ENZYMATIC DEGRADATION OF ADENOSINE TRIPHOSPHATE TO ADENINE BY CABBAGE LEAF PREPARATIONS[1]

MENDEL MAZELIS

WESTERN REGIONAL RESEARCH LABORATORY[2], ALBANY, CALIFORNIA

Apyrases, enzymes which hydrolyze ATP (adenosine triphosphate to form AMP (adenylic acid) and orthophosphate, have been described in potato (11) and citrus fruits (1). A phosphatase which will specifically dephosphorylate AMP has been found in potato (18); however, non-specific phosphatases will also dephosphorylate this compound (2). Enzymes specifically dephosphorylating yeast adenylic acid (A3P) are known in rye grass, wheat leaves, and barley (31). Nucleosidases which will hydrolyze adenosine to adenine have been demonstrated in potato (7), in wheat (29), and in soy bean leaves (22). This paper describes the enzyme complex in the cytoplasm of cabbage leaves which is capable of the breakdown of ATP to adenine. The route of breakdown appears to proceed to AMP by an apyrase reaction, followed by a dephosphorylation and hydrolysis to yield adenine. Two apyrases are present in the cytoplasm. An apyrase associated with the washed cytoplasmic particles has been found that is distinct from the apyrases of the soluble fraction.

MATERIALS AND METHODS

PREPARATION OF ENZYMES: Cabbage heads were purchased at local markets. The leaves were separated and washed with distilled water and stored in cellophane bags at 4° C. The leaves were used within 7 days of purchase. All of the following operations were carried out in the cold. The large midribs were removed and the leaf blades were cut into small pieces and blended with an equal volume of cold 0.5 M sucrose in a Waring blendor for 30 to 60 seconds with a voltage regulator control set at 60 to 80 volts. The blendor was rocked during this period to get all the leaf material into the blades. The homogenate was strained through cheesecloth and then centrifuged for 5 minutes at 1000 × G. The sediment was discarded and the suspension centrifuged for 20 minutes at 23,000 × G. The cytoplasmic supernatant solution was decanted and stored at −10° C. The particulate sediment was resuspended in 0.5 M sucrose and centrifuged again. The washed particulate material (WP) was resuspended in 0.5 M sucrose and used as an enzyme source. In most experiments it was recentrifuged and resuspended again before use. This material is called 2WP.

The frozen cytoplasmic solutions of several experiments, which in some instances had been stored for 90 days, were thawed and brought to 75 % saturation with solid $(NH_4)_2SO_4$ in the cold. The precipitate was recovered by centrifuging and dissolved in cold, distilled water. Insoluble material was removed by centrifugation. The solution was dialyzed overnight against several changes of cold distilled water. The dialyzed solution was clarified by centrifugation and the clear supernatant was lyophilized and stored at −10° C. A distilled-water suspension of the lyophilized cytoplasm (LC), usually 0.5 %, was made. The solution obtained after centrifugation was used as the enzyme source. Protein concentrations were determined spectrophotometrically (12) in the case of LC solutions and by micro-Kjeldahl analysis for N when WP and 2WP were used.

STANDARD ASSAY PROCEDURE: Acetate buffer was used at acid pH, collidine at neutral pH, and tris-(hydroxymethyl)-aminomethane at alkaline pH. Sixty micromoles of buffer of the desired pH was customarily used, with 5 micromoles of substrate, enzyme, and distilled water to a final volume of 1 ml. After incubating the reaction mixture at room temperature (with some shaking) for the desired time, the reaction was stopped by addition of 0.2 ml of 1.5 M perchloric acid. The resulting precipitate was removed by centrifugation. Inorganic phosphate was determined by the method of Fiske and Subbarow (19). Controls in which the LC or WP had been heated in boiling water for 5 minutes before use were frequently run. The amount of phosphate found in these controls was always negligible.

Paper chromatography was carried out by one-dimension paper chromatography with either the solvent system of Cohn and Carter (5) or with butanol : acetic acid : water (25).

