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10. There are two reliable precision time markers for the years 1884 and 1953 in the firn core. The 26 August 1883 Krakatoa volcanic eruption deposited particulates in the ice core at 14.90 m, and the nuclear explosions of 1953 deposited fission products at 4.35 m. Since these two markers bracket the time period to be studied, the error in dating is now reduced to ±7 years for the 69-year firn core between 14.90 and 4.35 m. Since the time period of my investigation is about 1909 to 1910, the uncertainty in dating a firn layer from this core corresponding to year 1910 is ±4 years. Samples from 10.0 to 11.7 m corresponding approximately to the time from 1920 ±4 to 1908 ±4 years were selected. An additional sample from 12.6 m was also examined. Details on the handling of ice core samples have been described (*9*). One-quarter of the ice core (10 cm in diameter) corresponding to 19.64-cm^2 area was cut into various lengths at the Institute of Polar Studies, Ohio State University. Sodium chloride was then added to the melted ice so as to produce a 2 percent NaCl concentration for conductive purposes. Usually, about ten slices of ice were cut covering a length of 17 cm. After these individual samples were studied for microparticles, the water from them was combined and filtered through special 0.45-μm filter paper. Half of this filter paper was subjected to high-sensitivity neutron activation analysis, and the other half was used to determine the particulate composition in the Polar Research Institute. The filter paper with the particulates was transferred into a specially cleaned high-purity synthetic silica tube, heated slowly to 400°C to destroy the organic matter from the filter, and sealed. These samples were irradiated with standards for 43 days at a thermal neutron flux of 2×10^{14} sec^{-1} cm^{-2}.
11. The procedure for experimental blanks was as follows: 100 ml of deionized water was filtered through a 0.45-μm Millipore filter in my laboratory; this filter was then processed in the same way as the samples. The only difference in the treatment of the blanks and samples was that the blank filter paper was not handled at the Polar Research Institute. The iridium content of the experimental blank was $(1.0 \pm 1.8) \times 10^{-14}$ g.
12. J. L. Barker and E. Anders [*Geochim. Cosmochim. Acta* **32**, 627 (1968)] quoted a factor of 3 for the enrichment at mid-latitudes over the South Pole. L. Machta (personal communication) suggests that it could be a factor of 5.
13. From none to all of the debris from nuclear explosions carried at ground level enters the stratosphere. G. J. Ferber (personal communication) suggests that the percentage entering the stratosphere is highly dependent on the meteorological conditions and explosive yield.
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16. The steady-state influx rate of iridium measured at the South Pole is 9×10^{-15} g cm^{-2} $year^{-1}$ or 4×10^5 tons of cosmic matter per year globally. This is higher by a factor of 4 than the value inferred from mid–Pacific Ocean sediments (*12*). Recent measurements of iridium in deep-sea cores corresponding to the Pliocene and Eocene-Oligocene periods have yielded accretion rates of 3.4×10^5 and 3.3×10^5 tons per year [F. Kyte and J. T. Wasson, *Lunar Planet. Sci.* **13**, 411 (1982)].

17. I am indebted to A. J. Barnard, Jr., J. D. Bonn, T. J. Hurley, H. A. Kaufman, and J. Volkert for support and encouragement; to B. J. Levin, Astronomical Council of the Academy of Sciences of the U.S.S.R., for his assistance in getting the Tunguska spheres; to A. A. Yavnel', Committee on Meteorites, U.S.S.R., for supplying the spheres; to L. Thompson and E. Mosley-Thompson for supplying the particulate matter from the Antarctic ice core and for many stimulating discussions; to A. Meyer and S. Gunn for support in the neutron irradiations; to O. K. Manuel for carrying out the reirradiation of iridium samples after chemical purification; to G. Downing for assistance; and to J. W. Larimer for helpful review.

