

sulted from behavioral studies. Glickstein *et al.* (6) observed an apparent recovery from hemianopia in monkeys after unilateral section of the optic tract. Subsequent section of the corpus callosum gave the appearance of reinstating the hemianopia. A similar observation had been reported in the early literature by Imamura (7) and Yoshimura (8) who noted a "hemiamblyopia" in dogs after unilateral lesions were made in the occipital cortex. The condition seemed to disappear with time. However, if the corpus callosum of these animals was subsequently sectioned, the visual defects were reinstated. In terms of the proposed crossed pathway, interpretation of these studies of cortical lesions and sectioning of the corpus callosum is relatively straightforward. However, problems of interpretation arise in the case of optic tract sectioning since, according to the classical understanding of retinal-geniculate anatomy, optic tract section alone should lead to a complete hemianopia.

Such a pathway could be important in interpreting interocular transfer of visual learning in cat and monkey. Myers reported that a visual discrimination, learned monocularly, is transferred to the untrained eye in cats with the chiasma sectioned. Such discriminations are not transferred if the corpus callosum is sectioned as well (9). A similar effect in the monkey has been described by Downer (10) and Sperry (11). These authors have assumed that the interhemispheric mechanism by which a contralateral trace is established in the chiasma-sectioned animal involves a cortico-cortical commissural route. The evidence presented here suggests that interhemispheric transfer may be brought about in part by a simple bifurcation of the visual projection pathway from the lateral geniculate nucleus.

There are two major possible sources of artifact in our experiment. The observed degeneration may have resulted from inadvertent damage to commissural neurons or their axons. However, the lesions were located far from the route of typical commissural fibers of the lateral gyrus. Moreover, our control case exhibited little or no degeneration in the lateral and suprasylvian gyri of the opposite hemisphere, a situation which further suggests that commissural damage did not occur. The second possibility for artifact, that of transneuronal degeneration, is unlikely

since contralateral degeneration was observed after survival periods as short as 5 days. Thus, we believe the anatomical evidence indicates the existence of a pathway crossing from the lateral geniculate nucleus of one hemisphere to the lateral and suprasylvian gyri of the other by way of the corpus callosum.

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12. This study was supported in part by USPHS grant MH 06722-02.

11 May 1964

Thermal Inactivation of the Primer in DNA-Dependent Synthesis of RNA in Animal Tissue

Abstract. *An enzyme preparation obtained from bovine lymphosarcoma tissue catalyzes the DNA-dependent synthesis of RNA. Native DNA is a more efficient primer in the reaction than heat-denatured DNA.*

The RNA polymerase of chicken embryos, like the bacterial enzyme, is soluble, and it is also dependent on the addition of DNA for maximal activity; the added DNA influences the rates at which each of the four ribonucleotides are incorporated (1).

In order to investigate further DNA-dependent RNA synthesis in animal tissues, we have studied the reaction with a soluble enzyme preparation obtained from bovine lymphosarcoma tissue (2). With this enzyme preparation, the synthesis of RNA requires DNA and all four ribonucleoside triphosphates. For example, in an experiment similar to that outlined in Table 1, 0.19 mμmole of uridylic acid was incorporated into an acid-insoluble form. When calf thymus DNA or the three unlabeled ribonucleoside triphosphates were omitted, less than 0.02 mμmole of uridylic acid was incorporated. In this experiment the reaction was inhibited 95 percent by the addition of 20 μg of ribonuclease, 84 percent by 20 μg of deoxyribonuclease, and 89 percent by 5 μg of actinomycin D.

Since DNA, unless it has been denatured, is a poor primer for DNA polymerase from calf thymus (3), native and heat-denatured DNA were compared as primers of the lymphosarcoma RNA polymerase. As shown in Table 1, native DNA is a better primer than heat-denatured DNA. The heat-denatured calf thymus DNA is still able to prime the RNA polymerase

from *Escherichia coli*, as has been reported (4). It is not considered likely that this primer specificity is the result of a nuclease preferentially degrading heat-denatured DNA; for, when the two enzymes are incubated together,

Table 1. Comparison of native and heat-denatured DNA as primers for RNA synthesis. The complete reaction mixture (0.5 ml) contained: 80 μM α-P³²-UTP (10) (3.1 × 10⁶ count/min per μmole); 160 μM each of CTP, GTP, and ATP; 8 mM MgCl₂; 4 mM MnCl₂; 2 mM 2-mercaptoethanol; 50 mM tris-maleate buffer at pH 8.0; DNA and enzyme. The amounts of DNA added, expressed as millimicromoles of deoxynucleotides, were: calf thymus DNA, 72; *H. influenzae*, 60 (5). The DNA preparations were denatured by heating at 100°C for 4 minutes followed by cooling in an ice bath. The amounts of enzyme protein added were, lymphosarcoma polymerase, 530 μg; *E. coli* polymerase, purified through the first ammonium sulfate step of procedure B (6), 49 μg. After incubation for 20 minutes at 38°C, the reaction was terminated by the addition of 0.2 ml of 7 percent perchloric acid, albumin (0.5 mg) was added, and the acidified mixture was centrifuged. The precipitate was washed twice with 3-ml portions of 1 percent perchloric acid, dissolved in 1.5 ml of 0.2N NH₄OH, decanted into metal planchets, and dried; the radioactivity was measured in a windowless Geiger-Muller counter.

Conditions	Uridylic acid incorporated		
	Lympho-sarcoma polymerase	<i>E. coli</i> polymerase	Both enzymes
Omit DNA	< 0.01	0.08	
Calf thymus DNA	0.56	0.68	1.39
Calf thymus DNA, heat-denatured	0.05	0.46	0.39
<i>H. influenzae</i> DNA	0.19	0.46	
<i>H. influenzae</i> DNA, heat-denatured	0.01	0.09	

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 m μ mole of uridylic acid was incorporated into RNA as compared to 0.011 m μ mole when heat-denatured DNA was substituted for native DNA. The incorporation in the absence of added DNA was 0.006 m μ mole.

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 enzy-

matic 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).

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10. Abbreviations: ATP, GTP, UTP, CTP, 5'-triphosphates of adenosine, guanosine, uridine, and cytidine.
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

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-

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 meta-

cercariae 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