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AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE



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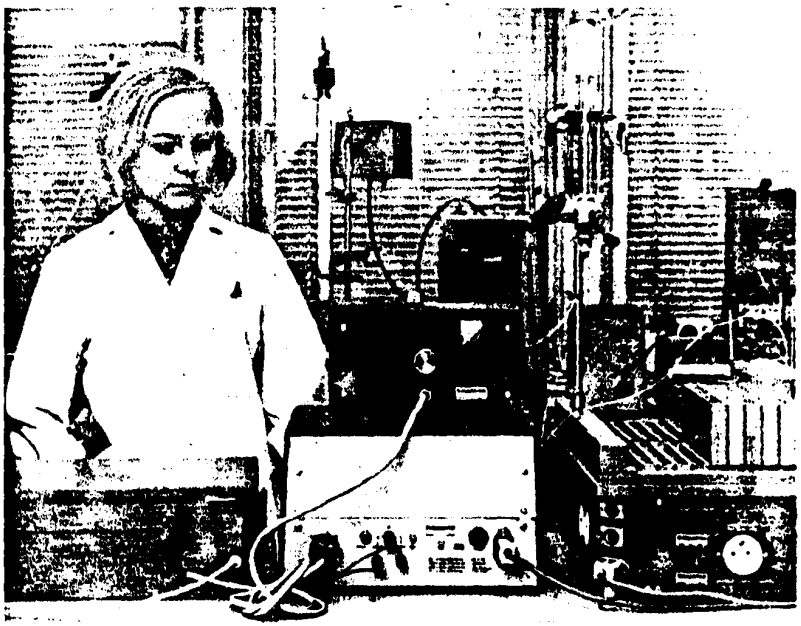

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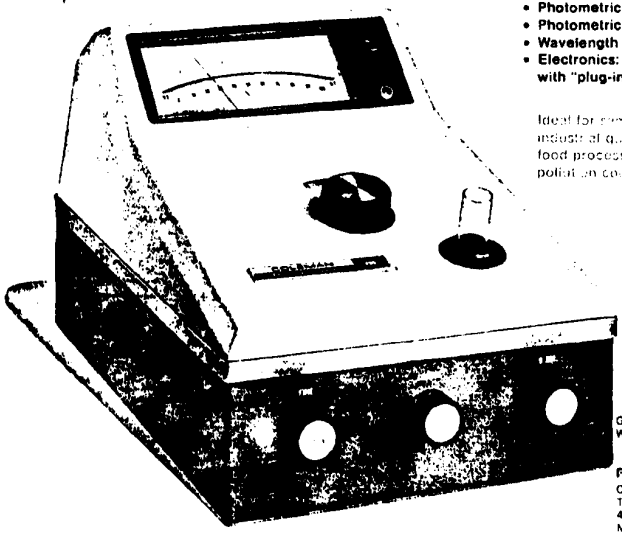

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COVER

Soft shell turtle (*Trionyx ferox*). The turtle is mainly a carnivore which may prey on a large number of animals but also scavenges. See page [illegible] [Leland LaFrance, Chicago Zoological Park, Brookfield, Illinois]

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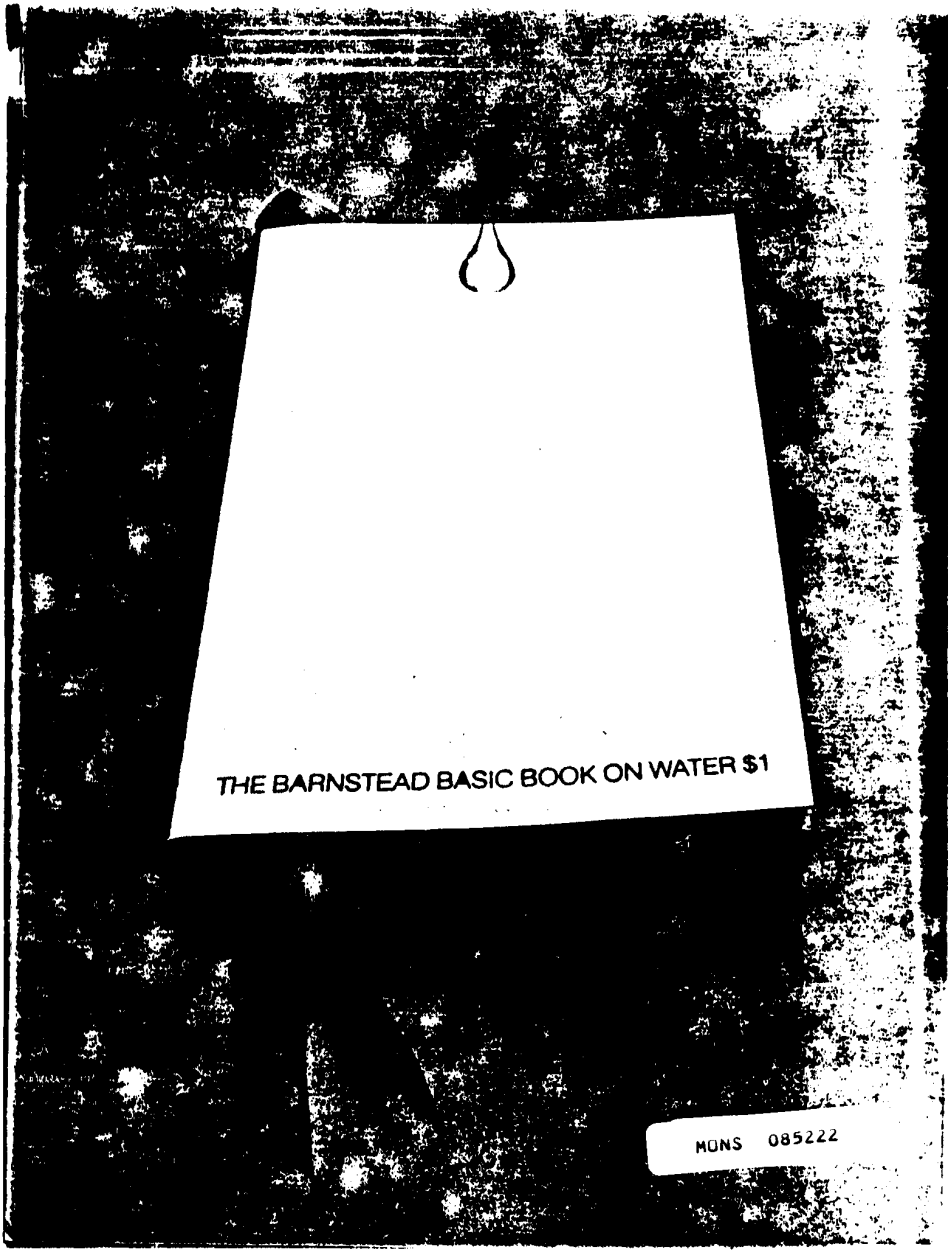
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Letters

Relations with South Africa

For a decade the Smithsonian Astrophysical Observatory (SAO), closely associated with Harvard College Observatory (HCO), has been contributing to the support of Boyden Observing Station in Bloemfontein, South Africa. Recently SAO has been asked to quadruple its support. Many members of HCO and SAO are upset by the use of government money to support a scientific facility which is operated according to the racist principles of South Africa's apartheid system. We have sent copies of the following letter, with 71 signatures, to Fred Whipple, director of SAO in Cambridge, and to S. Dillon Ripley, head of the Smithsonian Institution in Washington:

We have recently learned that the Smithsonian Astrophysical Observatory is planning to support observing trips by its staff members to Boyden Observatory in South Africa. Because of the racist policies of the South African government, we strongly oppose both the use of South African facilities by SAO and Harvard staff members and the continued financial support which SAO provides for Boyden Observatory.

Scientists in South Africa are not removed from apartheid. All facilities, scientific and otherwise, that a scientist uses there depend on cheap, black labor. Any scientist speaking out against apartheid would be banned from the country if he were a foreigner and imprisoned if he were South African.

Isolation is one of the few nonmilitary pressures the outside world can exert on South Africa. This isolation can be especially important in science because of the dependence of much of South Africa's economic growth on applied science. Visits even by scientists engaged in "pure" research contribute to the overall growth of the South African scientific community, which in turn contributes to the economic health of South Africa's apartheid system.

We believe in the internationalism of science, but the most basic ideal of internationalism—the spread of scientific knowledge and technological advances to *all* people, without national or racial barriers—is violated by South Africa's denial to blacks of equal education and economic benefits of modern technology.

Any professional visits to South Africa by American astronomers, especially with government support, lend legitimacy to that country's established system. Surely SAO's support could be used by observers at other southern hemisphere telescopes. The refusal by a prestigious establishment like the Smithsonian Institution to support research in South Africa would influence many American scientists to refrain from going to South Africa until its racial policies are changed.

The use of government money to support a facility which practices racial discrimination is probably in violation of the 1964 Civil Rights Act. We are currently looking into the possibility of legal action. We feel that cooperation with South African policies is certainly immoral. Members of the scientific community should know about the activities of SAO in South Africa and we hope they will join us in support of our endeavors.

JEFFREY HOFFMAN
Harvard College Observatory, Cambridge, Massachusetts

Your acceptance of an advertisement from the University of Natal at Pietermaritzburg does an injustice to the nonwhite members of the AAAS, since, because of their color, they would never be considered for such a position.

I would like to suggest that *Science* refuse to accept any more advertisements from institutions or organizations in the Republic of South Africa. By their very nature, these advertisements are insults to a portion of our membership.

THOMAS J. COX
2131 Rainbow Vista Drive, Tucson, Arizona 85712

On Paradigms

In "The Paradigm Concept" (14 May, p. 706), reviewing the second edition of Kuhn's *The Structure of Scientific Revolutions* and the proceedings of a colloquium, *Criticism and the Growth of Knowledge*, dealing with Kuhn's work, Shapere follows the majority of the essays in the second book in subjecting Kuhn's work to painstaking philosophical analysis. After two pages of such [illegible] concludes his review with

The point I have tried to make [illegible] merely that Kuhn's is a view which [illegible] the objectivity and rationality of the scientific enterprise; I have tried to show that the arguments by which Kuhn arrives at his conclusions are unclear and unsatisfactory.

Shapere may not regard "the objectivity and rationality of the scientific enterprise" as unarguable givens, but the sentence quoted implies that to deny them is a fault or flaw in Kuhn's thought. In the same issue of *Science*, Chargaff, a practicing scientist, discusses (p. 637) a situation curiously similar to the incommensurability, deprecated by Shapere, that is postulated by Kuhn as existing between paradigms and, in addition, challenges any uncritical acceptance of the objectivity and rationality of science. He writes:

It is almost impossible to retrace the course of the history of science to an earlier stage, for not only should we be required to forget much of what we have learned, but much of what a previous epoch knew or belived to know has simply never been learned by us. We must remember that the natural sciences are as much a struggle *against* as *for* facts.

Shapere points out in some detail the shifts in Kuhn's position and the logical problems engendered by such shifts. He examines the philosophical implications of that position and finds it wanting. (At this point it is difficult to resist speculation about which scientific or social scientific insight, principle, or law could have resisted the attack of the modern philosopher, armed with many more destructive weapons than constructive tools.) The paradigm concept is certainly open to philosophical attack and insofar as it is a philosophic construct should fail if found wanting philosophically. The paradigm concept is not strictly and solely a philosophic construct, however. It has at least two other layers. First, it describes occurrences in science and perhaps in other areas of knowledge. Second, repeated assertions by Kuhn (which are liberally quoted by Shapere) that in examining the scientific enterprise he is seeking psychological or sociological explanations force us to regard the paradigm concept, at least in part, as a construct (paradigm') *in* the social sciences *about* the natural sciences.

Chargaff in the section quoted earlier is only one of the scientists who currently describe their endeavor in Kuhnian terms. In "System and Hu-

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#70

manism in Social Science" (also 14 May, p. 661) Simpson, reviewing Friedrichs' *A Sociology of Sociology* and Gouldner's *The Coming Crisis of Western Sociology*, uses the word "paradigm" at least seven times and also refers explicitly to Kuhn's work. The widespread acceptance of Kuhn's ideas by scientists indicates that his insights illuminate the circumstances in which they actually practice science. His rejection by philosophers indicates that concepts that are clear to the research worker whose situation and environment they describe are unclear to the philosopher.

Nowhere, either in *Criticism and the Growth of Knowledge* or in Shapere's review, is the paradigm concept considered as a construct in the field of sociology. It is perhaps in this role that the paradigm concept will ultimately stand or fall. That Galileo recants or that Kuhn retreats has little to do with the descriptive and predictive power of their insights or with the productivity of the research that can go on under the aegis of their conceptual frameworks. Obviously, my approach here is intentionally Kuhnian. The acid test of the paradigm concept is not logic but effect, that is, its descriptive and predictive power. If the concept is effective it will survive and then the philosophers will have to find a way to deal with it.

WALTER J. FRASER

James Jerome Hill Reference Library, Saint Paul, Minnesota 55102

Indicators of Drug Effectiveness

One of the major problems in the practice of preventive medicine in developing countries is guaranteeing that drugs and biologicals are still effective at the time of use. This is especially true for live virus vaccines which must be kept within a narrow temperature range. Because of the hazardous conditions that sensitive pharmacologicals encounter in transit from the laboratory to the patient, many ineffective (and thus potentially harmful) drugs and biologicals are administered, or many effective drugs and biologicals are discarded because of a suspicious history.

Field workers desperately need a fail-safe indicator which either changes the color of the material or appears on the label (perhaps obliterating it) when temperature, time, or other factors have adversely affected the effectiveness of the material. Sterilization indicators which are routinely used in autoclaves operate according to the same principle.

If the United States were to adopt similar devices for drugs and biologicals, the immediate benefits would be international.

KIT JANSEN

Department of Preventive Medicine and Public Health, College of Medicine, Yonsei University, Seoul, Korea

Reform at the National Academy of Sciences

Page's editorial (14 May, p. 635) on the new Institute of Medicine of the National Academy of Sciences (NAS) raises some important issues that deserve further discussion. The most serious problem is whether the NAS or an institute within it can provide the kind of leadership that is needed to "encourage creative change" [Stewart Udall, quoted in C. F. Barfield, *Nat. J.* 3, 111 (1971)]. Clearly some of the innovations outlined by Page are attempts to improve the situation. Especially laudable is the intent to make a candidate's election to the Institute of Medicine contingent upon a commitment of time for public service. Perhaps this same stipulation should be extended to the entire Academy, which in the past has been a kind of exalted Phi Beta Kappa.

Unfortunately, such minor reforms do not go far enough. The most regressive feature of NAS—the election of members by a closed group—would be retained. This virtually assures that the institute and the NAS would remain unaccountable to the public and to the scientific community at large. I cannot help but feel that the problems of the poor and of minority groups will continue to be neglected under these circumstances.

The model provided by NAS is so deficient that a wholly different institution should be established to supply scientific and medical advice to the government. It should be designed to encourage inputs from competent scientists and nonscientists who are not necessarily members of the elite establishment.

D. MAX SNODDERLY, JR.

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Trouble



Conidiospores—really somatic cells—ride the wind. In effect, a single organism spreads genetically identical extensions of itself over a continent. Infection marks this "wind slot" from a narrow opening in the woods. Entire field was finally affected.
Mississippi State College photo



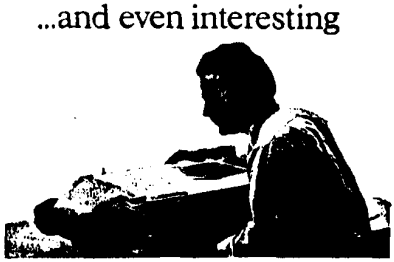
From spore to leafspot to new spore can take as little as 60 hours where and when dew is plentiful.
Mississippi State College photo



In 1970 Johnson grass, relative of corn, stayed healthy. Weeds and grass favor the blight by slowing the drying of the dew.



The mutant attacks the ears. The original race of Southern Corn Leaf Blight concentrated pretty much on leaves.



Corn, 1971

This is how Southern Corn Leaf Blight is being tracked this summer—by its color on KODAK AEROCHROME Infrared Film 2443 (ESTAR Base). (Useful also in estimating dwelling units in a congested central city and in many other kinds of "remote sensing.") This film, instead of trying to imitate the colors of the real world, works by shifting man's color vision a short way into the infrared. Has nothing to do with temperature sensing but something to do with the way the brain works and a lot to do with the spectral reflectance of green plants.

Fortnightly since June 14, cameras have been flying 11 miles above 200 sample sites, each 1 mile by 8 miles, that have been thoroughly studied on the ground. Film goes to the Laboratory for Applications of Remote Sensing at Purdue University, where the interpreters work. From their reports, judgments are made that relate Race T of the asexually reproducing fungus *Helminthosporium maydis* to the price of hamburgers, steaks, chicken, milk.

"T" stands for Texas, as in Texas male-sterile cytoplasm, an invention in genetic engineering that cuts out the heavy cost of detasseling the hybrid seed plants (so that they shall be pollinated only by the adjacent row of the male parent in the cross). This was a most fruitful idea until, early in 1970, there appeared in Florida a mutant of the previously unimportant *H. maydis* that specializes in Texas male-sterile cytoplasm. With the dewy nights and warm days of the 1970 summer proving ideal for the development of the fungus, Race T swept north. But hybrid corn from seed produced the costly old way kept its high yield and the cob strength that corn-handling machinery requires. Such seed, though, has become scarce.

This year each grower has needed all the information he could get for picking his own best strategy against this unfamiliar threat to profitable farming. Switch to soy beans? Take a chance on hybrid seed corn blended with a percentage produced by detasseling? Plant thickly or thinly? Deep or shallow? Weed control? Liming against soil acidity? Crop insurance? Hedge through the futures market? Participate in the program for taking some acreage out of production, specifically bottom land where dew is frequent? What a tangled web of biology, economics, and divination extends back from the meat counter to the not-so-simple farmer!

Never has the biologist's need to communicate by means of photographs been more strongly felt than in comparing findings on the Race T problem and getting the new-found facts out to all those individual decision-makers. See glimpses at left and right of images that deliver their impact via TV and press away from the cities and suburbs. A good communicator, Dr. Luther Farrar of Auburn University in Auburn, Ala., gave them to us with a little advice ▶



The toxin hits only corn tissue, but when it goes all the way to stem rot it opens the way for secondary organisms. Some of these might produce toxins for the animal kingdom.



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The Importance of Being Ernest Rutherford

Ernest Rutherford's good friend and colleague, A. S. Eve, once playfully depreciated his immense contributions to physics by charging that he rode the crest of a wave. Rutherford responded, "Well, I *made* the wave, didn't I?" The centennial of Rutherford's birth on 30 August of this year makes this an opportune time to look at that wave.

It has long been accepted that "great men" only accelerate scientific progress a bit, but this cliché does not seem to apply to the man hailed as the greatest experimentalist since Faraday. Consider radioactivity early in this century: the significant workers could be counted on one's fingers, and none of them had the range of interests or the fertile, intuitive mind that Rutherford had. He advanced the disintegration theory of radioactivity (with Frederick Soddy, in 1902 and 1903), proposed a nuclear structure to the atom (1911), and succeeded in artificially transmuting a nucleus (1919).

Although Rutherford was no mathematical physicist, there is a notable theoretical component to each of these major achievements. He was, moreover, the leading prophet (in 1920) for the existence of the neutron (and ^2H and ^3He as well, though all this was based on faulty experimental data!). Theoreticians who worked closely with his laboratory, with varying degrees of personal influence from Rutherford, included Soddy, Kasimir Fajans, Niels Bohr, C. G. Darwin, Ralph Fowler, Neville Mott, and George Gamow. Indeed, Rutherford was as great a research director as a discoverer, and Otto Hahn, George Hevesy, Hans Geiger, H. G. J. Moseley, James Chadwick, P. M. S. Blackett, Peter Kapitza, John Cockcroft, and E. T. S. Walton are only the most eminent of his distinguished pupil-colleagues.

For almost the last 20 years of his life Rutherford was director of the Cavendish Laboratory in Cambridge, England. There, he influenced the transition from little science to big science. Kapitza, Cockcroft, and Walton were among those responsible for introducing large machines and high costs into physics, and the electronic counters of C. E. Wynn-Williams and Geiger also presaged the new look. Although these departures from the "sealing wax and string" tradition were not entirely to his taste and Rutherford disliked looking for research funds, his "boys" generally acquired the equipment needed. As president of the Royal Society and later as chairman of the advisory committee to the government's Department of Scientific and Industrial Research, he was in a position to exert considerable influence throughout the "Establishment" of science.

A number of writers have pointed out that Rutherford said that the energy of the atom would never be harnessed. In effect, the criticism means that he failed to foresee a new physical phenomenon discovered a year after his death in 1937. In the early days of radioactivity, when Rutherford recognized that decaying radioelements eject alpha and beta particles, he actually wondered if larger chunks of the atom also broke off, but no evidence for what later was called fission could then be found. Still, with Chadwick's discovery of the neutron and with the discovery of fission by Hahn, Rutherford had a connection with applications of nuclear energy. We find his presence even in the beginnings of fusion research, as when in 1934, with M. L. E. Oliphant and Paul Harteck, he bombarded deuterium with deuterons. As a major participant in the transition from classical to modern physics, it would seem that the wave Rutherford created was even larger than he may have thought!—LAWRENCE BADASH, *Department of History, University of California, Santa Barbara*

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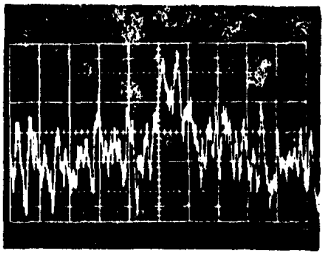

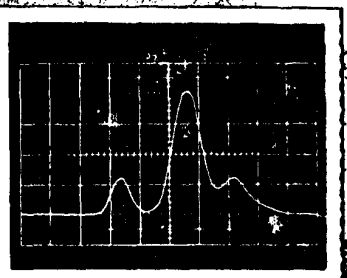

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Attempts to Map a Process Evolution of Peptide Biosynthesis

Synthesis of peptide antibiotics from thiol-linked amino acids parallels fatty acid synthesis.

Fritz Lipmann

In trying to establish a continuity between prebiotic evolution and present-day biochemistry, I have attempted to find surviving metabolic relics from earlier stages (*1*). The retention of inorganic pyrophosphate as phosphoryl donor and inorganic phosphate as phosphoryl acceptor have been proposed as examples of metabolic fossils in the evolution of energy transfer (*1, 2*).

In the domain of the transfer of genetic information I felt naïvely that, before translation into amino acid sequences could become the target of the genetic code, the extraordinary functional pliability of proteins would have to be established. In attempts to find a relatively simple process yielding peptides of defined composition, my attention was drawn to the recent preparation by Otani, Laland, and Kurahashi, and their co-workers, of ribonuclease-resistant systems for the synthesis in vitro of antibiotic polypeptides of reasonably long chain length (*3, 4*). Our studies proved to be rewarding because, in this mechanism, activation was found to yield thioester-linked amino acids as precursors in polymerization (*5*). This finding presented a link in process evolution between the condensation of two-carbon fragments to β-keto acids in fatty acid synthesis, and of amino acids to polypeptides in ribosomal protein synthesis, between which striking parallels had long been obvious (*6*).

Analogies between Fatty Acid Synthesis and Polypeptide Synthesis

During the isolation of a then new coenzyme for acetate activation, coenzyme A (CoA), a liver enzyme that *catalyzed acetylation of aromatic amines*, was used as a test system (*7*); Fig. 1 shows a thioester-linked reaction (*8*) yielding a peptide bond. Subsequently, Chantrenne (*9*) added hippuric acid synthesis, the benzoylation of glycine, to the family of CoA-thioester-linked reactions. In this way, carboxyl transfer from a thioester became more clearly connected with a peptide synthesis for which hippurate condensation had been considered a good model system. This peptide was among the first where the free energy change, ΔF, necessary to form a peptide bond was determined; it was found to be about +3 kilocalories (*10*). One of the more important results of these findings was that they strongly suggested that the carboxyl group would be the point of activation for amino acids in protein synthesis.

Although the initial energy donor for synthesis of acetyl-CoA was clearly adenosine triphosphate (ATP) (*7*), it took a long time to ascertain the details of the two-step reaction which yields acetyl-CoA by an energy transfer from ATP by pyrophosphate (PP_i) displacement (*11*). In eukaryote acetate activation, acetate-CoA ligase catalyzes the following two-step reaction:

$$\text{Acetate} + \text{ATP} \rightleftharpoons \text{acetyl}\sim\text{adenylate} + PP_i \quad (1a)$$

$$\text{Acetyl}\sim\text{adenylate} + \text{CoASH} \rightleftharpoons \text{acetyl}\sim\text{SCoA} + \text{adenylate} \quad (1b)$$

Acetate initially reacts with ATP to form an acetyl adenylate (*12*), followed by a second step (Eq. 1b) in which acetyl transfers from the acetyl-adenylate enzyme complex to the —SH group of CoA. As the result of a double transfer on the same enzyme, we find as end products acetyl-CoA, PP_i, and adenylate. A rather analogous reaction was eventually found to be responsible for the first step in amino acid activation. In prokaryotes and eukaryotes (*13*) the amino acid transfer RNA (tRNA) ligase catalyzes the following

$$\text{Amino acid} + \text{ATP} \rightleftharpoons \text{aminoacyl}\sim\text{adenylate} + PP_i \quad (2a)$$

$$\text{Aminoacyl}\sim\text{adenylate} + \text{tRNA} \rightleftharpoons \text{aminoacyl}\sim\text{tRNA} + \text{AMP} \quad (2b)$$

Here, different enzymes were required for the activation of different amino acids. As in acetate activation, the PP_i displacement of ATP in amino acid activation turned out to be the first step in a two-step reaction. The second step, however, involved an esterification to a reactive hydroxyl at the O-terminal adenosine of aminospecific tRNA's rather than to a thiol (*14*).

These parallels between the group activations for fatty acid synthesis and protein synthesis prepared us for finding analogies between the mechanisms of the two polymerizations, the repeating condensation steps of which are compared in Fig. 2 (*15*). The analogy between condensation in β-keto acid synthesis of carboxyl to methyl groups to yield CO-CH_2, and of carboxyl to amino groups to yield CO-

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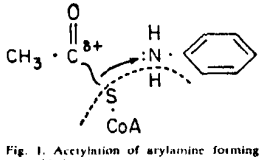

Fig. 1. Acetylation of arylamine forming a peptide bond.

NH· in peptide synthesis, was the topic of an interesting discussion at the 1959 Gatlinburg Conference, where I was very pleased that Professor Todd sided with my notion that the reactions that form these two linkages are quite similar (*16*). He went so far as to say that under certain conditions they are pretty much the same. Hydrolysis of β-keto acids by chymotrypsin may also be mentioned here (*17*).

Search for a Compact Mechanism of Polypeptide Synthesis

The studies on ribosomal protein synthesis (*18*) made us appreciate that the protein is a rather unusual chemical creation, composed as it is of various sequences of 20 different amino acids which have remained unchanged throughout the evolutionary process beginning with bacteria. Protein structure has the strange and useful property, depending on amino acid sequence, of giving rise to an inexhaustible variety of catalysts. We felt that this creation could not have been born, so to say, in one fell swoop. With the hope of finding remnants of evolutionary links, we looked in bacteria for a simpler mechanism of polypeptide synthesis and became interested in the biosynthesis of polypeptide antibiotics, particularly of gramicidin S (GS) and tyrocidine (Ty), the understanding of which had just begun to open up. After a brief period when it was thought that these polypeptides were manufactured on ribosomes (*19*), it appeared that their biosynthesis could be found in supernatant fractions of ribonuclease-treated bacterial homogenates centrifuged at high speed (*3, 20*). Extracts producing them can be obtained from certain strains of *Bacillus brevis*. Therefore, here seemed to exist a looked-for simpler biosynthetic mechanism. Indeed, since participation of nucleic acids was excluded, one had to expect that in this case a protein had to act as template. It was surprising, however, to discover here a process where initially thiol-linked amino acids polymerize in sequence on a polyenzyme system.

Table 1. Binding characteristics of complementary fractions in GS biosynthesis. Abbreviations: Phe, phenylalanine; Pro, proline; Val, valine; Orn, ornithine; Leu, leucine.

Amino acids bound	Sephadex G-200 fraction	Molecular weight	Remarks
Phe, D- or L-	Light	100,000	Contains racemase
Pro, Val, Orn, Leu	Heavy	280,000	Mol. wt./amino acid, 70,000

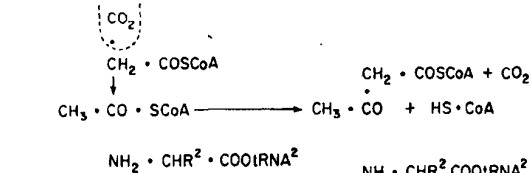

Fig. 2. Comparison of β-keto acid synthesis (upper equation) with peptide synthesis (lower equation).

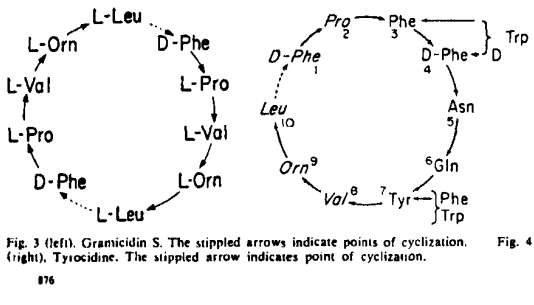

Fig. 3 (left). Gramicidin S. The stippled arrows indicate points of cyclization. (right). Tyrocidine. The stippled arrow indicates point of cyclization.

Fig. 4

Biosynthesis of Gramicidin S

The work of Saito, Otani, and Otani (*21*), of Kurahashi and his colleagues (*22*), and of Laland's group in Oslo (*23*) had laid a solid foundation on which to build. Both GS, the first to be studied, and Ty, an antibiotic similar to GS, are cyclized; their formulas appear in Figs. 3 and 4. The GS decapeptide is made up of two identical pentapeptides that cyclize head to tail (Fig. 3). In Ty the ten amino acids condense in a row and cyclize between starting D-phenylalanine and terminal leucine (Fig. 4). As general introduction, Fig. 5 illustrates the interrelation between formation of synthesizing enzyme and growth (*4*). Almost all spore-forming bacteria form antibiotics; these are produced only in the short period closely preceding sporulation, which is genetically linked to antibiotic production (*24*).

Before turning to details, I have to introduce the names of those responsible for all the work on GS and Ty in this laboratory. The early studies on GS were done by Wieland Gevers and Horst Kleinkauf, and Robert Roskoski, Jr., is largely responsible for the Ty analysis.

Tomino *et al.* (*4*) separated two complementary enzymes that function in the biosynthesis of GS; some basic facts are summarized in Table 1. (25) Figure 6 (*26*) is a Sephadex G-200 filtration profile for the GS system, showing the activation of amino acids by the respective peak fractions. The amino acid–dependent ATP⇌PP$_i$ exchange used for assay indicates that

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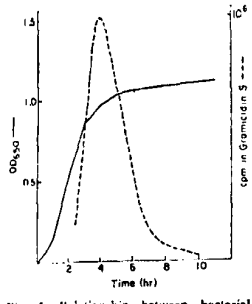

Fig. 5. Relationship between bacterial growth and enzyme production.

the primary activation in this system is of the type shown in Eq. 2a. When the enzyme that activates amino acids in the ribosomal system is filtered through Sephadex G-50, all protein-bound amino acid emerges as adenylate and links to added tRNA. In the antibiotic system, however, the filtration yields only half of the amino acid bound as adenylate (*25*); on precipitation with trichloroacetic acid (TCA), which destroys aminoacyl adenylates, the other half remains bound to the precipitate (*5, 27*).

The characteristics of this acid-stable protein-bonded amino acid are those of a thioester: it is acid stable but alkali labile, and is cleaved by mercuric acetate, a test for thioesters first used in biochemistry by Lynen *et al.* (*28*); it readily forms hydroxamate at slightly acid reaction (*29*); and it gives reductive cleavage with borohydride, which discharges the amino acid as the corresponding alcohol, and oxidative cleavage with performic acid. A reversible transfer from aminoacyl-adenosine monophosphate (AMP) had been postulated by our earlier finding (*5, 27*) that in addition to ATP↔PP_i exchange, all amino acids also catalyze an AMP↔ATP exchange. The secondary acceptor in the antibiotic system thus turned out to be an enzyme-bound thiol; from this the amino acid is fed into polymerization.

Mechanism of Peptide Synthesis

Charging all four amino acids to a preparation of the GS enzyme of 280,000 molecular weight (Table 2) causes them to become thioester-linked in equimolar proportions. However, so long as the complementary phenylalanine-charged enzyme, which has a molecular weight of 100,000, is not added, the four amino acids on the larger enzyme stay apart; polymerization is initiated by combination with the phenylalanine-charged enzyme (*25, 30*). Figure 7 presents a scheme of initiation found to be a transfer of thiol-linked D-phenylalaoine from the smaller enzyme to proline linked to the larger enzyme, which yields the thiol-linked D-phenylalanylproline dipeptide with amino terminal phenylalanine (*5, 27*).

The further elongation of the enzyme-bound polypeptide chains with one-by-one addition of amino acids is shown in Table 3 (*5*). Up to the fourth, ornithine, the [^{14}C]phenylalanine incorporation is seen to increase proportionately with every step. Thus, up to then, the chain is lengthening, and all growing intermediate peptides are simultaneously enzyme-bound, which was shown by isolation of their hydroxamates from the TCA precipitates (*27*). After the fifth amino acid, leucine, is added, a large amount of GS is liberated from the enzyme (last column in Table 3), indicating rapid cyclization between two pentapeptides because no peptides longer than pentapeptides were determined (*5, 27*), as confirmed by the Oslo group (*31*). Of

Table 2. Stoichiometric binding of amino acids to 85 percent pure GS heavy fraction (*30*). The amino acid bound as thioester was measured in the TCA precipitable material after Sephadex G-50 gel filtration as previously described (*39*).

Amino acid	Thioester bound (mole/mole enzyme)
[^{14}C]Proline	0.99
[^{14}C]Valine	1.04
[^{14}C]Ornithine	1.11
[^{3}H]Leucine	0.95
[^{14}C]Phenylalanine	0.00

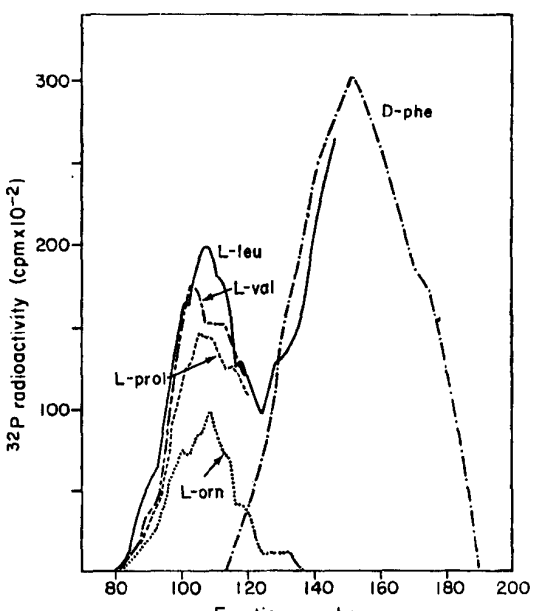

Fig. 6. Separation on Sephadex G-200 of fractions complementary in GS biosynthesis. The chromatography and amino acid–dependent ATP-^{32}PP$_i$ exchanges were carried out as described (*26*).

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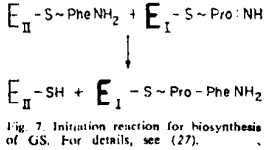

Fig. 7. Initiation reaction for biosynthesis of GS. For details, see (*27*).

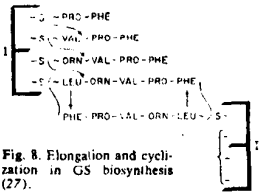

Fig. 8. Elongation and cyclization in GS biosynthesis (*27*).

great importance is the result of the last line in Table 3, namely, that when proline, the second amino acid, is omitted, but the following valine and ornithine are added, the enzyme-bound [^{14}C]phenylalanine incorporation is back to the stage when only phenylalanine alone was present. Thus, omission of one amino acid interrupts the progress of amino acid addition, similar to an amber mutation in ribosomal protein synthesis when an amino acid triplet in messenger RNA (mRNA) has undergone a mutation to a nonsense triplet (*32*). Although the template is a different one here, interruption of the sequence similarly leads to to premature termination. A preliminary scheme of elongation and cyclization is presented in Fig. 8 (*27*).

Table 3. Formation of protein-bound chains with increasing numbers of amino acids. For experimental details see Gevers *et al.* (*5*).

Peptide chains formed	Protein-bound thioesters (count/min)	GS (count/min)
[^{14}C]Phe	1,549	265
[^{14}C]Phe Pro	6,001	375
[^{14}C]Phe Pro-Val	10,005	1,531
[^{14}C]Phe Pro-Val-Orn	14,325	1,005
[^{14}C]Phe Pro-Val-Orn-Leu	2,029	25,409
[^{14}C]Phe- . . . . (Val, Orn)	1,610	376

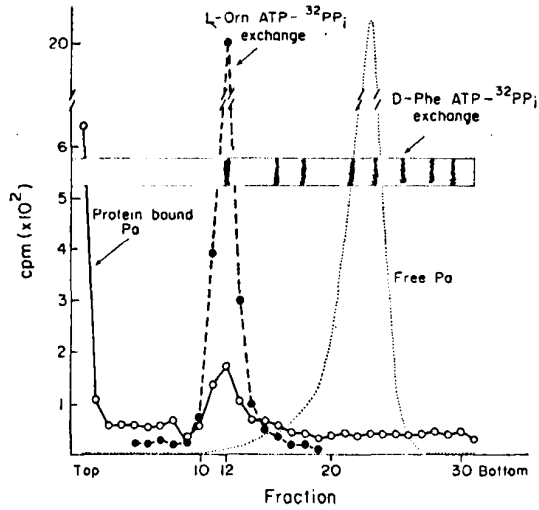

Fig. 9. Incorporation of pantothenic acid (*Pa*) into the GS heavy enzyme. For details see Kleinkauf *et al.* (*36*).

Table 4. Activity of the light fraction in GS biosynthesis after preliminary incubation with D-phenylalanine thiophenol. The latter thioester has been bound (charged) to the light fraction from the synthetic thioester and, in combination with the heavy fraction, activated with ATP and the four other amino acids including [^{14}C]proline, [^{14}C]valine, and [^{14}C]-leucine of equal specific activity has formed sizable amounts of GS, as measured by its content of ^{14}C.

Incubation	GS formed (μmole)
Light + D-phenylalanine	0
Light + D-phenylalanine thiophenol	5.0

Search for Pantothenic Acid

The discovery that enzyme-linked aminoacyl thioesters polymerize to enzyme-linked peptidyl thioesters introduced a closer resemblance to fatty acid synthesis. This led us to propose the possibility that 4′-phosphopantetheine, in analogy to its presence in the fatty acid–synthesizing polyenzyme system (*33, 34*), might take part in our polypeptide synthesis (*5, 27*). To test for the presence of pantothenic acid, we used a procedure analogous to that described by Vagelos *et al.* (*33*); they added radioactive pantothenic acid to wild-type *Escherichia coli* and found ample incorporation into acyl carrier protein. In our first experiments we failed to detect a sizable amount of pantothenic acid in our enzymes, presumably because of their late formation, at the end of the logarithmic growth period. In the meantime, the Oslo group, using the more sensitive microbiological assay, had found that pantetheine was present in the large enzyme fraction, 1 mole of pantetheine per mole of enzyme (*35*).

With a sample of *B. brevis* that grows somewhat better we have now detected (*36*) incorporation of pantothenic acid by the Vagelos method, but only in the larger enzyme (Fig. 9); there, with a rather pure preparation, the presence of phosphopantetheine was confirmed. As shown by Fig. 10, 1 mole of pantothenic acid per mole of enzyme was liberated for the micro-

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biological assay by the method of Pugh and Wakil (*37*); this single pantetheine is to be compared with the thioester links to four amino acids in the large enzyme (Table 2). It was quite pleasant to meet here, unexpectedly, part of CoA, the composition of which is shown in Fig. 11.

Transfer of Amino Acid from Chemically Prepared Thioester

In the hope of confirming the polypeptide synthesis from thioester links, Gordon Ryan prepared in our laboratory D-phenylalanine thioesters of *N*-acetylcysteine and of thiophenol by the procedures devised by Wieland and Köppe (*38*). Both of these were active as precursors of the D-phenylalanine in GS, whereas the nitrophenol ester was inactive (*30*). A representative experiment is shown in Table 4. After incubation with the thioester, the amino acid–charged small enzyme, isolated by ammonium sulfate precipitation with retention of enzymatic activity (*30*), was combined with a similarily precipitated large enzyme that had been incubated with the four other amino acids, including [^{14}C]proline and ATP. The ammonium sulfate precipitate retained only the thioester-bound amino acids but not aminoacyl adenylates.

Mechanism of Tyrocidine Biosynthesis

Figure 5 shows the sequence in Ty, numbered in the order of incorporation, including the exchangeable aromatic amino acids. This interchangeability was shown to be competitive by Mach and Tatum (*39*), using the living organism; incorporation depends entirely on the concentration of the different amino acids present in the medium, which we have confirmed (*40*) for in vitro synthesis.

The Ty enzyme system is not as easily obtained as that for GS. Fujikawa *et al.* (*41*) were the first to report a reliable method for preparing the Ty system, and we have used it, with a little modification, to prepare our extracts from *B. brevis*, Dubos strain (ATCC 8185) (*40*). The component enzymes of the Ty system are described in Table 5. As indicated in Fig. 4, biosynthesis again begins with D-phenylalanine bound and racemized by a light enzyme having a molecular weight of

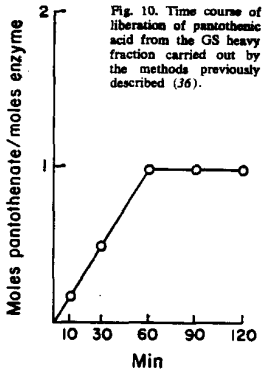

Fig. 10. Time course of liberation of pantothenic acid from the GS heavy fraction carried out by the methods previously described (*36*).

100,000. Proline, the next amino acid, is activated by a separate enzyme of intermediate size; the large enzyme activates and carries the eight other amino acids. The large enzyme also racemizes either phenylalanine or tryptophan in position 4. We can be brief with the description of the process because of the essential analogy to the GS system. The Sephadex G-200 filtration profile of a supernatant fraction is shown in Fig. 12 (*36*). The three fractions appear on this figure. The reason for the proline enzyme being so large (molecular weight 230,000) is not understood; it elutes after the heavy enzyme (molecular weight, 460,000) and often contaminates it. The light, phenylalanine-activating fraction elutes last. This diagram includes the assay for 4′-phosphopantetheine, which, we find, is present only in the large enzyme, in the ratio of 1 mole of pantetheine per mole of enzyme, and which, in this synthesis, carries eight thioester-linked amino acids (*36, 40*).

The mechanisms of activation and polymerization are the same in Ty and GS biosyntheses. All amino acids cause exchange of ATP↔PPi and ATP↔AMP (*40*), and ten ATP's are split to 10(AMP+PPi) with the formation of a decapeptide (*41*). Table 6

Table 5. Binding characteristics of complementary fractions in tyrocidine biosynthesis. Abbreviations as in Table 1; Asn, asparagine; Gln, glutamine; Tyr, tyrosine.

Amino acids bound	Fraction	Molecular weight	Mol. wt./ amino acid
Phe, D- or L-	Light	100,000	
Pro	Intermediate	230,000	
L-Phe, D-Phe, Asn, Gln Tyr(Phe)*, Val, Orn, Leu	Heavy	460,000	58,000

* When only Phe is present it will replace Tyr. Data from Roskoski *et al.* (*40*).

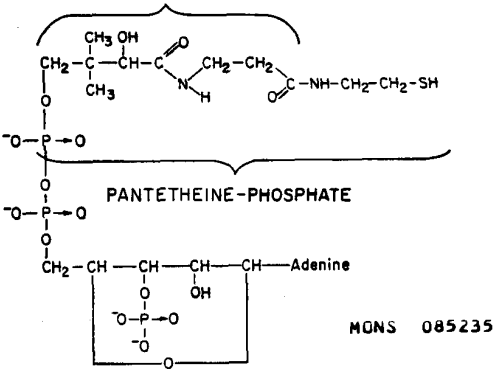

Fig. 11. Coenzyme A.

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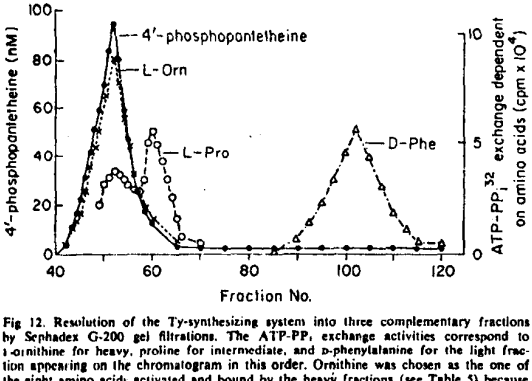

Fig. 12. Resolution of the Ty-synthesizing system into three complementary fractions by Sephadex G-200 gel filtrations. The ATP-PP$_i$ exchange activities correspond to L-ornithine for heavy, proline for intermediate, and D-phenylalanine for the light fraction appearing on the chromatogram in this order. Ornithine was chosen as the one of the eight amino acids activated and bound by the heavy fractions (see Table 5) because its activation is antibiotic-specific. Phosphopantetheine was determined as described (36).

