the various lines of evidence described here it is clear that an interplanetary magnetic field is always present, drawn out from the sun by the radially streaming solar wind. The field is stretched into a spiral pattern by the sun's rotation. The field appears to consist of relatively narrow filaments, the fields of adjacent filaments having opposite directions. At the earth's orbit the field points slightly below the ecliptic plane. The magnitude of the field is steady and near 5 gammas in quiet times, but it may rise to higher values at times of higher solar activity. A collision-free shock front is formed in the plasma flow around the earth. In the transition region between the shock front and the magnetopause the magnitude of the field is somewhat higher than it is in the interplanetary region, and large fluctuations in magnitude and direction are common. A shock front has also been observed in space between a slowly moving body of plasma and a faster, overtaking plasma stream.

Beyond the earth's orbit the solar plasma must continue to expand with the same velocity, carrying the interplanetary field with it (*26*). The angle between the spiral field lines and the radial direction from the sun continues to decrease until, at great distances, the field is perpendicular to the direction of plasma flow. The energy density of the plasma decreases with plasma number density as the inverse square of the radial distance until ultimately, between 10 and 100 astronomical units, the energy density of the plasma approaches that of the galactic medium. Instabilities produce a disordered outer region of plasma and field. Direct experimental investigation awaits a deep space probe capable of reaching and operating at a distance of 100 astronomical units.

The weak interplanetary field exerts considerable influence on the cosmic-ray protons. Study of these particles has been a particularly valuable tool in determining the large-scale properties of the interplanetary field. Precise measurement of the magnitude and direction of the field at a point in space and study of small-scale fluctuations can be accomplished only by means of a spacecraft magnetometer. The two methods are complementary, and both will be used in further study of the interplanetary field during the increasing solar activity of the next 5 years.

References and Notes

1. C. O. Hines, *Science* **141**, 130 (1963).
2. H. W. Babcock and H. D. Babcock, *Astrophys. J.* **121**, 349 (1955).
3. S. Chapman and V. C. A. Ferraro, *Terrest. Magnetism Atmospheric Elec.* **36**, 77, (1931); *ibid.*, p. 177; *ibid.* **37**, 147 (1932); *ibid.* **38**, 79 (1933).
4. H. Alfven, *Cosmical Electrodynamics* (Clarendon, Oxford, 1950).
5. L. Biermann, *Observatory* **77**, 109 (1957).
6. S. Chapman, *Smithsonian Contrib. Astrophys.* **2**, 1 (1957).
7. E. N. Parker, *Astrophys. J.* **128**, 664 (1958).
8. T. Gold, *J. Geophys. Res.* **64**, 1665 (1959).
9. J. A. Simpson, *Annals International Geophysical Year* (Pergamon, New York, 1956), vol. 4, p. 349; P. Meyer, E. N. Parker, J. A. Simpson, *Phys. Rev.* **104**, 768 (1956); D. A. Bryant, T. L. Cline, U. D. Desai, F. B. McDonald, *J. Geophys. Res.* **67**, 4983 (1962).
10. D. K. Bailey, *J. Geophys. Res.* **62**, 431 (1957).
11. E. N. Parker, *Astrophys. J.* **133**, 1014 (1961).
12. K. G. McCracken, *J. Geophys. Res.* **67**, 423 (1962); *ibid.*, p. 447.
13. ———, *ibid.*, p. 435.
14. K. I. Gringauz, V. V. Bezrukikh, V. D. Ozerov, R. E. Rybchinskii, *Dokl. Akad. Nauk SSSR* **131**, 1301 (1960); S. Sh. Dolginov, E. G. Eroshenko, L. N. Zhuzov, N. V. Pushkov, L. O. Tyurmina, *Proc. Intern. Space Sci. Symp., 1st, Nice* (1960), p. 863.
15. C. P. Sonett, D. L. Judge, A. R. Sims, J. M. Kelso, *J. Geophys. Res.* **65**, 55 (1960).
16. P. J. Coleman, L. Davis, C. P. Sonett, *Phys. Rev. Letters* **5**, 43 (1960).
17. J. P. Heppner, N. F. Ness, C. S. Scearce, T. L. Skillman, *J. Geophys. Res.* **68**, 1 (1963).
18. L. J. Cahill and P. G. Amazeen, *ibid.*, p. 1835.
19. J. W. Dungey, *Planetary Space Sci.* **10**, 233 (1963).
20. T. Gold, *Gas Dynamics of Cosmic Clouds*, Van De Hulst, Ed. (North-Holland, Amsterdam, 1955), p. 228; V. N. Zhigulev, *Soviet Phys. "Doklady"* (English Transl.) **4**, 514 (1959); W. I. Axford, *J. Geophys. Res.* **67**, 3791 (1962); P. J. Kellogg, *ibid.*, p. 3805.
21. M. Neugebauer and C. W. Snyder, *Science* **138**, 1095 (1962); C. W. Snyder, M. Neugebauer, U. R. Rao, *J. Geophys. Res.* **68**, 6361 (1963).
22. P. J. Coleman, Jr., L. Davis, Jr., E. J. Smith, C. P. Sonett, *Science* **138**, 1099 (1962).
23. C. P. Sonett, D. S. Colburn, L. Davis, Jr., E. J. Smith, P. J. Coleman, Jr., *Phys. Rev. Letters* **13**, 153 (1964).
24. J. H. Wolfe and R. W. Silva, paper presented at the 45th Annual Meeting of the American Geophysical Union, Washington, D.C., 1964; H. Bridge, A. Egidi, A. Lazarus, E. Lyon, L. Jacobson, paper presented at the 5th International Space Science Symposium, Florence, 1964.
25. N. F. Ness, C. S. Scearce, J. B. Seek, *J. Geophys. Res.* **69**, 3531 (1964).
26. L. Davis, *J. Phys. Soc. Japan* **17**, suppl. 2, 543 (1962); W. I. Axford, A. J. Dessler, Gottlieb, *Astrophys. J.* **137**, 1268 (1963); ———, *ibid.*, p. 193.
27. The variance, a measure of the fluctuation of field measurements in each 12-measurement average, is defined by Ness (*25*) as:

$$\delta X_{sc} = \left[\frac{1}{N} \sum_{i=1}^{N} (X_{se}^{i} - \overline{X}_{se})^2 \right]^{\frac{1}{2}}$$

where N is 12, X_{se}^{i} is an individual measurement of the component X_{se}, and $\overline{X}_{se}$ is the average of 12 measurements.

