stitute), H. G. Goodell (Florida State University), R. Herb (University of Bern, Switzerland), T. P. Ross, Jr. (Global Marine Inc.), J. A. Wilcoxon (Chevron Research Lab.), and R. L. Fleisher, R. L. Kolpack, and E. Vincent (University of Southern California) for contributing material for this study. Thanks are also due to J. Ingle, Jr., for useful suggestions regarding the manuscript, and to O. L. Brandy for making this research possible.

15 February 1968

Amino Acid Flux in an Estuary

Abstract. *Dissolved organic matter in York River estuary included 38 micrograms of free amino acids per liter. The highest concentrations were of glycine, serine, and ornithine. Of the 14 amino acids studied for uptake by planktonic bacteria, glycine, methionine, and serine had the greatest flux rates. The total amino acid flux represented from 1 to 10 percent of the daily photosynthetic carbon fixation.*

A few hundredths of a milligram of dissolved free amino acids (DFAA) per liter has been reported for the open ocean (*1, 2*), estuaries (*3*), and lakes (*4*). However, data on concentrations of the individual amino acids must be supplemented with flux data before the importance of these compounds in community energetics can be evaluated and their varying concentrations explained. Webb and Johannes (*5*) calculated a maximum replenishment time of 1 month from net zooplankton-excretion rates, but it is likely that the bacteria are removing the free amino acids even more rapidly (*6*). This uptake of substrates by bacteria has been studied in pure cultures (*7*), and the same general techniques have been modified for measurements of uptake of glucose and acetate by planktonic bacteria in natural waters (*6, 8*). Flux estimates of amino acids that we report are calculated from analyses of the DFAA and from simultaneous measurements of the uptake of 14 C^{14}-amino acids by the plankton of York River estuary, Virginia.

The methods of analysis of DFAA are those of Webb and Wood (*3*); the sample was desalted with a chelating resin in the Cu^{++} form, and the Technicon AutoAnalyzer system was used (Fig. 1). The bacterial uptake methods (Fig. 1) resemble those of Wright and Hobbie (*6*). The sample came from York River estuary (salinity, 20 per mille; 24.5°C); the control subsample was killed with 2 drops of Lugol's acetic acid solution. For each of the 14 amino acids, the subsampling procedure (Fig. 1) was done in duplicate. Amino acids were added in four concentrations: for example, 9, 18, 26, and 35 μg of alanine per liter; the highest concentration was also added to the killed blank. These bottles were incubated for 2.0 hours on a shaking table at 23°C—in the dark to prevent photosynthetic fixation of respired $C^{14}O_2$. Finally the plankton was filtered with HA Millipore filters (0.45 μ), and the activity was counted with a proportional counter.

Calculations were based on a modified Lineweaver-Burk equation:

$$(S_n + S_a)/v = (K/V) + [(S_n + S_a)/V]$$

The substrate concentrations S_n and S_a (micrograms per liter) are the natural quantities and the experimental added amounts, respectively; v is the measured velocity of uptake (micrograms per liter per hour), V is the maximum velocity of uptake (micrograms per liter per hour), K (micrograms per liter) is a constant indicating the affinity of the transport system for the substrate (*7*), and v equals the quantity $(S_n + S_a)$ times the fraction of the isotope taken up by the plankton per hour.

When plotted as $(S_n + S_a)/v$ versus $S_n + S_a$, the points fell along a straight line resembling the results of uptake experiments with glucose and acetate (*6*). The slope of the line was drawn from a calculation by least-squares regression; its inverse equals V. The K is calculated from the ordinate intercept (K/V), while the v at natural substrate concentrations, hereafter called v_n, is calculated from the equation when S_a equals zero. The turnover time T (hours), or the time required for the plankton to remove all the substrate, equals S_n/v_n.

Dissolved free amino acids were found in small quantities (Table 1), with glycine, serine, and ornithine in relatively high concentrations. Aspartic acid, threonine, alanine, and methionine were at intermediate levels, and the remainder were at low concentrations. The levels of concentration and the general order of abundance agree with results from York River estuary (*3*), Buzzards Bay (*2*), the Irish Sea (*1*), and certain lakes of northern Germany (*4*).

Table 1. Free amino acid concentrations (S_n), flux (v_n), removal time (T), and transport affinity constant (K) of the bacterial population in York River estuary, 6 September 1967. Surface samples (24.5°C) taken from the pier of Virginia Institute of Marine Science were incubated at 23°C. P-ala, phenylalanine.