CYTOPLASMIC SYSTEM: *Proof of ATP breakdown by an apyrase reaction*: LC was incubated with ATP or ADP (adenosine diphosphate) at pH 6.8 and aliquots of the reaction mixture were examined by paper chromatography after various time intervals. When ATP was the substrate and 5.5 mg. of LC were used, ADP was present after 5 minutes but AMP was absent. After 15 minutes only AMP and adenine could be detected on the chromatogram. With ADP as the substrate both AMP and adenine were present at the end of 5 minutes, and the ADP had completely disappeared. No trace of ATP was ever observed as a product starting with ADP. The above results would seem to exclude the possibility that a simple ATPase breakdown to ADP followed by an adenylic kinase reaction is occurring. If an adenylic kinase is present, then its reaction rate must be very slow compared to the ATPase to yield the above results.

Pathway from AMP to adenine: The breakdown of AMP to adenine could proceed either by a dephosphorylation to adenosine followed by a hydrolysis (7, 22, 29) or phosphorolysis (13) to adenine or by a

[1] Received October 6, 1958.

[2] A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

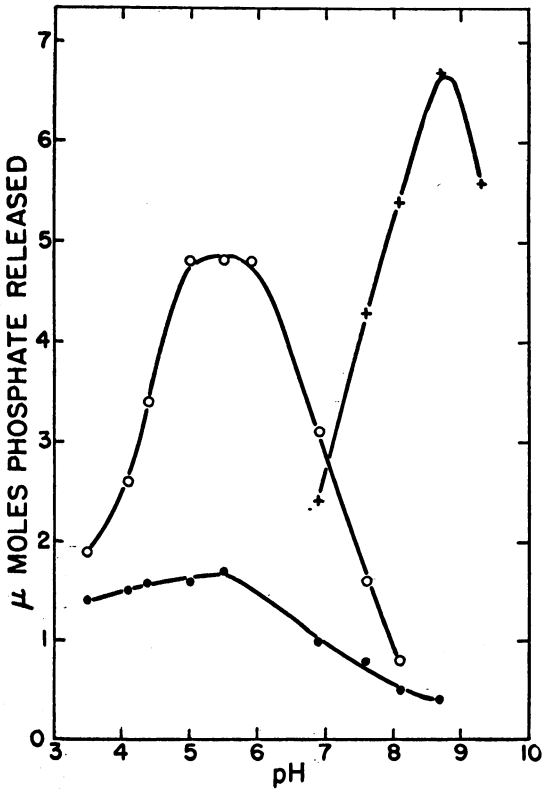
direct hydrolysis (9) or phosphorolysis (16) at the purine-ribose linkage to yield adenine and a phosphorylated ribose. There are several lines of evidence which indicate that the route from AMP to adenine is by way of a dephosphorylation to adenosine and then hydrolysis to adenine.

First, even though adenosine has never been found on any chromatogram in which adenylic nucleotides were converted to adenine, if LC is added to an adenosine solution, adenine is formed rapidly. This conversion can occur in the absence of phosphate. Thus a very active hydrolytic adenosine ribosidase is present. Studies of phosphatase activity, which will be discussed later, show that LC is capable of dephosphorylating AMP, A3P, and ribose-5-phosphate (R5P). Three milligrams of LC will dephosphorylate between 25 and 30 % of the added substrate in 30 minutes at neutral pH. Paper chromatography showed that adenosine was converted more rapidly than AMP to adenine at the same concentration. Thus if the conversion of adenosine to adenine is much more rapid than the breakdown of AMP to adenosine, as soon as adenosine is formed it would be converted to adenine, and a sufficient concentration of adenosine necessary for visualization on a paper chromatogram would never be accumulated. Roberts (29) has shown with wheat leaf preparations that incubation with AMP forms small amounts of adenosine compared to adenine, and concludes that the pathway from AMP to adenine in this tissue is via adenosine.

Second, at pH 8.1 AMP is not dephosphorylated, and concomitantly no adenosine or adenine is found. However, adenosine is hydrolyzed at this pH. It would seem to imply that the phosphate group must be removed before further hydrolysis can occur.

