Photoelectric Ecosystem

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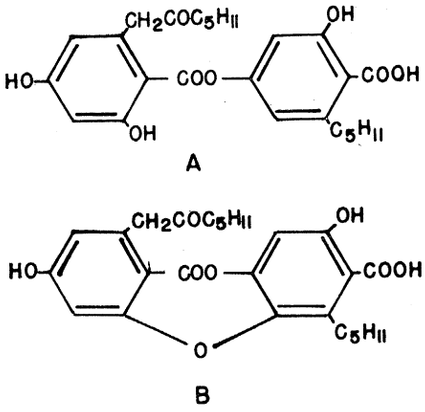

Fig. 1. Olivetoric acid, a depside (*A*), and physodic acid, a depsidone (*B*).

232 to 238 by comparison with an authentic sample from *Cladonia evansii* des Abb. On a paper chromatogram, the spot at R_F 0.52 fluoresces yellow in ultraviolet light (366 mμ) and colors yellow-brown with tetrazotized benzidine. Color reactions with alcoholic $FeCl_3$ (reddish brown) and dilute alkali (yellow), as well as microchemical tests in three reagents (*8*) were confirmatory. Chloratranorin mixed with atranorin would not have been distinguished by chromatography, color reactions, or microchemical tests, but the combined fractions gave a negative Beilstein test for halogen.

Fractions 391 to 407 contained olivetoric acid which gave the expected color reactions with alcoholic $FeCl_3$ (blue-red) and aqueous $Ca(OCl)_2$ (red). On the paper chromatogram, olivetoric acid appears as a double spot (R_F 0.61 and 0.73) due to base hydrolysis during development. It fluoresces deep blue in ultraviolet light (366 mμ) and turns red and then blue-red with tetrazotized benzidine. The results of microcrystal tests (*8*) in four reagents agreed with this identification (*9*). The infrared spectrum of olivetoric acid from *Cetraria ciliaris* confirms this identification and the ultraviolet spectrum is typical of an orcinol-type depside (*10*).

Fractions 408 to 412 contained a mixture of olivetoric acid and physodic acid (analysis by paper chromatography). Fractions 413 to 463, containing nearly pure physodic acid, were combined and again chromatographed to remove the last traces of olivetoric acid. The sample then gave the expected color reactions with alcoholic $FeCl_3$ (purple) and with aqueous $Ca(OCl)_2$ added to the sample in basic solution (red). Chromatographic comparison with physodic acid isolated from *Parmelia physodes* (L.) Ach. showed identical spots at R_F 0.41 which quenched ultraviolet light (366 mμ) and turned brown with tetrazotized benzidine. Characteristic crystals (*8*) were observed in two test solutions. The infrared spectrum (λ_{max} 3300, 1725, 1670, 1620, 1225, and 1145 cm^{-1}) confirmed the identification of this substance. The ultraviolet spectrum is typical of an orcinol-type depsidone (*10*).

Although lichen depsides and depsidones are structurally very similar, only one other pair of compounds, microphyllinic acid (a depside) and α-collatolic acid (a depsidone), differ at the site of the ether linkage alone. But there is some evidence that α-collatolic acid forms not from microphyllinic acid by dehydrogenation, but from the depsidone alectoronic acid by O-methylation. A depside which could give alectoronic acid directly by cyclization is unknown in nature. Erdtman and Wachtmeister and others have recognized that the secondary structural differences between known depsides and depsidones need not necessarily have occurred after cyclization (*3*). Future studies to reveal substances accompanying the major lichen metabolites are certain to give new clues to the biogenesis of these curious natural products.

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Photoelectric Ecosystem

Abstract. *A natural, self-maintaining photoelectric cell, composed of a blue-green algal mat and bacteria as a layered ecosystem, was isolated from a shallow marine bay in Texas near Port Aransas. In daytime the open-circuit potential across the ecological membrane was about 0.43 volt. The efficiency of conversion of light energy to organic potential energy before maintenance was 1.62 percent and to external electrical energy at optimum power loading was 0.016 percent, a flow analogous to a consumer population.*

This report concerns a living membrane in which the self-maintaining aspect of an ecological system (ecosystem), the electrochemical potentials of photosynthetic drive, and the dependency of organic and inorganic substances are combined.

