compound against three viruses was also demonstrated. Cytotoxicity to the cells was observed with 62 μg/ml of the compound after a 120-hour incubation period, but at 31 μg/ml chick embryo cells appeared normal and plaque development by the 3 viruses was completely inhibited. Furthermore when the concentration was reduced to 16 or 8 μg/ml, plaques definitely smaller than those of untreated control were found at 120 hours after the infection, although the number of plaques was not significantly reduced. It is evident from those results that FFB did not reduce the number of infectious centers but affected the size of plaques, probably due to inhibition of the formation of infectious particles. In contrast adamantyl compounds were reported to reduce the number of infectious centers induced by influenza virus(5). No inhibitory activity was observed with 4 μg/ml of FFB when measured by size and number of plaques, and the therapeutic index of the compound was calculated as 8.

With type 1 poliovirus the plaque inhibitory activity of the compound was tested in different host cells, monkey kidney and HeLa cells. The effect of this compound on poliovirus as well as myxoviruses was further tested by tube culture assay which presents air directly to the host-virus system. It is evident that in monkey kidney cells the virus was more sensitive to the compound than in HeLa cells. In both cell species with liquid overlay FFB was markedly less toxic. In chick embryo cells the higher concentration of Bacto-agar induced cytotoxicity, perhaps due to the relative anaerobioses of cells under agar(6).

Summary. A derivative of biguanide, N^1-furfurylbiguanide hydrochloride (FFB), has been found to possess an inhibitory effect in chick embryo fibroblasts or monkey kidney cells on the plaque formation of RNA viruses, such as Newcastle disease virus (NDV), the WSN strain of influenza A virus, vesicular stomatitis virus (VSV) and type 1 poliovirus either by drug-in-agar disc (diffusion) or drug-in-agar overlay (dilution) assay. Cytotoxicity of the compound was reduced when tested in tube cultures instead of plaque assays. Both toxic and effective concentrations of the compound decreased when the amount of Bacto-agar in the maintenance solution was increased, holding the same chemotherapeutic index. The data show that FFB can inhibit selected viruses in suitable host-virus systems and with sensitive methods of detecting virus.

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Received July 8, 1966. P.S.E.B.M., 1966, v123.

An Interferon-Like Viral Inhibitor in Body Fluids of Endotoxin-Injected Rabbits.* (31523)

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Several reports have shown that intravenous injections of Gram-negative bacterial endotoxins induce an interferon-like viral inhibitor in the blood of experimental animals(1,2,3). Recently Oh and Gill(3) reported that such

* Supported by a research grant from Medical Research Council of Canada (MA-1608).

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an inhibitor in the aqueous humor of rabbit eyes played an important role in induction of *in vivo* corneal resistance to Newcastle disease virus (NDV). They showed that intravenously administered typhoid endotoxin released an interferon-like viral inhibitor into the blood stream, and also allowed the passage of the inhibitor from the blood into the aqueous humor of eyes by disrupting the blood-aqueous humor barrier of the iris. They suggested that the passage of the viral inhibitor might occur from the blood to extravascular sites of various organs of endotoxin injected animals. The results presented here show such passage of a viral inhibitor from the blood into the cerebrospinal fluid (CSF), urine and ocular aqueous humor of rabbits following intravenous injection of typhoid endotoxin.

Methods. New Zealand white male rabbits weighing 2.0 to 2.5 kg were used. Under anesthesia with intravenous injection of sodium Nembutal (Abbott), they were injected intravenously with 200 μg of typhoid endotoxin(3) and at the same time bladders were emptied with sterile disposable feeding tubes, size 5 French (Becton, Dickinson & Co., Rutherford, N. J.). At various time intervals, specimens were collected from anesthetized rabbits in the following manners, and the rabbits were discarded: Blood was obtained by cardiac puncture, and the serum was separated after incubating at 37 C for 1 hour. Aqueous humor was aspirated from both eyes by a previously described technique (4). They were pooled for the titration of the inhibitor. CSF was collected by cisternal puncture with a 19-gauge spinal puncture needle, and urine by direct insertion of an 18-gauge hypodermic needle into the bladder. All specimens except for blood were centrifuged at 2000 rpm for 5 minutes immediately after collection, and the supernatant fluids were used for the titration. Aqueous humor, urine or CSF showing gross contamination with blood cells were not used for the study. To titrate the viral inhibitor, specimens were serially diluted in 1 ml of culture medium consisting of Medium 199 (Difco) plus 20% heat-inactivated calf serum (Difco) fortified with penicillin, streptomycin and nystatin.

