cell to the opposite side (Fig. 1*A*). Furthermore, it is reasonable to assume that the interior of the vacuole is essentially isopotential and that electrical events occurring locally at one part of the cell periphery are indistinguishable when recorded in the vacuole from similar events occurring at other points. Hence, the vacuolar potential can be expected to reflect the sum of electrical activity at the periphery during impulse conduction.

This interpretation was tested by comparing the shape of the vacuolar potential during normal impulse propagation with its shape during synchronous firing of the entire cell. Two external electrodes were used to monitor activity next to and opposite the stimulus site (*2* and *3* in Fig. 2 diagram) while the vacuolar potential was recorded with a third electrode (*1* in Fig. 2 diagram). When the stimulus consisted of a local inward current through the holding pipette, the external electrodes recorded asynchronous spikes and the internal electrode recorded a typical slow vacuolar action potential (Fig. 2*A*). When the stimulating current was drawn inward through the entire cell surface by an intracellular current-passing electrode, the externally recorded action potential was synchronous, and the vacuolar potential was significantly shortened in time course (Fig. 2*B*). With threshold amounts of current in a variation of the last-mentioned experiment, several degrees of asynchrony were obtained. The degree of temporal compression of the vacuolar potential was then closely related to the degree of peripheral firing synchrony.

Two primary conclusions have emerged thus far from the evidence presented here and in the preceding report. First, the luminescent flash of *Noctiluca* is triggered bioelectrically; and second, the flash-triggering action potential propagates actively in the peripheral cytoplasm.

Taken together, these two conclusions lead to a third, which also is subject to test, namely that luminescence is not initiated simultaneously throughout the cell, but is triggered asynchronously by the conducted action potential. Consideration of Fig. 2, *A* and *B*, trace 4, shows that the emission develops more rapidly and linearly during synchronous discharge of the cell than it does when the action potential is allowed to propagate. Figure 2*C* illustrates the reproducible nature of emission rate differences. During synchronous firing of the active membrane, luminescence appears to initiate simultaneously from all areas, causing a steeper rise of emission rate as recorded from the whole cell.

If luminescence is triggered locally during spread of the propagated potential, luminescence must spread over the cell with an advancing front whose velocity is similar to that of the triggering potential. This was experimentally substantiated by high-gain photometer display of flash initiation at selected portions of the cell periphery, while the cell was locally stimulated with current passed through the holding pipette. A 25× objective was focused to include in its field (and hence on the photocathode of the multiplier tube) limited portions of the cell periphery at the positions shown in Fig. 3. Photometer recordings of flashes from these areas were displayed with the oscilloscope sweep synchronized with the stimulus. Spatially correlated latency differences of emission (Fig. 3), similar in duration to those observed for the externally recorded action potential, were consistently observed.

The latency between local bioelectric activity and local light emission was examined by simultaneous electrical and photometric recording from small areas of the cell surface (Fig. 4). Latencies of emission ranged from 1 to 3 msec when measured from the active portion of the externally recorded potential. No significant local latency differences were found at diverse locations on the organism. This latency presumably represents the time required for the completion of the events which couple the luminescent chemistry to the active current flow of the action potential. It is similar in duration to that of vertebrate twitch muscle fibers (*7*).

Microscopic observations of *Noctiluca* by direct means (*8*) and with the aid of electronic image intensification (*9*) has shown that light emission is from numerous 1 to 2 μ sources distributed primarily in the peripheral cytoplasm (*10*). It is surmised that the action potential triggers luminescence locally at the level of individual organelles, and that the population of luminescent organelles is triggered asynchronously because of conduction latency of the propagated flash-triggering action potential.

