freely in the sea water and in a position to exploit a new food supply. Whether attack on fresh wood is possible can be tested under controlled laboratory conditions. Accordingly, dry "unconditioned" blocks of lumber, including Douglas fir, elm, hemlock, redwood, western red cedar, and western yellow pine, were exposed to healthy *Limnoria*. Each of these wood species was attacked within 24 hours at Friday Harbor, and within 2 to 3 hours at Naples. If "conditioned" wood is unnecessary in laboratory cultures, it is unlikely to be essential in the sea.

Finally, with reference to observation (iii), it was reported that *Limnoria* is unable to attack sterilized wood (*3*) and that animals living in sterilized wood survive no longer than controls kept without a food source (*2*). We find that healthy animals are quite capable of attacking and living in wood sterilized by autoclaving. In these tests the same six species of wood were used and the animals attacked them all within the same period of time (24 hours at Friday Harbor; about 2 hours at Naples). In all of these cases, growing populations were established in the absence of marine fungi (*7*).

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7. A full report on this whole problem, including consideration of marine wood-inhabiting bacteria and a discussion of the suggestion made by Becker and by Schafer and Lane that fungi might contribute to the nutrition of *Limnoria*, is in preparation.

8 September 1958

Zinc-65 in Foods and People

Abstract. Disposal of trace amounts of Zn^{65} is made in the Columbia River via Hanford reactor effluent water. The subsequent utilization of river water for irrigation permits the concentration of this radioisotope in farm produce and its eventual deposition in man. The Zn^{65} in irrigation water, in farm produce, and in individuals utilizing these materials has been measured.

Water from the Columbia River is used as a coolant for the Hanford reactors. The subsequent disposal of this water in the river introduces trace amounts of several induced radioisotopes, most of which have half-lives of the order of minutes to a few hours; however, the half-lives of some of these isotopes are sufficiently long to permit tracing the distribution of the isotopes into the food chains of the aquatic life in the river (*1*). Zinc-65 is the major long-lived radioisotope introduced into the river, and although it is present at a concentration far below the most conservative permissible limits, it exists in sufficient amounts to serve as a tracer; it is thus possible to follow its path from irrigation water through plants and animals to man.

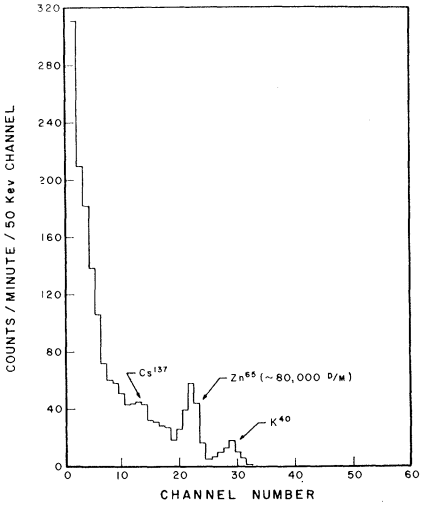

Fig. 1. Gamma-ray spectrum of an individual containing Zn^{65}.

Only a small fraction of the Columbia River water used for irrigation is obtained downstream from the Hanford project. The farm-produce and animal samples considered here were obtained from an irrigation project about 30 miles downstream from the Hanford reactors. By means of gamma-ray spectrometric techniques, measurable amounts of Zn^{65} were found in all the farm produce sampled from this location. The Zn^{65} concentrations found in milk, beef, and the various types of vegetables from this land are shown in Table 1. The concentration factor (Zn^{65} concentration in the sample/Zn^{65} concentration in the irrigation water) for each sample is also included.

With the exception of the beef, all of these samples were obtained during July and August 1957. The beef was obtained from an animal slaughtered in January 1957 after it had lived 1 year on the irrigation project. The fact that the pasture grass contained a relatively high Zn^{65} concentration as compared with the vegetables is probably related to both the manner and amount of irrigation as well as to the fact that some difference in uptake between the leaf and fruit portion of plants would be expected. The pasture grass was irrigated almost continuously, while the vegetables were irrigated only a few times during their growing season. In addition, the Zn^{65} may enter the grass by foliate absorption during irrigation as well as through the soil.

