Table 1. Effect of puromycin on MOC (milliliters of O_2 consumed per minute per 100 g of fat-free body weight). Each value represents the mean ± standard deviation of five rats.

Dose of thyroxine	Minimal oxygen consumption	
	Before puromycin	After puromycin*
0	1.50 ± 0.10	1.52 ± 0.10
0.1 mg	1.73 ± 0.11	1.77 ± 0.07

* Puromycin (100 mg/kg) was administered in two divided doses, 15 and 60 minutes before MOC determination [see (2)].

Table 2. Effect of puromycin on BMR (milliliters of O_2 consumed per minute per 100 g of fat-free body weight) at different ambient temperatures. Each value represents the mean ± standard deviation of seven to eight rats; T_4, thyroxine.

Treatment	(a) Ambient temperature (22°C)		(b) Thermoneutrality*	
	BMR	Rectal temperature	BMR	Rectal temperature
T_4, 0; before puromycin	4.88 ± 0.76	37.8 ± 0.2	3.34 ± 0.16	37.8 ± 0.2
T_4, 0; after puromycin	2.84 ± 0.24†	33.5 ± 0.2	3.27 ± 0.19	37.9 ± 0.1
P	< .001	< .001	> .5	> .5
T_4, 1 mg; before puromycin	6.44 ± 0.76	38.0 ± 0.3	5.54 ± 0.36	37.9 ± 0.05
T_4, 1 mg; after puromycin	4.76 ± 0.40†	36.0 ± 0.2	6.09 ± 0.53	38.1 ± 0.1
P	< .001	< .001	> .5	> .5

* Defined in text. † Q_{10} corrected values given in text.

milliliters of O_2 at standard temperature and pressure per 100 g of fat-free body weight (*5*) per minute; surface area corrections were not used for reasons given elsewhere (*6*). All rats were fed a low iodine diet (*3, 6*). The effect of puromycin on MOC of young adult rats receiving thyroxine (T_4) (0.1 mg kg^{-1} day^{-1} for eight consecutive days) was determined.

Contrary to our expectations, puromycin failed to decrease the MOC of anesthetized hyperthyroid and euthyroid rats (Table 1). In order to resolve the contradiction between these results and previous findings (*2*), we duplicated the original procedure. Therefore, the BMR was measured at an ambient temperature of 22°C in unanesthetized 4-week-old rats previously treated with 1 mg of T_4 per kilogram per day for ten consecutive days. Under these conditions, the original findings appear to be confirmed (Table 2, column a); puromycin depresses the BMR. However, puromycin significantly lowers rectal temperature in all rats, but rectal temperature was not reported in earlier publications (*1, 2*). When rectal temperatures are maintained within the normal range of 37.8° to 38.1°C (*3, 6*), by controlling the temperature of the test chamber, puromycin has no effect on the BMR of hyperthyroid and euthyroid rats (Table 2, column b).

Depression of the BMR by puromycin was observed in our experiments only in those rats whose rectal temperatures were permitted to fall to subnormal values. We suggest that a similar decline in rectal temperature might have occurred in the original experiments (*2*) carried out at a subthermoneutral ambient temperature (22°C). Valid comparisons between most reaction rates including O_2 consumption rates at different reaction temperatures requires a Q_{10} correction for these temperature differences. In order to make this correction, we used 2.3 as the Q_{10} of the major exothermic reactions of the rat (*6, 7*). Rectal temperature was used as an approximate index of core temperature (*5, 8*). After Q_{10} correction of the BMR of rats with subnormal rectal temperatures (Table 2, column a), there is no significant decrease in the BMR following puromycin treatment (Q_{10} corrected oxygen consumption of euthyroid, 4.06 ± 0.38; hyperthyroid, 5.61 ± 0.43).

While the earlier hypothesis concerning a possible relation between the rate of protein synthesis and the calorigenic effect of thyroid hormones ultimately may be correct, the finding of the present experiments, that puromycin lowers rectal temperature, appears to cast some doubt on the validity of the experimental conditions originally used to test this hypothesis.

