TABLE I. Cardiac Output, Blood Pressure, and Heart Rate of Pekin Ducks.

	Body wt, kg	Cardiac output, ml/min		Blood pressure, mm Hg			Total peripheral resistance units, U/kg	Heart rate, beats/min
		Per bird	Per kg	Systolic	Diastolic	Mean		
				Males (12)				
Mean	3.34	962.9	286.8	178.8	141.8	161.2	.5666	175
S.E.	.066	70.2	17.7	5.0	7.02	7.2	.3827	17.7
				Females (9)				
Mean	3.03	751.4	253.4	168.2	133.9	147.2	.62	185
S.E.	.115	90.3	31.2	9.8	10.7	8.9	.082	17.7
				Sex difference				
t	2.3*	1.9	.9	.9	.6	1.2	.13	.4

* Significant at .05 level.

the difference was not statistically significant. Total peripheral resistance in units per kg averaged 0.56 for males and 0.62 for females and the difference was not significant. It is pointed out that on a body weight basis cardiac output in ducks is higher than in chickens and of about the same magnitude as for turkeys. TPR tends to be lower in ducks than chickens.

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Antiviral Activity of N^1-Furfurylbiguanide Hydrochloride (FFB) *in vitro*. (31522)

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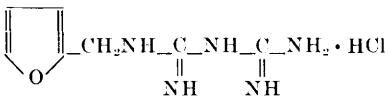
During recent years we have been investigating the antiviral activity of many biguanide derivatives, among which several derivatives have been found to possess activity against some myxoviruses(1,2). The activity of those biguanide derivatives prompted us to investigate the antiviral activity of a new derivative of biguanide, N^1-furfurylbiguanide hydrochloride (FFB), which primarily had been shown to have a marked inhibitory effect on the plaque formation of Newcastle disease virus by the agar diffusion assay. This report describes the activity of FFB chiefly against vesicular stomatitis, polio- and myxoviruses, tested both by the drug-in-agar disc (diffusion) and drug-in-agar overlay (dilution) methods.

Materials and methods. Compound. N^1-furfurylbiguanide hydrochloride (FFB) was kindly prepared and supplied by the Sumitomo Chemical Co., Ltd., Osaka. It is easily



soluble in water and its acute LD_{50} to mice was 254 mg/kg by intraperitoneal injection.

Viruses. The Miyadera strain of Newcastle disease virus (NDV), the WSN strain of

influenza A virus (WSN), the NIH strain of vesicular stomatitis virus (VSV) and the Mahoney strain of type 1 poliovirus were used in these studies. NDV and WSN were maintained by allantoic passage in 11-day chick embryos and titrated by the plaque counting method using chick embryo cells. VSV was grown in 48-hour cultures of chick embryo cells and the titration was carried out by the plaque counting method using chick embryo cells. The Mahoney strain of poliovirus was grown in the S_3 clone of HeLa cell tubes and the titer was expressed as the plaque number on HeLa cells.

Cells. Chick embryo cells were prepared from 11-day embryos by trypsinization according to the method of Dulbecco(3). The cells were grown in a medium consisting of Hanks' solution containing 0.5% lactalbumin hydrolysate and 5% calf serum for 2 days at 37°C.

For primary culture of cynomolgus monkey kidney cells the same medium was used but with 5% bovine serum substituted for the calf serum. The cells were used within 5 to 7 days when a confluent sheet formed.

The S_3 clone of HeLa cells was propagated in the medium consisting of 10% bovine serum and YLE solution (0.5% lactalbumin hydrolysate and 0.1% yeast extract in Earle's balanced salt solution with 0.45% glucose) and was used 3 to 4 days after subculture.

Media. The maintenance medium for chick embryo and monkey kidney cells was Earle's solution without serum modified to contain 0.5% lactalbumin hydrolysate. For the S_3 clone of HeLa cells, YLE solution containing 2% heat-inactivated horse serum was used. For studies on plaque formation, 1% Difco Bacto-agar and neutral red (1:20,000) were added to YLE solution supplemented with 2% heat-inactivated horse serum.

Plaque inhibition assays. Two plaque inhibition techniques were employed in these experiments. One was the drug-in-agar disc (diffusion) method, details of which have been already described(4). After a 2- to 3-day incubation period, cell monolayers in 2-ounce prescription bottles were inoculated with approximately 10^3 plaque-forming units (PFU) of test virus per bottle and returned to the 37°C incubator for a 90-minute virus adsorption period. Unadsorbed virus was then removed by washing twice with Hanks' solution. Cell layers were covered with 4 ml of agar medium and a drug-containing agar disc (10 mm in diameter, 3 mm in thickness) was placed on the agar overlay at the center of the bottle. After a 72-hour incubation period at 37°C, definite plaques appeared in normal culture, but with the drug-containing disc a distinct halo of deeply stained, living cells free of plaques was produced around the disc. The cytotoxic zone due to the compound could be easily distinguished from its inhibitory zone, because the latter was more deeply stained than the former.

