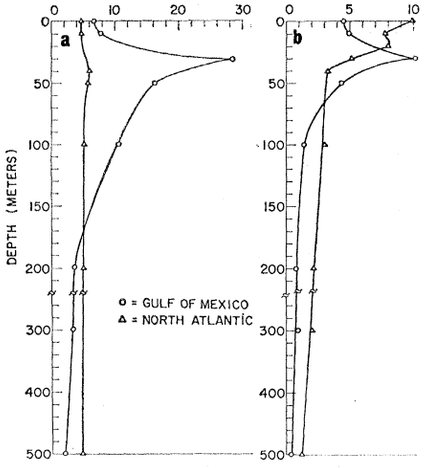

Fig. 1. Concentration-depth profile for methane (a; $\times 10^{-5}$ ml/liter) and the ethane-ethylene fraction (b; $\times 10^{-6}$ ml/liter). Gulf of Mexico samples from 28°59'N, 88°11'W; Atlantic samples from 52°35'N, 20°9'W.

adsorbed gases and to carry these gases into the chromatograph (*4*) for further separation and analysis (*3*).

For the low-molecular-weight hydrocarbons, the absolute sensitivity of the method is approximately 2×10^{-12} moles. On the basis of the 1-liter samples of water used by us, this sensitivity corresponds to about 1 part in 10^{13} by weight, or approximately 5×10^{-8} ml of dissolved gas per liter of sea water. At this lower limit, the precision of the method, on the basis of replicate measurements under laboratory conditions, is ± 10 percent (relative S.D.). For quantities of gas greater by a factor of 10 or more, the precision improves to ± 0.4 percent. Additional error incurred during field operations may increase this spread in uncertainty to as much as ± 1 percent. The chromatograph was calibrated with an artificial gas mixture containing known amounts of the hydrocarbons in question.

In most instances, analysis of our Atlantic samples at the Naval Research Laboratory entailed considerable time delay caused by shipment, some samples being analyzed from 2 weeks to 3 months after collection. The Gulf of Mexico samples, however, were analyzed within 2 to 6 hours of collection (*5*). Duplicate analyses were made of the Gulf samples: the first, immediately; the second, about 1 month later at the Naval Research Laboratory. All samples were stored in bottles having ground-glass stoppers. Duplicates did not differ significantly.

At the time of collection, several samples were treated with either sodium azide or mercuric chloride to retard bacterial action; a few samples were deliberately left untreated. Treatment with sodium azide had no noticeable effect, but mercuric chloride caused the complete disappearance of unsaturated hydrocarbons such as ethylene and propylene.

With all samples, concentrations of the C_1 to C_4 hydrocarbons generally tended to decrease with increase in depth, but in several samples the concentration profile showed a maximum at a depth of 30 to 50 m, where the concentration was from 4 to 15 times greater than at 500 m (Fig. 1). Figure 1a shows a dramatic increase in methane concentration near the surface for the Gulf water; the Atlantic samples showed only a slight increase in methane at the same depth. Figure 1b shows a similar trend for the ethane-ethylene fraction.

Some illustrative data for samples from both sources are shown in Table 1. In all instances, as one might anticipate, methane showed by far the highest concentrations. An interesting feature of these observations is shown by the propane-propylene data; in the 0- to 50-m range, the unsaturated-hydrocarbon concentration appears to exceed significantly the concentration of the saturated component. Individual ethane and ethylene concentrations, while not shown in Table 1, exhibit the same trend in the same range of depth.

In order to separate ethane from ethylene, a different chromatographic column was used, causing an increase in retention time of the other hydrocarbons; consequently, use of this column was limited during the period of production of these data. Data are still too few to permit conclusions concerning the possible significance of these values; the work continues.

J. W. SWINNERTON
V. J. LINNENBOM
Ocean Sciences and Engineering Division, U.S. Naval Research Laboratory, Washington, D.C. 20390

References and Notes

1. G. E. Hutchinson, *A Treatise on Limnology* (Wiley, New York, 1957).
2. F. A. Richards, J. D. Cline, W. W. Broenkow, L. P. Atkinson, *Limnol. Oceanog.* **10**, R185 (1965).
3. J. W. Swinnerton and V. J. Linnenbom, *J. Gas Chromatog.*, in press.
4. F & M model-700 equipped with dual hydrogen flame-ionization detectors.
5. Because of cooperation by the Gulf Coast Research Laboratory, Ocean Springs, Miss.

