The Biochemical Basis of the Fungus-Attine Ant Symbiosis

A complex symbiosis is based upon integration of the carbon and nitrogen metabolisms of the two organisms.

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The obligate symbiosis between the attine ants and the fungus which grows in their nests is a spectacular example of a mutually advantageous association of two very different types of organisms. In this article investigations which have elucidated the biochemical basis for this complex association are reviewed. It is the purpose of the article to trace the way in which a knowledge of the natural history of the attine ants permitted the formulation of questions which led to an understanding of the symbiosis in biochemical terms, and to show how this understanding has permitted the formulation of new questions of a more physiological and biological nature.

Natural History

The attine ants, commonly known as the fungus-growing ants, occur exclusively in the Western Hemisphere and are predominantly Neotropical in distribution. There is an extensive literature describing the natural history of this group (*1*, *2*). All of the species which comprise the tribe Attini culture a fungus in their nests and utilize this fungus as their primary and probably sole source of food. The substrate on which the fungus is grown varies with the genus. The simpler, more primitive genera use insect feces and insect carcasses as substrates for their cultures. The intermediate genera utilize a combination of plant debris and fragments of leaves and flowers cut from live plants. The most complex, most highly evolved, and most spectacular genera, *Acromyrmex* and *Atta*, culture their fungi almost exclusively on leaves and flowers cut from live plants. Species of these two genera are commonly known as leaf-cutting ants.

Identification of the fungus or fungi cultivated by the various attine species has been complicated by the fact that sporophores are not normally produced in the ants' nest, and their production in artificial culture has proved to be difficult. To date, only two ant fungi have been given names, after examination of their fruiting bodies (*1*). These are *Leucocoprinus* (or *Leucoagaricus*) *gongylophora*, cultivated by *Acromyrmex disciger* and possibly by *Atta*, and *Lepiota* n. sp., cultivated by the primitive attines *Cyphomyrmex costatus* and *Myrmicocrypta buenzlii*. Both are Basidiomycetes, and both are known only from attine nests.

The fungus gardens are fragile, spongelike structures consisting of many small pieces of substrate held together by the dense mycelial growth which covers them. The gardens constructed by *Atta* tend to be roughly hemispherical, with diameters ranging from 15 to 30 centimeters. A large *Atta* nest may contain hundreds of fungus gardens. The less conspicuous attines have fewer and smaller gardens. The fungus gardens are flourishing growths of a single fungus. This fungus is readily isolated in pure form by standard mycological plating procedures. When grown on standard culture media, such as potato dextrose or Sabouraud's dextrose agar, the attine fungi are seen to be rather slow-growing organisms which are readily overwhelmed by the common contaminants that complicate the lives of mycologists. However, Weber (*3*) has shown that if the ants have access to an agar culture of their fungus they can maintain it indefinitely even when it is adjacent to large areas of contamination. As soon as the ants are denied access to the fungus garden a rapid deterioration of the culture ensues, and contaminants replace the ants' fungus. Clearly, the growth of the ants' fungus in their nests is not fortuitous. The viability of the fungus depends directly upon the presence of the ants. It is this feature of the natural history of the attine ants—their ability to maintain a flourishing culture of a slow-growing fungus that cannot survive in nature independent of them—which has been the subject of research by my colleagues, Raymond Carman, John MacConnell, and Joan Martin, and me in the department of chemistry at the University of Michigan for the past few years. The behavior of the ants strongly implies a chemical basis for their success in culturing their food fungus in the face of possible competition from faster growing, more viable microorganisms present in the surrounding soil or brought into the nest on substrate or on the ants themselves (*1*).

Fungus-Culturing Behavior

Fresh leaves cut from live plants are the most commonly used substrate in the fungus gardens of *Atta* (*1*). A leaf fragment brought into the nest for incorporation into a garden is first cleaned and scraped. It is then cut into very small pieces, becoming quite pulpy and moist in the process, presumably from juices expressed from the leaf, and from saliva applied by the ants. An ant then holds the leaf particle to the tip of its abdomen and deposits a liquid fecal droplet on it. The leaf fragment is then inserted into the matrix of the fungus garden, and several tufts of mycelium are planted on it. The newly incorporated leaf fragment may receive several additional fecal applications from other ants. All attine species, regardless of their favored substrate, exhibit similar behavior. In all species the application of fecal material to the fungus garden, and to substrate prior to its incorporation into the garden, is characteristic (*1*, *3*, *4*). As Weber (*1*, *3*, *4*) has repeatedly emphasized, substances present in the fecal material and possibly also in the salivary material probably create environmental conditions favorable to the growth of

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the food fungus. It was the objective of our research to establish, in chemical or biochemical terms, precisely how the application of fecal material contributes to the enhanced viability of the fungus in the presence of the ants.