Table 6. Amino acid binding of three tyrocidine complementary fractions. The total amino acid bound to protein in the Sephadex G-50 effluent is noncovalently linked aminoacyl-adenylate and covalently linked thioester. Precipitation by TCA discharges the aminoacyl-adenylate but not the thioester.

Labeled amino acid	Amino acid bound (pmole)					
	Light		Intermediate		Heavy	
	Total	TCA	Total	TCA	Total	TCA
L-[^{14}C]Phenylalanine	9.5	4.7			29.0	14.4
L-[^{14}C]Proline			10.0	5.0		
L-[^{3}H]Ornithine					10.1	5.0
L-[^{14}C]Asparagine					10.7	5.3

Table 7. Formation of protein-bound nascent peptide chains by sequential addition of amino acids with [^{14}C] marker in the proline (*40*). Addition of phenylalanine causes just short of a threefold normal increment in proline incorporation because it is incorporated simultaneously into the three peptides presented in items 2, 3, and 4. The addition of the other amino acids with single presence causes approximately equal increments, except for glutamine, which is followed by a phenylalanine already present on the enzyme where the increment is about doubled. The dashes indicate peptide links formed after each addition; the newly added amino acids are italicized. In items 11, 12, and 13, omission of an amino acid, indicated by dots, stops further incorporation of [^{14}C]proline posterior to omission.

Peptide chains formed	Bound radioactivity (pmole)	Increment
1. . . .-[^{14}C]Pro	6	6
2. D-*Phe*-[^{14}C]Pro	20.5	14.5
3. D-*Phe* [^{14}C]Pro-*Phe*		
4. D-*Phe*-[^{14}C]Pro *Phe*-D-*Phe*		
5. D-Phe [^{14}C]Pro-Phe-D-Phe-*Asn*	27.6	7.1
6. D-Phe-[^{14}C]Pro-Phe-D-Phe-*Asn*-*Gln*	39.0	11.4
7. D-Phe-[^{14}C]Pro Phe D-Phe-*Asn*-*Gln*-Phe*		
8. D-Phe-[^{14}C]Pro-Phe-D-Phe-Asn-Gln-Phe-*Val*	43.5	4.5
9. D-Phe-[^{14}C]Pro-Phe D-Phe Asn-Gln-Phe-Val-*Orn*	49.3	5.8
10. D-Phe-[^{14}C]Pro-Phe-D-Phe-Asn-Gln-Phe-Val-Orn-*Leu*	54.7	5.4
11. . . .-[^{14}C]Pro(Pro,Phe,Asn,Gln)	7.0	
12. D-Phe-[^{14}C]Pro-Phe-D-Phe- . . . (Gln,Phe,Val,Orn,Leu)	21	
13. D-Phe-[^{14}C]Pro-Phe-D-Phe-Asn-Gln-Phe-Val- . . . (Leu)	43.7	

* This Phe waiting in position 7 does not cause an increment before Asn, 5, and Gln, 6, are added, indicating that binding out of sequence does not cause elongation.

shows that the three enzyme fractions yield Sephadex G-50 filtrates that contain half the amino acids in acid-unstable form bound as adenylates and half as acid-stable thioesters; in tests with phenylalanine, 3 moles are bound to the large enzyme for every mole of ornithine and asparagine. The latter appear only once in the sequence, whereas phenylalanine incorporates in three places; when it is the only aromatic amino acid added, it replaces tyrosine. This shows equimolarity of binding on the large enzyme, every site being charged independently. Furthermore, of the three phenylalanines, only the one on site 4 is racemized. These data indicate a strictly topographical incorporation.

To obtain synthesis, the three enzymes are combined; the light, charged with phenylalanine, initiates the chain by condensing D-phenylalanine to proline on addition to the intermediate enzyme. The D-Phe-Pro dipeptide transfers to the large enzyme which carries all the other amino acids to complete the decapeptide. In Table 7 an experiment analogous to that for GS in Table 3 is presented. The amino acids with marker in [^{14}C]proline only are added in succession and proceed in the direction of synthesis; every addition causes an increase in [^{14}C]proline incorporation approximately proportional to the number of places of amino acid addition (for details see the legend of Table 7). Eventually, when leucine, the tenth amino acid, is added, the decapeptide is released, but cyclization is rather slow.

In contrast to GS synthesis, a fair amount of decapeptide can be found attached to the enzyme. When, in the overall synthesis, phenylalanine is added as the only aromatic amino acid, it is incorporated in four places, one of which derives from the light fraction. When phenylalanine is left out (line 11, Table 7), only proline is incorporated, as if the other amino acids were not there. On omission of asparagine (line 12, Table 7), incorporation proceeds only to the level of Phe-Pro-Phe-Phe, and, by omitting ornithine, the one next to the last (line 13, Table 7), it stops with the preceding valine at the level of enzyme-bound octapeptide. In other words, polymerization proceeds sequentially without jumping; the large enzyme functions as template. It has been possible to show by differential marking that during biosynthesis of Ty nine different peptides and the initiating

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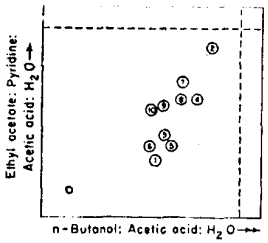
Fig. 13. Two-dimensional thin-layer chromatography of enzyme-bound peptides after alkaline liberation. The encircled residues indicate the number of amino acid residues in each peptide.



phenylalanine can be found bound to the enzyme. Figure 13 (*40*) shows their separation by two-dimensional thin-layer chromatography and their identification in ten different spots. This demonstration confirms the template-like activity of the large enzyme which takes over after initiation by transfer of D-Phe-Pro.

Preliminary Evidence for Gramicidin A Synthesis

The same organism that produces Ty also produces the gramicidins A, B, and C (*42*). Their constitutions are shown in Fig. 14. They have the same open-chain structure with a 15-amino acid sequence, and are distinguished only by amino acid replacements. We are in the process of exploring the synthesis of these gramicidins. Glycine and alanine, which are not present in Ty, have yielded TCA-stable activation products in our crude extracts, an indication that these gramicidin amino acid acids form thioesters with an enzyme protein (*40*). Karl Bauer, who recently joined us, is making further progress with this problem.

Summation

To complete the description of this new type of polypeptide synthesis, I present in Fig. 15 the structure of enzyme-bound 4'phosphopantetheine which takes part in fatty acid synthesis as well as in this peptide synthesis. In view of its alkali lability it appears to be esterified to a hydroxyamino acid in our enzyme proteins; it assays as in the acyl carrier protein, where its type of binding was proved more rigorously (*33*). The features of fatty acid synthesis which are repeated in our system are well brought out in Lynen's scheme in Fig. 16 (*34*). One sees the peripheral and central -SH groups; the latter is pantetheine-carried. When fatty acid synthesis begins, the peripheral -SH is charged with an acetyl residue which is the initiator, and the central -SH is charged by transfer of malonyl from CoA to pantetheine. There follows a condensation between the acetyl carboxyl and the CH_2 group of the malonyl, with liberation of CO_2, which yields an enzyme-bound acetoacetyl, written here as enol. Then, on the pantetheine, acetoacetyl swings around the polyenzyme through reduction and dehydration steps, until it reaches the butyric acid stage. A new round begins with the butyryl being translocated from pantetheine to peripheral thiol; a new malonyl is transferred to the liberated pantetheine-thiol, and condensation elongates to a six-carbon β-keto acid which continues the cycle and elongation. Translocation, to use the language of the ribosomal system, is from the acceptor to the donor site, where the peripheral thioester acyl is donated to a new malonyl on the acceptor site, and cycling goes on until a chain of 16 or 18 carbons is formed.

One realizes the close process similarity between fatty acid synthesis and polypeptide synthesis. However, in the former, one is dealing with a cyclic process where the same two-carbon unit is added in succession to the growing chain and the resulting β-keto acyl is reduced to fatty acid acyl until a certain length is reached. In antibiotic polypeptide synthesis one meets a synthetic machinery built on the same principle. However, the new combining units are different here, amino acids being added in a predetermined sequence: for this, a built-in template is required that dictates addition in the right order.

Antibiotic polypeptide synthesis, in terms of process evolution, is between fatty acid synthesis and the complex ribosomal polypeptide synthesis. It parallels features of the two reaction mechanisms; it runs in single steps through elongation to a product that grows

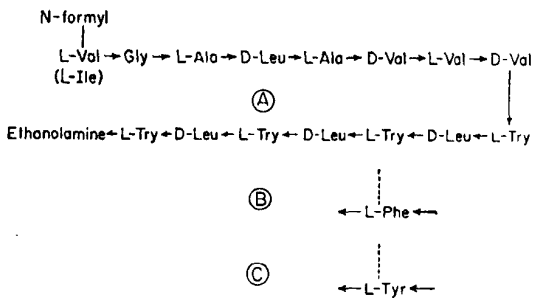


Fig. 14. Linear gramicidin (A, B, and C)

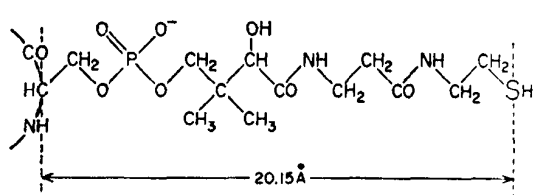


Fig. 15. Enzyme-bound 4'phosphopantetheine (*34*).

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vectorially by transpeptidation of thiol-linked peptides to amino groups of thiol-linked amino acids. One has to assume, and we have preliminary evidence that this assumption is valid, that the pantetheine performs a switch or translocation by alternative condensation and transthiolation. As illustrated in Fig. 17, the pantetheine is drawn as a central thiol-carrying arm charged with a growing chain. As a result of the events after initiation of GS synthesis, which were shown in Fig. 7, it starts donating a dipeptidyl (*30*) from pantetheine to the amino group of the succeeding amino acid to form a tripeptide, which thereby temporarily comes to sit on a peripheral thiol. The

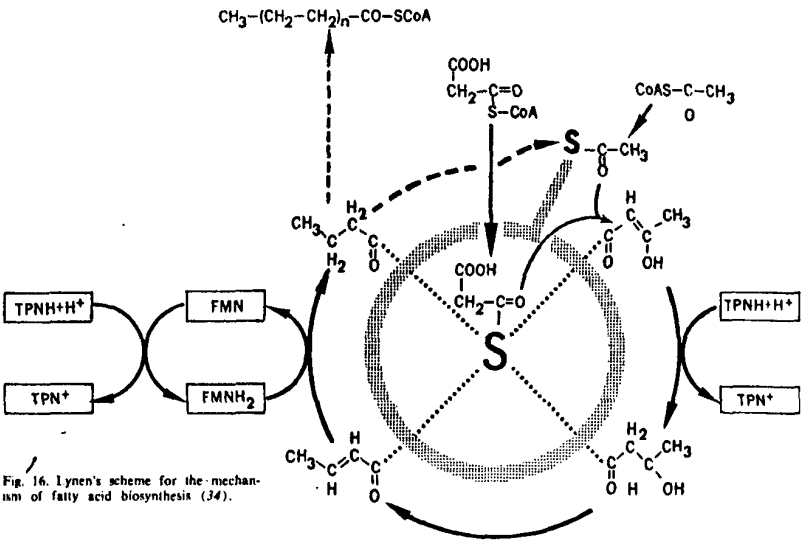
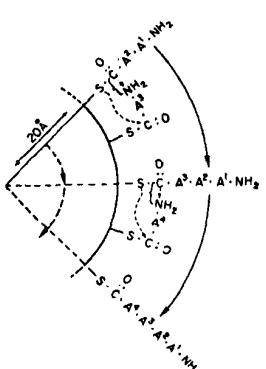


Fig. 16. Lynen's scheme for the mechanism of fatty acid biosynthesis (*34*).



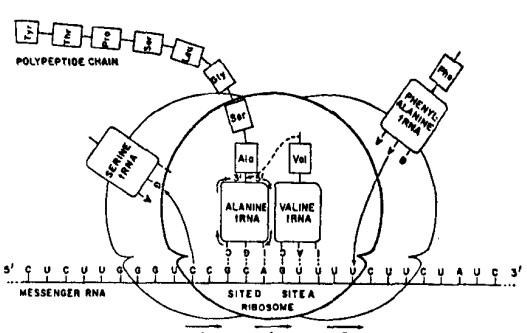


Fig. 17 (left). A schematic representation of transpeptidation and transthiolation, presumably mediated by enzyme-bound pantetheine in antibiotic peptide biosynthesis. Fig. 18 (right). Sequential addition of amino acids in ribosomal peptide synthesis, adapted from Crick (*43*) and discussed in (*18*).

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liberated pantetheine-SH performs a translocation by transthiolation of the tripeptide from peripheral acceptor-SH to its own, the donor thiol. The next step is a repetition of transpeptidation from pantetheine thioester to neighboring groups of peripherally bound aminoacyl to form a tetrapeptide. This is again transthiolated, and elongation continues to the pentapeptide, two of which cyclize from the decapeptide to GS. The length of the pantetheine is 20 Å, and it seems that this is an appropriate length to go round.

For comparison, the ribosomal system is presented in action in Fig. 18. The alternation of transpeptidation and translocation is transacted here by some kind of linear propulsion rather than rotation. The ribosomal mechanism is different in detail but serves the same purpose: the growing peptide chain condenses from tRNA to the amino group of a newly adding aminoacyl-tRNA on the acceptor site, and then is translocated to the donor site on the ribosome for continuation.

In antibiotic biosynthesis the construction is very compact; activation and polymerization are transacted on the same protein, which makes the whole machinery much smaller, and this system impresses like a missing link in the transition in process evolution from fatty acid to ribosomal protein synthesis. The less complex system, a descendant of fatty acid synthesis, has gained the ability to assemble a line of different amino acids in prescribed order. In both Ty and GS synthesis, the sequence of addition is guided by a protein template laid out on a polyenzyme system. In ribosomal synthesis, genetic information is translated by codon-anticodon interaction on the mRNA template, which accurately transmits the information to produce sequences of long polypeptide chains. A protein template is not suited to do this; its limits are probably 15 to 20 amino acid sequences.

The three systems have a common mode of polymerization. They fall into a category that I like to call head-growth polymerization (*15*). As seen in Fig. 19, in this mode the group that joins the chain is preactivated, and activation of the previously added group heading the chain is used for the elongation step. This leaves the activation at the new head intact so that it is already primed to do the next step. The

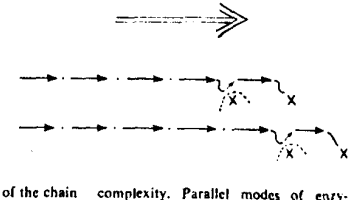

Fig. 19. Headward chain growth (*15*). The figure shows that the chain is headed by an activated annex, the displacement of which leads to addition of a new unit, whose preactivated head thereby becomes a new activated chain terminal.

mode facilitates the binding of the chain terminal to the template, thereby prompting the adding unit to settle next to it.

Concluding Remarks

This study served two purposes, and it might be worthwhile to discuss them separately.

1) The exploration of this enzyme mechanism was initiated as an attempt to connect backward from the present to a prebiotic evolution. For this purpose we embarked on a study of a relatively simple enzymatic mechanism of amino acid sequentialization which produced polypeptides in an RNA- and particle-free solution. There can be no doubt that the *B. brevis* strains produce the enzyme proteins that act as templates through a DNA-derived message on ribosomes. This does not exclude the possibility that before the takeover by genetic information transfer there may have existed a mechanism similar to the one described here to connect amino acids. Such medium-sized polypeptides could have been early representatives of a class of compounds exhibiting the remarkable functional versatility that developed fully in the synthesis of proteins.

As was mentioned, an amino acid transfer from synthetic aminoacyl thioesters to one of the GS enzymes has recently been found. We hope to continue such synthetic approaches for exploring the use of -SH-charged proteins or other polymers in amino acid polymerization, as exemplified by antibiotic synthesis.

2) The unexpected finding of aminoacyl thioesters as donors in enzymatic polypeptide synthesis which, as in fatty acid synthesis, was also linked to pantetheine as carrier, encouraged me to formulate the evolution of a scheme of operation to activate and connect subunits into polymers of increasing complexity. Parallel modes of enzymatic activity were grouped together in a preceding essay on the relation between direction and mechanism of polymerization (*15*). It does not seem to me too absurd to think of the possibility that some genetic background may appear for the development of such parallel modes. However, calling this a process evolution should not be construed to imply use of the word evolution in a Darwinian manner.

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44. Supported by PHS grant GM-13972.

Bacterial Differentiation

The cell cycle of *Caulobacter* is used as a model system for studying the molecular basis of differentiation.

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Living cells, whether they are components of complex tissues and organs or function as unicellular organisms, are not static units but can change morphologically, adapt to environmental conditions by biochemical alteration, or express different functions at different times in the life cycle. A major task of modern biologists is to understand the control of these cell changes. Recent investigations of the genetic control mechanisms which operate in microbial cells indicate that not all the genes in a given cell are translated into protein at the same time (*1*), and that environmental factors can and do influence gene expression (*2*). In a differentiating cell, undergoing a series of biochemical and morphological changes within a regulated time sequence, controls must also exist to ensure the organization and sequential expression of these events during the life cycle of the cell. A persistent notion among developmental biologists has been that organizational control functions are a regular component of the genetic composition of each cell. Important advances in the understanding of cellular differentiation would seem to lie in exploring systems of interacting regulatory genes involved in the programmed expression of well-defined characteristics.

The control mechanisms that are ultimately shown to regulate the process of differentiation in one type of cell may not be applicable to all cells. In both prokaryotes and eukaryotes, however, the observable changes accompanying cell development do reflect variations in the patterns of protein synthesis. It is possible that the complex genetic machinery of the eukaryote demands different or additional controls of protein synthesis (*3*). A full understanding of the regulatory mechanisms of differentiating prokaryotic cells, however, might provide a valid basis for extrapolation to cells of higher organisms. Most importantly, prokaryotic cells that undergo cellular differentiation are accessible to study by the established techniques of bacterial genetics. One such area in which significant contributions to the control of cell regulation have come is sporulation and germination in the genus *Bacillus* (*4*). In these studies it has become apparent that control at the level of selective messenger RNA production (transcription) represents the basic mechanism whereby the cell organizes its development (*5*, *6*). Clues as to the universality of this type of control mechanism may come from the exploration of other readily studied bacterial systems. It is in this context that we have attempted to develop a simple system, using the dimorphic *Caulobacter* bacteria, to study the regulation of cellular differentiation.

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Systems for Studying Bacterial Differentiation

Relatively few bacteria carry out a defined morphogenesis, other than cell division, during their normal life cycles. Still fewer bacterial species both differentiate and offer ease of genetic and biochemical manipulation. The criteria that make the use of bacteria reasonable as model systems for cell development include (i) a simple and well-defined differentiation pattern which can be studied in synchronized cell populations, (ii) the ability to grow the cells on defined media, permitting the correlation of biochemical events with morphological development, and (iii) the availability of mutants and a system for the exchange of genetic information in differentiating bacteria that offer

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possibility for study include *Bacillus*, *Streptomyces*, *Arthrobacter*, *Myxobacter*, and *Caulobacter*.

The spore-forming *Bacilli* fulfill all of these criteria and have commanded the greatest amount of attention; progress with this system has been extensively reviewed (*4*, *5*). These rod-shaped bacteria differentiate by forming heat-resistant and chemically resistent spores which can again become vegetative rods through the process of germination and outgrowth. Sequential changes occurring in a sporulating cell have been carefully defined and include, in addition to morphological alterations (*7*), the appearance of new enzymes (*8*), alterations in electron transport pathways (*9*), and the synthesis of spore-specific substances (*10*). Analysis of mutant organisms has demonstrated that genetic determinants govern these morphological and biochemical changes (*11*). The definition of the sequential expression of specific genes has been greatly facilitated because the outgrowth of cultures of spores to form vegetative cells proceeds synchronously (*5*). During outgrowth, the first gene product is new messenger RNA, followed by the formation of new proteins and enzymes (*5*, *12*). This control of sequential expression, which appears to be mediated at the level of transcription (*5*), has been demonstrated to be due to a new type of RNA polymerase which can be isolated from sporulating *Bacillus subtilis*. This polymerase differs from that of the vegetative cells in that the β-subunit of the core enzyme is altered along with an alteration in template specificity (*6*). Thus *Bacilli* offer important guidelines not only to the study of the control of differentiation of bacterial cells, but perhaps of eukaryotes as well.

Other groups of bacteria, such as *Streptomyces*, *Myxobacter*, and *Arthrobacter*, offer distinct advantages with respect to some of the above-mentioned criteria while they remain deficient in others. Of these groups, the *Streptomyces* can be grown on defined media and are particularly amenable to genetic study. Hopwood and Sermonti have extensively studied the genetics of these bacteria (*13*). The life cycle includes spore germination to form a vegetative cell that develops into a complex mycelium, then septates into aerial hyphae, and then produces single spores that can reinitiate the differentiation cycle. Only recently, however, have there been

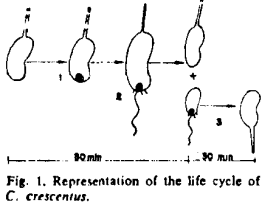

Fig. 1. Representation of the life cycle of *C. crescentus*.

studies on biochemical definition of the differentiation process by examination of mutants blocked in development (*13*). On the other hand, studies with *Myxobacter* have emphasized the isolation of morphological mutants in order to define macromolecular behavior during morphogenesis (*14*). This bacterium can be induced to change from the rod to microcyst form synchronously (*14*); but it cannot be grown on defined media, and a genetic system has not yet been developed. *Arthrobacter* presents an advantage distinct from *Streptomyces* or *Myxobacter* in that its simple differentiation process, which consists of a biochemically and morphologically well-defined sphere-rod transition, is under environmental control (*15*). The main drawback of this system, as in *Myxobacter*, is the absence of a genetic system; consequently no relation between genetic events and phenotypic response has as yet been demonstrated.

The purpose of this article is to demonstrate that *Caulobacter* fulfills the defined criteria and can therefore be used effectively to complement the *Bacillus* and other bacterial systems in providing further insight into control mechanisms operative during prokaryotic differentiation.

Properties of Caulobacter crescentus

Bacteria of the *Caulobacter* genus are aerobic, gram-negative organisms which, like *Pseudomonas*, metabolize hexoses via the Entner-Doudoroff pathway (*16*). A unique feature of *Caulobacter* bacteria is that they carry out obligatory well-defined morphogenic changes during their life cycles (*17*; Fig. 1). The *Caulobacter* are distinguishable morphologically in that they have a stalk at one pole of the cell (*18*). The wall of the stalk is continuous with both the lipopolysaccharide and mucopeptide layer of the cell wall (*19*, *20*). Under normal growth conditions a new stalked cell synthesizes an intracytoplasmic structure at the pole opposite the stalk (*16*, *19–21*), and this process represents the first step in a cycle of differentiation. It has been suggested that this structure is a membranous organelle that can be distinguished from cell mesosomes by the lack of continuity with the cytoplasmic membrane (*21*, *22*). The same type of membranous organelle is found at the stalked pole of the mature cell, as shown in Fig. 2, A and B. We are still unsure of the nature of this organelle, because electron microscopic analysis does not consistently reveal a discrete, membranous structure in the region of the polar organelle (*23*). The second occurrence in the differentiation process is the formation of a single flagellum and several pili also at the pole opposite the existing stalk (Fig. 1) (*19*, *20*). Binary fission yields a sessile stalked cell and a motile swarmer cell (Fig. 2C). The daughter swarmer cell must differentiate into a stalked cell before it is capable of cell division. This transition, the third step in the differentiation process, involves the deposition of new cell wall material at the point of attachment of the flagellum to the cell (*20*). The preexisting polar membrane structure now constitutes the interior of the newly forming stalk, where the flagellum can be found as an appendage at the tip of the stalk. Eventually the flagellum falls off and can be recovered in the medium (*24*). Autoradiographic experiments performed by Schmidt and Stanier (*20*) demonstrated that the site of stalk synthesis is restricted to the juncture of stalk and cell. Each step in the differentiation process, therefore, represents the synthesis of a specific structure (polar membrane, flagellum, pili, stalk) at a specific site in the cell, and at a specific time in the cell's life cycle.

The genome of *Caulobacter crescentus* is contained in approximately 3×10^{-8} microgram of DNA per cell (*25*), similar to *Escherichia coli*, a bacterium of approximately the same size. A unique feature of *Caulobacter* DNA is the existence of a satellite band that can be detected only in the stalked cell (Fig. 3). It is not known whether the satellite band is a piece of DNA with a lower guanine plus cytosine (G + C) content or one that has unusual bases. Chemical analysis of total

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Table 1. Characteristics of DNA bacteriophage φCbK and its host *Caulobacter crescentus* (*25*). C, cytosine; A, adenine; G, guanine; T, thymine; T_m, melting temperature.

Properties	φCbK	*C. crescentus*
Buoyant density in CsCl	1.53 g/cm³	
Size of head	195 by 64 nm	1 by 2 μm
Size of tail	275 nm	
DNA content	57 percent	3×10^{-4} μg/cell
DNA buoyant density in CsCl	1.721 g/cm³	1.718 g/cm³
DNA f_{gc}	63.55	
DNA T_m*	95°C	94°C
DNA base composition	C = 32.5; A = 17.4 G = 32.7; T = 17.4	C = 33.5; A = 16.5 G = 33.7; T = 16.4

* In saline sodium citrate.

stalked cell DNA labeled with ^{32}P revealed a G + C content of 67 percent, and all of the radioactive material migrated during electrophoresis as either adenine, cytosine, guanine, or thymine mononucleotides (*26*). The presence of the satellite band in stalked cells may therefore represent extra DNA with a low G + C content, which is either physically separated from the bulk of the nuclear DNA or replicated specifically during the stalked phase of the differentiation cycle.

Structural changes that occur during the *Caulobacter* life cycle include the synthesis of a new cell wall to form the stalk (*20*) and the temporally controlled appearance of a cell-wall receptor site for a DNA bacteriophage (*27*). In order to correlate such cellular alterations with biochemical events, D. Button, of this laboratory, is studying the chemical composition of the *C. crescentus* cell wall at different stages of the differentiation cycle. In general, the lipopolysaccharide (LPS) component of gram-negative cell walls contains a neutral sugar backbone structure coupled to a structure referred to as lipid A (*28*). The neutral sugar composition of *Caulobacter* LPS was analyzed in populations of swarmer cells and stalked cells and was found to be identical. The most abundant sugar is D-glucose; the remainder of this fraction, as determined by gas-liquid chromatography, includes D-glycero-D-mannoheptose, L-glycero-D-mannoheptose, D-galactose, D-mannose, and an unidentified polyhydroxy compound (*29*). The LPS also contains 2-keto-3-deoxyoctulosonate (KDO), but o-phosphorylethanolamine and glucosamine-phosphate, components of LPS in *E. coli* and *Salmonella*, cannot be detected (*29*). Further investigation of the lipid moiety showed that both β-hydroxymyristic acid and lauric acid are absent (*29*). We have concluded from these studies that, although *C. crescentus* is a gram-negative organism, it has no "lipid A" in its water-soluble LPS and that both *Caulobacter* cell forms have the same wall composition.



Fig. 2. Electron micrographs of *Caulobacter crescentus*: (A and B) Thin sections of osmium-fixed stalked cells of *Caulobacter* (*41*) (× 81,000; scale, 0.2 μm) [courtesy of Dr. P. G. Model]. A membranous structure (arrow) is evident as the base of the stalk in (A), but not in (B); (C) dividing cell negatively stained with phosphotungstate (× 22,000; scale, 0.2 μm) [courtesy of Dr. P. Atkinson].

Caulobacter Viruses

Seven distinct groups of lytic bacteriophages that specifically infect *Caulobacter* have been isolated and classified according to their nucleic acid content, host range, and serology (*30*). In addition, it has been reported that temperate bacteriophages could be induced from several *Caulobacter* strains (*31*). We have analyzed the physical characteristics of both a lytic RNA and DNA bacteriophage, and their relation to their dimorphic host, *C. crescentus*, during various steps in the infection process (*26, 27, 32*).

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The RNA bacteriophage φCb5 (*32*) is a small polyhedron 23 nanometers in diameter, whose sedimentation coefficient ($s_{20,w}$) is 70S, which is composed of a single molecule of single-stranded RNA and a protein coat assembled from two structural proteins. The phage appears to contain the genetic capacity to code for a coat protein subunit, a protein analogous to the R17 maturation protein, and an RNA polymerase not present in uninfected cells (*32*). Bacteriophage φCb5 differs from the *E. coli* RNA phages in that it specifically infects *Caulobacter*, contains histidine but lacks methionine in its coat protein, and is extremely salt-sensitive. During the infection process φCb5 specifically adsorbs to pili present on the *Caulobacter* swarmer cell (*33*). Susceptibility of the host cell to RNA phage infection during only a limited time in its life cycle poses unique problems in phage propagation, since at any given time in a log-phase culture susceptible swarmer cells compose approximately 15 percent of the population. Difficulties in obtaining liquid lysates of high titer were overcome by allowing small numbers of bacteria to grow for 12 to 16 hours in the presence of 10^{-8} phage per bacterium. Under these growth conditions phage-sensitive swarmer cells are continually being produced by the resistant stalked cell population (*32*).

The DNA bacteriophage φCbK was isolated in our laboratory and shown to be among the largest known bacterial viruses (*26*). The phage, composed of protein and DNA, has an elongated polygonal head 195 by 64 nm and a flexible tail 275 nm in length (Fig. 4). The properties of the double-stranded DNA from φCbK are similar to those of the host cell with respect to buoyant density, melting point, and G+C content (Table 1). We analyzed the mode of infection of *C. crescentus* with this bacteriophage and determined that the phage specifically infects the swarmer cell or a predivisional form with swarmer cell characteristics (*27*). Initial evidence that the phage required a specific host property for productive infection came from one-step growth experiments with synchronized populations of swarmer and stalked cells. A longer eclipse period was observed with the stalked cell than with the swarmer cell, suggesting that time was required for the formation of the sensitive cell type (*27*). The hypothesis that adsorption to stalked cells is reversible was confirmed by observations with wild-type cells and two classes of φCbK-resistant mutants; the phage is not eclipsed and remains viable (Table 2). Adsorption followed by DNA injection occurs only in the swarmer and predivisional cell forms. It appears, therefore, that the synthesis of a new phage attachment site that permits phage DNA injection illustrates another facet of differentiation in the *Caulobacter* life cycle. This phage receptor site apparently resides in the wall LPS (*29*). Sonically treated extracts of pure populations of swarmer and stalked cells were prepared and extracted with phenol (Table 3; *29*). The LPS was recovered in the aqueous phase, and protein was quantitatively recovered in the phenol phase. Bacteriophage φCbK was specifically inactivated by the LPS fraction of the swarmer cell, but not by the same fraction from the stalked cells. Therefore a structural moiety present in the swarmer cell appears to be absent or altered in the stalked cell. Antibodies were prepared against the LPS from wild-type cells and from the bacterial mutant "φ resistant I" which fails to adsorb phage (see Table 2). An antigen observed in the wild-type cell was not present in the phage-resistant mutant. The total LPS composition of "φ resistant I" was also determined, and it was found to be identical to wild type, with the exception that there was a 20-fold reduction in the amount of D-glucose present in this fraction (*29*).

Table 2. The ability of bacteriophage φCbK to absorb, infect, and inject its DNA into swarmer and stalked cells of wild-type and mutant host strains (*27*).

Cell type	Phage adsorption (%)	Phage eclipsed (%)	Phage ^{32}P DNA recovered in host cell (count/min)
Wild-type swarmer	90	80	558
Wild-type stalked	80	1	19
φ-Resistant I swarmer	0	4	96
φ-Resistant I stalked	0	3	62
φ-Resistant II swarmer	90		64
φ-Resistant II stalked	95		69

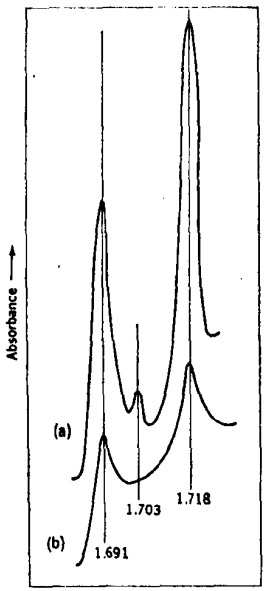

Fig. 3. Microdensitometer tracings of DNA in CsCl after 20 hours of centrifugation at 44,770 rev/min. (A) DNA isolated from stalked *Caulobacter* cells which banded at $\rho = 1.718$ g/cm^3 and $\rho = 1.703$ g/cm^3. (B) DNA isolated from swarmer *Caulobacter* cells which banded at $\rho = 1.718$ g/cm^3. *Clostridium perfringens* DNA ($\rho = 1.691$ g/cm^3) was used as a reference marker [from (*26*)].

Cell Synchrony

The study of an organized sequence of events requires populations of cells doing the same things at the same time. Swarmer and stalked cells can be separated by differential centrifugation (*17*), since the stalked cell offers greater frictional resistance in a centrifugal field than the swarmer cell does. At stationary phase, 95 percent of the cells are stalked. Remaining swarmer cells, as well as cell clusters (rosettes), are removed by low-speed centrifugation. Synchronous growth is

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initiated by incubating the stalked cells in relatively dilute cultures at 30°C. We have observed microscopically that by 80 minutes approximately 50 percent of the total cell population consists of motile swarmer cells. Simultaneous measurement of viable count showed that cell division occurs at 80 minutes (Fig. 5A).

Swarmer populations can be obtained when pure stalked cells go through one cell division, and 50 percent of the resulting population exists as swarmer cells. These are centrifuged at low speed, and synchronous growth is initiated by incubation at 30°C in fresh broth. Since we had determined the time required for stalked cells to divide and measured the time required for cell division starting with swarmer cells we deduced the interval required for the transition of swarmer cells to stalked cells (Fig. 5B). Swarmer cell populations, observed by microscopy and viable count measurements, divide 110 minutes after the start of growth. Swarmer cells develop stalks, therefore, in 30 minutes (*34*). Stove and Stanier followed the growth cycle of single clones of *C. crescentus* in microculture and showed that cell transition occurred in 25 to 30 minutes (*17*).

Table 3. Effect of extracts of the host cell wall on infectivity of bacteriophage φCbK measured as plaque-forming units (PFU). Bacteriophage φCbK was mixed with a known quantity of wall extract and its infectivity was determined. The control PFU was determined by infection of unfractionated cells (*29*).

Source of wall extract	φCbK infectivity (PFU)
Control	346
Stalked cells Aqueous phase (10 mg/ml)	320
Stalked cells Phenol phase (10 mg/ml)	368
Swarmer cells Aqueous phase (5.2 mg/ml)	7
Swarmer cells Phenol phase (5.2 mg/ml)	334

We have developed a technique for obtaining relatively pure populations of dividing cells based on the differences in buoyant density of cells in a mixed population. Cells in the log phase of growth in medium containing 0.2 percent glucose are layered on an equal volume of medium supplemented with 20 percent glucose in a simple glass centrifuge tube. Centrifugation for 3 minutes at 37,000*g* suffices to concentrate the dividing cells at the interface between the two glucose solutions. This technique obviates the need for using stationary phase cells in the synchronization process and thus reduces the metabolic fluctuations resulting from adaption of stationary phase cultures.

Most synchronization techniques yield populations of cells growing and dividing with a good deal less than perfect synchrony. The maximum concerted increase in cell number that we have observed is 80 percent (see Fig. 5). This degree of asynchrony, however, still permits us to observe the fundamental changes occurring in the *Caulobacter* life cycle. Synchronized cells, beginning with swarmers, were treated with ^{14}C-labeled amino acids at 10-minute intervals during the 110-minute differentiation cycle, and the newly synthesized proteins were analyzed by electrophoresis in polyacrylamide gels in the presence of sodium dodecyl sulfate (*35*). Thirty minutes into the cycle, at the time when swarmer cells have grown stalks and are no longer motile, a sharp change in the pattern of protein bands became apparent in stained gels and in autoradiograms (*24*). These differences most certainly represent changes in enzyme content and regulatory proteins, as well as in structural proteins. We are now attempting to correlate some of the bands with structural proteins peculiar to the morphogenesis observed in *Caulobacter*. Two of the major bands have been tentatively identified as flagella or pili protein (or both) by comparing gels of either whole swarmer or stalked cells with fractionated swarmer cells and flagella and pili purified from the medium of cultures in the log phase of growth (*24*).

We could have inferred from a gross study of *Caulobacter* morphogenesis that new structural proteins are produced during differentiation, and from a knowledge of molecular genetics we recognize that new enzymes are probably synthesized or activated (or both) for the purpose of building new cell structures. Cataloging the appearance or disappearance of proteins during differentiation permits us to follow cell changes. There still remains the crucial matter of defining the regulation of these changes at the molecular level. The focus of these studies on the defined development of *Caulobacter* is now directed toward an understanding of the specific inducers and regulators of differentiation.

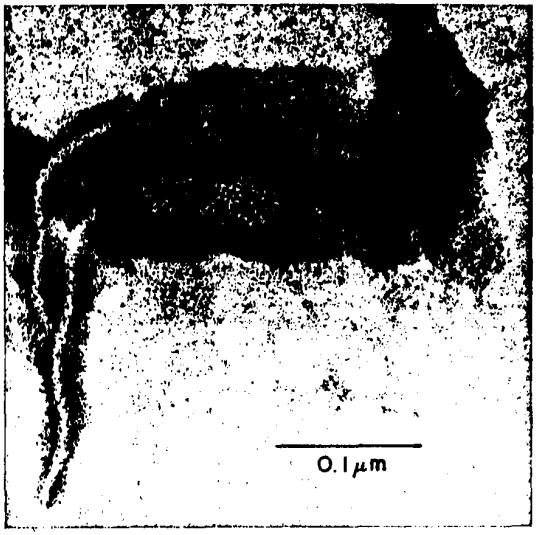

Fig. 4. Electron micrograph of bacteriophage φCbK negatively stained with uranyl acetate (× 440,000) [courtesy of Dr. A. K. Kleinschmidt].

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Assay for Differentiation in Caulobacter

Study of *Caulobacter* mutants that are blocked at various stages of morphogenesis is a promising approach toward understanding the relation between gene function and the concerted series of events that eventually result in differentiation. In order easily to identify differentiation mutants, we developed a technique with which to monitor the transition between *Caulobacter* cell types in synchronized cultures.

The differentiation assay is based on the fact that RNA and DNA bacteriophage receptor sites are synthesized at specific times in the cell's life cycle. RNA bacteriophage φCb5, labeled with radioactive adenine, is retained on Millipore filters only if combined with cells (*36*; Fig. 6A). Since φCb5 specifically combines with the pili present on swarmer cells (*33*), the swarmer cell loses the ability to retain labeled phage as it changes into a stalked cell and pili are lost (*34*). The time required for the transition of swarmer to stalked cells was deduced to be 30 minutes (see above). This time span was confirmed by the phage adsorption assay, because the binding of radioactive phage stopped at 30 minutes, along with the loss of cell motility observed microscopically. Thus the RNA phage adsorption assay reflects morphogenic changes occurring in *Caulobacter* and can be used to monitor simply and accurately the transition in synchronized cultures of swarmer to stalked cells.

A complementary phage assay is provided by measuring the appearance of the cell LPS receptor site specific for the injection of phage φCbK DNA labeled with [^{3}H]adenine (Fig. 6B). When phage was added to synchronized stalked cells, an increase in the ability to support injection of phage DNA occurred between 50 and 60 minutes. The structural changes occurring in the wall of a stalked cell preparing for cell division can thus be observed and defined by a simple radiochemical assay (*27*).

Caulobacter Mutants

Temperature-sensitive (Ts) mutants are of special value because they permit analysis of genes which control essential cellular functions, such as DNA, RNA, and protein syntheses, cell division, and cell wall synthesis (*37*). The transition of swarmer to stalk cells in *Caulobacter* is obligatory for survival, and therefore the processes of differentiation are essential functions of the cell. Approximately 70 Ts mutants of *Caulobacter crescentus* which are unable to form colonies at 37°C but grow normally at 23°C have been isolated (*38*). These mutants were isolated from cultures treated with the mutagen nitrosoguanidine and, in large part, are of independent origin. The isolation procedure, which excludes a selective step, was based on the method described by Hartwell for obtaining Ts mutants of yeast (*39*). The *Caulobacter* mutants vary in their ability to survive after exposure to the restrictive temperature, since they were never exposed to 37°C during the isolation steps. Mutants which are defective in DNA synthesis, RNA synthesis, and cell division, have been identified. These mutants can be used to study the relation between macromolecular synthesis and the expression of the differentiated state in synchronously growing cultures. Of primary interest, however, are mutants defective in the various aspects of the differentiation process. A mutant that we refer to as TsM continues to increase in mass after a shift to 37°C, but the viable count remains constant for 4 hours after the shift. During this period the cells, which can be observed by phase contrast microscopy, grow erratically, rarely divide, and form long, branched cells. DNA, RNA, and protein syntheses continue at near normal rates. In order to determine whether these cells are expressing functions connected with differentiation at 37°C, we measured their

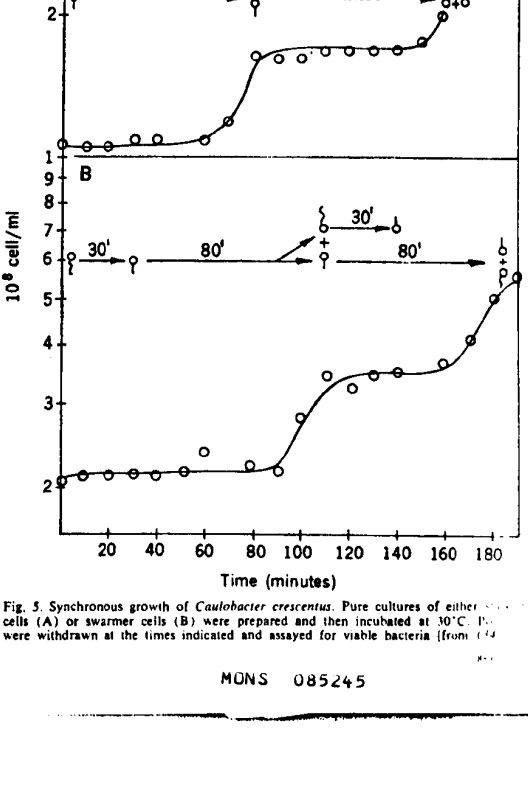

Fig. 5. Synchronous growth of *Caulobacter crescentus*. Pure cultures of either [illegible] cells (A) or swarmer cells (B) were prepared and then incubated at 30°C. [illegible] were withdrawn at the times indicated and assayed for viable bacteria [from [illegible]

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Table 4. Interspecific genetic exchange. The selective medium was minimal, str for Cross I; and minimal, pro for Cross II. CFU, colony-forming units.

Strains or crosses	Complete media (CFU)	Selective media (CFU)	Recombination or spontaneous mutation frequency
	Cross I		
CB15Str^{R}Bio$^+$	3.8×10^{10}	1.0×10^{8}	$1–2 \times 10^{-4}$
CV115Str^{R}Bio$^-$	8.6×10^{10}	0	0
CB15Str^{R}Bio$^+$ × CV115Str^{R}Bio$^-$	1.1×10^{11}	1.9×10^{6}	1.7×10^{-5}
	Cross II		
CV163Str^{R}Pro$^-$Vit$^-$ yellow	1.0×10^{9}	0	0
CB13Str^{R}Pro$^+$Vit$^+$ white	5.8×10^{9}	4.2×10^{3}	1×10^{-6}
CV163Str^{R}Pro$^-$Vit$^-$ yellow × CB13Str^{R}Pro$^+$Vit$^+$ white	1.0×10^{10}	1.5×10^{6}	1.5×10^{-4}

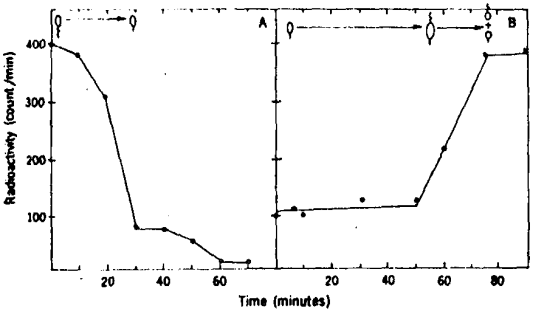

Fig. 6. Technique to monitor the transition between *Caulobacter* cell types in synchronous cultures. (A) Selective adsorption of [^{3}H]RNA bacteriophage φCb5 [data from (*14*)]. (B) Selective injection of bacteriophage φCbK [^{3}H]DNA.