Biosynthesis of Alkaloids

New and unexpected routes to the pyridine and piperidine rings have been discovered.

Edward Leete

Some organic compounds, particularly the α-amino acids and carbohydrates such as glucose and ribose, are found in all plants, and it seems probable that these ubiquitous compounds are formed by essentially the same metabolic reactions in all plants. However certain plants contain compounds known as alkaloids which to our knowledge appear to have no biological role in the plants which produce them. About 3000 different alkaloids have been isolated from about 4000 plant species (*1*). Some typical alkaloids are illustrated in Fig. 1. Until recently the chemical investigation of plants has been a rather random process, and I consider that tens of thousands of new alkaloids remain to be discovered in the vast plant kingdom. Alkaloids may be defined as naturally occurring organic compounds containing nitrogen, which is usually located in a heterocyclic ring. The nitrogen in alkaloids is present as an amino group, and this group causes solutions of alkaloids in water to be basic. Many nitrogen-containing compounds (such as nocardamine, gliotoxin) which are produced by microorganisms could be regarded as alkaloids, although one seldom finds such compounds discussed in treatises on alkaloids. The same alkaloid is sometimes found in quite unrelated species. Thus the fungus *Claviceps* produces a group of compounds known as the ergot alkaloids which are derivatives of lysergic acid. Lysergic acid derivatives have also been isolated from the

higher plant *Rivea corymbosa* (*2*). Basic heterocyclic compounds have also been isolated from animals and are sometimes referred to as animal alkaloids. One such example is samandarine found in the salamander, *Salamandra maculosa* (*3*).

Over 150 years ago, morphine, present in the opium poppy *Papaver somniferum*, was the first alkaloid to be isolated in a crystalline state. Since that time many organic chemists have been busy elucidating the structures of alkaloids. Eminent scientists spent many frustrating, but enjoyable, years carrying out degradative reactions on strychnine (Fig. 1) before they were able to ascertain its correct structure (*4*). The newest generation of chemists can well marvel at the patience and ingenuity of earlier organic chemists who did not possess the instrumentation, such as x-ray crystallography and infrared and nuclear magnetic resonance spectroscopy, now available for aiding in structure determination.

Soon after alkaloids were isolated, attempts were made to synthesize them in the laboratory. Many will recall the efforts of W. H. Perkin who, in 1856, attempted to obtain quinine by oxidizing allyltoluidine with potassium dichromate. The rationalization for his experiment was the following stoichiometric equation:

$$\underset{\text{allyltoluidine}}{2\ C_{10}H_{13}N} + 3\ O = \underset{\text{quinine}}{C_{20}H_{24}N_2O_2} + H_2O$$

The product from the reaction was dark brown and unpromising. Lesser chemists would have probably abandoned the experiment, but Perkin, at the age of 18, repeated the oxidation with crude aniline and, from the black oxidation mixture, isolated not quinine but the first synthetic dye *mauve*. The synthesis of quinine was not achieved until 1944 (*5*). Although many alkaloids have not yet been prepared in the laboratory, it is possible to devise plausible reaction sequences for their synthesis. The varied methods used for the laboratory synthesis of alkaloids usually depend on intermediates and reactions which could not possibly occur in the living plant. But there have been attempts to carry out alkaloid synthesis in aqueous solution at room temperature, as a simulation of conditions which prevail in the living plant, and some of these syntheses have been successful (*6*).

The author is professor of organic chemistry at the University of Minnesota, Minneapolis, and is currently an Alfred P. Sloan Foundation fellow.

Hypotheses of Biosynthesis

In general biochemists have ignored the alkaloids, and it has been left to organic chemists to suggest plausible ways in which the alkaloids could be produced in the living plant. Robinson in 1917 (*7*) was one of the first to propose that many of the alkaloids could be derived from the common α-amino acids by relatively simple reactions. The original ideas of Robinson have been amplified by many organic chemists over the years, and the chemical literature abounds in speculations concerning the biogenesis of alkaloids (*8*). However, until 1950 there was no experimental evidence supporting any of these hypotheses. Unsuccessful attempts were made to study alkaloid biosynthesis by feeding large amounts of a proposed precursor of an alkaloid to the plant in the expectation that the yield of alkaloid would be increased. Thus when lysine was fed to the opium poppy the amount of morphine increased (*9*), whereas the actual precursors of morphine (tyrosine and methionine) are quite unrelated to lysine. The lysine was in some way stimulating the enzymes which catalyze the formation of morphine in the opium poppy.

The general procedure that my colleagues and I have used to study alkaloid biosynthesis in vivo has been to administer to the alkaloid-producing plant a plausible precursor labeled with isotopic atoms. After a suitable period of time, the alkaloid is isolated from the plant and assayed for the isotope. In most of our work we have used precursors labeled with carbon-14, although more recently we have carried out investigations with compounds labeled with tritium and nitrogen-15. In some studies an intact plant growing hydroponically has been used, and the labeled organic compound has been added to the aerated nutrient solution in which the roots of the plant were growing. One can never be sure that the nutrient solution or the plant roots are free of microorganisms, and there is always the possibility that the labeled compound is modified or degraded by microorganisms before it is absorbed by the roots. Various techniques have been used to minimize this possibility. One method which has been successful with tobacco plants is to cut off all the old roots and allow new ones to grow into a sterile solution of the labeled compound. In other cases the roots have been washed with a germicide before immersion in a solution of the labeled compound. The method which we use most often in our current work is that of wick feeding, where cotton thread is passed through the stem of the plant by means of a fine sewing needle. Both ends of the thread are then placed in a 5-ml beaker which contains a solution of the labeled compound. The solution is usually rapidly absorbed into the plant in a few hours. Distilled water can then be added to the beaker to ensure that all the labeled compound is taken into the plant. This procedure has the advantage that one does not have to disturb a plant which is growing in soil, and feeding experiments have actually been carried out in the remote woods of northern Minnesota on *Lycopodium annotinum* (a club moss), a species difficult to cultivate in a greenhouse. Fine glass capillary tubes have been used in a similar way to introduce solutions of labeled compounds into the stems of *Ricinus communis* plants (*10*). Labeled compounds have also been injected directly into plants by means of a hypodermic syringe. A solution of radioactive tyrosine was injected into the bulbous stem of the peyote cactus *Anhalonium lewinii*, very little liquid exuding from the hole when the needle was removed (*11*). Compounds labeled with carbon-14 have also been injected into the seed capsule of the opium poppy (*12*). A method

