Table 1. Diffusion measurements on ethane, propane, and normal butane at 29.9°C.

Capillary length (cm)	Diffusion time (sec)	Concn. ratio, C_0/C_a	D (10^{-5} cm²/sec)
	Ethane		
2.412	81,000	2.10	1.56
2.413	79,620	2.07	1.54
2.407	82,380	2.20	1.64
2.475	83,100	2.11	1.60
Mean			1.59*
	Propane		
2.475	123,960	2.36	1.30
2.405	127,020	2.37	1.24
2.405	101,220	2.19	1.33
2.475	102,420	2.09	1.29
2.407	86,580	1.94	1.24
Mean			1.28†
	Normal butane		
2.475	162,000	2.38	0.96
2.475	178,980	2.49	0.99
2.407	177,420	2.70	1.04
2.475	116,220	2.06	1.05
2.407	116,720	2.07	1.10
Mean			1.03‡

* S.D. of mean, ± 0.01. † S.D., ± 0.02. ‡ S.D., ± 0.02.

We used a specially designed capillary of variable length having an internal diameter of about 0.1 cm. The capillary was made from a 50-ml syringe (*3*), with a flat barrel on one end. The close fitting stainless steel plunger that is a standard part of the syringe can be equipped with guide rods and a stopping device (Chaney adaptor) so that any desired length of capillary up to about seven centimeters may be used. By filling a known length of the capillary with an aqueous solution of a given hydrocarbon, the system is then ready for immersion in a temperature-controlled bath of pure water (Fig. 1).

The aqueous solutions were prepared by bubbling the hydrocarbon (*4*) gas through distilled water for about half an hour to ensure saturation. The solution was then allowed to stand for a day in a constant temperature air bath in order to achieve equilibrium.

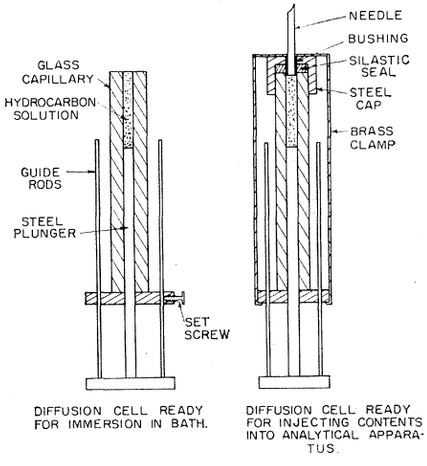

Fig. 1. Diagram of diffusion cell.

There are various sources of errors (*5*) in measuring diffusion coefficients such as mechanical sweeping of part of the solution out of the capillary as it is being immersed in the water bath. This error was eliminated by keeping an excess droplet of solution on the flat end of the capillary as it was carefully lowered into the bath. Gentle stirring in the water bath must be provided so that the concentration at the open end of the capillary remains effectively zero but this stirring must not cause appreciable convection currents. Wang (*2*) has discussed a procedure for checking this important experimental condition. The possibility of losing some of the hydrocarbons through the annular space between the plunger and the glass capillary was investigated and found to be negligible.

After an appropriate diffusion time had elapsed, the capillary was removed from the bath and equipped with a removable needle (Fig. 1) for injecting the contents directly into the analytical apparatus. This arrangement provides a distinct advantage over a capillary of fixed length. Tracer techniques were used by Wang in measuring solute concentrations, but in our method, hydrocarbon concentrations are measured with a hydrogen-flame ionization detector, thus eliminating the tracer apparatus. A short chromatographic column used with the detector consists of stainless steel tubing (0.3 cm by 1.5 m) packed with 60/80 mesh firebrick coated with 20 percent polymethylphenyl ether (six ring). By using a carrier gas that is approximately a 1:1 mixture of nitrogen and steam, the introduction of the liquid contents of the capillary simply produces additional steam and the detector reacts only to the hydrocarbons present. An analysis of 20-μl samples can be made in less than 1 minute with a precision of about 0.5 percent.

An example of the results of diffusion measurements (*6*) on ethane, propane, and butane at 29.9°C is given in Table 1 (*7*).