The Antibiotic Hypothesis

Weber (*1*, *4*) offered as a tentative hypothesis the suggestion that there might be present in the anal or salivary secretions of the ants antibiotic substances which prevented the growth of alien microorganisms in their fungus gardens. Perhaps as a consequence of man's inclination to celebrate the wisdom of the ant, this suggestion was so often cited that it came to be accorded the status more of a proved fact than of a tentative hypothesis. Indeed, our initial interest in the attine ants stemmed from our uncritical acceptance of this appealing theory and from our hope that a study of the symbiosis between the attine ants and the fungi which they culture in their nests might lead to the discovery of potentially useful antibacterial and antifungal substances. Unfortunately the theory appears to be incorrect (*5*). Despite repeated efforts we were unable to detect significant bacteriostatic or fungistatic activity in extracts of the ant *Atta colombica tonsipes*, of its fungus gardens, or of the remains of its fungus gardens after they had been cast out of the nest by the ants. The possibility remains that some secretion applied to the fungus gardens by the ants may contain substances which exert mildly deleterious effects on the growth rates of certain microorganisms, thereby influencing the composition of the bacterial or protozoan population of the garden, but we are forced reluctantly to conclude that no replacement for penicillin is likely to be discovered through a study of the fungus-attine ant symbiosis.

An Ecological View

Our initial probe of this symbiosis was designed around the preconceived notion that potent antibiotics played a major role in preventing alien microorganisms from growing in the ants' fungus cultures. Since our research was oriented toward the isolation of substances which were not there, it inevitably proceeded to an unproductive conclusion. Only after redefining the problem in more objective, general terms were we able to formulate meaningful and answerable questions which probed the basis for the association of the ants and their food fungi.

Table 1. The growth of the fungus cultured by *Atta colombica tonsipes* in defined media containing either a casein hydrolyzate rich in α-amino nitrogen or a casein peptone poor in α-amino nitrogen (*8*). Shaken liquid cultures (25°C, 7-day growth period).

Nitrogen source	Concentration (mg/ml)	Average dry weight of fungus (mg)
Casein hydrolyzate*	1.0	47
Casein peptone†	1.0	8
Casein hydrolyzate	2.0	91
Casein peptone	2.0	12
Casein hydrolyzate	4.0	110
Casein peptone	4.0	19

* Enzymatic Casein Hydrolyzate (*9*). † Peptone T (*9*).

The basic biological process being investigated in this work is interspecific microbial competition. Outside of the fungus chamber the ant fungus is a very poor competitor and is excluded from otherwise suitable substrates by better competitors. By contrast, within the fungus chamber it is such a successful competitor that it emerges as the dominant species. Thus, as a consequence of the fungus-culturing activities of the ants, the outcome of interspecific competition has been reversed.

An interesting feature of the attine ant fungus garden is revealed when the processes occurring there are contrasted with those which normally occur during the microbial decomposition of plant remains. In the normal succession which characterizes the decomposition of leaf litter there are several stages of colonization and exploitation by Fungi Imperfecti and Ascomycete fungi which preceed the Basidiomycete stage (*6*). Normally the Basidiomycetes do not appear until the litter is already considerably decomposed, compacted, and very moist (*6*). Yet, in the fungus garden, fresh leaf material is exploited by a Basidiomycete. Clearly, the ants have thoroughly disrupted the normal successional process.

The outcome of interspecific microbial competition is largely determined by the relative efficiencies with which each of the competing organisms sequesters substrate nutrients crucial to the growth of its competitors and itself. This capacity is closely associated with the relative growth rates (*7*). Our studies have shown that the outcome of competition in the fungus chamber has not been determined by the elaboration of potent antibiotic substances which drastically reduce the growth rate of alien fungi. Once we have cast the question in the context of competition and have expressed the outcome of our search for an antibiotic in such language, the corollary possibility that the ant secretions may contain substances which significantly increase the growth rate of their fungus becomes obvious. The possible role of growth-promoting substances was also anticipated by Weber (*1*, *3*, *4*). The possibility that the activities of the ants resulted in an enhanced growth rate for their food fungus suggested an experimental approach which would (i) establish the composition of the secretions being applied to the garden by the ants, and (ii) determine whether the addition of such materials to the natural substrate would alter it in such a way as to improve the growth characteristics of the fungus. This second objective in turn required an understanding of the metabolic characteristics of the fungus, and determination of the conditions which affect its growth rate.

