Inhibition of Hydroid Aging in Campanularia flexuosa

Abstract. *Hydranths of the colonial marine hydroid* Campanularia flexuosa *(phylum Cnidaria, class Hydrozoa, order Calyptoblastea) have a mean life span of approximately 7 days in intact colonies in culture. Hydranths then regress and are absorbed, and their cellular materials are utilized by the colony for continued growth. Hydranth life spans can be extended to 20 days in isolated hydranths if, repeatedly, the pedicel is damaged by pinching and is allowed to partially regenerate. This suggests that tissue damage and reorganization function to maintain the hydranth.*

The phenomenon of hydranth regression and replacement as an expression of aging in thecate hydroids is well documented (*1*). However, investigations thus far reveal few physiological or biochemical changes inherent to this aging process (*2*). Crowell (*3*) reported that hydranths of *Campanularia* do not grow once they are developed and do not regenerate parts which have been removed. This led to the conclusion that differentiation of *Campanularia* hydranth is "quick, thorough, and final" and indicates rapid, nonregulative differentiation (*4, 5*). This study reveals that the senescence of isolated hydranths can be inhibited by inducing repeated damage and tissue repair in hydranth pedicels. It is suggested that damage to regeneration of pedicel material in isolated preparations sustains hydranth organization; whereas, evolved synthesizing processes which develop new hydranths in intact colonies interact with old hydranth destruction to delimit finite hydranth maintenance.

Colonies of *Campanularia flexuosa* were grown in "Instant Ocean" (*6*) at 18°C and fed *Artemia* nauplii daily (*7*). Hydranths of various ages, with their pedicels intact, were removed from the colonies. Each was isolated in a depression slide and fed once per day. The proximal region of each isolated hydranth was crushed daily with microforceps. Isolated hydranths which were not subjected to such periodic trauma served as controls.

Table 1. Life spans of 34 isolated *Campanularia* hydranths maintained for 10 days, with daily induction of tissue repair. Overall experimental mean age attained is 14.68 days; $s^2 = .30$; $s = .55$. Pooled, control mean age attained for $N = 15$ was 8.65 days; $s^2 = .52$; $s = .72$. Numbers in parentheses are numbers of hydranths attaining the ages shown.

Hydranth age at time of isolation (days)	No. of hydranths isolated	Hydranth age attained
5	11	(1) × 10 days* (1) × 12 days* (9) × 14 days*
6	12	(2) × 11 days† (10) × 15 days†
7	7	(1) × 12 days‡ (6) × 16 days‡
8	3	(3) × 17 days
11	1	(1) × 20 days

* Mean for the group: 13.46 days. † Mean: 14.33 days. ‡ Mean: 15.43 days.

A tabulation of the experimental and control data appears in Table 1. Inspectional survey of the data suggests that the older the hydranths were at the time of isolation, the longer they might survive if pedicels were repeatedly damaged. However, a statistical analysis of variance indicates that hydranth age at the time of isolation is not significant ($\alpha = .05$) in regard to the duration of time for which isolated hydranths can be maintained. There is, however, a very evident blockage of the regular regression of hydranths and of colony formation as described by Nathanson (*8*) wherein materials from regressing hydranths are assumed to be utilized for new hydranth formation.

The concept of circulating, stimulatory substances existing in cnidarians is not new (*9, 10*). However, Barth (*10*) suggested that these substances are nutritive rather than regulatory. The nutritive nature of materials circulating in the coelenteron is supported by the fact that the maturation of isolated, young *Campanularia* hydranths is not prevented by the absence of possible circulating, regulatory materials (*11*). Such maturation is particularly significant when hydranth differentiation is minimal and tissues are relatively plastic. If coelenteron-circulated materials can regulate hydranth differentiation, that regulatory effect appears to occur early and to be limited to the determination of the site of hydranth formation, rather than regulating hydranth development per se.

Hydranth to colony connection is not necessary for an ordered regression of *Campanularia* hydranths to occur. Each hydranth appears to be a complete, functional unit. As a differentiated structure, hydranths are postmitotic and have a relatively fixed life span of approximately 7 days in intact colonies. Since constant blastema destruction of the pedicels of isolated hydranths inhibits hydranth regression, it is suggested that either (i) repeated damaging and reorganization of the pedicel sustains hydranth organization (*12*), thereby inhibiting senescence, or (ii) repeated reorganization removes by-products which accumulate and induce regression. The former mechanism suggests the existence of an aging-inhibitor or maintenance-promoter mechanism, whereas the latter suggests the existence of an aging-promoter or maintenance-inhibitor principle.

In intact colonies the ratio of regenerative and reorganization mass to total hydranth mass is much lower than in the isolated preparations. This suggests that if an aging promoter were present then gradual physiological decrements should be evident, even though the measurements of Strehler and Crowell (*2*) could not detect such. Neither the suddenness of the regression process nor the failure of regression materials to induce general, accelerated colonial regression favors the concept of an aging-promoter principle. Consequently, further inquiry should be directed toward determining the manner in which metabolites are used during pedicel reorganization which sustains hydranth organization and inhibits senescence.

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12. It is well known that embryonic tissue fluids

will enhance the growth of differentiated tissue or in other terms inhibit senescence. Regeneration in lower invertebrates also has considerable rejuvenation effects. See L. Haranghy and A. Balazs, in *Perspectives in Experimental Gerontology*, N. W. Shock, Ed. (Thomas, Springfield, Ill., 1966), p. 224.

