it can be shown that TDA potentiates depressor response to isoproterenol(2).

The experiments described herein were undertaken to examine the possibility of chemical inactivation of 5-HT by reaction with TDA. Inactivation has been demonstrated but evidence regarding mechanism is merely circumstantial. TDA does react with 5-HT *in vitro* and the reaction product in large doses has no measurable effect on blood pressure. It also blocks the effects of 5-HT on blood pressure in the rabbit but this may be a consequence of binding to receptor sites and not chemical combination with 5-HT. The presence of a blue color in arterial and venous plasma samples of animals treated with 5-hydroxytryptophane and TDA indicates that *in vivo* coupling can occur but does not exclude the possibility of concurrent binding to receptor sites.

The pyretogenic response to iproniazid and 5-hydroxytryptophane combinations was not modified by TDA possibly because TDA does not penetrate into central nervous system tissue in sufficient quantity, or because amount of 5-HT produced was sufficient to override a TDA blockade.

*Summary.* The tetrazo compound, tetrazotized diorthoanisidine (TDA) reduced or abolished depressor response to 5-HT in the rabbit but did not alter the pyretogenic effects of combined treatment with iproniazid and 5-hydroxytryptophane. The mechanism by which TDA modified blood pressure response to 5-HT is discussed.

1. Cafruny, E. J., Domino, E. J., *A.M.A. Arch. Neurol. & Psychiat.*, 1958, v79, 336.

2. Cafruny, E. J., Domino, E. J., Beck, L., *J. Pharmacol. and Exp. Therap.*, 1958, v124, 219.

3. Erspamer, V., *Pharmacol. Rev.*, 1954, v6, 425.

4. Horita, A., Gogerty, J. H., *J. Pharmacol. & Exp. Therap.*, 1958, v122, 195.

Received March 1, 1960. P.S.E.B.M., 1960, v104.

## Virus Nature of Infectious Pancreatic Necrosis in Trout. (25743)

KEN WOLF, S. F. SNIESZKO, C. E. DUNBAR AND E. PYLE

*Eastern Fish Disease Lab., U. S. Dept. of Interior Bureau of Sport Fisheries and Wildlife, Kearneysville, W. Va.*

Virus-caused hyperplastic diseases of fishes have long been recognized, and Zhdanov(1) included them with the pox group. They have been omitted from other classifications, and Smith(2) stated that "evidence for the existence of viruses affecting fish does not rest upon so secure a scientific foundation." Except for subsequent work on an infectious disease of a Pacific salmon(3), recognized virus diseases of fishes have been discussed in recent reviews(4,5). Infectious pancreatic necrosis (IPN) of trouts is an acute, virulent, and high-mortality disease occurring typically in very young eastern brook trout (*Salvelinus fontinalis*)(6,7,8). The pancreatic lesions are similar to those produced by Coxsackie viruses in mice(7), and like mice, older fish resist infection(8). On the basis of infectiousness of IPN, histological findings, and absence of other pathogens, a virus has been suggested as causative agent(6). This communication describes transmission, TC propagation, and some characteristics of an agent present in fish with IPN.

*Materials and methods.* 1. *Fish tissue* was cultivated according to methods of Wolf and Dunbar(9). Cultures were used after explants had developed extensive epithelial sheets, 3 to 4 days. 2. *Mammalian tissue and cell cultures.* HeLa cells (Gey) were cultivated in 80% SM 199 and 20% horse serum with antibiotics. Other tissue or cell cultures were obtained from commercial source,* and furnished with medium specified by supplier. 3. *TC inocula.* Moribund fish symptomatically diagnosed as having IPN were used fresh, after freezing and storage at −20°C, and after preservation in 50% glycerol at about 4°C. They were ground in sev-

* Microbiological Assoc., Washington, D. C.

