These results suggest two possibilities; the presence of repair 4 hours after the first irradiation, and alternatively, the differential sensitivity of the eye-antennal discs at different stages of cultivation. To examine these alternatives, eye-antennal discs were irradiated with single dose of 2,000 R 2 hours or 4 hours after explantation. When eye-antennal discs were irradiated with 2,000 R 2 hours after explantation, the differentiation of ommatidia was partially inhibited. However 2,000 R of X-ray had no inhibitory effect on the differentiation of ommatidia when given 4 hours after explantation. These results suggest that the organization of ommatidium-forming cells was inhibited by 2,000 R of X-ray when eye-antennal discs were irradiated at 0-2 hours after explantation. After 4 hours of cultivation eye-antennal discs showed no pronounced changes in morphology but they had a lesser sensitivity to X-ray and resulted in the full organization of ommatidium-forming cells following 2,000 R of X-irradiation.

Kuroda, Y. National Institute of Genetics, Misima, Japan. Effects of BUdR, actinomycin D and puromycin on the differentiation of eye-antennal discs of D. melanogaster in organ culture.

Eye-antennal discs dissected from mature third-instar larvae of the Oregon-R strain of D. melanogaster were cultured in chemically defined medium as described in the previous paper (1). In the medium supplemented with 10^{-4} mg/ml rubrosterone (an ecdysone analogue isolated from plants) a pronounced differentiation of ommatidia was observed in 92% of eye-antennal discs after 24 hours of cultivation at 28°C (2). When 10^{-5} M BUdR (5-bromodeoxyuridine, Sigma Chem. Co., crystaline) was added to the medium containing 10^{-4} mg/ml rubrosterone, eye-antennal discs showed differentiation of ommatidia similar to that in control cultures without BUdR. Similarly, the addition of 1 μg/ml actinomycin D (Daiichi Pure Chem. Co., Ltd.) to the medium containing 10^{-4} mg/ml rubrosterone also had no effect on the hormone-induced differentiation of ommatidia. The presence of 10 μg/ml puromycin (Nutritional Biochem Corp.) also did not inhibit the hormone-induced differentiation of ommatidia. These results are summarized in Table 1.

Table 1. Effects of BUdR, actinomycin D and puromycin on the differentiation of ommatidia in eye-antennal discs cultured in chemically defined medium containing 10^{-4} mg/ml rubrosterone

	No. of explants tested	No. of explants in which ommatidia differentiated	Percent of differentiation
Control	12	11	92
BUdR (10^{-5} M)	7	6	86
Actinomycin D (1 μg/ml)	14	11	79
Puromycin (10 μg/ml)	10	7	70

This suggests that the organization of ommatidium-forming cells in eye-antennal discs in organ culture promoted by an ecdysone analogue was not inhibited by inhibitors of RNA and protein synthesis and that the process of the formation of ommatidial cell clusters may be conducted by pre-existent macromolecules which were activated into their functioning by ecdysone analogue.

1. Kuroda, Y. and Tamura, S. 1956, Med. J. Osaka Univ., 7: 137. 2. Kuroda, Y., 1969, Japan. J. Genetics, 44, Suppl. 1: 42.