Third, no R5P has been found as a product of the reaction either by paper chromatography, or by use of a linked enzyme system composed of transketolase, phosphoribose isomerase, phosphotriose isomerase, and α-glycerophosphate dehydrogenase (8).

Specificity of the nucleosidase: Incubation of LC with different nucleosides, followed by paper chromatography revealed that only adenosine was hydrolyzed to adenine by cabbage LC. Uridine, inosine, xanthosine, and guanosine were untouched. The reaction was run in phosphate buffer to include a test for the presence of a nucleoside phosphorylase (13) also. These results are similar to those found with wheat leaves (29) in that only adenosine was hydrolyzed.



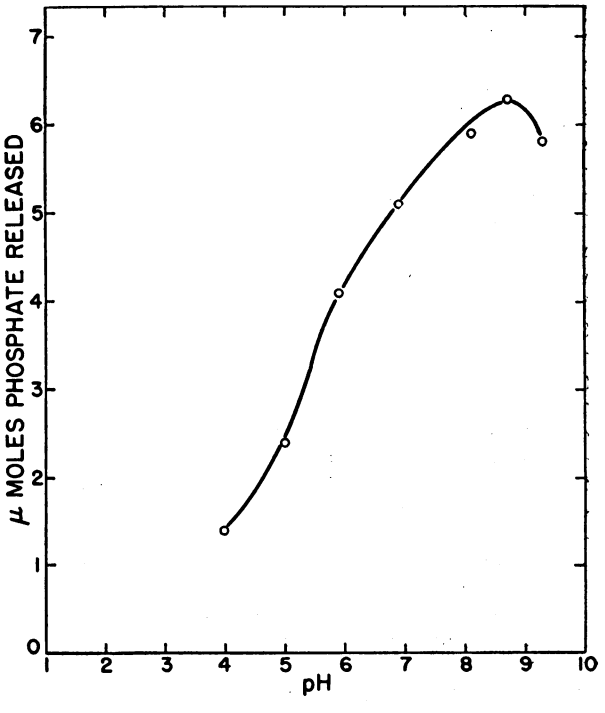


FIG. 1 (*left*). The amount of inorganic phosphate produced as a function of the pH with LC. ○ = ATP as substrate without Mg, x = ATP as substrate with 10 μM Mg^{++} present, ● = AMP as substrate, 1 to 1.2 mg LC were the enzyme source. The reaction time was for 15 minutes at room temperature with ATP as the substrate and for 30 minutes with AMP.

FIG. 2 (*right*). The amount of inorganic phosphate produced as a function of the pH with WP. Incubated at room temperature for 30 minutes with WP containing 1 mg N.

Soy bean leaves possess systems capable of hydrolyzing both pyrimidine and purine nucleosides (22).

pH optima of apyrase and AMPase activity: Two apyrases have been found, one with an acid pH optimum requiring no metal ion, and the other an alkaline pH optimum and needing Mg^{++} for activity. Figure 1 shows the curves of activity against pH for the apyrases. Each curve is a different experiment. Two inorganic pyrophosphatases (P-Pase) were also present with similar pH optima and metal requirements to the apyrases.

Certain differences between the acid apyrase and P-Pase in their response to pH were observed. On the acid side the pH optimum for the P-Pase was approximately 4.6 and for the apyrase was approximately 5.0. The breakdown of ATP had a broader peak than the curve for P-P hydrolysis. Above neutrality there was a very sharp drop in activity. Addition of Mg^{++} activated the breakdown of both P-P and ATP by enzymes having pH optima at 8.7.

The dephosphorylation of AMP reached an optimum in the region from pH 5.0 to 5.5. Above neutrality the activity dropped to low levels and could not be restored by Mg^{++}.

