arabinitol concentrations detected in the study of 113 people. D-Arabinitol was not found in serum from 54 of the 65 normal controls, and serum from the remaining 11 normal subjects had only trace amounts of D-arabinitol, with the highest concentration at 0.2 μg/ml. Three colonized patients with renal failure had serum D-arabinitol concentrations greater than 1.0 μg/ml: the highest concentration was 2.2 μg/ml. No other colonized patient, including six others in renal failure, had serum D-arabinitol concentrations greater than 1.0 μg/ml. Maximum serum D-arabinitol concentrations exceeded 1.0 μg/ml in 15 of the 20 patients with invasive candidiasis, that is, in 11 of the 12 with renal failure and in four of the eight with normal renal function.

To provide additional data for support of the peak eluting at 9.35 minutes found in the serum of patients with invasive candidiasis, we prepared peracetate derivatives of serums from four patients with high D-arabinitol levels. These derivatives and D-arabinitol peracetate were chromatographed on two columns with different liquid phases on SE-30 and Carbowax (20M). The peak of interest in each of the serum samples cochromatographed with the D-arabinitol derivative. The retention time on Carbowax at 240°C was 13.92 minutes and on SE-30 at 160° it was 11.61 minutes.

Accurate diagnosis of invasive candidiasis is essential for effective treatment. Our results show that D-arabinitol, a metabolite of *Candida* species, can be detected in serum by GLC at concentrations greater than 1.0 μg/ml in most patients with proved invasive candidiasis.

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Microbodies in Germinating Fern Spores: Evidence for Glyoxysomal Activity

Abstract. *Enzymes of the glyoxylate cycle, isocitrate lyase and malate synthase, are active during the germination of spores of the fern* Dryopteris filix-mas. *Increases in activity of both enzymes are correlated with the breakdown of lipid reserves. The occurrence of these enzymes suggests that the microbodies previously described in these spores are glyoxysomes.*

Fern spores are single-celled, haploid structures enveloped by a sculptured and impervious wall. Under appropriate conditions they can be induced to germinate and later develop into the prothallus or gametophyte. The morphological aspects of germination have been studied, and detailed descriptions of developmental events are available (*1*). Recently, however, research has been directed toward understanding the physiological and molecular mechanisms involved in spore germination (*2, 3*). The dry spore apparently contains stable messenger RNA's (mRNA's) that code for proteins essential to germination. When these stored messages are activated, hydrolytic enzymes are thought to be synthesized and to break down accumulated food reserves, thus providing the major source of carbon and energy for germination. Direct proof for the activity of hydrolytic enzymes during germination of fern spores and identity of the enzymes involved is lacking. However, in seeds of higher plants, enzymes involved in the degradation of stored materials are known, and their synthesis during germination depends on long-lived mRNA's previously transcribed during embryogenesis (*4*). Where lipid is the primary storage product the degradation of reserves in endosperm or cotyledons coincides with an increase in activity of glyoxylate cycle enzymes. These are compartmentalized in specialized microbodies, glyoxysomes, and participate in the catabolism of fatty acids and the generation of succinate that is converted to sucrose. This process of gluconeogenesis in seeds has been studied, and its importance as a primary metabolic pathway during germination is well known (*4, 5*).

Fern spores contain appreciable quantities of lipid, the amount in different species varying from 4 to 79 percent of the spore weight (*6*). The fatty acid composition of spore lipids generally is similar, qualitatively and quantitatively, to that of seed lipids. In addition, we reported the presence of microbodies in germinated spores of *Dryopteris filix-mas* (*7*), and these microbodies have been identified in spores of the fern *Polypodium vulgare* (*8*) and in the horsetail *Equisetum arvense* (*9*). The occurrence of these organelles in certain fern spores having appreciable quantities of lipid reserves suggested to us that glyoxysomal metabolism could be involved as a primary process in germination. Spore lipids might serve as food reserves, but no biochemical evidence has been presented for the involvement of the glyoxylate cycle or the participation of specific lipid-degrading enzymes. We report here that isocitrate lyase (E.C. 4.1.3.1) and malate synthase (E.C. 4.1.3.2), key enzymes of the glyoxylate cycle, are present during spore germination, and their activities coincide with the degradation of lipids.

