either with native or heat-denatured calf thymus DNA, the effect is roughly additive. In addition, prior incubation of either native or heat-denatured calf thymus DNA with lymphosarcoma RNA polymerase does not alter the capacity of the DNA to function as primer for the *E. coli* RNA polymerase.

This property is not unique to the lymphosarcoma polymerase, as is indicated by experiments with the chicken embryo RNA polymerase (*1*). With a reaction mixture containing native calf thymus DNA, 0.152 mμmole of uridylic acid was incorporated into RNA as compared to 0.011 mμmole when heat-denatured DNA was substituted for native DNA. The incorporation in the absence of added DNA was 0.006 mμmole.

Thus, it appears that for RNA synthesis in animal tissues the structural integrity of native DNA is required. The results reported for the DNA-dependent synthesis of RNA in bacterial systems are not inconsistent. Although denatured DNA primes bacterial RNA polymerase, the priming efficiency is reduced compared with that of native DNA (*4, 7*). And, significantly, biologically active RNA (that is, RNA which can stimulate amino acid incorporation into protein) has been synthesized only in a reaction primed by native DNA (*8*).

For DNA synthesis, the priming requirements are reversed. The DNA polymerase from calf thymus preferentially utilizes denatured DNA (*3*), and though DNA polymerase obtained from *E. coli* and *Bacillus subtilis* can utilize native DNA as primers, limited enzymatic degradation or heat denaturation can improve the priming efficiency of the DNA (*9*).

Taken together, these results in vitro suggest that, in vivo, while DNA may have to be altered in some way before DNA synthesis (replication) can occur, RNA synthesis (transcription) may require the rigid secondary structure of native DNA (*10*).

J. J. FURTH
PATRICIA LOH
Department of Pathology, University of Pennsylvania, Philadelphia

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11. This investigation was supported by a grant (GM-10390-01), and a research career development award (GM-K3-12,888) to J.J.F., from NIH.

23 March 1964

Persistence of Neorickettsiae helminthoeca in an Endoparasite of the Pacific Salmon

Abstract. *Silver salmon* (Oncorhynchus kisutch) *infected with the metacercariae of the "salmon poisoning" fluke* (Nanophyetus salmincola) *remain infected after they have migrated to sea. Metacercariae remain viable in such salmon for at least 33½ months. These metacercariae are capable of transmitting salmon disease* (Neorickettsiae helminthoeca) *to susceptible dogs throughout this period.*

Nanophyetus salmincola, an intestinal fluke, is a vector and reservoir of *Neorickettsiae helminthoeca* (*1*), the etiological agent of "salmon (poisoning) disease" in dogs, foxes and coyotes.

It has been assumed that the metacercariae are "resorbed" by the salmon on their migration to the Pacific Ocean (*2*). The disappearance of the metacercariae was assumed because of the lack of evidence of infection in ocean-caught salmon during these early investigations. Philip (*3*), after reviewing available literature, in 1955 reported "The implication is that cleansing must occur during the years that they [the salmon] are resident in salt water." Conversely, Bennington and Pratt (*4*) felt that "they were undoubtedly present since some [metacercariae] were recovered from such a salmon reportedly taken at sea."

Knowledge concerning the metacercarial persistence of *Neorickettsiae helminthoeca* was necessary for our study of the natural history of the disease. The general plan of the experiment was to collect highly parasitized fish from an enzootic area (Elokomin Salmon Hatchery near the mouth of the Columbia River) and transport them to a location where the fish could be maintained in salt water in an area free of infected fish and free of the snail host. The Bowman's Bay Marine Biological Station near Anacortes, Washington, provided a location which met the requirements, and silver salmon (*Oncorhynchus kisutch*) from the Elokomin hatchery were transported to the salt water ponds at the Bowman's Bay Station on 8 March 1958.

To determine the persistence of the metacercariae and of the *Neorickettsiae helminthoeca* within them, infected fish kidneys were fed to five dogs at the beginning of the experiment on 8 March 1958. All these dogs became sick and showed signs typical of salmon (poisoning) disease (*5*) and died. Adult flukes were demonstrated in the duodenum, and intracellular rickettsia bodies, as described by Cordy and Gorham (*6*), were demonstrated in stained sections of the lymph-nodes in each instance. These captive, salt-water fish were examined at approximately yearly intervals for viable metacercariae. After periods of 12, 24, and 33½ months from the beginning of the experiment, infected kidneys from these fish were fed to dogs to determine the infectivity of the metacercariae and to find out if such metacercariae contained *Neorickettsiae helminthoeca.* At the end of each period, the infectivity was tested in five dogs, 15 being used in all. All 15 dogs showed the typical signs of salmon (poisoning) disease and died. Necropsy revealed lesions characteristic of gross salmon disease; microscopic examination revealed the presence of the adult flukes and eggs in the small intestine. The presence of cytoplasmic rickettsial bodies in the reticulo-endothelial cells of lymph nodes was confirmed by histopathological examination. The possibility that the salmon had become infected with flukes after they had been transported to Bowman's Bay Station was excluded by holding fluke-free control silver salmon under the same conditions as the ex-

perimental salmon. Two control dogs were fed noninfected kidneys at 12, 24, and 33½ months. These dogs remained asymptomatic for at least 60 days. The same dogs were later proved susceptible to salmon disease when fed infected fish from the Elokomin River.