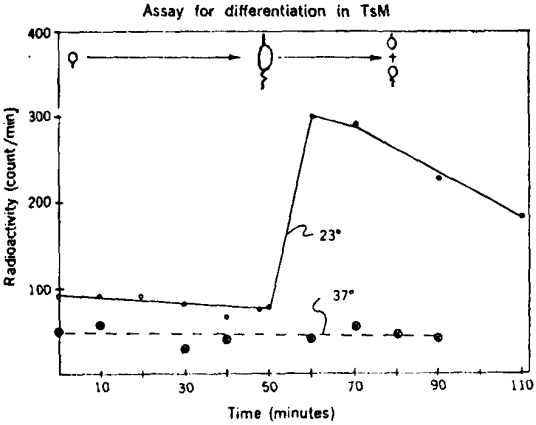

Fig. 7. Injection of bacteriophage φCbK [^{3}H]DNA during synchronous growth of TsM at 23° and 37°C.

ability to support bacteriophage φCbK DNA injection (Fig. 7). Synchronized populations of TsM were grown at both 23° and 37°C and challenged with radioactive phage at 10-minute intervals. The phage was unable to inject DNA at 37°C, whereas normal injection occurred at 23°C. With wild-type strains, phage DNA injection occurs normally at both temperatures. It appears, therefore, that TsM is unable to synthesize a phage receptor site at the restricted temperature, in addition to being unable to divide normally. Since normal *Caulobacter* cell division is dependent on cellular differentiation and one of the measurable determinants of the differentiation process is lacking at the restrictive temperature, TsM appears to represent a class of mutants blocked at some stage of differentiation.

Essential for the study of molecular events in differentiation is the demonstration of a mechanism of genetic exchange in the *Caulobacter* genus. Such a demonstration would permit us (i) to show that the apparent Ts mutants are a result of genetic alterations, (ii) to evaluate and localize different gene loci responsible for normal or aberrant differentiation phenotypes, and (iii) to ultimately map the gene loci controlling the cyclic differentiation process in *Caulobacter*. We have succeeded in mixing two populations of cells with different drug resistance and growth requirements and have obtained phenotypes showing markers from both parents (*40*; Table 4). Crossing was accomplished by mixing the cultures on the surface of solid, glucose-supplemented medium. Presumed mating types were grown together on solid media for 48 hours, eluted from the "mating plate," resuspended in distilled water, and then diluted and plated onto selective media. In one such cross, streptomycin-sensitive biotin-prototrophic (Str^{S}Bio$^+$) and Str^{R}Bio$^-$ genotypes were mixed, and Str^{R}Bio$^+$ progeny were selected. The exchange of genetic information occurred with an apparent frequency of 1.7×10^{-5}, while the frequency of spontaneous mutation of Str^{S}Bio$^+$ to Str^{R}Bio$^+$ was 2×10^{-8} and that of Str^{R}Bio$^-$ to Str^{R}Bio$^+$ could not be detected. In another cross, a strain resistant to streptomycin (Str^R), which cannot use proline (Pro$^-$) as a carbon source, requires vitamins (Vit$^-$) and is yellow (Str^{R}Pro$^-$Vit$^-$) was mixed with a strain sensitive to streptomycin (Str^S) which was proline and vitamin pro-

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trophic (Pro^-Vit^+) and white (Str^R-Pro^-Vit^+). "Recombinants" (Pro^+-Str^R) were selected for ability to utilize proline as a carbon source in the presence of *Streptomycin* medium containing a source of vitamins, and occurred with a frequency of 1.5×10^{-6}, while the spontaneous rate of mutation of Pro^+Str^R to Pro^+Str^R was 1×10^{-8}. In addition, the "recombinant," progeny of this cross were analyzed for the distribution of unselected vitamin (Vit) utilization and pigmentation (yellow or white) markers. Out of a total of 391 colonies examined the genotypes were distributed as follows: 23, $Pro^+Vit^-Str^R$, yellow; 334, Pro^+-Vit^+Str^R, yellow; 30, $Pro^+Vit^+Str^R$, white; indicating either a strong linkage between Pro and Vit markers or the presence of a partial diploid.

It appears that the genetic transfer may be mediated by cell conjugation, because filtrates or cell extracts prepared by sonic treatment, substituted reciprocally for each of the parents in these crosses, fail to yield any recombinant progeny. Although the mechanism of genetic exchange is still under investigation, it seems apparent that cell contact is required. It is possible that the *Caulobacter* swarmer cell, analogous to the piliated male *E. coli* cell, transfers genetic information.

Conclusions

The foregoing studies are intended to define a differentiation process and to permit genetic access to the mechanisms that control this process. In order to elucidate the basic mechanisms whereby a cell dictates its own defined morphogenic changes, we have found it helpful to study an organism that can be manipulated both biochemically and genetically. We have attempted to develop the studies initiated by Poindexter, Stove and Stanier, and Schmidt and Stanier (*16, 17, 20*) with the *Caulobacter* genus so that these bacteria can serve as a model system for prokaryotic differentiation.

The *Caulobacter* life cycle, defined in synchronously growing cultures, includes a sequential series of morphological changes that occur at specific times in the cycle and at specific locations in the cell. Six distinct cellular characteristics, which are peculiar to these bacteria, have been defined and include (i) the synthesis of a polar organelle which may be membranous (*21–23*), (ii) a satellite DNA in the stalked cell (*26*), (iii) pili to which RNA bacteriophage specifically adsorb (*16, 33*), (iv) a single polar flagellum (*17*), (v) a lipopolysaccharide phage receptor site (*27*), and (vi) new cell wall material at the flagellated pole of the cell giving rise to a stalk (*19, 20*). Cell division, essential for the viability of the organism, is dependent on the irreversible differentiation of a flagellated swarmer cell to a mature stalked cell.

The specific features of the *Caulobacter* system which make it a system of choice for studies of the control of sequential events resulting in cellular differentiation can be summarized as follows.

1) Cell populations can be synchronized, and homogeneous populations at each stage in the differentiation cycle can thus be obtained.

2) A specific technique has been developed whereby the progress of the differentiation cycle can be accurately measured by adsorption of labeled RNA phage or penetration of labeled phage DNA into specific cell forms. This technique can be used to select for mutants blocked in the various stages of morphogenesis.

3) Temperature-sensitive mutants of *Caulobacter* that are restricted in macromolecular synthesis and development at elevated temperatures have been isolated.

4) Genetic exchange in the *Caulobacter* genus has been demonstrated and is now being defined.

Two questions related to control processes can now readily be approached experimentally. (i) Is the temporal progression of events occurring during bacterial differentiation controlled by regulator gene products? (ii) Is the differentiation cycle like a biosynthetic pathway where one event must follow another? The availability of temperature-sensitive mutants blocked at various stages of development permits access to both questions. An interesting feature of the differentiation cycle is that the polar organelle may represent a special segregated unit which is operative in the control of the differentiation process. Perhaps the sequential morphogenic changes exhibited by *Caulobacter* are dependent on the initial synthesis of this organelle. Because the ultimate expression of cell changes are dependent on selective protein synthesis, specific messenger RNA production—either from DNA present in an organelle or from the chromosome—may prove to be a controlling factor in cell differentiation. We have begun studies with RNA polymerase purified from *Caulobacter crescentus* to determine whether cell factors or alterations in the enzyme structure serve to change the specificity of transcription during the cell cycle. Control of sequential cell changes at the level of transcription has long been postulated and has recently been substantiated in the case of *Bacillus* sporulation (*6*). The *Caulobacter* bacteria now present another system in which direct analysis of these control mechanisms is feasible.

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Women in Academia

A study of the hiring decision in departments of physical science.

Arie Y. Lewin and Linda Duchan

Recently, there has been a great deal of concern over the role of women in today's society. There have been numerous allegations of discrimination against women in many areas, especially in certain occupational and professional fields. A significant part of the controversy has involved alleged bias against women in academia.

Charges of discrimination against women in universities have been made both formally and informally. In the former category, Gruchow (*1*) cites 43 colleges and universities which have been charged with discriminatory policies against women under laws which call for the cancellation of federal contracts held by the schools. Bikman (*2*) investigated charges of discrimination at Columbia University and noted that women are admitted to the school and trained, but not hired; the few positions they do obtain are at levels relatively lower than those offered to men. Harvard University has been particularly singled out by women's rights groups for discriminatory practices (*3*). Informal or subjective evidence has also been widely publicized. Alexander (*4*) obtained evaluations of bias against women by female faculty members at Johns Hopkins University: they described the discrimination they experienced as being in the form of "unseen pressure." There are numerous other anecdotal accounts highlighting the alleged existence of discrimination against female faculty members (*5*).

Perhaps the most conspicuous discrimination is evident from salary differentials between men and women. Alexander reports that salaries for women employed in higher education are generally lower (*5*). Bayer and Astin concluded that salary disparities between men and women exist in academia across all ranks, work settings, and fields of specialization (*6*). Furthermore, a recent report in the field of chemistry indicates that "obvious differences in the earnings of men and women chemists" exist; these differences seem to carry over time, rather than being clustered in the earlier years of employment (*7*).

Even more illuminating are the statistics which show that women are being discriminated against in the awarding of research grants. In the Senior Postdoctoral Fellowship competition recently held by the National Science Foundation, 14 of the 395 applicants were women. Fifty-four grants were awarded—none went to women. In a pilot study of grants and awards given by the National Science Foundation to university researchers in a physical science discipline for the years 1964–1968, women were only awarded less than 0.03 percent of the grants although they comprise 5 to 8 percent of the scientists in the discipline. Furthermore, the mean dollar value of the awards received by women was smaller than that of those received by men. For the same discipline, however, no similar discrimination was observed in the awarding of grants by the National Institutes of Health (*8*). The American Chemical Society does not have a much better record: although no national award prohibits female applicants, the only one which has gone to a woman (with the exception of the Garvan Medal which is restricted to women) is an award in high school chemistry teaching (*9*). Similarly, out of the 827 Sloan Research Fellowships that have been awarded by the Alfred P. Sloan Foundation over the past 16 years, only one or two women were among the recipients.

Other inequalities facing women in academia have also been charged. Lack of job opportunities and fewer advancements are often cited (*10*). Other research has indicated that psychological and social barriers exist for women, especially in science. Specifically, White (*11*) concluded that it is difficult for women to attain "challenging interaction" with male colleagues, that men are hesitant about sponsoring women as protégés, and that women are denied the informal signs of recognition and belonging.

Perhaps the key to the problem may be found in the analysis of cultural influences. The dominant attitudes and norms concerning the role of the woman in our society are perceived to be incompatible with the concept of a successful professional woman. In the socialization process, children are conditioned as to which traits and professions

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are "masculine" and which are "feminine." Simmons alludes to this indoctrination that starts in and continues throughout the school years (*12*). Bird stresses some specific attitudes internalized by girls in our society, including the notion that it is wrong for them to excel over boys in school, and the traditional idea that "the real business of life is attracting a desirable husband" (*5*). Rossi highlights the different concerns parents express for their adolescent children—educational goals of their sons and dating patterns of their daughters (*13*). Graham points out the ambivalences in young women who are socialized into feeling that they must devote themselves to the affirmation of their femininity, rather than to preparation for a professional career. She further maintains that "women's low expectations for themselves so infect the society that both men and women refuse to think of women as generally likely to occupy important posts" (*14*). Finally, the attitudes concerning particular professions may deter women from training for them. The National Manpower Council reports that some qualified young women will not prepare for science and engineering, perceiving that these are "men's professions" (*15*). Similarly, Bernard concludes that the apparent discrimination against women is a result of the discrepancy between the supply of qualified women and the demand for them. She asserts that the demand for qualified women exists, but due to sociocultural influences working against women preparing for "masculine" careers, the supply is not sufficient (*16*).

Statement of the Problem

Previous studies have reported on bias against women already employed in academia; to our knowledge, however, there have been no studies of the discrimination which may occur at the time of hiring. Our study, therefore, focuses on possible discrimination at the time of the hiring decision.

The study involved the chairmen of all (179) graduate departments of a physical science discipline in American colleges and universities (*17*). Four different versions of a standardized résumé were constructed. In all cases, the applicant was married with two children and had held the title of assistant professor for 4 years. The undergraduate education was also identical for each applicant. The implied differential quality of graduate education—doctoral and postdoctoral training—was achieved by varying the universities attended; the better the implied graduate education, the better known the institutions involved. Research output was varied through the number of publications, the journals in which they appeared, and invited addresses at symposiums. The number and size of grants and awards were similarly treated. Finally, the higher the quality of the applicant, the greater was the prestige of the currently employing institution.

Two versions of an "average" quality résumé were used; they were identical except that one belonged to a female applicant, the other to a male. The third, an "excellent" résumé, was improved in all categories, and belonged to a woman. The final, a "superior" résumé, showed a female applicant outstanding in all areas. Our hypotheses were: (i) If bias exists after a woman has already been hired, it should also be evident at the time of the hiring decision, particularly when a woman is in competition with a male applicant. (ii) Specifically, department chairmen would exhibit preference for a male applicant over a female applicant with identical qualifications. (iii) Department chairmen would be less biased when considering an average male applicant with a superior female applicant.

The chairmen were randomly assigned to four groups, corresponding to the four different résumés. Each chairman was sent a résumé and a questionnaire on which to record his reactions to the résumé. The chairman was instructed to assume that there was an opening in his department for an associate professor, a position which could ultimately lead to tenure. He was further told to assume that the résumé was sent from a highly regarded colleague and that, as chairman, he was in a position to act on it.

The questionnaire was divided into two basic parts. The first section called for specific reactions to the résumé and information on factors involved in the hiring decision. The chairmen were asked to state their overall impressions of and inclination to hire the applicant. They were further asked to evaluate the applicant regarding the factors of age, educational background, and ability to attract research grants. Finally, they were asked if they would have responded differently had the résumé not come from a trusted colleague. Each chairman recorded his responses to the questions on a Likert-type five point scale which ranged from excellent to unsatisfactory. The second section of the questionnaire was aimed at getting background information for further classification of the data. It consisted of similarly scaled items concerning the chairman and makeup of the department faculty, as well as open-ended questions concerning the criteria and practices generally involved in hiring decisions.

Analysis of Results

One hundred and eleven (62 percent) of the questionnaires were returned, evenly distributed among the four groups in the survey population. Significance of differential ratings was measured by means of chi-square tests. The data was first submitted to an overall analysis comparing responses to each of the four résumés, and then further classified and analyzed under six headings: size of the full-time faculty; presence of women on the faculty; age of the chairman; length of time as chairman; geographical location of the school; and relative quality ranking of the institution. The analysis of results, however, is based on the responses to the average (male and female) and excellent (female) résumés only. This is because the superior female applicant had postdoctoral training in England, which was rated somewhat lower than the postdoctoral training of the excellent applicant. Therefore, for purposes of comparison, the excellent applicant is considered superior (and thus labeled) in the following discussion. The results, although not statistically significant, showed definite trends that confirm our hypotheses that discrimination against women does exist at the time of the hiring decision.

Comparison of responses to the varying quality résumés yielded results which showed a definite tendency for the chairmen to prefer an average male over an average female, but to recognize a superior woman. Specifically, of the two identical average applicants, the male was consistently rated higher on educational background and was more often considered positively for hiring. It is noteworthy that the identical résumés of male and female applicants conveyed the same general impression, yet on

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these more specific questions concerning education and hiring, the male was evaluated higher. As expected, the opposite tendency was found when the average male was compared to the superior woman. These results also held for the question concerning changes in responses if the résumé had come unsolicited—the chairmen were more inclined to change their minds on the average woman as opposed to the average man, but less likely to alter their responses on the superior woman in comparison with the average man.

The survey population was classified by geographical areas to test the belief that overall conservatism (viewed politically) might be a variable in the discrimination against women. Consequently, the chairmen were divided into two categories: east and west (considered to be more liberal) and middle west and south (considered to be more conservative). Chairmen from schools located in the east and west tended to evaluate the average male applicant higher than they did his female counterpart; no such distinction was evident in the responses of the chairmen from the middle west and south. Furthermore, although both groups of chairmen rated the superior woman higher than the average man, the differences were less pronounced in the responses of the chairmen from the eastern and western schools. Although not statistically significant, these findings indicate that political conservatism as we defined it is not an explanatory factor.

Departments in the survey population were ranked as to quality and classified into groups above and below the median (*18*). Analysis of the responses of chairmen from departments ranked above the median shows that, in the comparison of the identical average résumés, the male applicant was rated higher than the woman with the same qualifications. No difference was found in the responses from the chairmen from schools ranked below the median in quality. The superior woman was generally evaluated higher by respondents, but more so by the chairman from the lower-ranked schools. This may be because schools in this category receive fewer higher-quality applications. Somewhat surprisingly, the chairmen from the high-quality schools were more inclined to alter their responses concerning the superior woman, which implies that a recommendation from a trusted colleague is a more important factor to them than it is to chairmen from lower-ranked schools.

In order to determine whether the age of the chairmen might affect their responses, the respondents were divided into two age categories. In the comparison of the identical résumés, the younger group, surprisingly, evaluated the average male applicant higher than they did his female counterpart. The two applicants, however, were evaluated essentially equally by the older chairmen. When comparing the superior female applicant and the average male, the younger chairmen were more likely to recognize the female's qualifications and were more inclined to hire her. On the other hand, the younger chairmen also indicated a greater tendency to change their responses had the résumé come unsolicited.

It was also hypothesized that responses categorized in terms of the tenure of the respondent as chairman of his department would follow the same pattern as the data classified by age; the analysis confirmed this expectation. Those chairmen who were relatively new in their positions were more inclined to be biased against the average female than were those who had been department heads for a relatively longer period of time. In the comparison of the evaluations of the superior woman's résumé with that of the average man, it was found that both newer and older chairmen were more generally impressed with the superior female and evaluated her education higher. However, the younger chairmen showed a greater willingness to hire the female applicant, but placed greater weight on the issue of whether or not the application had come from a trusted colleague.

Two other variables were also subject to statistical tests—the size of the present department faculty, and whether or not it already included any female faculty members. The analysis of the responses showed no significant trends, which suggests that the size or the previous hiring of women are not factors in bias against hiring women.

Unsolicited Comments

Surprisingly, a significant number of unsolicited comments were returned with the questionnaires which had accompanied the résumés belonging to women. The chairmen seemed to be concerned with what the applicant's husband would do if she were hired. They questioned what the applicant would do with her children and what her personality was like. Further, and perhaps most important, concern was expressed over her compatibility with the existing staff and over potential difficulty in fitting in with the present makeup of the department.

Analysis of these comments leads us to the conclusion that women faculty in the physical sciences may be evaluated on the basis of different criteria than are males when they compete for positions in academia. These criteria appear to be based on personal values and attitudes reflecting widely held socially accepted beliefs regarding the role of a woman in the family and the perceived difficulties regarding her compatibility with male colleagues.

Conclusion

Although our study was conducted in only one physical science discipline, and although most of the individual tests did not yield statistically significant differences, the data consistently yielded a trend in the direction of the existence of discrimination against women in academia. This leads us to the tentative conclusion that when two equally qualified applicants are being considered for an academic position, a male would be chosen over a female. However, a woman with clearly superior qualifications, in competition with an average man, is likely to be recognized. The bias seems to hold especially for higher-quality schools, in departments with younger and newer chairmen, and for chairmen from schools located in the eastern and western parts of the country.

The underutilization of potentially qualified women in science represents lost opportunities to society and to science in terms of the overall goal of advancing science. To the extent that the bias reflects strongly held cultural norms, change will come slowly. However, structural changes within the institutions that employ and train women scientists may be achieved sooner through the active intervention of outside agencies, for example, the enforcement by the Department of Health, Education, and Welfare of the Equal Opportunities Act in colleges and universities (*19*). In addition, such federal grant-awarding agencies

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National Science Foundation and the National Institutes of Health could be given a mandate to make a special effort to identify and award grants and other forms of recognition to deserving women scientists.

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NEWS AND COMMENT

Vermont: A Small State Faces Up to a Dilemma over Development

Facing a period of substantial growth and intense development in the 1970's, we have the opportunity and hence the obligation to utilize the newer understanding of the science of ecology, and the improved knowledge concerning effective government organization, to provide a uniform, comprehensive approach by state government to assure development without destruction.—From the 1970 report of the Vermont Governor's Commission on the Environment.

Vermont is a relatively lightly populated, largely rural enclave lying between the Washington–New York–Boston megalopolis and its northern outpost, Montreal. The Green Mountains provide Vermont with ski runs in the winter and a scenic backdrop the rest of the year, and have formed a classic barrier to development. Now, however, geoeconomic pressures have grown intense and Vermonters are striving—in the mildly overheated prose of that key report—for "development without destruction."

Vermont's dilemma is exemplified in its experience with environmental and land use legislation. The state legislature, particularly in a prolific 1969-70 session, enacted a body of law that conservationists around the nation have applauded as being as enlightened and comprehensive as any state's. Two pieces of landmark legislation were a land use and development law (Act 250),* which, in effect, imposes state planning authority on major development projects, and a law creating a system of effluent charges, which is patterned roughly on water management practices in the Ruhr in Germany. In addition, the legislature in recent years has passed other legislation prohibiting open-dump burning, controlling shoreline and mobile-home development, and tightening regulations on pesticide use. The esthetics of the landscape were also considered in laws regulating billboards and junkyards. A major effort has been made to reorganize state agencies to deal more effectively with the new legislation. But despite this outpouring of legislation, critics are finding fault on the score of implementation, charging the legislature and state officials with underenforcement through underfinancing of technical and policing staff and through "permissive" administration.

For the casual visitor to Vermont, it is a bit difficult to understand the sense of urgency over environmental matters, since the state seems remarkably free of the population pressures and the kinds of pollution afflicting Vermont's neighbors to the south. The statistics appear to bear out this impression: Massachusetts, with a total area of 7800 square miles, has a population of 5.7 million, while Vermont, with 9300 square miles, has a population of 444,000, according to the 1970 census. Although Vermont's population rose by only about 55,000 in the 1960's, the increase was nearly five times the increase in the previous 10 years. With the rate of industrialization and the building of "second homes" for out-of-staters bounding upward in the 1960's, it appeared to Vermonters that the 1960's wrought a transformation in the landscape and the economy, and that change was running out of control.

The forces powering growth in Vermont are hardly unique. The extension of the federal interstate highway system and the building of turnpikes in Massachusetts and New York State began the process, and the building of Interstate 91 up the Connecticut River Valley opened southern and eastern Vermont to easy access from the south. Increasing population and affluence in the Northeast, as well as the skiing vogue, have given Vermont an all-season tourist industry and have caused the "second home" to proliferate. Industrial growth is centered in Burlington, the state's largest city in the northwest on Lake Champlain. By the end of the decade, the Burlington metropolitan area had a population of about 100,000, which is small even by the standards of New England cities, but it seemed to have reached critical size and to be exercising strong drawing power on the GE's and IBM's.

The results of development have also been predictable. With expectations primed by federal programs, Vermonters in the 1960's demanded expanded social services and investment in public education—both schools and higher education—also climbed steadily. The boom in the construction of second

* A good description of the land use bill is to be found in a chapter on Vermont in *Managing the Environment: Nine States Look for an Answer*, a report of a study sponsored by the Ford Foundation and directed by Elizabeth Haskell, a fellow of the Smithsonian Institution's Woodrow Wilson International Center in Washington, D.C.

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homes exerted new pressure for building roads and providing water and sewer services. As a consequence, the cost of state and local government rose rapidly and property taxes have skyrocketed. Higher taxes and rising land prices have accelerated trends in Vermont agriculture toward larger farming units and the breakup of the traditional pattern of farming by smallholders. Not surprisingly, frictions have developed between native Vermonters, who are anxious to see jobs created that will allow them and their children to continue to live in the state, and the "new" Vermonters—owners of second homes, retirees, industry executives, and many "counter-culture" colonists—who are likely to have different views and interests. A spirit of "Vermont for Vermonters" has begun to grow.

Wide Acceptance of Laws

Despite such friction, there seems to have been wide public acceptance of the environmental legislation in 1970. Most observers agree that this was based on concern about a "Florida style" real estate boom in the southern part of the state. Land on hill farms and mountain slopes was being sold off in acre and half-acre lots. Many of the new settlers seemed to be gripped by a chalet complex, seeking to build as high as possible to enjoy the view, and at the same time marring it. Bulldozing of roads and building sites created erosion problems in the thin and rocky soil, and apprehension grew about danger to water resources through damage to the holding capabilities of land at high elevations.

Awareness that big land development companies were moving in to exploit the market for second homes spurred state officials and the legislature to action. Vermont towns had a long tradition of local autonomy and meagre experience with major development problems. Planning and zoning laws were rudimentary in most towns, and observers say that this very lack of competence to cope with the problem made a state land use law more acceptable.

Impetus for the state law came from a report of a Commission on Environmental Control appointed by Governor Deane C. Davis. The governor, a Republican, had won reelection without putting much stress on environmental matters, but the land boom sharpened his concern. He put the authority of his office behind the land use and environmental legislation in 1970, and it passed with surprisingly little opposition. Environmental consciousness had been heightened in Vermont in the late 1960's by revelations of the extent of pollution in Lake Champlain on the state's western border. The International Paper Company plant at Ticonderoga, New York, had been one of the plants to pioneer the making of paper from wood pulp and had been dumping the resulting sludge into the lake since the turn of the century. Sewage from municipalities and from farms and summer homes along the shores had added to pollution, and oil spills from storage tanks and from oil barges plying the lake had reached serious proportions. Davis himself has noted that the fact that 1970 was the "year of environment" helped the cause.

Act 250 provides for the creation of a statewide land use plan to control major development of all types, with environmental as well as social and economic criteria being applied. The law's chief mechanism is its requirement of state permits for housing subdivisions of ten or more lots, or for commercial or industrial developments of more than 10 acres. Smaller developments are left to the discretion of local government. In cases where towns do not have adequate planning and zoning apparatus, however, state permits are required for developments larger than 1 acre.

Act 250 is administered by a statewide Environmental Board and nine district commissions, all with members appointed by the governor. There are three categories of objections under which district commissions may deny permits or impose restrictions. These are (i) effects on water and air pollution, (ii) impact on highways, school systems, and municipal services, and (iii) effects on natural beauty, historical sites, or irreplaceable natural areas. Applicants denied permits for development may appeal to the state Board and, ultimately, to the state's highest court.

The Environmental Board is in the process of producing a detailed planning document that is to govern land use throughout the state and decisively influence the future pattern of urbanization and industrialization.

One of the interesting features of Act 250 is that it incorporates many of the features of what Vermonters call "the 2500-foot bill." For some years there has been a campaign in progress for legislation to protect the extremely fragile ecology of the state's uplands and mountains. A University of Vermont biology professor, Hubert Vogelman, led the fight for the bill and at last won an important point when Act 250 classed any construction above 2500 feet for commercial, industrial, or residential purposes as "development" subject to the law.

Vermont's experience with its effluent charges program, popularly called the "pay to pollute" law, illustrates how conflict can develop after environmental legislation is put on the books. The state's lakes, rivers, and streams are classified for use according to quality, and the new bill, in effect, prohibits a discharge of wastes that reduce the quality of the "receiving water." The law provided that, after 1 July 1971, no person could discharge wastes, treated or untreated, into state waters without obtaining a permit. If officials found that proposed discharge would reduce the quality of waters, the permit was to be denied. The "pay to pollute" nickname of the law stems from the provision for temporary pollution permits under certain conditions. The temporary permits are authorized when applicants are in the process of adding approved pollution abatement facilities or attempting in good faith to find ways to create such facilities. During the period of the temporary permit, the payment of periodic pollution charges are required, in accordance with rates established by the Board.

No "License to Pollute"

The intent of the law, its advocates insist, is not to sell a "license to pollute," but to put pressure on polluters while, at the same time, giving them a reasonable time to meet pollution standards.

In practice, there has been a wrangle over how charges should be levied—on an annual basis or according to units of pollution. The issue has apparently been decided in favor of the latter method.

A much more politically troublesome debate developed, however, on the issue of how high to set effluent charges. Most Vermonters apparently assumed the law was aimed at industrial polluters and not at municipalities or individuals. It soon became clear that the law was blind in this respect. Federal funds for sewage treatment plants that towns had counted on did not materialize. And heavy pressure from industry, municipalities, and individual users moved the legislature to postpone the effective date of the law for a year.

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although polluters will have to take effective corrective measures before that time or face retroactive charges.

The framers of Vermont's new land use law intended to prod local governments to deal more effectively with planning and zoning problems. But the effect of the new law has been to shift power over development to the state government. Many Vermonters originally viewed the land use law as defending the state against land speculators and "summer people." Now they find that the law restricts the local land developer, as well as the big land company. In the same way, effluent charges hit not only the industrial polluter, but the farmer with a faulty septic tank. The signs are that many people are having second thoughts.

By American standards, Vermont has had a virtually static population and homogeneous society. Vermonters are slow to accept outsiders, and their feelings about individual rights and local self-determination make them view the state government's burgeoning role with suspicion. Old patterns of life are breaking up, and the process is painful to many Vermonters who feel that the outsiders, rather than themselves, are profiting from the changes. Vermonters are proud of their state's natural beauty, but, in practical terms, they resent seeing the cost of land and of decent housing soar out of reach, and they worry about the lack of jobs that would make it possible for their children to remain in the state. A sign of the times is a fairly widespread tendency to blame Act 250 for the current housing shortage in the state.

All of this has implications for environmentalists and is not lost on politicians. Serious consideration, for example, is being given to a system of preferential taxation, under which lower taxes would be levied on land kept in farming use than on land sold to developers. And, although environmentalists cannot be described as politically isolated, one state official who is generally sympathetic to the environmental cause may have shown which way the wind is blowing when he observed, "The trouble with your silver-haired, starry-eyed environmentalists is that they have only a small following and little clout."

The state's environmental activists seem to be facing up to the problem. At its last annual meeting, the Vermont Natural Resources Council, the leading statewide environmental organization, featured a panel on the operation of Act 250, and panelists dealt at some length with housing problems. The council is also the recipient of a $120,000 grant from the Ford Foundation to be used in a statewide program to inform the public on the purposes of Act 250 and to get the public more deeply involved in implementation of the act.

At this point, the question of how the new laws will operate in practice is still open and only time will tell whether the critics are correct in predicting that the laws will be administered permissively.

Vermonters, of course, should have no illusions that they will be left alone to settle their problems. Vermont exercises as strong an attraction in its region as an oasis in the Sahara. And as the state develops industrially, its interdependence with neighboring states will grow. For example, decisions must soon be made on a proposed East-West highway from Calais, Maine, to Amsterdam, New York, and its routing through Vermont will have a powerful effect on development. The public is more aware and perhaps more wary of new power plant construction in the state. A debate is now in progress over licensing of the first nuclear power plant constructed in the state, and Vermont faces early decisions on the location of other major power plants within its borders. Big power development as a local, state, and regional issue will be discussed in a second article on Vermont.

—JOHN WALSH

High Energy Physics: In-Group Talks Funds, Possible Closeouts

The Joint Committee on Atomic Energy (JCAE), the congressional overseer of the Atomic Energy Commission, has been putting pressure on the AEC Division of Research, and on the administrators of the six AEC-sponsored accelerator laboratories, to come to grips with one of their most sensitive problems: which accelerators should be shut down if money for high energy physics stays constant or dwindles again in fiscal year 1973.

Decisions on this issue will be made within the AEC and then by the Office of Management and Budget (OMB) by the time the Nixon Administration submits its fiscal 1973 budget to Congress next January. And when Congress reviews the Administration budget next spring, the JCAE will pass, among other things, on the fate of the big accelerators.*

In preparation for this process, the JCAE report on the fiscal 1972 budget made an unusual request—itself a masterpiece of ambiguity--which has set those who administer the six laboratories and hence direct high energy physics research in this country trooping to and from Washington like pilgrims flocking to Mecca.

The Committee asked the AEC to report back by 31 December 1971, with a "priority listing" of which accelerators should "be kept operating should future money be less than the minimum. . . ." But then it added a few sentences of praise for the six laboratories. It called them "highly deserving" of support "to keep them operating at their maximum level of productivity." It praised the "fundamental knowledge" that high energy physics provides to other fields, and it expressed the hope that the United States "remain in the forefront of this field."

No one knows for certain quite what the mention of minimum funding combined with lavish praise means; one school of thought is that JCAE is warning the labs to expect more cutbacks. However, it is clear that the second part of the passage, the praise of the six laboratories, has sent happy visions of sugar plums dancing through the

* The six accelerators sponsored by the AEC are: National Accelerator Laboratory, Batavia, Ill.; Brookhaven National Laboratory, Upton, N.Y.; Stanford Linear Accelerator, Palo Alto, Calif.; Argonne National Laboratory, Argonne, Ill.; Bevatron, Lawrence Berkeley Laboratory, Berkeley, Calif.; and the Cambridge Electron Accelerator, Cambridge, Mass.

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heads of some lab administrators. Among them, Wolfgang K. H. Panofsky, Director of the Stanford Linear Accelerator, reads the passage as an "exhortation" for the scientists to lobby for more money. "I interpret it positively to mean that 'if you push hard, we'll help you.'" And one AEC Division of Research staffer involved in compiling the requested report said that he personally views the passage as giving the physicists a chance to "make a strong case for high energy physics."

NAL Poses Problem

The other side of the coin, literally, is the possibility that fiscal 1973 will turn out to be the hardest year yet for the six accelerators. For then, the world's biggest accelerator, the 200-Gev National Accelerator Laboratory (NAL) will have begun routine operations. NAL says it will need $25 million in operating costs alone in fiscal 1973, not to mention capital equipment and construction costs. By 1975 or thereafter, when NAL is fully operational, it plans an operating budget of some $60 million—or, for comparison, half the total operating budget of $116 million for all AEC high energy physics for fiscal 1972.

At present, after years of declining budgets for high energy physics, there is no separate provision for obtaining money for NAL's operation. Unless Congress or the Administration is willing to deal out an overall increase in the high energy budget for NAL, the other five accelerators will have to pay for NAL from their own budgets. As one official said, the JCAE request merely gives the physicists a chance to decide among themselves "whether to cut their throats from left to right or right to left."

Most of the accelerator administrators contacted by *Science*—roughly half of those who have been in Washington recently—were unwilling to be named since the issue of throat-cutting is a sensitive one. When promised anonymity, they spoke frankly about the future. Although they seem divided on some issues, they appear to have reached a certain consensus on what to do if the worst hits, i.e., if the funding situation becomes bleak enough so that one of the accelerators would be forced to close down altogether.

Because it is so big, a glamorous focus of international scientific attention, and exciting experimentally (as well as being one of the best-managed large physics labs in the country) NAL, the consensus is, should be affected as little as possible by the vicissitudes of funding which will plague everybody else. At the moment, the world's highest energy accelerator is the 76-Gev machine in Serpukhov, U.S.S.R. At full energy NAL will operate at 200 Gev and above; it will attain at least ten times the energy level of the next biggest United States accelerator, the 33-Gev machine at Brookhaven National Laboratory. NAL, then, is considered relatively immune.

Like NAL, the Stanford Linear Accelerator Center (SLAC) occupies a unique international position as the highest energy electron accelerator. It runs at 22 Gev and draws 20 AEC user groups from all over the country; the next highest energy machines include a 2-Gev linear electron accelerator in Orsay, France, a 2-Gev installation in Kharkov, U.S.S.R., and the 7.5-Gev electron synchrotron, known as DESY, in Hamburg, West Germany. Most laboratory administrators agree that SLAC is a national asset. In addition, SLAC has taken a share of the funding cuts. For example, it requires all staff to take 1 day off per month without pay, which stretches the payroll about 5 percent further. For fiscal 1973 and beyond it would be difficult to foresee any drastic reduction or closeout at SLAC.

The consensus that Brookhaven should remain is only slightly less unanimous. One or two of the administrators polled by *Science* argued that the 33-Gev alternating gradient synchrotron is as regional a facility as some of the smaller accelerators, since a fair percentage of the user groups there come from Columbia University and the East Coast area generally. But most administrators agreed that Brookhaven is an international asset almost as much as SLAC, since its current research is considered competitive with ongoing research at the 28-Gev proton synchrotron at CERN, in Switzerland. In addition, they argue, Brookhaven has already taken its share of the punches, having closed down its 3-Gev Cosmotron in 1966, and abandoned a major modification program last year at a cost of about 200 staff members. Current staff is estimated at about 1020.

This leaves the Cambridge Electron Accelerator (CEA), the 12.5-Gev zero gradient synchrotron (ZGS) at Argonne National Laboratory, and the 6.2-Gev Bevatron at the Lawrence Berkeley Laboratory in Berkeley, Calif. All of these have been variously described as "useful, but older" machines, and "doing very good work, but not at the frontier" and as "local" facilities, associated primarily with one geographic area or one or two institutions.

One criterion the administrators apply to these lower energy, older machines is the extent to which they have become diversified by moving into new areas of research where they can claim to be unique. In this regard the Berkeley Bevetron has been changing, and is the most promising candidate to survive. (It announced last week that it can speed nitrogen nuclei to a total level of 36 Gev, an example of heavy ion acceleration that could lead to applications in biology and medicine, including cancer research).

CEA and Argonne

The situation at the Cambridge accelerator—whose budget is a tiny $2.15 million compared to the others—is more ambiguous. By valiantly preparing to run a projected 2- to 3-year series of colliding beam experiments, and by cutting out all other work, CEA is making a dramatic attempt at diversification. In addition, CEA has agreed to let the AEC reclassify it as an "experiment" instead of as an "accelerator"—in an attempt to save CEA from the financial axe. But on the other hand, there are indications that the Office of Management and Budget may be far less agreeable to exempting CEA, as well as the other accelerators. In 1970, for example, the $2 million Princeton-Penn Particle Accelerator (PPA), already cut back by the AEC, was abruptly declared "obsolescent" as part of an Administration-wide economy move and funds ran out on 30 June 1971 (see *Science*, 2 July 1971). A similar fate could await CEA.

A number of laboratory administrators believe that the AEC Division of Research, which oversees the six accelerators, will make a strong pitch to keep the CEA open at least another 2 or 3 years, and try to arrange an option to renew its lease on life then. They are willing to confirm—although hesitant to comment further—that the two chief rivals for extinction are the Argonne and Berkeley accelerators. However, there is quite a lot of agreement that diversification is a plus on the balance sheet, and that Berkeley is carrying out a rather conspicuous program of

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diversification. None of those contacted were willing to deny, and some actually confirmed, that, by this reasoning, Argonne could be the least likely to survive.

The Argonne spokesmen contacted by *Science*, however, reply that the two lower energy accelerators—Berkeley and CEA—will be, so to speak, at the bottom of the list. They say that their lab is doing very good and useful work, and that their gigantic neighbor, NAL, should not be a factor in a decision concerning Argonne. In addition, one said, "There's always talk like that but I don't take it seriously . . . we're doing better than most accelerators."

At this point in time, the AEC Division of Research is still hoping to satisfy the JCAE request without naming candidates for possible shutdown. They hope to reply "positively" with estimates of "good" levels of funding to keep all six accelerator laboratories viable. They will probably agree with the lab administrators' desire for a step-function increase in funds for fiscal 1973; Panofsky, for example, mentions a need for 15 percent more money, which would mean about 17 million more dollars for high energy physics.

But in a wider context, the JCAE request has set the laboratories' and AEC's officials buzzing over the issue of future funding. But it also points up the weakness of the current system for deciding research priorities. Two additional accelerators, one at Cornell and one at Stanford, are not even being formally considered in this process because they are funded by the National Science Foundation and not reviewed by JCAE. In addition, the $17 million increase which the physicists glibly mention as "adequate" for 1973 is more than four times all AEC funding for computer research and mathematics, and 30 percent more than total support of medium energy physics. Finally, there is a distinct chance that the entire AEC budget may be cut in fiscal 1973, in which case trade-offs will have to be made between reactor development, nuclear weapons, and high energy physics. The Joint Committee has handed the physicists—faced with high unemployment rates and years of declining budgets—a much-longed-for opportunity for special pleading on their own behalf. But it has not opened the way to any broader discussion of priorities in science funding.

—DEBORAH SHAPLEY

PCB's: Leaks of Toxic Substances Raises Issue of Effects, Regulation

Sharp criticism of federal regulatory agencies has followed in the wake of a bizarre contamination of poultry feed marketed in a part of southeastern United States. The controversy reached full steam on 18 August when the Food and Drug Administration (FDA) meekly acknowledged that a shipment of some 60,000 eggs contaminated with toxic polychlorinated biphenyls (PCB's) escaped federal inspectors and apparently got into the stomachs of Washington, D.C., area residents. The announcement came on the heels of FDA's seizure of another batch of PCB-contaminated eggs (75,000) on 13 August—almost a month after the initial discovery that PCB's had gotten into fish meal eaten by millions of chickens in ten southeastern states.

Besides possibly threatening human health through direct contamination, PCB's are considered a long-term potential hazard to the environment. In the United States, PCB's are produced solely as a heat transfer mechanism and an insulating fluid, particularly in cooling systems and big power transformers. In response to mounting evidence that PCB's are a potential hazard, the single U.S. manufacturer of PCB's, Monsanto Chemical Company, in order to restrict their release into the environment has limited the chemicals' uses to sealed systems. In the past, Monsanto widely produced PCB's as an additive in such things as sealants, rubber, paints, plastics, adhesives, printing ink, and insecticides.

Infiltration of PCB's into the environment occurs mainly in three ways:

- From accidental leaks in industrial equipment, such as heat-transfer systems;
- Through the weathering or friction wearing of the many materials that have PCB's as an ingredient. (Since they are fire resistant, PCB's usually remain intact even as a waste product.)
- Through interaction with food products in their uses as an ingredient in substances like paint and plastic.

Before the fish meal contamination incident, however, PCB's were virtually unknown except to a few scientists and some professional environmentalists. Critics have now become quite vocal in warning about the threat of PCB's, and, more important, they have expressed doubt about the ability and desire of federal agencies to effectively shield the consumer and the environment from the potential long-term dangers of thousands of synthetic chemicals.

The controversy is fueled by a basic disagreement among the participants as to the precise threat PCB's pose to human health and the environment. Federal officials, while acknowledging a problem, do not feel that PCB's are a serious enough hazard to justify major governmental action. Research on composition of PCB's and toxicity is very incomplete. The actual composition of their product is known only by Monsanto. Present evidence indicates that severe injury or death from short-term exposure to PCB's is unlikely. Like so many other environmental hazards, the potential PCB danger is long-term, low-level exposure, perhaps making it difficult for "crisis prone" regulatory agencies to respond in a big way now. Except for isolated accidents, federal officials contend, PCB's in their present application will not get into the environment or the food chain in harmful amounts.

From an industrial standpoint, PCB is considered very valuable, mainly for its incredible persistence as a chemical, being capable of withstanding temperatures of up to 1600°F (870°C). Like DDT—another, related chlorinated hydrocarbon—PCB's are fat-soluble but do not dissolve in water. For these reasons, industry holds that PCB's are in fact "safety" chemicals that are needed in populated areas to reduce the chances of accidental explosions, especially in big power transformers. It is this very persistence of PCB's that makes them a

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NEWS & NOTES

• **ARMSTRONG STEPS INTO ACADEME:** Neil A. Armstrong, the first man to set foot on the moon, will assume the post of full professor at the University of Cincinnati's College of Engineering on 1 October. Armstrong has been NASA's deputy associate for aeronautics since July 1970. In his new job, he will be closely associated with Paul Hergett, head of the university's observatory, who is also a former consultant to the U.S. space program.

• **FDA X-RAY GUIDE:** The Food and Drug Administration has issued a booklet to guide health personnel in the prudent use of diagnostic x-rays. The publication places particular emphasis on factors that doctors untrained in radiology should consider when referring patients for x-rays. *X-Ray Examinations . . . A Guide to Good Practice* was jointly produced by the FDA Bureau of Radiological Health and the American College of Radiology. It is available for 35¢ from the Superintendent of Documents, Government Printing Office, Washington, D.C. 20402.

• **NEW SOCIETY:** Scientists interested in a multidisciplinary approach to health and disease formed a group called the Society for Environmental Geochemistry and Health on 1 July. The object of the society is to further knowledge about the effects of geochemical environment on both plant and animal life. President of the group is Delbert D. Hemphill of the University of Missouri's Environmental Health Center.

• **STANDARD REFERENCE JOURNAL:** The National Bureau of Standards has decided on a new procedure to provide more effective dissemination of the evaluated property data and critical reviews produced by the National Standard Reference Data System (NSRDS). The American Institute of Physics (AIP) and the American Chemical Society (ACS) have agreed to publish jointly the NSRDS output, and the material will appear in the *Journal of Physical and Chemical Reference Data*, to appear quarterly, beginning early next year. Offprints from the journal and book-length compilations will also be published through the new arrangement. Subscriptions will be obtainable through the AIP or the ACS.

great concern to environmentalists. PCB's have been detected throughout the world in fish, birds, waterways, and humans. In a study released by the Environmental Protection Agency (EPA), PCB's were detected in samples of human fatty tissue with concentrations as high as 250 parts per million (ppm).

Despite Monsanto's assurances that the present uses of PCB's are safe, critics say the recent fish meal incident vividly illustrates that use of PCB's in any form is too risky to permit. Leading the fight against PCB's are consumer advocate Ralph Nader and New York Democratic Congressman William Fitts Ryan. They argue that, even if Monsanto can stop PCB's from getting into the environment, products containing them are in wide use and will continue to disperse PCB's in the environment in harmful amounts. The government should act now, Nader asserts, to ascertain where PCB's have been used before they cause serious damage to the environment and get into the food chain.