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Acquisition of Digestive Enzymes by Siricid Woodwasps from Their Fungal Symbiont

Abstract. *Larvae of the woodwasp,* Sirex cyaneus, *contain midgut digestive enzymes that enable them to utilize the major fungal and plant polysaccharides found in their food. At least two classes of enzymes, the C_x-cellulases and the xylanases, are not produced by the larvae. Instead, larvae acquire these enzymes while ingesting tissue of* Amylostereum chailletii, *the fungal symbiont that occurs in the wood on which the larvae feed.*

Woodwasps (*1, 2*) maintain a close association with a fungal symbiont both as larvae and adults. Adult female woodwasps oviposit in dying and dead standing trees. As eggs are laid, the wood is simultaneously inoculated with a mass of fungal oidia which are maintained in special pouches associated with the egg-laying apparatus. The fungus permeates the surrounding wood, and the larvae tunnel into this, ingesting both wood and fungal hyphae. Although attempts to study the enzymatic characteristics of the gut fluids of woodwasps have given ambiguous or conflicting results (*3, 4*), Müller demonstrated significant digestion and assimilation of wood constituents, including cellulose and hemicellulose, by larvae of *Sirex gigas* and *S. phantoma* (*4*).

We have found (Table 1) that the midgut of *S. cyaneus* larvae contains enzymes active against a number of plant polysaccharides, including microcrystalline cellulose (the cellulase complex), carboxymethyl cellulose (C_x-cellulase), xylan (xylanase), pectin (pectinase), and amylose (amylase). The midgut of *S. cyaneus* also contains enzymes active toward laminarin, a β-1, 3-glucan representative of a widely distributed class of fungal cell wall polysaccharides. The enzymes are present in larvae that have been reared on balsam fir chips permeated by the mycelium of the fungal symbiont, *Amylostereum chailletii* (*5*), as well as larvae collected from their natural galleries in the trunks of standing balsam fir trees. This same suite of carbohydrases is present in both the culture fluid of *A. chailletii* and in an extract of balsam fir wood permeated by the fungus. These results suggested that *S. cyaneus* might be acquiring essential digestive carbohydrases when it ingests fungal tissue and fungal secretions along with the wood it consumes. In agreement with this idea is the observation that larvae fare poorly when they are fed a diet of symbiont-free balsam fir chips, extracts of which are virtually enzyme-free (Table 1). Larval mortality is high after only a week, and the level of midgut enzymes decreases dramatically (*6*).

In order to test the hypothesis that the enzymatic activity of the larval gut fluid is due to ingested fungal enzymes, we have purified the C_x-cellulases and xylanases from larvae feeding on *A. chailletii*-infested balsam fir chips and from the culture fluid from *A. chailletii* growing on microcrystalline cellulose in a defined medium, and have compared isoelectric points (*p*I) of the insect- and fungus-derived enzymes. C_x-cellulase and xylanase were chosen for detailed comparison since *Sirex* larvae have been shown to assimilate significant portions of cellulose and hemicellulose, which make up nearly 70 percent of the dry matter of balsam fir wood (*4, 7*). Although the presence of enzymes active against pectin, starch, and laminarin suggests that woodwasp larvae have the capacity to utilize polysaccharides other than cellulose and hemicellulose, the limited quantities of starch and pectin in balsam fir wood (*8*) and the sparse growth of *A. chailletii* mycelium in the wood surrounding larval tunnels (*9*) indicate that these are minor sources of nutrients for the larvae.

C_x-Cellulase and xylanase activities were detected in comparable fractions

Table 1. Enzymatic activity toward various polysaccharides of extracts of midguts (tissue plus contents) of *S. cyaneus* larvae and of extracts of cultures of their symbiotic fungus *A. chailletii*. Each value is the mean ± standard error of the mean. The number of replicates was five in all cases except that only a single replicate was made for larvae cultured for 1 week on sterile balsam fir chips (BFC) (*6*). A unit of activity is the amount of enzyme required to liberate 1 μm of maltose equivalents per hour under the conditions of the assay (37°C, *p*H 5, incubation volume 1.0 ml). Substrates were microcrystalline cellulose (MC), carboxymethyl cellulose, larchwood xylan, citrus pectin, α-amylose, and seaweed laminarin (*19, 20*).