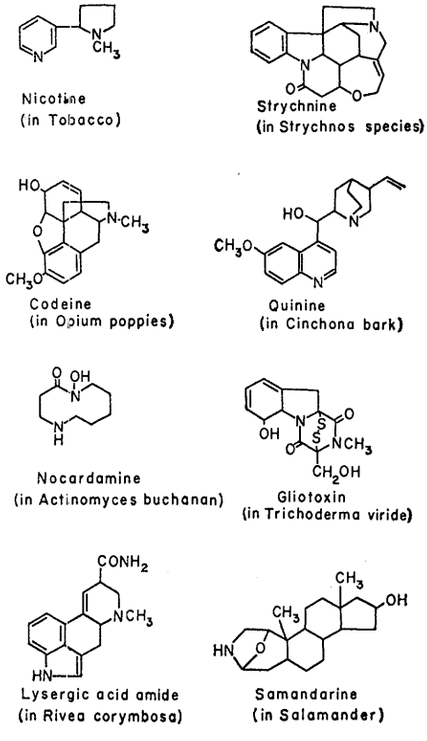

Fig. 1. Typical alkaloids.

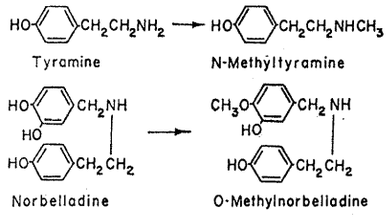

Fig. 2. Methylations catalyzed by isolated enzymes.

which has received little attention is the direct absorption of organic compounds through the leaves (*13*). I have sprayed the leaves of *Digitalis purpurea* plants with an equeous solution of mevalonic acid-2-C^{14} and have observed excellent translocation of the mevalonic acid into the plants.

In the extensive work with sterile cultures of excised roots (*14*), the environment in which the roots are growing can be very well controlled, and one can be sure that the nutrient solution is free of microorganisms. However, such experiments can be successful only if the roots are the site of alkaloid synthesis. Often alkaloids are synthesized only in the aerial parts of a plant.

Quite large organic molecules such as tryptophan and nicotine can enter a plant by way of its root system, and, in the examples reported, the size of a labeled compound has not prevented its absorption by a plant. However, we can say very little about translocation of labeled compounds inside plants. The lack of incorporation of a given compound into an alkaloid may be due to the fact that the administered labeled compound was unable to reach the site of alkaloid synthesis. In this kind of experiment alkaloid synthesis should be occurring at the time when the labeled compound is being fed to the plant. To determine whether alkaloid synthesis is taking place at a particular stage in development, a plant may be grown in an atmosphere containing radioactive carbon dioxide, and alkaloids produced during growth may be examined for radioactivity. Rapoport is ambitiously attempting to determine the radioactive intermediates between such atmospheric carbon dioxide and the nicotine formed in *Nicotiana* plants by methods which have proven successful in the study of photosynthesis. This is probably the ideal way to study alkaloid biosynthesis, since there is no interference with the normal physiology of the plant if the amount of carbon-14 fed does not cause radiation damage to the plant (*15*).

The incorporation of carbon-14 or other isotope into an alkaloid after administration of a labeled organic compound to a plant does not necessarily mean that the administered compound is a precursor of the alkaloid. It is always possible that the labeled compound is degraded into smaller fragments which are then utilized for alkaloid synthesis. For this reason the use of uniformly or randomly labeled compounds in the study of metabolic pathways has its limitations. Investigations with such compounds can provide preliminary information which should then be followed by work with specifically labeled precursors. Even when it is shown that a specifically labeled compound results in the formation of an