Besides the fish meal incident, there have been two other hazardous leaks of PCB's in the past 2 years. One of these accidents, in Japan in 1968, resulted in 300 people developing a severe skin disease and in babies showing symptoms of chlorobiphenyl poisoning. Indeed, the preliminary research, though incomplete, makes Nader's anxiety about PCB's potential danger quite plausible. University of California research ecologist Robert Risebrough, one of the foremost authorities on PCB's, says that while the effects of PCB's on humans are undetermined, the contaminants in PCB's are "among the most poisonous compounds known." Tests of PCB's by the Environmental Protection Agency's Florida laboratory revealed the chemical killed one-half of the test population of pink shrimp exposed to 1 ppm and similarly killed samples of white shrimp. These tests, EPA's Thomas Duke told *Science*, indicate that PCB's are a "potential threat to the environment." Monsanto, however, has reported that its own tests have shown "no adverse effects" to rats or other laboratory species fed PCB's in amounts up to 100 ppm. This conflicts with tests repeated by others that have linked repeated dosages of PCB's to liver damage in mice and lower fertility in birds.

In fact, the recent contamination at the North Carolina plant that manufactures fish meal was detected only after the hatchability of eggs from chickens fed the meal began to diminish alarmingly.

The PCB's were traced to a leak in a pipe in the cooling system at the fish meal plant. Although the leak began in late April, PCB's were allowed to drip into the fish meal until the defect was discovered in mid-July. In the intervening period of 2½ months, approximately 16,000 tons of fish meal had been distributed to more than 60 companies in ten states. One of the purchasers of the fish meal, Holly Farms, the nation's largest poultry producer, voluntarily slaughtered 77,000 fowl after discovering PCB's in amounts above those deemed safe, reportedly as high as 40 ppm. In the meantime, the U.S. Department of Agriculture undertook its own tests of poultry that had been fed the fish meal. On 29 July, USDA said that its tests turned up no evidence that chickens given the contaminated meal were unsafe. The chickens in the ten-state area, USDA reported, were "wholesome," and the consumer could continue to eat chickens "with confidence." But this did not explain why chickens at Holly Farms contained unsafe amounts of PCB's. In response to this apparent disparity, Harry Mussman, director of USDA's laboratory service division, told *Science* that the department "surmised" that Holly Farms got an "extremely contaminated" batch of fish meal while the other producers did not.

Contaminated Eggs Seized

Two weeks later, on 13 August, FDA, under prodding from associates of Nader, revealed that it had seized over 75,000 eggs because tests had detected excessive amounts of PCB's in eggs from chickens that had consumed the contaminated fish meal. On 16 August—a month after the leak was detected—USDA took its turn and "detained" more than 50,000 pounds of frozen-egg products in which the level of PCB's was high. When FDA seized the 75,000 contaminated eggs, a spokesman said they knew of no incident in which contaminated eggs had reached consumers but could offer no assurances that this had not happened. Unwilling to stop there, associates of Nader and Ryan conducted their own ad hoc investigation and, to their dismay, discovered that contaminated eggs had reached the consumer. The FDA, on 18 August, confirmed that a shipment of 60,000 contaminated eggs had reached the retail market and apparently had been consumed in the Wash-

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ington, D.C., area after this information had been released by Ryan's and Nader's associates.

The disclosure prompted both Ryan and Nader to blast the government's handling of the entire fish meal incident as one of the most flagrant examples of misregulation by federal agencies.

In denying that it has lagged on PCB's the FDA points out that since January it has been conducting studies to determine the toxicity of PCB's and their effect on humans. An FDA spokesman said that the test results should be known "within a year." For its part, EPA is doing some research at two field laboratories. Since the beginning of the year USDA, according to Mussman, has conducted a routine surveillance of poultry for PCB's and other related chemicals.

Perhaps some of the difficulties in regulating PCB's can be attributed to the confusing, overlapping jurisdictions of federal agencies. For example, FDA has jurisdiction over PCB's in whole shell eggs and in fish meal, USDA has control over cracked eggs and their products—such as mayonnaise—and poultry, and EPA is in charge of PCB's once they get into the air and water.

In the recent incident, FDA had to recall the contaminated fish meal and locate where it was sold and then, in turn, supply USDA with a list of the fish meal purchasers, so that USDA could check the affected chickens. When associates of Nader raised the possibility of contamination of egg products, they had to go to FDA for testing whole eggs and to USDA for cracked eggs and frozen poultry products.

Such a confusing arrangement makes it almost inevitable that some of the contaminated eggs would slip through this network. As for the eggs that did reach the consumer, an FDA spokesman says there is no immediate danger since "any potential health hazard would come from continued consumption of PCB's over an extended period of time."

PCB's intrusion into the environment is difficult to regulate because of a lack of federal laws and because no one is quite sure how much PCB's have been produced. EPA and FDA officials point out that at present they have no legal authority to halt Monsanto's present uses of PCB's. Last year PCB's in pesticides were banned by USDA's pesticide regulation division, now a part of EPA. According to a spokesman, FDA has, in the past, indicated to Monsanto that it would not allow PCB's to be used in food as an additive.

It is virtually impossible to determine the exact amount of PCB's already present in the environment and where the chemical might be concentrated because no one, except Monsanto, knows what amounts of PCB's have been made. A National Academy of Science panel estimates that, in 1968 alone, 5 billion grams of PCB's were produced in the United States, in addition to those made by PCB's manufacturers in Europe, the Soviet Union, and Japan. Monsanto has refused repeatedly to make available, even to government officials, production and sales figures for PCB's because it regards this information as a "trade secret." Monsanto is backed up by its industrial counterparts and by a law that permits a company in certain situations to withhold information that might seriously jeopardize its competitive advantage. A National Academy of Sciences panel, in disagreeing with Monsanto's refusal to release the important figures, noted "it is not only competitive concerns alone that determine the less than candid posture assumed by industry concerning production figures." A bill now before Congress would give federal agencies the authority to get production figures from companies manufacturing hazardous chemicals.

In a further effort to cut down on PCB's released into the environment, Monsanto recently built a special incineration unit at Sauget, Illinois, to destroy waste PCB's. It has also told government officials that it will not sell PCB's for use in power transformers and cooling systems that will be used near foodstuffs. In the fish meal plant, Monsanto reportedly claims that it was misled by the name—East Coast Terminal, Inc.—into believing that the PCB's sold for cooling purposes would not be used near food products. Monsanto now refuses to comment on PCB's for publication. The reason, a company spokesman told *Science*, is that there are "many investigations under way" of Monsanto and PCB's, and the company does not want "misinterpretations" about Monsanto's production of PCB's.

Associates of Nader and Ryan admit, in part, that their strong criticism of the federal handling of PCB's via the fish meal incident stems from their disagreement with the way federal agencies respond to environmental problems. They dislike what they feel is the slow response of federal agencies to potential hazards like PCB's. The

EPA Moves on Refuse Act

The Environmental Protection Agency (EPA) has told its regional offices to notify laggard industries that they face possible legal action if they fail to explain why they haven't applied for waterway discharge permits under the recently resuscitated Refuse Act of 1899.

The deadline for filing permit applications was 1 July. Detailed follow-up data are required by 1 October. But so far, says EPA administrator William Ruckelshaus, applications received by the Army Corps of Engineers (which administers the program) have leveled off at about 18,000, although at least 40,000 firms are supposed to be affected by the permit program.

The EPA is still conducting the industrial waste studies on which to base the standards it had planned to have ready in early July. Officials explain that this task has taken longer than expected because it was found that laying down specific limitations on effluents for each of the 20 designated industrial categories would be too complex and rigid. Instead, the agency is working on more flexible "guidelines," which will aid regional EPA directors in interpreting federal-state water quality standards and in advising companies on how to conform.

Meanwhile, Ruckelshaus has urged regional offices to proceed with enforcement actions, using the knowledge and equipment already available to them.

Ruckelshaus told *Science* recently that press reports to the effect that EPA was backing down on its standards were wrong. Nevertheless, he said, some industries "read these stories and think we're easing off. Then we have to start all over rattling sabres at them."—C.H.

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government is geared more to reacting to a crisis, they hold, than to taking the type of preventive action necessary to head off disasters before they occur. Federal officials point out that, as far as they are concerned, PCB's are a rather new problem that they do not consider as "top priority." Since PCB's had not in the past caused any major catastrophes that can be likened even to the fish meal incident, most government officials were not aware of PCB's until recently.

The FDA regards the fish meal contamination as an accident that is not likely to occur again. Blaming FDA for the fish meal incident is "like asking a police department to be responsible every time a crime occurs," one FDA official told *Science*. But critics claim that federal agencies have had sufficient warning about PCB's. In the last year, they say, Campbell Soup Company, a New Jersey firm, discovered soups contaminated with PCB's, and certain Ohio dairymen lost their licenses to sell milk when PCB's found their way into silage consumed by their cows.

Might Enter Food Chain

Risebrough points out that in "our throwaway society" PCB's, because of their past wide use, will eventually become wastes and thereby enter the environment. It is believed that the PCB's could make their way to the sea through drainage systems, spillages, and the like, and eventually into the food chain.

No tolerance levels have been determined, Risebrough says, for human food supplies. As an interim guideline, FDA set PCB limits of 5 ppm as safe for human consumption of poultry, and USDA pronounced that chickens below this level in the fish meal incident were "wholesome." Critics claim the 5 ppm guideline is "unfounded" and "arbitrary." "Five parts per million is just a number," Risebrough told *Science*. "I'm sure FDA pulled it out of the air."

FDA officials reply that the interim guideline is based on the "best scientific information available." The guideline was set for the fish meal contamination, FDA says, and can provide an adequate "margin of safety" for the "short-term exposure expected to occur."

"It's not complete," an FDA official told *Science*. "But you got to go on what you have. We can't be held accountable for every goddam chemical!"

—JOE PICHIRALLO

APPOINTMENTS

Edward J. Bloustein, president, Bennington College, to president, Rutgers University. . . . **Stanley S. Bergen, Jr.**, senior vice president for medical and professional affairs, New York City Health and Hospital Corporation, to president, College of Medicine and Dentistry of New Jersey. . . . **Richard J. Grosh**, dean, Schools of Engineering, Purdue University, to president, Rensselaer Polytechnic Institute. . . . **John D. Isaacs**, professor of oceanography, Scripps Institution of Oceanography, University of California, San Diego, appointed director, university-wide Institute of Marine Resources. . . . **J. Thomas Grayston**, dean, School of Public Health and Community Medicine, University of Washington, to head, Health Sciences Center and vice president for Health Affairs at the university. . . . **Thomas P. Logan**, assistant professor of dentistry, Meharry Medical College, to dean, School of Dentistry at the college. . . . **William C. Wesley**, professor of education, University of Evansville, to dean, School of Education at the university. . . . **John C. Guyon**, chairman, chemistry department, University of Missouri, Columbia, to chairman, chemistry department, Memphis State University. . . . **Thomas H. Kirschbaum**, professor of obstetrics and gynecology, University of California, Los Angeles, to chairman, obstetrics, gynecology and reproductive biology department, Michigan State University. . . . At Iowa State University, **Robert F. Brodsky**, manager of systems test and deployment, Aero Jet-General Corporation, California, to chairman, aerospace engineering department and **Richard G. Barnes**, professor of physics, to chairman, physics department. . . . **James A. Luker**, acting dean, College of Engineering, Syracuse University, appointed dean of the college. . . . **Alfred A. H. Keil**, head, ocean engineering department, M.I.T., to dean, School of Engineering at M.I.T. . . . **Charles A. Berg**, associate professor of mechanical engineering, M.I.T., to chairman, mechanical engineering department, University of Pittsburgh. . . . **Max R. Culver**, vice president for academic affairs, Kansas Wesleyan University, to chairman, sociology department, Capital University. . . . **Stanley Deutsch**, professor of anesthesiology, University of Chicago Pritzker School of Medicine, to head, anesthesiology department, University of Oklahoma Medical Center. . . . **Guido H. Daub**, acting chairman, chemistry department, University of New Mexico, appointed chairman of the department. . . . **Willis E. Sibley**, professor of anthropology, Washington State University, to chairman, anthropology department, Cleveland State University. . . . **Robert M. Boynton**, director, Center for Visual Science, University of Rochester, to chairman, psychology department at the university. . . . **John W. Schermerhorn**, dean health sciences division, Northeastern University, to dean, School of Allied Health Professions, University of Texas Southwestern Medical School. . . . **John W. Warner**, Undersecretary of the Navy, appointed director, Office of Ocean Affairs, Department of Defense. . . . **Warwick E. Kerr**, professor of genetics, University of São Paulo, Brazil, appointed president, board of directors, Brazilian Association for the Advancement of Science. . . . **Paul D. Hurd, Jr.**, former professor of entomology, University of California, Berkeley, to chairman, entomology department, Smithsonian Institution. . . . **Hubert L. Rosomoff**, chairman, neurological surgery department, Albert Einstein College of Medicine, to chairman, neurological surgery department, University of Miami School of Medicine. . . . **David A. Prince**, acting chairman, neurology department, Stanford University School of Medicine, elevated to chairman of the department. . . . **Paul D. Sturkie**, professor of physiology, Rutgers University, to chairman, environmental physiology department, College of Agriculture and Environmental Science, at the university. . . . **Robert Rothstein**, professor of biology, Agricultural and Technical College, State University of New York, Farmingdale, to chairman, medical laboratory technology department at the college. . . . **Joseph F. Traub**, professor of computer science, University of Washington, to head, computer science department, Carnegie-Mellon University.

Erratum: In a footnote to the review of M. M. Gerassimov's *The Face Finder* (173, 712 (1971)), the misspelling of Julius Kollman's name is erroneously attributed to a bibliography (1962) by W. M. Krogman. The error occurs in a different authority's work. Kollmann established methods (1898) for building on a skull a face recognizably correct.

Erratum: In the report "DDT administered to neonatal rats induces persistent estrus syndrome" by W. L. Heinrichs *et al.* (13 Aug., p. 642), line 4 in the last paragraph of column 1, page 643 should read "secretion of luteinizing hormone (LH)."

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Book Reviews

Quantitative Primate Studies

Baboon Ecology. African Field Research. STUART A. ALTMANN and JEANNE ALTMANN. University of Chicago Press, Chicago, 1971. viii, 220 pp., illus. $12.

Since Washburn's and DeVore's baboon studies of the early 1960's most field studies of primate behavior have been influenced by anthropological interests. The research questions have been based on the hypotheses that social behavior and organization are adaptations to ecological conditions; that therefore they covary with habitats; and that insight into the correlations between primate social systems and habitats will permit inferences from known habitats of early man to his unknown social life. The ecological orientation paraleled a similar new trend in ethology and was accepted by most zoologist monkey watchers.

The proposed aims required that the same primate populations be studied by both an ecologist and a student of behavior. As it has turned out, most field studies have been done by single workers who have been unable to cope with both tasks. The typical field report of the last decade focuses on the social interactions of the resting group. It then describes the habitat in qualitative terms, presents travel maps and a list of food species, and ends with speculations on the assumed local adaptiveness of the social system.

The Altmanns here set a new standard by devoting a full, heavily quantitative monograph to the ecology of a primate population. The monograph reports original fieldwork on the yellow baboons (*Papio cynocephalus*) of the Amboseli Reserve and extensively reviews ecological knowledge on the other baboon species. As quantitative treatment reveals the space-time patterns of feeding, drinking, and sleeping, some commonsense expectations are shaken by surprising facts. For example, the rate of alarm reactions is proportional to the time spent in a home range quadrat, not to the frequency with which the quadrat is entered. This suggests a steady rate of alarms rather than an alarming effect of a newly entered and therefore risky locality. In the descriptive sections the authors avoid declaring that an obviously adaptive group response is typical. It becomes clear that responses to apparently similar situations vary greatly and often inexplicably. In the same vein, the Altmanns treat migrating males not as a class but by presenting differing individual histories.

The authors' mathematical skills, acquired in their earlier work on communication processes, are here applied to the problems of home range use. Data are analyzed and reanalyzed in search of meaningful parameters and of ways to circumvent that pervading difficulty of fieldwork, biased samples. The reader looking merely for the usual type of descriptions may find that certain subjects are mathematically overtreated. He will soon realize, however, that the authors do more than just study baboons. With this book primate field research begins to test its own methods.

Speculations are set apart in the last 15 pages of the book. The major concept is the "utility" of a given section of the home range, which is composed of its resource value and its risk rate. "The perpetuation of the group depends upon its ability so to allocate the distribution of its activities among the parts of the home range that the net utility of the range to the animals is positive, or at least, non-negative" (p. 199).

Primate field research aims at an ecology of social systems. So far, we have had little more than a primate sociology. The Altmanns have now replaced the description of primate habitats by an analysis of interactions with the habitat. This ecology is "social" in such aspects as the avoidance of predators. As it concerns foraging, it is still predominantly an ecology of the individual. The third and most difficult step, it seems, will have to reveal the *social* strategies of resource exploitation.

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Sea Animals

Explorations in the Life of Fishes. N. B. MARSHALL. Harvard University Press, Cambridge, Mass., 1971. xiv, 204 pp., illus. $6.50. Harvard Books in Biology, No. 7.

This book's title is indeed descriptive of its content. Its author is one of the world's outstanding ichthyologists; he says he began the book when he gave a series of lectures at Harvard in the spring of 1963, but what he offers the reader are the experiences of a lifetime of research into the many and varied adaptations of the largest class of vertebrate animals. His fascination is with the animals of the deep sea, those vast reaches of the oceans "where sunlight is perpetually faint or lacking," where the pressure is high and food is scarce. The largest part of the book (chapters 3 and 4) deals with the ways in which fishes cope with these contingencies.

We are led into the problems of the deep sea, though, through a broad overview of the success of teleosts in fishes that deals lightly with feeding adaptations and reproduction and then proceeds to considerations of hydrodynamic design including neuroanatomical and physiological specializations for cruising and high-speed swimming.

While deep sea fishes and their peculiar adaptations occupy about a third of the book, its nearly equally large final portion deals with convergent evolution. Examples from invertebrate phyla are given here, but fishes still provide the principal illustrative matter to explain how environmental forces shaped form and function, eventually to result in similar features among unrelated groups of animals. Surface feeders, for instance, whether the mosquito-fish *Gambusia* or flying fish, have small, upturned jaws and their pelvic fins originate anterior to the dorsal for reasons of skimming the surface with the snout. Certain fast-swimming sharks and tunas have silhouettes that can practically be superimposed, but more remarkable still, they both have retia mirabilia in their red muscles to enable them to operate several degrees above their ambient temperature. A number of advantages thus accrue to these large elasmobranch and teleost predators alike; to single out only two among many important metabolic reactions, nerve impulse transmission and digestion are speeded up.

At the very end of the book Mar-

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shall speculates on the convergence of natural and man-made systems and makes some sketchy remarks about symbols and processes in nature and culture. One may throw out interesting asides about such matters in a lecture, as Marshall obviously did, but in print a three-page treatment of these matters is not adequate. Apart from this, he deals with his large and complex subject in a lively fashion; there are many terse descriptions, and the style is such that fairly complex details are conveyed with ease. The illustrations are well worth mentioning; they are mostly fine-lined drawings on the margins of the pages, an arrangement which makes for a particularly attractive book. *Explorations in the Life of Fishes* certainly should be in the libraries of skin-in and skin-out biologists alike.

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Mollusca

Terrestrial Slugs. N. W. RUNHAM and P. J. HUNTER. Hutchinson University Library, London, and Hillary House, New York, 1971. 184 pp., illus. Cloth, $6; paper, $2.50. Biological Sciences series.

Although mollusks are one of the largest groups among the invertebrates, there are few books on them that would interest and assist the reader with broad or general interests. This small volume on the widely distributed group called "slugs" should serve to stimulate interest in the use of slugs for teaching and research purposes. Well-organized and informative, it brings together much salient information on these animals—information that has been somewhat buried in scattered papers and volumes not readily available to most persons. The text covers a wide range of topics, such as general features of the animals; their classification; foods and feeding habits; respiration; blood composition and circulation; excretion, reproduction, development, and growth; locomotion and mucus production; sensory and nervous anatomy with their functional relations. All of the morphological material is illustrated with good text figures. The ecology section includes a description of methods for sampling, discussions of the responses of the animals to humidity and temperature and of seasonal variation in aggregation and dispersal, and an analysis of what factors are important in their ecology.

A concluding chapter discusses "slugs as pests." Useful information, much of it dealing with the more recent literature, is reviewed. In England slugs are a serious garden pest, and they are not welcome in areas under cultivation in many other parts of the world. On the whole, this chapter is well written and contains pertinent material. Some helpful citations are missing, for example an excellent review of methods of snail control in A. R. Mead's *The Giant African Snail* (University of Chicago Press, 1961), but after all the book is not a manual on slug control and actually there are as yet no good methods, as the authors clearly indicate.

One of the noteworthy features of the book is the excellent series of well-integrated references. The 21 pages of citations will serve as a useful tool for anyone wanting to work in a wide field of interests in which slugs would serve for field and laboratory studies. It seems almost pedantic to point out that there are references that might have been added to assist those wanting additional information. A few that come to mind that could be helpful are as follows: The veronicellids, as is stated, are poorly known, but they are widespread in the tropics; some of a series of papers by Hans Hoffman could have been cited. The preservation of slugs is important and should at least have been mentioned; among others a paper by Hubricht (*Nautilus* **64**, 90 [1951]) would be helpful. The role of galactogen is discussed, and in this connection a reference to the work of E. M. Goudsmit and G. Ashwell (1965) would be timely. With regard to the parasite and predator relationships, the compilation of S. V. Wild and A. E. Lawson (*J. Conch.* **20**, 252 [1937]) on the enemies of land and freshwater mollusks of Britain would be a useful reference; also, an account of the more recent serious spread of rat lungworm (*Angiostrongylus cantonensis*), which also has slugs as hosts in many regions, was published in 1969 in a book by Alicata on parasites of man and animals in Hawaii.

Terrestrial Slugs will be helpful not only to those involved in classroom and laboratory programs; agencies concerned with control of slugs also will find a wealth of useful basic information in it.

HENRY VAN DER SCHALIE
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Crucial Numbers

Geography and a Crowding World. A Symposium on Population Pressures upon Physical and Social Resources in the Developing Lands, University Park, Pa., Sept. 1967. WILBUR ZELINSKY, LESZEK A. KOSINSKI, and R. MANSELL PROTHERO, Eds. Oxford University Press, New York, 1970. xvi, 602 pp., illus. $10.95.

Ironically, a three-year delay makes the publication of these conference proceedings more timely. The book outclasses this year's hundred bad books on the subject of a crowding world. It is coherent and broad-minded —better still, mind-stretching.

The conference topic, "population pressure upon resources," eluded the attempt at a single definition, but the participants did arrive at a consensus that it entails not one worldwide threshold of overpopulation, but three thresholds, each of them now critical somewhere. There is a lower threshold of insufficient population: "A certain degree of demographic pressure is necessary for man to seek to improve the utilization of his resources" (Tricart). There is an intermediate threshold above which one goes from extensive exploitation (long fallow) to intensive systems of cultivation which conserve or enhance environmental possibilities (soil fertility, streamflow processes, variability of species, and so on—see the contribution of Boserup or Mabogunje); and there is a more widely discussed upper threshold beyond which food crises recur, and at which urban assimilation capacities become critical (Beaujeu-Garnier, C. G. Clarke). As societies move along this path of development, they seem to go through an "optimum period" between the middle and upper thresholds, "in which one may have the impression of prosperity for one or two generations" (Pierre George).

The thresholds of population density or carrying capacity are not everywhere the same, and some geographical environments are more tolerant or more resilient than others. Many regions of "population pressure" are at the intermediate threshold, where a cultural and technical transformation must take place. "The passage from one equilibrium to another is precarious and always accompanied by serious tensions" (Tricart).

The conference was an attempt to restore a sense of priorities among geographers, by focusing on the interaction of fundamental human pro-

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—the production of enough food (and other goods), its equitable distribution, and rates of reproduction. For centuries these questions were recognized as the vital ones in "political economy." Since 1900, they have been progressively zoned out of the offspring social sciences—economics, political science, sociology—whose practitioners, ever more ramified, have lost their capacity to cope with earthy variables such as mosquitoes, microbes, genes, proteins, soils, and rainfall (Philip Porter). Meanwhile, the bulk of geographers and ethnologists have dispersed into far corners of the globe to stake out private scholarly claims. (There have been individual exceptions, of course, of the stature of Isaiah Bowman, Owen Lattimore, Ruth Benedict, and Gilbert White.) The 1967 conference was a major step toward reversing the trend toward exotic fragmentation, and "reintegrating" geography. The participants were of a dozen nationalities, with strong input from America, France, and Poland. Several were elder statesmen of their faculties, who formulated crucial problems of distribution of wealth and the ecological balance long before these hit the comic strips, *Fortune*, and the *National Geographic*. The geographical coverage of the contributions is remarkable: de Planhol compares societies along the mountain backbone of Eurasia; Brookfield compares social systems in Malagasy, Mauritius, and New Guinea; Kempton Webb reports ten years of study of northeast Brazil, and Gerhard Sandner of Costa Rica. Urban population structures are discussed for cities of India, Japan, and Jamaica. The historical perspective is also broad: Pokshishevskii takes the millennial view, Bonasewiez and Taeuber a scale of generations; and William Vogt reminds us of our short collective memory ("What ever happened to Krilium?"—the miracle fertilizer reported at the AAAS meeting of 1951). The bibliographies extend the horizons still farther.

In contrast to any textbook of world geography, this volume is an impressive answer to the question (fair enough), What are geographers good for? If they continue in this direction, putting first things first, posing the questions carefully, comparing, exploring the political implications, then Pierre George's comment is indeed prescient: "It is not surprising any longer that geography is considered by some to be a dangerous discipline."

The medium does not do full justice to the message. As with other "proceedings," one doesn't read the 600 pages cover to cover. But anyone who dips into this work frequently enough or long enough becomes another Jacob wrestling with the angel. He confronts the issue of his own relevance and the problem of reintegrating or collapsing the fragmented "sciences."

Is there no new medium for capturing the magnificent spectacle of 40 people thinking? A good scholarly symposium is a Woodstock and wants sharing. In this particular case, revolutions, political and technological, are the substance. Yet, as the editors comment, "despite deep anxieties and a lack of agreement on remedial strategies, there was almost no gnashing of teeth and crying of havoc." The humanity of this work, in the midst of the clamor, calls for a more powerful means of expression.

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Psychoanalytic Theory

Freud and Philosophy. An Essay on Interpretation. PAUL RICOEUR. Translated from the French by Denis Savage. Yale University Press, New Haven, Conn., 1970. xviii, 574 pp. $15. Dwight Harrington Terry Foundation Lectures, vol. 38.

This book, with its many insights into Freud, psychoanalysis, religion, and the theoretical structure of psychoanalytic psychology, is both stimulating and frustrating. The conceptual framework is difficult and the argument somewhat elusive, being a mixture of Hegelianism and phenomenology with some cogent comments about the causal nexus of behavior as distinguished from the reasons that are often given for behavior. The distinction attempted between causes and reasons is not spelled out in the usual terminology employed in the philosophy of science. Rather, the terms "archeology" and "teleology" are used to express these concepts. Although Ricoeur, a philosopher and professor at the University of Paris, disarmingly disclaims expertise as a psychoanalyst, or even having been psychoanalyzed, it is apparent that his theoretical knowledge about Freudian analysis (to which he restricts his discussion) is greater than that of most psychiatrists and a great many analysts.

Ricoeur's concern is not with the therapeutic efficacy of psychoanalysis but rather with the implications of the Freudian view of man, which in effect he sees as a particular view of the development of culture. He has constructed his exposition in three "books": the "problematic," in which questions are raised about Freudian philosophy, principally through the analysis of language and its variations in terms of "ordinary usages"; the "analytic," composed of the author's insights from his reading of Freud utilizing the rules of symbolic transformation and language; and the "dialectic."

It is in the dialectic section that the philosophical problems most relevant to the current scene in psychoanalysis emerge, problems that are of particular concern to the psychologist with an interest in problems of theory or the philosophy of science and that should also concern clinicians who blindingly use theoretical notions with few questions regarding the nature, validity, or generalizability of the theories they use. Here perplexing and unresolved problems arise, such as the disparity between giving reasons why a given child or adult has followed particular pathological lines of development and demonstrating the necessity of the development's having taken that particular route. Our theories can give us reasons, but these reasons never achieve a state of sufficiency. Hence the possibility for an endless multiplication of reasons, none of which are compelling. What this means is that psychoanalytic theory—like other psychological theories—is left at the level of lacking a causal network of explanation.

Perhaps the problem resides in our inability to get at sufficient numbers of independent variables that may then achieve causal status in a nomological system. During the course of psychoanalytic therapy relying on a reconstructive model many significant variables are elicited. Yet still we can provide only insightful explanations which are based on alleged and inferred reasons for development and behavior but which can be rejected. Theory formation in psychology has come a long way since the proposed hypothetical-deductive model, but even employing a probabilistic-statistical model we arrive only at a partial theory which at best has many difficulties in terms of confirmation or disconfirmation. The original strict criteria for a scientific theory set forth by Karl Popper are

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not strictly adhered to at present. The variables are too complex and too numerous to be easily subsumed under a theory adequate to produce more than fragmentary confirmations or disconfirmations. In the absence of an ability to know what variables are significant, and to what extent, prior prediction concerning lines of development becomes at least hazardous, if not mere speculation. Particularly is this so when, as in psychoanalysis, we are not dealing with discrete variables such as certain physical attributes. Lacking the ability to make predictions, and ideally "point predictions," we lack a means to a tight theory which can abandon many of its untusable components as well as its disconfirmed parts while retaining enough openness not to foreclose useful concepts.

Ricoeur's treatment of these issues relies heavily on the English philosophers of language and their style of exegesis. This is most intriguing to me, yet also most disappointing. Ricoeur makes almost no mention of the philosophers who appear to be exerting the most groundbreaking and compelling influence these days in the philosophy of science. Especially is this so with regard to the structure of scientific theories. Over the past decades there has been monumental work on this subject, but it is given only nodding acknowledgment in this book. Such philosophers as Popper (mentioned once, on p. 363), Herbert Feigl (mentioned in one footnote), and Stephen Toulmin (pp. 359 and 363 and one footnote) are examples. The work of Imre Lakatos, Paul Feyerabend, and the late Arthur Pap receives no mention whatsoever. One waits disappointingly and in vain for Ricoeur to deal with the insights of these men into the nature of scientific theory, explanation, and nomological networks.

I have no personal acquaintance with today's scene in Paris in the philosophy of science, but I think it fair to presume that the work of the philosophers mentioned cannot have been ignored there. I can only speculate that these neglected "philosophies" do not appeal to the author, with his predilection for the analysis of language and the philosophers of meaning. To appraise Ricoeur's book requires an appraisal of the philosophy of "ordinary language" as well as of his attempt at a synthesis of dialectic philosophy based on his reading of Freud. This is all highly stimulating, and provides exposure to a perspective on Freud and psychoanalysis I have not had before. I was left wishing for an opportunity to sit down with Ricoeur and personally explore many of the issues he has raised in his book and also those raised in this review.

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Books Received

Acoustics. B. J. Smith. Elsevier, New York, 1970. vi, 138 pp., illus. $9.45. Environmental Physics Series.

Advances in Behavior Therapy. Proceedings of a conference, 1969. Richard D. Rubins, Herbert Fensterheim, Arnold A. Lazarus, and Cyril M. Franks, Eds. Academic Press, New York, 1971. xviii, 302 pp., illus. $14.50.

Advances in Metabolic Disorders. Vol. 5. Rachmiel Levine and Rolf Luft, Eds. Academic Press, New York, 1971. xvi, 374 pp., illus. $18.50.

Advances in Photochemistry. Vol. 8. J. N. Pitts, Jr., George S. Hammond, and W. Albert Noyes, Jr., Eds. Wiley-Interscience, New York, 1971. x, 366 pp., illus. $22.50.

Air Pollution Control. Part 1. Werner Strauss, Ed. Wiley-Interscience, New York, 1971. x, 452 pp., illus. $19.95. Environmental Science and Technology Series.

The Analysis of Intelligence. J. P. Guilford and Ralph Hoepfner. McGraw-Hill, New York, 1971. xiv, 514 pp., illus. $16.95.

The Anatomy of a Scientific Institution. The Paris Academy of Sciences, 1666–1803. Roger Hahn. University of California Press, Berkeley, 1971. xiv, 433 pp. + plates. $12.

Antigens, Lymphoid Cells, and the Immune Response. G. J. V. Nossal and G. L. Ada. Academic Press, New York, 1971. xx, 324 pp., illus. $17.50. Immunology Series.

The Application of Metabolic and Excretion Kinetics to Problems of Industrial Toxicology. Jerzy Piotrowski. Prepared under the Special Foreign Currency Program of the National Library of Medicine. Superintendent of Documents, Washington, D.C., 1971. iv, 166 pp., illus. $2.25.

Archaeological Survey in the Lower Yazoo Basin, Mississippi, 1949–1955. Philip Phillips. Peabody Museum of Archaeology Ethnology, Cambridge, Mass., 1970. In two parts. Part 1, xviii + pp. 1–572, illus. Part 2, x + pp. 573–1000, illus. Paper, $30. Papers of the Peabody Museum, vol. 60.

Archaeology. Discoveries in the 1960's. Edward Bacon. Praeger, New York, 1971. xiv, 294 pp., illus. $12.50.

Associative Information Techniques. Proceedings of a symposium, Warren, Mich., September 1968. Edwin L. Jacks, Ed. Elsevier, New York, 1971. x, 224 pp., illus. $15.50.

Astronomy. Richard Whittington. Hubbard, Northbrook, Ill. 1971. 48 pp., illus. $2.95.

Atomic and Molecular Orbital Theory. Peter O'D. Offenhartz. McGraw-Hill, New York, 1970. xiv, 370 pp., illus. $14.50.

Atoms, Molecules and Chemical Change. Russell H. Johnsen and Ernest Grunwald. Prentice-Hall, Englewood Cliffs, N.J., ed. 3, 1971. xiv, 368 pp., illus. $10.95.

Autonomy and Biogenesis of Mitochondria and Chloroplasts. A symposium, Canberra, December 1969. N. K. Boardman, Anthony W. Linnane, and Robert M. Smillie, Eds. North-Holland, Amsterdam, and Elsevier, New York, 1971. xii, 512 pp., illus. $38.50.

Basic Principles of Spectroscopy. Raymond Chang. McGraw-Hill, New York, 1971. x, 304 pp., illus. $14.95.

Behavior Therapy and Beyond. Arnold A. Lazarus. McGraw-Hill, New York, 1971. xiv, 306 pp. $8.95.

Cambrian of the New World. C. H. Holland, Ed. Wiley-Interscience, New York, 1971. viii, 454 pp. + plates. $29. Lower Palaeozoic Rocks of the World, vol. 1.

Campustown U.S.A. at Midcentury. Mark Graubard. Campus Scope, Minneapolis, 1971. viii, 300 pp. Paper $3.95.

Cell Biology. Robert M. Dowben. Harper and Row, New York, 1971. xiv, 570 pp., illus. $12.95.

Cell Fine Structure. An Atlas of Drawings of Whole-Cell Structure. Thomas F. Lentz. Saunders, Philadelphia, 1971. xiv, 438 pp., illus. $11.25.

Chemical Mutagens. Principles and Methods for Their Detection. Alexander Hollaender, Ernst Freese, Kurt Hirschhorn, and Marvin Legator, Eds. Plenum, New York, 1971. Vol. 1, xxviii + pp. 1–310, illus. + index. Vol. 2, xxii + pp. 311–610, illus. + index. $17.50 each.

A Collation of Anthroponetry. John W. Garrett and Kenneth W. Kennedy. Aerospace Medical Division, Air Force Systems Command, Wright-Patterson Air Force Base, Ohio, 1971. Vol. 1, A–H, viii + pp. 1–1110. Vol. 2, I–Z, index, x + pp. 1111–2176. $12 each volume.

Comprehensive Biochemistry. Vol. 26. Extracellular and Supporting Structures. Part C. Marcel Florkin and Elmer H. Stotz, Eds. Elsevier, New York, 1971. xii + pp. 595–898, illus. $21.50.

The Contemporary American Family. William J. Goode, Ed. Quadrangle, Chicago, 1971. viii, 302 pp. Cloth, $8.95; paper, $2.95. A *New York Times* Book.

Contemporary Nursing Practice. A Guide for the Returning Nurse. Signe Skott Cooper. McGraw-Hill, New York, 1970. xii, 348 pp., illus. $7.50.

A Critical Review of the Techniques for Testing Insecticides. James R. Busvine. Commonwealth Agricultural Bureaux, Slough, England, ed. 2, 1971. xiv, 346 pp., illus. $13.

Data Structures. Theory and Practice. A. T. Berztiss. Academic Press, New York, 1971. xiv, 442 pp., illus. $14.50. Computer Science and Applied Mathematics series.

Decision and Stress. D. E. Broadbent. Academic Press, New York, 1971. xiv, 522 pp., illus. $21.

(*Continued on page 946*)

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Reports

Racemization of Amino Acids in Marine Sediments

Abstract. *Isoleucine, one of several amino acids isolated from a suite of well-dated deep-sea cores, shows a progressive increase in the degree of racemization with the age of the sediment. Amino acids in sediments show an initial rate of racemization almost an order of magnitude faster than the rate observed for free amino acids at a comparable pH and temperature. The observed kinetics depend on a variety of diagenetic processes, but it appears that the ratio of alloisoleucine to isoleucine is a reliable indicator of age for samples less than 400,000 years old; for older samples the results are more ambiguous. Isoleucine is racemic in samples older than about 15 × 10⁶ years.*

Proteins, peptides, and amino acids can undergo a variety of biological and nonbiological reactions in the geologic environment. Biological amino acids, the basic components of proteinaceous matter, are primarily of the L form, and with time these optically active enantiomers convert to an equilibrium mixture of the D and L forms. This phenomenon has now been observed in several apparently diverse environments: in fossil calcareous organisms from former nearshore marine sediments (*1–3*); in nearshore fjord-type sediments (*4*); and deep-sea marine sediments (*2*, *5*). In fossil shells from several Recent, Pleistocene, and Tertiary deposits from the southeastern United States, racemization was found to increase with the increasing age of the fossil, with complete racemization found in fossils older than the Pliocene (*1*, *2*). Fossil corals from raised reef deposits on Barbados likewise showed increased racemization in fossils from older terraces, though in situ contamination apparently occurred in some samples (*3*). Kvenvolden *et al.* (*4*) found that racemization in the sediments of Saanich Inlet had proceeded to about 10 percent of the amount at equilibrium in the first 9000 years of diagenesis. Hare and Mitterer (*2*) found that racemization of isoleucine in an Antarctic deep-sea core increased with depth in the core and that, when compared to the sedimentation rates determined from paleomagnetic data, the rate of racemization of isoleucine was in good agreement with the rates extrapolated from high-temperature pyrolysis data on actual shell material. Recently Bada *et al.* (*5*) have found a progressive increase in the ratio of alloisoleucine to isoleucine with depth in a core from the Mid-Atlantic Ridge. High-temperature pyrolysis experiments on solutions of free L-isoleucine were used to calculate a sedimentation rate of 0.42 cm per 1000 years for this core.

In this work we report data on the racemization of L-isoleucine in a suite of cores of deep ocean sediments; the ages of all samples analyzed are known independently. We offer here a mechanism to explain the observed kinetics of racemization, and we shall reinterpret existing data (*5*) in light of this mechanism.

The racemization of L-isoleucine produces D-alloisoleucine (*6*); because of their two adjacent asymmetric carbon atoms, these diastereomers have slightly different chemical properties and are resolvable in the standard ion-exchange procedure of amino acid analysis. Thus the measurement of the extent of racemization of isoleucine is comparatively simple. It has been shown (*1*, *2*, *4*, *5*, *7*) that isoleucine is one of the slowest amino acids to racemize, so it provides an index of the degree of racemization of other amino acids in a sample (*8*).

We have analyzed the foraminifera fraction (> 74 μm) rather than the gross sediment, the rationale being that the mineral test would be most resistant to contamination from outside sources. Samples are ultrasonically cleaned, washed, wet-sieved with distilled water, air-dried, and weighed. They are then dissolved in twice the stoichiometric amount of concentrated HCl, and a slight excess of acid is added to make the final solution 6*N* HCl. Hydrolysis is performed in sealed Pyrex test tubes under N_2 for 22 hours at 110°C. Desalting techniques used

Table 1. Cores analyzed [see (*22*) for a discussion of dating by ^{230}Th]. Cores V23-110 and V24-28 have only faunal analyses for their age determinations. Core V23-110 is judged to be about 2.0 × 10⁶ years old at the core bottom (821 cm) on the basis of the presence of *Globorotalia multicamerata*, *Sphaeroidinella dehiscens*, and left-coiling *Pullenlantina primalis* at the 800-cm level, and the abundance of discoasters below 780 cm (*26*). Core V24-28 is entirely Upper Pleistocene in age, and faunal analyses and correlations with other cores indicate an age of about 400,000 years at the core bottom, 910 cm (*27*). All cores listed have been obtained on cruises of Lamont-Doherty Geological Observatory research vessels, with the exception of core CH96-G12, which was obtained by the Woods Hole Oceanographic Institution.

Core	Latitude	Longitude	Water depth (m)	Approximate water temperature (°C)	Method of dating	Reference
V12-122	17°00'N	74°28'W	2730	4.0	^{14}C, ^{230}Th, fauna	(28, 29)
A179-4	16°36'N	74°48'W	2965	4.0	^{14}C, ^{230}Th, fauna	(28)
V16-205	15°24'N	43°24'W	4045	2.4	Magnetic reversals, fauna	(28)
V16-39	24°43'S	4°45'W	4510	2.4	Magnetic reversals, fauna	(28)
V12-18	28°42'S	34°30'W	2935	2.9	Magnetic reversals, fauna	(28)
RC8-93	29°22'S	105°14'W	3157	2.0	Magnetic reversals	(28)
V23-110	17°38'N	45°52'W	3746	2.4	Fauna	(26)
CH96-G12	30°16'N	43°19'W	4014	2.2	^{230}Th, fauna	(5, 14)
V24-28	15°19'N	77°57'W	2274	4.1	Fauna	(27)
RC5-12	26°35'N	56°29'W	5104	1.5	Fauna	(26)
A167-21	29°49'N	76°35'W	1455	~5	Fauna	(30)
V18 BBD No. 2	17°56'S	154°08'W	~ 800	~5	^{14}C	This work

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Table 2. Alloisoleucine, isoleucine, and the alloisoleucine to isoleucine ratio in hydrolyzates of foraminifera from deep-sea cores.

Depth in core (cm)	Approximate age ($\times 10^3$ yr)	Alloisoleucine (nmole/g)	Isoleucine (nmole/g)	Alloisoleucine / Isoleucine
		A179-4		
30	0.013	2.69	64.84	0.042
100	.042	3.82	39.72	.096
100	.042	2.62	28.20	.073
175	.073	6.15	54.20	.114
250	.105	6.50	37.0	.172
493	.169	11.30	55.40	.204
493	.169	12.80	65.60	.195
600	.250	13.27	35.22	.377
700	.295	12.90	35.10	.368
		V23-110		
0-1	> 0	2.1	35.7	.059
21-22	~ 0.05	3.7	41.6	.089
42-43	~ .10	6.0	39.5	.152
62-63	.16	7.7	37.5	.205
62-63*	.16	4.1	17.7	.232
81-82	.20	8.0	31.6	.253
101-102	.25	7.7	27.0	.285
151-152	.38	12.2	41.4	.295
211-212	.53	13.0	38.3	.339
301-302	.75	11.6	33.4	.347
405-406	1.01	8.2	21.4	.383
405-406*	1.01	7.2	19.6	.367
499-500	1.24	10.4	29.7	.350
591-592	1.48	5.2	10.6	.491
682-683	1.71	11.8	26.0	.459
791-792	1.90	9.5	15.0	.633
		V12-122		
40-41	0.017	5.2	72.8	.071
98-99	.041	8.0	66.7	.120
98-99*	.041	7.1	44.2	.161
290-291	.121	11.8	56.4	.209
290-291*	.121	15.6	62.4	.250
464-465	.193	8.5	34.6	.255
505-506	.210	10.0	37.2	.269
551-552	.230	10.0	44.6	.224
650-651	.271	12.0	38.9	.309
745-746	.310	8.9	28.7	.310
850-851	.355	7.8	25.5	.306
930-951	.395	11.5	28.4	.405
1055-1056	.438	10.1	28.5	.354
1097-1098	.456	11.0	33.7	.326
		V16-205		
375	.70	7.18	16.85	.43
375	.70	10.45	22.41	.46
861	1.80	10.24	16.83	.609
		V16-39		
290	0.700	5.56	15.38	.387
730	1.80	4.71	7.47	.63
		V12-18		
720	1.80	3.56	6.73	.53
		RC8-93		
1087	2.20	1.90	2.99	.637
		CH96-G12		
400	~ 0.10	2.85	18.08	.158
		V24-28		
5-6	~ .003	1.13	72.78	.016
413-416	~ .170	9.82	44.50	.220
		V18- BBD No. 2		
Dredge haul	.010	2.4	33.5	.072
Dredge haul	.010	1.3	17.9	.073
		RC5-12		
317-320	~ 70	4.60	3.44	1.33
317-320	~ 70	3.07	2.27	1.35
317-320	~ 70	1.14	1.42	0.80†
		A167-21		
300	~ 50	3.61	4.89	.74†
		JOIDES samples		
Leg 7, site 62-1, Upper-Upper Miocene		2.56	2.35	1.08
Leg 7, site 64-1, Lower-Middle Miocene		3.9	3.3	1.18
Leg 6, site 47-2, Lower Paleocene		1.55	1.23	1.26

* Hydrolyzed for 46 hours. † Not subjected to ultrasonic treatment.

are those of Hare (9). The sample size is usually on the order of 200 mg of foraminifera, thus reagent blanks are kept to a minimum. Negligible amounts of all amino acids except serine and glycine are found in blanks carried through the entire procedure. All cores analyzed are described in Table 1; data are given for the location, depth, bottom water temperature, and method used for the determination of the absolute age. The rate of racemization is sensitive to temperature (*1, 2, 5, 7*); all cores discussed have bottom-water temperatures in the range from 2.2° to 4.0°C. The present-day temperature is taken as an estimate of the average thermal condition of the cores; we have not corrected any of the data for temperature differences between cores because of other uncertainties. For large temperature differences (≥ 3°C), some correction would be necessary.