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Symbiosis: On the Role of Algae Symbiotic with Hydra

Abstract. *Green algae living within gastrodermal cells of hydra photosynthetically incorporate $C^{14}O_2$. About 10 percent of the carbon fixed by the algae is released to the hydra where it is assimilated into animal cell components. The specific activity (counts per minute per microgram of protein nitrogen) of hydra animal tissue is 50 to 100 times greater than that of algae-free controls exposed to $C^{14}O_2$. Analyses were facilitated by a new method for rapid separation of hydra tissue layers.*

A variety of aquatic invertebrates contain unicellular algae as hereditary symbionts (*1*). The importance of these foreign, intracellular, self-reproducing "plasmids" (see *2*) to the host animal is not known. There is radioautographic evidence that carbon-14 photosynthetically incorporated by the algae in some coelenterates appears in the host tissues (*3*). The metabolic fate of this material in the animal tissues has not been previously studied because of the difficulty of separating the animal tissues from the algae. Using green hydra and a new method for rapid separation of ectodermal (algae-free) and gastrodermal (algae-laden) tissues, we have shown that a portion of the carbon-14 photosynthetically assimilated by the algae is released and incorporated into the major chemical components of the animal.

All experiments were carried out with asexually reproducing *Chlorohydra viridissima* (Schulze, 1927) (*4*) at ambient laboratory temperatures (22° to 24°C) and in continuous light (2750 lu/m², Sylvania Cool White fluorescent). Most of the gastrodermal cells of this species contain 15 to 25

unicellular green algae (Chlorophyceae), each 3 to 6 microns in diameter. Animals were maintained in the laboratory by mass culture methods (*5*) and an artificial culture solution (*6*). Algae-free (albino) *C. viridissima* were obtained by growing green individuals for 7 to 9 days in culture solution containing 0.068*M* glycerine (*7*). Animals used for experiments were of similar developmental stage taken from logarithmically growing stock cultures; they were fed daily except on the day prior to experimentation.

The rate of accumulation of carbon-14 by hydra tissues was measured as follows: replicate groups of five green hydra each were placed in 5 ml of culture solution in Warburg flasks (Fisher-6-437E) and exposed to $C^{14}O_2$ evolved from 25 μl of a $NaHC^{14}O_3$ solution (about 200 μc/ml) in the sidearm. Albino *C. viridissima* treated similarly served as controls to demonstrate animal fixation of $C^{14}O_2$. At intervals of 1, 3, 5, 6, 12, 24, and 48 hours groups of green and albino hydra were removed from the flasks and immediately washed by four serial transfers through clean culture solution. Tissue layers of the green hydra were separated by first cutting off the hypostome just below the tentacle bases and then placing the remaining body tube in unbuffered culture solution (*6*) adjusted to *p*H 2.5 with HCl. In 15 to 30 seconds the ectodermal and gastrodermal cell layers became detached from the supporting mesolamella. Longitudinally oriented epitheliomuscular elements caused the ectoderm to contract toward the base of the animal while, simultaneously, contraction of the unrestrained circular and oblique elements caused the gastrodermis to elongate. After about 1 minute the layers were joined loosely at the base of the animal and could easily be teased apart with glass needles. The needles and glassware were coated with Beckman Desicote to keep tissues from sticking to them. Solutions at *p*H values below 2.5 macerated the tissues, while solutions at higher *p*H values were not as effective. In contrast to other methods for separation of hydroid tissue layers (*8, 9*), our method combines speed, clean separation, and elimination of tedious manipulation. Less than 1 percent of the total fixed carbon-14 was lost during the process. The acid *p*H eliminated contamination of tissues from free $C^{14}O_2$.

For assay of radioactivity, gastrodermal and ectodermal tissues were pooled into separate groups, dried on aluminum planchets, and counted with a gas flow counter. Replicate counts were corrected for background and averaged. Whole albinos were assayed in the same fashion after treatment with 0.1*N* HCl to evolve unincorporated $C^{14}O_2$.

