order to produce reasonably consistent results.

It was of interest to determine the origin of this phosphorus and its distribution

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was therefore examined. Results are shown in Table I. The total phosphrus (TP) was measured on a sample of material the bulk of which was extracted in turn:

(a) twice with cold 10% trichloracetic acid;
(b) for one hour with boiling alcohol and then for one hour with boiling ether;
(c) for 15 min with 5% trichloracetic acid at 90°C.

After each stage the phosphorus content was measured. These measurements represented:

(a) the acid-insoluble phosphorus (AIP);
(b) the acid-insoluble non-lipid phosphorus (AINLP);
(c) the protein phosphorus (PP).

TABLE I

PHOSPHORUS DISTRIBUTION IN FLAGELLA

Origin of material	*TP%*	*AIP%*	*AINLP%*	*NAP% (by difference)*	*NAP% (by UV absorption)*	*RNAP%*	*DNAP%*	*PP%*
Polytoma uvella	0.77	0.64	0.59	0.52	0.50	—	—	0.07
	0.82	0.87	0.86	0.80	0.74	0.72	0.11	0.06
	0.72	—	0.56	0.51	0.62	0.57	0.02	0.05
	0.63	0.49	0.46	0.40	0.35	0.79	0.09	0.06
Perch sperm	0.24	0.21	0.19	0.05	0.04	—	—	0.14
	—	—	0.20	—	0.04	—	—	—
					0.22*			
					0.26*			
Trout sperm	0.25	0.19	0.17	0.03	0.05	—	—	0.14
	0.41	0.37	0.23	—	0.03	—	—	—
					0.02**			
					0.08**			
Char testes	0.33	0.31	0.38	0.32	0.32	0.21	0.14	0.06
	0.43	—	0.45	0.30	0.41	0.26	0.17	0.05
					0.42*			
					0.42*			

* Includes acid-soluble nucleotide phosphorus.
** Estimation carried out on trichloracetic acid precipitate of flagella washed by dialysis (see text).

The nucleic-acid phosphorus (NAP) was represented by the difference between (b) and (c). The nucleic acid phosphorus was also determined by measuring the UV absorption of the extract, obtained with hot trichloracetic acid, by the method proposed by LOGAN, MANNELL AND ROSSITER[9]. Ribonucleic acid phosphorus (RNAP) was also determined in the extract by the orcinol method as described by DAVIDSON AND WAYMOUTH[10], and deoxyribonucleic acid phosphorus (DNAP) by the diphenylamine method as described by the same authors in the same paper.

It appeared that substantially all the phosphorus arose as a result of the presence of ribonucleic acid. Since the cells themselves only contain 1.6% total phosphorus and no cell lysis was ever observed to take place during the preparation of *Polytoma* flagella, it is impossible to account for the presence of some 7% of nucleic acid as impurity.

When the preparations from fish sperm were examined the unexpected result

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was obtained that nucleic acid appeared to be absent in these cases. Preparations from the char testes contained a small quantity of both ribonucleic acid and deoxyribonucleic acid. It is most likely that this was due to the impure nature of the specimens, since these particular preparations were of doubtful purity (see earlier). In order to be certain that the nucleic acid was not lost in the washing or precipitation stages of the preparation, semen was washed by dialysis. The heads, which had become detached from the flagella, were removed and the total nucleotide – nucleic acid in the suspension measured by extracting with hot trichloracetic acid and determining the UV absorption. A proportion of this absorption was almost certainly due to nucleotides from the seminal fluid, but even so the results were still considerably lower than the results for *Polytoma*. In fact, two specimens of trout semen were washed by dialysis, the heads spun away, and an equal volume of cold 10% trichloracetic acid added. This was to remove any nucleotide phosphorus; and when the precipitate was extracted with hot 5% trichloracetic acid, only a very small amount of UV-absorbing material (corresponding to 0.02% and 0.08% nucleic acid phosphorus) was obtained.

Sulphur and cystine. Material used for these determinations was extracted first with hot trichloracetic acid to remove any nucleic acid. Calculations were then made on the basis of the weight of nucleic acid-free material.

Sulphur analyses were performed by WEILER AND STRAUSS of Oxford.