The algal mat studied occurs in shallow marine bays of southern Texas. The vertical zonation, growth, and morphology of one group of such mats have been described by Sorensen and Conover (*1*). The sheaths of the filaments in the top layer had become stained with an iron complex which gave them a dark appearance; this top layer acted as a shield that reduced light intensity for lower layers. Maximum growth occurred below this top layer and the growth decreased with depth until the layers of sediment began a centimeter or less below the surface. In the area where mat and sediment meet, the filaments were decomposed.

The dominant species in the mat may differ. The mat which became stabilized in the laboratory experiments contained blue-green algae, such as *Microcoleus cthonoplastes* (Mert.) Zanard. and a few other sparsely represented species, *Schizothrix* and *Anacystis*. *Desulfovibrio, Beggiatoa,* and other bacteria occurred in the sediments. In the gross relation of the upper oxidized, photosynthetically active zone to the lower reduced and regenerative zones, the mat ecosystem is like some other aquatic systems with sharply defined epilimnetic and hypolimnetic zones, such as the Black Sea, except more compressed. Field measurements indicated a potential between top and bottom of the mat of 0.5 v.

Microcosms were set up by transferring small sections of the mat from outdoor culture ponds to finger bowls

which were placed under fluorescent light for 12-hour periods alternating with 12-hour periods in the dark. The light energy available to the mat was measured with a Cambridge large-surface thermopile and a Schott RG-8 filter for estimating the ratio of visible to infrared light. The mat was adapted at 1.25 cal/cm^2 per hour of light energy. When measurements were made, the mat had been in place without visible change for 6 months.

Diurnal measurement of changes in *p*H and oxidation potential were made with a Beckman Zeromatic *p*H meter in the upper liquid phase (13 cm deep), and platinum wire coils were placed in upper fluid and lower black ooze in order to provide an electrochemical outlet from the oxidized and reduced layers maintained by the ecosystem (Fig. 1). In addition to these platinum-platinum potentials, platinum-calomel measurements were made in the water and under the mat. Diurnal measurements were made of open circuit potentials and of properties under maximum power loading.

Photoecosystems of the size of test-tubes were obtained by cutting a plug from the mat with a cork borer. A platinum foil was placed as the negative grid under the mat, and a coiled platinum wire was placed on top. Rates of carbon assimilation and respiration per area of mat were obtained with the *p*H-CO_2 method; the relation of *p*H to CO_2 (*2*) was calibrated empirically.

When power was drawn from the platinum grids, voltage and current diminished to a lower value, depending on the current demand. By varying the external resistance load, various voltage, amperage, and power drains were arranged for the same conditions of light. The responses of potential and power to various external loads (Table 1) were somewhat similar to those of a selenium-barrier photocell (*3*). Optimum loading for maximum power was 24,000 ohm, which included the internal resistance of the algal mat of about 1500 ohm. An efficiency-power curve (from data in Table 1) would illustrate the general case for low efficiency with maximum power (*4*). The resistance of the mat taken in the reverse direction was 10 to 20 times larger.

A representative sequence of observations made through a 24-hour period included a day with open circuit (Fig. 1*b*) and one under maximum power drain (Fig. 1*c*). With the onset of light, the oxidation potentials rose rapidly in the upper layer but only slightly in the lower layer, so that the difference across the membrane reached a near-maximum in several hours (Fig. 1*b*). When the light went off, the respiratory processes rapidly depleted the oxygen, and related changes in the inorganic system lowered the oxidation potential of the surface at a rate somewhat analogous to that of an electrical capacitance. After several hours, a leveling-off state was reached at a lower redox potential across the membrane. The upper side of the ecosystem thus remained more oxidized than the lower side, even late at night and after several days of continued darkness.

Circuit loading at optimum output from the mat for several days resulted in a near-steady state diurnal fluctuation of power (Fig. 1*c*), with 4.99 cm^2 of platinum (0.355 mm in diameter) in each electrode in a bowl of 289 cm^2 area. The total current was 9.023 coul/m^2 per day; the average voltage was 0.083 v, and the power output was 0.18 cal/m^2 per day.

In the small tube with a larger ratio of platinum electrode (2.01 cm^2) to photosynthetic area (1.52 cm^2) a current of 1421.3 coul/m^2 for each 12 hours of light and an average voltage (platinum-platinum) of 0.070 v, the efficiency was 0.016 percent, the power output being 23.75 cal/m^2 per 12 hours of light.