Figure 1 shows the relatively constant rate of appearance of carbon-14 in the ectodermal and gastrodermal cell layers of green hydra and in the intact whole albino controls. The large incorporation of label by the gastrodermis (upper curve) was undoubtedly the result of photosynthesis by the symbiotic algae in this tissue layer. The most striking feature is the relatively rapid appearance of carbon-14 in the green hydra animal tissue (ectoderm, middle curve) when compared to the relatively slow labeling rate of the albinos (lower curve). The ectoderm accumulated 12.3 $\pm$ 3.2 percent of the total carbon-14 incorporated into the whole green hydra; this is far more than would be expected from animal fixation alone, as demonstrated by low levels of radioactivity in the albinos.

The appearance of carbon-14 in green hydra animal tissue (ectoderm) beyond that accumulated by animal fixation can be interpreted as a result of (i) direct passage of labeled photosynthetic products from the symbiotic algae or (ii) passage of a cofactor, such as reduced triphosphopyridine nucleotide, which in turn might endow the animal tissues with additional "reducing power" for animal fixation of $C^{14}O_2$.

Preliminary chemical (*10, 11*) and radiochromatographic (*12*) analysis of labeled green hydra ectoderm after very short exposures to $C^{14}O_2$ supports the first alternative.

To determine the fate of the labeled products accumulated by the ectoderm, the distribution of radioactivity in different chemical fractions was analyzed and compared with the distribution of carbon-14 in albinos. Animals were labeled and green hydra tissue layers separated as described above. Ectoderm, gastroderm, and whole albino controls were fractionated by differential solubilities (*13*).

Table 1 shows that after 48 hours' exposure to $C^{14}O_2$, the percentage distribution of carbon-14 among fractions of green hydra ectoderm is similar to that of albinos. This distribution indicates that once material from the algae enters the animal cells it is probably handled like most animal cell metabolites and distributed accordingly. In both tissues the largest percentage of label appeared in the residual protein fraction (insoluble in hot trichloroacetic acid and alcohol insoluble); the specific activity (counts per minute per microgram of protein nitrogen) was 50 to 100 times greater in green hydra ectoderm than in albino tissues as a result of transfer of material from the algae. The fractionation of gastroderm, where photosynthetic fixation predominates, illustrates the pattern of plant cell $C^{14}O_2$ incorporation after 48 hours. The marked difference between the hot-TCA-soluble, alcohol-insoluble and hot-TCA-alcohol-insoluble fractions of the green hydra gastroderm and the green hydra ectoderm shows that the ecto-

Table 1. Percentage distribution of carbon-14 among different fractions of *C. viridissima* tissues. Replicate values are given for each fraction. TCA, trichloroacetic acid.

Albino-hydra (intact)	Green hydra	
	Ectoderm	Gastroderm
Cold-TCA-soluble fraction		
19.4, 23.6	29.0, 27.4	21.3, 22.0
Alcohol-soluble fraction		
14.1, 16.0	24.0, 27.6	34.3, 29.1
Alcohol-insoluble, hot-TCA-soluble fraction		
7.5, 8.3	4.7, 4.4	27.6, 27.1
Alcohol, hot-TCA-insoluble fraction		
59.0, 52.0	41.5, 40.5	18.0, 16.8

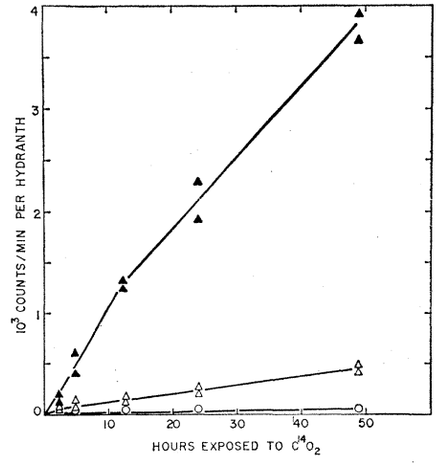

Fig. 1. Rate of accumulation of carbon-14 by *C. viridissima* tissues: green hydra gastroderm (solid triangles), green hydra ectoderm (open triangles), and whole albinos (circles).

derm derived from the separation procedure was not contaminated by algae.