The combined cystine-cysteine was estimated using the method of SULLIVAN, HESS AND HOWARD[11], the protein being first hydrolysed in a sealed tube by heating at 110°C for 16 hours with concentrated hydrochloric acid diluted to 20% with formic acid. Hydrolysis under these conditions reduces the decomposition of cystine which tends to take place, especially in the presence of carbohydrate[12]. The protein hydrolysate was taken to dryness over phosphorus pentoxide and caustic potash and, after being dissolved in 0.1 *N* hydrochloric acid, was treated with 0.2% sodium amalgam to reduce the cystine to cysteine.

TABLE II

SULPHUR AND CYSTINE-CYSTEINE IN NUCLEIC ACID-FREE FLAGELLA

Source of material	*S content* (%)	*Cystine-cysteine in hydrolysate (g acid from 100 g dry material)*
Polytoma uvella	0.36	—
	0.50	—
	0.59	—
	—	0.81
	—	0.87
Char testes	—	1.12

Proline and hydroxyproline. The presence of proline had been observed in the hydrolysate obtained from *Polytoma* flagella. No hydroxyproline was found but this could well have been missed. When heavily loaded one-dimensional chromatograms were run and sprayed with 0.2% isatin in 4% acetic acid in *n*-butanol[13] with the specific intention of detecting any hydroxyproline, no signs of this amino acid were obtained. These chromatograms confirmed the presence of proline.

Tyrosine and tryptophan. Any nucleic acid was first removed by extraction with

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hot trichloracetic acid and the tyrosine and tryptophan estimated in the extracted preparations by the UV absorption method following the recommendations of BEAVEN AND HOLIDAY[14]. Corrections were made for the haze of the solution and the accuracy of these corrections checked by measuring the point at which the curves of the material in 0.1 *N* caustic soda and in 0.1 *N* hydrochloric acid crossed. In all cases this point, the isobestic point, fell within the limits prescribed (2768 A to 2810 A). Fig. 4 shows the absorption of a specimen from perch sperm, the graph being typical of all the specimens examined. The analyses are summarized in Table III.

TABLE III

TYROSINE AND TRYPTOPHAN IN FLAGELLAR PROTEIN

Results in g amino acid from 100 g protein assuming a protein nitrogen of 16.0%

Source of material	*Isobestic point (mμ)*	*Tyrosine content*	*Tryptophan content*
Polytoma uvella	278	3.35	2.34
	277	3.35	2.61
	277	3.60	2.45
Chlorogonium elongatum	277	3.62	2.22
Polytomella caeca	279	3.28	2.26
	277	3.92	2.32
Perch sperm	279	3.73	2.68
Char sperm	277	4.26	2.63

Some observations regarding solutions of flagella

Precipitated *Polytoma* flagella easily redissolve in distilled water at pH 7. Material from sperm does not dissolve so readily under the same conditions. The difference may be due to the presence of nucleic acid in the former.

There were no obvious consequences of adding neutral adenosine triphosphate to *Polytoma* flagella solutions in distilled water and certainly no noticeable precipitation took place (*cf.* actomyosin solutions).

Some sedimentation experiments on material from *Polytoma* have been kindly carried out by Mr. N. K. PEACOCK. It was found that at pH 7 in the absence of salt sedimentation occurred mainly in the form of two components with values of $S_{20} \times 10^{13}$ around 3.1 and 3.3. These values varied considerably with concentration. If salt was present however, these two fractions associated to form to a single component with $S_{20} \times 10^{13} = 7.2$.

The apyrase activity of flagella

In connection with the energetics of flagellar and ciliary motion it was of interest to examine the enzymic properties of flagellar preparations with adenosine triphosphate (ATP) as substrate.

Measurement of apyrase activity. A solution of the flagella (0.5 ml) was incubated with buffer (0.5 ml) and activator (0.2 ml) for 3 min at 37°C. The reaction was then started by the addition of the substrate (0.4 ml) which contained 1 mg terminal phosphorus per 2 ml of solution. After the specified period 2 ml cold 10% trichloracetic acid were added, the precipitate removed and the phosphate estimated in the supernatant by the GOMORI method[8]. Controls were carried out by adding the trichloracetic acid

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before the substrate. Activities are expressed as Q_p values[15] and a nitrogen:protein ratio of 1:6 was assumed.

Optimal conditions for enzymic activity. The pH optimum for the splitting of ATP in 0.1 *M* borate was determined using 0.05 *M* magnesium sulphate as activator. Fig. 5 shows that the curve obtained had a maximum at pH 7.4. All further experiments were carried out in pH 7.4 0.1 *M* borate buffer.

Fig. 6 shows the effect of incubation time on the breakdown of ATP. The curve is not linear even up to 15 min, but to give reasonable values for measurement 15 min incubation was normally used and linearity assumed.