Amino acid	S_n (μg/liter)	T (hr)	K (μg/liter)	v_n (μg/liter hr^{-1})
Gly	16.85	39.8	9.2	0.423
Met	1.31	7.6	0.6	.171
Ser	4.91	36.9	81.4	.133
Ala	1.49	15.4	7.7	.096
Asp	1.92	33.9	69.7	.056
Val	0.86	17.4	12.4	.050
Thr	1.50	51.0	32.3	.029
Leu	0.63	28.0	27.5	.023
Glu	1.00	42.9	56.0	.023
Ile	0.57	46.5	24.9	.012
Lys	.95	82.2	86.5	.012
Tyr	.75	81.8	230.5	.009
Pro	.58	63.3	34.3	.009
Arg	.55	93.5	158.4	.006
His	.95			
Orn	3.17			
P-ala	0.18			

Aquatic bacteria in estuaries appear to have transport systems for every amino acid tested. The uptake kinetics of a mixed population can be measured only if all the uptake systems have similar characteristics (*8*); for example, a low K, or one dominant species. In the York River experiment, in other runs in Pamlico River estuary (salinity, 12 per mille), and in freshwater ponds near Raleigh, North Carolina, the kinetics of uptake of the isotopes were similar to uptake by transport systems in pure cultures of bacteria (*6, 7*). The specificity of the amino acid transport systems has not been extensively tested, but the uptake of aspartic acid was unaffected by additions of the other common amino acids; glutamic acid did interfere to some extent.

Glycine, methionine, and serine have the highest flux, with valine, alanine, and aspartic acid in an intermediate group. The importance of the DFAA flux, rather than concentration alone, as a measurement of ecological significance is illustrated by data on methionine and threonine. Although they were present in similar concentrations, me-

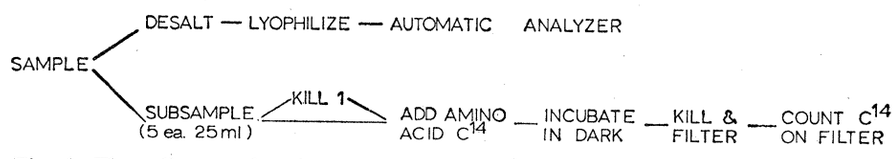

Fig. 1. Flow diagram for the measurement of dissolved free amino acids and their flux in York River estuary, 6 September 1967.

thionine moved eight times faster than threonine through the DFAA pool.

Loss of the C^{14}, as respired CO_2, introduces an error in these estimates. In pure-culture experiments, this loss was about double the retention in the bacteria (*9*). On 24 September 1967 a 25-ml water sample from Pamlico River was incubated in the dark for 2.0 hours with 0.100 μc of aspartic acid–C^{14}. After killing of the plankton, the water was acidified with HCl and then bubbled with CO_2. The gas evolved during incubation, plus the sparged CO_2, was passed through a $SnCl_2$ trap into an evacuated 1-liter Cary ion chamber, and the activity (microcuries) of C^{14} was measured with a Cary electrometer. About 0.004 μc was retained on the filter and 0.006 μc was respired. In uptake experiments, corrections for this error would not affect K but would lower T and increase V and v_n; thus the flux estimates (Table 1) are minimum values; the true flux may well be more than twice as high.

To refine measurements of DFAA flux, more amino acids need to be tested, measurements must be made at different seasons and depths, and accurate respiration corrections must be applied. However, comparison of our determinations of flux with published values of primary production indicates the order of magnitude of the process relative to total carbon flux through the ecosystem. Our York River DFAA data convert to a flux of 9.4 μg of carbon per liter per day; this is 1.3 percent of the surface rate of primary production reported for the same area (*10*). Corrections must be made for respiratory losses; it would then appear that we could expect DFAA flux rates to be within the range of 1 to 10 percent of primary production.