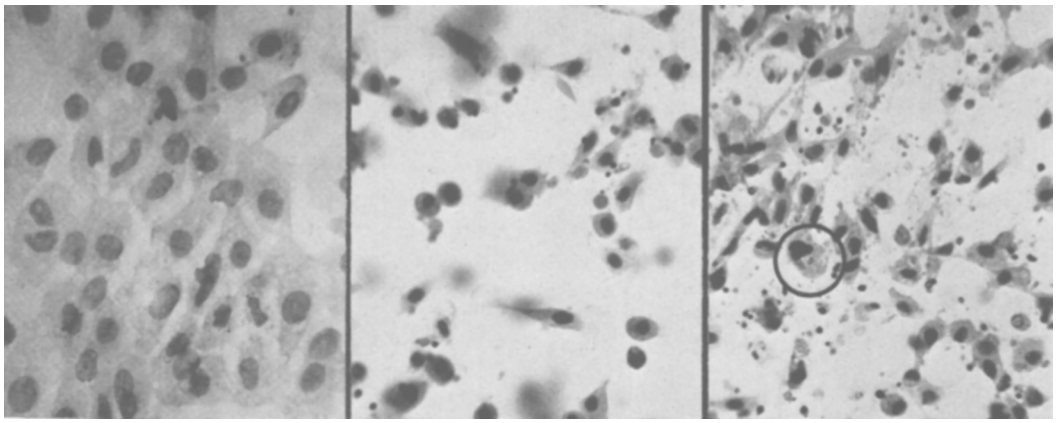

FIG. 1. (Left) Control culture showing normal epithelial sheet from explant of eastern brook trout caudal fin. (Center) Inoculated culture showing degeneration which followed introduction of virus of infectious pancreatic necrosis. (Right) Inoculated culture with advanced necrosis of the cell sheet. Circled area contains cell in which there is an object that is similar to cytoplasmic inclusion bodies seen in histological preparations of pancreas from infected fish. ×280.

eral volumes of SM 199 or Earle's BSS, centrifuged at 12,000 or 26,000 G for 10 to 15 minutes at about 4°C, then filtered through asbestos (Seitz) or porcelain (Coors P3). Inoculum was .01 ml or, more commonly, .1 ml/culture. 4. Two *infectivity trials* were conducted with susceptible fish at 12.5°C, and survivors of each given follow-up feedings of virulent material. A single lot of eastern brook trout was used at 4 weeks and 8 weeks of age. For first trial duplicate jars of 100 4-week-old fish were fed TC harvests of 2 "strains" of IPN in 8th transfer representing $2.6 \times 10^{-10}$ dilution of primary inocula. Control fish received no IPN, but were otherwise treated identically. Observation was carried out for 30 days when mortality ceased. One hundred and fifty fish which survived exposure to infection were distributed to 2 jars, and 2 other jars each received 90 control fish. All were fed ground victims of IPN. At start of second trial fish were 8 weeks old and considerably larger. They were distributed among 12 troughs, 53 g, average 190 fish/trough. The 12 troughs were randomly subdivided into 4 groups of 3 troughs each. Fish in first group were fed with TC harvest IPN in 6th transfer ($1.1 \times 10^{-8}$ dilution of primary inoculum). Fish in second group were fed pooled ground IPN victims, and fish in third group received a Seitz-filtrate of same pool of ground material. A group of 3 control troughs received no IPN. Five weeks after infection, mortality had almost stopped and observations terminated. Eighty survivors which had been fed IPN victims were distributed equally to 4 jars. Two of these jars were held for possibly recurrent mortality. The 2 remaining jars and 2 jars each with 20 formerly uninfected fish were given follow-up feedings of ground IPN victims. Mortality from all phases was sampled and examined by histological section and/or tissue culture inoculation.

*Results.* 1. *Propagation and cytopathic effect (CPE) of IPN.* Epithelium from gill, swim bladder, spleen, kidney, and caudal fin of eastern brook trout was consistently destroyed by filtered inocula prepared from fish with IPN. Caudal fin was used for most work, because it was easiest to prepare in uniform explants free of debris. CPE was evident in 18 to 24 hours after primary inoculation, and complete destruction followed in 1 to 4 days (Fig. 1). Fibroblast-like cells were more resistant but underwent some granulation and darkening. CPE usually began as darkening of peripheral cells but was also common as foci within sheets. Necrotic areas enlarged and met, and sheets were usually obliterated. Objects similar to inclusion bodies were found (Fig. 1), but definitive work has not been done. Inocula were prepared from 8 outbreaks of this disease, and all produced characteristic CPE. Log dilutions of primary inocula always produced CPE at 1 ×