13 February 1967

Symbiosis: Effects of a Mutualistic Fungus upon the Growth and Reproduction of Xyleborus ferrugineus

Abstract. Xyleborus ferrugineus *beetles developed from asymbiontic eggs through the adult stage on a sterilized meridic diet, but the resulting adults reproduced only when a mutualistic fungus was inoculated into the diet. Beetles with bacterial symbionts still required the fungus for reproduction on a second meridic diet.*

As a nutritional substrate for insects, wood is especially deficient in vitamins, sterols, and related growth factors. Because species of insects successfully attack and reproduce in the wood of most trees, their sources of vitamins, sterols, and related growth factors in such situations is of interest. Wood-infesting beetles in the families Scolytidae and Platypodidae are closely associated with microbes in their brood galleries. The associated microbes have been strong suspects as sources of the nutrients that are absent in wood but that are required by these beetles.

Francke-Grosmann (*1*) recently reviewed the extensive knowledge of the interrelationships among ambrosia beetles (Platypodidae and certain species of Scolytidae), their microbial symbionts, and their woody substrate. This knowledge lacked proof that microbes produce specific nutrients essential to the beetles.

In studies of the nutritional interrelationships among the ambrosia beetle *Xyleborus ferrugineus* (Fabricius), its microbial symbionts, and the woody substrate, we have found that the insects developed from asymbiontic eggs through the adult stage on a sterile meridic diet (I) similar to that described by Chippendale and Beck (*2*), but eggs produced by these asymbiontic adults were not viable.

The females tunneled and fed extensively in the sterile medium, but oviposited viable eggs only after a mutualistic fungus (*Fusarium solani*) was inoculated into the diet. This fungus was isolated consistently from the oral mycangia (organs for the transport and

perpetuation of symbionts) in the beetles. The fungus in vitro produced characteristic *Cephalosporium* and *Fusarium* types of conidia on a range of media. It grew in the monilioid (ambrosia) form on the walls of the brood tunnels in the meridic diet. This fungus was always isolated from brood tunnels of the beetle in the host tree.

Adults (free of all but bacterial symbionts) tunneled and fed extensively for many days in the sterilized elaborate basic diet (II) of our research colleagues (*3*), but again oviposited viable eggs only after the *Fusarium* was inoculated into the medium. Beetles (with *Fusarium* and bacteria) produced eggs which developed through the adult stage on a diet (III) of only ground sapwood of *Populus* plus 4 percent Bacto-agar.

We concluded that the *Fusarium* synthesizes chemicals that are essential to reproduction by the insect. The limited production of nonviable eggs by the asymbiontic beetles on the elaborate diets I and II would negate ovipositional stimulation as the sole role of the *Fusarium*.

Our experimental procedures and more detailed results were as follows: Adults (ten females, one male) developed from 87 surface-sterilized eggs within 30 days (experiment 1) on a sterilized meridic diet (I) containing the ingredients used by Chippendale and Beck (*2*), excluding alfalfa juice, sorbic acid, and sodium alginate. Adults of experiment 1 tunneled and fed as long as 35 days in the medium, but the ten females produced a total of only two nonviable eggs. Eleven larvae hatched from 51 surface-sterilized eggs on diet I when vitamins were omitted (experiment 2). All larvae died during early instars.

This mortality of insect larvae on a diet deficient in vitamins was consistent with existing knowledge of insect nutrition. Three adults developed from 51 surface-sterilized eggs on diet I excluding the vitamins, but with 4 g of ground, freeze-dried *Fusarium* fungus added per batch of diet (experiment 3). Fourteen additional eggs in this experiment hatched, but the larvae died. The adults of experiment 3 tunneled and fed extensively for 25 days (experiment terminated) in the diet, but produced no eggs. We believe that the extremely small number of adults produced and the nonfertility of the adults in experiment 3 were attributable to only a limited amount of freeze-dried *Fusarium* being added to the medium.

In experiment 4, seven adults developed from 87 surface-sterilized eggs on diet I. The adults lived up to 60 days in this diet, but produced no eggs. A pure culture of the *Fusarium* then was inoculated into the medium containing the seven previously asymbiontic females of experiment 4, and they began to oviposit within 7 days after the inoculations (experiment 5).

Adults (12 females) from surface-sterilized pupae yielded viable eggs when placed on a second sterilized meridic diet (II) that was inoculated with a pure culture of the *Fusarium* fungus (experiment *6*). Diet II used ground sapwood of *Populus* as the basic component, and was described in detail by Saunders and Knoke (*3*). The eggs produced in experiment 6 hatched, and 18 progeny adults (all males) were present in the medium within 30 days. The adult females used in this experiment were unmated; such females of this species always yield all-male progeny. Compared to a mean of 1.5 progeny adults (all males) per maternal female on diet II plus the viable *Fusarium fungus* (experiment 6), a mean of 6.0 female and 0.5 male progeny per mother beetle was produced in 30 days by 24 females (nonsterilized) from a stock laboratory culture of *X. ferrugineus* and its symbionts, when the beetles were placed on diet II (experiment 7). The differences in progeny produced per female in experiments 6 and 7 would indicate that *Fusarium* and bacteria are not the only important symbionts of these beetles. Twenty-six female beetles from surface-sterilized pupae produced a total of only two eggs (no hatch) in 30 days on diet II (experiment 8). Pure cultures of the *Fusarium* fungus were inoculated into the medium in tubes containing 11 of the 26 beetles after 30 days (experiment 9). These 11 beetles produced a mean progeny of 5 adults (all males) during the next 30 days. The other 15 females from experiment 8 were left as controls on the original diet II, free of *Fusarium fungus*, for another 30 days, and they yielded no eggs.

