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.

NEAL E. ARMSTRONG
HOWARD T. ODUM*
Institute of Marine Science, University of Texas, Port Aransas

References and Notes

1. L. O. Sorensen and J. T. Conover, *Publ. Inst. Marine Sci. Univ. Tex.* **8**, 61 (1962).
2. R. J. Beyers and H. T. Odum, *Limnol. Oceanog.* **4**, 499 (1959); R. J. Beyers, J. L. Larimer, H. T. Odum, R. Parker, N. E. Armstrong, *Publ. Inst. Marine Sci. Univ. Tex.*, in press.
3. E. Billig and K. W. Plessner, *Phil. Mag.* **40**, 568 (1949).
4. H. T. Odum and R. C. Pinkerton, *Am. Scientist* **43**, 331 (1955).
5. Aided by a grant from the National Science Foundation (NSF G13160 on Ecological Microcosms). We thank Dr. Jack Myers for suggestions and assistance and Dr. Francis Drouet for algal identifications.
* Present address: Puerto Rico Nuclear Center, Rio Piedras, Puerto Rico.

9 October 1963

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

cytopathic changes in tube cultures of domestic and cottontail rabbit embryos, but onset is late and only a small proportion of cells are affected. No cytopathic effects were produced during 14 to 21 days of observation in cultures of rat or mouse embryo; in cultures of kidneys from hamsters, human embryos, rhesus or cynomolgus monkeys; in cultures of human embryo fibroblasts; in cultures of bovine conjunctiva; or in cultures of Hep-2 cells.

Strain 443 is highly heat resistant, infectivity being unaffected by exposure of the virus to 60°C for 30 minutes. There is only a 3- and 5-day delay in the time of appearance of cytopathic effects by virus heated for 30 minutes at 65° and 70°C, respectively. Overnight exposure to 20 percent chloroform did not significantly reduce the infectivity, nor did 90-minute exposure to 20 percent ether. The infectious particles passed a Selas 03 filter.

The virus apparently contains DNA, since incorporation of $10^{-4}M$ bromodeoxyuridine or $2 \times 10^{-4}M$ iododeoxyuridine in the culture medium protected rabbit kidney cultures against 10^5 to 10^6 infectious doses of virus ($TCID_{50}$), the protection by the latter being prevented by simultaneous incorporation of $3 \times 10^{-4}M$ thymidine.

Fluorescent-antibody staining of infected cultures showed early nuclear staining in early stages of infection, but discrete, fluorescing cytoplasmic granules were occasionally seen during later stages of infection. In cells that were vacuolated, the vacuoles were outlined by specific fluorescent stain, but the nucleus was not stained.

Preliminary electron microscopic studies of thin sections of infected cells showed intranuclear and cytoplasmic viruslike particles closely resembling those reported with polyoma virus and SV-40. Negatively stained extracellular virus particles were essentially indistinguishable from polyoma and SV-40 viruses with regard to size and capsid structure (*1*).

The virus produces hemagglutination of guinea pig erythrocytes at 4° and 20°C, but only after treatment of the culture fluids with the receptor-destroying enzyme (RDE) of *Vibrio cholerae*, followed by heating at 56°C for 30 minutes. Receptors for the virus on guinea pig erythrocytes are destroyed by RDE treatment, but not by exposure to the virus. Nonspecific inhibitors of hemagglutination in various serums are destroyed by RDE or 0.25 percent potassium periodate. The periodate treatment, and subsequent absorption of the serums with guinea pig erythrocytes, have been used routinely to prepare serums for hemagglutination inhibition (HI) tests.

Table 1. Representative patterns of serologic reactivity of Kansas cottontails to RKV and Shope viruses. The rabbits were bled on arrival, challenged by dermal scarification with Shope virus, and bled again 3 to 4 months later. Titers are given as reciprocals; 0 = less than 10; (10) = partial reaction at 1:10; NT = not tested.

Rabbit number	Presence of tumor when captured	Response to challenge with Shope virus *	Antibody titers before and after challenge			
			Complement-fixing antibody to Shope virus		HI antibody to RKV virus	
			Before	After	Before	After
763	—	I	0	0	NT	<40
794	—	I	0	0	<40	20,480
800	—	I	0	0	2560	10,240
775	—	S	0	16	<40	<40
799	—	S	0	>20	<40	80
777	—	S	0	(10)	640	640
819	+	I	80	160	<40	10,240
826	+	I	160	NT	2560	NT

* I, immune; S, susceptible.