Some Metabolic Characteristics of the Fungus

Through the use of synthetic culture media we demonstrated that the growth characteristics of the food fungus cultured by the ant *Atta colombica tonsipes* are strongly dependent upon the form in which nitrogen is supplied to the fungus (*8*). The most important finding was that the fungus grew very poorly in culture media in which the nitrogen was supplied as a protein, such as casein, lactalbumin, or zein, but grew very well in media in which the nitrogen source was a hydrolyzate of these same proteins. The fungus even exhibited significantly different growth rates in media in which the nitrogen was supplied in the form of hydrolyzates of the same protein which differed in the extent of hydrolysis (Tables 1 and 2). When the nitrogen source was Enzymatic Casein Hydrolyzate (*9*), a hydrolyzate consisting primarily of free amino acids and of peptides of low molecular weight, the fungus grew significantly better, both in shaken liquid cultures and on solid agar cultures, than when the nitrogen source was Peptone T (*9*), a tryptic digest of casein composed largely of soluble polypeptides of fairly high mo-

Table 2. The growth of the fungus cultured by *Atta colombica tonsipes* in defined media containing either a casein hydrolyzate rich in α-amino nitrogen or a casein peptone poor in α-amino nitrogen (*8*). Solid agar cultures (25°C, 21-day growth period).

Nitrogen source	Concentration (mg/ml)	Area covered by fungus (mm^2)
Casein hydrolyzate	1.0	44
Casein peptone	1.0	17
Casein hydrolyzate	2.0	65
Casein peptone	2.0	14

lecular weight and containing only small quantities of free amino acids and simple peptides.

From these experiments we have concluded that the fungus cultured by *Atta colombica tonsipes* lacks the full complement of proteolytic enzymes necessary to make effective use of polypeptide nitrogen. This metabolic limitation is of considerable ecological significance, since it means that the fungus will be at a considerable competitive disadvantage when it is required to live on a substrate in which the nitrogen is present predominantly as polypeptide. Fresh leaf material would, therefore, seem to be a most inappropriate substrate for this fungus, since most of the amino acid nitrogen in leaves is present as protein. Clearly the ability of the ants to grow their fungus on fresh leaf material implies that their fungus-culturing activities effectively compensate for the critical metabolic limitations of their fungus.

Composition of the Fecal Material

Since the form of nitrogen available to the fungus greatly affects its growth, a determination of the nitrogenous components of the ants' fecal material is obviously pertinent (Table 3) (*8*). In the rectal fluid of *Atta colombica tonsipes*, allantoic acid and allantoin are the two major nitrogenous components. In addition, ammonia and free amino acids are present in significant quantities. All 21 of the common natural amino acids are present in the rectal fluid (*8*). The six amino acids—glutamic acid, histidine, arginine, proline, lysine, and leucine—make up 82 percent (by weight) of the total (*8*).

It is evident that when the ants defecate on their fungus gardens they are supplementing the culture medium with nitrogenous substances which greatly stimulate the growth of the fungus. However, any beneficial effect will necessarily be short-lived, since, as soon as the supplemental nutrients are consumed, the fungus will again find itself in a very unfavorable competitive position in which it must sustain itself on a culture medium in which the nitrogen is in the form of protein. What the fungus culture most needs is a supplemental supply of proteolytic enzymes. We therefore examined the possibility that either the saliva or the fecal material contained proteolytic enzymes.

No protease activity could be detected in homogenates of the salivary glands, mandibular glands, maxillary glands, or postpharyngeal glands, all of which might be regarded as reasonable sources of a secretion applied to the leaf particle. However, significant protease activity was detected in the contents of the midgut and rectum (*8*, *10*). Seven attine species were examined (*10*), and in every case the level of protease activity was several times higher in the rectal fluid than in the contents of the midgut (Table 4).

