Photoresponses in colorless *Paramecium*[1]

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Summary. Two species of colorless *Paramecium, P. caudatum* and *P. tetraurelia,* were suspended in a saline solution. They accumulated in the shaded region when they were introduced into a half-shaded glass tube and illuminated. 520 nm light was most effective in stimulating the accumulation.

It used to be generally accepted that colorless species of *Paramecium,* such as *P. caudatum* and *P. tetraurelia,* do not exhibit locomotor responses to ordinary visible light[3,4], though some early workers pointed out that they showed negative responses to very strong light[5,6]. We recently examined photoresponses in specimens of *P. bursaria,* which contain hundreds of symbiotic green algae, *Chlorella,* and found that they showed locomotor responses to ordinary visible light even after the algae were completely removed from the cells[7]. The *Chlorella*-free, colorless *P. bursaria* accumulated in the dark region (photodispersal[8]) in contrast to the normal, *Chlorella*-containing specimens which accumulated in the light region (photoaccumulation[8]). We, therefore, examined the photobehavior exhibited by specimens of colorless *Paramecium* in a half-shaded glass tube, which was previously employed for demonstrating photoaccumulation in *P. bursaria.* We found that the 2 species of colorless *Paramecium* showed photodispersal as *Chlorella*-free *P. bursaria* did.

Material and methods. Two species of colorless *Paramecium* (*P. caudatum*; kyk 201, *P. tetraurelia*; 51 S VII) were cultured in a wheat-straw infusion at 21 °C under a fixed illumination cycle (12 h light, 12 h dark). All the experiments were performed on specimens obtained from the cultures 3 h after the light was turned on[9]. The specimens were gently washed with a standard saline solution (1 mM KCl, 1 mM $CaCl_2$, 1 mM tris-HCl buffer; pH 7.2) and equilibrated in the solution for more than 20 min prior to experimentation. In order to examine the photoresponse of *Paramecium* and its spectral sensitivity, specimens suspended in the saline solution were introduced into a glass tube (4 mm in inner diameter, 40 mm in length) one half of which was covered by a black foil. The tube was placed horizontally under a constant light (4000 lux; white light, 4×10^{18} quanta m^{-2} sec^{-1}; monochromatic light) for 2 min[7]. The number of specimens present in the shaded region of the tube was counted and expressed as a percentage of the total number of specimens in the tube, to indicate the degree of photodispersal. Monochromatic light (half-band width less than 18 nm) was obtained by combined use of interference and cut filters. Thermal radiation from the light source (650 W halogen lamp) was eliminated by placing an infrared-impermeable filter and a plastic vessel containing water in front of the light source. All the experiments were performed at 22 °C ± 2 °C.

Results. As shown in figure 1, more than 90% of the specimens introduced into the half-shaded glass tube accumulated in the shaded region of the tube (photodispersal) during a 2 min exposure to white light (4000 lux). The photodispersal was consistently observed in lights intensities varying from 1200 to 10,000 lux. A spectral sensitivity curve for the photodispersal was obtained by plotting the degree of photodispersal (percentage of specimens present in the shaded region) exhibited by the specimens for each monochromatic light wavelength. As shown in figure 2, the curves were essentially identical with each other in these 2 species. Both showed a major broad peak at about 520 nm and a minor peak at about 680 nm.

Discussion. Both species of colorless *Paramecium* (*P. caudatum* and *P. tetraurelia*) showed apparent locomotor responses to visible light of moderate intensity, and accumulated in the shaded region (photodisperal). The photodispersal seems to be caused mainly by the photophobic response exhibited by the specimens at the light-dark border. The fact that the spectral sensitivity curves for the photodispersal are identical in these 2 species seems to suggest that *Paramecium* possesses a photoreceptor mechanism common to different species.

Behavioral or locomotor responses to ordinary visible light in *Paramecium* are so faint and unstable that they have long been overlooked by many investigators[3,4]. Even slightly

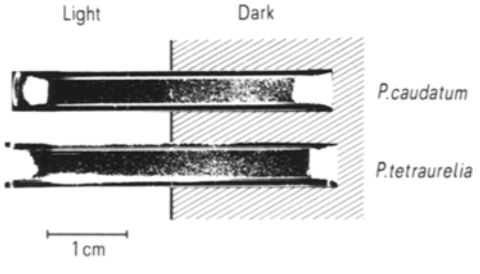

Figure 1. Distribution of *Paramecium* in a half-shaded glass tube during its exposure to white light (4000 lux) for 2 min. Right half of the tube was covered by blackened foil. Both ends of each tube were plugged by silicon-rubber stoppers. Each photograph was taken under dark-field illumination just after the foil was removed. *Paramecia* are seen as white dots in the tube.

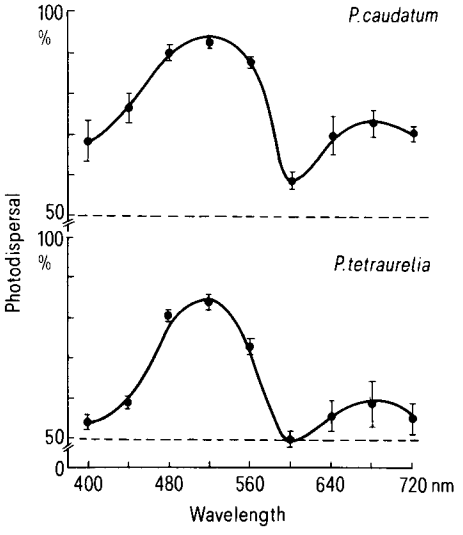

Figure 2. Spectral sensitivity curves for photodispersal in *Paramecium.* Degree of photodispersal was expressed by the percentage number of the specimens in the tube which accumulated in its shaded region during exposure to monochromatic light (4×10^{18} quanta $m^{-2}sec^{-1}$) for 2 min. 50% indicates that the specimens distribute uniformly in the tube. Vertical line on each plot shows the SEM obtained from 3 series of measurements.

rough handling of the specimens during experiments easily destroyed their photoresponsivity.