Data for the alloisoleucine to isoleucine ratio in these samples are given in Table 2; age assignments are given by interpolation or extrapolation from dated horizons. We report the absolute abundances of the amino acids with some caution, for consistent percentage recovery is not easily attained (*10*). Duplicate analyses indicate an uncertainty of up to ± 30 percent for the absolute abundance of any amino acid and an uncertainty of ± 5 percent in the alloisoleucine to isoleucine ratio. In some cases the ratios scatter even more than this in successive levels in a core, particularly in samples from core V12-122.

Racemization during acid hydrolysis is a potential problem (*4, 11*); extended hydrolysis (46 hours) significantly increases the alloisoleucine to isoleucine ratio in three out of four samples tested. However, little or no alloisoleucine is observed in samples of living corals analyzed by identical techniques (*3*); samples of living foraminifera have not been analyzed (*12*), but the presence of alloisoleucine in the sample from the top of core V23-110 is probably due primarily to the finite age of the top of the core. The youngest sample analyzed, from core V24-28, shows an alloisoleucine to isoleucine ration of ~ 0.015.

The kinetics of racemization in simple systems have been observed to obey first-order reversible rate laws (*5*); for the racemization of isoleucine, the equation is:

$$\text{L-isoleucine} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{D-alloisoleucine} \quad (1)$$

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where λ_I and λ_A are the characteristic rate constants of isoleucine (I) and alloisoleucine (A), respectively. Alloisoleucine is more abundant than isoleucine in the equilibrium mixture of the two diastereoisomers. The equilibrium ratio has been found to be between 1.25 and 1.40 in high-temperature heating experiments (*1, 2, 7, 11*) and in old fossils at normal environmental temperatures (*1, 2*). There is no definite evidence of a temperature dependence of this ratio, although a slight one might be expected (*13*). We use a value of 1.25 for calculations here. Thus the ratio of the rate constants is fixed: $\lambda_I/\lambda_A = 1.25$. For the equation

$$\frac{dI}{dt} = -\lambda_I(I) + \lambda_A(A) \qquad (2)$$

$$\frac{X_e - X}{X_e} = e^{-t\,\ln\lambda_A/t} \qquad (3)$$

The quantity X_e is the equilibrium ratio of alloisoleucine to (alloisoleucine + isoleucine), or $1.25/(1.25 + 1.0) = 0.556$; X is the value of this ratio at time t (*14*). If the data fit this model a straight line for the plot of the logarithm of $(X_e - X)/X_e$ as a function of time would result; the data are plotted in Fig. 1, and clearly such a straight-line relation does not exist. The plot is linear to an alloisoleucine to isoleucine ratio of about 0.25 (approximately 400,000 years at these temperatures), and then the rate of racemization decreases rather abruptly. Similar conclusions can be drawn about racemization in fossil corals (*3*) and fossil *Mercenaria* (*1, 2*); the change in slope occurs at about 50,000 years at the higher temperatures. Contamination of older samples could give the curve this shape, but we feel that the major explanation for this nonlinearity comes from an understanding of the complexity of the diagenetic reactions that fossil amino acids undergo.

When the foraminifera are dissolved and the hydrolysis step is omitted, only free amino acids and small peptides are detected. In all cases, we observe that the alloisoleucine to isoleucine ratio is significantly higher in these free amino acids than in the total hydrolyzate (*15*). The free alloisoleucine to isoleucine ratio (Table 3) reaches a value of 1.0 in samples as young as 5000 years; this ratio increases up to a value of 1.35 to 1.40 in samples of Miocene age (*3*). Significant racemization of free amino acids was first observed in younger fossil *Mercenaria* samples (*1, 2*).

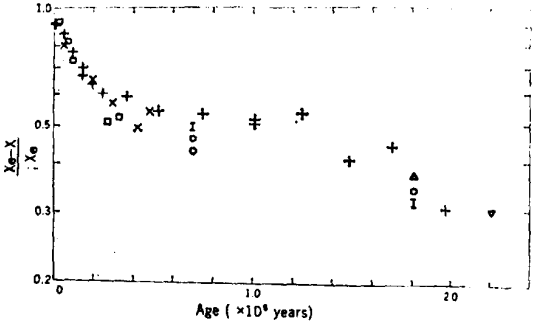

Fig. 1. Plot of $(X_e - X)/X_e$ as a function of time in deep-sea sediments. +, Core V23-110; □, core A179-4; ×, core V12-122; ○, core V16-205; △, core V12-18; ▽, core RC8-93; I, core V16-39.

Free amino acids represent a very small fraction of the total amino acids in young fossils (*1, 2*), but the ratio of free amino acids to total amino acids grows slowly with time, approaching a value of 1.0 (*16*). Almost all the free amino acids in a fossil are derived from the breakdown of proteins, and their extensive racemization is apparently a consequence of the natural hydrolysis of peptide bonds at the slightly alkaline pH's of the carbonate environment (*17, 18*). Hydrolysis under these conditions does not produce completely racemic amino acids, however, and, once liberated, these free amino acids continue to racemize at their own characteristic rates. Laboratory pyrolysis experiments indicate that racemization in calcareous matrices can be inhibited by a deficiency of water (*2*), further suggesting the link between the hydrolysis and racemization mechanisms. These experiments also indicate that amino acids are leached from the fossil, so that the rate of removal of amino acids from the matrix will also affect the abundances of alloisoleucine and isoleucine in the fossil (*19*). The free amino acids leached from both corals and foraminifera have alloisoleucine to isoleucine ratios of 0.95 to 1.3 whereas the hydrolyzates of the material leached have alloisoleucine to isoleucine ratios nearly identical to the ratios found in the hydrolyzates of the residual sam-

Table 3. Free amino acids in selected samples.

Depth in core (cm)	Alloisoleucine (nmole/g)	Isoleucine (nmole/g)	Alloisoleucine / Isoleucine
	V23-110		
0–1	0.4	0.3	1.3
42–43	3.7	1.5	2.13
62–63	4.1	4.1	1.0
81–82	6.2	5.7	1.1
151–152	2.7	2.6	1.04
405–406	6.9	6.9	1.0
591–592	1.4	1.5	0.93
791–792	1.7	1.8	.94
	V12-122		
40–41	0.3	1.1	.27
505–506	6.8	7.2	.97
650–651	7.7	7.9	.98
850–851	7.6	7.6	1.0
1055–1056	9.1	8.4	1.1
	V24-28		
5–6	~ 0.32	0.32	~ 1.0
	JOIDES samples		
Leg 7, site 64-1, Lower-Middle Miocene	1.6	1.1	1.45
Leg 6, site 47-2, Lower Paleocene	1.46	1.09	1.33

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ples. The total amino acid content of the foraminifera studied here varies from about 1500 nmole/g in the youngest samples to about 500 nmole/g in Lower Pleistocene samples, most of the decrease coming in the first 200,000 years of diagenesis. Free amino acids accumulate in the fossils up to a concentration of about 350 nmole/g when the alloisoleucine to isoleucine ratio in the total hydrolyzate is in the range from 0.25 to 0.30. The abundance of free amino acids slowly decreases beyond an age of about 400,000 years. From the shape of the curve in Fig. 1, we conclude that rapid racemization during early diagenesis is a consequence of natural hydrolysis and the accumulation of nearly racemic free amino acids in the fossil. As indicated by the ratio of free amino acids to total amino acids, hydrolysis is not completed within 500,000 years at these temperatures; beyond this time, the rate of racemization is probably controlled by slow hydrolysis, continued racemization of free amino acids, and some racemization of bound amino acids. The exact mechanism of this latter process is somewhat obscure, but evidence that it occurs does exist (*18*). Presumably terminal amino acids would be free to racemize. Our data suggest that alloisoleucine is more abundant in the total (hydrolyzed) amino acids than in the free amino acid fraction, and earlier results (*1, 2, 4*) also suggest that racemization of amino acids does occur while they are bound. A simple calculation of the alloisoleucine to isoleucine ratio in the "peptide" fraction

$$\frac{(A)_{total} - (A)_{free}}{(I)_{total} - (I)_{free}} \qquad (4)$$

is vulnerable to the uncertainties of percentage recovery discussed above, but it appears that the kinetics of racemization in the "bound" fraction are nearly linear and not too different from the rates based on laboratory experiments (*5*). Ideally one should measure the extent of racemization only after all free amino acids have been removed (*4*).

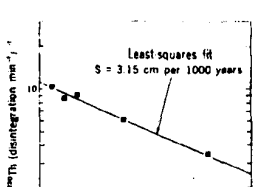

Fig. 2. Least-squares plot of excess ^{230}Th as a function of depth for core CH96-G12. *S*, sedimentation rate.

Extrapolation of our data would lead to the conclusion that the racemization of isoleucine would be complete within 15×10^6 to 20×10^6 years. Analyses of several high-carbonate samples from the JOIDES (Joint Oceanographic Institutions for Deep Earth Sampling) Deep Sea Drilling Program, all stored frozen since collection, support this extrapolation (see Table 2). The fact that the alloisoleucine to isoleucine ratio for the free amino acids in the Lower Middle Miocene sample is somewhat higher than that for the total hydrolyzate suggests that true equilibrium has not been reached. The Paleocene sample is in equilibrium.

As the overall abundance of amino acids decreases, the potential effect of contamination increases. Contamination can obviously have the effect of indicating an "apparent" rate of racemization that is slower than the real rate (*4, 20*). Analyses of Eocene and Cretaceous samples (Table 2) indicate that "nonequilibrium" ratios can be found if the sample is not correctly prepared. The Eocene sample consisted mostly of small (< 74 μm) particles that could not be ultrasonically cleaned because of the loss of starting material that is inherent to this procedure. The Cretaceous sample, however, was rich in coarse, well-preserved foram tests which could be subjected to the standard vigorous ultrasonic treatment. Two analyses of hydrolyzates of this sample showed alloisoleucine to isoleucine ratios of 1.33 and 1.35, whereas a third sample that was merely rinsed with H_2O and dilute HCl showed a ratio of 0.80. None of the Lamont cores examined here have been stored frozen, and contamination during storage could be responsible for some of the scatter in the data. Because our extrapolation is supported by the JOIDES data and because we are able to observe racemic mixtures in samples that have not been stored or prepared in an unusual manner we are confident that contamination during storage has not systematically affected the results. The fact that we do see the effects of contamination points out the difficulties of detecting racemic mixtures in samples that have been exposed to terrestrial processes (*3, 4, 20, 21*).

The nonlinear kinetics and the initial rate of racemization that we have observed are not in agreement with the recent work of Bada *et al.* (*5*), and we will offer additional evidence that their data support the mechanism that we have discussed. They observed alloisoleucine to isoleucine ratios increasing monotonically to a value of 0.157 in the 500-cm depth of the core they examined, CH96-G12. An age of about 1.2×10^6 years was assigned to the bottom of the core (518 cm) on the basis of an extrapolation of the results of laboratory measurements of the rates of racemization of free isoleucine in buffered solutions at elevated temperatures (*5*). The alloisoleucine to isoleucine ratios were measured on hydrolyzates of total sediment samples. Our data show that the time constant for the racemization of free isoleucine is not an alloisoleucine to isoleucine ratio of peptide-bound amino acids. We observe an alloisoleucine to isoleucine ratio of

Table 4. Data on uranium and thorium isotopic composition of selected samples from core CH96-G12. Calcium carbonate samples were analyzed gasometrically; U and Th analyses were performed on totally dissolved samples by the techniques of Ku (*22*); dpm, disintegrations per minute; ppm, parts per million.

Depth (cm)	$CaCO_3$ (% by weight)	U (ppm)*	$^{234}U/^{238}U$	^{230}Th (ppm)*	^{230}Th (dpm/g)	^{230}Th excess† (dpm/g)
30	73			3.93 ± 0.25	10.8 ± 0.4	10.4 ± 0.4
60	71	0.63 ± 0.06	1.05 ± 0.11	3.76 ± 0.19	9.16 ± 0.25	8.65 ± 0.36
90	62	0.71 ± 0.04	1.03 ± 0.07	5.16 ± 0.26	9.68 ± 0.36	9.15 ± 0.40
200	79	0.46 ± 0.05	0.93 ± 0.12	2.22 ± 0.12	6.49 ± 0.20	6.16 ± 0.21
400	76	0.60 ± 0.02	0.92 ± 0.03	3.62 ± 0.20	3.87 ± 0.15	3.47 ± 0.25

* On a total sediment basis. † ^{230}Th excess = $^{230}Th_{total}$ (dpm/g) − U^{234} (dpm/g). ‡ Calculated on the assumption that [U] (ppm) = 2.18 ± .22 (average of four other samples) and $^{234}U/^{238}U = 1.00$; we were unable to measure [U] in this sample because of very low recovery.

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0.15 to 0.16 in samples about 125,000 years old, which would mean that core CH96-G12 has a sedimentation rate almost an order of magnitude greater than suggested by Bada *et al.* (*5*). Since the magnetic record is rather ambiguous (*5*), no definitive independent age estimates are available for this core. We undertook to obtain such information in order to provide a basis for comparison with our work. By the method of excess ^{230}Th (*22*) we conclude that core CH96-G12 has a sedimentation rate of about 3.1 cm per 1000 years, rather than the 0.42 cm per 1000 years proposed by Bada *et al.* Data are given in Table 4 and plotted in Fig 2. Calculations on the basis of excess ^{230}Th ($CaCO_3$-free) or excess $^{230}Th/^{232}Th$ give slightly higher sedimentation rates but somewhat poorer graphical plots. The coccolith *Emiliania huxleyi* (formerly *Coccolithus huxleyi*) is abundant at the 200- and 400-cm levels of core CH96-G12 (*23*), thus suggesting a sedimentation rate of at least 1.5 cm per 1000 years, since *E. huxleyi* made its evolutionary appearance about 250,000 years before the present (*24*). A high sedimentation rate for this core is not unreasonable since it is a core with a high-carbonate content (Table 4) taken from the floor of the Atlantis fracture zone, where localized "ponding" effects could be operative (*25*). The magnetic record of core CH96-G12 shows no reversals of inclination (*5*), thus suggesting that the age of the bottom of the core could be less than 690,000 years. Since core CH96-G12 has a high carbonate content, results of gross sediment analyses probably are similar to those that we have reported here for foraminifera. We analyzed the foraminifera from the 400-cm level of core CH96-G12 and found an alloisoleucine to isoleucine ratio of 0.158, as compared to the value of 0.127 found for the hydrolyzate of the total sediment samples (*5*). Without further analyses, it is difficult to attribute too much significance to the difference between the two results, although in situ contamination could be responsible for the difference. The data that we have obtained for core CH96-G12 put the results of Bada *et al.* (*5*) in substantial agreement with our own. If core CH96-G12 had as slow a sedimentation rate as originally calculated (*5*), we would predict that the "nonlinear" phenomenon would have been observed.

The above discussion indicates that the racemization of protein-derived amino acids in the geologic environment is not a simple process. The kinetics of racemization observed in hydrolyzates of fossil or sediment samples depend on a variety of time constants, none of which is easily evaluated. The rate of racemization of amino acids in both free and bound fractions, the rate of hydrolysis of peptide bonds, and the rates of diffusion of native or contaminating amino acids into or out of the system are all disguised in the simple mathematical expression of Eq. 2. Faced with the large number of parameters that can affect the apparent rate of racemization, we have chosen an entirely empirical approach and analyzed samples of known absolute age. In the present state of the art, reasonably reliable age estimates are available back to 400,000 years at deep-sea temperatures, and future work should concentrate on resolving the ambiguity of older samples. In any event, the use of racemization as a chronological tool will depend on a thorough understanding of the diagenetic reactions of amino acids in the particular system under study.

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6. Strictly speaking, this process is not racemization but epimerization. We use the term "racemization of isoleucine" rather loosely, referring only to the rearrangement about the α-carbon atom of the amino acid. We use the extent of epimerization of isoleucine as a measure of the degree of racemization of all the amino acids.
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8. We are presently determining the degree of racemization of other amino acids in these cores, in cooperation with K. A. Kvenvolden and E. Peterson.
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10. Tracers of norleucine, an amino acid not found in proteins, have been added to most of the samples. Generally recovery is between 70 and 90 percent, but occasionally recoveries as low as 20 percent are observed. Because of these problems, we do not place too much emphasis on the absolute abundance of any amino acid; ratios of amino acids are not susceptible to this problem, for the difficulty is entirely one of mechanical losses in the desalting, rather than selective degradation of particular amino acids.
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12. Analyses of 35 other core tops by similar techniques also show little or no alloisoleucine (< 1 percent) (K. King, personal communication).
13. By analogy with isotopic equilibrium, a simple 1/*T* relation might be expected

$$(Allo/Iso)_{eq} = 1 + -109/T \text{ (in °K)}$$

The constant 109 is fitted to the observed data. Equilibrium ratios no lower than 1.25 have been observed at 165°C, and ratios no higher than 1.4 have been observed at ambient temperatures.
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16. Exactly when this ratio reaches this value is not known, nor is the exact nature of the bound amino acids in ancient fossils very clear (*1*, *2*, *9*, *11*). We refer to the bound amino acids as "peptides" only very generally, for we have not confirmed the presence of peptides in the older samples.
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Computer Derivation of Some Dolphin Echolocation Signals

Abstract. *Recent advances in radar theory have given rise to a straightforward method of sonar signal design. The method involves computer maximization of a signal-to-interference ratio. The procedure has been used to derive sonar signals that can accurately measure target velocity. When two dolphins were placed in a situation conducive to the utilization of such signals, their waveforms were similar to those that had been theoretically derived.*

In active sonar and radar systems, information about a reflecting object is obtained by investigating the effect of the target upon transmitted waveforms. For many situations, it has been found that echoes can be adequately described as delayed, doppler-distorted versions of the transmitted signal. Such a description is analytically convenient, since the returned waveform is easily represented in terms of range and target velocity.

An autocorrelation process is generally used to test for the presence of a target with specific range and velocity. A correlation process can be defined as the product of two waveforms, integrated over time. For autocorrelation, an echo is multiplied by a signal identical to itself, and the product is integrated over the time duration of the signal. Since echo delay and doppler distortion are unknown a priori, the receiver must hypothesize them. For a correct hypothesis, the receiver experiences its maximum possible response. If a sonar signal is very sensitive to the range (or velocity) hypothesis, an incorrect guess about delay (or doppler distortion) will result in a comparatively small receiver response.

When received echoes are immersed in additive white noise, the autocorrelation process is optimal in the sense that it maximizes the ratio of output signal power to expected output noise power (for correct hypotheses). Correlation processors are usually implemented either by multiplication of stored (hypothetical echo) waveforms with an echo, followed by time integration, or by passing received signals through a bank of linear, time-invariant filters with appropriate impulse responses.

$$\mathrm{SIR} = \frac{E_s\,[\chi_{vu}^{WB}(0,1)]^2}{\binom{N_o}{2}\int |v(t)|^2 dt + E_s \int_0^\infty \int_{-\infty}^{\infty} p(\tau,s)\,[\chi_{vu}^{WB}(\tau,s)]^2 d\tau ds}$$

Fig. 1. Signal-to-interference ratio.

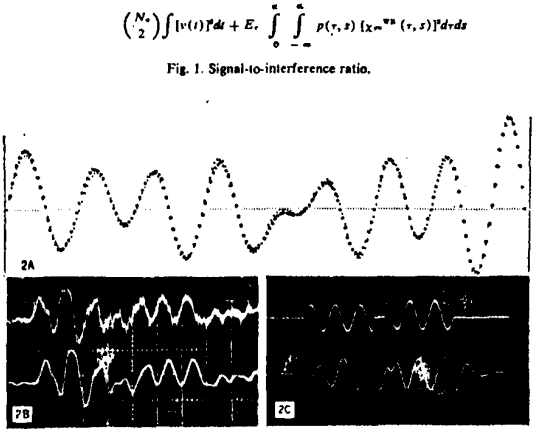

Fig. 2. (A) A computer-derived signal-filter pair for optimal velocity resolution. Heavy dots denote the signal function $u(t)$; small dots denote $v(t)$, the time-reversed filter impulse response. Note that the two functions almost coincide. (B) Top signal is an 8-msec signal used by one of two dolphins that had been newly introduced to a tank. Time scale is 1 msec per large division. Bottom signal is the same signal as above, low-pass filtered with high-frequency cutoff at 3 khz. (C) Top signal is a sinusoid with discontinuous amplitude. Bottom signal is a band-pass version of the above waveform.

It is sometimes advantageous to perform *cross* correlations rather than autocorrelations. An echo can be correlated with waveforms other than delayed, doppler-distorted versions of itself in order to obtain more accurate information about target motion or to better separate the target from a reverberatory environment. Cross correlation sacrifices signal-to-noise ratio for the sake of other benefits. For example, a slight decrease in maximum receiver response can sometimes be traded for better velocity resolution. The trade-off between maximum receiver response and other desirable system properties can be expressed by the formation of a signal-to-interference ratio.

Signal filter design by signal-to-interference ratio maximization can result in sonar waveforms that resemble certain dolphin signals. The purpose of these dolphin wave'orms can then be interpreted, and some tentative conclusions about dolphin signal processing can be deduced.

If a real, wide-band sonar signal $u(t)$ is reflected from a planar target traveling at velocity v_T, then the energy-normalized echo is written $s^{1/2}u[s(t+\tau)]$. The doppler scale factor s equals $(1 + v_T/c)/(1 - v_T/c)$, where c is the speed of sound. The translation variable τ describes the time delay experienced by the signal from transmission to reception (t). If the echo is processed by a linear filter with impulse response $v(-t)$, then the filter output is

$$\chi_{vu}^{WB}(\tau,s) = s^{1/2}\int_{-\infty}^{\infty} v(t)u[s(t+\tau)]dt \qquad (1)$$

$\chi_{vu}^{WB}(\tau,s)$ is called the wide-band uncertainty function of the signal-filter pair $u(t)$, $v(t)$. $[\chi_{vu}^{WB}(\tau,s)]^2$ is known as the wide-band cross-ambiguity function.

If a target is surrounded by an array of spurious planar reflectors (clutter) at various ranges and velocities, then the distribution of the unwanted reflectors can be written $p(\tau,s)$, where τ and s are measured relative to the desired echo. The target return is then written simply as $u(t)$, and $p(\tau,s)$ is a probability distribution function describing the clutter. If $p(\tau,s)$ were concentrated at $s=1$ and $\tau=\tau_0$, for example, then the clutter would consist of a single unwanted reverberation received τ_0 seconds after the target echo.

The power of the filter response to the desired signal (the reflection from

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the target at $\tau = 0$, $s = 1$) is proportional to $|\chi_{ww}(0, 1)|^2$, while the expected power of the response to clutter (2) is

$$\int_0^\infty \int_{-\infty}^{\infty} p(\tau, s) \cdot |\chi_{ww}(\tau, s)|^2 d\tau\, ds$$

In addition to signal and clutter returns, there is always a certain amount of additive noise that would be received even if no signal were transmitted. If the expected noise power per unit frequency interval is a constant ($N_0/2$) for all frequencies, then the expected filter response to the noise (2) is

$$(N_0/2) \int_{-\infty}^{\infty} [v(t)]^2 dt$$

By defining interference as the expected sum of system response to noise and to clutter, a signal to interference ratio (SIR) can be written (2–4) as shown in Fig. 1, where E_s is signal energy and E_c the total energy of clutter returns.

An optimal signal-filter pair is defined as one which maximizes SIR. Given a particular clutter distribution $p(\tau, s)$, along with E_s, E_c, and $N_0/2$, an optimal signal and filter can be found by using a computer (2–4). In order to utilize a computer, however, it is necessary to write the signal and filter functions in terms of a sum of orthonormal components,

$$u(t) = \sum_{i=1}^{N} a_i \phi_i(t)$$
$$v(t) = \sum_{i=1}^{N} b_i \phi_i(t) \qquad (2)$$

where

$$\int_0^T \phi_i(t)\phi_j(t)\, dt = \begin{cases} 1, & i = j \\ 0, & i \neq j \end{cases} \qquad (3)$$

In Eq. 3 it is assumed that the signal is time limited to the interval [0, T].

It is possible to simulate a clutter distribution $p(\tau, s)$ in order to design a signal with certain resolution properties (5). A velocity resolvent signal, for example, has a very small filter response for $s \neq 1$, compared with the maximum response $|\chi_{ww}(0, 1)|^2$. A velocity resolvent signal can be derived by placing clutter uniformly along the s-axis of the τ, s plane (except at the point $s = 1$).

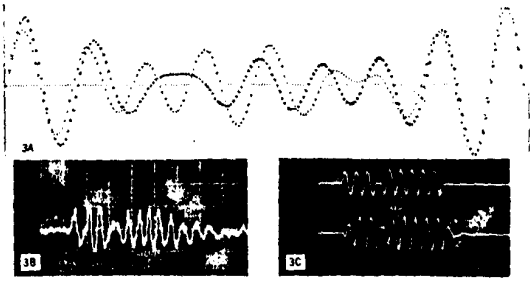

Fig. 3. (A) Another computer-derived signal-filter pair for optimal velocity resolution. Heavy dots denote the signal $u(t)$; light dots denote $v(t)$, the time-reversed filter impulse response. (B) A signal used by one of two dolphins that had been newly introduced to a tank. Time scale is 2 msec per large division. (C) Top signal is a sinusoid with discontinuous amplitude. Bottom signal is a band-pass version of the above waveform.

Maximization of the SIR for such a contrived clutter distribution would yield a signal-filter pair such that $[\chi_{ww}(0, s)]^2$ was small for most values of $s \neq 1$.

A computer maximization of SIR for clutter distributed uniformly in s has been accomplished. The orthonormal components ($\phi(t)$) consisted of five time-limited sine functions (6). Two resulting signal-filter pairs are shown in Figs. 2A and 3A, and are associated with the same value of SIR. The existence of two solutions indicates that waveform pairs which maximize SIR are not necessarily unique. The problem is complicated by the existence of many waveform pairs that provide local maxima of the SIR. Out of several such solutions, the waveforms given here provide the largest value of SIR. There is no guarantee, however, that these waveforms represent a global maximum. The waveforms in Fig. 2A are plots on [0, T] of $u(t) = .172 \sin(3\pi t/T) + .408 \sin(11\pi t/T) + .544 \sin(15\pi t/T) + .608 \sin(17\pi t/T) - .373 \sin(18\pi t/T)$; $v(t) = .140 \sin(3\pi t/T) + .434 \sin(11\pi t/T) + .498 \sin(15\pi t/T) + .598 \sin(17\pi t/T) - .432 \sin(18\pi t/T)$. The waveforms in Fig. 3A are pictures of $u(t) = .483 \sin(13\pi t/T) + .129 \sin(14\pi t/T) + .595 \sin(15\pi t/T) - .243 \sin(16\pi t/T) + .581 \sin(17\pi t/T)$; $v(t) = .492 \sin(13\pi t/T) - .314 \sin(14\pi t/T) + .367 \sin(15\pi t/T) + .023 \sin(16\pi t/T) + .724 \sin(17\pi t/T)$ (7).

Since clutter is likely to be distributed in range as well as velocity, it would seem that range clutter, as well as clutter at $\tau = 0$, should be taken into account. The inclusion of range clutter, however, is not really necessary. The cross-ambiguity functions of the illustrated solutions reveal that for $0.98 \leq s \leq 1.02$,

$$\max_{\tau} [\chi_{ww}(\tau, s)]^2 \sim [\chi_{ww}(0, s)]^2$$

In other words, moving targets with velocities less than about 35 miles per hour are not likely to be confused with stationary targets at a different range. The signals' cross-ambiguity functions thus indicate that there is little to be gained by including range clutter at $\tau \neq 0$. If range clutter were added, different waveforms would result. Such waveforms would needlessly sacrifice signal-to-noise ratio and doppler resolution if targets of interest travel at speeds less than 35 miles per hour (30 knots) (8).

A narrated tape recording of dolphin (*Tursiops truncatus*) echolation signals was supplied by D. K. Caldwell (University of Florida, St. Augustine). The recording contained many different waveforms. Occasionally, however, a specific pulse shape was repeated many times with relatively little variation. One such situation was observed when two young females had been newly introduced to a tank. Typical waveforms are shown in Figs. 2B and 3B. The illustrated signals were produced as isolated waveforms (rather than in a train of closely spaced pulses). The signals have substantially longer time durations than are usually associated with dolphin echolocation (9, 10). Waveforms having such long duration

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have, however, been obtained by Ayrapet'yants *et al.* (*11*), who classified them as echolocation pulses.

The waveforms in Figs. 2B and 3B are similar to the velocity resolvent signals derived by SIR maximization.

Velocity-sensitive echolocation waveforms are well suited to a situation characterized by (i) unfamiliarity with the environment and (ii) a second (moving) animal in the tank. With no information about environmental reverberation, a reasonable way to detect a moving target in stationary clutter is to use a waveform that recognizes the target by its motion. In radar theory (*12*), such a strategy is commonly called moving target indication.

A laboratory simulation of the dolphin waveforms can be accomplished by band-pass filtering a time-gated sinusoid. The bottom signals in Figs. 2C and 3C were obtained by band-pass filtering the waveforms immediately above them. In order to simulate the waveform of Fig. 2B, the sinusoid of Fig. 2C (top signal) must have a frequency of $f_0 = 1$ khz and be band-pass filtered with high- and low-frequency cutoffs $f_H \sim 2$ khz, $f_L \sim 0.8$ khz, respectively. For Fig. 3C, $f_0 = 0.86$ khz, $f_H \sim 3.43$ khz, and $f_L \sim 0.62$ khz.

It is interesting to compare the above frequencies with the frequency range that dolphins actually have at their disposal. According to Johnson (*13*), *Tursiops* is capable of utilizing frequencies between $f_L \sim 0.1$ khz and $f_H \sim 150$ khz. Most of the signal energy in Figs. 2B and 3B is therefore restricted to a comparatively narrow band. It is the *ratio* of bandwidth to carrier (centroid) frequency, however, that is important to deciding whether a signal is narrow band or wide band (*6*, *14*). By such a criterion, the dolphin signals are extremely wide band. Because of the large bandwidth-to-carrier frequency ratio, the doppler effect must be described by a compression factor s rather than by a frequency shift.

The reason for the dolphins' use of such a narrow frequency range is an open question. A possible explanation is that the signal is meant to detect objects at comparatively large distances. Because attenuation in water increases with frequency, a low-frequency waveform with small bandwidth is most likely to retain its structure and strength over long distances. Since the maximum output of a correlation processor is proportional to echo energy, a signal with comparatively long time duration is also to be recommended for long-distance propagation (if correlation is used).

It has been shown that optimum wide-band waveforms for velocity resolution are similar to certain dolphin signals. The optimum waveforms were derived by assuming a cross-correlation processor, that is, a filter whose output is the integrated product of input signals with a time function that may not be identical to the waveform that is sought. The resemblance between Figs. 2A and 2B, and 3A and 3B, would seem to suggest that *Tursiops truncatus* uses a generalized form of correlation processing. Similar evidence, for autocorrelation processing, has been found for the little brown bat (*Myotis lucifugus*) (*6*, *15*) and the red bat (*Lasiurus borealis*) (*6*).

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6 May 1971

Fate of Air Pollutants: Removal of Ethylene, Sulfur Dioxide, and Nitrogen Dioxide by Soil

Abstract. *The ultimate sink for many air pollutants is unknown. Data are presented here in support of the idea that reaction with soil, through microbial or chemical means, can remove ethylene, other hydrocarbons, sulfur dioxide, and nitrogen dioxide from the air.*

Large quantities of ethylene, an air pollutant and plant hormone, are produced in the United States each year. Table 1 presents data on the relative amounts of ethylene produced by natural sources and human activities. In

Table 1. Ethylene emissions from major sources in the United States, 1966

Source*	Quantities utilized ($\times 10^6$ tons)	Emission factor†	Ethylene emissions ($\times 10^6$ tons)
Coal combustion	486	0.001	0.5
Fuel oil combustion	235	.001	.2
Motor fuels			
Gasoline	280	.05	14.0
Diesel	27	.009	0.2
Jet	28	.001	.03
Refuse burning			
Good incineration	8	.000025	.0002
Poor incineration	16	.015	.24
Open burning	56	.001	.06
Ethylene production from industrial leakage‡	11.2	0.01	0.1
Vegetation§	2000	.00001 ‖	20

* Values for sources except leakage from industry obtained from (*8*). † Estimated values; information from (*9*). ‡ From (*9*). § Assumes a ton of vegetation per acre and an area of 3×10^6 square miles for the United States. ‖ Calculated on the assumption of 0.5 nl of ethylene produced per gram (fresh weight) per hour, 24 hours a day, 365 days a year.

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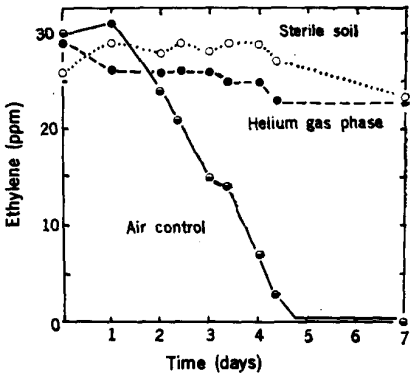

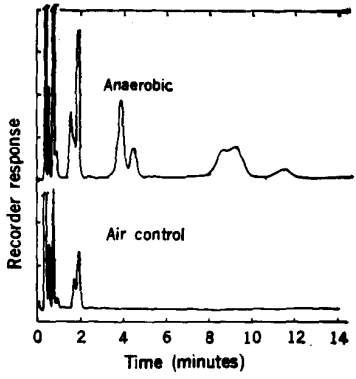

Fig. 1 (left). Effect of soil on concentrations of ethylene in air. Soil samples of 50 g were either autoclaved or used as controls and incubated in 2.5-liter desiccators in the presence of air or helium. Fig. 2 (right). Gas chromatographs showing reduction in the concentrations of hydrocarbons from dilute auto exhaust in the presence of soil after 5 days. The initial distribution of hydrocarbons was similar to that of the anaerobic sample.

1966 15×10^6 tons (13.5×10^6 metric tons) of ethylene were released as a pollutant, whereas only 11.2×10^6 tons were produced as a raw material for industry, and the amount produced by plants was only 0.13 percent of that produced by man. As a comparison, estimates for the total production of hydrocarbons, sulfur dioxide, nitrogen dioxide, and carbon monoxide are 32×10^6, 33×10^6, 20.6×10^6, and 100×10^6 tons, respectively (*1*). However, even though large quantities of ethylene are produced, concentrations in nonurban areas do not increase and remain low—< 5 parts per billion (ppb) (5.75 $\mu g/m^3$). Two mechanisms for ethylene degradation are known: oxidation by ozone and reaction with an oxide of nitrogen in the light (*2*). The purpose of this report is to show that, in laboratory experiments, soil acts as an important sink for ethylene through microbial degradation, and for sulfur dioxide and nitrogen dioxide primarily through chemical action.

Soil samples (50 g for ethylene, 250 g for sulfur dioxide and nitrogen dioxide) were placed on petri plates in desiccators (2.5-liter capacity for ethylene, 10-liter capacity for sulfur dioxide and nitrogen dioxide) equipped with sleeves on their lids. Gases were introduced and removed through a rubber vaccine cap fitted on the side arm of the lid collar. Soil samples for ethylene uptake were obtained from Beltsville and Frederick, Maryland, and soil samples for sulfur dioxide and nitrogen dioxide uptake were taken from Waltham, Massachusetts. The concentrations of ethylene and other hydrocarbons were measured by flame-ionization gas chromatography on an alumina column, sulfur dioxide was measured according to the method of Adams *et al.* (*3*) with the use of a fixed gas volume, and nitrogen dioxide was measured according to the evacuated bottle method of Saltzman *et al.* (*4*).

Figure 1 shows the effect of sterilization (110°C for 15 minutes) and anaerobiosis (helium gas phase) on the uptake of ethylene by soil. Ethylene uptake by soil does not occur in the absence of oxygen. A ratio of oxygen to nitrogen of 2 percent to 98 percent (by volume) satisfied the oxygen requirement, and 1 percent oxygen resulted in a rate of ethylene uptake equal to half the maximum rate. A ratio of carbon dioxide to nitrogen of up to 10 percent to 90 percent had no effect on the rate of ethylene consumption. Inhibition of ethylene uptake was also observed when the soil was sterilized with ethylene oxide, an indication that the effect of autoclaving was not due to thermal modification of the soil structure. As shown in Fig. 1, a 24-hour lag in uptake occurred after ethylene was added to the gas phase. A similar lag was not observed when subsequent amounts of ethylene were added. The fact that bacteria are known to consume hydrocarbons, including ethylene (*5*), and the fact that ethylene uptake by soil is heat-sensitive, requires oxygen, and occurs after a lag period suggests that microorganisms are the major sink for ethylene. The ability of soil to reduce ethylene concentrations is extremely effective. The soil atmosphere in Beltsville was free of measurable amounts of ethylene (5 ppb is the limit of detection), whereas concentrations in the air above vary from 10 to 40 ppb.

Figure 2 shows that soil is able to absorb a number of hydrocarbons in the presence of oxygen. The soil sample completely removed some hydrocarbons and partially removed others from a sample of dilute auto exhaust. A curve similar to the one labeled "anaerobic" was observed when the experiment was initiated. Although we were unable to identify all the peaks, we know that the normal composition of hydrocarbons is reported to be 27 percent acetylene, 20 percent ethylene (third peak from the left), 8 percent propene, 7 percent butane, 5 percent toluene, 5 percent of the various isomers of pentane, and various amounts of 42 other gases (*6*).

The soil was also able to remove sulfur dioxide and nitrogen dioxide. The rate of removal of these gases was faster than that observed for ethylene. Sulfur dioxide was reduced from 100 to 8 parts per million (ppm) in 15 minutes in the presence of soil. Autoclaving the soil prior to the experiment reduced the rate of uptake to 20 ppm after 15 minutes. The reaction with nitrogen

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dioxide was slower. Concentrations of nitrogen dioxide dropped from 100 to 3 ppm after 24 hours, and autoclaving the soil reduced the uptake to 13 ppm after 24 hours. These results indicate that the major portion of sulfur dioxide and nitrogen dioxide is removed by some chemical reaction. However, a microbial contribution cannot be entirely excluded.

Uptake of carbon monoxide by soils was recently demonstrated by Inman *et al.* (*7*). They found that their soil samples reduced carbon monoxide concentrations from 120 ppm to near zero within 3 hours.

Extrapolating from our laboratory experiments, we calculated that 7×10^6 tons of ethylene may be removed from the air in the United States by means of microbial degradation. Since the rate of production (15×10^6 ton/year) exceeds uptake by soil by a factor of 2, other mechanisms of degradation are probably important. The uptake of sulfur dioxide and nitrogen dioxide was more rapid that that of ethylene. Our calculations yield values of 6×10^9 tons of nitrogen dioxide and 4×10^{10} tons of sulfur dioxide per year. These values are large enough to accommodate all the sulfur dioxide and nitrogen dioxide produced in the United States (sulfur dioxide = 32×10^6 tons, nitrogen dioxide = 33×10^6 tons) each year.

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Eight-Membered Cyclosilicate Rings in Muirite

Abstract. *The determination of the crystal structure of muirite, $Ba_{10}(Ca,Mn,Ti)_4Si_{17}O_{51}(Cl,OH,O)_{10} \cdot 4H_2O$, revealed the presence of discrete cyclic silicate anions, $(Si_8O_{24})^{16-}$, formed by the condensation of eight silicate tetrahedra. This first reported occurrence of eight-membered rings is of particular interest, because rings with eight tetrahedra are reported to be energetically less stable than rings with six tetrahedra, which have been found in many minerals.*

Muirite has been found with many other barium-containing silicate minerals in metamorphic rocks in Fresno County, California. Its composition has been given as $Ba_{10}Ca_2MnTiSi_{10}O_{30}(OH,Cl)_{10}$. On the basis of the chemical analysis and the x-ray data supplied with the original description, it is not apparent to which group of silicates muirite belongs (*1*). Because of its tetragonal symmetry the formulation $Ba_8CaTiO_4(Si_2O_{12}) \cdot 3H_2O$, with four-membered silicate rings, has been proposed (*2*).

A crystal of muirite (*3*) of approximately isometric shape (0.2 by 0.2 by 0.2 mm) was used for data collection on an automatic four-circle diffractometer with graphite crystal–monochromatized AgKα radiation (wavelength $\lambda = 0.56083$ Å). From the usual processing and averaging (*4*) of 10,476 measured intensities 978 unique observed structure factors F_{obs} were obtained (maximum $\sin\theta/\lambda = 0.75$ Å^{-1}). No absorption correction was applied to the observed data (absorption coefficient $\mu = 53$ cm^{-1}; the maximum corrections would have been ± 8 percent on F_{obs}^2). The unit cell parameters (the figures in parentheses represent the standard deviations in units of the least significant digit) are $a = 14.030(10)$ Å and $c = 5.635(4)$ Å. Because no systematic absences of intensities have been observed, the space groups *P4/mmm*, *P4mm*, *P422*, *P$\bar{4}$m2*, and *P$\bar{4}$2m* are possible (*1*). The structure was solved by Patterson methods in space group *P4/mmm* and refined by full-matrix least-squares analysis to a discrepancy index R of 0.065 for all observed reflections. In the calculation the X-sites were assumed to be populated only by calcium atoms. The relatively low value of the isotropic temperature factor B (Table 1) for the X-sites reflects partial occupancy by Ti and Mn, as also evidenced by the chemical analysis (*1*). The occupancy factors of 0.5 for Ba(3) and O(2) (Table 1) are based on the Fourier maps and on the least-squares refinement. They may possibly be related to either a lower-symmetry space group for muirite (*P4mm*) or to the occurrence of a superstructure, since reflections on highly overexposed precession photographs indicate a doubling of the c cell constant. Because of the inconclusive evidence for either of these possibilities, we are reporting here the structure in space group *P4/mmm* and are ignoring the superstructure reflections. Thus the statistical occupation of these two atomic sites either may reflect true disorder or may mean that we are describing an averaged structure. The fact that the temperature factors of most of the atoms (Table 1) in the structure are fairly high is another indication that some kind of disorder is present.

The eight-membered rings, $(Si_8O_{24})^{16-}$, of silicate tetrahedra, centered around the origin of the unit cell (Fig. 1), are the most striking and unusual feature of this crystal structure. The eight-membered rings are connected to neighboring rings both in the a and the c directions by the trigonal prismatic coordination polyhedra around the X-sites (populated by Ca, Mn, and Ti), thus forming a polyhedral three-dimensional framework of tetrahedra and trigonal prisms of composition $(Ca,Mn,Ti)_4Si_8O_{24}(OH,O)_4$. The spaces around this framework are filled with barium and chlorine atoms and H_2O molecules, so that the correct chemical and structural formula of muirite must be $Ba_{10}(Ca,Mn,Ti)_4Si_8O_{24}(Cl,OH,O)_{12} \cdot 4H_2O$ with one formula unit per unit cell. The assignment of particular atomic species to the different equipoints (Table 1) is based on the following: (i) the observed interatomic distances, (ii) the scattering factors giving the best agreement between observed and calculated structure factors, (iii) the multiplier refinement, and (iv) partly on the original chemical analysis (*1*).

The main differences between the original formulation (*1*) and the one arrived at here are that in this formulation: (i) there are only eight silicon atoms per unit cell, and the structure does not contain any space in which additional silicon atoms would fit; (ii) there is more chlorine per formula unit; and (iii) although most of the O(h4) atoms must be OH groups (in

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virtue of considerations of the bond strengths), some of them must be oxygen atoms, because the chemical formula would otherwise not be balanced.

Eight-membered rings have not been known up to now to occur in cyclosilicates. Electrostatic energy calculations (5) have shown that eight-membered rings should be energetically less stable than six-membered rings, which are found in many mineral crystal structures. However, they should be more stable than either three- or four-membered rings (5), which have been described previously in a number of minerals. The reason for this apparent paradox may be that three- and four-membered cyclosilicate rings pack more efficiently in the crystal structures than eight-membered rings. The presence of the large cations (barium) and large anions (chloride) in muirite may also be a stabilizing influence: these ions fill the large open spaces between the bulky eight-membered rings.