TABLE I

EFFECT OF VARIOUS METALS ON CYTOPLASMIC APYRASE ACTIVITY AT DIFFERENT pH

pH	PROTEIN	ACTIVITY			
		−METAL	$+Mg^{++}$	$+Mn^{++}$	$+Ca^{++}$
	mg				
5.0	1.2	5.6	4.9	3.7	5.6
6.9	1.8	6.0	3.5	1.7	3.2
8.1	1.2	1.0	4.6	0.7	1.1

* Micromoles of orthophosphate produced in 15 minutes.

Metal effects on apyrase: Table I shows that only Mg^{++} activated the apyrase in the alkaline region. An interesting observation is the inhibition of apyrase activity by Mg^{++} at neutral pH. Replicate experiments showed that 0.01 M Mg^{++} inhibited the apyrase activity from 38 to 55 % at pH 6.9. P-Pase was not affected under these conditions. Below pH 6.9 there was little inhibition by Mg^{++} or Ca^{++}.

Inhibition studies of apyrase: Apyrase activity was inhibited 90 % by 10^{-3} M molybdate at pH 6.9 or lower. At the acid and alkaline optima for the apyrases, 5×10^{-2} M F^- is very good as an inhibitor. Incubation of the enzyme preparation with EDTA (ethylenediaminetetraacetic acid) for 15 minutes prior to addition of the substrate had no effect on the acid apyrase. At neutral pH the effect of added F^- decreased. Only 60 % inhibition was obtained with the same concentration of F^- which gave 90 % inhibition at the acid and alkaline optima.

Specificity of LC for phosphate and pyrophosphate esters: Table II is a summary of the ability of LC preparations to hydrolyze various phosphate esters

TABLE II

SUBSTRATE SPECIFICITY OF CABBAGE LC

SUBSTRATE	ACTIVITY*	
	pH 5.0	pH 8.7**
ATP***	6.0	7.0
ADP***	4.4	2.8
AMP	1.7	0.4
A3P	3.0	0.3
UTP***	6.4	2.2
Uridylic acid	2.2	0.4
R5P	1.9	0.4
Glucose-6-PO_4	2.3	0.2
Hexosediphosphate	4.9	1.0
Phosphoglycoric acid	2.9	0.5
Na β-glycerophosphate	3.2	0.1
Inositol monophosphate	1.6	0.1
Flavin mononucleotide	2.9	0.4
Na P-P***	8.3	9.0

* Expressed as μM orthophosphate released by 1 mg protein in 30 minutes except as noted.

** Includes 10 μM Mg^{++} in reaction mixture.

*** Incubated for 15 minutes.

to give inorganic phosphate. At pH 5.0 a large number of phosphate esters are hydrolyzed. Pyrophosphate esters such as ATP, ADP, UTP (uridine triphosphate), and P-P are very readily attacked and monophosphate esters are also hydrolyzed. Note that the pyrophosphate compounds were incubated for only half as long as the simple phosphate esters.

Substituted pyrophosphates and phosphodiesters were used as substrates and the presence of cleavage enzymes determined by the appearance of orthophosphate through the action of the acid phosphatase. Table III gives the results. Uridine diphosphoglucose, DPN DPNH (oxidized and reduced diphosphopyridine nucleotide, respectively), and ribonucleic acid were cleaved at both pH 5.0 and at 6.9. The higher pH was more effective except in the case of the ribonucleic acid. Since pH 5.0 is the optimum for the phosphatase activity this would imply that the cleavage enzymes work much more rapidly at the

TABLE III

ABILITY OF CABBAGE LC TO CLEAVE PHOSPHATE DIESTERS AND PYROPHOSPHATE DERIVATIVES

SUBSTRATE**	ACTIVITY*	
	pH 5.0	pH 6.9
Uridine diphosphoglucose	1.4	1.9
DPN	3.5	4.4
DPNH	5.3	6.6
L-β-Glycerophosphorylethanolamine	0.5	0.6
Ribonucleic acid	1.6	0.8

* Micromoles of orthophosphate released in 60 minutes with 2.8 mg protein.

** Five μM in each case except with ribonucleic acid, where 1 mg was used.

higher pH. L-*a*-Glycerophosphorylethanolamine was not cleaved at either pH. The DPNH was not oxidized prior to cleavage. A nucleotide pyrophosphatase similar to that in potato (17) and a ribonuclease seem to be present.