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Genetic Recombination in the Dinoflagellate Crypthecodinium cohnii

Abstract. *Genetic recombination in dinoflagellates has been detected with the use of chemically induced carotene-deficient mutants of* Crypthecodinium cohnii.

We report the first unequivocal demonstration of genetic recombination in dinoflagellates with carotene-deficient mutants (*1, 2*) of *Crypthecodinium cohnii* (Seligo) Chatton in Grassé, 1952 (*3*), a nonphotosynthetic marine species. Because dinoflagellates possess chromosomes which remain condensed throughout their cell cycle (*4*) and contain large amounts of DNA per cell (*2, 5*), speculation as to their chromosome structure and nuclear organization has received increasing attention (*2, 6*). The occurrence of sexual reproduction in dinoflagellates has been a subject of conjecture (*7*). Until recently only incomplete cytological evidence existed for the presence of a sexual cycle.

Using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, we have produced (*1, 2*) mutant clones that are deficient in β- and γ-carotenes—the only pigments present in wild-type cells (*1*)—at frequencies (*2*) consistent with the vegetative cell being haploid. At least two phenotypes of carotene-deficient mutants have been isolated (*1*); albino clones (pig-1, pig-3, pig-4, pig-5, and pig-13) lack measurable carotenes, and cream-colored clones (pig-2 and pig-10) contain about one-tenth the carotenes of the wild type (*8*).

In media depleted of nitrogen and phophorus (*9*) we have observed that pairs of motile cells sometimes fuse while swimming. The cytological studies of von Stosch (*10*), in which he observed in *Gymnodinium pseudopalustre* Schiller and *Woloszynskia*

Table 1. Results of mixing carotene-deficient mutants of *Crypthecodinium cohnii*.

Mixing	Plates scored (No.)	Colonies (No.)		Yellow colonies as % of total
		Yellow	White	
pig-1 × self	24	0	2216	0
pig-3 × self	25	0	1747	0
pig-4 × self	25	0	1949	0
pig-5 × self	24	0	984	0
pig-13 × self	23	0	3618	0
MassX*	24	96	1590	5.69
pig-1 × pig-3	19	0	1338	0
pig-1 × pig-4	29	0	2616	0
pig-1 × pig-5	26	0	1150	0
pig-1 × pig-13	21	211†	1744	10.8*
pig-3 × pig-4	25	71	1137	5.80
pig-3 × pig-5	23	63	1384	4.36
pig-3 × pig-13	23	0	3670	0
pig-4 × pig-5	22	150	951	13.6
pig-4 × pig-13	26	107	1204	8.16
pig-5 × pig-13	23	15	1537	0.966

* Mixture of all of the above mutants. † Only cream-colored recombinants were obtained.

apiculata von Stosch isogamous cell fusion and meiotic division of the zygote, and our observations on fusing cells of *C. cohnii* are consistent with the presumption that recombination is associated with cell fusion.

In a medium (*9*) containing low concentrations of nitrogen and phosphorus we inoculated equal numbers of the mutant clones pairwise. As controls we grew each one of the mutants separately, and we also mixed all of the clones in the same flask under the low nitrogen and phosphorus conditions. After incubation, cells were counted, diluted, and plated on a solid medium (*11*). Colored and albino colonies were counted. Table 1 shows the results of mixing our pigment-deficient mutant clones along with the two types of controls. No yellow colonies were observed in any of the five albino clones grown separately. It is very improbable that the high number of yellow and cream-colored colonies (713 pigmented colonies out of a total number of 18,321 colonies) can be due to reversion in view of the total absence of yellow colonies in the 10,514 colonies which we counted to look for revertants. Therefore we feel justified in using genetic recombination to explain our results. The numbers of yellow colonies observed in the mixings of two different mutants or in the mixing of all mutants cannot be explained by reversion or suppression. A mass mixing of all five mutant clones or the pairwise mixing of pig-3 × pig-4, pig-3 × pig 5, pig-4 × pig-5, pig-4 × pig-13, and pig-5 × pig-13, all produced recombinant yellow-colored colonies with a frequency of 0.966 to 13.6 percent of the viable colonies. The experimental procedure allows all of the mixed cells to form colonies; thus the numbers presented express a minimum figure for genetic recombination frequencies. The relatively high frequency of recombinant progeny found in some of the mixtures would suggest recombination by independent assortment if these are nuclear mutations. Recombinant phenotypes are obtained with pig-3 × pig-4, pig-3 × pig-5, and pig-4 × pig-5; this indicates that mating types (+ or − types) do not exist in *C. cohnii*. Of the four pairwise mixings with pig-1, three of the mixings gave no wild-type recombinant colonies and the mixing of pig-1 × pig-13 gave 10.8 percent cream-colored colonies. Pig-3 × pig-13 did not yield recomibnant colonies. At this time we have no explanation for these last results.

