References and Notes

1. W. A. Atchley and N. V. Bhagavan, *Federation Proc.* **21**, 370 (1962).
2. Kindly supplied by Dr. Leon Kind.
3. H. E. Bowen, *J. Immunol.* **68**, 429 (1952).
4. A. H. Coons and M. H. Kaplan, *J. Exptl. Med.* **91**, 1 (1950).
5. G. Friou, S. C. Finch, K. D. Detre, *J. Immunol.* **80**, 324 (1958).
6. We thank David A. Wood, director of the Cancer Research Institute, for his interest and support, and Lynne Mahoney and Betty Woodside for their technical assistance. This work was supported by grant C-5060 from the U.S. Public Health Service, the Martha and Spencer Love Foundation, the Vivian and Herbert Brede Fund, the Lehman Fund, and a Various Donors Fund.
7. T. J. Gill, H. W. Kunz, E. Friedman, P. Doty, *Federation Proc.* **21**, 32 (1962).

6 July 1962

Fallout Radionuclides in Euphausiids

Abstract. Radioanalyses by gamma-ray spectrometry have been carried out on 37 samples of *Euphausia pacifica* obtained from a network of stations off the Oregon coast. Data on euphausiids taken from this area in November 1961 and in March and April 1962 are compared to show the variation in concentration of zirconium-95 and niobium-95, ruthenium-103, and cerium-141, with time and location. A mixture of zirconium-95 and niobium-95 was the most abundant fission product noted.

A large portion of the radioactive debris from the testing of nuclear devices falls into the ocean. The fission products Zr^{95}, Nb^{95}, Ru^{103}, and Ce^{141} occur in particulate form in sea water (*1*). They are produced in considerable quantity, and result in percentages of 14.6, 12.9, 12.5, and 10.6, respectively, of the total radioactivity from a nuclear detonation 45 days after the blast (*2*). These radionuclides were the principal fallout gamma emitters found in the samples examined in my study. Zinc-65, though ubiquitous in this part of the ocean, is introduced mainly by the Columbia River where it is contained in the effluent from the Hanford, Washington, nuclear reactors (*3*). In general, the gamma-ray spectra of organisms taken near the mouth of the Columbia River showed strong Zn^{65} peaks (*4*), but my research indicates there is no correlation between Zn^{65} and fallout peaks. No additional gamma emitters, other than relatively weak natural potassium-40, were observed in the spectra which extended out to about 3.0 Mev.

Particulate radionuclides tend to be concentrated at the second trophic level (*5*). The euphausiid, *Euphausia pacifica,* is a filter feeder of this level (*6*) and appears to be a good indicator of radioactive fallout in the ocean because it is an effective concentrator of most radionuclides. Euphausiids are the shrimp-like crustaceans (known as "krill" by Norwegian whalers) which comprise the food of certain whales in polar regions. *Euphausia pacifica* is a species found in more temperate waters. Because of its great abundance, its use as forage by many predators, and its extensive diurnal vertical migrations, it is an important vehicle for the transport of radioactivity in the ocean.

The euphausiids were collected at depths ranging from 200 meters to the surface during 30-minute tows with a 6-foot midwater trawl (Isaacs-Kidd). The specimens were preserved in formalin and freeze-dried. After freeze-drying, which gave a weight reduction factor of about 7, gamma spectra were made with the low background anticoincidence instrument (*7*) at General Electric's Hanford Laboratories. Counting time was 30 minutes per sample.

Figure 1 shows the distribution of the three fission products in euphausiids in early November 1961, about 2 months after the beginning of the Russian nuclear tests. The highest concentration occurred 45 miles off Astoria, Oregon. This radioactivity must have crossed the Pacific Ocean in the atmosphere, because the North Pacific Current is too slow to account for its presence at this time. The mixture of Zr^{95} and Nb^{95} in the sample taken 45 miles from the mouth of the river is more than two orders of magnitude greater than that of the sample taken 15 miles from the mouth. Figure 2 shows the virtual disappearance of the "hot spot" by April 1962 and a general lessening of radioactivity. A similar series of measurements made in January 1962 indicates that this change occurred gradually.

Table 1. Results of analyses of two euphausiid samples collected in mid-March 1962. Samples No. 1 and No. 2 were located at 34°19′N, 120°48′W and 32°49′N, 123°54′W, respectively. The lower radioactivity is probably caused by a latitudinal effect in the fallout pattern.

Fission product	Content of sample No. 1 (pc)	Content of sample No. 2 (pc)
Zr^{95}-Nb^{95}	10.6 ± 1.4	8.8 ± 1.3
Ru^{103}	0.2 ± 1.2	0.2 ± 1.2
Ce^{141}	2.3 ± 1.4	3.6 ± 1.4

In general, the amount of Ce^{141} varied directly with the Zr^{95}-Nb^{95}, but Ru^{103} did not show any great fluctuations during the 5-month period covered by these observations.