As yet no pathogenicity for laboratory animals has been demonstrated with the RKV virus grown in tissue culture. Intranasal, intracerebral, subcutaneous, and intravenous inoculation, and inoculation onto scarified skin or oral and lingual mucosa of domestic and cottontail rabbits, both infant and adult, did not produce any disease which could be attributed to the inoculation. Occasional rabbits developed mild pyogenic skin infections or pneumonia, but these conditions were not reproducible, and virus was not recovered from any of the lesions. Hemagglutination-inhibiting antibody regularly developed; and virus was generally recovered, in low titer, from the liver, spleen, lung, and skin from 7 to at least 28 days after subcutaneous inoculation. No disease was observed over a 5-month period in suckling mice or hamsters inoculated by various routes.

Although the RKV virus was recovered from Shope papillomata and resembles papilloma virus in many respects, there is much evidence to indicate that they are different agents.

1) RKV virus grown in tissue culture does not induce papillomata on the skin of domestic or cottontail rabbits, and does not immunize against Shope virus. Conversely, many extracts of cottontail warts produced papillomata but they did not induce an increase in HI antibody to RKV virus and they did not yield RKV virus in tissue culture. In a group of serums supplied by Richard E. Shope, no HI antibody to RKV virus was present in six domestic or two cottontail rabbits bearing papillomata induced by a variety of inocula, but all of three tumorous cottontails that Shope had inoculated with a pool of Kansas rabbit tumor extracts, concentrated by ultracentrifugation, were positive.

2) In the wild Kansas rabbits studied in our laboratory, there was no correlation between the presence of HI antibody for RKV virus and either complement-fixing antibody or immunity to Shope virus (Table 1) (*2*). The papilloma suspensions used in the immunity tests were from Kansas cottontail rabbits from the same shipments, and the suspension used to challenge No. 794 and No. 819 subsequently was found to contain RKV virus.

3) HI antibody to RKV virus was found in serums of 13 (28 percent) of 46 cottontails trapped in Maryland; this area is thought to be free of Shope virus, because papillomata are not encountered and the rabbits are uniformly susceptible to inoculation.

The incidence of antibody in the Maryland cottontails is roughly comparable to that in the Kansas rabbits; 14 (52 percent) of 27 Kansas rabbits were positive on capture. In contrast, no HI antibody was found in domestic rabbits from several breeding stocks, although many rabbits from one of the colonies had a low concentration of serum inhibitor which was resistant to RDE but sensitive to periodate treatment.

Hemagglutination by RKV virus is not inhibited by potent antiserums to polyoma, SV-40, bovine papilloma, canine papilloma, K virus, Kilham rat virus, or H-1 virus. No antiserum was available to test for relationship to

rabbit oral papilloma virus, but the pathogenicity studies indicate that they are distinct. The properties of the RKV virus clearly differentiate it from Virus III and rabbitpox virus.

The physical and chemical properties of the virus, the type of cytopathic effect, hemagglutination characteristics, and electron microscope findings clearly indicate the relationship of the RKV virus to the papovavirus group (*3*). Since the Shope virus belongs to this group, the possibility was considered that the RKV virus is a serologically distinct strain of rabbit papilloma virus; this possibility cannot be eliminated by present data, but seems unlikely in view of the serologic identity of all previously studied strains of rabbit papilloma virus (*4*) and the fact that RKV virus grown in tissue culture did not produce tumors.

JANET W. HARTLEY
WALLACE P. ROWE
National Institutes of Health, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

References and Notes

1. The electron micrographic studies were done in collaboration with C. F. T. Mattern and Wendell A. Daniel, and will be reported in detail elsewhere.
2. The serologic and challenge experiments with Shope virus were done by D. O. White and R. J. Huebner (D. O. White, R. J. Huebner, W. P. Rowe, R. Traub, *Austral. J. Exptl. Biol.* **41**, 41 (1963).
3. J. L. Melnick, *Science* **135**, 1128 (1962).
4. R. E. Shope, personal communication, 1963.
5. We thank Mrs. Joan B. Austin for invaluable assistance in developing the hemagglutination and hemagglutination-inhibition tests.