Spores of *D. filix-mas* were collected in Hanover, New Hampshire, in July 1976 and 1977 and stored at 5°C. They were sifted through lens paper, divided into 100-mg lots, and germinated in petri dishes lined with filter paper moistened with 10 ml of water and one drop of wetting solution (Aerosol OT). Spores were maintained in a controlled room at 25°C and exposed daily to 12 hours of illumination (~ 2675 lu/m^2) provided by a combination of incandescent bulbs and Cool White fluorescent tubes. At various times germinated spores were collected, blotted between filter paper, and

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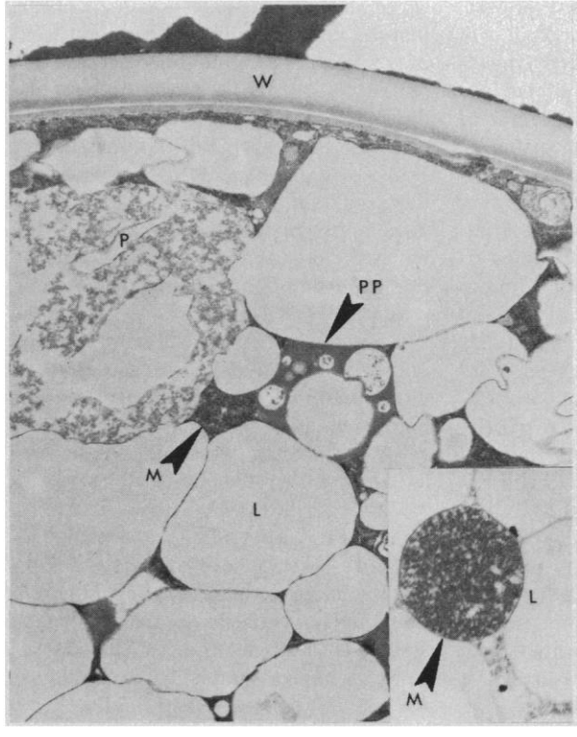
weighed, and samples were counted in a hemacytometer. All subsequent steps were carried out at 4°C. Spores were ground by hand with a mortar and pestle or in a glass tissue homogenizer in buffer (*10*), and the crude homogenate was filtered through three layers of Miracloth before centrifuging at 270*g* for 10 minutes in a Sorvall RC 5 centrifuge. Usually the 270*g* supernatant was used to determine total enzyme activity, but for some determinations a particulate fraction also was assayed for this activity. The particulate fraction was obtained by centrifuging the 270*g* supernatant at 10,800*g* for 30 minutes and suspending the pellet in 2 to 3 ml of homogenizing buffer. Levels of activity were similar in both fractions. Enzymes were assayed on a Gilford recording spectrophotometer (model 2000). Isocitrate lyase, malate synthase, and catalase were assayed as described previously (*10, 11*). Total lipids were estimated gravimetrically (*12*), and protein was determined according to the Lowry procedure (*13*). Spores for electron microscopic examination were prepared and processed in the usual manner (*7*).

An ultrastructural examination of the spore after 4 days of germination (Fig. 1) showed numerous, large lipid bodies, protein granules, and smaller microbodies in close association with the stored material. The lipid bodies were similar to those observed in sectioned endosperm or cotyledons of "fatty" seeds (*14*). Some of them contained electron-opaque material but others were electron-transparent and resembled vacuoles. The latter type also were found in tomato cotyledons (*15*) and were interpreted to be lipid bodies that had lost most of their contents. The microbodies were both regular and irregular in outline and were closely appressed to the lipid bodies. They were bounded by a single membrane and consisted internally of a uniform fibrillar matrix. In most respects they were morphologically indistinguishable from other plant microbodies. They often contained small electron-opaque areas and membrane-bound inclusions of cytoplasm. Crystalline inclusions observed in microbodies from a variety of plants and plant tissues have not been detected in spores at this stage of development.