Ten beetles from the stock cultures (with symbionts) produced a mean progeny of three eggs, two larvae, one pupa, and eight adults in 30 days on diet II minus the yeast extract component (experiment 10). The omission of the yeast extract from diet II would make the insect largely dependent upon its symbionts for vitamins and other special growth factors.

Thirty-four beetles from the stock laboratory culture were fed in diet II plus 1.2 percent sorbic acid for 28 days to free the insects of most, if not all, loosely associated microbes (experiment 11). No *Fusarium* grew in this diet, and the beetles did not oviposit. The beetles were transferred to a diet (III) of sterilized ground aspen sapwood containing 4 percent Bacto-agar, and they yielded a mean progeny of 4.0 individuals in 30 days. Diet III was about as similar to intact wood as any artificial substrate could be. The reproduction of the beetles in this medium of ground sapwood (diet III) indicated that the microbes closely associated with these insects can provide most, if not all, of the vitamins and other complex growth substances essential to the growth and sexual development of the beetle.

The eggs used in experiments 1 to 4 were surface-sterilized through the following technique: (i) washed twice for 1 minute in sterile, distilled water; (ii) submerged for 4 minutes in 0.1 percent $HgCl_2$; (iii) rinsed twice for 1 minute in sterile, distilled water; (iv) washed for 30 seconds in 70 percent ethanol; and (v) rinsed once for 1 minute in sterile, distilled water. Hatch was poor, but the percentage of sterilization was always 100 percent when series of treated eggs subsequently were plated on several media.

Beetles used in experiments 6 and 8 transformed from pupae that were surface-sterilized through the following technique: pupa was (i) submerged in 0.1 percent $HgCl_2$ for 4 minutes; (ii) rinsed twice for 1 minute in sterile, distilled water; (iii) rinsed once for 30 seconds in 70 percent ethanol; and (iv) rinsed once for 1 minute in sterile, distilled water. The treated pupae then were placed singly on sterilized 2 percent water agar in a petri dish for transformation to adults. The surface of the agar of each plate was checked microscopically for growth of microbes from the pupa. Most adults from the treated pupae still bore bacteria (apparently from the intestinal tract of the pupa), but the adults were free of yeasts and other fungi.

The *Fusarium* fungus growth, added to diet I, experiment 3, was prepared as follows: plugs of the fungus were cut from the edge of actively growing colonies [on PDA (*4*) in petri dishes] with a sterile No. 3 cork borer. Five plugs of fungus were placed in 50 ml of Difco nitrogen-free, yeast-base liquid medium (containing 1.0 g of added ammonium acetate per liter), each in a

250-ml Erlenmeyer flask. The cultures (still) were held for 4 weeks at room temperature in the light. The entire culture contents were then freeze-dried at 150°F. This freeze-dried material was added to the diet for experiment 3.

DALE M. NORRIS
JOCELYN KING BAKER
Department of Entomology,
University of Wisconsin, Madison

References and Notes

1. H. Francke-Grosmann, in *Symbiosis*, S. M. Henry, Ed. (Academic Press, New York, 1967), vol. 2, pp. 141–205.
2. G. M. Chippendale and S. D. Beck, *Entomol. Exp. Appl.* **7**, 241 (1964).
3. J. L. Saunders and J. K. Knoke, *Science*, in press.
4. Potato-dextrose-agar medium.
5. Approved for publication by the director of the Wisconsin Agricultural Experiment Station. This study was supported in part by a research grant (AI 06195-03) from NIH.

3 April 1967

Urinary Metabolites in Congenital Hyperuricosuria

Abstract. *The excretion of oxypurine metabolites in urine of patients with congenital hyperuricosuria exceeds, on a creatinine basis, that observed in any previously recognized metabolic anomaly. The ratio of hypoxanthine to xanthine is from 2 : 1 to 3 : 1 and results from increased hypoxanthine excretion, in contrast to other hyperuricosuric conditions where ratios of less than one have been reported. Administration of allopurinol (a xanthine-oxidase inhibitor) reduces the excretion of uric acid but results in an equivalent increase in xanthine and hypoxanthine. These features appear to be unique to congenital hyperuricosuria.*

Lesch and Nyhan have recently described a familial disorder of purine metabolism in which hyperuricemia is associated with evidence of central nervous system dysfunction (*1*). Since the original report, many cases have been recognized and congenital hyperuricosuria now appears to be one of the more common "inborn errors of metabolism."