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References and Notes

1. R. Eckert, *Science*, this issue.
2. R. Werman, *J. Gen. Physiol.* **46**, 517 (1963).
3. J. J. Chang, *J. Cellular Comp. Physiol.* **56**, 33 (1960).
4. Ion substitution experiments were performed by Miss C. Quinn. Chloride action potentials have been described in the fresh-water algal cells *Chara* [C. T. Gaffey and L. J. Mullins, *J. Physiol.* **144**, 505 (1958)] and *Nitella* [L. J. Mullins, *Nature* **196**, 986 (1962)].
5. W. A. Rushton, *Proc. Roy. Soc. London Ser. B* **124**, 201 (1937); H. Rosenberg, *Trans. Faraday Soc.* **33**, 1028 (1937); A. L. Hodgkin, *J. Physiol.* **90**, 183 (1937).
6. Unpublished data obtained with electron microscope in collaboration with Dr. H. S. DiStefano.
7. A. Sandow, *Yale J. Biol. Med.* **25**, 176 (1952).
8. E. B. Harvey, *Carnegie Inst. Wash. Publ. No. 251* (1917), p. 235.
9. G. T. Reynolds *et al.*, *Biol. Bull.* **125**, 389 (1963).
10. The relationship of these luminescent organelles to the "scintillon" crystals found in the luminescent dinoflagellate *Gonyaulax polyedra* described by R. De Sa, J. W. Hastings, and A. E. Vatter [*Science* **141**, 1269 (1963)] is not yet clear.
11. Dr. J. W. Hastings first called my attention to *Noctiluca* as a preparation potentially useful for the study of excitation-response coupling. I thank him for discussions during this investigation.
12. Supported by NSF grants G-21529 and GB-1908, PHS grant B-3664, and in part by an ONR grant to the Marine Biological Laboratory.

7 December 1964

Body Composition and Coat Color Correlation in Different Phenotypes of "Viable Yellow" Mice

Abstract. *The "viable yellow"* (A^{vy}-) *mouse genotype produces phenotypes with "clear yellow," "black spotted," and "agouti" coat color. "Agouti"* $A^{vy}a$ *mice gain weight at a lower rate and contain significantly less fat and water than "clear" and "spotted"* A^{vy}- *mice but have similar nonfat dry weights. Between 4 and 28 weeks of age, the length of the tail increases at the same rate in all phenotypes.*

Heterozygous "viable yellow" $A^{vy}a$ mice have a variable coat color pattern ranging from clear yellowish orange through various degrees of black spotting to complete agouti which is indistinguishable from mice of the wild-type *A*- genotype. Heterozygous "lethal yellow" $A^{y}a$ mice have a clear yellowish orange coat color and deposit excessive fat in the carcass and liver (*1*). Homozygous $A^{vy}A^{vy}$ mice are viable and fertile (*2*), whereas homozygous $A^{y}A^{y}$ mouse embryos die about the time of implantation (*3*).

"Agouti" $A^{vy}a$ mice appear with a frequency of about 10 percent in litters from all $A^{vy}a \times aa$ and $A^{vy}A^{vy} \times aa$ matings in the VY/Wf stock, derived from (C3H/Di × C57BL/6J) F_1 hybrids (*4*). "Clear" yellow mice are al-

Table 1. Composition of the carcass and liver from various A^{vy}- mouse phenotypes at 28 weeks of age ($N = 5$). Increase in tail length between 4 and 28 weeks of age. Mean ± standard error.

Phenotype	Sex	Wet weight (g)	Water (g)	Fat (g)	Nonfat dry weight (g)	Increase in tail length between 4 and 28 weeks of age	
						cm	*N*
				Carcass			
Clear	M	58.1 ± 1.8	24.9 ± 0.9	22.0 ± 1.0	11.2 ± 0.9	3.4 ± 0.3	13
	F	58.7 ± 2.4	20.6 ± 1.2	27.1 ± 1.2	11.0 ± 2.1	2.2 ± 0.1	14
Lightly spotted	M	58.5 ± 2.3	25.2 ± 1.0	22.4 ± 0.9	10.9 ± 1.1	3.1 ± 0.1	15
	F	57.2 ± 2.4	20.3 ± 0.4	27.6 ± 1.3	9.3 ± 1.2	2.2 ± 0.1	15
Heavily spotted	M	56.4 ± 1.4	24.4 ± 0.7	22.1 ± 2.2	10.7 ± 0.6	2.8 ± 0.1	15
	F	52.7 ± 2.5	18.7 ± 0.7	24.1 ± 1.6*	9.2 ± 1.1*	2.2 ± 0.1	16
Agouti	M	44.7 ± 3.7	20.3 ± 0.9	12.9 ± 2.5*	9.7 ± 0.9*	3.0 ± 0.2	15
	F	44.5 ± 3.3	17.3 ± 0.8	19.2 ± 2.3	8.0 ± 0.9	2.3 ± 0.1	15
				Liver			
Clear	M	3.4 ± 0.3	1.8 ± 0.2	0.6 ± 0.1	0.9 ± 0.04		
	F	2.8 ± 0.4	1.6 ± 0.2	0.4 ± 0.1	0.7 ± 0.1		
Lightly spotted	M	3.5 ± 1.1	1.9 ± 0.2	0.6 ± 0.1	1.0 ± 0.04		
	F	2.8 ± 0.3	1.7 ± 0.1	0.4 ± 0.1*	0.8 ± 0.1*		
Heavily spotted	M	3.4 ± 0.2	1.8 ± 0.1	0.6 ± 0.1	1.0 ± 0.04		
	F	2.3 ± 0.2	1.2 ± 0.1	0.3 ± 0.1	0.8 ± 0.05		
Agouti	M	2.0 ± 0.2	1.0 ± 0.2	0.2 ± 0.05	0.7 ± 0.05		
	F	1.8 ± 0.2	0.9 ± 0.1	0.2 ± 0.03	0.8 ± 0.1		