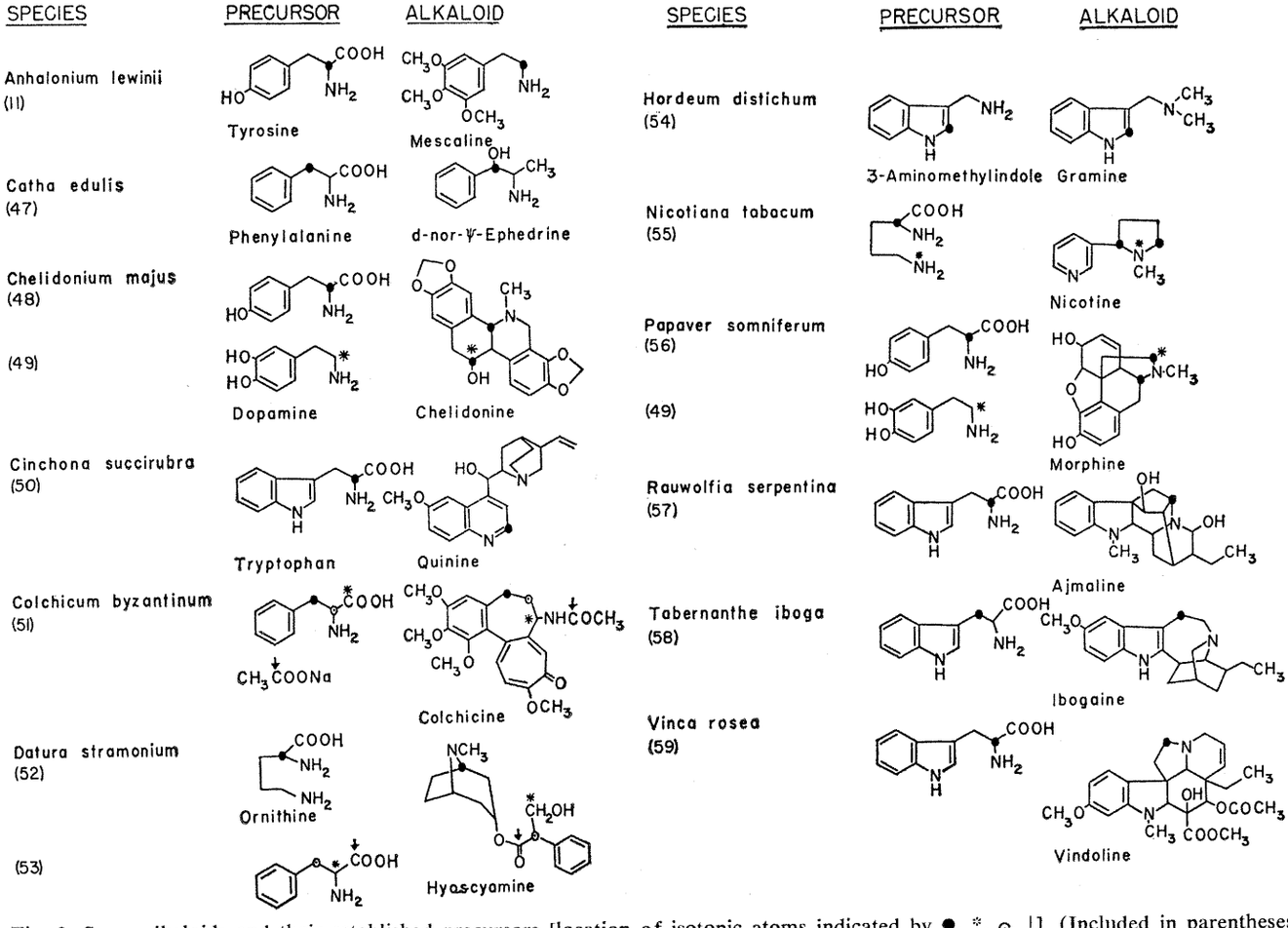

Fig. 3. Some alkaloids and their established precursors [location of isotopic atoms indicated by ●, *, ○, ↓]. (Included in parentheses are the reference numbers of the sources of the data.)

alkaloid labeled at specific positions, in agreement with a preconceived biogenetic scheme, one must proceed with caution in interpreting the data. Suppose that an alkaloid F is formed from carbon dioxide by way of the intermediates A, B, C, D, and E. A compound X could be incorporated into the alkaloid if the plant is able to convert it to any of the intermediates in this biosynthetic sequence, whereas compound X may not play any role in the normal biosynthesis of the alkaloid. We actually know very little about the capacity of plants to carry out chemical reactions which do not take place in a normal plant.

Isolation of Enzymes

Very little work has yet been reported on the isolation of enzymes which control alkaloid synthesis in plants. An enzyme has been isolated from the roots of germinating barley *Hordeum vulgare* which catalyzes the transfer of a methyl group from *S*-adenosyl-L-methionine to tyramine affording *N*-methyltyramine (*16*). Mudd and co-workers have also obtained from *Neriine bowdenii* a cell-free enzyme (*17*) which catalyzes the *O*-methylation of norbelladine. These two reactions are illustrated in Fig. 2. Mothes (*18*) was able to show that radioactive morphine was obtained when uniformly labeled tyrosine-C^{14} was added to the aqueous latex obtained from the opium poppy.

Some of the recent results obtained in my laboratory by feeding specifically labeled compounds to alkaloid-producing plants are summarized in Fig. 3. A relatively small number of α-amino acids—ornithine, lysine, phenylalanine, tyrosine, and tryptophan—serve as direct precursors of many alkaloids. Peripheral *O*- and *N*-methyl groups are derived from the *S*-methyl group of methionine. Acetic acid takes part in the biosynthesis of some alkaloids.

The structures of the alkaloids are so varied that it is not possible to formulate a single hypothesis concerning the biogenesis of all of them. In this review I will illustrate several ways in which pyridine and piperidine rings are produced in nature.

Nicotine is the best known alkaloid containing a pyridine ring, and it occurs in most *Nicotiana* species. It is, however, found in quite unrelated species: *Asclepias syriaca* (milk weed), *Atropa belladonna* (deadly nightshade),

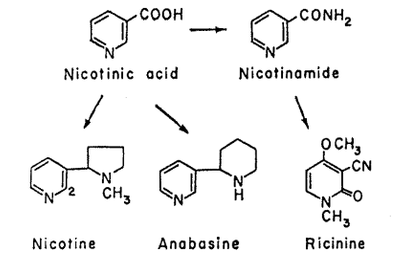

Fig. 4. Alkaloids derived from nicotinic acid.

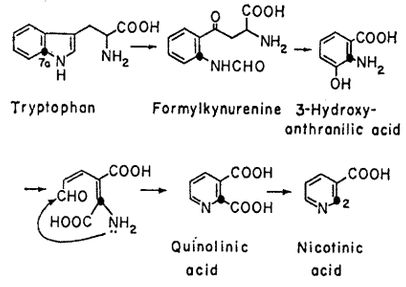

Fig. 5. The biosynthesis of nicotinic acid from tryptophan.

Equisetum arvense (horse tails), and *Lycopodium clavatum* (club moss). There has been much work on the biosynthesis of nicotine and its isomer anabasine (Fig. 4), the main alkaloid of *Nicotiana glauca* (wild tobacco). Dawson and his co-workers established that nicotinic acid is a precursor of the pyridine ring of these two alkaloids, the carboxyl group being lost at some step in the biosynthesis (*14, 19*). Nicotinic acid was also found to be a precursor of ricinine, an alkaloid found in *Ricinus communis* (castor bean). In this case the carboxyl group of nicotinic acid ultimately becomes the cyano group of ricinine, nicotinamide apparently being an intermediate (*20*). Thus by studying the origin of the pyridine or pyridone rings of these alkaloids, important information has been obtained concerning the origin of nicotinic acid in higher plants. When we commenced our investigations the only known biological source of nicotinic acid was tryptophan. The acid is produced by the sequence of reactions illustrated in Fig. 5. This pathway operates in some microorganisms, such as *Neurospora*, and in animals (*21*). Nicotinic acid labeled at C-2 is obtained from tryptophan labeled at C-7*a*. If the same sequence of reactions occurs in the tobacco plant we would expect nicotine isolated from a plant which had been fed tryptophan-7*a*-C^{14} to be labeled also at C-2 in the pyridine ring. In actual fact no activity was detected in the nicotine (*22*). The failure of tryptophan to serve as a precursor of nicotinic acid or related compounds in other higher plants has also been observed (*23*).

Six years ago I (*24*) and Griffith and Byerrum (*25*) showed that the administration of acetic acid-2-C^{14} to *Nicotiana* plants led to the formation of radioactive nicotine and anabasine. An appreciable amount of the radioactivity was located in their pyridine rings. On the other hand, there was no activity in the pyridine ring of nicotine when acetate-1-C^{14} was fed to tobacco plants. Other investigators studying the biosynthesis of ricinine found that acetate-2-C^{14}, succinate-2,3-C^{14}, glycerol-1,3-C^{14}, and glycerol-2-C^{14} were also excellent precursors of the pyridone ring of this alkaloid (*10, 26*). However, the administration of acetate-1-C^{14} or succinate-1,4-C^{14} to the castor bean yielded ricinine which had most of its activity located on the cyano group. The significance of these results could not be evaluated until methods were developed for the systematic degradation of nicotinic acid and ricinine to determine activity on each of the carbon atoms.