The high specific activity of carbon-14 in green hydra ectoderm, and its broad distribution in the host's major chemical fractions, supports the hypothesis that the symbiotic algae are of nutritional significance to the host. This view is also supported by recent quantitative growth studies (*11*, *14*) which show that symbiotic algae exert a favorable influence on the growth and well-being of their hosts, especially under regimes of limited food or starvation. We are currently investigating the manner in which the carbon supplied by the algae aids the host. This material might be used as a general supplemental carbon source or, alternatively, a small part, possibly in the form of essential cofactors or vitamins, might be required for enzymatic activities of the animal (*15*).

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Insecticides: Effects on Cutthroat Trout of Repeated Exposure to DDT

Abstract. *Cutthroat trout were periodically exposed to* p,p′*-DDT, in acetone solution or in the food. Excessive mortality occurred only in lots treated with high concentrations of DDT, probably as a result of decreased resistance to nonspecific stressors. Surviving fish in these lots were significantly larger than those in the control lot, or in the lots treated with low concentrations of DDT. The number and volume of eggs produced was not reduced by DDT, but mortality among sac fry appeared to be highest in the lots treated with high concentrations. The data suggest that the sublethal concentrations of DDT ordinarily encountered in the environment are unlikely to damage a fishery.*

The Fish-Pesticide Research Laboratory of the Bureau of Sport Fisheries and Wildlife organized an experiment at the National Fish Hatchery at Jackson, Wyoming, to measure some long-term effects of DDT on the Snake River cutthroat trout, (*Salmo clarki lewisi*). This study began in December 1960 and terminated in September 1962.

Eleven lots of yearling fish were used; each lot was composed of 636 fish and was divided into three sublots located randomly in raceways. Lot I included untreated control fish. Lots II to VI were exposed for 30 minutes, once every 28 days, to a bath of DDT (*1*) in acetone solution, at concentrations of 1.0, 0.3, 0.1, 0.03, and 0.01 parts per million, respectively. Lots VII to XI were given DDT once a week in a pelleted diet, in doses of 3.0, 1.0, 0.3, 0.1, and 0.03 mg/kg body weight, respectively. At 4-week intervals throughout the experiment the surviving fish were weighed to determine growth. Samples of fish were withdrawn according to a schedule and were either frozen for subsequent chemical analysis or fixed in Bouin's solution for histopathological examination. Sampled fish, and those suffering mortality during the experiment, were weighed, measured, sexed, and examined for gross pathology. Microhematocrit measurements were made on blood from sampled fish. Reproductive success was determined during artificial spawning in 1962. The effects of DDT on mortality and pathology, growth, residue levels, and reproduction will be considered separately (*2*).

Cumulative mortality over the course of the experiment is shown in Fig. 1. For simplicity, the data from the sublots have been combined and are presented as the total for each lot. Little difference in mortality occurred among sublots receiving identical treatment. To determine if mortality in the treated lots differed significantly from that in lot I, *t*-tests were made at 2-month intervals.

Three months after the start of treatment, mortality in lot VII was significantly higher than that in lot I ($p < .05$). After 4 months, mortality differed also in lots II and VIII ($p < .05$, $< .02$, respectively). At 6 months it differed in lots III and IV ($p < .001$, $< .01$, respectively). Cumulative mortality in the remaining lots did not differ significantly from that in lot I during the 20-month experimental period.

Mortality rates, calculated by dividing the number of deaths during a month by the number of living fish in the lot, varied for each lot throughout the study. The control lot, and the lots treated with low concentrations of DDT, displayed peaks of mortality at about the 6th, 11th, and 18th months after the initial treatment. The lots treated with the highest concentrations displayed peaks at about the 6th and 18th months.

The periodicity of the death rate in all lots suggests that additional factors were involved in the increased mortality of the fish exposed to higher concentrations of DDT. One such factor was disease. Lesions due to disease were most common in dead fish from the control lot and lots treated with the lower concentrations of DDT. Fish that were obviously sick survived for considerable periods, but were the first to succumb during the periods of high mortality. When fish in the lots treated with higher concentrations showed signs of illness, they seldom survived long, perhaps indicating that the DDT had reduced their resistance to disease. Another stressor operative during part of this study was artificial spawning. Mortality was highest among fish from lots II, III, VII, and VIII, again indicating reduced ability to withstand stress.

Histopathological examination of sampled fish in apparent good health, and of fish which died, revealed no