*$N = 4$.

most always of the $A^{vy}A^{vy}$ genotype; however, a few "clear" yellow mice are heterozygous ($A^{vy}a$) as indicated by pedigrees and breeding tests. "Spotted" $A^{vy}a$ mice include yellow mice with a few black hairs or a few small black spots ("lightly spotted"), with many areas of black spotting, and mice which are completely agouti-colored except for small yellowish areas ("heavily spotted"). It is not difficult to distinguish "black spotting" from "sootiness" in the VY/Wf stock.

Since "agouti" $A^{vy}a$ mice of both sexes appeared to gain weight more slowly than the other phenotypes, 15 mice of each sex and each phenotypic category were weighed weekly, when they were between 4 and 28 weeks of age, to the nearest 0.5 g. Weighing was always done on the same day of each week and at the same time of day to avoid diurnal fluctuations in weight. Tail-length measurements from the tip of the tail to the anus were made to the nearest 0.25 cm at the beginning of the experiment and every 4 weeks thereafter as a rough indication of possible differences in rate of bone growth among the different phenotypes.

Four to six mice were kept in clean stainless steel cages (15 cm by 30 cm) with sterilized white pine shavings. The cages were changed weekly, and Old Guilford mouse breeder diet and water were always available. The temperature was maintained at 24.4° ± 1.2°C and relative humidity at about 45 to 55 percent.

The more rapid weight gain of the "clear" and "spotted" phenotypes as compared with the "agouti" phenotype is illustrated in Fig. 1. The "lightly spotted" and "heavily spotted" categories are arbitrary divisions with considerable overlap and heterogeneity in coat color pattern. If "lightly spotted" mice are physiologically more similar to the "clear" yellow animals while "heavily spotted" mice are more similar to "agouti" mice, the slightly different rates of weight gain of the "clear" and "spotted" classes may be true differences. However, since these mice were not inbred, it is not possible to determine whether these differences are due to actual phenotypic effects, genetic heterogeneity, or chance.

At 28 weeks of age five animals from each of the eight sex-genotype categories were killed with chloroform after being weighed. The livers were removed and weighed to the nearest 0.01 g. Carcasses and livers were immediately immersed in toluene in separate glass jars. Simultaneous fat and water determinations were performed on each carcass and liver as described previously (*5*). Nonfat dry weight was determined by subtracting total fat and water content from the wet weight.