The relatively high Zn^{65} concentration in milk as compared with that in the pasture grass indicated that a large amount of Zn^{65} is taken from the feed into the blood stream of the cow and translocated into the milk. The low Zn^{65} concentration found in the beef samples (Table 1) may be explained by the fact that the animal was slaughtered in late winter and had been fed on essentially Zn^{65}-free foodstuffs for 3 to 4 months prior to that time. Measurements of Zn^{65} in the same milk supply during January and February of 1958 showed about 10 percent of the value listed in Table 1. This again can be explained by the animals' relatively Zn^{65}-free diet during the winter months.

A second animal which had spent its entire life in the same location was slaughtered in March of 1958 and was

Table 1. Concentrations of Zn^{65} in farm produce.

Sample	Concentration ($\mu\mu c/g$)	Concentration factor (produce/water)
Pasture grass	82.9	440
Beef, flesh	5.23	28
Beef, fat	1.48	7.9
Beef, bone	5.80	31
Milk (cow)	4.88	26
Black-eyed peas	0.55	2.9
Tomatoes	0.46	2.4
Okra	0.39	2.1
String beans	0.29	1.5
Corn	0.16	0.83
Grapes	0.089	0.47
Irrigation water	0.188	

Table 2. Concentrations of Zn^{65} observed in the various organs of a beef animal.

Sample	Concentration ($\mu\mu c/g$)
Flesh	10.7
Fat	2.22
Bone	13.4
Ovaries	4.07
Hide	3.91
Kidney	5.98
Lung	5.11
Brain	2.74
Pancreas	7.27
Blood	0.86
Hair	28.6
Thymus	3.79
Liver	11.5
Horn	3.59
Hoof	2.59

subjected to a much more extensive Zn^{65} analysis. The Zn^{65} concentrations found in the various organs of this animal are listed in Table 2. It may be noted that the Zn^{65} concentration in the flesh, fat, and bone samples are about twice those observed in the samples of the previous year (see Table 1). It is also apparent that hair, liver, and bone concentrate zinc to a greater extent than the other organs.

The observation of these Zn^{65} concentrations in farm-produce samples suggested the possibility that Zn^{65} could be measured in individuals obtaining their food supply from these irrigation projects. For the measurement of Zn^{65} in people, a 3- by 5-in. sodium iodide crystal detector operated in a shielded room similar to the installation developed at Argonne National Laboratory (*2*) was used. The gamma-ray spectrum of an individual who consumed approximately 0.1 and 0.7 kg, respectively, of the meat and milk per day from the sources listed in Table 1 is shown in Fig. 1. This Zn^{65} photopeak area represents about 3.6×10^{-2} μc or 80,000 disintegration/min.

Twelve other individuals were measured whose diet did not include food from the irrigation project but did in some cases include drinking water whose source was the Columbia River. The gamma-ray spectra of most of the individuals whose drinking water originated in the Columbia River show a small Zn^{65} photopeak. The Zn^{65} content of these individuals was estimated to be between 5000 and 10,000 disintegration/min. The gamma spectra of individuals who receive neither their water nor their food supply from the Columbia River or its irrigation projects showed no detectable Zn^{65} photopeaks. The Zn^{65} found in all samples of foods, in fodder, or in individuals could be traced either to Columbia River drinking water or to food from the lower irrigation projects. It is believed that, with a sensitive total-body counting device of the Argonne National Laboratory type (*2*) Zn^{65} could be measured in most individuals who receive their drinking water from the Columbia River downstream from Hanford, provided that some purification step in the local water treatment does not remove the isotope.

Zinc-65 has been reported (*3*) in individuals living near the Pacific Proving Grounds and was shown to be a result of contamination from nuclear tests; however, Zn^{65} from nuclear tests has not been observed in foods raised in this country. The presence of Zn^{65} in the amounts observed here in no way constitutes a hazard. Even the value of 3.6×10^{-2} μc of Zn^{65} in the individual whose diet included the meat, milk, and drinking water of Table 1, is less than 0.01 percent of the total permissible body burden for this isotope (*4*). Also, even the highest Zn^{65} value in Table 1 (for pasture grass) is less than 5 percent of the maximum permissible concentration for human foods (*4*).