The first morphological evidence of germination in *Dryopteris* spores was the rupture of the spore coat after 4 days of imbibition. This was followed by the emergence of the rhizoid by day 5 and its subsequent elongation during the 14-day germination period that we studied. The prothallial cell was first observed breaking through the spore coat after 6 to 7 days, and by day 12 it had developed into a filament. An analysis of changes in the amount of total lipid during the time morphological activities were occurring showed that catabolism of spore lipid was correlated with development of the rhizoid and prothallial filament. Approximately 40 percent of the weight of *Dryopteris* spores was lipid, and during 14 days the quantity of storage lipid was reduced to 20 percent. Gemmrich (*16*) determined that, in spores of the fern *Anemia phyllitides*, lipids were broken down during the early stages of germination. A correlation of their subsequent metabolic changes with developmental events suggested that lipids were the main reserves of energy for early cell development. In these and other studies (*5, 8, 17*) in which a decrease in lipid reserves has been reported during spore germination the two assumptions generally made are (i) that the enzymes necessary for metabolizing lipids are present and active during fern spore germination and (ii) that the pathways for converting lipid reserves to energy are similar to those for seeds of higher plants.

In order to support or refute these assumptions we initiated experiments to determine whether enzymes of the glyoxylate cycle were present during spore germination. The time sequence for the development of enzyme activity was determined by making daily measurements of the changes in isocitrate lyase and malate synthase content in germinating spores (Fig. 2). Detection of enzyme activity during fern spore germination provides the first evidence for involvement of the glyoxylate cycle in this process. Activity of both enzymes increased sharply, peaked between day 4 and day 5, and then proceeded to de-



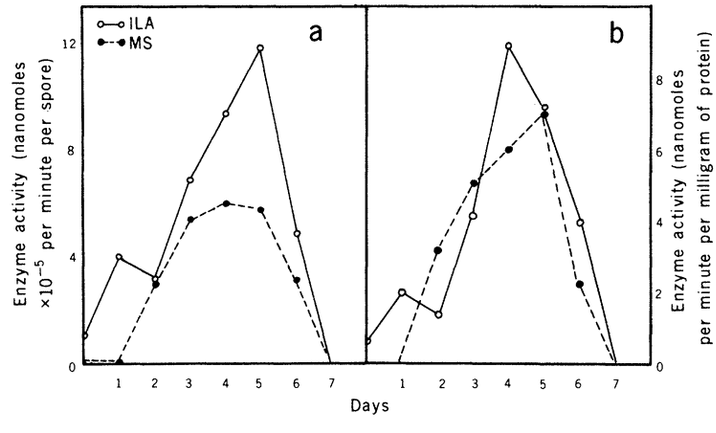


Fig. 1 (left). Spore of *Dryopteris filix-mas* showing spore wall (*W*), protein bodies (*P* and *PP*), and lipid bodies (*L*) (× 8500). (Inset) Microbodies (*M*) are both irregular and spherical in shape (× 12,700). Fig. 2 (right). Time course of the changes in isocitrate lyase (*ILA*) and malate synthase (*MS*) activity during the germination of fern spores. Results are expressed as activity per spore (a) and activity per milligram of protein (b).

crease rapidly, not being detectable on day 8. This characteristic pattern was noted whether results were calculated as activity per spore or as activity per milligram of protein. In both instances enzyme activity was barely detected in the dry spores. During the first 24 hours of germination no change was noted in the level of malate synthase although isocitrate lyase activity increased. For the next 48 to 96 hours activity of both enzymes increased. Differences in the temporal pattern of increase in the activity of malate synthase and isocitrate lyase were observed. However, both enzymes displayed significant increases from day 2 to day 5 of spore germination. A rapid decline in activity took place between day 5 and day 7. These data indicate that the activity of glyoxylate cycle enzymes increases during the very early stages of spore germination before the spore coat ruptures and the rhizoid emerges. More importantly, enzyme activity is at its highest during the time lipids are being rapidly degraded. As the storage material is metabolized enzyme activity declines.