The average Si–O distance in the silicate tetrahedron is 1.629 Å, whereas the individual distances are as follows: Si–O(1), 1.658(9) Å; Si–O(2), 1.654-(11) Å; and 2 × Si–O(3), 1.601(8) Å (2 × refers to the fact that two of the Si–O distances in the silicate tetrahedron are symmetrically equivalent). The deviations of the individual Si–O distances from the mean are significant. Moreover, their variation is consistent with the extended electrostatic valence rule (6), according to which

$$d(\text{Si–O})_{\text{calc}} = [d(\text{Si–O})_{\text{mean}} + 0.091\,\Delta p_0]$$

where $d(\text{Si–O})_{\text{calc}}$ is the calculated Si–O distance within the tetrahedron (in angstroms), and Δp_0 is the difference between the bond strength of the individual oxygen atom and the mean bond strength of all oxygen atoms within the tetrahedron. Since O(1), O(2), and O(3) have Pauling bond strengths of 2.13, 2.31, and 1.71 valence units, respectively, the values calculated for the Si–O(1), Si–O(2), and Si–O(3) distances are 1.644, 1.661, and 1.606 Å, respectively, in reasonable agreement with the observed values. The angles Si–O(1)–Si and Si–O(2)–Si are, respectively, 121(1)° and 154(1)°. The angle of 121° is one of the smallest ever observed for a bridging oxygen atom in a condensed silicate. According to simple d-p π-bonding theory (7), this value would mean that the Si–O(1) distance should be considerably longer than the Si–O(2) distance. Since that is not the case, this model of d-p π-bonding does not seem to apply to this silicate.

Table 1. Positional and thermal parameters for muirite (C.N. = coordination number; estimated standard deviations in units of the least significant digit are given in parentheses).

Atom	Equipoint	C.N.	Refined coordinates			B (Å²)
			x	y	z	
Ba(1)	4n	10	0.5000	0.1656 (1)	0.0000	1.15 (3)
Ba(2)	1a	16	.0000	.0000	.0000	3.3 (1)
0.5 × Ba(3)	2h	8	.5000	.5000	.2447 (8)	1.76 (7)
Ba(4)	4k	11	.2671 (1)	.2671	.5000	1.14 (3)
X (= Ca,Mn,Ti)	4m	6	.3635 (3)	.0000	.5000	0.5 (1)
Si	8p	4	.2654 (3)	.1030 (3)	.0000	0.97 (6)
O(1)	4i	3	.2074 (11)	.0000	0.000	1.7 (3)
0.5 × O(2)	8r	4	.1801 (9)	.1801	.0643 (31)	1.3 (3)
O(3)	16u	4	.3289 (5)	.1141 (5)	.2345 (14)	1.9 (2)
O(h4) (= OH, O)	4j	4	.5000	.0000	.2419 (38)	3.7 (4)
O(w5) (= H_2O)	4k	3	.0977 (11)	.0977	.5000	4.7 (5)
Cl(1)	4o	5	.5000	.2777 (4)	.5000	1.6 (1)
Cl(2)	4j	5	.3525 (3)	.3525 (3)	.0000	1.5 (1)

The trigonally prismatic six-coordination around the X-sites is the second unusual feature of the muirite structure [4 × X–O(3), 2.244(8) Å; and 2 × X–O(h4), 2.338(13) Å]. In predominantly ionic compounds, such as muirite, the six-coordinated cations tend to have an octahedral environment. Trigonally prismatic coordinations usually are found in compounds with covalent bonding character such as MoS_2, WC, and AlB_2. None of the three kinds of atoms that are populating the X-sites (Ca, Mn, or Ti) appears to have been known to occur in such coordination.

The coordination polyhedra around the Ba(1) atom [4 × Ba(1)–O(3), 2.834(8) Å; 2 × Ba(1)–O(h4), 2.753-(12) Å; 2 × Ba(1)–Cl(1), 3.226(3) Å; and 2 × Ba(1)–Cl(2), 3.340(3) Å] and the Ba(4) atom [2 × Ba(4)–O(2), 3.001(15) Å; 4 × Ba(4)–O(3), 2.756-(8) Å; 1 × Ba(4)–O(w5), 3.362(12) Å; 2 × Ba(4)–Cl(1), 3.271(6) Å; and 2 × Ba(4)–Cl(2), 3.288(2) Å] are typical for this cation, both with regard to coordination numbers and interatomic distances. The other two barium atoms are coordinated atypically. The Ba(2) atom is surrounded by 16 oxygen

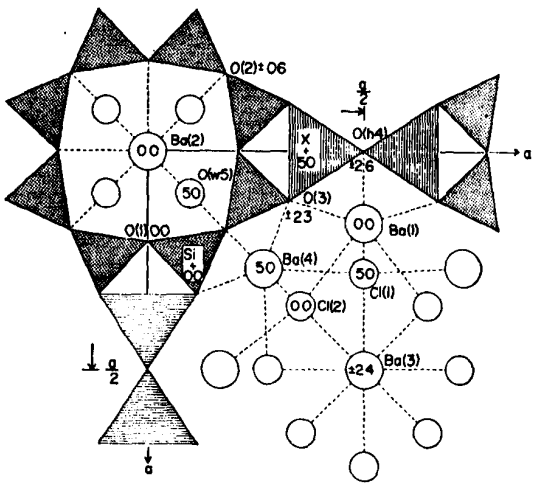

Fig. 1. Crystal structure of muirite viewed parallel to the [001] direction. The coordinations around silicon and X are shown as polyhedra. The bonds to the barium atoms are indicated by broken lines. The heights of the atoms are indicated in $z \times 100$.

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atoms at an average distance of 3.335 Å [4 × Ba(2)–O(1), 2.910(16) Å; 4 × Ba(2)–O(2), 3.592(9) Å; and 8 × Ba(2)–O(w5), 3.419(7) Å]. Eight chlorine atoms form square antiprisms around Ba(3) which share with each other a square face [4 × Ba(3)–Cl(1), 3.435(5) Å; and 4 × Ba(3)–Cl(2), 3.235(3) Å]. This brings the Ba(3) atoms to within 2.76 Å of each other. However, the Ba(3) atoms have an occupancy factor of 0.5, which means that statistically only every other Ba(3)-site is occupied, and therefore the Ba(3) atoms actually do not have to be in this close contact.

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Early Silurian Spore Tetrads from New York: Earliest New World Evidence for Vascular Plants?

Abstract. *Several taxa of abundant cutinized trilete spores from earliest Silurian shale in New York predate by almost an entire period vascular land plant megafossils. Paleoecological evidence suggests that these spores may represent vascular land or semiaquatic plants but a bryophytic origin cannot be precluded on the basis of spore characters. An algal origin is considered unlikely.*

Extracted samples of the Medina Group (Lower and Middle Llandovery, basal Silurian) of western New York have yielded abundant spore tetrads that may provide some of the earliest evidence for the presence of vascular land plants. This record, to our knowledge, is preceded only by the recent discovery of rare triradiate spores, tracheids, plant tissues, and cuticle fragments from the Middle Ordovician (Llanvirnian Sarka Formation) of the Bohemian Massif (*1*). If the Bohemian remains can be authenticated, they will provide the earliest evidence of vascular plants (*2*).

Sandstone and shale of the Medina Group are exposed in a section along the Niagara River, about 11 km to the north of Niagara Falls at the Robert Moses Power Plant site. In this region, the Medina Group overlies the Late Ordovician Queenston Formation. The basal unit of the Medina is the Whirlpool Sandstone ("White Medina"); above it (in ascending order) are the Power Glen Formation ("Gray Medina") and the Grimsby Formation ("Red Medina"). The uppermost Medina Group unit is the Thorold Sandstone, above which is the Neahra Shale.

Berry and Boucot (*3*) review the available evidence for correlation of these Silurian units. Neither the Medina units nor the Queenston or Thorold formations of this area have yielded zonable invertebrate megafossils (*3*). All are considered to be largely nonmarine, estuarine, or very nearshore marine on the basis of their physical characteristics, their lithofacies relationships with richly fossiliferous, zonable, wholly marine units occurring to the west on the northern rim of the Michigan Basin, or the extreme rarity and restriction to certain limited horizons of marine invertebrate megafossils. Microfossil evidence (discussed below) and the presence of infrequent marine invertebrates (*4*) suggest that the shales of the Medina Group were indeed deposited in a marine environment, although possibly near shore in a situation unsuited for the presence of abundant marine shelly invertebrate megafossils.

Shale from the Whirlpool, Power Glen, and Grimsby of the Medina Group, as well as from the Queenston and Neahga, were extracted for plant spores and other organic microfossils, by using several of the chemical and other methods described in detail by Gray (*5*) (Table 1). No organic microfossils of any type were recovered from three extracted samples of the Queenston; marine organic microfossils including scolecodonts, acritarchs, and chitinozoans were, however, recovered from the Neahga [see also (*6*)].

The three Medina Group samples provided spore tetrads (*6*) and other organic microfossils in a hash of organic material, much of which appears to be highly carbonized. Other organic objects suggestive of plant origin, such as cuticular tissues of the type common in abundantly sporiferous Late Silurian samples from Gotland (*7*), were not found in any of the samples. The Gotland samples, however, have spore tetrads reminiscent of the simple tetrahedral type described herein, in addition to single spores with triradiate laesurae.

The Medinan spore tetrads are most often dark brown, or even opaque, and quite brittle. They are arranged in the conventional tetrahedral tetrad configuration (Fig. 1) common to the development of most modern pollen and to some spores of both vascular and nonvascular plants, which indicates that the precursor spore mother cell underwent meiotic division. The individual spores are arranged in two planes and appear to be at the apices of a pyramidal tetrahedron. Where individual spores have broken away from the tetrad, a generally distinct, clearly marked triradiate scar is present, with the simple rays extending to the equator of the spore. The tetrads range in size from approximately 18 to 50 μm, most being in excess of 20 μm, and have a smooth, faintly scabrate, or microspinous surface (Figs. 1 and 2). Some few appear to be reticulate (Fig. 1). A denser marginal coloration on some of the spores suggests real differences in wall thickness or even the possibility of an equatorial thickening. These morphologic variations appear to indicate a greater diversity of spore taxa than have been previously recorded from the Llandovery. Some clusters of spore tetrads may have come directly from sporangia without breaking up into individual tetrad units, although such clusters may also indicate clumping of the type that is known to occur among the pollen of certain modern plants (*8*).

Spore tetrads are common to abundant as compared with other organic microfossils in both the Whirlpool and Power Glen formations; in the Grimsby, however, spore tetrads are rare compared with other microfossils (Table 1). The other recognizable organic micro-

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fossils (acritarchs, scolecodonts, chitinozoans) are considered to be of marine origin. Their presence, if indigenous, supports the conclusion that the Medina shales are marine, although the generally low abundance of most forms and the scarcity of marine invertebrates argue for a nearshore origin (9).

Are the spore tetrads the reproductive structures of vascular plants of terrestrial or perhaps semiterrestrial origin? Are any deductions possible from the microfossil assemblage or from the environmental occurrence and morphology of the spores that support their reference to early vascular plants?

There are no credible pre-Silurian megafossils attributable to vascular plants. Spores, tracheids, plant tissues, and cuticle fragments have, however, been reported from the Middle Ordovician of Bohemia (*1*), although this occurrence is yet to be authenticated. Within the Silurian, the few megafossils attributed to vascular plants commonly lack definitive criteria such as cutinized spores, tracheids, and epidermal cuticle with stomates; such remains are known only from Late Silurian occurrences in Britain (*10*), Czechoslovakia (*11*), Podolia (*12*), and New York (*13*). However, dispersed spores with distinct triradiate scars whose vascular plant origin is commonly assumed are more abundant and more widely recorded from the Silurian of Pennsylvania [(*14*); see also Table 1, Bloomsburg Formation], New York (*6, 15, 16*), Virginia (*17*), Libya (*18*), Britain (*19*), Spain (*20*), Algeria (*21*), and Gotland and Scania (*7*). Although additional Silurian vascular plant megafossils may be discovered, it is a reasonable assumption from their known very limited fossil record that dispersed Silurian spores will always outnumber megafossil remains, just as younger spores and pollen occur more commonly than coeval plant megafossils because they are produced more abundantly and are dispersed more widely in marine and nonmarine sedimentary rocks. The earliest, generally accepted evidence for a vascular land flora is the presence of a few isolated spores from the Lower Silurian (near the Early Llandovery–Middle Llandovery boundary) of Libya (*18*). The recently reported occurrence of spores and other microscopic remains attributed to land vascular plants in the Middle Ordovician of Bohemia (*1*) predates acceptable megafossil remains by possibly 40 million years. The scarcity of credible vascular land-plant megafossils can-

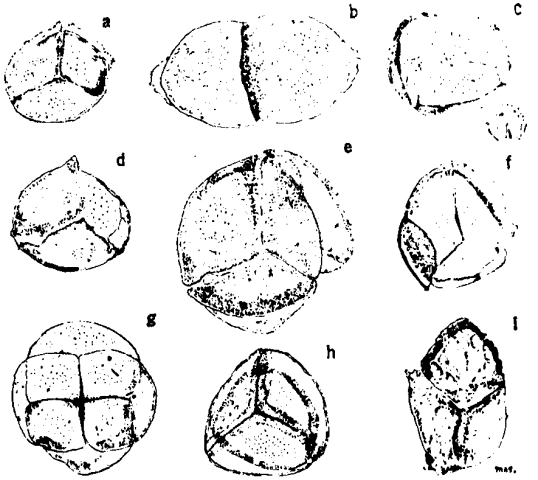

Fig. 1. Organic microfossils from the Medina Group (Lower Llandovery) of New York (× 750). Specimens illustrated are all from the Power Glen Formation. (a, c–i) Plant spores. (b) Dyad, of unknown affinity. (a, d) Single spores with distinct triradiate scar: (a) with remnants of possible perispore; (d) part of proximal surface bounded by triradiate scar broken away. (c) Spore with distinct triradiate scar, an apparent equatorially thickened wall, and small surface clavae shown in greater detail in generalized enlargement. (e, f) Spores with remnants of other members of the spore tetrad still adhering; (e) appears to have surface clavae similar to those of (c). (g–i) Spore tetrads in tetrahedral configuration: (i) tetrad with surface reticulum.

not in itself constitute prima facie evidence that widespread and abundant dispersed spores do not have a vascular plant source.

In attempting to determine the affinities of dispersed Silurian spores with vascular plants, lack of supporting evidence from vegetative and other remains throws the burden of proof mainly on spore characters. Neither the presence of spores in tetrad groups, nor the presence of a proximal triradiate scar, nor the presence of a cutinized cell wall that will withstand the rigors of preservation and chemical extraction is definitive evidence that isolated spores were produced by vascular plants (*2*), although the correlation between these features and the spores of living vascular land plants is high (*22*). Certain algae, especially among the red algae (Rhodophyta; subclass Florideae) produce tetrasporangia with four nonmotile spores in tetrahedral configuration that may bear rudimentary triradiate scars. Although phycologists have expressed skepticism that any *living* red algae (*23*) possess spores capable of preservation similar to the Silurian spores here described, it would be unwise to conclude that Silurian shallow-water or nonmarine algae possessing such wall structures did not exist. The known record of fossil red algae provides little help, for all Paleozoic red algae are lime-secreting forms. Coralline algae belonging to the Rhodophyta are common in the Late Cretaceous and Tertiary, but only one genus is known from the Silurian (*24*). The known fossil record together with certain highly specialized reproductive features suggests a very long geologic history for the red algae (*25*), for which the spores described here might provide some evidence. Nor can the possibility of a bryophytic origin for these Silurian spores be ignored. This group produces cutinized spores in tetrahedral tetrad or individual spores may display a prominent triradiate scar. Bryophytes have a megafossil record that stretches back into the middle Paleozoic (*26*), although spores attributed to bryophytes are

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recognized in the early Mesozoic. Both records probably poorly reflect the geologic history of this group of nonvascular land plants. Although it is tempting to conclude that the abundant, simple Early Silurian spore tetrads from the Medina Group represent the dispersed reproductive structures of primitive vascular plants, a more comprehensive survey of algal and bryophytic reproductive structures, both living and fossil, is necessary before a completely definitive statement is possible concerning the affinities of our Silurian material on the basis of spore morphology alone.

Although many Silurian spores, including our Medinan specimens, are unaccompanied by confirming plant remains (tracheids, cuticle), Evitt (*16*) finds cuticle fragments in the Maplewood Shale (New York), where spores are also reported by Cramer (*15*), and similar fragments plus spores and tracheids in the Williamson Shale (New York), both of Late Llandovery age and only a little younger than our Medinan material. If the complex of characters taken to define the vascular plant condition did not arise until the Silurian (*27*), it would appear that they arose almost simultaneously. On a theoretical basis, however, one might speculate that in the shallow-water marine or semiterrestrial state, which may have



Fig. 2. Microphotograph of a spore tetrad (magnification, approximately × 1200). [Harrison Howard]

preceded full land entry by many millions of years, cutinized spores able to withstand periodic desiccation and thus ensure reproduction might well have appeared prior to either the vascular system or the cutinized epidermal wall so necessary for wholly land existence. In this connection, the apparent absence of cutinized spores in the wholly aquatic modern algae, as well as the loss through reduction of a cutinized wall in pollen of living vascular plants whose pollen operates completely submerged (*8*, *28*), is noteworthy. Since cutinization primarily assures against desiccation, there is no functional reason for wholly submerged spores or pollen to be cutinized. The presence of a cutinized wall in our Early Silurian spores strongly suggests that they belonged to either terrestrial or semiaquatic plants, which, in the latter case, bore their sporangia out of water.

If a widespread, nearshore, shallow marine or nonmarine primitive vascular flora existed during the Early Silurian, as we hypothesize, its remains might be expected to be extremely abundant in sedimentary rocks from localities representing that environment, as opposed to samples from areas either farther away on the dry land or farther offshore in deeper water. However, previously reported rare Llandovery through Ludlow age spore occurrences are from relatively offshore marine environments; common to abundant latest Silurian (Pridoli age) spores, by contrast, are from relatively nearshore marine environments. None represents wholly continental environments. This coincidence raises the possibility (*29*) that the published record of Silurian spore occurrences to date reflects environmental control (and inadequate sampling) as much as evolution and development of the Silurian flora in time. The examination of over 75 diverse Early and Late Silurian rocks from New York, Pennsylvania, Ohio, and Sweden [Gotland

Table 1. Age, location, and organic microfossil content (c, common; r, rare) of extracted Silurian rock samples from New York and Pennsylvania.

Age	Formation	Locality	Results
Ludlow, Late Silurian	Bloomsburg Formation	Mount Union Section, Mount Union, Pa. Field 8, Laboratory 562	Spores (r) Acritarchs (r) Chitinozoans (c)
Late Llandovery, Early Silurian	Neahga Shale	Robert Moses Power Plant Section, Niagara Falls, N.Y. Field 36; Laboratory 517	Acritarchs (c) Chitinozoans (c)
Early-Middle Llandovery, Early Silurian	Grimsby Formation "Red Medina"	Robert Moses Power Plant Section, Niagara Falls, N.Y., green beds at base of Grimsby. Field 35; Laboratory 516	Spore tetrads (r) Acritarchs (c) Chitinozoans (r) Scolecodonts (r)
Early Llandovery, Early Silurian	Power Glen Formation "Gray Medina"	Robert Moses Power Plant Section, Niagara Falls, N.Y., about 2 to 2.5 m above Field 33. Field 34; Laboratory 515	Spore tetrads (c) Acritarchs (c) Scolecodonts (r)
Early Llandovery, Early Silurian	Whirlpool Sandstone "White Medina"	Robert Moses Power Plant Section, Niagara Falls, N.Y., about 2.5 to 3 m above Queenston Formation contact. Field 33; Laboratory 514	Spore tetrads (c) Acritarchs (c) Scolecodonts (r)
Richmondian, Late Ordovician	Queenston Formation	Robert Moses Power Plant Section, Niagara Falls, N.Y., fifth green bed below Medina Group contact (about 3.3 m). Field 30; Laboratory 511	Blank
Richmondian, Late Ordovician	Queenston Formation	Robert Moses Power Plant Section, Niagara Falls, N.Y., second green bed below Medina Group contact (about 1 m). Field 31; Laboratory 512	Blank
Richmondian, Late Ordovician	Queenston Formation	Robert Moses Power Plant Section, Niagara Falls, N.Y., first green bed below Medina Group contact. Field 32; Laboratory 513	Blank

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and Scania (7)] for organic microfossils tends to substantiate our interpretation of the importance of [illegible] control in the occurrence of Silurian spores, although the absence of *any* organic microfossils from many rocks where we had predicted the occurrence of spores if environment of deposition rather than evolution was the critical factor can best be explained by post-depositional alteration (*30*). Abundant spores in our Llandovery and Ludlow age samples and in other latest Silurian age samples are known only from shallow-bottom or nearshore marine environments. Truly nonmarine Early Silurian environments have not yet yielded spores, but unmetamorphosed rocks of this age capable of providing spores have not been studied by us or reported in the literature.

The possibility of environmental control of Silurian spore occurrences related to water depth and shoreline proximity is further substantiated by the inverse relationship between abundant Silurian spores, both Early and Late, and marine organic microplankton (*9*). We find that abundant spores occur in rocks deposited either landward of those containing abundant and diversified marine microplankton or in rocks consistent with very shallow-bottom conditions. Strata conventionally accepted as marine or giving evidence of more offshore or deeper water environments yield abundant marine microfossils, including acritarchs, chitinozoans, or scolecodonts, but either no spores or rare spores. The results of our preliminary work suggest that these two groups of organic microfossils have occurrence patterns that are largely mutually exclusive and that may be correlated with marine versus nonmarine or shallow marine environments (*29*).

We suggest, therefore, that the known progressive increase in the frequency and taxonomic diversity of trilete spores that occurs from the Early Silurian to the Late Silurian–Early Devonian may be largely a function of biofacies correlated with geologic environment rather than a function of organic evolution as now concluded (*19, 22*). If the orderly evolutionary sequence that appears to be represented among Silurian spores is an environmental artifact and if the Silurian spores represent vascular plants, it may yet be found that Early Paleozoic vascular plants show a trend similar to the evolutionary history of many animal groups, which exhibit far greater diversity and morphological richness in their early rather than their later history. It remains for future work to determine whether or not plant spores will be recovered in sufficient abundance from nonmarine Early Silurian or older beds to warrant the conclusion that plants had already invaded the land.

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Homograft Rejection Delayed by Treatment of Donor Tissue in vitro with Antilymphocyte Serum

Abstract. *Treatment of rabbit corneal tissue in vitro with pooled rabbit serum delays the onset of corneal homograft rejection in the host. Addition of antilymphocyte serum results in a further significant delay in the onset of rejection. The mechanisms by which such treatment of donor tissues may modify the antigenic content of the material are discussed.*

The use of antilymphocyte serum (ALS) (*1, 2*), as well as other immunosuppressive agents (*2, 3*) in the treatment of donor animals to modify the homograft reaction is well documented in the literature. We now report the effect of treatment of corneal donor tissue with ALS in vitro prior to its use for penetrating keratoplasty.

Horse antiserum to rabbit lympho-

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Table 1. Effect of in vitro treatment of donor corneal grafts on date of onset of homograft rejection.

Treatment	Animals (No.)	Mean onset of reaction (days)	Significance
HARLS (5 percent) in rabbit serum	15*	19.5	$P < .01$†
Rabbit serum (100 percent)	10	14.9	$P < .01$†
Horse serum (5 percent) in rabbit serum	10	14.2	$P < .01$†
Direct transplantation	7	9.7	

* Excluding three animals that have remained clear for more than 180 days. † The delay in rejection between the 5 percent HARLS group and the serum groups is significant at $P < .05$.

cytes (HARLS) was prepared as previously described (*4*). It had agglutinating titers of 1 : 4000 for lymphocytes and 1 : 16 for red cells. Albino rabbits weighing 3.0 to 5.0 kg were used. After enucleation the entire cornea and a rim of sclerae were removed from the donor eye. The donor tissue was then placed in one of three solutions at 4°C or transplanted directly. The solutions were 100 percent pooled rabbit serum in which complement had been inactivated, 5 percent HARLS in rabbit serum, and 5 percent horse serum in rabbit serum. After being soaked for 24 hours at 4°C, the donor tissue was placed epithelial side down in a paraffin block with a fashioned trough. A 6-mm corneal button was trephined, and with the host anesthetized with pentobarbital a penetrating keratoplasty was performed with a continuous 8-0 black silk suture. Clean, but not sterile technique was used. The animals received daily topical applications of atropine ointment and antibiotic drops until the suture was removed on the 10th day after the operation. On the 14th day all the corneal grafts were successful and skin grafts were then performed between the same pairs of donors and recipients in order to achieve a uniformly high rate of corneal graft rejection (*2, 4, 5*). The skin grafts were placed subcutaneously in the ventral abdominal wall. The animals were examined daily from the day of skin grafting for evidence of corneal homograft rejection. Because of their deep location, the skin grafts were not directly observable, but the presence of the graft and the inflammatory response of the surrounding tissue verified its continued presence. Soaked corneas from each group were frozen in liquid freon, suspended in liquid nitrogen, sectioned, and stained with fluorescein-labeled rabbit antiserum to horse γ-globulin (*6*).

The results reported here are those observed during the 6 months after skin transplantation (Table 1). The mean onset of corneal graft rejection was 9.7 days in the group in which tissue was transplanted directly (control group). It was prolonged to 14.9 days when the corneal buttons were first soaked in rabbit serum ($P < .01$) and to 14.2 days when 5 percent horse serum in rabbit serum was used ($P < .01$). Soaking the tissue in 5 percent HARLS in rabbit serum delayed the mean onset of rejection to 19.5 days in those that rejected the graft. This delay is significant when compared to that for the control group ($P < .01$) and when compared to that for the group treated with rabbit serum ($P < .05$). Furthermore, three animals in the HARLS group retained clear grafts for 6 months whereas none in any of the other groups did. These results are suggestive but not conclusive ($.05 < P < .10$).

Studies with fluorescein-labeled antiserum to horse γ-globulin failed to demonstrate binding of this protein to the donor tissue. As observed with the light microscope the structure of the cornea appeared normal, with all cell layers present and intact.

All immunosuppressive agents, including ALS (*7*), used to prevent homograft reaction in cases of organ transplantation have serious side effects associated with their use. Recently, we reported (*2*) the efficacy of treatment of donor animals in modifying corneal homograft rejection and suggested that organs from patients treated with ALS could serve as treated donor material. Extending this work we have treated the donor material in vitro and significantly delayed the onset of rejection without exposing the donor animal to any immunosuppressive therapy. Soaking of the donor material in pooled serum somewhat delayed the onset of rejection, and adding 5 percent ALS to the soaking solution resulted in grafts that remained clear for an additional period of time.

The intriguing question which remains is, "Why does previous treatment of donor tissue in vivo or in vitro modify the homograft reaction?" It has been postulated by Guttman and Lindquist (*3*) that the effectiveness of the immunosuppressive agent is dependent upon the destruction of nonparenchymal cells of hematopoietic origin, which are the stimuli to the effector mechanisms of rejection. In this work donors of renal allografts were treated with a variety of immunosuppressive agents (cyclophosphamide, methotrexate, and procarbazine), and a significant reduction in the amount of kidney transplant rejection compared to the known control series was noted. We do not believe that this mechanism is operative in our experiments because the avascular cornea contains few nonparenchymal cells.

On the other hand, Guttman *et al.* (*1*) have suggested that ALS may act on the organ to be transplanted by coating or binding available antigenic sites. It is also possible that the treated donor tissue acts as a repository of ALS which is slowly released and continually bathes the regional lymph nodes preventing recognition of the foreign material (*8*). The failure to demonstrate the binding of HARLS in the donor tissue by the fluorescent antibody technique does not negate the possibility of the binding of smaller quantities of γ-globulin than are detectable by this technique. But it would seem unlikely, under these circumstances, that the donor button could act as a repository of ALS which would continuously bathe the regional lymph nodes.

Another possibility is that soaking of the tissue prior to skin grafting may allow soluble antigens, if present, to diffuse into the media. Earlier work by Kuwahara (*9*) on a lamellar heterograft system demonstrated that soaking of the donor button in recipient serum reduced the apparent antigenicity of the graft. Stocker *et al.* (*10*), on the basis of work by Kuwahara, have used donor material stored in autologous serum for human transplantation, and they express their clinical impression that the results achieved with this material were more favorable than ordinarily could have been expected in this particular group of cases.

As determined with the light microscope, the donor material appeared completely intact. Kobayashi (*11*) has recently reported the results of his ultrastructural investigation of rabbit corneas soaked in dialyzed rabbit serum. He concludes that morphologically cor-

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ness soaked in serum for 72 hours are the most satisfactory for penetrating keratoplasty. The exact mechanism by which our soaking regimen decreases the apparent antigenicity of the donor material is not clear, and our results suggest that more than one mechanism may be operable.

The demonstration of the effectiveness of the treatment of corneal buttons with ALS in modifying the homograft reaction should be immediately applicable in man in those cases where graft rejection is expected to occur, that is, in patients who have already had graft reaction, highly vascularized corneal tissue, and so forth.

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Calcium Requirement for Melanophore-Stimulating Hormone Action on Melanophores

Abstract. The calcium ion is specifically required for the action of melanophore-stimulating hormone on melanosome dispersion within lizard (Anolis carolinensis) *melanophores in vitro. The response to this hormone is directly related to the concentration of the Ca^{2+} ion. Lithium, choline, rubidium, and cesium will replace the sodium and potassium of Ringer solution if Ca^{2+} is present. Calcium ions are not required for melanosome dispersion itself, since theophylline or dibutyryl cyclic adenosine monophosphate reversibly darkens lizard skins in the absence of calcium.*

Melanophore-stimulating hormone (MSH) initiates the movement (dispersion) of melanosomes from a perinuclear (aggregated) position out into the dendritic processes of vertebrate melanophores (*1*). This leads to a rapid darkening of the skin of most poikilotherms and may precede and be prerequisite for melanin synthesis (*2*). A number of in vitro studies have been directed toward understanding the possible ionic requirements for MSH action. It has been reported that there is an "absolute" requirement for the sodium ion in the action of MSH on frog (*Rana pipiens*) melanophores (*3*). Not only was the calcium ion reported not to be required for melanophore responses to MSH, but, indeed, the response was said to be enhanced in a Ca^{2+}-free medium (*3, 3a*). Others (*4*) have suggested that melanosome dispersion within frog melanophores results from an increase in the intracellular level of calcium ions and a simultaneous decrease in intracellular potassium ions. Fingerman has implicated a role for Ca^{2+} ions in the hormonal control of crustacean chromatophores (*5*). Horowitz (*6*) showed that melanosome aggregation within melanophores of the lizard *Anolis carolinensis* was facilitated by the removal of Ca^{2+} ions from Ringer solution. Novales (*3, 3a*) on the other hand, reported that there is a sodium requirement for MSH action on melanophores of this lizard.

Table 1. Response (change in reflectance) of *Anolis* skins to MSH (6×10^{-4} g/ml), theophylline (10 m*M*), or dibutyryl cyclic AMP (DcAMP, 10 m*M*), in the presence or absence of calcium ion (1 m*M*). Each value represents the maximal mean percent reflectance change, ± standard error, of the skins comprising each experimental group. (Number of skins in parentheses.)

NaCl, EDTA (5 m*M*), MSH (6)	0 ± 0.67
NaCl, Ca^{2+}, MSH (6)	39 ± 3.69
NaCl, EDTA (5 m*M*), theophylline (6)	58 ± 2.31
NaCl, Ca^{2+}, theophylline (6)	58 ± 3.58
NaCl, EDTA (5 m*M*), DcAMP (8)	49 ± 2.54
NaCl, Ca^{2+}, DcAMP (8)	52 ± 1.44

In the present report we demonstrate a specific Ca^{2+} ion requirement for MSH action on melanophores of the lizard *Anolis carolinensis*. We also show that neither sodium nor potassium ions are necessary for MSH action and, in addition, that the Ca^{2+} ion requirement is for an early event in MSH action, since this divalent cation is not necessary for melanosome movements per se. The in vitro response of the integumental melanophores of *Anolis* to MSH or other agents under varying ionic conditions was studied by photometric reflectance methods as described originally for frog skin (*1*) and modified slightly for *Anolis* skin (*7*). Melanosome dispersion in response to MSH results in a change from a bright green to a dark brown color and this change can be measured as a decrease in reflectance from the outer (epidermal) surface of isolated skins. Conversely, melanosome reaggregation resulting in a lightening of skins is correlated with an increase in reflectance.

As reported previously (*3, 3a*), MSH darkens *Anolis* skins immersed in Ringer (NaCl, 111 m*M*; $NaHCO_3$, 2 m*M*; KCl, 2 m*M*; $CaCl_2$, 1 m*M*) solution (Fig. 1). Skins residing solely in sodium chloride (120 m*M*) or in an equivalent amount of NaCl plus KCl (2 m*M*) are almost totally unresponsive to MSH. The addition of the Ca^{2+} ion (1 m*M*) to the NaCl solution permits a response equal to that of the Ringer control group of skins (Fig. 1). The degree of darkening of lizard skins to MSH is clearly directly related to the concentration of the Ca^{2+} ion present (Fig. 2).

These results indicated that either the Ca^{2+} ion is needed directly for MSH action or else its role is permissive to the action of the Na^+ ion. Other monovalent cations were substituted for sodium to determine whether the Na^+ ion is specifically required in addition to the Ca^{2+} ion for MSH action. Melanophore-stimulating hormone failed to darken skins in isotonic solutions (120 m*M*) of either lithium or choline chloride. The presence of Na^+ (1 m*M*) or K^+ (1 m*M*) ions in combination with either Li^+ or choline ions failed to reestablish a response of the melanophores to MSH. There was, however, a near

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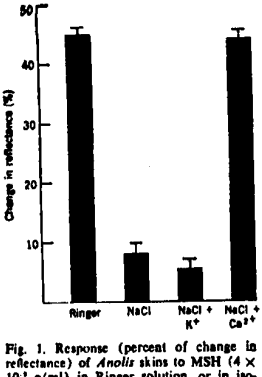

Fig. 1. Response (percent of change in reflectance) of *Anolis* skins to MSH (4×10^{-7} g/ml) in Ringer solution, or in isotonic NaCl (120 m*M*), or isotonic NaCl containing either 2 mmole of K^+ or 1 mmole of Ca^{2+}. Values represent the maximal responses, ± standard errors of the means, of the 16 skins representing each experimental group.

maximal response of the skins to MSH when Ca^{2+} (1 m*M*) was added to either lithium or choline chloride isotonic (120 m*M*) solutions. Similar results were obtained with rubidium or cesium ions in place of the sodium ion. Only the Ca^{2+} ion (1 m*M*) added to isotonic solutions of these monovalent cations reestablished the response of

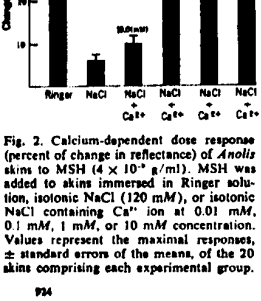

Fig. 2. Calcium-dependent dose response (percent of change in reflectance) of *Anolis* skins to MSH (4×10^{-7} g/ml). MSH was added to skins immersed in Ringer solution, isotonic NaCl (120 m*M*), or isotonic NaCl containing Ca^{2+} ion at 0.01 m*M*, 0.1 m*M*, 1 m*M*, or 10 m*M* concentration. Values represent the maximal responses, ± standard errors of the means, of the 20 skins comprising each experimental group.

melanophores to MSH. The reported inhibition of MSH in a sodium-free potassium Ringer solution and the implication, therefore, of a sodium requirement for MSH action on *Anolis* melanophores as suggested previously (*3*, *3a*), can now be explained simply as a further demonstration of a Ca^{2+} requirement for MSH action.

Other divalent ions were substituted for Ca^{2+} to determine the specificity of the Ca^{2+} requirement for MSH action. Strontium, barium, or beryllium ions (1 m*M*) could replace Ca^{2+} and permit melanosome dispersion in response to MSH. These other ions, however, are of limited significance, being generally unavailable under normal physiological conditions. The other divalent cations (Mg^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Cu^{2+}) studied either failed to substitute for Ca^{2+} or were apparently lethal to the melanophores. Either phosphate or bicarbonate, but not sulfate, anions could replace the chloride ion of Ringer solution and allow full MSH expression, but only if Ca^{2+} was present. Thus the Ca^{2+} ion is the only ion normally present within Ringer solution which is specifically required for MSH action. High concentrations of $CaCl_2$ (120 m*M*) mimic the action of MSH by dispersing melanosomes and darkening *Anolis* skin.

The immediacy of the Ca^{2+} ion requirement for MSH action is clearly shown in Fig. 3. Skins only darken minimally in the absence of the Ca^{2+} ion. Addition of Ca^{2+} (1 m*M*) to the Ca^{2+}-free Ringer causes a rapid and maximal darkening of skins. Transfer of maximally darkened skins to Ca^{2+}-free Ringer (but still containing MSH, as before) results in a complete reversal (or lightening) of the darkened skins. The minimal darkening response of lizard skins to MSH in isotonic NaCl (Fig. 1) or Ca^{2+}-free Ringer (Fig. 3) could be totally inhibited or reversed by adding EDTA (ethylenediaminetetraacetic acid, 5 m*M*) to the skins (Table 1). Ouabain (0.01 to 0.5 m*M*) did not inhibit the response of *Anolis* skins to MSH.

Melanophore-stimulating hormone stimulates an increase in cyclic adenosine 3,5′-monophosphate (cyclic AMP) within dorsal frog (*Rana pipiens*) skin (*8*). This intracellular second messenger (*9*), or its dibutyryl derivative, darkens frog (*10*, *11*) and lizard (*12*, *13*) skins directly, suggesting that this nucleotide may play a normal role in melanophore regulation. Methylxanthines such as caffeine and theophylline darken frog (*3*, *11*) and lizard (*12*) skins, apparently by increasing intracellular levels of cyclic AMP through inhibition of cyclic AMP phosphodiesterase (*14*).

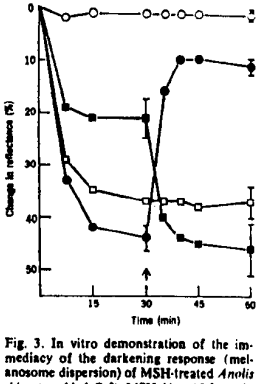

Fig. 3. In vitro demonstration of the immediacy of the darkening response (melanosome dispersion) of MSH-treated *Anolis* skins to added Ca^{2+}. MSH (4×10^{-7} g/ml) was added to skins immersed in Ringer solution (● + □) or in a Ca^{2+}-free Ringer solution (■). One group of skins (○) was maintained as an unstimulated Ringer control. At 30 minutes (arrow), Ca^{2+} (1 m*M*) was added to the Ca^{2+}-free Ringer skins (■) and at the same time one group of Ringer MSH-darkened skins (●) was transferred to Ca^{2+}-free Ringer (containing an identical concentration of MSH, as before). Each point on the graph is the mean of 16 measurements of reflectance. Vertical lines indicate the standard errors.

Although the present results clearly establish a Ca^{2+} requirement for MSH action, the Ca^{2+} ion is not required for melanosome dispersion per se, since theophylline darkens lizard skins in Ca^{2+}-free isotonic (120 m*M*) solutions of NaCl, LiCl, RbCl, CsCl, or isotonic (240 m*M*) sucrose. In addition, either theophylline or dibutyryl cyclic AMP will reversibly darken *Anolis* skins (Table 1) even when Ca^{2+} ions are removed and EDTA (5 m*M*) is added, thus ruling out the possibility that these agents mediate their actions by releasing Ca^{2+} from bound intracellular pools.

It would appear then that the Ca^{2+} requirement for MSH action is related to an increase in cyclic AMP which is then responsible for melanosome dispersion. It is not known how MSH increases melanophore cyclic AMP

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levels. It could do so by stimulation of adenyl cyclase activity, or, as suggested elsewhere (*15*), by an inhibition of cyclic AMP phosphodiesterase activity. It would appear that Ca^{2+} must play a role in regulating the activity of one of these two enzymes, since Ca^{2+} is not required for cyclic AMP–stimulated melanosome dispersion. The Ca^{2+} requirement for MSH action on melanophores is consistent with a similar cation requirement for the lipolytic action of adrenocorticotropic hormone (ACTH) on isolated fat cells (*16*) or the stimulation of adenyl cyclase activity in adrenal cortical microsomal fractions (*17*). The similarity in structure of these two hormones might suggest a similar mechanism of action.

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3 MPTI MHER 1971

Endothelial Projections as Revealed by Scanning Electron Microscopy

Abstract. *Scanning electron micrographs of the endothelium of the pulmonary artery reveal that the entire surface is covered by a meshwork of irregular projections which vastly increase the surface area. The size and density of the projections suggest that they may function to direct an eddying flow of plasma along the endothelial surface.*

The endothelial lining of blood vessels is remarkable not only for its extensiveness and thinness but also for its position in direct communication with blood. Previous studies of the structure and function of endothelium have concentrated on the selective transport of fluid and solutes in capillaries, the structural studies having focused on cell junctions, pinocytotic vesicles, and endothelial flaps (*1*). Recent evidence indicates that enzymes on or near the surface of endothelial cells are actively engaged in the metabolism of circulating substances, such as the adenine nucleotides and polypeptide hormones, which are degraded rapidly but which are not retained by the cell itself (*2*).

Observation of the endothelial surface by scanning electron microscopy emphasizes a very different aspect which has been overlooked in transmission electron micrographs. The surface is covered by a profuse array of minute, irregular, fingerlike projections. The projections are distinguished from the regular palisades of microvilli which are characteristic of many vertebrate and invertebrate epithelia and from flaps or marginal folds.

Figures 1 and 2A show the inner surface of the pulmonary artery of a

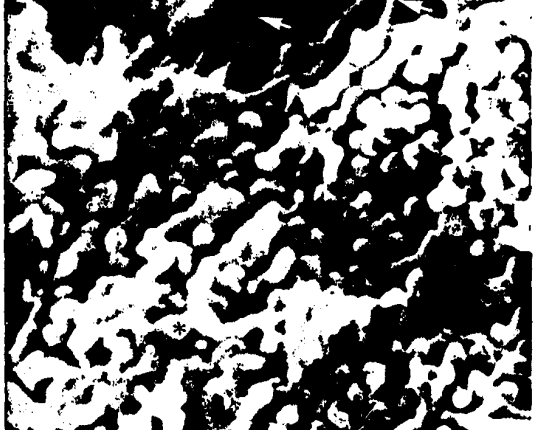

Fig. 1. Scanning electron micrograph. The endothelial projections are approximately 250 to 350 nm in diameter and vary in length from 300 to 3000 nm. They may take the form of knobs or longer arms, some of which branch or bud (black arrow). They are densest over the main body of the cell (*) but extend laterally to overlap adjacent cells (white arrows) (× 14,000).

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dog examined by scanning electron microscopy (*3*). The lining is thrown into a series of projections which occur over the entire surface of each endothelial cell. Reference to thin sections in the transmission electron microscope confirms the presence of these projections (Fig. 2, B and C), but their numerical prominence and functional importance can only be appreciated in a three-dimensional scanning survey of the entire surface.

The projections are approximately 250 to 350 nm in diameter and 300 to at least 3000 nm long, as estimated from the scanning electron micrographs. Some of the shorter projections come to blunt ends whereas others end in knoblike processes. Longer projections may bud or branch. The branches may occasionally fuse with other projections or with the main surface of the cell. Measurements of the diameters of the projections are in good agreement, as estimated from the transmission and from the scanning electron micrographs.



Fig. 2. (A) Low-power scanning electron micrograph of the inner surface of the dog pulmonary artery. The endothelial cells are denoted by an asterisk, the cell outlines being clearly distinguishable. The main body of each endothelial cell is richly covered by an array of irregular projections which thin out toward the borders of the cell (× 3000). (B) Detail of a group of endothelial projections showing the apparent variability in diameter. Note pinocytotic vesicles (asterisk) associated with the projections (× 75,000). (C) Portion of an endothelial cell with nucleus (*N*). Fibrillar strands (*F*) are present in the subnuclear cytoplasm. Endothelial projections (arrows) occur both on the main body of the cell and on the tenuous peripheral portions (× 29,000).

The projections contain pinocytotic vesicles and ribosomes (Fig. 2, B and C). Mitochondria are situated at the base of some. Fibrillar structures are evident in the body of the cell, especially beneath the nucleus (*4*), and may extend to the base of the projections (Fig. 2C).

The projections occur in concentrations of up to seven per square micrometer (Fig. 1). The denser clusters are found over the body of the cell. Numbers become smaller near cell junctions. Projections near cell junctions extend toward, and intertwine with, the adjacent cell, but we have no evidence of the fusion of a projection of one cell with that of another.

The long axis of some projections may parallel the main surface of the cell. Both the density of the clusters and the tendency of long branches to reflect back on the main surface of the cell would appear to make it unlikely that formed elements of blood could come in contact with the body of the cell.