PARTICULATE SYSTEM: *Conversion of ATP to adenine*: WP converted ATP and ADP to AMP very rapidly at neutral pH. The AMP was then slowly hydrolyzed to adenine. Adenosine was not detected on any paper chromatogram. Time experiments with ADP as the substrate indicated as before that adenylic kinase was not involved in this conversion. With ATP as substrate, ADP and AMP are detected after a short time. ATP then disappears, followed by ADP leaving AMP and adenine. Lyophilized WP retained the complete system which could not be extracted by water. Repeated washing of the particles in 0.5 M sucrose during preparation showed that even particles washed 4-times had good apyrase activity. Continued washing appeared to lower the rate of conversion of AMP to adenine. The ability to convert adenosine to adenine also was gradually lost, but adenosine was still not observed as an intermediate from AMP to adenine under these conditions. Figure 2 shows apyrase activity as a function of pH.

Unlike the soluble system, there is an enhancing effect of Mg^{++} at any pH. The optimum is at pH 8.7.

Magnesium ion and effect of inhibitors: Table IV shows the effects of addition of Mg^{++}, F^-, molybdate, and EDTA on the apyrase activity at different

TABLE IV

EFFECT OF Mg^{++}, F^-, MOLYBDATE, AND EDTA ON THE PARTICULATE APYRASE WITH VARYING pH

pH	N	Activity*				
		Control	+10 μM Mg^{++}	+50 μM F^-	+1 μM Molybdate	+5 μM EDTA
	mg					
5.0	0.8	2.5	4.0	0.8	0.6	2.8
6.9	0.8	5.0	6.8	2.2	3.0	3.2
8.7	0.3	5.5	6.2	4.6	3.6	0.5

* Micromoles of orthophosphate released in 30 minutes.

pH values. The particulate enzyme has considerably different properties from either of the soluble apyrases. It is 75 % inhibited at pH 5.0 by F^-, and molybdate, 40 to 50 % inhibited at pH 6.9, but only slightly inhibited at its optimum pH. Mg^{++} stimulates to a significant degree at all pH levels. Pre-incubation of the particles with EDTA does not remove the residual activity at pH 5.0, inhibits 36 % at pH 6.9, but completely inhibits at pH 8.7. This may be related to the requirement that the EDTA be in the ionized form to act as an inhibitor.

Substrate specificity of WP: 2WP had relatively slight activity toward simple phosphate esters at both pH 5.0 and 6.9. If enough time is allotted, however, considerable hydrolysis of AMP will take place. Table V shows that of the simple esters only hexose

TABLE V

SUBSTRATE SPECIFICITY OF 2WP

Substrate	Activity*	
	pH 5.0**	pH 6.9***
ATP	1.8	4.2
UTP	2.0	5.9
AMP	0.4	0.7
R5P	0.5	0.7
Hexosediphosphate	1.2	1.8
Na β-glycerophosphate	0.9	—
Na P-P	2.9	3.7
Inositol monophosphate	0.5	0.4
Phosphoglyceric acid	0.7	1.1

* Micromoles of orthophosphate from 2WP containing 0.6 mg N.
** Incubated 30 minutes at room temperature.
*** Incubated 15 minutes at room temperature.

diphosphate and phosphoglyceric acid are hydrolyzed to any extent at either pH. ATP, UTP, ADP, and P-P were hydrolyzed very readily. UTP appeared to be a better substrate than ATP at either pH.

DISCUSSION

The observation of Naganna et al (24) that cabbage possesses 2 inorganic pyrophosphatases distinguishable by their pH optima and Mg^{++} activation was confirmed. Soluble apyrases have also been demonstrated which are active in combination over a wide range of pH. The cytoplasmic particles of cabbage leaves have been shown to contain apyrase and pyrophosphatase activity. Particulate apyrases have been found in yeast (20), brain (21), and chick embryos (32). Rafter (27) has recently shown that mouse liver particles possess pyrophosphatase activity. Since all isolated enzymatic reactions must eventually be integrated into the physiology of the cell as a whole, one is justified in speculating as to the function these particular enzymes perform in the economy of the cell.