Recent investigations have indicated that the electrophysiological characteristics of *Paramecium* membranes are essentially similar to those of metazoan excitable cells[10]. Thus *Paramecium* is potentially a valuable organism for the study of mechanisms underlying light-excitation processes in the membrane.

1 The work was supported by grants from Mitsubishi Foundation and from Ministry of Education of Japan to Y.N. (144006, 411802, 411808, 510902, 511201).
2 The authors would like to thank Drs T. Ikawa, F. Fukui, K. Kobayashi and S. Ishizaka for many suggestions and helpful discussions. To whom reprint requests should be addressed.
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The effect of chronic marginal vitamin C deficiency on the α-tocopherol content of the organs and plasma of guinea-pigs

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Summary. Chronic marginal vitamin C deficiency lasting 270 days induced hypertriglyceridaemia, an abrupt fall of ascorbic acid in all organs and a significant decrease of α-tocopherol in the liver and the lungs in guinea-pigs.

Experiments in which a deficiency of vitamin C was ameliorated by the administration of vitamin E, and vice versa, were suggestive of a synergic effect of the 2 vitamins[1-3]. The plasma level of vitamin E is known to be affected by changes in triglyceridaemia and cholesterolaemia[4-7]. We undertook this study to ascertain whether the assumed synergism between vitamins C and E and changes in the lipid metabolism caused by a prolonged latent vitamin C deficiency[8] induce changes in α-tocopherol levels in the blood and organs of guinea-pigs exposed to marginal vitamin C deficiency over a long period.

Material and methods. We induced a vitamin C deficiency lasting 270 days in 18 growing male guinea-pigs[9]; 20 animals served as controls. Both groups were fed a scorbutogenic diet[10] containing an adequate amount of α-tocopherol (47 mg/kg diet). The deficient group received 0.5 mg oral ascorbic acid/animal/day vs 5 g ascorbic acid/kg diet received by controls. After 16 h of fasting the animals were anesthetized with ether and killed. Blood was collected by cardiac puncture. Blood samples were assessed for concentrations of vitamin C[11], α-tocopherol[12] and triglycerides[13]. The amount of vitamin C[14], α-tocopherol[15] and total fats[16] was determined in organs. The results were evaluated by Student's t-test.

Results. Food intake and weight curves were virtually the same in both groups (body weight at the end of experiment: controls 866±31 g, deficiency 836±28 g). Deficient animals showed a significantly increased weight of the liver (controls 28.6±1.7 g, deficiency 35.5±2.5 g; $p < 0.05$) and a decreased weight of the testes (controls 4.7±0.2 g, deficiency 3.6±0.3 g; $p < 0.01$). The average content of fat in organs was not significantly changed except for a distinct fat accumulation in the liver of experimental animals. Chronic vitamin C deficiency elicited a rapid rise of triglyceridaemia, exceeding almost 4-fold the values of controls (controls 1.7±0.3, deficiency 6.4±0.7 mmoles/l; $p < 0.001$).

Vitamin C concentration in the plasma and organs of the deficient group decreased significantly to 4-10% of the values found in controls (table).

Prolonged vitamin C deficiency resulted in a decrease of α-tocopherol concentration in the liver, lungs and kidneys to approximately a half of the control values; its concentration in testes, epididymal fat and blood plasma did not change significantly (table).

Discussion. The marked decrease of α-tocopherol in some organs of the guinea-pigs exposed to prolonged marginal vitamin C deficiency can be attributed only to the interaction of ascorbic acid with α-tocopherol as both groups of guinea-pigs consumed the same amount of food and hence also the same amount of α-tocopherol. A direct interaction of free ascorbate and tocopherol radicals was observed in

The effect of chronic marginal vitamin C deficiency on the content of vitamin C and α-tocopherol in the organs and plasma of guinea-pigs

	Vitamin C (μmoles/kg)		α-Tocopherol (μmoles/kg)			
	Control, n = 20	Deficiency, n = 18	Control	n	Deficiency	n
Liver	1561.1±41.4	68.3±4.1	26.7±3.3[a]	12	14.2±3.9[a]	11
Lung	1768.3±69.1	99.3±7.8	20.9±4.3[b]	13	10.2±2.3[b]	11
Kidney	524.0±13.3	36.5±2.7	3.6[c]		2.1[c]	
Testes	1229.3±31.6	120.1±6.5	8.7±2.3	12	10.8±1.9	10
Plasma (μmoles/l)	129.7±15.7	11.4±1.1	25.0±3.5	13	28.9±3.5	14

Mean values ± SEM; vitamin C content in all tissues of deficient guinea-pigs is significantly lower than in controls, $p < 0.001$; [a,b] Significantly different from controls, $p < 0.05$; [c] α-Tocopherol in the kidneys was determined in a pooled sample.