Finally, it was established that *Atta colombica tonsipes* does excrete active proteolytic enzymes onto its fungus garden. We demonstrated this by recovering from the garden a substrate, which had no proteolytic activity of its own, after the ants had prepared it for incorporation into their garden. In captivity *A. colombica tonsipes* readily utilizes cornflakes as a substrate for its fungus cultures, treating them, to all appearances, exactly like leaves. Untreated cornflakes have no protease activity. Pieces of cornflakes recovered from a garden immediately after the ants had completed their entire substrate preparation behavior were soft, moist, and tacky. Homogenates of such treated cornflakes had significant protease activity. Thus we have demonstrated that the ants' fecal material provides their fungus with both a nutrient supplement and a proteolytic enzyme supplement.

In addition to examining the rectal fluid of seven attine species for protease activity (Table 4), we examined the midgut and rectal contents of 15 non-attine species (Table 5) (*10*). The contrast between the attines and non-attines is clear-cut. In most of the non-attines, no activity whatsoever could be detected in the rectal fluid. In the five species in which some activity was detected it was much less than that of the midgut. In no case was rectal activity greater than, or even comparable to, midgut activity. To the extent that this survey is representative it appears that excretion of fecal material exhibiting significant protease activity is characteristic of, and peculiar to, the attine ants (*11*). Since none of the attines examined are representatives of primitive genera, we cannot be certain whether protease excretion is characteristic of the entire tribe of Attini or of only the more advanced genera. A study of the fecal material of primitive attines and non-attine myrmicines believed to be closely related to the attines might provide results pertinent to an understanding of the evolutionary origins of the attines. It might reveal whether the excretion of proteolytic enzymes was a characteristic of attine progenitors which permitted the fungus-growing habit to develop, or whether it was a characteristic which developed after the first fungus-growers had appeared, and which played a role in directing subsequent evolution and speciation.

Table 3. Nitrogenous components of the rectal fluid of *Atta colombica tonsipes* (*8*).

Substance	Range (μg per ant)	Average value (μg per ant)
Allantoic acid	1.4–2.5	1.9
Allantoin	0.7–2.0	1.3
Free amino acids		0.46
Ammonia	.07–0.14	.10

Most digestive enzymes in insects are produced in the midgut, the primary site of digestion. Presumably, therefore, the proteolytic enzymes in the rectal fluid of the attine ants are simply digestive enzymes from the midgut which have accumulated in the rectum, although their production by rectal microbial symbionts has not been ruled out. The contrasting enzymatic properties of the fecal material of the attine and non-attine ants raises two basic questions. What is the fate of the digestive enzymes in those insects which do not excrete them, and how are the midgut enzymes in the attines spared from this fate? Neither question can be answered at present.

Biochemical Basis for the Symbiosis

Garrett has identified five factors which contribute to a high degree of competitive ability in a fungus (*12*). These are (i) a high inoculum potential, permitting the fungus to overcome substrate resistance readily; (ii) rapid hyphal growth, favoring rapid and extensive colonization and coverage of

the substrate; (iii) good enzyme production, favoring rapid utilization of nutrients; (iv) antibiotic production, reducing competition; and (v) tolerance to antibiotics produced by other organisms. The application of fecal material to the substrate prior to its incorporation into the garden obviously enhances the competitive position of the fungus by compensating directly for its metabolic limitations. The nutrient supplement provides for the short-term needs of the newly planted fungus and thereby accelerates the initial phases of growth. The protease present in the fecal material supplements the enzymes produced by the fungus and facilitates the breakdown and subsequent utilization of substrate nutrients.

The entire repertoire of the ants' fungus culturing behavior can be interpreted in terms of the criteria for competitive ability enunciated by Garrett. The process of scraping, scarring, and macerating the leaves breaks down the leaf's barrier to fungal infection; this has the same effect as increasing the inoculum potential of the fungus. The cleaning and scraping process probably also serves to remove parasitic fungi and incidental spores which may have been on the leaf while it was still attached to the plant. Such organisms are frequently among the fungi observed growing on newly fallen leaves (*6*). The maceration of the leaf liberates nutrients from the leaf tissue, facilitating their utilization by the fungus as soon as it is planted. The maceration process also liberates cellular enzymes which supplement those from the fungus and from the ant, and which very probably contribute to autolytic degradation of potential nutrients in the leaf. Finally, the ants' practice of planting several tufts of mycelium on the newly incorporated leaf fragment has the same effect as rapid colonization and coverage of the substrate through rapid hyphal growth by a newly inoculated fungus. The net effect of all the activities is to enhance the competitive ability of the fungus to such a degree that, in the narrow ecological niche encompassed by the ants' fungus chamber, it emerges as the dominant fungus which excludes all competitors.