In our own experience, without making an intensive search for cases, four boys in four families have been identified within a year, and two additional close relatives probably have the disease. The mothers of two of the children are sisters, supporting the suggestion that inheritance is linked to the X chromosome (*2*).

The predominant oxypurine metabolites of nucleic acid metabolism have been measured in 24-hour collections of the urine in four of these childen (Table 1). Uric acid was measured by the uricase method. Hypoxanthine and xanthine were first partially purified by passing the urine through a column of Dowex 50 to remove uric acid and urea. The two oxypurines were separated on a second column of Dowex that had been calibrated with standard solutions of xanthine and hypoxanthine. Xanthine was eluted first with 0.15*N* HCl, and hypoxanthine was then eluted with 0.6*N* HCl. The concentrations were assayed with xanthine oxidase (*3*). The excretion of individual and of total oxypurines has been related to creatinine excretion for 24 hours. Body weight could also have been a standard without altering the interpretation.

The four normal children excreted somewhat larger amounts of uric acid per gram creatinine than adults, who usually excrete less than 500 mg (Table 1). However, the excretion of hypoxanthine and xanthine remains low with xanthine distinctly higher, as in adults. In the gout cases, and these may be considered representative (*4*), the excretion of uric acid is increased but that of the other oxypurines is normal. The same is true in the hyperuricosuria reflecting the rapid degradation of nucleic acids, as, for example, in granulocytic leukemia (Table 1).

The observations previously reported, and those we present, suggest that the major catabolic pathways to uric acid are from adenylic acid via adenosine and inosine to hypoxanthine, and from guanylic and xanthylic acids to xanthosine and xanthine. Tracer experiments indicate that normally adenine is the primary precursor for urinary hypoxanthine (*3*). Urinary xanthine exceeds hypoxanthine by severalfold in normal individuals, suggesting that xanthylic acid or guanylic acid (or both) may make the major contribution to uric acid. Support for this concept is found in patients with xanthinuria, in whom there is a congenital deficiency of xanthine oxidase (*3*). In addition to a sharp reduction in the synthesis of uric acid, the conversion of hypoxanthine to xanthine is reduced, and this permits a clearer interpretation of the relative rates of synthesis. Xanthine excretion is two or more times that of hypoxanthine. Possibly the difference in excretory rates of hypoxanthine and xanthine lies in the relatively more effective incorporation of adenine than of guanine into nucleic acids (*5*). This provides a mechanism for reutilization and conservation of the adenine moiety.

Thus the observations on the four children with congenital hyperuricosuria are simpler to intrepret. The children excreted amounts of uric acid far exceeding, based on creatinine excretion, those previously observed. They also excreted so much hypoxanthine that the usual ratio of hypoxanthine to xanthine was reversed. This anomaly could have resulted from a reduced efficiency in the reutilization of adenylic acid and its derivatives. An alternative hypothesis is that inosinic acid is synthesized in such large amounts that the bulk is converted to uric acid without being incorporated into nucleic acids. Some of this ino-

Table 1. Urinary metabolites. The oxypurines excreted are expressed as milligrams per gram of creatinine.

Patient	Allopurinol treatment (mg)	Oxypurines excreted		
		Uric acid	Hypoxanthine	Xanthine
		Hyperuricosuria		
C.W.*	None	2500	78	38
	100	1900	550	570
	150	1300	740	870
	200	550	670	910
	250	260	1100	2000
R.K.†	None	2700	70	28
	100	820	1000	700
J.A.†	None	2600	90	30
	100	970	1200	650
D.C.†	None	2800	100	43
		Leukemia		
S.W.(*7*)	None	900	3.2	17
	800	200	7.0	120
A.S.(*7*)	None	1300	4.7	11
	800	400	7.0	27
		Gout ‡		
	None	1290		
	300	500	190	
	None	1260	15	
	300	378	76	
		Normal children §		
	None	670 (475–880)	6 (3.5–9)	21 (10–33)
		Normal adults §		
	None	370 (270–580)	1.5 (.4–3.5)	30 (13–45)

* Average of two 24-hour urine collections. † Average of three 24-hour urine collections. ‡ Hypoxanthine and xanthine were not determined separately. Values in milligrams per 24 hours. Creatinine excretion in the adult approximates 1 to 1.5 g/24 hr. § Averages of four normal children (5 to 12 years) and five normal adults. Range of values in parentheses.