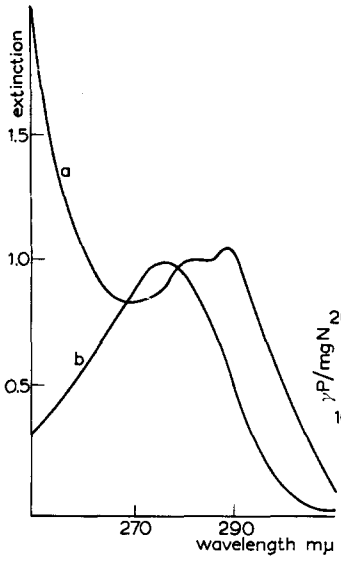

Fig. 4.

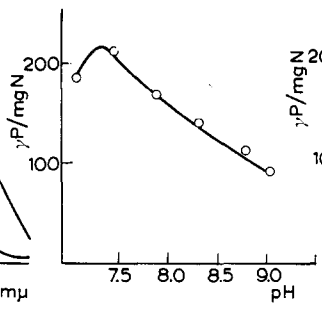

Fig. 5.

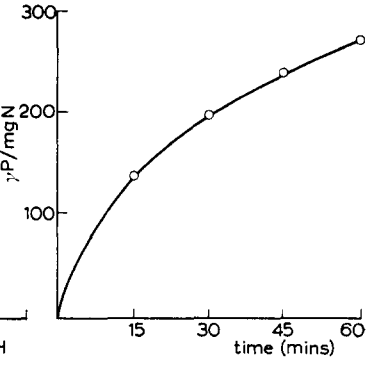

Fig. 6.

Fig. 4. Extinction of a perch sperm flagellar protein solution of concentration 1 g dry protein/litre; (a) in 0.1 *N* NaOH, (b) in 0.1 *N* HCl.

Fig. 5. Activity of unprecipitated *Polytoma* flagella in 0.1 *M* borate, 15 min incubation at 37°C, ATP substrate, Mg^{++} activator.

Fig. 6. Breakdown of ATP by unprecipitated *Polytoma* flagella suspension with time. pH 7.4, 0.1 *M* borate, 37°C, Mg^{++} activator.

Calcium, manganese, and magnesium, in the form of a 0.05 *M* solution of their salts, were all tried as activators. An unprecipitated *Polytoma* flagella suspension at 37°C, with ATP as substrate, had a Q_p value of 10 with no activator, 28 with Ca^{++}, 45 with Mn^{++}, and 68 with Mg^{++}.

Purity of preparations. It was important to be certain that the observed enzymic activity was not due to impurities. Thus in the case of *Polytoma* the wash liquor used in the preparation of the material, the heads, and flagella which had undergone precipitation three times, were all examined for activity. Activity was absent in the wash liquor and was less in the heads than in the flagella (and in any case the preparations were apparently free from heads), whilst thrice-precipitated material still possessed considerable activity as shown in Table IV.

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TABLE IV

Q_p VALUES, AT 37°C, TOWARDS ATP, OF REPRECIPITATED *Polytoma* FLAGELLA

No. of precipitations	0	1	2	3
Q_p values	73	41	53	89*

* May be considerably inaccurate, since by this time the sample was extremely dilute.

In addition, the supernatants obtained in the second and third precipitations contained no nitrogen and no activity, indicating that the activity was completely precipitated with the flagella.

Two preparations of trout sperm flagella were also examined. The final wash liquors were devoid of activity whilst the flagellar suspensions had Q_p values at 37°C of 92 and 98. In the former case the activity of the heads was also measured, the Q_p value being 32.

Specificity of enzyme. In order to examine the specificity of the flagellar enzyme samples of the same preparation were incubated with ATP, adenosine diphosphate (ADP) adenylic acid (AMP), and sodium β-glycerophosphate, respectively. ATP and ADP were split to about the same extent but negligible phosphate was released from the other two substrates. Thus Q_p values, at 37°C with Mg^{++} activator, of an unprecipitated *Polytoma* flagella suspension towards ATP, ADP, AMP, and β-glycerophosphate were 68, 70, 4, and 5, respectively. Five preparations all gave similar results. A specimen of trout sperm flagella also had no activity when examined with respect to AMP and β-glycerophosphate.

The metal activation characteristics of the ADP breakdown were the same as for ATP breakdown.