The foregoing discussion has defined in ecological and biochemical terms the contribution of the ants to the fungus. It is possible to describe the contribution of the fungus to the ants in similar terms. The fungus cultured by the attines is a cellulose-decomposer (*13*). By cultivating as a food crop a cellulose-degrading organism the ants gain access to the vast cellulosic reserves of the rain forest for indirect use as a nutrient. What termites have accomplished by their endosymbiotic association with cellulose-degrading microorganisms, the attine ants have achieved through their more complex ectosymbiotic association with a cellulose-degrading fungus. In biochemical terms, then, the contribution of the fungus to the ant is the enzymatic apparatus for degrading cellulose. The symbiosis of the attine ants and their food fungi can be viewed as a metabolic alliance in which the carbon and nitrogen metabolisms of the two organisms have been integrated.

Table 4. Proteolytic enzyme activity of the midgut and rectal contents of attine ants (*11*).

Species	Activity* per ant	
	Midgut	Rectum
Atta colombica tonsipes Santschi	55–185	355–550
Atta cephalotes L.	110	505
Atta sexdens L.	100	350
Acromyrmex octospinosus (Reich)	55†	235
Acromyrmex lobicornis Emery	60†	810
Sericomyrmex urichi (McCook)	125	500
Trachymyrmex septentrionalis Forel	55	715

* Activity was determined by means of the Azocoll procedure. The rectal or midgut contents was incubated for 20 minutes at 37°C in a test tube containing 15 mg of Azocoll (*14*), 1 ml of 0.05*M* phosphate buffer (*p*H 6.65 ± 0.03), and 2 ml of water. After the incubation mixture was filtered through a sintered glass funnel, the absorbance at 580 mμ of the filtrate was determined. Activity is expressed in terms of the number of nanograms of fungal protease (Sigma, type VI) which exhibit comparable activity. † Entire midguts were used instead of gut contents. The dissected midguts were homogenized by hand, and the protease assay was carried out on the entire homogenate.

Table 5. Proteolytic enzyme activity of the midgut and rectal contents of non-attine ants (*11*).

Species	Activity* per ant	
	Midgut	Rectum
Subfamily Formicinae		
Acanthomyops claviger (Roger)	200	≦ 5
Lasius alienus (Foerster)	100	≦ 5†
Lasius pallitarsis (Provancher)	145	≦ 5
Polyergus breviceps Emery	195	≦ 5
Formica montana Emery	100	≦ 5
Formica ulkei Emery	140	≦ 5
Formica obscuripes Forel	235	≦ 5
Formica pergandei Emery	60–125	≦ 5–20
Subfamily Myrmicinae		
Myrmica monticola Wheeler	90	≦ 5
Myrmica emeryana Forel	300	≦ 5
Myrmica brevinodis Forel	70	25
Crematogaster cerasi (Fitch)	200	≦ 5
Crematogaster lineolata (Say)	185	25
Aphaenogaster treatae Forel	1800	40
Aphaenogaster rudis Emery	600	20

* Activity was determined as described in the first footnote of Table 4. † Entire rectums were used instead of rectal contents. The dissected rectums were homogenized by hand, and the protease assay was carried out on the entire homogenate.

Summary

The natural history of the fungus-growing ants provides a spectacular example of a symbiotic association of two very different types of organisms. An anthropomorphic description is difficult to resist. The ants are efficient and industrious farmers. Their single crop is a fungus, grown on a substrate of leaves in carefully fertilized, well-tended gardens. Virtually every facet of the ants' behavior and life cycle has been shaped by their association with the fungus they culture. A characteristic feature of the ants' gardening technique is the application of their fecal material to the garden and to substrate being prepared for incorporation into the garden. We have established the biochemical significance of this behavior. The fecal material contains proteolytic enzymes which compensate for a deficiency of such enzymes in the fungus. In addition, the nitrogenous components in the fecal material facilitate the initial growth of the fungus. In biochemical terms, then, one can say that the ants contribute their enzymatic apparatus to degrade protein and the fungus contributes its enzymatic apparatus to degrade cellulose. As in the case of so many other natural symbiotic and parasitic associations, the basis is an integration of complementary metabolic capabilities and deficiencies.