Analysis of the data in Table 1 indicates that "clear" and "spotted" A^{vy}-

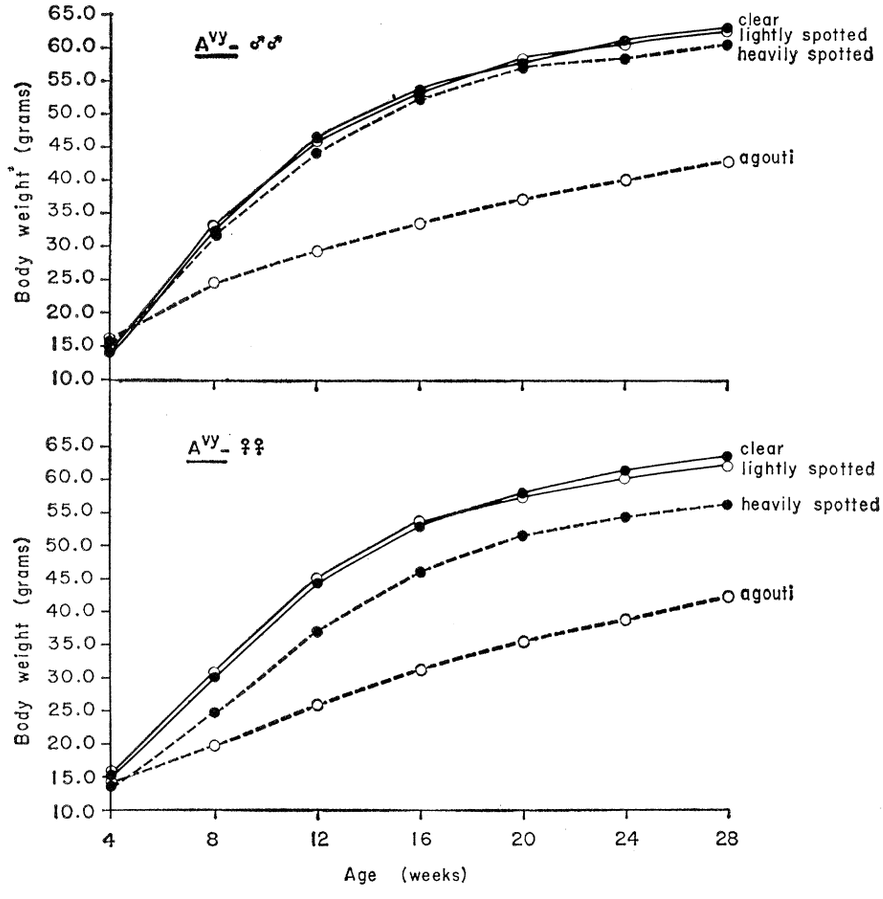

Fig. 1. Rate of gain in weight between 4 and 28 weeks by male and female A^{vy}- mice.

mice are heavier than "agouti" A^{vy}- mice because of a larger fat and water content of the carcass and liver. Differences between the mean fat and water content of "clear" and "agouti" carcasses and livers (data for both sexes pooled in each category) were significant ($P < .01$), while the non-fat dry weights of carcasses and livers of the two phenotypes were not statistically different ($P > .05$). There was no difference in the rate of bone growth, as indicated roughly by increase in tail length, between the "clear" and "agouti" phenotypes (Table 1).

Variability of expression of the $A^{vy}a$ phenotype and the correlation of coat color with body and liver composition suggest that A^{y} and A^{vy} alleles have similar effects on the synthesis of hair pigment as well as on fat metabolism. However, the characteristics of the "agouti" $A^{vy}a$ phenotype indicate that these effects of the $A^{vy}a$ genotype are more easily modified than those of the $A^{y}a$ genotype.

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References and Notes

1. G. L. Wolff, *Genetics* **48**, 1041 (1963).
2. M. M. Dickie, *J. Heredity* **53**, 84 (1962).
3. G. G. Robertson, *J. Exptl. Zool.* **89**, 197 (1942); G. J. Eaton and M. M. Green, *Genetica* **33**, 106 (1963).
4. Breeding stock kindly supplied by Dr. M. M. Dickie.
5. G. L. Wolff and B. Bakay, *Proc. Soc. Exptl. Biol. Med.* **112**, 524 (1963).
6. The technical assistance of Cecil Congleton, Archie McDuffie, Mrs. Julia Lewis, and Barbara Resnick is gratefully acknowledged. Presented in part at the meeting of the Genetics Society of America, Boulder, Colo., 24–26 August 1964. This investigation was supported by PHS research grants GM 10112 and CA 06927.