Q_p values obtained. During the course of the work the activities of a considerable number of *Polytoma* preparations were examined. Q_p values, measured in pH 7.4 0.1 *M* borate, at 37°C, with Mg^{++} activator ranged from 60 to 100.

Two preparations from trout sperm had Q_p values of 92 and 98.

Some structural aspects

During the routine checking of preparations numerous electron micrographs of flagella were taken. Many of these gave fleeting indications of detail below the level of the constituent fibrils, but in only a few was detail sufficiently distinct to give reliable information. One of these, a picture of *Polytoma* flagella, is reproduced in Fig. 7. The fibrils appear to be constituted of rows of particles of diameter about 175 A. From measurements made on these pictures ASTBURY *et al.*[16] have inferred that the fibril width is practically equivalent to that of four rows of particles. The detail in the electron micrographs is not clear enough to confirm this, and no more than two rows have been clearly distinguished in any one fibril. Between the fibrils themselves there appear to be other particles (or particle aggregates), larger than the ones forming the main mass of material, situated on sites about 750 A apart.

Sedimentation measurements, described earlier in this paper, indicate that the particles seen in the electron microscope suffer further breakdown when a solution of flagellar material is formed.

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Fig. 7. Electron micrograph showing the disintegration of *Polytoma* flagella into particles.

DISCUSSION

The chemical constitution

As long ago as 1892 MIESCHER[17] obtained plasmolysed flagella from salmon sperm and showed that the suspension contained more than 50% lipid whilst the remainder, which precipitated on the addition of alcohol, was proteinaceous and contained 1.37% sulphur. Salmon sperm cytoplasm, which is mainly flagella in origin, has also been investigated by FELIX *et al.*[18], and the presence of phosphatases, lipid, 9.5% ribonucleic acid, and several free amino acids, has been reported. ZITTLE AND O'DELL[19] fragmented bull sperm and examined the constitution of the heads, mid-pieces, and tails. The tails were found to contain 23% of lipid which was probably mainly from the sheath and, on a lipid-free basis, 0.5% phosphorus, 3.5% deoxyribonucleic acid, 13.6% nitrogen, 1.5% sulphur, 0.88% cystine and 1.1% ash. No particular significance was attributed to the small amount of deoxyribonucleic acid, which presumably arose as the result of head contamination.

The results presented here do not conflict with those of MIESCHER and those of ZITTLE AND O'DELL. Alcohol precipitation gave rise to a preparation which was mainly protein and contained little polysaccharide or mucopolysaccharide. Lipid was absent in this alcohol precipitate, since no signs were observable in the X-ray diagram, and alcohol-ether extraction caused little alteration, up or down, in the phosphorus content. While intact sperm flagella contain a fair amount of lipid, probably in the sheaths, *Polytoma* flagella do not seem to possess much, since by precipitation virtually all the material was removed from a flagella suspension. Although FELIX and co-workers[18] have reported the presence of alkaline phosphatase and ribonucleic acid in their preparations from sperm, these substances were not found in our material. No claim, however, was made to have isolated pure flagella preparations, and a close agreement between the two sets of results is perhaps not to be expected.

An examination, principally by X-rays, of algal flagella was reported some time ago from this laboratory[20]. These early findings that flagella gave a new and well-defined X-ray diagram have not been substantiated and the only X-ray diffraction pattern obtained during the work reported here is described earlier in this paper. The discrepancy has not yet been finally cleared up but present indications are that the early preparations must have been impure.

The absence of hydroxyproline, the infra-red absorption, and the X-ray diffraction pattern show that the material does not belong to the collagen group of proteins.

Ribonucleic acid appears to be present in the flagella of *Polytoma* but absent from the tails of fish sperm. In the former case it does not seem possible that the result can be due to contamination. The cell bodies contain only 1.6% *total* phosphorus and, in order to account for the 0.7% nucleic acid phosphorus in the flagellar preparations, would have to contaminate the material to the extent of some 50% to 100%. Neither is it likely that the presence of culture medium can explain the observation. This was washed away and a good deal more than a trace would have to have been left behind to account for some 8% of the final preparation. The ribonucleic acid must be present in the living algal flagellum. On the other hand there seems no doubt about the absence of this substance from fish sperm tails.