JOHN E. HOBBIE
CLAUDE C. CRAWFORD
Department of Zoology, North Carolina State University, Raleigh
KENNETH L. WEBB
Virginia Institute of Marine Science, Gloucester Point

References and Notes

1. Y. K. Chau and J. P. Riley, *Deep-Sea Res.* **13**, 1115 (1966).
2. A. Siegel and E. T. Degens, *Science* **151**, 1098 (1966).
3. K. L. Webb and L. Wood, in *Automation in Analytical Chemistry, Technicon Symposium 1966* (Mediad, White Plains, N.Y., 1967), vol. 1.
4. J. Brehm, *Arch. Hydrobiol. Suppl.* **32**, 313 (1967).
5. K. L. Webb and R. E. Johannes, *Limnol. Oceanog.* **12**, 376 (1967).
6. R. T. Wright and J. E. Hobbie, *Ecology* **47**, 447 (1966).
7. G. N. Cohen and J. Monod, *Bacteriol. Rev.* **21**, 169 (1957).
8. R. F. Vaccaro and H. W. Jannasch, *Limnol. Oceanog.* **12**, 540 (1967).
9. R. D. Hamilton and K. E. Austin, *Can. J. Microbiol.* **13**, 1165 (1967).
10. B. C. Patten and B. F. Chabot, *Chesapeake Sci.* **7**, 117 (1966). Surface production rates, reported in gram-calories per square centimeter per day for eight experiments conducted between 24 June and 14 August 1963, were converted to micrograms of carbon per liter per day by multiplication by 176. This factor was derived by use of the following data: a water-column depth of 6.1 m [from B. C. Patten, *Oak Ridge National Laboratory ORNL-3634* (1965)] and a standard of 112 kcal/mole CO_2 (reduced). Surface production rates ranged from 404 to 1460 ($\bar{x}$ 735) μg of carbon per liter per day. Integrated values for the total water column were about twice these values.
11. Aided by the Office of Water Resources Research, U.S. Department of the Interior, and NSF grants GB-5678 and GB-6064. Contribution No. 1 from Pamlico Marine Laboratory; contribution No. 267 from Virginia Institute of Marine Science.

7 February 1968

Electron Microscopy of Unstained Biological Material: The Polytropic Montage

Abstract. *With use of an electronic picture-scanning device and a digital computer, electron micrographs taken of a specimen along several different directions can be superimposed to form a montage that is more informative than the component images. Preliminary results indicate that one may thus study unstained, unshadowed biological material at high resolution.*

Because of their low inherent contrast in the electron microscope, isolated biological particles or tissue sections are usually prepared for observation by staining, negative staining, or shadowing with some electron-dense material. While such techniques have been of great value for observation of features coarser than 20 Å, finer details are thus destroyed, obscured, or simply not enhanced. Clearly, if recent improvements in electron microscopes are to be fully utilized in biology, contrast must be enhanced without drastic molecular alteration of the specimen or obscuration by extraneous material.

One might think that sufficient contrast could be achieved by use of high-contrast emulsions and developers in making and printing electron micrographs. But the problem is not merely one of amplifying a weak "signal" representing the faint image of a specimen; there is also a form of "noise" due to fixed inhomogeneities in the specimen-support film (*1*). Photographic increase of the contrast serves only to amplify the noise along with the signal; the benefits are quite limited.

Relying upon known or anticipated symmetries in their specimen material, Markham's group (*2*) superimposed the photographic images of corresponding (symmetrically equivalent) regions within the same micrograph to make a montage in which the image, or signal, was reinforced while the background noise was largely canceled. The results are striking, but the method is applicable only to highly symmetric structures such as the simpler viruses.

I have developed another kind of montage to reinforce weak image signals which requires no specimen symmetry. The several component images that are to form the montage are obtained by tilting the specimen in the electron microscope (*3*) and taking transmission micrographs of the same area along several different directions (hence the term polytropic). To produce such a montage, the component images appearing in the original micrographs must be transformed, by simple linear operations, into images that are superimposable. If the images are reduced to numerical data with a scanning device, transformation may be by high-speed computer. Besides providing adequate contrast for the study of unstained biological material, the polytropic montage enables one to discern three-dimensional relations; a single set of component images can be combined so as to reinforce the specimen details occurring in any plane of interest.

A dilute suspension of tobacco mosaic virus and colloidal gold in distilled water was sprayed on a carbon-parlodion film and air-dried. The specimen mount supporting the film was a platinum disk (*4*) having a 70-μ viewing hole at its center. Two diametrically opposite notches cut in its edge allowed the disk to be rotated by a special wrench. The specimen holder was specially designed to hold the disk at a fixed angle of tilt, 20 deg from horizontal. By rotating the disk within its own plane, about its own axis, one changed the specimen orientation relative to the vertical electron beam. During a series of micrograph