Two samples of euphausiids taken in California waters in mid-March 1962 were also examined (Table 1). The lower radioactivity which they displayed is probably caused by a latitudinal effect in the fallout pattern.

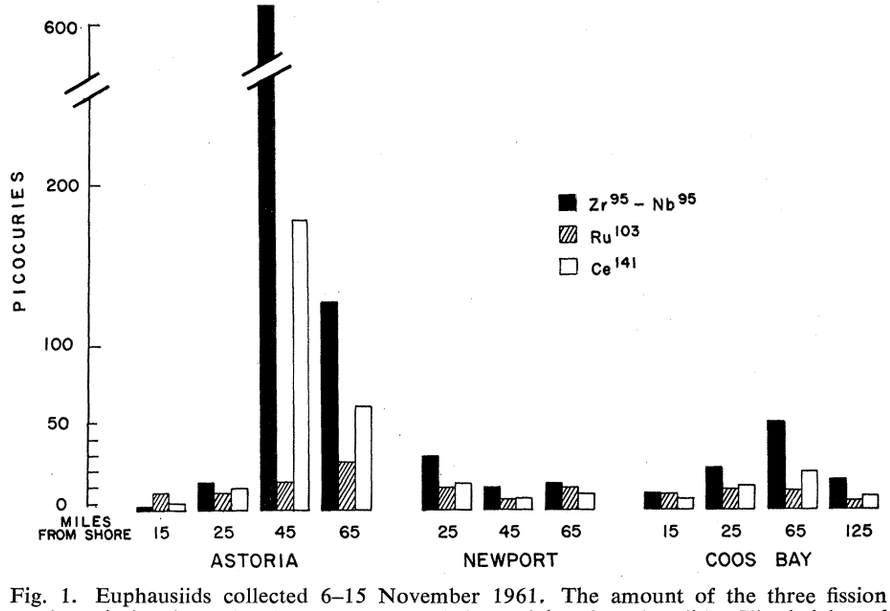

Fig. 1. Euphausiids collected 6–15 November 1961. The amount of the three fission products is in picocuries (pc) per gram of dry weight of euphausiids. The heights of the bars are proportional to the radioactivity, which varies from 2.5 to 618 pc. A wet weight of about 30 g of the sample was the minimum required. The distance from shore is in nautical miles due west from Astoria, Newport, and Coos Bay, Oregon.

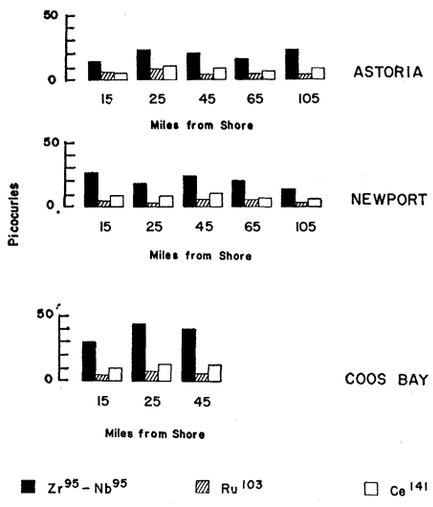

Fig. 2. Euphausiids collected 26 March to 6 April 1962. See legend of Fig. 1 for details.

Radioanalyses of euphausiids taken at the same series of stations (Figs. 1 and 2) in the summer of 1961 (before Russian tests) failed to show any fallout radioactivity at the same level of detection, although Zn^{65} was plentiful. The conclusion is that the three fission products taken up by the euphausiids were part of the airborne fallout associated with the Russian tests (*8*).

CHARLES OSTERBERG
Department of Oceanography, Oregon State University, Corvallis

References and Notes

1. Greendale and Ballou, *USNRDL Document No. 436* (1954), cited by R. Revelle and M. B. Schaefer, in "The effects of atomic radiation on oceanography and fisheries," *Natl. Acad. Sci.–Natl. Res. Council, Publ. No. 551* (1957), p. 12.
2. N. E. Brezhneva, V. I. Levin, G. V. Korpusov, N. M. Manko, E. K. Bogachova, *Proc. U.N. Intern. Conf. Peaceful Uses At. Energy, 2nd Geneva* **18**, 219 (1958).
3. *Hanford Atomic Products Operation Rept. No. HW-68435*, I. C. Nelson, Ed. (1961), p. 22.
4. C. Osterberg, *Limnol. Oceanog.*, in press.
5. L. Berner, Jr., R. Bieri, E. D. Goldberg, De C. Martin, R. L. Wisner, *Limnol. Oceanog. Suppl.* **7**, lxxxii (1962).
6. L. A. Ponomareva, *Dokl. Akad. Nauk. SSSR* **98**, 153 (1954), translated by V. O. Pahn.
7. R. W. Perkins, J. M. Nielsen, R. N. Diebel, *Rev. Sci. Instr.*, **31**, 1344 (1960).
8. This investigation was carried out during the tenure of a predoctoral fellowship from the division of general medical sciences, U.S. Public Health Service. Permission has been received from the graduate school of Oregon State University to publish data which are to appear in a Ph.D. thesis now in progress. The technical assistance of R. W. Perkins of Hanford Laboratories is gratefully acknowledged, as is the cooperation of Edward Brinton of Scripps Institution of Oceanography, who furnished the two plankton samples from off the southern California coast. Special thanks are due N. Kujala and L. Hubbard for collecting the bulk of the biological material, and to Sue Borden for carrying out the electronic data reduction program.