The persistence of a sub-microscopic disease agent in an endoparasite has been studied by Shope (*7*) who reported that swine influenza virus persisted for at least 2 years in the immature lungworms of swine.

Recently, we recovered a Chinook salmon (*Oncorhynchus tshawytscha*) returning to the Elokomin River of Washington with "sea lice" still attached. (These lice drop off soon after the salmon return to fresh water). This fish was marked at the Elokomin River Hatchery and was 5 years of age; presumably, it was returning after having spent 4 years in the ocean. Viable metacercariae were seen in the kidneys of this fish on microscopic examination. The kidneys of the fish were fed to a susceptible dog which became sick and died of typical salmon disease. Adult flukes capable of transmitting rickettsiae were demonstrated on postmortem examination. It was unlikely that this fish was reinfected on its return migration because the presence of "sea lice" indicates recent return from salt water and it is known that the cercariae have to remain in the fish tissue for approximately 10 days before they become infective for the dog.

We conclude that the metacercariae of *Nanophyetus salmincola* containing *Neorickettsiae helminthoeca* can persist for at least 33½ months in fish in sea water.

R. KEITH FARRELL
United States Department of Agriculture, Animal Disease and Parasite Research Division, Fur Animal Disease Laboratory, Washington State University, Pullman
MARILYN A. LLOYD*
Washington State Department of Game, Olympia
BRIAN EARP†
Washington State Department of Fisheries, Olympia

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* Present address: Sacred Heart Hospital, Spokane, Wash.
† Present address: J. R. Clark Company, Salt Lake City, Utah.

25 March 1964

Tetracycline Fluorescence in Permeability Studies of Membranes around Intracellular Parasites

Abstract. *Certain protozoa, bacteria, and viruses when phagocytosed by host cells become surrounded by an intracytoplasmic boundary. This membrane prevents the fluorescent antibiotic tetracycline from entering the parasites when it is added to the medium, since they show no fluorescence, whereas extracellular parasites are immediately visible. As soon as the host cell dies, the intracellular parasites also become visible. This indicates that the boundary probably is of host origin. This phenomenon provides a means for selective permeability studies of such boundaries. A similar exclusion of tetracycline from certain extracellular parasites is seen in the presence of whole serum.*

It was reported previously (*1*) that tetracycline is selectively accumulated by mitochondria of living cells in tissue culture and in vivo, but that nuclei, vacuoles, interparticulate cytoplasm, and cell boundaries appear dark when the tetracycline distribution is observed by fluorescence microscopy. This indicates that of the four boundaries involved, two are very permeable (cell and mitochondrial membrane) and two are slightly permeable (nuclear and vacuolar membrane) to the drug.

For the study of intracellular parasites reported herein, host cells were allowed to attach to coverslips in small petri dishes containing the appropriate medium. After the cells adhered, a suspension of parasites was added so that phagocytosis could take place. Five minutes before observation a tetracycline solution, adjusted to *p*H 7 (final concentration, 50 to 200 μg/ml), was added. The coverslip was then applied to a tissue culture chamber or placed on a slide and observed under a microscope (*2*). Photomicrographs were made of the same preparation; first by phase contrast to localize the parasites, then by fluorescence microscopy to determine whether the parasites fluoresced. In each case it was shown that the extracellular parasites or the internal parasites in moribund host cells did fluoresce.

The results obtained in typical preparations are illustrated in Figs. 1 to 5. Inclusion *Blennorrhea* (*3*) was used as a representative large, complex virus, *Salmonella typhosa* (strain Ty 2) as a bacterium capable of intracellular multiplication, *Escherichia coli*, strain S, and *Bacillus cereus* (recently isolated) as representatives of Gram-negative and Gram-positive bacteria, which could be stained with tetracycline and were capable of being phagocytosed, and *Toxoplasma gondii* (*3*) as an intracellular protozoan.

Figure 1, *A* and *B*, show human synovial cells, infected with inclusion *Blennorrhea* virus suspended in Eagle's

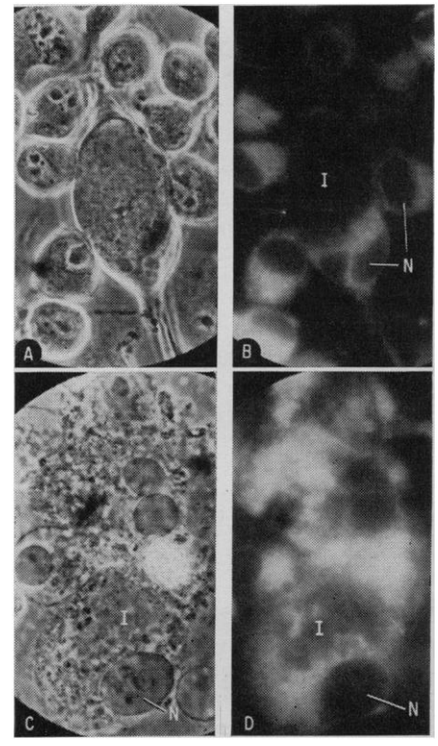

Fig. 1. Photomicrographs of *Blennorrhea* in living human synovial cells, shown (*A*) by phase contrast, and (*B*) after staining with fluorescent tetracycline; (*C*) and (*D*) show the same, but moribund cells. *I*, Intracellular inclusion body; *N*, nucleus.