Radioactive anabasine isolated from *Nicotiana glauca* plants which had been fed acetate-2-C^{14} or glycerol-2-C^{14} was degraded by oxidation of the anabasine with potassium permanganate (*27*).

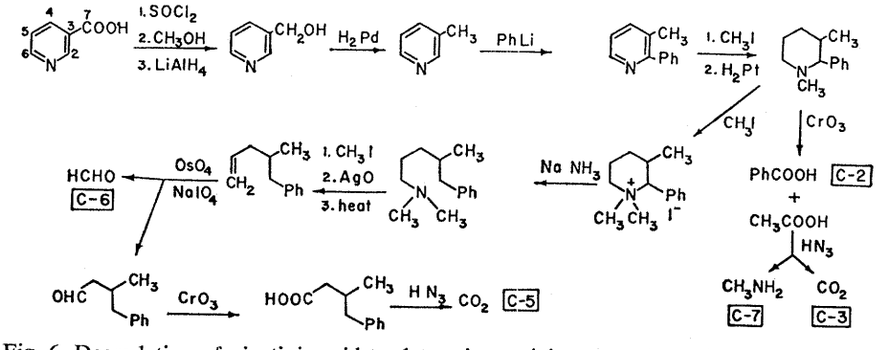

Fig. 6. Degradation of nicotinic acid to determine activity on each of the carbon atoms.

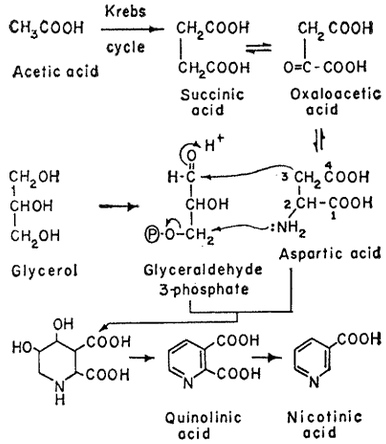

Fig. 7. Biogenetic scheme for the formation of nicotinic acid in higher plants and some microorganisms.

The nicotinic acid so obtained was then degraded by the scheme illustrated in Fig. 6. Activity at each of the carbons except C-4 was determined directly. Activity at C-4 was determined by difference. A quite different degradative scheme for nicotinic acid has been published by Christman *et al.* (*28*). Complete degradations have also been carried out on radioactive ricinine derived from acetate, succinate, and glycerol (*10, 26, 29*). The results obtained from these degradations are all consistent. It was found that acetate-2-C^{14} leads to the same pattern of labeling as succinate-2,3-C^{14}, and it is suggested that the acetate is converted to succinate or a closely related metabolite prior to its incorporation into nicotinic acid. The results indicated that carbons-4, -5, and -6 of nicotinic acid were derived from a three-carbon compound closely related to glycerol. The other carbons were derived from succinate or a closely related compound. A tentative scheme for the biosynthesis of nicotinic acid based on these tracer results is illustrated in Fig. 7. It is suggested that the heterocyclic ring is formed by a condensation between glyceraldehyde-3-phosphate and aspartic acid. The resultant piperidine derivative then undergoes dehydration and dehydrogenation to yield quinolinic acid, which is then decarboxylated affording nicotinic acid. Quinolinic acid is indeed a precursor of nicotinic acid in corn and castor bean plants (*30*). Therefore the administration of aspartic acid-3-C^{14} to tobacco should result in the formation of nicotine labeled at C-3 of the pyridine ring. In actual fact only about 50 percent of the activity of the pyridine ring was located at this position (*31*). This result can be rationalized by postulating that the administered aspartic acid-3-C^{14} is metabolized by way of the Krebs cycle to the symmetrical succinic acid. Aspartic acid which could then be resynthesized from this succinic acid would have radioactivity equally distributed between C-2 and C-3. In *Escherichia coli* glycerol and succinic acid are also precursors of nicotinic acid (*32*). Exciting results were recently obtained by Mothes *et al.* studying the biosynthesis of nicotinic acid by *Mycobacterium tuberculosis* (*33*). By feeding aspartic acid-1,4-C^{14}-N^{15}, they were able to show that the pyridine nitrogen was derived from the amino group of aspartic acid and the carboxyl group was derived from the γ-carboxyl of aspartic acid. Glycerol also served as a precursor of nicotinic acid in this same organism. Thus it seems quite likely that nicotinic acid is produced by the same sequence of reactions in *E. coli, M. tuberculosis, Ricinus communis*, and *Nicotiana* species. Since the proposed precursors of nicotinic acid, glyceraldehyde-3-phosphate and aspartic acid, are such active metabolites, it seems unlikely that the actual mechanism of the formation of nicotinic acid by this new route will be determined until the enzymes responsible for its synthesis are isolated.

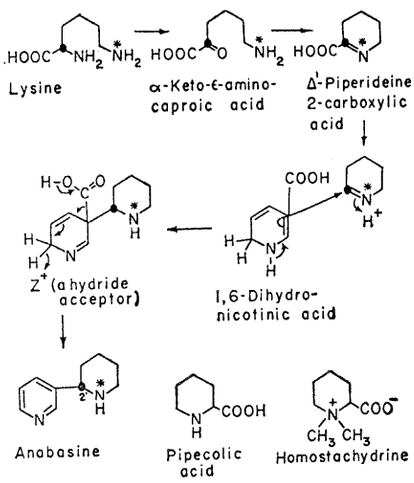

Fig. 8. Biosynthesis of the piperidine ring of anabasine.

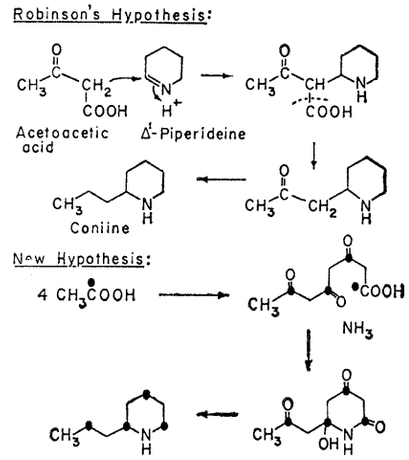

Fig. 9. Old and new biogenetic schemes for coniine.