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Carcinogen-Induced Immune Depression: Absence in Mice Resistant to Chemical Oncogenesis

Abstract. *Administration of 3-methylcholanthrene 6 days before antigenic challenge depressed the immune response to sheep red cells in young adult mice (C3Hf/Bi strain) sensitive to the oncogenic effect of the drug (100 percent local tumors 240 days after carcinogen). The drug was not immunodepressant in the I strain, which is relatively resistant to its carcinogenic effect (11 percent local tumors 240 days after carcinogen).*

A relative resistance to local tumor development after subcutaneous injection of 3-methylcholanthrene (MC) in oil was described in mice of the I strain (*1*). Since MC and other carcinogenic aromatic hydrocarbons depress lymphohemopoietic tissues (*2*) and reduce immune responses (*3*) in mice, it is of interest to study the effect of MC on the immune response to sheep red cells in the relatively resistant mouse strain I and in the carcinogen-sensitive strain C3Hf/Bi.

Table 1. Incidence of local tumors in 35-day-old mice 240 days after subcutaneous injection of 0.1 mg of 3-methylcholanthrene (MC) in 0.1 ml of corn oil.

Strain	Treatment	Mice with tumors per number of treated mice
I	None	0/50
I	Oil	0/40
I	MC	10/87 (11 percent)
C3Hf/Bi	None	0/50
C3Hf/Bi	Oil	0/60
C3Hf/Bi	MC	50/50 (100 percent)

Since the demonstration of tumor-specific antigens in many experimental tumors (*4*), immunodepression has been considered a possible factor in carcinogenesis by favoring the development of antigenic tumor cells (*5*). It has been proposed that the evolutionary value of immune mechanisms (especially cell-mediated immunity of the homograft type) may be related to a general homeostatic mechanism directed toward the recognition and destruction of abnormal (malignant) cells (*6*). Malignant development may occur when the immunological surveillance is bypassed. One possible mechanism of this bypassing may be the immunodepressive effect of the oncogenic agent itself, since immunodepressive effects have been described for physical (*7*), chemical (*3*), and viral carcinogens (*8*).

I now report that no significant immunodepression was observed when MC was administered 6 days before antigenic stimulation with sheep red cells in the relatively resistant I strain. In contrast, MC was a potent immunodepressor in the carcinogen-sensitive C3Hf/Bi mice under comparable conditions. The incidence of tumors was 100 percent in the C3Hf/Bi mice and 11 percent in the I mice (Table 1). Both mouse strains are derived from the colony of the late Dr. J. J. Bittner and are highly inbred. In my experiments all the animals were 35-day-old males.

Sheep red blood cells (0.2 ml of 10 percent suspension in saline) were administered intraperitoneally 6 days after injection of MC or oil vehicle alone, and the response was measured 4 days later by a direct hemolysis plaque-forming technique (*9*). Controls also included mice injected with red cells alone. The plaque-forming technique was used to measure hemolysin production by single cells in suspension. The results are expressed as number of hemolysis plaque-forming cells (PFC) per total number of nucleated cells in spleen (PFC per spleen in Table 2) and as PFC per million nucleated spleen cells. The number of PFC in nonimmunized animals ranged from zero to 120 per total number of spleen cells (mean, 88), and the results are corrected for background counts. Neither MC nor oil alone contributed to background counts.

Methylcholanthrene had no depressive effect on the response of the I mice to sheep red cells. The magnitude of the response in the MC-treated and control groups was comparable. Analogous results were obtained in four other experiments in which the MC dose ranged from 0.2 to 1.0 mg. By contrast, MC produced a marked depression of the immune response in C3Hf/Bi mice. Comparison of oil- and MC-injected groups showed that the reduction of PFC per 10^6 nucleated cells was 85 and 88 percent when results were expressed as PFC per total number of nucleated cells in spleen. A reduction of 16 percent of the total nucleated cells per spleen was observed in the MC group. Comparable results have been obtained (*10*) for circulating hemolysin and agglutinin responses. Both responses to sheep red cells were depressed in C3Hf/Bi mice treated with MC and were not modified in the I strain. Similarly, 0.1 mg of MC at birth produced marked immune depression in C3Hf/Bi mice when the response to the red cells was tested at

Table 2. Immune response to sheep red cells in mice (35-day-old males, 12 animals per group) measured as direct hemolysis plaque-forming cells (PFC). Results are expressed as means ± standard errors. Treatment consisted of 0.1 mg of 3-methylcholanthrene (MC) in 0.1 ml of corn oil injected subcutaneously. Oil indicates oil injection alone. Six days later mice were given intraperitoneal injection of 0.2 ml of 10 percent sheep red cells in saline. Response was measured 4 days after this injection.

Strain	Treatment	10^6 nucleated cells per spleen	10^6 PFC spleen cells	10^3 PFC per spleen
I	None	200 ± 14.2	162 ± 30.1	32.4 ± 6.9
I	Oil	188 ± 21.2	110 ± 33.6	20.6 ± 8.8
I	MC	205 ± 16.9	178 ± 28.4	36.4 ± 6.1
C3Hf/Bi	None	161 ± 15.0	111 ± 21.2	17.8 ± 6.8
C3Hf/Bi	Oil	153 ± 18.2	104 ± 29.6	15.9 ± 6.8
C3Hf/Bi	MC	130 ± 10.1	16 ± 6.0	2.0 ± 0.5