Item	Enzymatic activity [units per milligram (dry weight) of dissolved solids in extract]					
	Cellulase complex	C_x-Cellulase	Xylanase	Pectinase	Amylase	Laminarinase
Larvae						
Collected from natural galleries in balsam fir	0.33 ± 0.13	8.86 ± 6.20	11.59 ± 7.21	8.47 ± 5.30	6.06 ± 2.62	14.23 ± 8.91
Cultured 1 week on BFC permeated by *A. chailletii*	0.48 ± 0.01	20.17 ± 0.59	33.41 ± 3.97	11.03 ± 1.11	4.18 ± 1.30	12.91 ± 3.14
Cultured 1 week on sterile BFC	0.00	0.09	1.39	0.97	2.36	1.62
Culture fluid from *A. chailletii* growing on MC	0.33 ± 0.03	7.45 ± 0.33	9.24 ± 0.33	9.76 ± 0.46	9.48 ± 0.26	8.47 ± 0.38
BFC permeated by *A. chailletii* mycelium	0.37 ± 0.00	1.58 ± 0.65	6.78 ± 2.53	1.50 ± 0.74	2.54 ± 0.00	5.65 ± 2.12
Sterile BFC	0.00	0.00	0.00	0.00	0.02 ± 0.01	0.02 ± 0.01

when gut extracts and fungus culture fluids were subjected to a purification scheme involving ion exchange chromatography on DEAE-Sepharose CL6B, gel filtration on Sephadex G75, and chromatofocusing on Polybuffer Exchanger 94 (*10*). Both the larval midgut and the fungus culture extracts yielded two major C_x-cellulases and three major xylanases. The first cellulase from both sources eluted from the preparative isofocusing column at *p*H 4.9, and the second cellulase at a *p*H below 4.0 (*11*). The first xylanase eluted at *p*H 5.7, the second at *p*H 5.3, and the third at *p*H 4.5.

A further comparison of insect and fungal enzymes isolated from the preparative isofocusing columns was made by subjecting the cellulase and xylanase fractions to analytical isofocusing on ultrathin polyacrylamide gels (*12*). This procedure revealed that several proteins were present in each of the cellulase and xylanase fractions (Fig. 1). We did not determine whether the observed protein multiplicity reflected enzyme multiplicity or simply incomplete purification. However, the point of overriding importance is that the patterns seen in each of the cellulase and xylanase fractions from the fungal extract are identical to those seen in the comparable fractions derived from the insect extract. These experiments demonstrate that the C_x-cellulases and xylanases present in the gut of *S. cyaneus* larvae are identical to (or indistinguishable from) the enzymes produced by the fungus *A. chailletii* and confirm our hypothesis that the woodwasp larvae acquire essential digestive enzymes by the ingestion of their fungal symbiont.

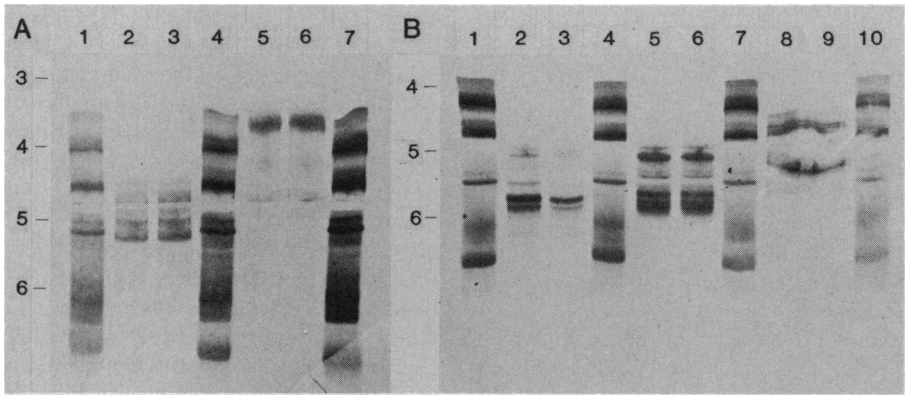

Fig. 1. Isofocusing gels of C_x-cellulases and xylanases of *A. chailletii* and *S. cyaneus* obtained from preparative chromatofocusing. The *p*H values are indicated to the left of each figure. (A) C_x-cellulases. Lanes 1, 4, and 7 contain *p*I (isoelectric point) marker proteins (*12*); lanes 2 and 5 are the fungal C_x-cellulases; lanes 3 and 6 are the insect C_x-cellulases. The *p*I values for bands in lanes 2 and 3 are 4.5, 5.1, and 5.3; in lanes 5 and 6, 3.0 and 3.7. (B) Xylanases. Lanes 1, 4, 7, and 10 are the marker proteins; lanes 2, 5, and 8 are the fungal xylanases; lanes 3, 6, and 9 are the insect xylanases. The *p*I values for bands in lanes 2 and 3 are 4.9, 5.2, 5.4, and 5.5; in lanes 5 and 6, the *p*I's are 4.5, 4.8, 4.9, 5.1, 5.3, and 5.5; in lanes 8 and 9 the *p*I's are 4.4, 4.5, and 5.0.