7 November 1963

Stretch Receptor-Like Organs in the Fly Larva: Their Possible Role in Growth Regulation

Abstract. *Typical insect stretch receptors and receptor-like organs present in larvae of* Sarcophaga bullata *and other flies contain associated cells which are suspected of passing secretory products into "target" organs by way of strands of connective tissue. In the fly larva, such organs are attached to leg rudiments, tracheal discs, muscles, the genital disc and gonads, and may be concerned with the neuroendocrine control of metamorphosis of these structures.*

Throughout the insect orders there are a series of stretch receptor organs associated with the peripheral nerves (*1–5*). These organs are characterized by the presence of at least one multipolar neuron, together with a number of other cells whose nuclei are very conspicuous; all are enclosed within a common connective tissue sheath. A diagram of such an organ taken from the fly *Sarcophaga* is shown in Fig. 1. Nerves and connective tissue strands leave the organs to pass to the surrounding tissues, mainly muscles. There appears to be considerable uncertainty about the exact nature of the second type of cell, whose nuclei are arbitrarily termed "associated cell" nuclei in Fig. 1. These nuclei have been considered by Finlayson and Lowenstein (*2*) to be connective tissue nuclei, and by Osborne (*6*) to be neurilemma cell nuclei. Beckel (*7*), for the lepidopteran *Hyalophora*, described a series of "scolopophorous organs," some of which he considered to be homologous with the stretch receptor organ of Finlayson and Lowenstein (*1*). He did not find multipolar neurons within the organs and he considered that the many cells present were oenocytes.

In the fly *Sarcophaga bullata*, there are, in addition to the typical stretch receptor organs (*5*), other organs similarly attached to peripheral nerves in which there are no neurons, only associated cells and their nuclei being present. Occasionally, neurons and associated cells have also been found alongside each other, but apparently within separate connective tissue sheaths. Both of these variants suggest that there is functional independence of the neuron and "associated cell" nuclei in the typical stretch receptor.

In *S. bullata*, connective tissue strands (not nerves) from these stretch receptor-like organs have been traced not only to leg rudiments [Osborne (*4*) noted nerves passing from the stretch receptors in *Phormia*] but also to the disc of the external genitalia, and to pupal tracheal discs. It was suggested by Whitten (*5*) that rather than being neurilemma cells, the associated cells of the fly receptor organs might have a secretory function and, furthermore, that the connective tissue strands issuing from the organs may not be randomly distributed, but be in some way associated with the nuclei contained within the organ. It has since been noted that channels, apparently filled with secretory material, are present within these connective tissue strands in at least the late larva, and at the time of puparium formation. These channels are similar in appearance to those described within the epi-

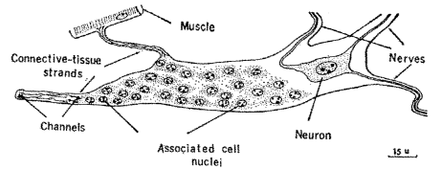

Fig. 1. Diagram of a typical stretch receptor organ from the larva of *Sarcophaga bullata* (Diptera Cyclorrhapha). Stretch receptor-like organs are similar except for the absence of the neuron and related nerves.

neurium of the dorsal nerves of *Sarcophaga* and other fly larvae (*8*). When observed with phase contrast optics, the channels appear as dark lines; observed with dark-field optics, they appear as opaque lines. The channels of the dorsal nerves, plus their contained material, appear to pass directly to their target organ; the channels of the receptor-like organs appear to do likewise.

The receptor-like organs, as well as the stretch receptor organs themselves with their multipolar neurons, are always associated with the lateral segmental nerve branches. It has also been found that in *Sarcophaga*, a terminal branch of the last dorsal nerve connects with a stretch receptor-like organ. A receptor-like organ has even been found indirectly attached to the larval gonads. In the fly larva [for example, *Drosophila* (*9*)], the gonads have been considered to lie embedded within the fat body, unattached to other structures. However, in *Sarcophaga* it has now been found that the gonads are, in fact, attached to a fine

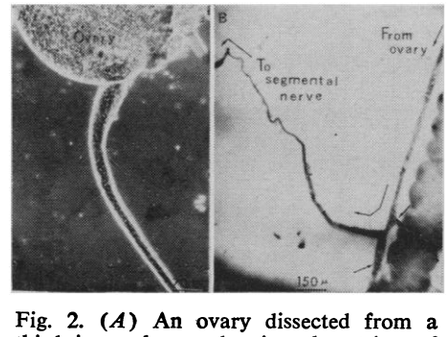

Fig. 2. (*A*) An ovary dissected from a third instar larva, showing the string of cells contained within the connective tissue sheath which is continuous with that over the ovary. (*B*) The string of cells showing attachments to a Malpighian tubule. The narrowing portion of the strand at top left passes into the ventral muscle mass and connects with a segmental nerve and receptor-like organ. Dissected in methylene blue; viewed with phase-contrast optics.