It is difficult to conceive a role for the presence of apyrase in both soluble and insoluble cell fractions at the present time. A role for apyrase in the fermentation of yeast was shown by Meyerhof (20). The function of the apyrase was to provide a phosphate acceptor at the 1,3-diphosphoglyceric acid step. Engelhardt (6) believes that this role of apyrase must be considered in all those cases where adenylic nucleotides are involved as phosphate acceptors and not as an energy source. Racker (26) has made the interesting speculation that the non-specific phosphatase activities of cell-free preparations may be examples of group transfer reactions that have developed into simple hydrolyses. By this reasoning, apyrase could be an enzyme or complex of enzymes that customarily

transfer adenyl or adenosine diphosphoryl groups to some acceptor and transfer them to water under the present experimental conditions because of either lack of acceptor or through an artifact of preparation.

One may conjecture that the function of inorganic pyrophosphatase may be to act in a linked synthetic system to affect the biosynthesis of compounds under unfavorable equilibrium conditions. Many reactions involving ATP as one of the reactants have pyrophosphate as an end-product. The synthesis of DPN (15), and flavin-adenine dinucleotide (30); the activation of acetate (10), sulfate (4, 28), and carbon dioxide (3); the formation of intermediates in carbohydrate (23) and phospholipid (14) synthesis are all examples in which P-P is an end-product. Often the equilibrium is unfavorable for synthesis, and the hydrolysis of the P-P formed could be used to drive the reaction to completion.

Summary

Water extracts of lyophilized ammonium sulfate fractions of the cytoplasm from cabbage leaf homogenates are capable of readily degrading ATP completely to adenine. Washed cytoplasmic particles and suspensions of lyophilized particles can also carry out this degradation. The pathway of breakdown appears to be by means of an apyrase reaction to adenylic acid, followed by a relatively slow dephosphorylation to adenosine followed by a rapid hydrolysis to adenine.

The cytoplasm has 2 apyrases which can be distinguished by pH optima and response to Mg^{++}. One enzyme is an acid apyrase with a pH optimum at 5.0 requiring no added Mg^{++}. The other is an alkaline apyrase with a pH optimum at 8.7 and a requirement for added Mg^{++}. Associated with each apyrase is the ability to split inorganic pyrophosphate. In the case of the acid apyrase, it has been shown that these activities are associated with different enzymes which can be distinguished by differences in pH optima and response to Mg^{++}. The soluble cabbage leaf preparation has also been shown to contain a very active hydrolytic adenosine ribosidase, ribonuclease, and nucleotide pyrophosphatase in addition to an active acid phosphatase.

The possible functions of apyrase and pyrophosphatase in cell metabolism are discussed.

I would like to thank Mr. Lawrence White and Miss Geraldine Secor for carrying out the micro-Kjeldahl analyses.