Smaller amounts of the radioisotopes Cr^{51} and Sc^{46} have been detected in samples of pasture grass but have not been found in farm produce.

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23 September 1958

Studies on 4-Keto-L-Proline

Abstract. The administration of ketoproline to chick embryos resulted in an increase in the free hydroxyproline. This phenomenon is explained by the inhibitory action of ketoproline on the catabolism of hydroxyproline as well as by the conversion of the former to the latter. Ketoproline was found to be reduced to hydroxyproline by the supernatant fraction of rat-kidney homogenate in the presence of a reduced pyridine nucleotide.

Since hydroxyproline occurs uniquely in collagen in the animal, studies on the biogenesis and metabolism of this compound have a great significance in the understanding of the biochemistry of collagenous tissues. In the course of a survey of compounds structurally related to hydroxyproline for their ability to affect the metabolism of this imino acid, the administration of 4-keto-L-proline (*1*) to chick embryos was found to produce an increase in free hydroxyproline.

For these studies 5 mg of free 4-keto-L-proline in 0.2 ml of water was placed in the air space of a 12-day-old chick embryo through an opening in the shell. After 24 hours an 80-percent ethanol extract was prepared from the whole embryo and hydroxyproline was assayed colorimetrically by a modification (*2*) of the method of Neuman and Logan (*3*). The results are shown in Table 1. It can be seen that ketoproline caused a five- to sixfold increase in free hydroxyproline without affecting the proline level. Similarly, when 15 mg of ketoproline was administered subcutaneously to rats (200 to 250 g), a prolonged elevation of the blood hydroxyproline level was observed, an increase from 0.55 to 1.0 mg per 100 ml being maintained for a period of several hours. Although administration of 5 mg of hydroxyproline produced a comparable increase, this lasted for less than 2 hours. The mechanism by which ketoproline produces the increase in free hydroxyproline in vivo was found to be twofold: (i) inhibition of hydroxyproline destruction and (ii) conversion of ketoproline to hydroxyproline.

A strain of *Achromobacter* grown on hydroxyproline as the sole source of carbon metabolized hydroxyproline extensively. However, when increasing amounts of ketoproline were added to the incubation mixture, destruction of hydroxyproline was diminished (Table 2). Proline was also found to antagonize the metabolism of hydroxyproline under similar conditions. However, the metabolism of proline by proline-adapted *Achromobacter* was not appreciably affected by ketoproline. Similar observations were made when mammalian liver or kidney mitochondria were used in place of the bacteria. Neither the bacteria nor the mitochondria were able to convert ketoproline to hydroxyproline in any detectable amounts.

When ketoproline was incubated with a well-dialyzed soluble fraction of rat-kidney homogenate, it was reduced to

Table 1. Effect of ketoproline on free proline and hydroxyproline levels in chick embryos. Twelve-day-old embryos received either 0.2 ml of saline or 5 mg of ketoproline in 0.2 ml of water. After 24 hours, 80-percent ethanol extracts of the embryos were assayed for hydroxyproline (*3*) and proline (*6*).

Treatment	Wet wt. of embryo (g)	Free hydroxyproline (μg)	Free proline (μg)	Free hydroxyproline/proline free
Control (av. of 6)	5.2	112.1	206.2	1.84
Ketoproline administered (av. of 4)	6.3	627.2	218.7	0.35

Table 2. Effect of increasing concentrations of ketoproline on hydroxyproline metabolism by *Achromobacter*. One milligram of hydroxyproline was incubated for 30 minutes with 9.4 mg (dry weight) of hydroxyproline-adapted *Achromobacter* (*7*).

Ketoproline/hydroxyproline	Hydroxyproline remaining (μg)	Inhibition (%)
0	97	
1	340	29
2	420	36
3	585	54
4	690	66
5	780	76