This surprising difference between the two types of flagellum is not easily accounted for. The algal cells were grown in an organic broth and it is at least conceiv-

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able that the living flagellum has adsorbed on to itself nucleic acid from the medium. This point would be cleared up by an examination of flagella from cells grown in mineral solutions. Such growths, however, give only small yields, and as yet insufficient material has been obtainable for the determination to be carried out. Alternatively, since ribonucleic acid is usually considered to be associated with protein synthesis, the difference between the two types of flagella might be interpretable on this basis. It is possible that the algal type of flagellum possesses a "backbone" of ribonucleic acid for use in any renewing and repairing which takes place. On this thesis the sperm tails, which have only a short useful life, would not be repaired and would contain no nucleic acid "backbone". The whole matter is at present under investigation.

Material from five different flagellates has substantially the same tyrosine and tryptophan content. It is reasonable to suppose that this is a result of chemical similarity between the proteins of the different preparations. Such a similarity is, of course, only to be expected, but it is reassuring to put this expectation on a factual basis, however slight. Of perhaps greater interest is the dissimilarity between these proteins and those from the flagella of the bacteria, *Proteus vulgaris* and *Bacillus subtilis*, which contain negligible cystine and tryptophan and less than 1% of tyrosine[21].

Some energy considerations

The question of the ability of the flagellum to split adenosine triphosphate, and thereby obtain the energy for the work which it carries out, is of importance. Protein material possessing ATPase activity has been obtained from *Paramecium aurelia*[22], from sperm cell homogenates[23], and from salmon sperm cytoplasm.[18]

In none of these cases was it shown that the activity was located on the vibratile organ. NELSON[24] made a considerable advance by fragmenting bull sperm and showing that the tail fraction split ATP more successfully than the heads or mid-pieces. His work may be criticized perhaps because of the rather violent conditions necessary to break up the spermatozoa (cooling in a deep freeze, followed by grinding). A liberation of cellular ATPases at this point could well give rise to misleading results. The work on *Polytoma* does not suffer from the drawbacks inevitably associated with spermatozoa and particularly with the mammalian type. The flagella are easily detached (too easily sometimes) and can be purified by reprecipitation. There is no possibility of contamination by ATPases from seminal fluid.

Q_p values at 37°C are between 60 and 100, which are only one tenth to one hundredth that of myosin. The values are quite sufficient, however, for the observed motion as the following calculation shows.

For a spherical cell of diameter 10 μ the work required to move it through a distance of 100 μ/sec against the viscous resistance of water at 20°C is given by Stokes' Law and is approximately $1.0 \cdot 10^{-8}$ ergs. This work is the main work the cell must perform. A flagellum, 25 μ long with a mass made up of eleven fibrils of minimum diameter 300 A and of density 1.3 g/cc weighs about $2.5 \cdot 10^{-13}$ g. For a Q_p value of 50 each gram liberates $6.0 \cdot 10^{-7}$ moles of terminal phosphate/sec from ATP with a free energy of 11,000 cal/mole. The total energy released in a system with two flagella is thus about $1.5 \cdot 10^{-7}$ ergs. It is likely that the enzyme *in vitro* is working at less than its *in vivo* value. Thus with an efficiency of only a few percent the flagella can liberate sufficient energy to propel the cell.

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SUMMARY

1. Preparations of flagellar material have been obtained from some members of the Chlorophyceae and from the spermatozoa of perch, brown trout, and char.

2. The preparations consisted mainly of non-collagenous protein which the infra-red absorption indicated to be of the α-type.

3. Carbohydrate was determined on material from *Polytoma uvella* and was found to be present to the small and variable extent of 0.6 % to 6.2 %. There was negligible hexosamine.

4. In the *Polytoma* preparations there was 0.6 % ribonucleic acid phosphorus, but ribonucleic acid was absent from the fish sperm tails.

5. Paper chromatography of *Polytoma* flagella hydrolysates showed that these contained aspartic acid, glutamic acid, histidine, lysine, arginine, cystine, serine, glycine, threonine, alanine, tyrosine, valine, phenylalanine, the leucines, and proline.

6. The protein obtained from *Polytoma* contained 0.8 % cystine-cysteine.

7. Flagellar protein from five different organisms had substantially the same tyrosine and tryptophan content. Values for the former amino acid were 3.6 $\pm$ 0.3 g from 100 g protein, and for the latter 2.4 $\pm$ 0.2.

8. The flagellum was found to possess a small apyrase activity. In pH 7.4 0.1 *M* borate buffer with Mg^{++} as activator, Q_p values at 37°C, with ATP as substrate, were between 60 and 100 for preparations from *Polytoma* and trout sperm.

9. At one stage in the disintegration of flagella the fibrils seem to be formed of particles of diameter about 175 A.

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