Literature Cited

1. Axelrod, B. Citrus fruit phosphatase. Jour. Biol. Chem. 167: 57–72. 1947.
2. Axelrod, B. Transphosphorylation (phosphotransferases, mutases, and kinases). In: Encyclopedia of Plant Physiology, W. Ruhland, ed. Vol. IX. Pp. 222–236. Springer-Verlag, Berlin 1958.
3. Bachhawat, B. K. and Coon, M. J. Enzymatic activation of carbon dioxide. I. Crystalline carbon dioxide-activating enzyme. Jour. Biol. Chem. 231: 625–635. 1958.
4. Bandurski, R. S., Wilson, L. G. and Squires, C. L. The mechanism of "active sulfate" formation. Jour. Amer. Chem. Soc. 78: 6408–6409. 1956.
5. Cohn, W. E. and Carter, C. E. The separation of adenosine polyphosphates by ion exchange and paper chromatography. Jour. Amer. Chem. Soc. 72: 4273–4275. 1950.
6. Engelhardt, V. A. Adenosine triphosphatase properties of myosin. Adv. in Enzymol. 6: 147–191. 1946.
7. Heppel, L. A. and Hilmoe, R. J. Phosphorolysis and hydrolysis of purine ribosides by enzymes from yeast. Jour. Biol. Chem. 198: 683–694. 1952.
8. Horecker, B. L., and Smyrniotis, P. Z. Transketolase from liver and spinach. In: Methods in Enzymology, S. P. Colowick and N. O. Kaplan, eds. Vol. 1. Pp. 371–375. Academic Press, New York 1955.
9. Hurwitz, J., Heppel, L. A. and Horecker, B. L. The enzymatic cleavage of adenylic acid to adenine and ribose 5-phosphate. Jour. Biol. Chem. 226: 525–540. 1957.
10. Jones, M. E., Lipmann, F., Hilz, H. and Lynen, F. On the enzymatic mechanism of coenzyme A acetylation with adenosine triphosphate and acetate. Jour. Amer. Chem. Soc. 75: 3285–3286. 1953.
11. Kalckar, H. M. Adenylpyrophosphatase and myokinase. Jour. Biol. Chem. 153: 355–367. 1944.
12. Kalckar, H. M. Differential spectrophotometry of purine compounds by means of specific enzymes. III. Studies of the enzymes of purine metabolism. Jour. Biol. Chem. 167: 461–475. 1947.
13. Kalckar, H. M. Enzymatic synthesis of nucleosides. Fed. Proc. 4: 248–252. 1945.
14. Kennedy, E. P. and Weiss, S. B. The function of cytidine coenzymes in the biosynthesis of phospholipides. Jour. Biol. Chem. 222: 193–214. 1956.
15. Kornberg, A. Reversible enzymatic synthesis of diphosphopyridine nucleotide and inorganic pyrophosphate. Jour. Biol. Chem. 182: 779–793. 1950.
16. Kornberg, A., Lieberman, I. and Simms, E. S. Enzymatic synthesis and properties of 5-phosphoribosylpyrophosphate. Jour. Biol. Chem. 215: 389–402. 1955.
17. Kornberg, A. and Pricer, W. E., Jr. Nucleotide pyrophosphatase. Jour. Biol. Chem. 182: 763–778. 1950.
18. Kornberg, A. and Pricer, W. E., Jr. On the structure of triphosphopyridine nucleotide. Jour. Biol. Chem. 186: 557–567. 1950.
19. Leloir, L. F. and Cardini, C. E. Characterization of phosphorus compounds by acid lability. In: Methods in Enzymology, S. P. Colowick and N. O. Kaplan, eds. Vol. III. Pp. 840–850. Academic Press, New York. 1957.
20. Meyerhof, O. The origin of the reaction of Harden and Young in cell-free alcoholic fermentation. Jour. Biol. Chem. 157: 105–119. 1945.
21. Meyerhof, O. and Geliazkowa, N. The rate of anaerobic glycolysis of various hexoses in mammalian tissues. Arch. Biochem. 12: 405–434. 1947.
22. Miller, G. W. and Evans, H. J. Nucleosidase from higher plants. Plant Physiol. 30 Suppl.: xxxvii. 1955.

