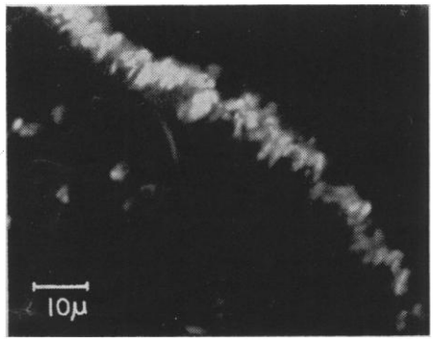

Fig. 1. Cross section of *Dugesia dorotocephala* showing fluorescence of epidermal rhabdites and subepidermal rhabdite clusters. The preparation was from an animal fixed in 100-percent ethyl alcohol; it was cleared and embedded in paraffin and sectioned at 5 μ. The section was then deparaffinized and mounted in nonfluorescing immersion oil for observation under the fluorescence microscope.

The substances used were 95-percent ethyl alcohol, 5-percent neutral formalin, 0.05*N* NaOH, 0.1*N* NH_4OH, and 50-percent H_2O_2. The appearance of fluorescence seemed to coincide with death of the animal. Observation was facilitated by applying pressure on the cover slip and flattening the animal. The red fluorescence was localized within rod- or crescent-shaped structures at the surface and just below the surface of the epidermis. The identification of these structures as rhabdites was based on their location, shape, and size (*2*). Their length varied from 2 to 8 μ, and their width from 1 to 3 μ. These structures could be seen as refractive bodies in the epidermis of the living animal under the phase-contrast microscope. The identity of the rhabdites was further checked by observing fluorescent rhabdites in acetone-frozen and dried sections, and in sections of animals fixed in 100-percent ethyl alcohol, cleared in benzene, and embedded in paraffin. Although some porphyrin may have been extracted by the alcohol, deparaffinized sections under the microscope clearly revealed fluorescent rhabdites perpendicular to the surface of the animal (Fig. 1). In the subepidermis, rhabdites were noted in clusters, which were identified as rhabdite-forming gland cells. Fluorescent rhabdites were retained in a saccular gland cell when the whole animal was placed in 0.05*N* NaOH; the cells separated and flowed freely when slight pressure was placed upon the cover slip. This treatment allowed observation of the number and shape of rhabdites within the somewhat swollen gland cells (Fig. 2).

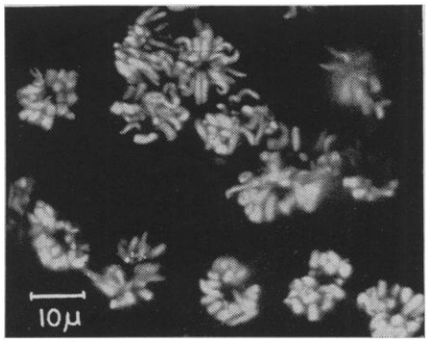

Fig. 2. Cluster of fluorescing rhabdites within gland cells of *Dugesia tigrina* from a squash of the whole animal treated with 0.05*N* NaOH, and observed under the fluorescence microscope.

Sections were stained with acid dyes such as eosin, orange G, picric acid, and fast green. The rod-shaped structures which exhibited the red fluorescence also showed the marked eosinophilia and acidophilia described by other investigators as characteristic of rhabdites (*3*, *4*). Rhabdites did not stain with basic dyes. such as methylene blue and toluidine blue.

Homogenates of planarians were made in a Potter homogenizer, and a drop of homogenate was examined under the fluorescence miscroscope. Red fluorescence was clearly visible in rhabdites isolated from other cellular materials. Fluorescence was also visible in clusters of rhabdites and in some smaller "subrhabdite" units, which may be structural precursors of rhabdites. These "subrhabdites" could also be observed within some gland cells after treatment with 0.05*N* NaOH. The fluorescence of the rhabdites in the homogenate appeared without any chemical pretreatment of the homogenate. When homogenates or whole planarians were subjected to HCl (1*N* or 2.5*N*), an extraction of red fluorescing material occurred. Examination under the microscope revealed an extraction of fluorescence from the rhabdites. The acid extract contained uroporphyrin as analyzed by the paper chromatography method of Nicholas and Rimington (*5*).

Rhabdites were observed to stain intensely with bromphenol blue, indicating protein in rhabdites; this confirmed observations of Prenant (*3*) and Pedersen (*4*) made on several other species of planarians. A linkage between protein and uroporphyrin or between a metal and uroporphyrin may account for the lack of fluorescence in the rhabdites of living animals. Such a linkage would eliminate the alternating double-bond structure upon which the fluorescence of porphyrin depends. On the other hand, the uroporphyrin may be present as a nonfluorescing porphyrinogen (*6*).

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13 April 1961

Aging in Irradiated and Nonirradiated Hydras

Abstract. Evidence is presented that as hydras become older they bud more slowly and become more susceptible to adverse conditions. In these studies, a 2000-roentgen dose of x-radiation did not seem to affect the rate of these changes. The mean number of tentacles did not change with age.

