rechromatography in the EdBzf system, #4 appears to lose its persulfate-staining properties. This observed change in staining behaviour has not been noted for the other compounds tested (#2 through #6). There is tentative evidence that the presence of compound #4 in urine may be related to the use of tobacco.

1. Salzman, N. P., Brodie, B. B., *J. Pharmacol. and Exp. Therap.*, 1956, v118, 46.

2. Clare, N. T., *Australian Vet. J.*, 1947, v23, 340.

3. Walkenstein, S. S., Seifter, J., *J. Pharmacol. and Exp. Therap.*, 1959, v125, 283.

4. Ross, J. J., Jr., Young, R. L., Maass, A. R., *Science*, 1958, v128, 1279.

5. Block, R. J., Durrum, E. L., Zweig, G., *Paper Chromatography and Paper Electrophoresis*, 2nd Ed., 1958, p362, Academic Press, Inc., New York.

6. Smith, I., *Chromatographic Techniques*, 1958, Interscience Publishers, Inc., New York.

7. Goldenberg, H., White, D. L., presented before Metropolitan-L. I. Subsection, New York Section, Am. Chem. Soc., 1957.

8. Fishman, V., Goldenberg, H., unpublished data.

9. Lin, T. H., Reynolds, L. W., Rondish, I. M., Van Loon, E. J., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 602.

Received February 23, 1960. P.S.E.B.M., 1960, v104.

Effects of Tetrazotized Diorthoanisidine on Depressor Response to 5-Hydroxytryptamine in the Rabbit. (25742)

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Diazo and tetrazo salts react *in vitro* with 5-hydroxytryptamine (5-HT) to form blue colored azo dyes(1). If similar reactions can occur *in vivo* these salts should block the pharmacological actions of 5-HT unless the coupled reaction by-product retains 5-HT activity. Results of previous studies indicate that the tetrazo salt, tetrazotized diorthoanisidine (TDA) is an adrenergic blocking drug (2). The blue colored complex formed by reacting TDA with 5-HT does not possess 5-HT activity on blood pressure of dogs or rabbits even in doses equivalent to 200 μg of 5-HT. We, therefore, decided to determine whether TDA could block some of the effects of exogenously administered 5-HT. Rabbits were used in all experiments since 5-HT produces a hypotensive response in this species. Other workers have shown that sympatholytic agents can modify the pressor effects of 5-HT but do not significantly alter its depressor action in the rabbit(3).

Methods. Albino rabbits weighing 2-3 kg were used. The technic of Horita(4) utilizing the pyretogenic effects of 5-hydroxytryptophane in animals pretreated with iproniazid was used as an index of central effects of 5-HT. Rectal temperatures were recorded at various time intervals in unanesthetized control animals and in others pretreated with TDA. Blood pressure responses to 5-HT, l-epinephrine and l-norepinephrine were recorded continuously from a carotid artery of atropinized, vagotomized rabbits before and after treatment with TDA. These animals were anesthetized with pentobarbital (25-30 mg/kg). Supplementary doses were given as needed.

Results. Pyretogenic action of 5-HT. Iproniazid was administered subcutaneously in 50 or 100 mg/kg doses. Two to 3 hours later 20 mg/kg of 5-hydroxytryptophane were injected intravenously. Rectal temperatures of rabbits so treated were found to be increased markedly within one hour. According to Horita and Gogerty(4) this response is the resultant action of 5-HT accumulation in brain tissue brought about by monoamine oxidase inhibition with iproniazid. A total of 14 rabbits received iproniazid and 5-hydroxytryptophane treatment. Seven of them also were injected intravenously with 20 mg per kg doses of TDA immediately before or following 5-hydroxytryptophane administration. No apparent differences in rectal temperature

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curves, either in rate of ascent or absolute rise, between the 2 groups were noted. Maximum temperature levels were reached 2-3 hr following 5-hydroxytryptophane administration. Mean levels were 41.2 for animals which did not receive TDA and 41.1 for those treated with TDA. Control values for the 2 groups were 39.1 and 38.8, respectively. All but one of the animals died at or near the peak of temperature rise. Arterial blood samples from animals treated with TDA and 5-hydroxytryptophane were blue-colored probably because of coupling between circulating TDA and 5-hydroxytryptophane with formation of an azo dye. In 4 additional experiments TDA (20 mg/kg) alone had no significant effect on rectal temperatures measured up to 4 hours after administration.