23. MUNCH-PETERSON, A., KALCKAR, H. M., CUTOLO, C. and SMITH, E. E. B. Uridyl transferases and the formation of uridine triphosphate. Nature 172: 1036–1037. 1953.
24. NAGANNA, B., VENUGOPAL, B. and SRIPATHI, C. E. Occurrence of alkaline pyrophosphatase in vegetable tissues. Biochem. Jour. 60: 224–225. 1955.
25. PUTMAN, E. W. and HASSID, W. Z. Sugar transformation in leaves of *Canna indica*. I. Synthesis and inversion of sucrose. Jour. Biol. Chem. 207: 885–902. 1954.
26. RACKER, E. Mechanism of action and properties of pyridine nucleotide-linked enzymes. Physiol. Rev. 35: 1–56. 1955.
27. RAFTER, G. W. A pyrophosphatase activity associated with mouse liver particles. Jour. Biol. Chem. 230: 643–648. 1958.
28. ROBBINS, P. W. and LIPMANN, F. Separation of the two enzymatic phases in active sulfate synthesis. Jour. Biol. Chem. 233: 681–685. 1958.
29. ROBERTS, D. W. A. The wheat leaf phosphatases. II. Pathways of hydrolysis of some nucleotides at pH 5.5 Jour. Biol. Chem. 222: 259–270. 1956.
30. SCHRECKER, A. W. and KORNBERG, A. Reversible enzymatic synthesis of flavin-adenine dinucleotide. Jour. Biol. Chem. 182: 795–803. 1950.
31. SHUSTER, L. and KAPLAN, N. O. A specific b nucleotidase. Jour. Biol. Chem. 201: 535–546. 1953.
32. STEINBACH, H. B. and MOOG, F. Localization of adenyl pyrophosphatase in cytoplasmic granules. Jour. Cell. and Comp. Physiol. 26: 175–183. 1945.

GROWTH AND DEVELOPMENT OF ISOLATED PHYCOMYCES SPORANGIOPHORES[1]

HANS E. GRUEN[2]
BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY, CAMBRIDGE, MASSACHUSETTS

The large sporangiophores of Phycomyces (Order Mucorales) have long been favorite objects for research in several areas of fungus physiology. However, these asexual reproductive structures differ greatly in development from the vegetative mycelium, and none of the information now available on the nutritional requirements and metabolism of entire Phycomyces colonies applies to the sporangiophores themselves.

In order to achieve an understanding of the metabolic processes involved in the growth and tropistic responses of sporangiophores, it seemed imperative to study these structures separately from the mycelium.

The feasibility of removing Phycomyces sporangiophores intact from the mycelium has been mentioned several times in the literature. Laurent (13) determined certain osmotic quantities of isolated sporangiophores. Burgeff (3) excised sporangiophores for his hybridization experiments, and thought that they remained turgid because the basal ends were apparently plugged with cytoplasm. Grehn (8) made a few observations on the growth of isolated Phycomyces sporangiophores as part of his studies on the sporangiophores of several mucoraceous fungi. Isolated immature sporangiophores placed horizontally on malt agar blocks, with their bases and apical portions in air, stopped growing for a few hours due to "wound shock," but then resumed growth by first forming sporangia. The morphology of these specimens was normal, and there was no regeneration of mycelium at the base; their phototropic and geotropic sensitivity was retained. In a parallel experiment with an isolated sporangiophore wedged between agar blocks (basal and apical portions in air) Grehn found that the growth rate was reduced by almost 50 % when compared with that of intact sporangiophores, and that the final length (61 mm at 39 hours after resumption of growth) was less than normal (98 mm in 32 hours). No other data are given. Grehn thought that sporangiophores treated in the manner described must obtain all their nutrients and water through the cell wall, which was somewhat lighter and more transparent at the contact area. He speculated that a lack of water might be an important, although not necessarily the sole cause of the reduction in growth. When isolated sporangiophores were embedded in small gypsum blocks placed on nutrient agar most of them produced branches after 18 to 24 hours.

Isolated sporangiophores were used by Roelofsen (14) in his "iron lung" experiments, and by Johannes (10) in work on vital staining with fluorescent dyes. Other reports in the literature indicate that intact sporangiophores of Pilobolus, a genus related to Phycomyces, can also be removed from the mycelium. For instance, Bünning (2) studied the elastic extension of the cell wall of isolated sporangiophores of *Pilobolus kleinii*.

However, in none of this work, apart from Grehn's limited observations, were the isolated sporangiophores actually grown, nor were they maintained for any length of time.

In preliminary experiments the author (9) found that isolated Phycomyces sporangiophores could be grown for a considerable time on water or other liquid substrates. This finding, recently confirmed

[1] Received revised manuscript October 6, 1958.
[2] Aided by a postdoctoral fellowship grant from the American Cancer Society.