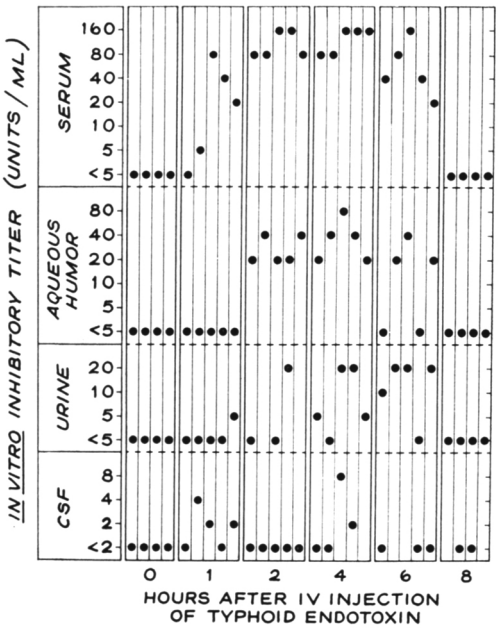

FIG. 1. An interferon-like viral inhibitor in serum, aqueous humor, urine and cerebrospinal fluid (CSF) following intravenous injection of 200 μg of typhoid endotoxin. Each vertical column represents a rabbit. Titer of urine or CSF of some rabbits is not shown due to the failure of obtaining the the specimen or the specimen was contaminated with blood.

They were placed into tubes of primary rabbit kidney cell cultures. Following incubation at 37 C for 20 hours, the cultures were washed twice with 2 ml of phosphate buffered saline (PBS) at pH 7.2 and inoculated with 10 $TCID_{50}$ of the Indiana strain of vesicular stomatitis virus (VSV) in 1 ml of Medium 199, 10% heat-inactivated calf serum and the antibiotics. Cytopathic effect (CPE) was recorded 24 hours later. Inhibitory titers expressed as units/ml were determined as reciprocals of the highest dilutions of specimens which inhibited CPE completely.

Results and discussion. As shown in Fig. 1, aqueous humor, urine, CSF and serum showed inhibitory activity against VSV some time after the injection of typhoid endotoxin. The inhibitor could be detected in serum, urine and CSF as early as 1 hour and in aqueous humor at 2 hours after the endotoxin injection. No inhibitory activity was demonstrated in any specimens obtained after 8 hours. The highest peak titer of inhibitor

(160 units) was detected in the serum at 2, 4 and 6 hours and the lowest peak titer (8 units) in the CSF at 4 hours after the endotoxin injection. At no time was the inhibitory titer of the aqueous humor, urine and spinal fluid higher than that in the serum of the same rabbit. Intravenous injection of pyrogen-free normal saline (Baxter, Alliston, Ontario, Canada), used as a diluent for the endotoxin, did not induce the inhibitor in rabbits. Pretreatment of cells with either 200 μg of typhoid endotoxin, normal rabbit serum or a mixture of both failed to protect the cells from the virus. Normal aqueous humor, CSF or urine had no suppressive effect.

The properties of the inhibitor detected in aqueous humor, urine and CSF were identical to those in serum which were reported previously(3): The inhibitor induced resistance in kidney cells by acting on the cells rather than by inactivating the virus. It was effective in suppressing CPE produced by not only VSV but also NDV, western equine encephalitis and influenza A viruses, and its effect was species specific. The protective effect against VSV was partially abolished by heating it at 56 C for 1 hour and completely abolished by heating at 70 C for 1 hour. Its activity was greatly diminished by keeping it at pH 2.0 or by incubating with crystalline trypsin at a final concentration of 0.01% at 37 C for 1 hour. All inhibitory activity remained in the supernatant fluid after centrifugation at 104,500 × *g* for 3 hours. These properties are similar to those of virus-induced interferon(5) with the exception of acid sensitivity.

The inhibitor in aqueous humor, urine and CSF appears to be derived from the blood since (a) properties of the inhibitor in these specimens are identical to those of the inhibitor in serum, (b) the titer of the inhibitor in any of these specimens is never higher than that in serum of the same rabbit, and (c) the inhibitor in the aqueous humor or urine following injection of endotoxin into the anterior chamber or urinary bladder is either not detectable or present at a negligible degree.