18 November 1964

Interspecific Transfer of the "Sex-Ratio" Agent of Drosophila willistoni in Drosophila bifasciata and Drosophila melanogaster

Abstract. *The maternally transmitted "sex-ratio" condition in several species of* Drosophila *appears to be due to infection by a microorganism of the genus* Treponema. Drosophila bifasciata *is an exception, since no microorganism has been found in the "sex-ratio" strains of this species. Normal* D. bifasciata *can be infected by injection of the hemolymph of a "sex-ratio" strain of* D. willistoni *containing treponemas. The progenies of the infected* D. bifasciata, *up to and including the F_4 and F_5 generations, have numerous treponemas in their hemolymph. Their progenies are, however, not unisexual, although both females and males are infected. The hemolymph of these* D. bifasciata *injected in* D. melanogaster *females causes typical "sex-ratio" symptoms in the progenies of the latter.*

A cytoplasmically inherited "sex-ratio" condition has been found in several species of *Drosophila,* namely, *D. bifasciata* (*1*), *D. prosaltans* (*2*), *D. willistoni* and *D. paulistorum* (*3*), *D. nebulosa* (*4*), *D. equinoxialis* (*5*), and perhaps others. The females carrying this condition produce mainly or exclusively daughters in their progenies. About 50 percent of the eggs deposited by "sex-ratio" females, presumably representing the male zygotes, die. Malogolowkin and Poulson (*6*) and Malogolowkin, Poulson, and Wright (*7*) showed that the agent responsible for the "sex-ratio" condition can be transmitted to females originally free of this condition, by injection of the oöplasm of the eggs of "sex-ratio" females, or by injection of their hemolymph (*8*). Poulson and Sakaguchi (*9*) then discovered that the infective agent is a microorganism belonging to an apparently new species of the genus *Treponema.* Treponemas can be seen in the hemolymph of the flies from the "sex-ratio" strains of all the species tested, excepting *D. bifasciata.* The agent responsible for the "sex-ratio" condition in the last named species remains to be found by microscopic observation. While the treponemas apparently responsible for the "sex-ratio" condition can be transferred by injection from species to species, the "sex-ratio" agent of *D. bifasciata* appears uninfectious (*10*), either inter- or intraspecifically. It cannot be easily inactivated by x-rays (*11*) or γ-rays, as it can be in *D. willistoni* (*12*). Poulson and Sakaguchi (*13*) showed, however, that *D. bifasciata* can be infected with the "sex-ratio" agent of *D. willistoni.* The experiments described here are concerned with transfer to *D. bifasciata* of the infectious agent in the hemolymph of *D. willistoni* and its subsequent testing in *D. melanogaster.*

A "sex-ratio" strain of *D. willistoni* used in this study was obtained from B. Sakaguchi; it is descended from the original culture of Ch. Malogolowkin. The normal strain of *D. bifasciata* was collected by Moriwaki, Okada, Ohba, and Kurokawa at Akkeshi, Japan, in 1952. Females of *D. bifasciata* were injected with the hemolymph of the "sex-ratio" *D. willistoni;* the females were transferred at 4-day intervals to fresh culture bottles. The proportions of the females and males were determined in the successive broods. Several pair matings were arranged from each brood, and in the F_2, F_3, F_4, and F_5 progenies the frequencies of the two sexes were determined. The hemolymphs of at least five females were examined under a phase-contrast microscope in most lines in F_2, F_3, and F_4 generations. All the experimental cultures were kept at 25°C.

The eight injected females of *D. bifasciata* produced a total of 372 females and 164 males in the F_1. The percentage of males is, consequently, 30.6 percent, which is significantly different from 48 percent of males in the control cultures. The total count in the F_2 generation was 549 females and 277 males, or 33.5 percent males; the counts in F_3, F_4, and F_5 were 2427 females and 826 males, 4356 females and 1631 males, and 4064 females and 2433 males, which means that the frequencies of the males were 25.4, 27.2, and 37.5 percent, respectively. This is consistently and significantly below the control frequency of males. Detailed data (*14*) show that some males appeared in almost all broods, at least in those in which an appreciable number of flies were produced. The numbers of the

Table 1. The hemolymph of the progenies of *D. bifasciata* injected with "sex-ratio" of *D. willistoni.*

Sex	Flies examined (No.)	Treponemas		
		+	—	Few
	F_2 generation			
♀	67	67	0	0
♂	10	10	0	0
	F_3 generation			
♀	81	74	2	5
	F_4 generation			
♀	187	150	16	21
♂	16	12	3	1

Table 2. Tests in *D. melanogaster.*

Species of *Drosophila*		Progenies	
Donor	Recipient	Unisexual	Normal
bifasciata	*melanogaster*	102	14
willistoni	*melanogaster*	11	0