Nature's manifest diversity and its underlying unity are recurring themes in biology. What has made the research described in this article particularly enjoyable has been the obvious counterpoint of these two antithetical attributes. My associates and I decided to study a spectacular and dramatic natural phenomenon. Yet we discovered nothing that was fundamentally new about the factors which govern the association of species in natural communities. We found, instead, that this unique system is a result of combinations and variations of familiar biological principles and practices. There is something satisfying about that.

References and Notes

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14. A product of Calbiochem.

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Laser Raman Spectroscopy

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The field of Raman spectroscopy has increased greatly in activity in the last few years primarily as a result of the development of reliable, high-power, continuously operating lasers (*1*). The study of many new fundamental processes in solids, liquids, and gases has been made possible by the use of lasers and modern light-scattering techniques. In this article I shall describe the techniques of laser Raman spectroscopy as well as some of the fundamental physical processes which can be and have been studied by their use.

Theory

Raman-scattered light is usually quite weak (typically 10^{-6} or less of the incident intensity) and is shifted from the laser frequency by some characteristic excitation frequency of the substance. Raman scattering can occur from such excitations as molecular vibrational modes, lattice vibrational phonon modes in solids, plasma modes in solid-state and gas plasmas, spin wave modes in magnetic solids, and from transitions between energy levels of an atom.

Raman scattering may be thought of as arising from an induced polarization **P** of the medium by the electric field **E** of the laser beam (*2*). This polarization is written as

$$\mathbf{P} = \alpha \cdot \mathbf{E} \tag{1}$$

where α is the polarizability tensor of the medium which has a static component α_0 and, to a first order, small time-varying components α_n which oscillate at the fundamental excitation frequencies of the medium, ω_n. Equation 1 is then

$$P = (\alpha_0 + \sum_n \alpha_n \sin \omega_n t)\, E_i \cos \omega_i t = \alpha_0 E_i \cos \omega_i t + \tfrac{1}{2} E_i \sum_n \alpha_n [\sin(\omega_i + \omega_n)t - \sin(\omega_i - \omega_n)t] \tag{2}$$

Both energy and momentum are conserved in the scattering process and may be written as

$$\mathbf{k}_i - \mathbf{k}_s = \mathbf{q}_n \tag{3a}$$

and

$$\omega_i \pm \omega_n = \omega_s \tag{3b}$$

where $\mathbf{k}_i$ and $\mathbf{k}_s$ are the momenta and ω_i and ω_s are the frequencies of the incident (i) and scattered (s) photons, respectively, $\mathbf{q}_n$ is the momentum change, and ω_n is the frequency of the excitation.

Equation 2 describes the familiar situation of an amplitude-modulated radio wave whose two side bands occur at $\omega_i - \omega_n$ and $\omega_i + \omega_n$. In Raman scattering, these side bands correspond to the Stokes and anti-Stokes components, and are associated with the emission and absorption, respectively, of a quantum of some elementary excitation. The intensity of these components depends on the number of excitations,

$$n_\omega = [\exp\left(\frac{\hbar\omega}{kT}\right) - 1]^{-1}$$

present in thermal equilibrium, being proportional to $(n_\omega + 1)$ for the Stokes components and to n_ω for the anti-Stokes components. Here $\hbar$ is Planck's constant, T is the absolute temperature, and k is the Boltzmann constant. At low temperatures, n_ω approaches zero, and the anti-Stokes intensities become very small while the Stokes components still remain.

Experimental Methods

In early studies of Raman spectroscopy investigators used the excitation lines from a high-pressure mercury arc lamp; this technique had the disadvantage of requiring long exposure times for the photographic detection and there is also the difficulty of separating the weak Raman components from the Hg emission spectra. With the use of a laser as a Raman excitation source the spectral source has a much higher purity, intensity, polarization, and directionality. With the rapid development of lasers in recent years a wide variety of wavelengths have been made available for use in specific experiments. Table 1 lists a number of lasers that are commercially available for use in Raman spectroscopy, together with their useful output wavelengths and powers.

When the frequency of laser excitation is close to some absorption band

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