Piperidines from Lysine

The amino acid lysine serves as a precursor of the reduced pyridine ring (piperidine) in several alkaloids and other natural products. Some time ago I showed that the administration of lysine-2-C^{14} to *Nicotiana glauca* plants afforded anabasine which was labeled solely at C-2′ of the piperidine ring (*34*). Recently we have carried out feeding experiments with lysine-2-C^{14} labeled with N^{15} on the α- or ϵ-nitrogen (*35*). The results indicated that the piperidine nitrogen was derived only from the ϵ-amino group of lysine. This result is consistent with the hypothesis that the piperidine ring of anabasine is formed from lysine by way of α-keto-ϵ-aminocaproic acid (Fig. 8). I consider that this keto acid then cyclizes to Δ^1-piperideine-2-carboxylic acid. Decarboxylation affords Δ^1-piperideine which could condense with 1,6-dihydronicotinic acid to yield ultimately anabasine as illustrated in Fig. 7. Other piperidines which are derived from lysine are pipecolic acid (*36*) found in various plants and microorganisms, and homostachydrine (*37*) found in *Medicago sativa* (alfalfa).

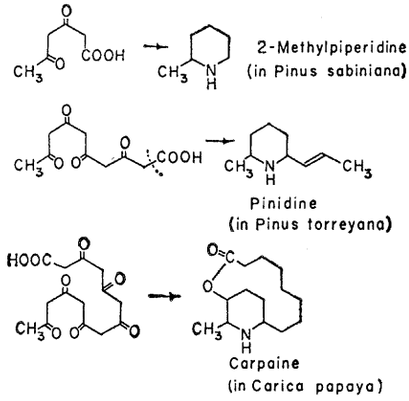

Fig. 10. Some piperidine alkaloids which are plausibly derived from acetate.

Piperidines from Acetate

Until recently it has been generally accepted that the piperidine ring of coniine, one of the main alkaloids of *Conium maculatum* (hemlock) was also derived from lysine. Robinson (*7*) had suggested that coniine and related alkaloids are produced by a condensation between Δ^1-piperideine and acetoacetic acid (Fig. 9). Decarboxylation followed by reduction of the side-chain then yields coniine. Initial tracer experiments (*38*) were apparently consistent with this hypothesis. Uniformly labeled lysine-C^{14} was fed to hemlock plants, and radioactive coniine was formed. However the alkaloid was not degraded to determine the distribution of radioactivity. On the other hand, I administered lysine-2-C^{14} to hemlock by various methods and observed but little incorporation of activity into the crude hemlock alkaloids. I thus considered a new scheme for the biogenesis (Fig. 9) of coniine whereby both the side-chain and the piperidine ring are formed from a poly-β-ketoacid derived from four acetate units. Tracer experiments confirmed this hypothesis (*39*). When acetate-1-C^{14} was fed to hemlock radioactive coniine was obtained, the activity being equally distributed between the four positions indicated with heavy dots in the formula of coniine (Fig. 10). Several other piperidine alkaloids are also plausibly formed from acetate derived poly-β-ketoacids (Fig. 10). The fatty acids are of course formed by the linear combination of acetate units, and it seems that these piperidine derivatives arise by some deviation in the normal biosynthesis of fatty acids.

Pyridines from Mevalonic Acid

The pyridine ring of the alkaloid actinidine (Fig. 11) is probably formed by yet another biosynthetic route. This alkaloid occurs in the plant *Actinidia polygama*, along with the nitrogen-free compound matatabilactone (*40*). Skytanthine, having the same carbon skeleton as actinidine, but with the pyridine ring reduced, is found in *Skytanthus acutus* (*41*). These compounds are apparently terpenes being readily constructed from two isoprene units (Fig. 11). The biological precursor of the isoprene unit is mevalonic acid, and this compound is an excellent precursor of skytanthine in *S. acutus* (*42*).

Several other pyridine compounds

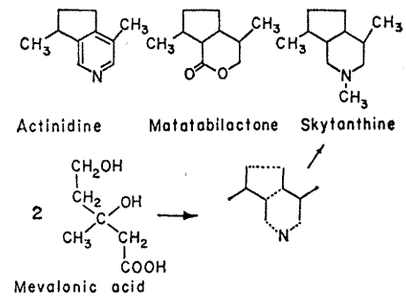

Fig. 11. Terpenoid pyridine and piperidine alkaloids.

found in nature do not fit readily into any of the previously mentioned schemes. 3-Methoxypyridine is found in *Equisetum arvense* (*43*), and I suggest that it is formed by the cyclization and dehydration of an amino pentose such as 5-amino-5-deoxy-D-xylose as illustrated in Fig. 12. The cyclization of such amino sugars to pyridine derivatives has been observed in vitro (*44*). The important vitamin pyridoxine (Fig. 12) is a pyridine derivative, but little is known of its precursors. Another pyridine derivative whose origin appears to be unknown is 3-hydroxypicolinic acid (Fig. 12), part of the cyclic polypeptide etamycin which is produced by certain *Streptomyces* species (*45*). Three different biogenetic schemes (*46*) have been suggested for gentianine, an alkaloid found in various *Gentiana* species. I favor a fourth scheme (Fig. 12). The carbon skeleton is built up from a six-carbon poly-β-ketoacid derived from three acetate units, a one-

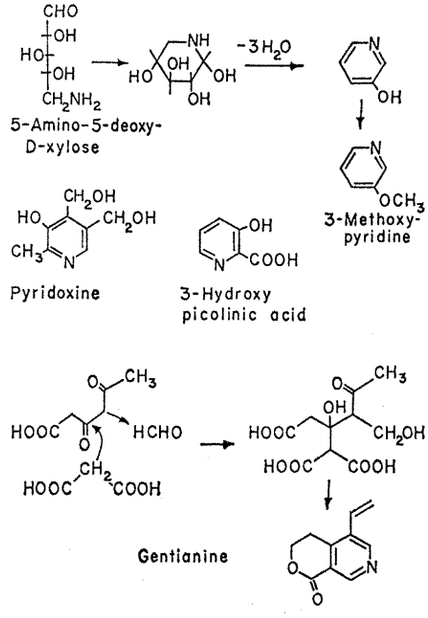

Fig. 12. Miscellaneous pyridine derivatives of unknown origin.

carbon fragment (represented here as formaldehyde), and malonic acid.

In this review the methods which are being used for studying the biosynthesis of alkaloids in plants have been described. Many of the biogenetic hypotheses proposed almost 50 years ago by Sir Robert Robinson have now been shown to be correct. However, new and unexpected biosynthetic routes to the pyridine and piperidine rings have been discovered. Many problems remain to be solved and we are only just beginning to understand the relation of alkaloid biosynthesis to other metabolic reactions which occur in the living plant.