The reactions at the platinum electrodes which provide the mechanism for electrochemical coupling to the photosynthesis of the ecosystem have

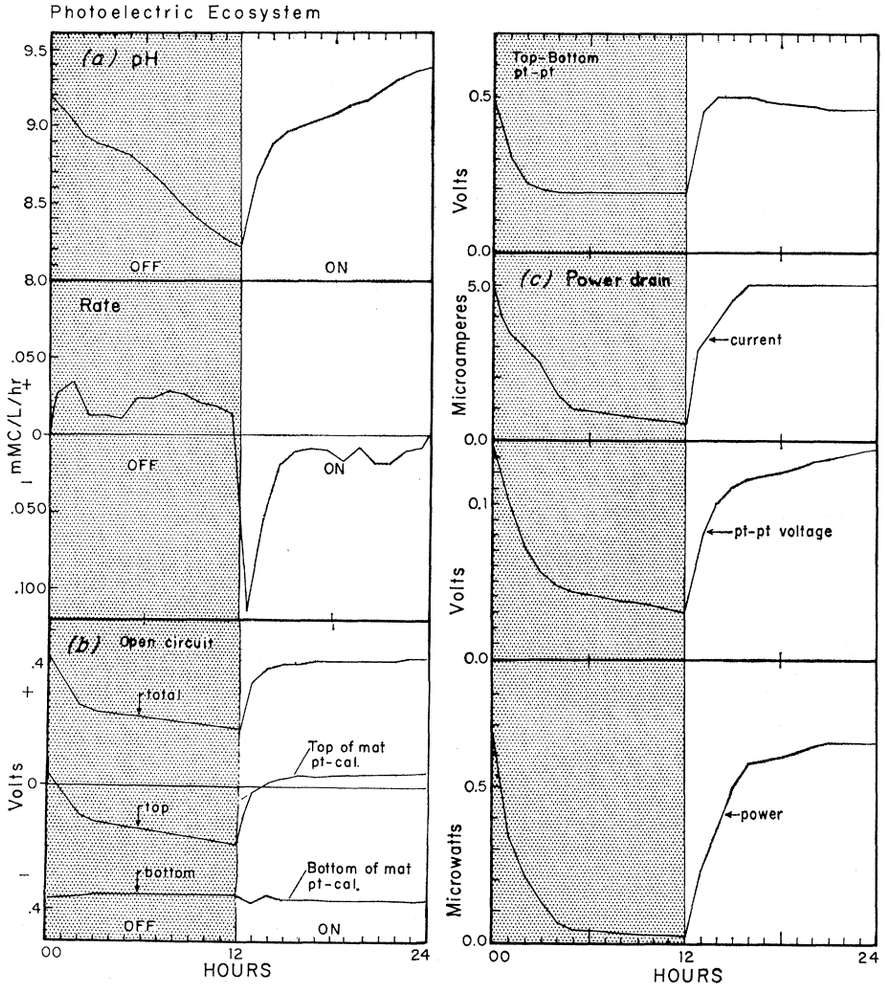

Fig. 1. Diurnal record of variables in the blue-green mat ecosystem with a 12-hour day and a 12-hour night. (*a*) The *pH* in the water above the mat and the rate of carbon metabolism computed empirically from the *pH* changes. (*b*) Open circuit voltages (electron voltmeter) with saturated calomel reference electrodes puncturing the mat (bottom left) and across the intact membrane with laterally inserted platinum wire (top right). (*c*) Electrical current, top to bottom potential difference, and external power drain under the loading for maximum power shown in Table 1.

Table 1. Voltage and amperage output with increase in load resistance under fluorescent light.

External resistance (kohm)*	E.M.F. (mv)	Current (μa)	Power (10^{-9} watt)
Short	0	30†	0
0.52	4	17	68
0.97	15	16	240
1.5	20	15	300
10.5	90	8	720
22.5	140	6	840
100.5	230	2	460
Infinite	430	0	0

* Includes meter resistance (520 ohm) but does not include mat internal resistance (1500 ohm). † Momentary.

not yet been determined. Undoubtedly, they include reactions such as the ferrous-ferric, manganous-manganic, sulfide-sulfate, and nitrate-nitrite changes that are already known to affect platinum electrodes in the hypolimnetic reduced waters of lake and marine ecosystems.

The results were obtained with polarized electrodes. Momentary reversal of voltages temporarily increased power output several times. When the small tubes were connected in series, voltages were additive, as with dry cells in series.