$10^{-4}$, but at $1 \times 10^{-6}$, some inocula failed to evoke CPE. Material from 2 sources was passaged 8 and 9 times during period of waiting for seasonally available host fish. Final dilutions were $3.6 \times 10^{-10}$ and $2.6 \times 10^{-11}$, but latter passages with one "strain" were accompanied by reduced CPE, and it was difficult to distinguish inoculated from uninoculated cultures. Inocula were customarily left in cultures throughout incubation. If cultures were merely exposed to inocula for 15 minutes, then washed repeatedly, CPE still occurred. 2. *Characteristics of agent of IPN.* The agent was inactivated at 60°C for 1 hour. Agent stored in victim fish for 4½ years at −20°C and 2½ years in 50% glycerol at 4°C retained its infectivity. It consistently produced CPE in rainbow trout (*Salmo gairdneri*) caudal fin epithelium. In 2 attempts with brown trout (*Salmo trutta*) and 1 with bluegill (*Lepomis macrochirus*) caudal fin epithelium, CPE failed to occur. HeLa cells inoculated with this agent and incubated at 19°, 25° and 37°C, were resistant. Similarly at both 19° and 37°C, agent did not produce CPE in following tissue and cell cultures: human embryonic intestine (Henle), human adult liver (Chang), MAF—human embryo skin and muscle (Gray), KB—human carcinoma nasopharynx (Eagle), Sarcoma 180—mouse sarcoma (Foley), human heart adult (Girardi), monkey heart (Salk), hamster kidney and monkey kidney. 3. *Infection experiment with 4-week-old fish.* Loss in inoculated and control portions during first 5 days of experiment was about equal. Cause was not determined but was not unusual for such young fish. Starting at 7 days, usual incubation period, inoculated fish exhibited whirling and other abnormal behavior characteristic of IPN. Mortality increased in infected portion, but mortality in controls dropped (Fig. 2). Dead fish from inoculated jars showed symptoms of the disease, and IPN was confirmed by histological section. A filtered inoculum prepared from one such dead fish produced typical and rapid CPE in susceptible tissue culture. An additional feeding of infected food was followed by a second, but slight, peak in mortality. At termination, 23% of inoculated fish and 8.5% of control

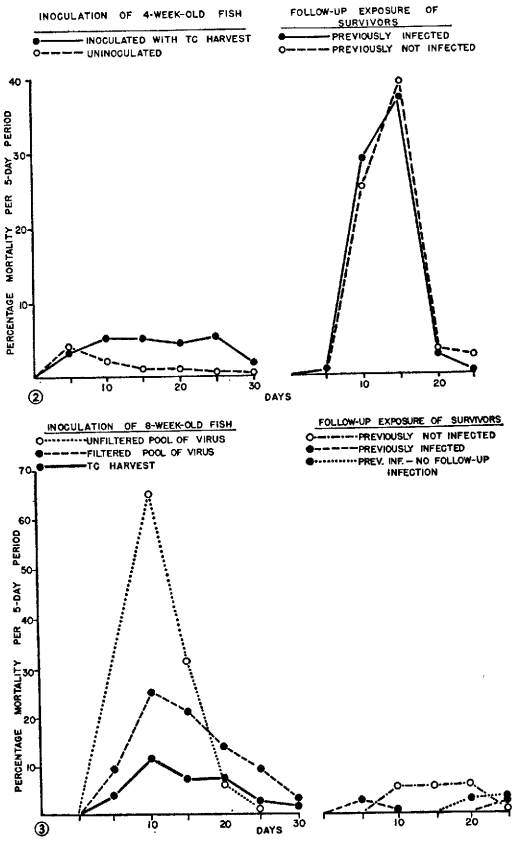


FIG. 2. (Left) Effect of feeding 8th TC transfer of virus of infectious pancreatic necrosis to 4-wk-old eastern brook trout, and (Right) effect of feeding freshly ground victims of an epizootic to the survivors.