The correlation between lipid catabolism and glyoxylate enzyme activity is common to germinating "fatty" seeds. There the conversion of fat to carbohydrate is enhanced by increases in activity of glyoxylate cycle enzymes. By the time enzyme activity disappears, lipid reserves have been largely depleted and photosynthetic activity in the seedling is capable of providing a continuing source of energy and materials for subsequent growth.

In our study of germinating fern spores, isocitrate lyase and malate synthase activity could not be detected after 7 days of germination. By that time the prothallial cell had protruded from the spore and appeared to be photosynthetically competent. Mature chloroplasts with compact grana have been described in growing filaments of *D. filix-mas* and *P. vulgare*. Our evidence indicates that in fern spores—as in "fatty" seeds of higher plants—the glyoxylate cycle functions in the early stages of development. The decline of enzyme activity is coincident with the establishment of the prothallus as a free-living, autotrophic plant.

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Circular Forms of DNA Synthesized by Rous Sarcoma Virus in vitro

Abstract. *Electron microscopic analysis of the DNA product synthesized by detergent-disrupted preparations of Rous sarcoma virus in vitro revealed the presence of several interesting molecular forms including covalently closed circular DNA. The identification of such circular DNA indicates that virions of retroviruses contain all the components necessary to facilitate the complete synthesis of mature forms of viral DNA and therefore provide a useful system to delineate the molecular mechanisms involved in their synthesis.*

Information concerning the structure and mechanism of synthesis of retrovirus DNA has been obtained from studies conducted both in vitro and in vivo (*1*). Studies in vivo have contributed predominantly to a description of the structure of the intracellular forms of retrovirus DNA preceding integration, presumably because of logistical difficulties in obtaining sufficient amounts of viral DNA precursors for analysis. Studies in vitro, however, have provided considerable information that has guided our current thinking regarding the details of retrovirus DNA synthesis. For example, information concerning the nature of the primer molecule (*2–5*), site of initiation of DNA synthesis (*6–8*), genomic terminal redundancy (*8–12*), hydrolysis by ribonuclease H (*13, 14*), and elongation of 5′ initiated DNA at the 3′ end of the viral genome (*15, 16*) was obtained by studies in vitro in which investigators used detergent-disrupted preparations of Rous sarcoma virus (RSV) as well as reconstructed reactions containing purified retroviral genomic RNA and reverse transcriptase.

Recently, the DNA product synthesized by detergent-disrupted preparations of both murine (*17*) and avian retroviruses (*18*) has been demonstrated to exhibit infectivity. Studies on the structure of the DNA molecules present in the infectious preparations indicated the presence of molecules analogous to the linear forms identified in the cytoplasm of cells shortly after infection (*19, 20*). These molecules were double-stranded in nature, consisting of genome-length minus-strand and subgenomic fragments of plus-strand DNA, and represented virus-specific DNA molecules similar in structure to linear duplex DNA known to serve as the immediate precursor of the mature, covalently closed circular forms of retrovirus DNA identified in virus-infected cells (*17–22*). These findings indicated that the retrovirus reverse transcriptase was capable of transcribing the single-stranded viral RNA genome into the precursor of the mature, covalently closed circular forms of retrovirus DNA in vitro. In this report we demonstrate the presence of circular mature forms of retrovirus DNA in the DNA product synthesized by detergent-disrupted preparations of the avian retrovirus RSV in vitro, thereby suggesting that the virus particle contains all the components necessary to facilitate the synthesis of covalently closed circular DNA.

Viral-specific DNA was synthesized by detergent-disrupted preparations of RSV in vitro under enzymatic conditions that facilitate genome-length and infectious DNA synthesis as previously described (*18*). Single-stranded DNA molecules of approximately 7,500 to 10,000 nucleotides in length were isolated by

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