Values for net carbon photosynthesis and 12-hour night respiration of the system (Fig. 1*a*) were 0.353 g of CO_2 and 0.291 g of CO_2 per square meter per 12 hours, respectively, giving a total estimated gross production of 0.644 g of CO_2 per square meter per 12 hours. The efficiency with respect to visible light was 1.62 percent. The electrochemical power take-off was only 1 percent of the gross production, a figure comparable to the power drain of some consumer populations.

The number of moles of oxidizing-reducing substances which participate in the diurnal process may be obtained from the photosynthetic and respiratory rates obtained from carbon values above (0.008 mole of CO_2 per square meter per 12 hours). By the end of the day, about 5.504 kcal/m^2 free energy of net photosynthesis had been stored in the system (688 kcal/mole being used for photosynthesis). The electrochemical mat potential change of 0.43 v with Faraday's constant indicates a free energy available electrically as 0.079 kcal/m^2, 1.43 percent of the photosynthetic produce.

The open circuit voltage change of 0.43 v developing during the day represents the reversible free energy momentarily available to other circuits whether electrical or biological. In a duplicate bowl, a population of corixid water bugs became established during the 6-month adaptation period. The numerous bugs were eating the blue-green algae directly, as indicated by stomach examination. In this bowl the layered blue-green mat with its high free energy did not develop, and the mat was replaced with balls and loose aggregations of blue-green algae. The bowl containing the water bugs and the mat delivering electrochemical power represent potentially competing circuits.

The half-volt potential across the thin mat system may have geochemical significance in maintaining an electrophoretic gradient moving cations down and anions up. The mat may pump needed nitrates and phosphates to the upper algae in this way. The system may also be suitable for encapsulation for tests of closed and balanced ecosystems in space.

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New Papovavirus Contaminating Shope Papillomata

Abstract. *A virus having the characteristics of the papovaviruses was isolated from several naturally occurring cottontail papillomata. Serologic and animal inoculation studies indicate that it is not Shope papilloma virus, but a previously undescribed, nonpathogenic agent of cottontail rabbits.*

In March 1960, we acquired a group of freshly trapped cottontail rabbits (*Sylvilagus* sp.) from Kansas; many of these animals bore cutaneous papillomata. In attempts to develop a tissue culture assay system for Shope papilloma virus, a cytopathic virus was detected in several of these tumors. Although the virus shares many properties of the Shope virus, it appears to be a previously undescribed agent present as a "passenger" in the tumors. Because of the type of tissue culture used and the character of cytopathic effects produced, the virus is tentatively designated as the rabbit kidney vacuolating (RKV) virus.

The initial isolation (strain 443) was obtained from a pool of extracts of papillomata from three rabbits; this pool had been stored in the frozen state for 3 years. Primary monolayer tissue cultures of domestic rabbit (*Oryctolagus cuniculus*) kidney were maintained in a medium consisting of Eagle's basal medium with 10 percent fetal bovine serum, penicillin, and streptomycin. Four tube cultures were inoculated with 0.1 ml of the extract. All cultures, 12 to 16 days later, showed focal areas of cells with vacuolated cytoplasm resembling the cytopathic change produced by the simian vacuolating virus SV-40. Within several days, a second type of cytopathic change developed that consisted of necrotic cells floating above the cell sheet. This change closely resembled that produced by polyoma virus, and eventually most of the cell sheet showed this change. Similar cytopathic effects were reproduced without difficulty on serial passage of culture fluids. The virus has been reisolated from the pooled extract of papilloma in each of three attempts.

Papillomata from 16 rabbits were similarly tested in rabbit kidney cultures, and viruses apparently identical to strain 443 were recovered from four of the extracts. The incubation periods before cytopathic effects appeared ranged from 10 to 23 days.

Strain 443 was used for determining the viral properties. The virus produced cytopathic effects rapidly and to the greatest extent in cultures of domestic rabbit kidney, maintained in Eagle's basal medium with antibiotics and containing either 5 percent horse serum, heated at 56°C for 30 minutes, or commercial calf serum from which the globulin had been removed. The time of appearance of cytopathic effects ranged from 3 to 5 days with the maximum dosage to 24 to 46 days with the lowest effective dosage.

Plaques about 0.5 to 1 mm in diameter are produced after 11 days in rabbit kidney cultures overlaid with nutrient agar. The virus also produces