No data are available that allow an estimate of the relative importance to the larvae of polysaccharide digestion products liberated in the gut and those that might have been generated through decay of the wood prior to ingestion. However, Müller's (*4*) demonstration that *S. gigas* and *S. phantoma* assimilate 22 and 31 percent, respectively, of the cellulose they ingest indicates that structural polysaccharides digested in the gut are an important source of carbon to these insects.

It has been recognized since the investigations of Buchner (*2*) and Cleveland (*13*) that microbial symbionts can play an important role in the digestive processes of insects. It is now well established that permanent populations of hindgut protozoa are responsible for cellulose digestion in wood roaches and in the lower termites (*14*), and that hindgut bacteria contribute to the digestion of refractile plant polysaccharides in the rhinoceros beetle (*15*) and American cockroach (*16*). The ingestion of active fungal enzymes, first demonstrated by Martin and Martin (*17*) in their studies of cellulose digestion by the fungus-growing termites, and now shown to occur also in the siricid woodwasps, constitutes an alternative mechanism by which insects exploit the digestive capabilities of microorganisms. Recently, a significant role for acquired fungal cellulases in the digestive processes of the amphipod, *Gammarus fossarum*, has been proposed (*18*). In this system, the fungi which are the source of the enzymes are not obligate symbiotic associates of *Gammarus*, but rather are normal colonizers of the aquatic detritus that constitutes the major food of this invertebrate. We anticipate that additional examples of the acquisition of di-

gestive enzymes will be uncovered by further studies of insect-microbial associations and of the utilization of refractory plant materials by arthropods.

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5. Basidiomycetes, Aphyllophorales, Stereaceae; the strain of *A. chailletii* used in all experiments was a single-cell isolate obtained from the intersegmental sacs of an adult female of *S. cyaneus*.
6. Most of the larvae died before the end of the first week when fed symbiont-free balsam fir chips. It was necessary to pool the surviving larvae to obtain sufficient material for a single series of enzyme assays.
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10. Larval midguts, in groups of 10 to 20, were homogenized and centrifuged. The extract was desalted on a Pharmacia PD-10 column and soluble proteins were dialyzed against 50 m*M* acetate, *p*H 5.0. Cell-free fungal culture fluid was concentrated in a Pellicon Cassette (Millipore). Proteins were precipitated with ethanol at −20°C and dialyzed against acetate buffer, *p*H 5.0. Larval and fungal extracts were treated similarly thereafter. Cellulases and xylanases were fractionated on a column (2.6 by 40 cm) of DEAE-Sepharose CL6B equilibrated with 5 m*M* acetate buffer, *p*H 5.0, and eluted with a 0 to 500 m*M* linear NaCl gradient. Four major peaks of xylanase activity were eluted from the column at concentrations of 35 m*M*, 80 m*M*, 165 m*M*, and 345 m*M* NaCl. Two major cellulases emerged from the column at concentrations of 295 m*M* and 370 m*M* NaCl. Active fractions were further fractionated on a column (2.6 by 40 cm) of Sephadex G75 eluted with 50 m*M* acetate buffer, *p*H 5.0. Isoelectric focusing on an ion exchanger (chromatofocusing) was performed on a column (1 by 40 cm) of Polybuffer Exchanger 94 (Pharmacia) equilibrated with 25 m*M* imidazole HCl, *p*H 7.4, and eluted with 0.0094 m*M* Polybuffer 74 (Pharmacia) per *p*H unit per milliliter, *p*H 4.
11. The second cellulase fraction did not elute from the column, since the *p*H gradient was set to run from *p*H 7 to 4. This cellulase was eluted with an NaCl solution. That the *p*I values of the proteins in this fraction are less than *p*H 4 was confirmed by polyacrylamide gel isofocusing (Fig. 1).
12. Analytical isofocusing was performed on 0.1-mm polyacrylamide gels (Servalyt Precotes, Serva Fine Biochemicals) with a nominal *p*H range of 3 to 6. Focusing was performed for 2000 volthours (final field strength of 100 V/cm) at 10°C in a Desaga flatbed apparatus. Gels, fixed in 20 percent trichloroacetic acid, were stained with silver nitrate [C. R. Merril, D. Goldman, S. A. Sedman, M. H. Ebert, *Science* **211**, 1437 (1981)]. Amyloglucosidase, glucose oxidase, soybean trypsin inhibitor, β-lactoglobulin A, and bovine carbonic anhydrase B (Serva Fine Biochemicals) were used as *p*I marker proteins.
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21. We thank A. Sakai, R. VandeKopple, G. Keevil, and E. Weatherbee for field assistance, the University of Michigan Biological Station and the Matthaei Botanical Gardens for use of their facilities, and the National Science Foundation for grants PCM-78-22733 and PCM-82-03537 to M.M.M.