References and Notes

1. H.-G. Boit, *Ergebnisse der Alkaloid-Chemie bis 1960* (Akademie-Verlag, Berlin, 1961).
2. A. Hofmann and H. Tscherter, *Experientia* **16**, 414 (1960).
3. G. Weitz and E. Wölfel, *Acta Cryst.* **15**, 484 (1962); E. Wölfel, C. Schöpf, G. Weitz, G. Habermehl, *Chem. Ber.* **94**, 2361 (1961).
4. L. H. Briggs, H. T. Openshaw, R. Robinson, *J. Chem. Soc.* **1946**, 903 (1946); R. B. Woodward, W. J. Brehm, A. L. Nelson, *J. Am. Chem. Soc.* **69**, 2250 (1947).
5. R. B. Woodward and W. von E. Doering, *J. Am. Chem. Soc.* **66**, 849 (1944).
6. E. E. Van Tamelen, *Fortschr. Chem. Org. Naturstoffe* **19**, 242 (1961).
7. R. Robinson, *J. Chem. Soc.* **111**, 876 (1917); *The Structural Relations of Natural Products* (Clarendon Press, Oxford, 1955).
8. I accept with enthusiasm the suggestion of F. R. Stermitz and H. Rapoport, *J. Am. Chem. Soc.* **83**, 4045 (1961) that the word biosynthesis should describe in vivo experimental studies on the mode of formation of natural products, while the word biogenesis should be applied to the hypothetical schemes suggested to rationalize the formation and structures of natural products.
9. O. V. Bogdashevskaya, *Fiziol. Rast.* **5**, 185 (1958).
10. P. F. Juby and L. Marion, *Biochem. Biophys. Res. Commun.* **5**, 461 (1961); *Can. J. Chem.* **41**, 117 (1963).
11. E. Leete, *Chem. Ind. London* **1959**, 604 (1959).
12. A. M. Kuzin and V. I. Merenova, *Biochimiya* **19**, 616 (1954).
13. K. Mothes and G. Trefftz, *Naturwissenschaften* **41**, 382 (1954); E. C. Humphries, *Proc. Fertiliser Soc. Soc. Anal. Chem.* **48**, 1 (1948).
14. R. F. Dawson, D. R. Christman, R. C. Anderson, *J. Am. Chem. Soc.* **75**, 5114 (1953).
15. W. L. Alworth, R. C. DeSelms, H. Rapoport, *ibid.* **86**, 1608 (1964); W. L. Alworth, A. A. Liebman, H. Rapoport, *ibid.* **86**, 3375 (1964).
16. J. D. Mann, C. E. Steinhart, S. H. Mudd, *J. Biol. Chem.* **238**, 676 (1963).
17. H. M. Fales, J. D. Mann, S. H. Mudd, *J. Am. Chem. Soc.* **85**, 2025 (1963).
18. G. Kleinschmidt and K. Mothes, *Z. Naturforsch.* **14b**, 52 (1959), *Arch. Pharm.* **293**, 948 (1960); L. Meissner and K. Mothes, *Phytochemistry* **3**, 1 (1964); J. W. Fairbairn and G. Wassel, *ibid.* **3**, 583 (1964).
19. R. F. Dawson, D. R. Christman, A. D'Adamo, M. L. Solt, A. P. Wolf, *J. Am. Chem. Soc.* **82**, 2628 (1960); M. L. Solt, R. F. Dawson, D. R. Christman, *Plant Physiol.* **35**, 887 (1960).
20. E. Leete and F. H. B. Leitz, *Chem. Ind. London* **1957**, 1572 (1957); G. R. Waller and L. M. Henderson, *J. Biol. Chem.* **236**, 1186 (1961).
21. W. A. Krehl, L. J. Teply, P. S. Sarma, C. A. Elvehjem, *Science* **101**, 489 (1945); G. W. Beadle, H. K. Mitchell, J. F. Nyc, *Proc. Natl. Acad. Sci. U.S.* **33**, 155 (1947); Y. Nishizuka and O. Hayaishi, *J. Biol. Chem.* **238**, 3369 (1963); R. K. Gholson, I. Ueda, N. Ogasawara, L. M. Henderson, *ibid.* **239**, 1208 (1964).