It has been suggested by Brien (*1*) that individual hydras are theoretically immortal. He bases this suggestion on the fact that there seems to be no noticeable decrease in the budding rate of individuals maintained under observation for long periods (*2*). Also, in each individual hydra there is a continual replacement of the entire cell population (*1*, *3*), and it may be that this produces a continual rejuvenescence. The facts that stimulation of the rate of cellular replacement by repeated injury seems to increase the

Table 1. Number of buds produced by offspring minus the number produced by parents ($\bar{x}$)

Group	Generation	$\bar{x}$	t	p
Control	4	−0.06	0.20	0.8
Control	8	.42	1.56	.07
Control	12	.63	3.07	$<.005$
Irradiated	4	.54	1.1	.15
Irradiated	8	.765	1.88	$<.05$
Irradiated	12	.53	1.59	.07

Table 2. Number of buds produced by irradiated hydras minus number of buds produced by controls ($\bar{x}$).

Group	Generation	$\bar{x}$	t	p
Parents	4	0.10	0.28	0.7
Parents	8	−0.24	.65	.5
Parents	12	.14	.47	.65
Buds	4	.50	1.37	.2
Buds	8	.20	0.79	.2
Buds	12	.06	.155	.9

resistance of hydras to adverse conditions (*4*) and that interstitial cells from a normal hydra can restore an irradiated one (*5*) support the notion of rejuvenescence.

However, several investigators have argued that aging does take place in *Hydra*. Hase and Grosz (*6*) found that the number of tentacles increases with age; Vishnevs'kii (*7*) found that the time required for successive buds to develop increases with age; and Hyman (*8*) noticed that both irregular budding and depression occur more readily in older individuals. Hase reported populations of *Hydra oligactis* and *H. vulgaris* that lived only 167 and 337 days, respectively. However, Pearl and Miner (*9*) examined Hase's data and found that the death rate was constant with increasing age. Therefore, death was probably due to environmental accidents.

It has not yet been adequately demonstrated by direct comparison between "old" and "young" hydras that there is no difference between them. In an attempt to throw light on this matter, the budding rates and tentacle numbers of parent hydras and of their youngest buds in a line of successive first buds were compared, both under normal conditions and under radiation stress.

Two groups of 20 *Hydra littoralis* lacking buds and gonads were selected at random from a large male clone and reared by Loomis's culture method (*10*) in individual 10-ml beakers each containing 5 ml of water. One group was irradiated with a dose of 2000 r of x-rays. Detached buds were isolated daily. A dose of 2000 r was chosen because this was the highest dose that did not decrease the budding rate.

The number of buds produced by each 4th-, 8th-, and 12th-generation offspring in each line of successive first buds was compared with the number of buds produced by the corresponding original parent over a 15-day period, and the mean differences were evaluated by Student's t test. Presumably fewer cell divisions occurred in the history of a bud produced by one of the parents than in that of one of the buds derived from a bud, several generations removed, of a parent. Thus, the performances of "old" (the original parents) and of "young" hydras were compared during the same period of time and under the same conditions. The results are summarized in Table 1.

As the original parents in the nonirradiated group became older, their budding rate became progressively slower compared with that of young hydras. At the 12th generation the difference was highly significant ($p < .005$). The irradiated hydras followed this same trend between the 4th and 8th generations ($p < .05$), but in these experiments the results are not conclusive at the 12th generation ($p = .07$). These results seem to support the hypothesis that older hydras bud more slowly than younger ones.

There was no significant difference between the budding rates of irradiated and nonirradiated hydras, compared group by group (Table 2), but there was some indication that doses of from 100 to 2000 r stimulate budding during the first week after irradiation.

The number of tentacles of each hydra in these experiments was recorded daily, and comparisons were made between the mean numbers of tentacles for the various groups. In contrast to the results of Hase and Grosz, no significant differences were found. Also, there was no increase with time in the mean number of tentacles of the original parents. We conclude that the number of tentacles does not increase with age in *Hydra littoralis*.

At the end of the first month of the experiments the hydras suffered from a depression possibly caused by traces of detergent left in the glassware when it was washed. Thirty-two percent of the original parents but only 8 percent of the youngest buds (in generations 2 through 6) became badly depressed. Six days later 7.5 percent of the original parents but only 2.5 percent of the youngest buds had died. This increased susceptibility of older hydras supports the hypothesis that aging takes place (*11*).

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Radiation Dosimeter Utilizing the Thermoluminescence of Lithium Fluoride

Abstract. A dosimeter, with little wavelength dependence and large useful energy range for electromagnetic radiation, which is simple to use and read, has been developed. It appears to have applications in personnel monitoring as well as radiation research.

A radiation dosimeter has been developed which utilizes the thermoluminescence properties of lithium fluoride crystals. The principles involved in such dosimetry have been previously described by Daniels *et al.* (*1–3*). A CaF_2 : Mn thermoluminescent dosimeter has recently been developed by Schulman *et al.* (*4*).

The phenomenon of thermoluminescence in crystals may be described as "frozen-in" or stored luminescence which is unavailable at normal temperatures but which may be recovered by heating. It is due to the trapping of electrons displaced by high-energy radiation. The amount of thermoluminescence stored in a given crystal is proportional to the amount of previous exposure to ionizing radiation. For dosimetry purposes the thermoluminescence must be stable in the temperature range of normal use, that is, it must have a high activation energy relative to room temperature. Several materials, including CaF_2, LiF, $CaCO_3$ (calcite), and SiO_2, have the capacity for such high-temperature thermoluminescence.

Lithium fluoride has a high-temperature thermoluminescence particularly suited for dosimetry purposes. As a