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Is Thymosin Action Mediated by Prostaglandin Release?

Abstract. *Treatment of spleen cells derived from adult thymectomized mice with thymosin fraction 5 resulted in a rapid and dose-dependent stimulation of the release of immunoreactive prostaglandin E_2. The release of prostaglandin E_2 was associated with induction of theta antigen and was totally inhibited by indomethacin. In contrast, prostaglandin E_2 release from spleen cells from intact donors was inhibited by treatment with fraction 5. The data support the concept that prostaglandin E_2 mediates the effects of thymosin fraction 5 on lymphocytes.*

Prostaglandins have been implicated as possible mediators of the biological activity of thymic factors. Studies in our laboratory showed that an analog of prostaglandin E_2 (PGE_2) was able to mimic the action of thymic factors in the induction of theta antigen and in the appearance of serum thymic-like activity (STA) when it was given to adult thymectomized mice (*1*, *2*). Moreover, the action of thymosin on theta antigen and on STA appearance was completely abrogated by the administration of indomethacin, a potent inhibitor of prostaglandin synthetase. In the experiments reported here we investigated the mode of action of thymosin on lymphocytes and examined the relation between thymosin and prostaglandins. Our results show that thymosin induces an early and dose-dependent release of high concentrations of PGE_2 by lymphocytes collected from thymectomized mice.

For these experiments we used 4- to 8-week-old male C57BL/6J mice obtained from ENEA-Casaccia, Rome, Italy. Spleen cells from thymectomized or intact mice were incubated with 100 μg of thymosin fraction 5 (Fr5) (Hoffmann–La Roche, Nutley, New Jersey) with or without indomethacin. At various time intervals, the amount of PGE released was measured by using the radioimmunoassay described by Jaffe *et al.* (*3*). As early as 5 minutes after incubation, spleen cells obtained from thymectomized mice and treated with Fr5 released markedly higher concentrations of PGE_2 than untreated spleen cells or spleen cells from thymus-intact mice (Fig. 1A). This difference persisted at least for 1 hour. Conversely, spleen cells from intact donors showed a slight inhibition of the PGE release after exposure to Fr5. Indomethacin inhibited the release both from Fr5-stimulated spleen cells from thymectomized mice and from intact donors.

In a second experiment, spleen cells from thymectomized and intact mice were separated on sodium metrizoate–Ficoll solution (Lymphoprep) and incubated with various concentrations of Fr5. After 15 minutes (that is, at the time of full PGE release) specific PGE_2 release was measured by radioimmunoassay. Thymosin Fr5 increased the PGE_2 release by lymphocytes from thymectomized mice in a dose-dependent fashion (Fig. 1B). Conversely, in normal splenic lymphocytes, small amounts of Fr5 stimulated a slight PGE_2 release, whereas high concentrations of the factor (10 to 100 μg/ml) inhibited the spontaneous release of PGE_2. Furthermore, most of the PGE released by splenocytes belonged to the PGE series. Treatment with indomethacin inhibited PGE_2 release.

Figure 2 shows the relation between the induction of theta-antigen in vitro and the release of PGE_2 by lymphocytes derived from thymectomized mice and incubated with various amounts of Fr5. The presence of theta antigen was evaluated by measuring the concentrations of azathioprine (AZ) required for inhibiting splenic, spontaneous rosette-forming cells, according to a modification of Bach's technique (*4*). Theta-positive cells were defined as those in which rosetting was inhibited by 1.5 μg of AZ. High concentrations of Fr5 (100 μg/ml) induced the presence of theta antigen and stimulated the greatest release of PGE_2, whereas low levels of the factor (1 to 5 μg/ml) were unable to induce theta antigen and produced only a limited amount of PGE_2.