Although our scanning electron microscopic studies of endothelial surface projections are limited to the lining of the pulmonary artery of the dog, transmission micrographs indicate that these structures occur in much smaller vessels of a wide number of species and are a general feature of the intima of blood vessels. The projections may be similar to the surface specializations in the vessels of the pecten of the bird's eye (*5*) and to endothelial microvilli in the vessels of the rat Gasserian ganglion and testis (*6*). In addition, published transmission electron micrographs (*7*) of capillaries of the pancreas (cat) and myocardium (mouse) and unpublished micrographs of capillaries in lung (rat) (*8*) show projections arising from the main body of the endothelial cell; circular structures within the lumen are presumably transverse sections of endothelial projections.

The low-power scanning electron micrograph shows projections over the entire endothelial surface and no preferential localization at the peripher of

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the cell. While marginal folds may occur in addition, the structures which we describe here and which we have compared by scanning and transmission electron microscopy are not folds but rather projections with a circular profile in transverse section. It is unlikely that these fingerlike projections function in conjunction with the process of pinocytosis by engulfing plasma as suggested for the marginal folds (*1*). Although the projections may reflect onto and fuse with the main body of the cell, their cylindrical shape would make them no more efficient than an apposed thumb and forefinger for holding water. On the other hand, the projections could possibly enmesh small chylomicra.

The functions of the endothelial surface projections are not known. Clearly, the projections vastly increase the surface area of endothelial cells and cannot fail to affect fluid dynamics. Their density and the irregular meshwork which they form are such that they could have the effect of producing an eddying flow of cell-free plasma along the surface of the cell body. This possibility is of special interest in relation to large vessels with a high flow such as the pulmonary artery, where nutrient capillaries enter the adentitia and outer media but do not penetrate to the endothelium. A retarded flow of plasma along the surface of the cell could provide conditions of flow and pressure favorable for the exchange of metabolites and, possibly, for the metabolism of circulating hormones.

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3. Dogs were anesthetized with sodium pentobarbital (25 mg per kilogram of body weight) administered intravenously. A portion of the pulmonary artery near the pulmonary valve was removed, immediately dropped into 0.25*M* glutaraldehyde in 0.05*M* cacodylate buffer (pH 7.4) containing 0.17*M* sucrose, and prepared for transmission electron microscopy. For scanning electron microscopy, material was transferred from the cacodylate buffer to 1 percent OsO_4 in the same buffer. The small tissue blocks were frozen in liquid Freon 12 cooled by liquid nitrogen and dehydrated under vacuum. The specimens were then attached to glass cover slips with the luminal surface of the endothelium uppermost. The material was shadowed by evaporation of a gold and platinum alloy on the surface and examined in a Philips AMR 900 microscope.
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9. Supported by the Florida Heart Association, PHS research grant HE 12843, NSF grant GB-12117X, and the Council for Tobacco Research. We thank Dr. S. B. Mohl, Advanced Metals Research, Inc., Burlington, Massachusetts, for taking the scanning electron micrographs, and Research Division, Electron Microscopy Laboratory, Veteran Administration Hospital, Miami, Florida, for use of the Philips EM 300.

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Plasmalemma: The Seat of Dual Mechanisms of Ion Absorption in Chlorella pyrenoidosa

Abstract. Dual mechanisms of absorption of rubidium were demonstrated in a nonvacuolate unicellular alga, Chlorella pyrenoidosa, *in both the light and the dark. The two mechanisms were sensitive to metabolic inhibitors. At high concentrations rubidium enhanced the respiration of* Chlorella *cells. The findings support the conclusion that the mechanisms of rubidium absorption in both the low and high concentration ranges are active processes and reside in the plasmalemma.*

Epstein *et al.* (*1*) discovered that the rate of ion absorption by plant cells followed two distinct patterns possibly due to two different mechanisms, one operating over a concentration range of the ion up to 1 m*M*, and the other extending from 1 m*M* upward. Later, a theory proposed by Laties and his coworkers (*2*, *3*) suggested that the first mechanism is located at the plasmalemma and the second at the tonoplast. It is further held that ions in the high concentration range enter the plasmalemma by diffusion. However, this theory has been questioned by others (*4*–*6*) who hold that both mechanisms are located in the plasmalemma and operate in parallel. Welch and Epstein (*6*) consider that the Laties theory contradicts the classical view of the semipermeability and ion selectivity properties of the plasma membrane.

Dual patterns of absorption have been recorded for a number of ions in tissues of several higher plants (*4*, *7*) and for carbohydrate in microorganisms (*8*). Studies made so far with *Chlorella pyrenoidosa* relate to the membrane potentials and the kinetics of potassium influx (*9*, *10*). Dual patterns of rubidium absorption in *C. pyrenoidosa* are reported here. It is further demonstrated that both mechanisms reside in the plasmalemma and that absorption of rubidium in the high concentration range (1 to 50 m*M*) is metabolically mediated.

The alga *C. pyrenoidosa* was grown in aerated nutrient culture (*11*) at 20°C, under 5000 lu/m² of fluorescent light. The cells were washed thrice with deionized distilled water before use. The procedures for incubation and measurement of ion absorption were those

Table 1. Effects of metabolic inhibitors on rubidium absorption by *Chlorella* cells from 0.2 and 10 m*M* RbCl in the light and the dark. The inhibitors (10^{-5}*M*) were presented along with RbCl. Values are means ± standard errors of the means.

Treatment	Rubidium absorption (nmole/mg per hour)			
	0.2 m*M* RbCl		10 m*M* RbCl	
	Light	Dark	Light	Dark
Control	25.5 ± 0.4	9.5 ± 0.1	37.0 ± 2.8	23.1 ± 0.9
Antimycin A	12.7 ± .8	2.5 ± .3	28.8 ± 1.2	8.0 ± .9
m-Cl-CCP	21.2 ± 1.2	7.6 ± .6	29.8 ± 1.1	15.3 ± .1
p-CF_3O-CCP	21.5 ± 0.6	7.9 ± .7	30.1 ± 1.8	14.5 ± .1

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reported for isolated leaf cells (*12*). The absorption medium contained 50 mg dry weight equivalent of cells per 50 and 25 ml, in the low (0.01 to 0.5 m*M*) and high (1 to 50 m*M*) concentration ranges, respectively, and ^{86}RbCl. The flasks containing the experimental solution were shaken in a reciprocating water bath at 20° ± 2°C. At the end of the absorption period, the cells were rapidly centrifuged and washed thrice with equimolar unlabeled RbCl at 5°C, and air-dried triplicate samples were radioassayed. The *p*H of the experimental solution ranged from 6.5 to 7. All experimental solutions contained 0.1 m*M* $CaCl_2$. For experiments in which the effects of metabolic inhibitors were studied, the inhibitors were presented along with RbCl.

Efflux studies were carried out by first allowing the cells (500 mg dry weight equivalent) to absorb from 250 ml of 0.2 or 10 m*M* ^{86}RbCl for 4 hours under light. The cells were rapidly centrifuged and resuspended in unlabeled RbCl. Portions (10 ml) of the suspension were centrifuged, and the radioactivity retained in the cells was measured. The suspensions were vigorously aerated during the absorption and efflux.

Table 2. Effects of different concentrations of RbCl on the respiration of *Chlorella* cells. Respiration was measured manometrically in Warburg flasks, containing 50 mg dry weight equivalent of cells, over a period of 3 hours. Values are means ± standard errors of the means.

RbCl concentration (m*M*)	Respiration (μl of O_2 per 50 mg of cells)		
	1 hour	2 hours	3 hours
Control	43.4 ± 2.3	74.1 ± 5.8	105.8 ± 4.7
0.1	42.7 ± 0.6	73.1 ± 0.8	103.8 ± 1.3
0.5	44.8 ± 2.2	79.9 ± 1.9	113.0 ± 1.8
10.0	44.4 ± 2.2	87.7 ± 4.9	125.7 ± 5.8
30.0	45.6 ± 2.3	93.3 ± 1.7	130.3 ± 2.1

Respiration of *Chlorella* cells was measured manometrically in Warburg flasks, each of which contained 50 mg dry weight equivalent of cells, and RbCl as a variable, in a final volume of 4.4 ml.

The absorption of rubidium by *Chlorella* follows two patterns, one in the low and the other in the high range (Figs. 1 and 2). Furthermore, the dual uptake pattern is observed in both the light and the dark. The first mechanism has a high affinity for the ion [Michaelis constant (K_m) = 0.038 m*M* and maximum velocity (V_{max}) = 28.6 nmole/mg per hour in the light; and K_m = 0.182 m*M*, V_{max} = 11.11 nmole/mg per hour in the dark]. The V_{max} for uptake of rubidium in the dark, in the low concentration range, is very close to that reported for the uptake of potassium (*4*). The mechanism in the high concentration range, however, has a low affinity for rubidium (K_m = 6.45 m*M*, V_{max} = 86.9 nmole/mg per hour in the light; and K_m = 12.38 m*M*, V_{max} = 56.49 nmole/mg per hour in the dark). Absorption of rubidium from 0.2 and 10 m*M* RbCl is inhibited by antimycin A, carbonyl cyanide chlorophenyl hydrazone (*m*-Cl-CCP), and *p*-trifluoromethoxyphenyl hydrazone (*p*-CF_3O-CCP), especially in the dark (Table 1). Antimycin A is found to be most inhibitory among the inhibitors used. Absorption of rubidium from 10 m*M* RbCl in the dark is nearly equally inhibited by the two phenylhydrazones. The results of efflux studies show that there is very little loss of rubidium absorbed either from 0.2 or from 10 m*M*

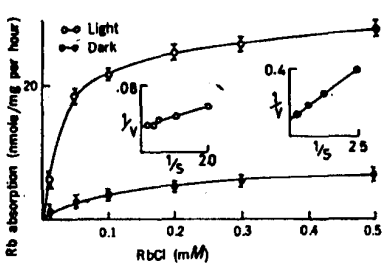

Fig. 1 (above). Absorption of rubidium by *Chlorella* in the low concentration range, in the light and the dark. Insets: Reciprocal plots of the rate of uptake *V* (in nanomoles per milligram per hour) versus substrate *S* (in millimoles per liter). Vertical bars represent standard deviations of the mean.

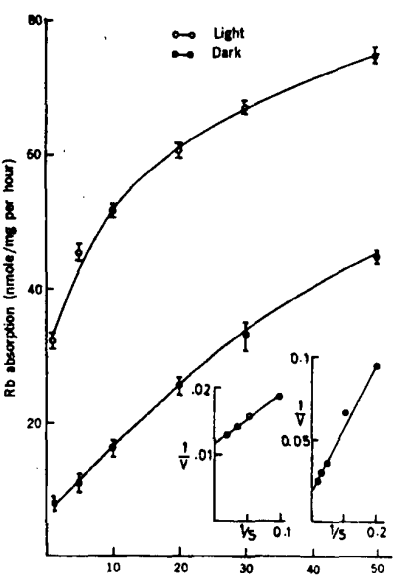

Fig. 2 (right). Absorption of rubidium by *Chlorella* in the high concentration range, in the light and the dark. Insets: Reciprocal plots of the rate of uptake *V* (in nanomoles per milligram per hour) versus substrate *S* (in millimoles per liter).

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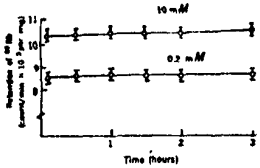

Fig. 3. Efflux of rubidium absorbed by *Chlorella* from 0.2 and 10 m*M* RbCl. *Chlorella* cells (500 mg dry weight equivalent) were allowed to absorb from ^{86}RbCl for 4 hours under light and were then transferred to 1 liter of unlabeled RbCl of equimolar concentrations. Samples were drawn at different times to measure the radioactivity retained in the cells.

RbCl, even at 3 hours (Fig. 3). Respiration of *Chlorella* is significantly enhanced by 10 and 30 m*M* RbCl (Table 2).

A dual isotherm for the absorption of a given ion points to two transport systems and raises the question of the location of each system, whether they operate in parallel at the same membrane or function consecutively across the plasmalemma and tonoplast. The evidence in support of the sequential transport through the two membranes is derived mainly from the absorption isotherms of nonvacuolate root tips and vacuolated segments of corn roots (*13*). Nonetheless, it has been admitted that the distinctions made on this basis cannot be absolute since the subapical cells differ from the apical ones for reasons other than vacuolation. Furthermore, the conclusion that the compartments into which the ions move are subcellular in origin is drawn from the kinetic analysis of ion absorption by multicellular systems (*3*, *13*), which consist of cells differing morphologically and physiologically between themselves. The unicellular alga, *C. pyrenoidosa* is nonvacuolate (9) and hence devoid of an inner tonoplast membrane. The mechanisms for the dual pattern of rubidium absorption observed in this system therefore must reside in the plasmalemma. Chloroplasts are known to accumulate ions. However, these are functionally and anatomically different from the vacuoles. The occurrence of a dual pattern of absorption by *Chlorella* cells in the dark is evidence that the chloroplast is not implicated in the two ion-absorption mechanisms.

It has been contended that ions at high concentrations readily negotiate the plasmalemma by diffusion (*2*). However, sensitivity to metabolic inhibitors and nonexchangeability of the ion clearly support the view that the uptake, in the low and high concentration ranges, is energy-dependent. The energy for the operation of dual mechanisms in both the light and the dark (Figs. 1 and 2) is presumably derived from photosynthesis and respiration. The implication of respiration with rubidium absorption in the high concentration range (Table 2), which has been observed in higher plants (salt respiration) (*14*, *15*), suggests that ion absorption by the second mechanism is not diffusive.

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Receptive Field Organization of Units in the First Optic Ganglion of Diptera

Abstract. *Centripetal spike potentials were recorded from two classes of units (transient and sustained) in the intermediate chiasma of flies. On-off units were characterized by a transient discharge after the onset and cessation of a light spot presented within its elliptical receptive field. Receptive fields of sustaining units were composed of three roughly circular regions arranged adjacently along a line; stimulation of the center region elicited a sustained discharge, whereas stimulation of either adjacent region elicited an off discharge. Adjacent regions antagonized the central region, for stimulation of either inhibited the discharge resulting from stimulation of the central region.*

The retina and first optic ganglion of flies have highly ordered structures. The retina is comprised of an array of several thousand ommatidia, each consisting of eight retinular cells; the first optic ganglion is composed of a corresponding array of synaptic compartments, called cartridges, each containing endings of six retinular cell axons and several second-order neurons (*1*, *2*). Within each cartridge numerous synaptic contacts are made between the six retinular cell axons and two second-order neurons (type I monopolar cells) which send their axons to the second optic ganglion (*2*). Furthermore, the presence of elements conceivably capable of supporting interaction between neighboring cartridge elements (*2–4*) suggests that more than simple summation may occur within the first optic ganglion. Although slow potentials (*5*, *6*) have been recorded from the cartridge region of flies, the fact that no spike potential has ever been observed raises the question of how neural signals are transmitted to the second optic ganglion. Experiments were performed which show that, indeed, spike potentials are carried by fibers en route to the second optic ganglion. Two types of fibers were identified whose spatial and temporal characteristics of discharge indicated complex integration mechanisms.

Several species of flies, principally *Phaenicia sericata*, were studied, and no species differences were observed. A specimen was prepared for an experiment by removal of a small triangular flap of exoskeleton from the posterior surface of the head capsule, which exposed one optic lobe. The preparation was placed at the center of a reflecting sphere (2 m in diameter) upon which stimulus patterns were projected. Tungsten microelectrodes (noise level 20 μv, peak-to-peak) were placed in the intermediate chiasma, and centripetal discharges representing two distinctly different classes of units were recorded (*7*).

One type is an on-off unit which responds to a diffuse light pulse with a transient discharge following the onset and cessation of stimulation. The other type is a sustaining unit which is quiescent in the dark, as is the on-off unit, but a diffuse light pulse elic-

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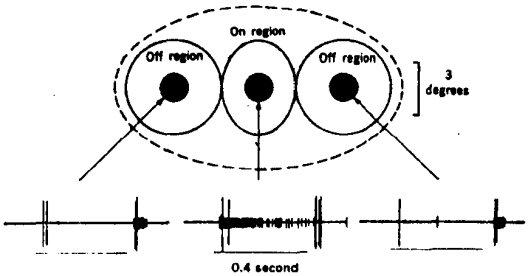

Fig. 1. Receptive field organization of the on-off and sustaining units. The three records of simultaneously recorded on-off and sustaining units correspond to different location of the 1.5° stimulus spot (dark spots) and characterize the receptive field organization of both units. Whereas the on-off unit responds in a transient fashion (on-off) regardless of the stimulus location within the receptive field, the sustaining unit responds with a maintained discharge upon stimulation of the on region and with a pure off discharge upon stimulation of either adjacent off region. In this case the receptive fields of both units were coincident and are schematically represented by the dashed (on-off unit) and solid (sustaining unit) lines.

an on discharge which slowly adapts to a steady rate dependent upon the light intensity. Frequently a unit of each type is recorded simultaneously with one electrode (Fig. 1), and in all cases the amplitude of the spike potential of the on-off unit exceeds that of the sustaining unit. Although 102 on-off and 74 sustaining units were studied, the relative number of each type does not reflect their true proportion, for on-off units were easier to observe and isolate. Another property which differentiates the two units is the threshold, for the sustaining unit is, on the average, 1.3 log units more sensitive to a small spot of light placed at its receptive field center.

The receptive field configuration of each unit was investigated by the presentation of a 1.5° light spot at each point of a 7 by 11 grid having a 1.5° spacing between grid points. Three representative records of the 77 taken during a typical experiment in mapping the receptive field are shown in Fig. 1.

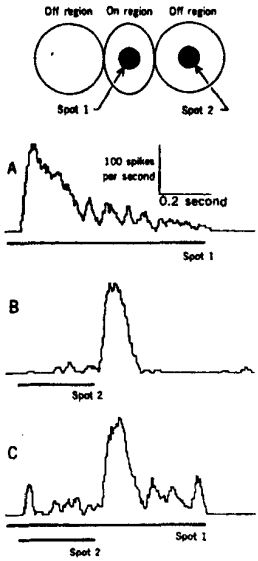

Fig. 2. Inhibitory influence exerted through the off regions of the sustaining unit. Poststimulus time histograms A and B represent the average discharge behavior resulting from stimulation of the on region (spot 1) and off region (spot 2), respectively. Note that some discharge occurred during the presentation of spot 2 and was presumably due to scattered light. Histogram C represents the average discharge behavior when both regions are stimulated in the temporal order indicated and reveals the inhibition resulting from stimulation of the off region. The histograms were computed from 12 trials, and the bin width was 4 msec. Both spots were 1.5°, and spot 2 was 0.9 log unit more intense.

An on-off and a sustaining unit were recorded simultaneously; the dark spots represent the approximate size and location of the three stimuli relative to the schematized outlines for the receptive fields (*8*) of both units. The on-off unit has an elliptical receptive field with average half-sensitivity angles (θ) of 5.5° and 4.2° along the major and minor axes, respectively. Furthermore, the orientation of the major axis for all on-off units studied corresponds to the mediolateral axis (*10*) of the eye. The receptive field is homogeneous in that the discharge rate and not the discharge pattern is dependent upon the stimulus location.

In contrast, the receptive field of the sustaining unit is not homogeneous but, instead, consists of three functionally distinguishable regions each being roughly circular and arranged adjacently along a mediolateral axis line. Stimulation of the center on region elicits a sustained discharge characteristic of stimulation with diffuse light (Fig. 1); however, stimulation of either adjacent region elicits an off discharge. Consequently, the sustaining unit also possesses an elliptical receptive field which, like that of the on-off unit, is always oriented with its major axis roughly along the mediolateral axis of the eye. The average half-sensitivity angles of the on region along the major and minor axes are 2.4° and 2.7°, respectively, which compares favorably with the 3° average half-sensitivity angle for retinular cells in the frontal part of the eye (*11*).

On the basis of the discharge patterns evoked by stimulating the on and the off regions and on the basis of the pinched appearance of the on region along the major axis (*12*), antagonism between the on and off regions was suspected. Experiments were performed to test this possibility by the simultaneous presentation of 1.5° light spots to both the on and off regions. In Fig. 2 poststimulus time histograms A and B represent the average discharge elicited by stimulation of the on region (spot 1) and off region (spot 2), respectively; poststimulus time histogram C is the average discharge behavior when both stimuli are presented in the temporal order indicated. The inhibitory influence of stimulation of the off region is readily apparent, and both off regions are equally effective. However, to reveal such significant antagonism as this, it is necessary to make the intensity of spot 2 as

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much as 1 log unit greater than spot 1.

The sizes of receptive fields of both units reveal that they integrate the activity of several cartridges and that they do so more extensively along the mediolateral axis of the eye. However, the complex temporal characteristics of both units indicate that more than simple summation of the retinular cell signals occurs in the first optic ganglion. The spatial and temporal characteristics of the discharge of the sustaining unit suggest that the sustained discharge reflects the excitatory influence of the retinular cells converging on a single cartridge while the off discharge reflects a rebound from inhibition (*13*) resulting from the excitation of neighboring cartridges along the mediolateral axis. The discharge characteristics of the on-off unit are not so easily explained. The receptive field organization of sustaining units is similar to that of eccentric cells in *Limulus* and of certain retinal ganglion cells (on center) in the vertebrate eye, for, like both, lateral inhibition can be demonstrated and antagonistic discharge (off) can be elicited by stimulation of the surround region of the receptive field (*14*). However, in contrast to the concentric receptive field organization of the eccentric and retinal ganglion cells that of the sustaining unit is not circularly symmetric.

The existence of centripetal discharges in the intermediate chiasma establishes that spike potentials are a mode of neural communication between the first and second optic ganglia; however, only two different types of units have been identified on the basis of their discharge behavior to various stimuli (spatial, temporal, spectral, polarization) (*15*), and at least six centripetal fibers, associated with each cartridge, have been identified anatomically (*4*, *16*). It would appear that either the units unaccounted for do not support impulse traffic, or they do and their discharge behaviors are either not sufficiently different from those of the on-off and sustaining units as to be distinguished with the types of stimuli available or their signals cannot be observed with the techniques used. The anatomical study of Trujillo-Cenóz (*16*) revealed two large fibers (3 to 4 μm) associated with each of the many bundles of fibers comprising the intermediate chiasma, and these fibers correspond to the axons of the type I monopolar cells of each cartridge. It is, therefore, possible that one large fiber of each pair is responsible for the on-off discharge while the other mediates the sustained discharge; however, it is difficult to account for the disparity in spike size between the two types of units. Furthermore, Autrum *et al.* (*6*) have recently recorded hyperpolarizing slow potentials without evidence of spikes from neurons in the first optic ganglion which they believe are monopolar cel. These observations are difficult to reconcile unless the micropipette renders the cell incapable of spike generation or unless spikes do not invade the cell body. The situation is further complicated by a recent report by Strausfeld and Braitenberg (*4*) that a type II monopolar cell associated with each cartridge sends collaterals to adjacent cartridges along the +Y and −X axes where they terminate on one of the type I monopolar cell processes, for the axis of interaction implied by this anatomical observation is roughly perpendicular to the axis of interaction manifested by the elliptical receptive fields of the on-off and sustaining units. Although the functional and structural correlates of the first optic ganglion remain unsettled, it is clear that profound transformations are made on the retinular cell signals.

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7. Stainless steel microelectrodes were also used, thereby making it possible to deposit a small amount of iron at the recording site which upon histological examination was found in the intermediate chiasma.
8. The outlines reflect only the relative shape and size of the receptive field of the on-off and sustaining units. A more absolute measure is given by the angular sensitivity which is the reciprocal of the stimulus intensity required to elicit a criterion response (one spike) as a function of the angular orientation of the stimulus relative to the receptive field center of the unit.
9. The separation, measured in degrees of arc, between points having half the maximum sensitivity and lying on an axis line of the receptive field is called the half-sensitivity angle along that axis. If the receptive field is circularly symmetric, then one half-sensitivity angle suffices.
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13. Inhibition and off discharge are frequently associated; for example, see R. Granit, *Sensory Mechanisms of the Retina* (Oxford Univ. Press, London, 1947); C. A. G. Wiersma and T. Yamaguchi, *J. Exp. Biol.* 43, 409 (1967); M. L. Wolbarsht, H. G. Wagner, E. F. MacNichol, Jr., in *The Visual System: Neurophysiology and Psychophysics*, R. Jung and H. Kornhuber, Eds. (Springer-Verlag, Berlin, 1961), p. 163; R. Granit, *Receptors and Sensory Perception* (Yale Univ. Press, New Haven, 1955). The strength of the off discharge or postinhibitory rebound depends greatly on the species and the level of the nervous system.
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Serum Dopamine-β-Hydroxylase: Decrease after Chemical Sympathectomy

Abstract. *Dopamine-β-hydroxylase is an enzyme that is localized to catecholamine-containing vesicles in sympathetic nerves and the adrenal medulla, and is also found in the serum. Treatment of rats with 6-hydroxydopamine, a drug which destroys sympathetic nerve terminals, leads to a decrease in serum dopamine-β-hydroxylase activity. The decrease is not due to an effect on the adrenal medulla or to an increase in circulating inhibitor or inhibitors of enzyme. These data represent evidence that at least a portion of the circulating dopamine-β-hydroxylase activity arises from sympathetic nerve terminals.*

Dopamine-β-hydroxylase (DBH), the enzyme that catalyzes the conversion of dopamine to norepinephrine (*1*), is localized to the chromaffin granules in the adrenal medulla (*2*) and to the catecholamine-containing vesicles in

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sympathetic nerves (*3*). This enzyme is released with catecholamines when the isolated perfused adrenal gland is stimulated with acetylcholine (*4*) and when the nerves to the isolated perfused spleen are stimulated (*5*).

A DBH activity in the serum of both man and experimental animals has been described (*6*). Serum DBH activity is similar to that of the adrenal enzyme as judged by cofactor requirements, oxygen requirement, K_m (Michaelis constant) for substrate, and response to cupric ions (*6*). When rats are stressed by means of forced immobilization, a procedure that increases catecholamine excretion (*7*), there is an increase in serum DBH activity, and the increase occurs even in the absence of the adrenal glands (*8*).

Our study was undertaken to determine the source of serum DBH activity. If this circulating enzyme is derived from the adrenal medulla, the sympathetic nerves, or both, it might prove to be an easily measured index of the activity of these tissues. 6-Hydroxydopamine (6-OHDA) causes a partial destruction of sympathetic nerve terminals (*9*), while not affecting the adrenal medulla. Serum DBH activity in the rat decreases after treatment with 6-OHDA, indicating that at least a portion of the serum DBH originates in sympathetic nerve terminals.

All animals used were male Sprague-Dawley rats (160 to 200 g) (from Hormone Assay Labs, Chicago). They were either normal or sham-operated, or they had been subjected to surgical removal of the adrenal medulla. The adequacy of adrenal demedullation was verified by examination of sections of the adrenal glands stained with hematoxylin and eosin.

6-Hydroxydopamine hydrobromide (Regis Chemical Company, Chicago, Illinois) was dissolved in 0.001*N* HCl within 15 minutes of injection, and each treated animal was injected intravenously by the tail vein with either 34 or 68 mg of 6-OHDA (calculated as base) per kilogram of body weight. This injection was followed by 0.5 ml of saline. Control animals received the same volume of HCl and saline intravenously. Animals were killed by decapitation; blood was collected (on ice) and centrifuged; serum was separated and was either assayed for DBH activity the same day or was immediately frozen for assay the next day (*10*).

Purified bovine adrenal DBH was prepared by a modification of the method of Geffen *et al.* (*11*). Catecholamines were assayed by methods previously described (*12*). Lactate dehydrogenase activity in the serum was determined as described by Kornberg (*13*) by measuring the oxidation of reduced nicotinamide adenine dinucleotide, with 20 μl of rat serum in each assay. The results were expressed as change in absorbance at 340 nm per minute per milliliter of serum. The DBH activity was measured by a sensitive enzymatic assay (*14*) modified for the determination of serum activity described in detail elsewhere (*6*). A unit of DBH activity represents 1 nmole of octopamine formed per milliliter of serum per 30 minutes of incubation.

Normal rats were given two injections of 6-OHDA 24 hours apart and were killed 72 hours after the first injection, a time at which adrenergic nerve terminals have been observed, in electron microscopic studies, to be degenerating after the doses of drug used (*9*). In repeated experiments, serum DBH activity was reduced 18 to 25 percent after this treatment (Fig. 1A). In no experiment was there a significant difference between the effects of the two doses of the drug which were used. As has been reported by other investigators, a 95 percent reduction in cardiac norepinephrine was found 3 days after the injection of 6-OHDA (*9*).

Although 6-OHDA in the concentrations used does not affect concentrations of catecholamine in the adrenal glands (9), animals in which the adrenal medulla had been removed were treated with two doses of drug to

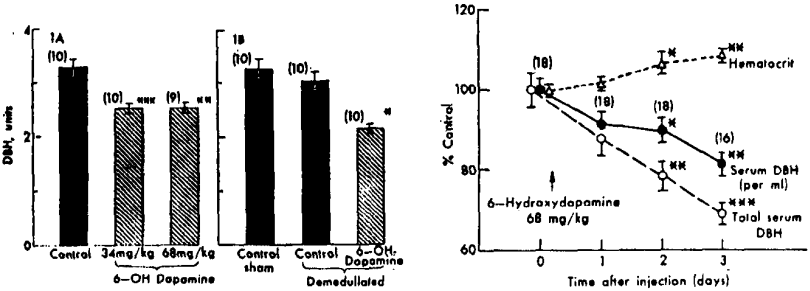

Fig. 1 (left). (A) Effect of two intravenous injections, 24 hours apart, of 6-hydroxydopamine (6-OHDA) of either 34 or 68 mg/kg on serum DBH activity 72 hours after the first injection. All serum samples were diluted with three volumes of water and assayed with 1 m*M* tyramine as substrate in the presence of 32 μM $CuSO_4$, with a 30-minute incubation period and internal standards of 40 ng of octopamine hydrochloride (*6*, *8*). (B) Effect of two injections of 6-OHDA (68 mg/kg) on serum DBH activity on rats in which the adrenal medulla had been removed. The number of animals in each group is given in parentheses. * $P < .02$ compared with control. ** $P < .01$ compared with control. *** $P < .001$ compared with control. Fig. 2 (right). Relative values of serum DBH expressed both as total serum activity and activity per milliliter of serum are shown at 24, 48, and 72 hours after single intravenous injection of 68 mg of 6-OHDA per kilogram. Relative hematocrit values are also shown. The number of animals in each group is given in parentheses. * $P < .05$ compared with control. ** $P < .01$ compared with control. *** $P < .001$ compared with control.

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rule out the possibility that the changes in serum DBH activity might be due to an effect on the adrenal medulla. The treatment resulted in a decrease of serum DBH activity of the same magnitude as that seen in normal rats (Fig. 1B). As in previous studies (*8*), no significant difference was found between serum DBH activity in sham-operated animals and that in the adrenal-demedullated animals 12 days after operation (Fig. 1B), demonstrating that the adrenal medulla is not necessary for the maintenance of serum DBH activity.

Inhibitors of DBH have been found in many tissues. These inhibitors are thought to act by binding to the copper in the enzyme molecule, and the effect of inhibitors can be blocked by addition of cupric ions to tissue homogenates (*15*). Cupric ions also increase the activity of serum DBH (*6*). Because of the possibility that the decrease in DBH activity after treatment with 6-OHDA might be due to an increase in serum inhibitor (or inhibitors), pooled rat serums from a control group and from a group of animals treated with two doses of 6-OHDA (68 mg/kg) were mixed with purified bovine adrenal DBH in the absence of cupric ion. Serums from the treated animals had shown a 25 percent decrease in DBH activity, but no increase in the inhibition of pure DBH was seen in its presence. Control serums inhibited purified DBH 48.3 ± 4.4 percent and that from treated animals inhibited 47.4 ± 2.3 percent.

The time course of the effect of a single injection of 6-OHDA on serum DBH activity was examined in two separate experiments. In each of these experiments serum DBH, expressed as activity per milliliter of serum was reduced significantly by 72 hours after the injection. The results of these experiments are shown in Fig. 2. Because rats treated with 6-OHDA develop diarrhea and become dehydrated, hematocrits were determined on blood from all animals. Hematocrits increased 10 percent during the 3 days after drug treatment (Fig. 2). Therefore, the magnitude of the change in serum DBH activity after treatment with 6-OHDA had been underestimated because of a reduction in total serum volume. It is possible to calculate relative values for total serum DBH activity if the assumption is made that red cell mass remains constant after drug treatment. With the use of the data presented in Fig. 2, a decrease in total serum DBH of 31 percent 3 days after a single injection of 6-OHDA was calculated. In these same experiments serum lactate dehydrogenase activity was determined in samples from control animals and from animals treated 72 hours earlier with 6-OHDA. Lactate dehydrogenase activity was increased in the treated animals from 2.13 ± 0.18 to 2.85 ± 0.21 (mean ± S.E.M., $P < .05$), demonstrating that the decrease of DBH after treatment with the drug was not a nonspecific phenomenon affecting all serum enzymes.

Because serum DBH activity still appeared to be decreasing 72 hours after 6-OHDA (Fig. 2), a long-term experiment was carried out. Rats were treated with 68 mg/kg twice within 24 hours and again with 68 mg/kg 1 week after the first injection. They were killed 2 weeks after the first injection, at a time that there is a continued depletion of norepinephrine in the heart and spleen (*9*). Two weeks after treatment was begun, serum DBH was reduced 20 percent in treated animals from 3.13 ± 0.31 to 2.49 ± 0.12 units (mean ± S.E.M., $P < .02$).

The "chemical sympathectomy" achieved with 6-OHDA, like the "immunosympathectomy" induced by anti-nerve growth factor (*16*), is only partial. The effects of 6-OHDA on norepinephrine stores vary greatly from organ to organ (*9*), and it has been reported that sympathetic nerves in rat mesenteric blood vessels are resistant to the effects of the drug (*9*). Even if most of the circulating DBH activity is derived from sympathetic nerve terminals, the incomplete nature of the sympathectomy achieved with 6-OHDA may explain the relatively small magnitude of the changes in serum enzyme activity found. Furthermore, that the vascular nerve terminals may be a major source of serum DBH is suggested by (i) immunofluorescent studies that have shown DBH to be present in nerves in the walls of blood vessels (*17*), (ii) evidence that vessels are a major source of the total norepinephrine excreted (*18*), and (iii) the resistance of vascular sympathetic nerve endings to destruction by 6-OHDA. It is also possible that only a portion of the serum enzyme activity is derived from sympathetic nerves, or that the decreases in activity in serum 3 days after drug were not larger because the circulating enzyme has a long half-life. This latter possibility is less likely as an explanation, however, because no further decrease in serum DBH was seen 2 weeks after treatment with 6-OHDA.

Our results demonstrate that a decrease in serum DBH activity occurs after a partial chemical sympathectomy. This finding is further evidence that the source of at least a portion of the serum DBH activity is the sympathetic nerve terminal, and that this easily measured, circulating enzyme activity might be useful as a measure of activity of the sympathetic nervous system both in normal physiologic responses and in certain disease states.

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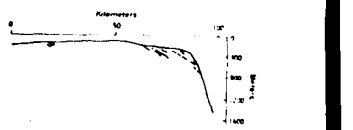

Fig. 2. Seismic cross section derived from records along line *G* of Fig. 1.

Orbitolina, a Cretaceous Larger Foraminifer, from Flemish Cap: Paleoceanographic Implications

Abstract. *The Tethyan larger foraminiferal genus* Orbitolina *has been found in the easternmost part of the western North Atlantic continental shelf at 46°30′N. All other known occurrences of the genus in North America are south of 33°N. The species is* Orbitolina conoidea *Gras; its abundance in a grain-supported limestone indicates a tropical neritic environment and precludes the influence of Arctic waters in the Flemish Cap region in Early or Middle Cretaceous times.*

Flemish Cap is the easternmost element of the Atlantic continental margin of North America. Although it is separated from the Grand Banks of Newfoundland to the west by the deep water channel of Flemish Pass, the water depth over the central part of the cap is less than 150 m (Fig. 1). Dipping sedimentary strata have been detected on the flanks of Flemish Cap. A fragment of fossiliferous limestone dredged from the southern slope of Flemish Cap (*X* in Fig. 1) contains abundant *Orbitolina conoidea* Gras, a Lower Cretaceous larger foraminifer of unquestionable Tethyan affinity.

The limestone was dredged from a depth of approximately 1480 m during a cruise of the C.S.S. *Hudson* in 1967 (*1*). The dredge haul consisted mainly of large angular slabs of limestone and well-rounded cobbles and pebbles of granite and gneiss. On the southern slope of Flemish Cap, limestone has been recovered in quantity only in dredge hauls from depths greater than 270 m (*2*), where seismic profiler results and bottom photographs indicate outcrops of layered rocks. The ubiquitous occurrence of well-rounded specimens of igneous rocks indicates an ice-rafted origin for this fraction of the samples.

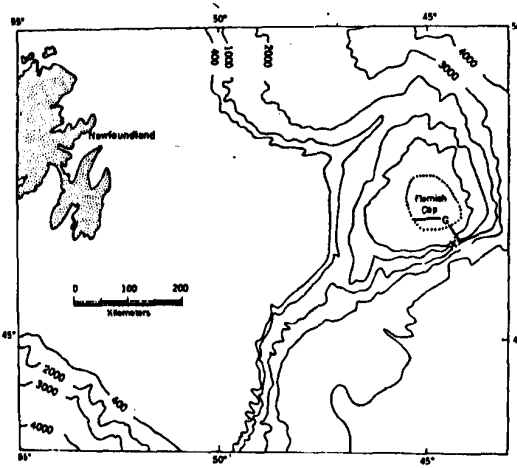

Fig. 1. Bathymetric map of the Flemish Cap area; isobaths are in meters. Letter *X* indicates sample location. Line *G* denotes the location of the seismic cross section in Fig. 2.

Seismic profiling was conducted over Flemish Cap in 1969 from C.S.S. *Hudson* and C.N.A.V. *Sackville* (*2*); a single-channel recording system was used, in conjunction with an air-gun energy source. The seismic profiler results show that the top of Flemish Cap is a smooth erosional surface, with a central area of seismically "hard" basement encircled by a zone of outward-dipping strata. The inferred extent of the central basement area is indicated by the dotted line in Fig. 1. One of the seismic profiler traverses, line *G* (Figs. 1 and 2), is in the vicinity of our sample station. A seismic cross section derived from the records along this line is shown in Fig. 2. The bottom profile is smooth, except for a minor step at the contact between the truncated strata and the basement media. Truncation of the layered rocks is also obvious on the slope of Flemish Cap.

The thin sections illustrated in this report (Fig. 3) were cut from a fossiliferous limestone specimen, which measured approximately 10 by 7 by 1.5 cm. This grain-supported limestone is extremely rich in specimens of the larger foraminifer *Orbitolina conoidea* Gras (*3*). In the thin sections examined, the density of tests is often about 40 per square centimeter. The form is generally conical. The very distinct marginal zone is about 0.04 mm thick. The radial zone is narrow and, at base, does not generally cover more than one-third of the diameter. The thick central reticulate zone includes a small amount of fine detrital particles. Calcite eyes are not seen. The megalospheric embryonic apparatus is apical and is surrounded by a number of periembryonic chambers. Because of the random orientation of the specimens of *O. conoidea* in the rock sections, few measurements

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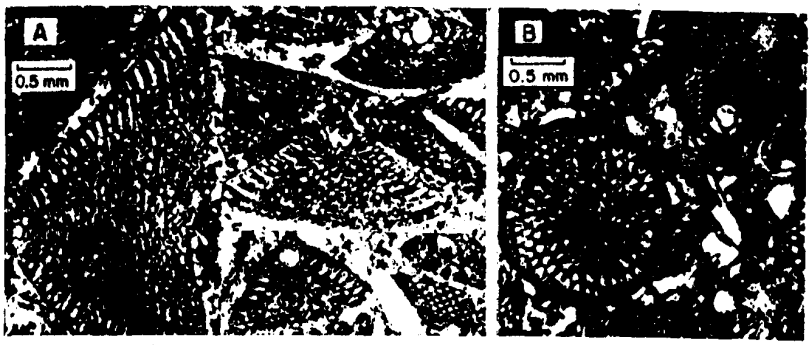

Fig. 3. *Orbitolina conoidea* Gras in grain-supported limestone from Flemish Cap. (A) Axial, near-axial, and oblique sections. (B) Near-basal and oblique sections.

be made of axial sections of individuals. The measurements of seven megalospheric and seven microspheric specimens are summarized in Table 1.

Orbitolina is fairly common in Lower Cretaceous limestones (Trinity Group) of the southern United States, but most of the species seem to be indigenous (*4*, *5*) and none of them resemble the Flemish Cap species *O. conoidea* Gras. To our knowledge, this is the first report of a North American *Orbitolina* north of 33°N. The species is locally abundant in other areas, especially in western Europe and the Middle East. The known distribution of *Orbitolina* is shown in Fig. 4; different species range from Neocomian to probable Maestrichtian, but the abundance of the genus is greatest in Aptian-Albian. The reported range of *O. conoidea* is Neocomian to Cenomanian, with the maximum development in Aptian. The restriction of *Orbitolina* to the Tethyan or Mediterranean biogeographic province will be obvious from Fig. 4. An association of *Orbitolina* with rudistan reefs has also been frequently reported (*5–8*).

Southwest of Flemish Cap, from the Grand Banks of Newfoundland, two subsurface sections with about 1445 m of Mesozoic and Cenozoic sediments have recently been described (*9*, *10*). The Lower and Middle Cretaceous sediments were interpreted as nearshore clastics containing few planktonic Foraminifera, which have been identified as primitive globigerinids, mostly of Tethyan affinity but with occasional boreal elements (*9*). Our Flemish Cap lime-

Table 1. Measurements (in millimeters) of *Orbitolina conoidea* Gras from Flemish Cap.

Value	Diameter	Height	Diameter / Height	Larger diameter of proloculus
	Megalospheric generation (seven specimens)			
Maximum	2.9	1.3	2.6	0.18
Minimum	0.9	0.4	1.7	0.12
Mean	1.6	0.8	2.1	0.16
Coefficient of variation	40.8%	40.2%	13.9%	13.6%
	Microspheric generation (seven specimens)			
Maximum	4.2	1.7	2.8	
Minimum	3.1	1.2	2.4	
Mean	3.5	1.4	2.6	
Coefficient of variation	11.8%	12.9%	4.8%	

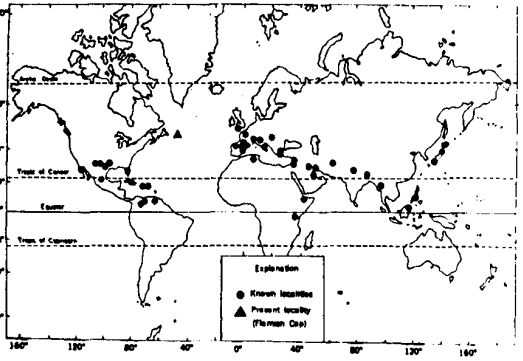

Fig. 4. Geographic distribution of *Orbitolina* [after Douglass (*4*), with additional data from Mébes (*5*)].

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stone belongs to a different facies. Planktonic and smaller benthonic Foraminifera are rare and poorly preserved. The striking abundance of *Orbitolina* is, on the other hand, characteristic of a reef facies (*7*).

The presence of the Tethyan larger Foraminifera indicates a "warm water" neritic environment for the Flemish Cap area during Early or Middle Cretaceous times. Paleomagnetic data support the conclusion that the North Atlantic Ocean (north of 40°N) was almost fully open by Middle Cretaceous time (*11*). The presence of a tropical Mediterranean (possibly forereef) fauna would, however, preclude the influence of Arctic waters and certainly the dominance of a cold current in the Flemish Cap region. The existence of a warm transoceanic gyre would, of course, be advantageous for the distribution of identical species of tropical larger Foraminifera along comparable latitudes on both sides of the North Atlantic. Thus, the water-mass distribution in the North Atlantic about 100 million years ago was very different from that of the present. The Labrador Current is a very significant component in the North Atlantic Ocean of today, and between 45° and 60°N the mean isotherms for surface water swing northward through several degrees of latitude from the western to the eastern side (*12*). Instead of this cold current, a northern branch of a Cretaceous "Gulf Stream" system might have been flowing in the Flemish Cap region. The idea of a major change in the water-mass relationships in this area after Middle Cretaceous times agrees well with the suggestion of Berggren and Hollister (*13*) that the North Atlantic region became linked with the Arctic region about 60 million years ago, at which time a boreal biogeographic province was established in the Atlantic Ocean.

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Eye Marks in Vertebrates: Aids to Vision

Abstract. *Lines leading forward from the eye may function as aiming sights in many small vertebrates. The chief evidence is the correlation of distribution and positions of eye-lines in various vertebrate groups with predatory feeding habits. Dark patches around the eye may serve to reduce glare in species in bright environments. Facial patterns often have multiple functions.*

Several functions have been suggested for the characteristic colors and markings of species, including camouflage (*1*, *2*), social signals (*3*), mimicry (*4*), and thermoregulation (*5*). We suggest that, in addition, the patterns of circles and lines about the eyes of vertebrates may enhance their vision and enable predaceous species to locate and capture prey more effectively.