The inhibitor in aqueous humor was shown to originate from the blood following the breakdown of the blood-aqueous humor barrier in ciliary processes of the iris by intravenous injection of typhoid endotoxin(3,4). It is not certain, however, whether the inhibitor in CSF and urine in the endotoxin-injected rabbits is brought from the blood through the same mechanism as above. It should be noted that by the use of colloidal dyes, Eckman *et al*(6) showed the disruption of the blood-brain barrier in rabbits by intravenous injection of *E. coli* endotoxin.

The inhibitor in aqueous humor of endotoxin-injected rabbits was found to play an important role in the induction of *in vivo* corneal resistance to NDV(3). The significance of the presence of interferon-like viral inhibitor in urine and CSF of endotoxin-injected rabbits is not clear. In view of the findings that endotoxin rendered resistance to brains of experimental animals against several viral infections(7,8,9) and also an interferon-like viral inhibitor was detected from the CSF of human meningitis patients with Gram-negative bacteria(10,11), it may be worthwhile to explore the role of interferon-like viral inhibitor present in the CSF and urine in the pathogeneses of viral infections in the brain and urinary tract.

Summary. An interferon-like viral inhibitor in serum, ocular aqueous humor, urine and cerebrospinal fluid (CSF) of rabbits was titrated in primary rabbit kidney cell cultures with vesicular stomatitis virus (VSV) after intravenous injection of 200 μg of typhoid endotoxin. The inhibitor was detected in the serum, urine and CSF as early as 1 hour and in the aqueous humor at 2 hours after the endotoxin injection. No inhibitory activity was demonstrated in any specimen obtained after 8 hours. The highest peak titer of inhibitor was detected in the serum and the lowest in the CSF. The experimental data indicate the passage of the viral inhibitor from the blood into the aqueous humor, urine and CSF of rabbits following intravenous injection of typhoid endotoxin.

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Received July 11, 1966. P.S.E.B.M., 1966, v123.

Influence of Route and Concentration of Ethanol Upon Central Depressant Effect in the Mouse. (31524)

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Hiestand *et al*(1) reported that a fixed dose of ethanol, administered intraperitoneally to mice in increasing concentration, produced an increasing rate of mortality. Similar observations were made by Aston and Cullumbine(2), who found an inverse relationship between the concentration and the LD50 of ethanol solutions administered intraperitoneally to mice. The present report describes the relationship between the concentration of ethanol, administered orally, intraperitoneally and intravenously, and its hypnotic effect in mice, as measured in terms of potentiation of a fixed dose of hexobarbital.

Methods. Male albino mice of the Holtzman strain weighing from 19 to 40 g were housed in stainless steel cages with exterior dimensions of 24 × 19.5 × 19.0 cm in aggregates of approximately 8 per cage. Their diet consisted of Rockland Chow and water. The lighting of the animal quarters was automatically controlled to provide 12 hours of artificial light alternating with 12 hours of darkness. Room temperature was maintained at 74°F. The mice were kept in their quarters at least 3 weeks prior to experimentation to allow adequate environmental adaptation.

Hexobarbital sodium was administered in a dose of 75 mg/kg intraperitoneally *(i.p.)* as a 1% solution which was made isotonic with sodium chloride. Ethanol solutions of 30, 50, 70 and 90% (vol/vol) were made up in water with sodium chloride added to make the solutions isotonic. These solutions were administered by the oral *(p.o.)*, intraperitoneal *(i.p.)* or intravenous *(i.v.)* route in combination with the barbiturate and sleeping times were recorded. All doses of alcohol reported are expressed in terms of absolute ethanol.

The mice were divided into 3 major groups on the basis of the route of ethanol administration. One group received 3 g/kg ethanol *p.o*,. 5 minutes prior to hexobarbital. Another group received 2 g/kg ethanol *i.p.* simultaneously with hexobarbital. A third group received 1.5 g/kg ethanol *i.v. via* the dorsal tail vein, 4 minutes after barbiturate administration. Sleeping times were measured as the time elapsing between the loss of righting reflex and the point at which the anesthetized mice righted themselves.

Statistical procedures employed in this report were those outlined by Burn *et al*(3). The significance of differences between the mean sleeping times was evaluated by application of the 'Student' t-test. The letters S.E. in this paper refer to the standard error of the mean.

Results. The results are given in Table I. The 3 control series, in which hexobarbital was given by the *i.p.* route, provided mean sleeping times of 33.5, 36.0, and 41.5 minutes. These values were not statistically homogeneous, indicating that day-to-day variation in sleeping time was significant. An attempt to reduce this error was made by insuring that all treatments, within one group,