Depressor responses to 5-HT. Effects of TDA on depressor response to intravenous administration of 5-HT were studied in 18 rabbits. Response was partially or completely blocked by 10 mg/kg doses of TDA in 14 animals. Larger doses (20-40 mg/kg) were required to produce significant blockade in the remaining 4 animals. A typical experiment is illustrated in Fig. 1.

Panel A of Fig. 1 records the depressor effect of a 10 μg/kg dose of 5-HT. Pressor responses to a 2 μg/kg dose of epinephrine and a 3 μg/kg dose of norepinephrine are also shown. Panel B illustrates alterations in response induced by a 10 mg/kg dose of TDA given intravenously. Only a slight depressor response was obtained on administration of 5-HT 30 minutes following TDA injection. At this time carotid blood pressure level was only slightly lower than that prior to TDA administration. Pressor responses to epinephrine and norepinephrine could still be obtained (Panel B) but were diminished in magnitude. In most experiments TDA produced a sustained lowering of mean blood pressure ranging from 5-30 mm of Hg. The blocking action usually persisted for duration of an experiment (4-6 hr).

The blockade produced by TDA appeared to be competitive and could be overcome by increasing dose of 5-HT. One of 3 equivalent responses to 20 μg/kg injections of 5-HT is shown in panel A of Fig. 2. Accentuation of

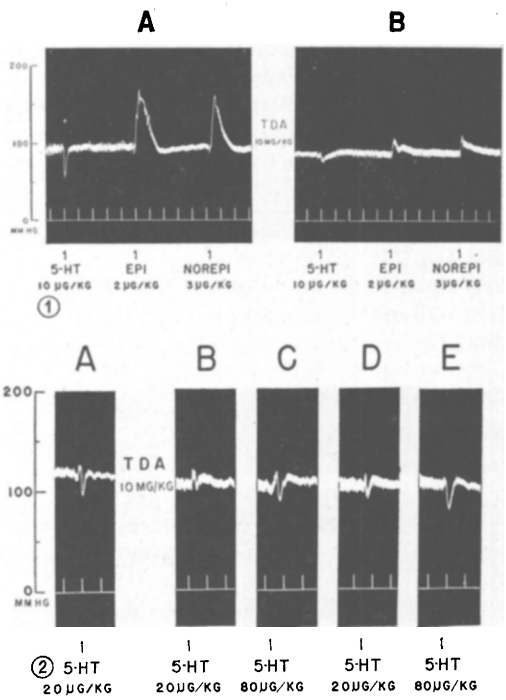

FIG. 1. Blockade of depressor effect of 5-hydroxytryptamine and pressor effects of l-epinephrine and l-norepinephrine by TDA. Time base in min. 35 min. period between A and B.

FIG. 2. Competitive blockade of depressor effects of 5-hydroxytryptamine by TDA. Time base in min. Periods between responses A-B, 1 hr; B-C, 10 min.; C-D, 2 hr; D-E, 10 min.

the initial pressor component and elimination of the depressor effect of 5-HT one hour after TDA administration are shown in panel B. A 4-fold increase in dose 10 minutes later restored depressor component (panel C). Partial antagonism was still evident on administration of the initial 20 μg/kg dose of 5-HT 2 hours after TDA was given (panel D). Readministration of the large dose of 5-HT 10 minutes later again overcame the blockade produced by TDA.

Effects of phenoxybenzamine. Phenoxybenzamine in doses which completely abolished or reversed pressor response to l-epinephrine in 4 rabbits did not significantly affect depressor response to 5-HT.

Discussion. Our data show clearly that TDA can reduce or abolish depressor response to exogenous 5-HT. The receptors involved are not ordinary adrenergic sites since they are not inactivated by phenoxybenzamine and

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)

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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-

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