22. E. Leete, *Chem. Ind. London* **1957**, 1270 (1957); L. M. Henderson, J. F. Someroski, D. R. Rao, P. L. Wu, T. Griffith, R. U. Byerrum, *J. Biol. Chem.* **234**, 93 (1959).
23. E. Leete, L. Marion, I. D. Spenser, *Can. J. Chem.* **33**, 405 (1955).
24. E. Leete, *Chem. Ind. London* **1958**, 1477 (1958).
25. T. Griffith and R. U. Byerrum, *Science* **129**, 1485 (1959).
26. G. R. Waller and L. M. Henderson, *Biochem. Biophys. Res. Commun.* **5**, 5 (1961); J. M. Essery, P. F. Juby, L. Marion, E. Trumbull, *Can. J. Chem.* **41**, 1142 (1963).
27. A. R. Friedman and E. Leete, *J. Am. Chem. Soc.* **85**, 2141 (1963); **86**, 1224 (1964).
28. D. R. Christman, R. F. Dawson, K. I. C. Karlstrom, *J. Org. Chem.* **29**, 2394 (1964).
29. U. Schiedt, G. Boeckh-Behrens, A. M. Delluva, *Z. Physiol. Chem.* **330**, 46, 58 (1962).
30. L. A. Hadwiger, S. E. Bodiei, G. R. Waller, R. K. Gholson, *Biochem. Biophys. Res. Commun.* **13**, 466 (1963).
31. T. Griffith, K. P. Hellman, R. U. Byerrum, *Biochemistry* **1**, 336 (1962).
32. M. V. Ortega and G. M. Brown, *J. Biol. Chem.* **235**, 2939 (1960).
33. D. Gross, H. R. Schütte, G. Hubner, K. Mothes, *Tetrahedron Letters* **1963**, 541 (1963).
34. E. Leete, *J. Am. Chem. Soc.* **78**, 3520 (1956).
35. E. Leete, E. G. Gros, T. J. Gilbertson, *ibid.* **86**, 3907 (1964).
36. N. Grobbelaar and F. C. Steward, *ibid.* **75**, 4341 (1953); P. H. Lowy, *Arch. Biochem. Biophys.* **47**, 228 (1953); A. Meister and S. D. Buckley, *Biochim. Biophys. Acta* **23**, 202 (1957); L. Fowden, *J. Exptl. Botany* **11**, 302 (1960).
37. A. V. Robertson and L. Marion, *Can. J. Chem.* **37**, 1043 (1959).
38. U. Schiedt and H. G. Höss, *Z. Naturforsch.* **13b**, 691 (1958); *Z. Physiol. Chem.* **330**, 74 (1962).
39. E. Leete, *J. Am. Chem. Soc.* **85**, 3523 (1963); **86**, 2509 (1964).
40. T. Sakan, A. Fujino, F. Murai, Y. Butsugan, A. Sazui, *Bull. Chem. Soc. Japan* **32**, 315 (1959).
41. C. Djerassi, J. P. Kutney, M. Shamma, J. N. Shoolery, L. F. Johnson, *Chem. Ind. London* **1961**, 210 (1961); G. C. Casinovi, J. A. Garbarino, G. B. Marini-Bettòlo, *ibid.* **1961**, 253 (1961).
42. G. C. Casinovi and G. B. Marini-Bettòlo, Abstracts A, meeting of International Union Pure and Applied Chemistry, London (1963), p. 285.
43. R. H. F. Manske and L. Marion, *Can. J. Res.* **B20**, 87 (1942).
44. H. Paulsen, *Angew. Chem. Intern. Ed. Engl.* **2**, 555 (1963).
45. J. C. Sheehan, H. G. Zachau, W. B. Lawson, *J. Am. Chem. Soc.* **79**, 3933 (1957).
46. R. Thomas, *Tetrahedron Letters* **1961**, 544 (1961); T. R. Govindachari, K. Nagarajan, S. Rajappa, *Experientia* **14**, 55 (1958); E. Wenkert, *J. Am. Chem. Soc.* **81**, 1474 (1959).
47. E. Leete, *Chem. Ind. London* **1958**, 1088 (1958).
48. ———, *J. Am. Chem. Soc.* **85**, 473 (1963).
49. ——— and J. B. Murrill, *Tetrahedron Letters* **1964**, 167 (1964); A. R. Battersby and R. J. Francis, *J. Chem. Soc.* **1964**, 4078 (1964).
50. N. Kowanko and E. Leete, *J. Am. Chem. Soc.* **84**, 4919 (1962).
51. E. Leete and P. E. Németh, *ibid.* **82**, 6055 (1960); E. Leete, *ibid.* **85**, 3666 (1963); E. Leete, *Tetrahedron Letters* **1965**, 333 (1965); A. R. Battersby, R. Binks, D. A. Yeowell, *Proc. Chem. Soc.* **1964**, 86 (1964); A. R. Battersby, R. Binks, J. J. Reynolds, D. A. Yeowell, *J. Chem. Soc.* **1964**, 4257 (1964).
52. E. Leete, *J. Am. Chem. Soc.* **84**, 55 (1962); *Tetrahedron Letters* **1964**, 1619 (1964).
53. M. L. Louden and E. Leete, *J. Am. Chem. Soc.* **84**, 1510, 4507 (1962).
54. B. G. Gower and E. Leete, *ibid.* **85**, 3683 (1963).
55. E. Leete, E. G. Gros, T. J. Gilbertson, *Tetrahedron Letters* **1964**, 587 (1964).
56. E. Leete, *Chem. Ind. London* **1958**, 977 (1958); *J. Am. Chem. Soc.* **81**, 3948 (1959); A. R. Battersby and B. J. T. Harper, *Chem. Ind. London* **1953**, 364 (1958); A. R. Battersby, R. Binks, B. J. T. Harper, *J. Chem. Soc.* **1962**, 3534 (1962).
57. E. Leete, *J. Am. Chem. Soc.* **82**, 6338 (1960).
58. ——— and M. Yamazaki, *Tetrahedron Letters* **1964**, 1499 (1964).
59. E. Leete, A. Ahmad, I. Kompis, unpublished work.
60. I thank the National Science Foundation and the U.S. Public Health Service for grants (NSF-GB-363, NIH-MH-02662) which have supported my work on alkaloid biosynthesis.

Animal Communication

A communication network model for languages is applied to signaling behavior in animals.

Thomas A. Sebeok

The term "semiotic," in its earliest sense equivalent to symptomatology, was introduced into philosophical discourse at the end of the 17th century by John Locke to label one of the three branches of contemporary science, to wit, the doctrine of signs. The real founder and first systematic investigator of the field, however, was the subtle and profound American philosopher, Charles Sanders Pierce. The unique place of semiotic among the sciences—not merely one among the others, "but an organon or instrument of all the sciences"—was insisted on by Charles Morris who, in 1938, proposed to absorb logic, mathematics, and linguistics entirely within semiotic. "The whole science of language," the logician Rudolf Carnap then reaffirmed in 1942, "is called semiotic," and, in 1946, Morris introduced further refinements when he distinguished among pure semiotic, which elaborates discourse about signs; descriptive semiotic, which focuses on actual signs; and applied semiotic, which utilizes knowledge about signs for the accomplishment of various purposes. In 1962, the anthropologist Margaret Mead proposed a variant, "semiotics," as a term which might aptly cover "patterned communications in all modalities," that is, for the global study of the interactional and communicational context of the human use of signs and the way in which these are organized in transactional systems involving all of the senses (*1*). "Zoosemiotics" was then coined (*2*) to identify a very rapidly expanding discipline within the behavioral sciences, one which has crystallized at the intersection of semiotics, the science of signs, and ethology, a field which Niko Tinbergen characterized, in the first book ever written on the subject, as "the objective study of behavior," but which he more recently—and more fairly—redefined as "the biological study of behaviour" (*3*). Zoosemiotics has not only emerged as a dominant theme in ethology, but "data on animal communication have contributed a thread of continuity that, in some ways and at some times, has seemed to be the principal axis of synthesis in the entire field of animal behavior" (*4*).

Modern developments in the study of animal communication stem largely from Charles Darwin (*5*). They received substantial impetus from the classic investigations of K. von Frisch, and were placed in their present academic frame by K. Z. Lorenz, Tinbergen, W. H. Thorpe, and many others. The period from Darwin until the end of the last decade has been conveniently summarized by Kainz (*6*), whose book may be complemented by a series of easily accessible review articles and a recent, semi-popular, survey of the field

The author is professor of linguistics and chairman of the Research Center in Anthropology, Folklore, and Linguistics at Indiana University, Bloomington.