FIG. 3. (Left) Effect of feeding 6th TC transfer of virus of infectious pancreatic necrosis, ground victims of the disease, and a bacteria-free filtrate of ground victims to 8-wk-old eastern brook trout. Mortality among uninoculated controls was less than 2%. Its cause was not determined, and curve was not plotted. (Right) Response of some surviving fish to a follow-up feeding of ground victims from 2 epizootics.

fish died. IPN was not found among controls. When given follow-up feeding of infected fish, 57% of controls and 56.5% of previously infected fish died. 4. *Infection experiment with 8-week-old fish.* Initial loss from unknown causes did not occur. Typical IPN behavior was seen in all troughs of infected fish after usual incubation period, and followed by a rapid rise in mortality rate (Fig. 3). At termination, 86% of fish fed unfiltered material died, and loss was 60% among fish fed from filtered portion of this pool. Mortality among TC harvest-fed fish

totalled 30%. IPN was confirmed in all inoculated lots by histological section but could not be found in control fish. Fish were then 13 weeks old. Twenty-five days after the follow-up feedings of infective material 6 of 40 previously uninfected fish had died. Two of 40 formerly infected fish died after the follow-up feedings, but an equal number died in the jars which were not reinfected (Fig. 3).

*Discussion.* The agent of IPN demonstrated a high degree of specificity and produced CPE only in trout tissue. In contrast, Sanders and Soret propagated eastern equine encephalomyelitis in embryonic fish(10). When primary inocula were diluted CPE was variable or even absent at $10^{-6}$. IPN was passaged in TC with concomitant CPE at dilutions of $10^{-11}$ and $10^{-10}$, therefore, *in vitro* propagation of agent must have occurred. Such TC harvests were used to infect fish, but were associated with a low rate of mortality. Lowest mortality resulted from material which had been passaged most. When 2 portions of a virus pool were used to infect fish, the filtrate produced less mortality than the unfiltered portion. Presumably, filtration reduced number of infective particles. If low mortality from TC harvests was due to a low population of infective virus, TC passages could have been mere dilution of original virus. A more likely explanation was that TC passage resulted in virus propagation, but that it was accompanied by alteration. Two facts supported this latter hypothesis. First, through repeated transfer CPE diminished somewhat but could not be a toxicity phenomenon at dilutions used and with specificity shown. Secondly, reisolation of agent from fish infected with TC harvest was accompanied by usual severe CPE. It seemed more likely, therefore, that alteration of virus occurred in TC. Few 13-week-old fish died following infection, confirming reported resistance of older fish(8).

*Summary.* A filterable, heat labile agent has been found in salmonid fishes with presumptively diagnosed and histologically confirmed infectious pancreatic necrosis. In presence of penicillin, streptomycin, and mycostatin, agent produced CPE in cultures of tissues from susceptible host fishes, but not in cultures from a non-host fish or mammals. Isolates of agent were passaged 8 and 9 times, and last dilutions significantly exceeded extinction point noted in original inocula. Sixth and 8th TC passage material was infective for susceptible trout and produced typical disease syndrome. Inocula prepared from such victim fish also evoked characteristic CPE in fish tissue culture. Agent has thus far survived 4½ years storage at –20°C, and 2½ years in 50% glycerol at 4°C. These facts support the proposition that IPN is a virus-caused disease.

The authors are indebted to Dr. R. E. Lennon, S. W. Watson, T. J. Parisot, and R. G. Piper for reviewing the manuscript.

1. Zhdanov, V. M., Opredyelityel Virusov Cheloveka E. Zhivotnik, *Izdatyelistvo Akademii Meditzinskik Nauk SSSR,* Moskva, 1953.

2. Smith, K. M., *The Virus, Life's Enemy,* Cambridge Univ. Press, 1948.

3. Guenther, R. W., Watson, S. W., Rucker, R. R., *U. S. D. I., Fish and Wildlife Service,* Spc. Sci. Rpt. Fish No. 296, 1959.

4. Watson, S. W., *Trans. Am. Fish. Soc.,* 1954, v83, 331.

5. Nigrelli, R. F., *Ann. N. Y. Acad. Sci.,* 1952, v54, 1076.

6. Wood, E. M., Snieszko, S. F., Yasutake, W. T., *A. M. A., Arch. Path.,* 1955, v60, 26.

7. Snieszko, S. F., Wood, E. M., Yasutake, W. T., *ibid.,* 1957, v63, 229.

8. Snieszko, S. F., Wolf, K., Camper, J. E., Pettijohn, L. L., *Trans. Am. Fish. Soc.,* 1959, v88, 289.

9. Wolf, K., Dunbar, C. E., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 455.

10. Sanders, M., Soret, M. G., *Trans. N. Y. Acad. Sci.,* 1954, v17, 19.

Received March 1, 1960. P.S.E.B.M., 1960, v104.