27 July 1962

RNA: A Marker in Embryonic Differentiation

Abstract. A modified fluorochrome technique which identifies nucleic acids differentially in tissues permits following production of RNA in the embryo. Compact primordial chick embryo cells show a sequence to RNA production as differentiation begins: First in the nucleus, then in the perinuclear area, then in the cytoplasm. RNA increases in the cytoplasm with cell growth and continues as the cells merge to form an epithelium.

The incredible complexities of biological systems thwart any simple description of embryonic differentiation. Customarily progressive diversification of the embryo is studied in relation to some "end-point," such as the appearance time and activity of an enzyme or other biological substance, or some change in morphology of cells or tissues. Whatever the milepost chosen, studies should attempt to localize and identify this "end-point" in tissues, to follow it through its lifetime or the organism's (embryo to adult), and to quantitate it biochemically. In the course of studies in a laboratory on the metabolism of nucleic acids and protein in the chick embryo it became necessary to localize RNA in cells and tissues. By means of a modification of a fluorochrome method for differential histochemical identification of nucleic acids, the sequence of early changes seen in primordial chick embryo cells was studied. Intracellular appearance and distribution of RNA was used as the "end-point."

The embryos studied were from a pure strain of chicken, the sex-linked Hallcross (*1*). Cleaving eggs were removed from hens' uteri for study at varying times before organization of the primordial cells into blastoderm epithelium occurred. Stages of cleavage of fertilized eggs which remained in hens' oviducts or uteri were estimated from the times the preceding eggs were laid. It was thus possible to recapitulate the formation of an epithelium by the highly condensed, compact primordial cells, from the time when they were little more than nuclei until they merged into a continuous epithelium.

Conventional staining techniques which localize RNA histochemically lack the sensitivity, reproducibility, and sharp differentiation between RNA and DNA which is afforded by fluorescent histochemical techniques (*2*). The best-known fluorochrome techniques, originated by Strugger (*3*) and studied more recently by Armstrong (*4*) and by von Bertalanffy (*5*), employ acridine orange. Under appropriate conditions acridine orange is conjugated by the nucleic acids and gives a brilliant polychromatic differentiation between DNA, which fluoresces green, and RNA, which fluoresces red. The following method, adapted for embryonic tissues, is a modification of that of von Bertalanffy (*5*) and Bertalanffy (*6*) for detecting malignant cells.

Tissues were fixed in Carnoy's solution at 4°C for 6 to 12 hours, washed in three changes of absolute alcohol over 2 hours at 4°C, cleared in cedarwood oil, and embedded in paraffin; sections were mounted and cut at 3 microns' thickness, and the paraffin was removed with xylol. The sections were hydrated rapidly, in 80, 70, and 50 percent alcohols, successively and then they were placed in 0.002*M* $MgSO_4$. Once the sections were hydrated, the presence of 0.002*M* $MgSO_4$ in all subsequent solutions resulted in better polychromatic differentiation, cell integrity, reproducibility, and stability. Thus, every solution in the following steps contained 0.002*M* $MgSO_4$. Sections were then placed in 1 percent acetic acid for 1 minute, rinsed in $MgSO_4$ solution, stained for 3 minutes in 0.01 percent acridine orange in .067*M* Na_2HPO_4 and KH_2PO_4 buffer (*p*H 6), and rinsed 5 minutes or more (until excess aridine orange had been removed) in the *p*H 6, .067*M* phosphate buffer. After 2 minutes in 0.1*M* $CaCl_2$ solution, sections were again rinsed in phosphate buffer, mounted in a drop of buffer with a thin coverslip, and sealed with paraffin.

Observations were made with a Leitz (Ortholux) microscope and fluorescence equipment. Light from a 200-watt, high-pressure mercury lamp was passed through a blue BG-12 (Schott) filter to transmit light of 4150 Å wavelength, and fluorescence was observed through a suppression filter OG-1 (Schott) mounted in the barrel of the microscope. Photomicrographs were taken on 35-mm high-speed Ektachrome color film with an Exacta single-lens reflex camara.

Companion control sections for every section examined were treated with ribonuclease (*7*). Any fluorescence present after ribonuclease treatment was presumed to be due to substances (notably mucopolysaccharides)