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8. Cells of the mutants in a nitrogen atmosphere were extracted repeatedly with acetone, ethanol, and methanol. The colorless extracts were combined, concentrated, and transferred to *n*-hexane and scanned for pigments with a Bausch and Lomb 505 spectrophotometer. No carotenoids were detected in the visible range of the spectrum in the albino clones.
9. Mixing conditions included incubation for 15 days at 27° ± 0.5°C, a light-dark (hours) cycle (LD 6 : 18), a 125-ml erlenmeyer flask with 30 ml of medium. One liter of medium consisted of: 342 m*M* NaCl, 28 m*M* $MgSO_4$, 5 m*M* $CaCl_2$, 9 m*M* KCl, 10 ml of F metals, 0.79 m*M* disodiumglycerophosphate, 0.15 m*M* $(NH_4)_2SO_4$, 15 m*M* sodium acetate, 8×10^{-5}*M* histidinehydrochloride, 8.2×10^{-9} *M* biotin, 2.96×10^{-6}*M* thiaminhydrochloride, 7.49×10^{-10}*M* vitamin B_{12}, 22 m*M* glucose, 9.7×10^{-4}*M* betainehydrochloride, 8 m*M* 2(*N*-morpholino)ethanesulfonic acid, *p*H 6.6 with NaOH. The F metal mixture contained 6 m*M* nitrilotriacetic acid, 30 m*M* NaOH, 0.18 m*M* $Fe(NH_4)_2$, 0.08 m*M* 5-sulfosalicyclic acid, the *p*H being adjusted to 3.8 with HCl.
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11. Recombinants were detected as follows: 350 cells from each mixing were plated on 1 percent agar-solidified medium (the nitrogen and phosphorus contents were ten times that of the mating medium) containing *N*-lauryl-sarcosine (200 parts per million) [R. C. Tuttle and A. R. Loeblich III, *J. Phycol.* **10** (Suppl.), 21 (1974)]. Plates were incubated for 2 weeks at 27°C, exposed to light from an incandescent light (0.2 watt/m²).
12. We thank J. R. Allen, L. C. Klotz, and L. A. Loeblich for reading the manuscript. Supported in part by NIH predoctoral traineeship GM 00036 and NIH grant GM 19,519.

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Pulmonary Platelet Aggregates Associated with Sudden Death in Man

Abstract. *Six human cases of unexpected sudden death were found to have many platelet aggregates in the small arterial vessels of the lungs. Postmortem examination revealed no other significant findings.*

Silver *et al.* (*1*) reported that small intravenous injections of arachidonic acid in rabbits caused accumulation of platelet aggregates in pulmonary blood vessels and sudden death. Their experiment appears to constitute a pathogenetic model for the unreported human entity which we now describe (*2*).

The subject cases (five women and one man), ranging from 17 to 40 years of age, were apparently in good health until immediately before death (*3*). In three instances, the terminal episode was observed and featured severe respiratory difficulty, lasting from less than a minute to about 30 minutes. The remaining in-