Eye-lines are well-defined marks, usually very dark and narrower than the eye. The dorsal borders are usually more or less straight and uninterrupted (Fig. 1A). The ventral margins may be straight, but some are curved, demarcating a facial patch. The eye-lines extend from the margin of the eye or, more rarely, from or through an eye circle (Fig. 1B) toward, above, or below the bill tip or snout; sometimes they become narrower along their path. Some are positioned so that the line of sight is along the upper edge of the eye-line. However, often a line from the center of the pupil bisects the dark eye-line. Another frequent pattern is that of light over dark lines, which could be used as a line of sight in at least two ways; either the dark line or the line margins could be followed (Fig. 1B). The area above the eye-line might regulate light falling on the eye-line. Many birds lift the feathers above the eye-line forming an eye-brow (Fig. 1C). In birds with eye-lines the area above the line is frequently light in color. Increased light falling on the dark line from this area might increase visual acuity.

If the eye-lines serve as lines of sight in tracking and capturing swiftly moving prey, their presence, position, and type should be related to feeding habits. These lines should occur more frequently in species that feed on rapidly moving prey. We grouped North American songbirds by feeding habits in six categories, ranging from rank 1 in which more than 50 percent of the diet consisted of flying insects or other swiftly moving animal life. There was a + .94 rank-difference correlation between the presence of eye-lines and feeding on swiftly moving prey [see (*6*)]. Also we compared 50 species of insectivorous vireos and warblers with 44 species of granivorous and insectivorous sparrows (Emberizinae, closely related to warblers). We assigned all 94 species to categories based on the complexity of eye markings. We considered a dark line, as shown in Fig. 1A, simple; light over dark stripes with a broken or unbroken eye circle we considered complex (Fig. 1B). The most complex eye markings, as depicted in Robbins *et al.* (*7*), were found in all North American vireos and 32 out of 36 northeastern North American warblers, but in only four North American sparrows ($P < .01$).

Many species of birds having eye-lines show variations in the direction of the eye-line which are apparently

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adaptive. As a general rule (Fig. 1), bird eye-lines point just below the tip of the bill. Some grebes (Fig. 1D) and mergansers (*Mergus* sp.) have a line slanting downward from the eye to the tip of the opened lower mandible. They may use the lines in sighting on prey before diving or when swimming under water. The birds probably open their bills and look down the eye-line toward their prey. Many sandpipers have eye-lines which point below the bill tip or, in the case of some with decurved bills, to it (Fig. 1E). These birds have medium to very long bills which would probably interfere with direct sighting on the very small invertebrates on which they feed. On the other hand, it is possible that the upward angle of the eye-line in herons is used as a line of sight that helps them correct for light refraction by the water. They strike at the apparent image, but they hit the fish (*6*).

If eye-lines serve as sighting devices it would be expected that smaller members of a group would be more likely to have such lines than larger members because the prey-capturing apparatus of the former is relatively smaller and the prey moves more swiftly in relation to their size. Among the North American frogs depicted in Conant (*8*) and Stebbins (*9*), almost all ranids and hylids longer than 2½ inches lack eye-lines, whereas almost all below this size have them ($P < .01$). Among eastern North American salamanders (*8*) there is a similar trend, smaller species more frequently having eye-lines ($P < .01$); however, such eye-lines are relatively uncommon in salamanders. Do predaceous young have eye-lines more often than the much larger adults? This seems to be the case among some ambystomid salamanders; the lines are present in the young of some species and absent in adults. The same trend occurs in certain lizards (for example, skinks).

The eye-line or groove (or both) in fishes is almost always unicolored and typically leads from the eye to the snout. If predation is broadly defined as feeding on discrete objects and includes biting of seaweed, then eye-lines or grooves occur only in predaceous species of fish, although only a minority of such fish have eye-lines. In general, nonpredatory species such as filter feeders, suckers, and grazers (but not browsers) do not have eye-lines (*10*). The pickerels (*Esox* spp.), which have a teardrop mark (Fig. 1F), typically wait for prey by maintaining steady postures near the water surface where they probably sight down the teardrop on small species of fish in submerged aquatic vegetation beneath them; then they plunge downward and snap up a victim. The pigmented eye grooves of the northern pike (*Esox lucius*), which converge toward the tip of the spatulate snout, may enhance binocular vision and function as aiming sights (*11–13*). These sighting grooves also occur in the American chameleon (*Anolis carolinensis*), and Polyak (*11*) suggests that the black stripes around and in front of the eyes of purple martins (*Progne subis*) resemble these sighting grooves.

Refinement of the accurate strike faculty increases with the probability of missing prey and may reach a zenith in the tree snakes. For example, the vine snake (*Oxybelis aeneus*) has a very precise eye-line in a very narrow groove, which tapers to a point (Fig. 1H). The narrow head in this and other arboreal forms increases binocularity in aiming along such a path; as far as we know, such features are never this precise in terrestrial snakes. Vine snakes probably have the opportunity for comparatively fewer strikes at prey than terrestrial snakes since they are partially anchored to limbs and, more important, living where prey can easily drop away through space. Another species of tree snake (*Dryophis mycterizans*) not only has a pigmented groove but is further specialized for accurate striking by the addition of a keyhole-shaped pupil and a temporal fovea (*14*).

Several functions might be served by eye-lines and grooves exclusive of, or in addition to, aiming at prey. The

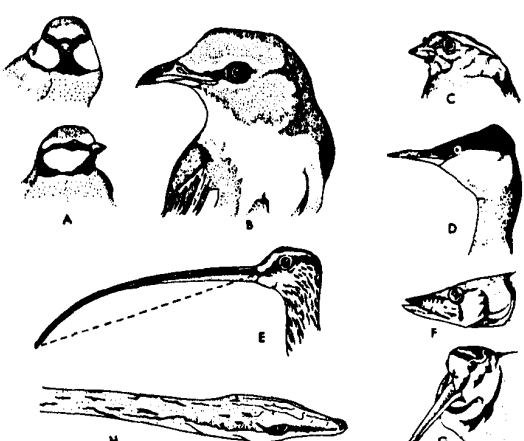

Fig. 1. (A) Simple eye-line of the partially insectivorous blue tit (*Parus caeruleus*). This is the most common type of eye-line in vertebrates. The eye-line in this species is wider than it is in other avian examples, but it was illustrated because it was the only species for which we could find both frontal and lateral photographs. (B) Tracing of close-up photograph showing the combined eye circle and eye-line of the yellow-throated vireo (*Vireo flavifrons*). (C) Raised yellow feathers above the eye-line in a white-throated sparrow (*Zonotrichia albicollis*) which may cast light along the line of sight. (D) Red-necked grebe (*Podiceps griseigena*) showing eye-line slanting downward. Such a line also occurs in some other piscivorous birds. (E) Long-billed curlew (*Numenius americanus*) showing direction of eye-line forward of center of pupil to bill tip. (F) Teardrop mark of the pickerel (*Esox americanus*) associated with downward dashes at prey. (G) Rearward pointed eye-line of the European woodcock (*Scolopax rusticola*), probably used in sighting of predators coming from behind. Associated with 360° vision plus front and back binocularity. (H) Head of the arboreal vine snake (*Oxybelis aeneus*) showing eye-line and groove.

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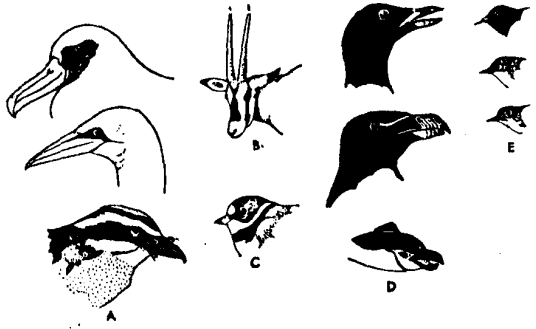

Fig. 2. (A) Facial patches and stripes associated with reduction of glare in very bright habitats. Laysan albatross (*Diomedia immutabilis*) (top), northern gannet (*Morus bassanus*) (middle), and great kiskadee (*Pitangus sulphuratus*) (bottom). (B) In the beisa oryx (*Oryx beisa*) eye-lines may hide eyes and further decrease visibility to potential predators by interrupting the beast's outline; also, the pattern may decrease glare on the line of sight toward clumps of grass in the animal's usually bright open habitat, and the pattern may be important as a social signal. (C) The semipalmated plover (*Charadrius semipalmatus*) has no sighting line; stripes are probably mainly for disruption of the head pattern (hiding the eye) and for aiding species recognition. (D) Alcids and penguins are very highly specialized for catching fish. The lines about the eyes and heads of virtually all birds of these two groups could not be for sighting on prey; they probably serve intra- and interspecific recognition. (E) Male (top), female (middle), and first-year male (bottom) American redstart (*Setophaga ruticilla*), showing an interaction of selection for recognition of sex, species, and age as well as a complex eye-line and eye circle associated with feeding.

Old World bittern (*Botaurus stellaris*) points its bill up and fixedly stares down a subocular stripe in reaction to mammalian predators. The stripes pointing rearward in the European woodcock (*Scolopax rusticola*) (Fig. 1G) may also serve as sighting lines toward predators. The woodcock is well known for its rearward binocular vision. Duke-Elder (*15*) suggests that in the goliath heron (*Ardea goliath*) the eye groove increases binocularity; his photograph reveals the eye sighting along this groove. A dark line along part of this groove may decrease glare, aid aiming, or both.

We have presented evidence for the association of the presence and position of eye-lines as aids in feeding. A line drawn through the center of the eye to the farthest extent of the eye-line usually bisects the eye-line. Further, the eye-line is directed toward the point where prey would be expected. No other function that we know of would require such precise positioning (Fig. 1, A to H), although there may be secondary functions.

Now we consider some facial marking other than eye-lines. Light-colored circles around the eye, variable in width according to the species, are more common in birds feeding in reduced light. Such circles probably act as light-gathering devices (Fig. 1B). On the other hand, dark patches or, less commonly, broad black stripes around, across, or near the eye are often found in diurnal birds living in very bright (open water) and bright (open land) environments (Fig. 2A). Some mammals have such patches which almost certainly act as reducers of glare.

Other types of markings on the head may have various other functions including hiding the eye from predators (*2*) or disrupting the head pattern as camouflage (*1*). In the beisa oryx (*Oryx beisa*) (Fig. 2B) eye-lines may hide eyes and further decrease visibility to potential predators by interrupting the animal's outline; also, glare may be decreased along the black line of sight when the animal feeds in its bright open habitat. Another suggested function of facial markings in this species is mimicry of the antlers, which probably enhances their effect as social signals (*16*). This is a good example of the multiple functions of such eye markings. The head pattern of the short-billed semipalmated plover (*Charadrius semipalmatus*) is probably mainly disruptive (Fig. 2C). Facial marks in the alcids and penguins are probably used only as social signals (Fig. 2D). Eye-lines are absent. Smith (*17*) found that eye circles of certain Arctic gulls, which vary in color in sympatric species, were part of a pattern of contrast of eye and head that forms the basis for species recognition. Certain facial markings in other species have similar functions.

Mutually exclusive demands for facial adaptations may override selective pressures for eye circles and eye-lines, or a compromise may be reached. Figure 2E shows the faces of three plumages of the American redstart (*Setophaga ruticilla*). These birds live in the understory of deciduous forest where there is subdued light. The first-year male and the adult female have complete or nearly complete eye circles which may increase light entering the eye. The black-headed male has no eye marks, very likely because of the demands of age, species, and sexual recognition.

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Vitamin A: Concentration in the Rat Liver Golgi Apparatus

Abstract. Vitamin A compounds (principally as retinyl esters) are concentrated in Golgi apparatus fractions from rat liver. The amounts vary with the vitamin A status of the liver and show an inverse relation to the concentration of ubiquinone. The results suggest a specific role of the Golgi apparatus in the mobilization or action, or both, of vitamin A compounds.

Vitamin A compounds are essential dietary constituents for man and vertebrates. In many parts of the world, their deficiency is a major cause of juvenile disease and blindness (*1*, *2*). Problems of vitamin A deficiency are partially averted through mechanisms of storage and slow release of the vitamin. During periods of sufficiency, excess vitamin A is stored in the liver where levels reach several hundred times the minimum daily requirement (*1*, *2*). The stored vitamin is then secreted from the liver into the circulation from which it is withdrawn at the many sites where the vitamin plays a functional role. The liver also provides the main store of vitamin A alcohol (retinol), which is important to vision and other body processes. Thus an understanding of the mechanism of hepatic sequestration and secretion of vitamin A compounds is important to an understanding of vitamin A nutrition and molecular function.

High concentrations of ubiquinone in fractions of Golgi apparatus from rat liver (*3*) are accompanied by large amounts of a compound with an absorption maximum at 328 nm. Similar extracts from endoplasmic reticulum or mitochondrial fractions reveal none of this compound on a protein equivalent basis. In this report, we show that the compounds absorbing at 328 nm are derived principally from vitamin A esters concentrated in the Golgi apparatus. Previous studies of the distribution of vitamin A in rat liver (*1*, *4*) have not considered the Golgi apparatus. Yet the hepatocyte Golgi apparatus is a major component in the mechanism for secretion of lipoproteins into the blood (*5*). We suggest that this functional role may be more general and include fat-soluble vitamins.

Livers of male Holtzman rats were used for preparing subcellular fractions. Animals deficient in vitamin A were obtained by feeding weanling rats, without restriction, a test diet (General Biochemicals) deficient in vitamin A until their weight plateaued (5 to 6 weeks). Purified Golgi apparatus, endoplasmic reticulum, and mitochondrial fractions were obtained as described previously (*6*).

Two procedures were used to determine vitamin A compounds. Large quantities (from more than 50 mg of Golgi apparatus protein) were determined spectrophotometrically after an extraction procedure similar to that employed for determination of ubiquinone (*3*) except that the vitamin A was eluted in the fraction containing 1 to 2 percent diethyl ether. Smaller quantities (from less than 10 mg of Golgi apparatus protein) were determined after alkaline hydrolysis, either the Carr-Price reaction (*7*) or a fluorometric method being used (*8*). Protein was determined according to the method of Lowry *et al.* (*9*).

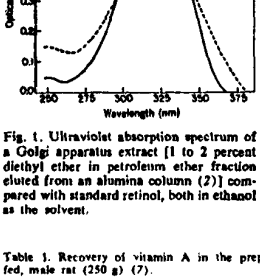

Fig. 1. Ultraviolet absorption spectrum of a Golgi apparatus extract [1 to 2 percent diethyl ether in petroleum ether fraction eluted from an alumina column (*2*)] compared with standard retinol, both in ethanol as the solvent.

When Golgi apparatus extracts were fractionated on alumina columns, the 1 to 2 percent diethyl ether eluate showed an ultraviolet absorption spectrum characteristic of vitamin A compounds (Fig. 1). With the Carr-Price reagent, the spectrum characteristic of vitamin A reaction products (absorption maximum at 620 nm) was obtained.

Retinol and retinyl palmitate standards were cochromatographed with partially purified vitamin A from Golgi apparatus extracts. Silica gel G thin-layer plates were developed in a mixture of cyclohexane and diethyl ether (80 : 20, by volume) or benzene. Spots were visualized with antimony trichloride reagent or observed in ultraviolet light (*10*). Most of the vitamin A of the Golgi apparatus extracts had an R_f similar to that of retinyl palmitate. Other thin-layer chromatography solvent systems used were those listed by Stahl (*10*) and the R_f's are provided in that reference. No attempt was made to identify specific retinyl esters. Neither the characteristic ultraviolet spectrum nor the Carr-Price reaction were obtained with extracts similarly prepared from equivalent amounts (on a protein basis) of purified mitochondria or endoplasmic reticulum fractions.

Table 1. Recovery of vitamin A in the preparation of a Golgi apparatus fraction from a fed, male rat (250 g) (*7*).

Fraction	Vitamin A content as retinol	
	Micrograms	Micrograms per milligram of protein
Total homogenate	1150	0.43
Unbroken cells, cell fragments, and nuclei	597	.62
Supernatant (2000*g*)	488	.44
Large particle fraction (sedimenting at 2000*g* with the Golgi apparatus and containing mitochondria, endoplasmic reticulum fragments, lysosomes, and plasma membrane)	96	.37
Purified Golgi apparatus	13	1.48

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Table 2. Vitamin A content (as retinol) of whole livers and Golgi apparatus fractions of rats, under varying conditions of age, sex, and dietary condition, determined by means of the Carr-Price reaction (6).

Age (days)	Weight (g)	Sex	Dietary condition	Vitamin A content (μg)		Vitamin A concentration (μg/mg of protein)		Percent of total vitamin A in Golgi apparatus fraction
				Whole liver	Golgi apparatus fraction	Total homogenate	Golgi apparatus fraction	
60	272 ± 22	Male	Fed	1915 ± 455	17.4 ± 0.3	1.4 ± 0.3	3.5 ± 0.7	1.0 ± 0.4
	244 ± 8	Male	Starved	1650 ± 160	55.6 ± 7.0	1.7 ± .2	6.7 ± 1.4	3.4 ± .9
	208 ± 2	Female	Fed	2070 ± 400	18.5 ± 0.5	2.1 ± .5	6.1 ± 0.3	0.9 ± .02
100	419 ± 4	Male	Fed	3920 ± 340	17.2 ± .1	2.3 ± .2	2.5 ± 1.0	4 ± .03
	328 ± 33	Female	Fed	7025 ± 125	25.7 ± 1.1	5.5 ± .3	8.0 ± 1.0	.4 ± .02

In recovery experiments, in which the fluorometric method was used (*8*), the bulk of the vitamin A was recovered in the supernatant and the fraction containing unbroken cells (Table 1). This agrees with previous studies (*4*). However, among the membranous fractions, the greatest concentration was in the Golgi apparatus. If one assumes a recovery of 30 to 40 percent for Golgi apparatus (*6*), as much as one-fourth to one-third of the total particulate vitamin A of the liver cell appeared within the Golgi apparatus fraction. A significant portion of the remainder was found in the heterogeneous membrane fraction containing mitochondria, lysosomes, endoplasmic reticulum, and plasma membrane which sedimented at 2000*g* with the Golgi apparatus. However, no vitamin A compounds were detectable spectrophotometrically in extracts of purified mitochondrial or endoplasmic reticulum fractions.

To relate the vitamin A content of the Golgi apparatus to the vitamin A status of the animal, Golgi apparatus fractions were prepared from a number of rats of both sexes and of various ages and dietary backgrounds (Table 2). As reported previously (*1*, *2*), rats accumulate more vitamin A in their livers with increasing age, females accumulating more than males. With the older females, the concentration of vitamin A in the Golgi apparatus reached about 8 μg per milligram of protein, and the fractions were noticeably yellow before extraction. With old males, the concentration of vitamin A in the Golgi apparatus was about 2.5 μg per milligram of protein, but the percentage of vitamin A in the Golgi apparatus fraction was approximately the same for both males and females. The latter was due to compensating differences in the total amounts of vitamin A in the livers. Similar relations were observed in comparing younger males and females, but the proportion of the total vitamin A recovered in the Golgi apparatus fraction was increased nearly threefold to about 1 percent of the total (Table 2). When male rats weighing 250 g were fasted for 48 hours, the vitamin A content of the Golgi apparatus exceeded that of females of comparable age. Under these conditions, approximately 3.5 percent of the vitamin A of the liver was recovered in the Golgi apparatus fraction.

In parallel experiments, vitamin A was not detected in Golgi apparatus fraction or total homogenates prepared from livers of rats deficient in the vitamin (indicated by a weight plateau), although the ubiquinone content of the Golgi apparatus fraction (9.6 nmole per milligram of protein) was increased nearly fourfold over that of control animals that had been fed similar diets supplemented with vitamin A. A similar reciprocal relation between ubiquinone and vitamin A concentrations has been observed in whole liver, the increase of ubiquinone in vitamin A deficiency being most pronounced in the crude microsomal fraction rather than in the mitochondrial fraction (*11*).

The results show that the concentrations of vitamin A in the Golgi apparatus reflect both the rate of supply to the circulation and the total vitamin A content of the liver. For example, starvation is known to increase the mobilization of reserve lipids and the secretion of very low density lipoproteins into the blood; a similar effect on vitamin A compounds might be expected (*12*). Thus a role of the Golgi apparatus in the mobilization and transport of vitamin A compounds is suggested. We do not discount the possibility that vitamin A participates in some regulatory function at the level of the Golgi apparatus. Retinol is a membrane-active substance that changes the properties of cytoplasmic membranes (*2*, *13*, *14*). Additionally, interest has focused on effects of vitamin A on sulfated mucopolysaccharides (*1*, *2*, *15*) and lysosomal membranes (*1*, *2*, *14*), both of which are derived from the Golgi apparatus (*16*). Yet a role of the Golgi apparatus in vitamin A mobilization seems clearest. Perhaps the vitamin A is carried to the blood stream via the same Golgi apparatus–secretory vesicle–plasma membrane export route postulated for secretion of plasma lipoproteins (*5*). A similar functional role for Golgi apparatus–derived vesicles as transporters of vitamin A–containing photopigments was suggested previously for photosensory cells in the eyes of a pulmonate snail, *Helix aspera* (*17*).

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Suppression of Food Intake with Intragastric Loading: Relation to Natural Feeding Cycle

Abstract. Rats were infused through chronically implanted intragastric tubes with 100 percent of their normal total daily food intake. The infusion was given either continuously over 24 hours or divided into discrete meals programed to simulate the rats' natural eating pattern. The same diet was also available for consumption by mouth. In neither case did the animals completely stop eating. During slow infusions excessive consumption ranged from 30 to 50 percent. During simulated meal infusion of the same total quantity of diet, they compensated far better, overeating by only 2 to 18 percent. Periodic filling of the stomach between scheduled meals was no more effective than a continuous slow infusion. Therefore, factors related to the natural feeding cycle make a significant contribution to the effectiveness of food in maintaining satiety and controlling food intake.

It is generally accepted that mammals control food intake under a variety of conditions to meet caloric requirements. Thus rats eat more in the cold, less in the heat (*1*), and compensate for [illegible]ng by overeating when refed and [illegible] dilution of the diet by increasing intake (*2*, *3*). However, it has been suggested that need for calories may not be the sole factor controlling food intake, because when 100 percent of food normally supplied by mouth is supplied by stomach load, animals do not compensate by precisely reducing food intake (*4*). This finding has led workers to postulate that oral factors may play an important role in controlling food intake (see *4*, *5*).

It is possible, however, that the observed failure to regulate during intragastric infusions may have resulted from the temporal distribution of the infusions. For example, Thomas and Mayer (*6*) used either a slow continuous infusion or an infusion which began when the rat began to eat a meal. In each case total caloric intake significantly exceeded the preinfusion intake.

We report here a study which shows that rats will more precisely compensate for intragastric loads if they are given in discrete meals at times which closely approximate their normal eating habits.

Four separate experiments, using a total of 23 rats, were carried out. All rats had previously been implanted with chronic nasopharyngeal tubes (*7*) permitting direct injection of a nutritionally adequate liquid diet into the stomach (*8*). The same diet was also available for the rats to eat. Infusion of the diet into the stomach was accomplished by using a Holter peristaltic pump which delivered diet at a preset rate and which was controlled automatically by behavioral programing equipment. Water was always available.

Our first experiment was designed to determine the degree to which rats

Table 1. Mean daily food intake, meal sizes, and number of meals before and during slow continuous infusion and infusion in discrete meals simulating each animal's normal meal pattern. The two rows for each animal are the two trials under each load condition. A period of at least 5 minutes without eating was used to separate meals.

Rat No.	Body weight (g)	Preinfusion			24-hour slow infusion			Discrete meal infusion		
		Mean oral intake (ml)	Mean number of meals	Mean meal size (ml)	Oral intake (ml)	Number of meals	Mean meal size (ml)	Oral intake (ml)	Number of meals	Mean meal size (ml)
62	278	30	10	3.0	9	6	1.5	3	3	1.0
					9	2	4.5	2	2	1.0
53	279	35	17	2.1	17	7	2.4	5	7	0.7
					12	6	2.0	4	6	0.66
45	289	33	12	2.75	12	4	3.0	4	4	1.0
					23	6	3.8	6	4	1.5
40	320	36	22	1.63	20	13	1.54	3	2	1.5
					21	4	5.25	4	2	2.0
39	324	33	12	2.75	12	5	2.4	3	4	0.75
					10	4	2.5	4	2	2.0
38	370	50	29	1.7	16	9	1.77	1	1	1.0
					29	14	2.07	5	2	2.5
30	280	27	10	2.7	11	3	3.6	4	3	1.3
					10	4	2.5	5	4	1.2

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Table 2. Total food intake in milliliters, as a function of type of infusion: slow continuous infusion versus discrete infusions of equal volume occurring either 10 or 90 minutes before access to food.

Rat No.	Preinfusion intake	Infusion 10 minutes before door opens	Infusion 90 minutes before door opens	Continuous 24-hour slow infusion
66	30	5	16	17
64	30	1	11	9
63	27	1	13	7
61	35	0.5	11	10
57	37	2	5	12
56	39	8*	15	16

* Infused only 34.0 ml owing to pump malfunction.

failed to reduce food intake when the total daily caloric requirement was supplied directly to the stomach by a continuous intragastric infusion. Six rats each received a single continuous infusion for a 24-hour period. The amount infused was the animal's maximum intake during three consecutive preinfusion days. In spite of the fact that the rats received 100 percent of their preinfusion intakes by stomach tube, all six animals overate by 30 to 50 percent.

It is possible that this failure to adjust intake precisely was because a single 24-hour period was not sufficient time for rats to become accustomed to food being injected directly into the stomach. It has been shown that dogs take a number of days to adjust to intragastric loads (*9*). Accordingly, in our second experiment four additional rats were given a continuous 24-hour infusion for 5 to 14 consecutive days. The results showed that no gradual compensation occurred and that oral intake remained as high after 14 days as it did after 24 hours. During the infusion period, all animals gained weight which was gradually lost when the infusion was stopped.

One possible explanation for these findings is that while food was infused at a constant rate, the diurnal caloric needs of the animal probably were not constant. For example, it is well known that rats eat in meals and that these meals follow periodic bursts of activity and energy expenditure (*10*). Meal-taking behavior may be a response to changing metabolic demands which slow infusion at a constant rate would not always satisfy.

Our third experiment was designed to test this hypothesis. Instead of a constant infusion of food, we attempted to infuse discrete meals at times which closely approximated the rat's normal eating pattern. To determine when rats ate, the feeding behavior of each rat was recorded on an event recorder continuously for 24 hours, using a drinkometer which detected licking of the liquid diet from the spout of an inverted graduated cylinder. Once each day the total food intake was measured and the event records were analyzed. At least four consecutive days of meal-taking patterns were used to create a pattern which simulated the animal's own. In order to infuse those meals, a punched-tape programing device was used. Holes were cut in strips of 16-mm film, which was run through a modified Gerbrands variable interval timer. Each time the microswitch encountered a hole in the tape, a meal equal in volume to the average meal size on the preinfusion day on which food intake was maximum was delivered through the intragastric tube. Each rat received 2 days of discrete meal infusions and 2 days of continuous 24-hour infusions. Total volume infused under both conditions was approximately equal. The order in which these were given was randomized for each rat. Infusions were separated by a number of days sufficient to allow food intake to return to preinfusion levels.

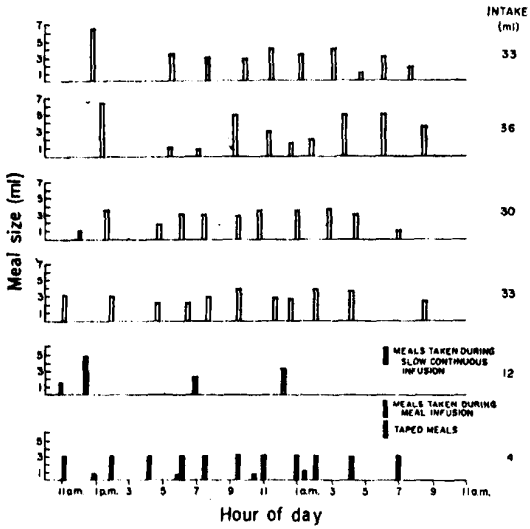

Fig. 1. Meal-taking before and during intragastric loading in a single animal. The top four lines show the temporal pattern of meal-taking for four consecutive days before intragastric infusion. The height of each bar shows the size of the meal, and its location on the abscissa shows its time of occurrence. The fifth line (hatched bars) shows meals taken during a day of continuous slow infusion. The bottom line shows the pattern of simulated meal infusion (solid bars) and meal-taking (stippled bars) during simulated meal infusion. The numbers at the right are the oral consumption for each day. The experimental chamber was completely darkened between 6 p.m. and 6 a.m.

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The results of this experiment, shown in Table 1 and Fig. 1, indicate that infusion according to the rat's individual eating pattern always produced a greater suppression of oral intake than did a continuous 24-hour slow infusion. Examination of the pattern of eating during the infusions suggested a mechanism by which reduction of intake was achieved. During both slow continuous and intermittent meal infusions, the number of meals taken over the 24-hour period was substantially reduced. It seems likely, therefore, that the infused diet provided metabolic signals which postponed the onset of a meal by maintaining the level of some metabolite above a critical threshold. On the other hand, the sizes of meals were reduced only by discrete meal infusion.

The results of this experiment strongly suggest that periodic rapid increases in caloric needs which were not offset by the slow infusion resulted in the regulatory failure in our first experiment. Our third experiment, however, did not rule out the possibility that the suppression of intake when rats were loaded discrete meals simply resulted from filling the animals' stomachs periodically with food. In order to disengage stomach loading per se from stomach filling when the animal was hungry, a fourth experiment was carried out.

In order to control more precisely the time at which an animal would eat a meal, a device was constructed and programed so that every 3 hours a door opened allowing access for 15 minutes to the liquid diet. When animals had adapted to the 3-hour schedules the infusions were begun, while the animals were maintained on the same schedule. Each of six animals was given three intragastric infusions: a continuous slow 24-hour infusion, an infusion 10 minutes before the door opened, and an infusion 90 minutes before the door opened. Animals were loaded meals equal in size to the mean meal size taken on the preinfusion day on which intake was the highest. Table 2 clearly shows that the suppressing effect of loading discrete meals observed in experiment 2 is not the result of periodic stomach filling. In fact, loading meals 90 minutes before animals were due to eat does not suppress oral intake any more effectively than does slow infusion. This experiment therefore strongly suggests that intragastric loading is more effective in suppressing food intake if it occurs when the animal is ready to eat a meal.

Furthermore, it seems that neither continuous slow infusions nor loads which begin once eating is in progress are as effective in suppressing food intake as are loads which anticipate a spontaneous meal. For example, Thomas and Mayer (*6*) infused 50 or 75 percent of rats' total daily intakes either continuously or during spontaneous meals. Calculations based on their data show that each milliliter of intragastric load reduced food intake 0.75 ml under either condition. In our experiments, infusions of 100 percent of the rats' total daily intakes reduced consumption 0.61 ml per milliliter of intragastric load during slow infusions and 0.86 ml per milliliter of intragastric load during simulated meal infusions. Thus, when equated on the basis of a milliliter of suppression of oral consumption per milliliter of infusion, loading which anticipates spontaneous feeding is the most effective means of suppressing food intake by intragastric infusion. Nevertheless, it is possible that complete suppression was not obtained because oropharyngeal stimulation may be necessary for complete satiation. This is suggested by Snowdon's (*3*) demonstration that in rats obtaining all of their food by intragastric self-injection, intragastric intakes were 62 to 76 percent of oral intakes.

The major conclusion to be drawn from this study is that the effectiveness of suppression of oral intake depends not only on the total amount of food infused but also on the temporal distribution of the infusions relative to the time when the animal is about to eat. This finding would indicate that factors other than the total caloric intake may play a more important role in determining satiety than some of the earlier infusion studies had suggested (*4, 5*). Finally, these data may have some clinical application where enteral nutrition is the preferred route of hyperalimentation (*11*).

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Chesapeake Bay: A Second Look

In a recent issue of *Science* (*1*), Holden wrote an article on the Chesapeake Bay, describing some of the uses made of the bay and the different, sometimes conflicting, demands made on the estuary by these activities. Holden compiled several impressive statistics on the areas of oyster bars, clam bars, and beaches that have been closed because of "pollution," as well as statistics on declining oyster production. These figures may leave the reader with the impression that the Chesapeake Bay is "on the ragged edge of becoming badly polluted," as the Chesapeake Bay Foundation has stated. For the most part, Holden's statistics are "accurate," but they are strongly reminiscent of the comic sportscaster's account of partial baseball scores: "In the game

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in Detroit between the Tigers and the Baltimore Orioles, it was the Detroit Tigers 5!" From this report, one cannot tell whether the Tigers won or lost. From Holden's account, one cannot begin to objectively assess either the possible harmful effects these activities have had on the bay, or the general state of health of the bay.

Apparently in order to establish the once pristine condition of the bay, Holden begins her story with an often-quoted description by Captain John Smith: "Heaven and earth seemed never to have agreed better to frame a place for man's commodious habitation." This "commodius habitation" is, according to Holden, threatened by pollution whose "ominous signs may be read in seasonal fish kills" and other menacing indicators. It is ironic that in a seldom-quoted account of one of his voyages on the unspoiled, "commodious" Chesapeake Bay, Smith recorded (*2*):

> . . . that abundance of fish, lying so thick with their heads above the water as for want of nets we attempted to catch them with a frying pan . . . neither better fish, nor variety of small fish had any of us ever seen in any place so swimming in the water . . . and some we have found dead upon the shore.

Smith was describing a fish kill in the pristine waters of the upper Potomac (Patawomek) estuary, a fish kill long before the "march of progress" imperiled the health of the bay.

Officials of the Fish and Wildlife Administration of the State of Maryland have no evidence that fish kills have increased either in frequency or in severity since Smith wrote his account. In fact, fish kills are a superficial yardstick for measuring pollution, one whose application may lead to false conclusions.

Holden points out that other "ominous signs may be read in . . . the 28,000 acres of oyster beds and 39,000 acres of clam beds that have been closed due to pollution." The 20,877 acres of oyster bars that were closed in 1970 represent less than 7 percent of the total area of productive oyster bars in Maryland waters, and of these 20,877 acres, 15,007 acres were closed because of pollution (coliform bacteria counts). The remaining 5870 acres were closed because of a Maryland regulation requiring a "buffer zone" around sewage treatment plant discharges. The total area of oyster bars closed each year during the past decade has ranged from more than 27,000 acres in 1963 to less than 10,000 acres in 1965, and has averaged about 20,000 acres. The total area has not increased in the past 4 years. The 39,000 acres of clam beds that were closed represent about 13 percent of the total clam bottom in Maryland waters. The total closed area has decreased over the past 5 years.

Holden made the following statement concerning oyster production:

> The supply of oysters has been severely depleted in recent decades, largely because of overexploitation. In the early 1900's, the annual take from 300,000 acres of oyster bars was between 8 and 10 million bushels. Now the harvest is between 2 and 3 million bushels.

Although statistics on seafood harvests prior to 1929 are not very reliable, there is no doubt that oyster production has declined since the early days of commercial oystering in the bay. At that time, oystermen were harvesting an accumulated resource, a resource virtually untapped except for the small quantities of oysters taken by Indians and settlers. Because bars were literally stripped clean, early harvest figures frequently included seed oysters and empty shells. Modern catch figures include only marketable oysters.

The decline in the bay's oyster production since the turn of the century is attributable to approximately 100 years of overfishing. Maryland's production fell to less than 1.5 million bushels in the 1961–62 season. Many of the bars had been depleted, and the rate of natural recruitment was not sufficient to bring them back. In 1961, Maryland reenacted a seed production program. By the 1965–66 season, production began to climb rapidly, and since 1966, Maryland has led the nation in oyster production by a substantial margin. Over the past 4 years, Maryland's harvest has ranged from more than 3 million bushels for the 1966–67 season to about 2.5 million bushels in 1970–71. Production this past season was limited by the market, not by the crop. Increased oyster production awaits the creation of new markets and new processing techniques.

Since 1963, the value of Maryland's total catch by commercial fishermen has been increasing at the rate of about $1.5 million a year. Over the past decade, Maryland's annual commercial catch of striped bass, averaging more than 3 million pounds, has led the nation. Sport fishermen are estimated to take more than twice this number. Maryland leads the nation in the production of soft-shell clams, and the Chesapeake Bay is the nation's leading producer of blue crabs.

The general trend of the total catch and the total value of the Chesapeake Bay's commercial fisheries has been upward since the 1930's. The success of individual fisheries has fluctuated quite wildly over the same period. Large natural fluctuations of estuarine fisheries are common and may be greater than fluctuations caused by fishing. No other causes of fluctuations have been documented. The Chesapeake Bay remains one of this nation's richest fishing grounds.

When we read statements of the deterioration of the bay, we are reminded of Mark Twain's cable to the Associated Press after he read of his death: "The reports of my death are greatly exaggerated." Several of the bay's tributaries are, in layman's language, "dying." To be more nearly correct, they are much too "alive." Large populations of blue-green algae thrive in the nutrient-rich Potomac and in Baltimore Harbor and Back River. Blue-green algae are also, according to the Environmental Protection Agency in Annapolis, Maryland, beginning to appear in the Gunpowder and the Bush rivers, two small tributaries of the upper bay. The presence of these algae, following the construction of sewage treatment plants on these tributaries, could have been predicted. The adjacent upper bay is still "healthy," but the levels of nutrients, whose primary source is the Susquehanna River, are near the upper limit. The phytoplankton production is high, but the grazing rate is also high, thus preventing a buildup of undesirable algae.

The Chesapeake Bay is certainly threatened with change by man's activities. Some changes have already occurred, but, by most currently accepted standards, the main portion of the bay is healthy. It is because of its healthy state that it bears close watching. This is not to suggest that we are necessarily using the correct yardsticks. Large harvests of shellfish and finfish are poor indicators of a healthy estuary if the shellfish and finfish cannot be consumed because of high concentrations of pesticides or heavy metals. Sewage, pesticides, herbicides, and sediments pose the greatest "threats" to the bay. These by-products of man's activities

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have the most demonstrable effects in leading to "less desirable" conditions in the estuary. Studies of the effects of thermal discharges have failed to document any substantial damage from present inputs.

Perhaps the most disturbing aspects of Holden's articles are contained not in her "floating statistics," but in the following three sentences: "Although the bay has long been a laboratory for marine scientists, no comprehensive plan has yet been developed to protect its bounty and order its progress. . . . Is the bay dying and if so what can be done to save it? Decisive action will have to await more detailed understanding of the bay." Holden's implication that scientists should devise a master plan for the management and utilization of this great natural resource is perilous, and her statement that there is presently not sufficient scientific information for any decisive action is erroneous. There are certainly countless unanswered scientific questions—there always will be. But the general features of many of the important processes in the bay are known and understood, and scientific predictions can be made. In many respects, scientific information has developed at a faster rate than management's ability to utilize it. Decisions of how to manage the Chesapeake Bay, of how to "order its progress," require not only scientific inputs, but social and economic inputs as well. Management problems rarely have unequivocal answers. They are very frequently value judgments, and natural scientists have no peculiar talents for making such decisions. Science cannot solve all of man's problems, environmental or otherwise. It cannot incontestably determine either what uses of the bay are most important, or even what uses are most desirable. Through science we can learn to understand the bay and even, in part, to control it, but science cannot unequivocally and decisively determine the ways in which we *should* control it. Scientists can, however, help to design and implement the actions necessary to the attainment of the desired ends, once those ends have been selected. Decisive action does not "await a more detailed understanding of the bay." It awaits a crisis. And there is little evidence to suggest that such "decisive action" will be predicated on scientific knowledge.

The Chesapeake Bay has served her many masters well, and she continues to do so. The bay is healthy, but it requires nonsimplistic management.

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Chemical Defense Mechanisms and Genetic Polymorphism

Although this comment is not intended to be critical of the admirable article of Whittaker and Feeny (*1*), it does aim to be complementary in that it emphasizes a point crucial to their argument which they overlooked. Evolution of the type they described does not take place at the specific level, but within species. Thus in order to substantiate their thesis, particularly for passive defense mechanisms, it is essential to show that chemical differences between individuals in polymorphic or continuously varying populations convey differential advantages as far as protection is concerned. Admittedly, they did briefly mention *Bufo regularis*, *Strophanthus sarmentosus*, and *Nezara viridula*, but none of these examples fulfills the criteria required.

The impasse can be expressed another way. Where the same form of a character occurs in all members of one species, but in none of a closely related one, it is not possible to avoid confounding the character with the species. This is, of course, the ideal state of affairs for the taxonomist. Yet, when we consider a species monomorphic for a secondary substance, we have no means of telling whether a particular nonparasite could and would attack that species if the putative defensive substance were not present.

The only way around this difficulty is to examine species polymorphic for secondary substances; that is, we must examine species in which some individuals contain secondary substances while others, in the same interbreeding population, do not. If we find that it is only, or predominantly, the form not containing the secondary substance which is attacked by the parasite or eaten by the animal, only then are we justified in concluding that the secondary substance is protective or defensive.

The differential eating of the acyanogenic forms of *Lotus corniculatus* L. (*2*, *3*) and *Trifolium repens* L. (*3*) is clearly established, and so, undoubtedly, cyanogenesis in these species can be regarded as a defensive mechanism, although it is likely to be more important to the seedling than to the adult (*4*). Sickle-cell anemia and malaria is now the classic example (*5*); the genetic systems associated with interactions of vascular plants and fungal pathogens are equally elegant (for example, *Linum usitatissimum* (flax) and *Melampsora lini* (flax rust) (*6*), in spite of the fact that in most cases the exact chemical differences between resistant and susceptible host plants are inferred rather than proven. For an example where quantitative rather than qualitative differences are important, it is clear that the concentration of chlorogenic acid in potato tubers determines the differential palatability of three potato varieties to some slug species (*7*). Thus there is good evidence that secondary plant substances do act as defensive mechanisms at the intraspecific level.

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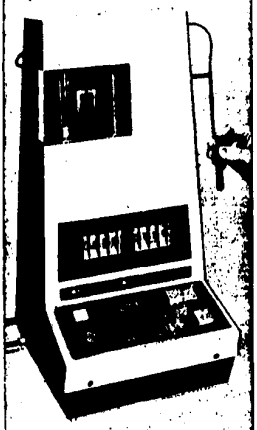

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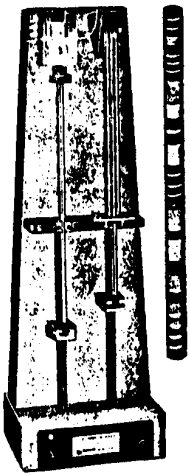

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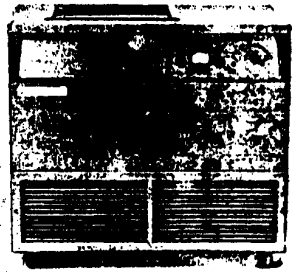

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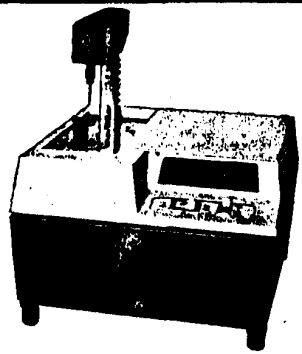

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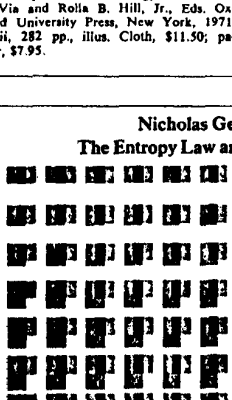

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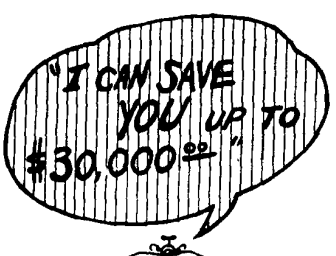

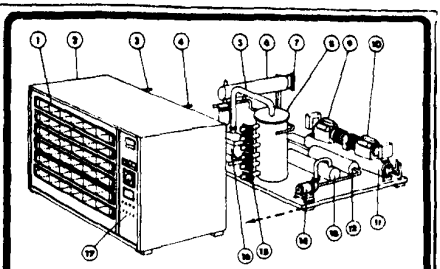

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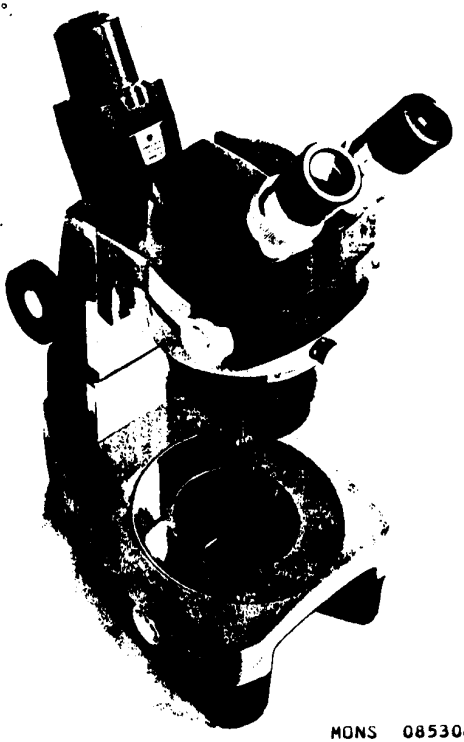

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