The other method of plaque inhibition was the drug-in-agar overlay (dilution) one. Up to infection, this procedure was exactly the same as that of the drug-in-agar disc method. After unadsorbed virus was removed, agar overlay containing the compound in different concentrations was added and plaque development was observed at 72 and 120 hours after infection.

Tube culture assay. Two-hour pretreated and control cells in culture tubes were inoculated with viruses at a multiplicity of 2:1 to 20:1. After a virus adsorption period, unadsorbed virus was then removed by washing 3 times and the treated cell layers were covered with the compound-containing medium while the control cell layers were overlaid with

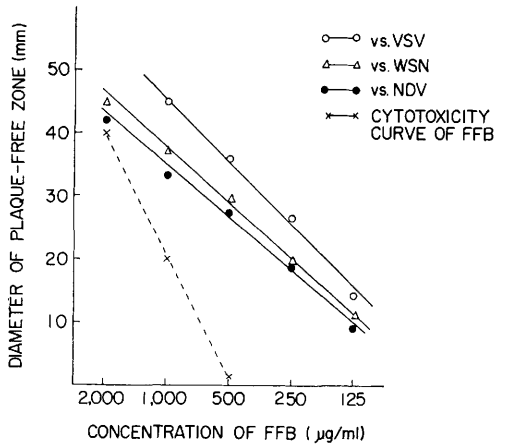
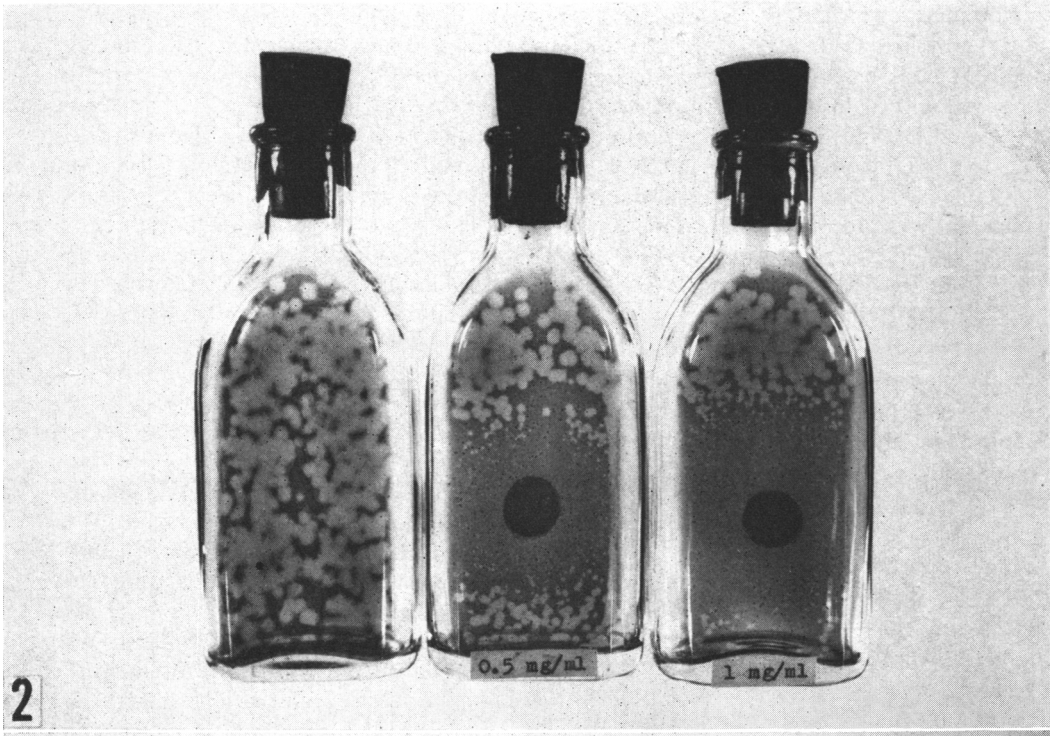


FIG. 1. Inhibitory effect of FFB* on plaque formation of myxoviruses obtained by diffusion assay in chick embryo cells.

* N^1-furfurylbiguanide hydrochloride.

regular maintenance medium. The infected cells were scraped in the medium at 2-hour intervals, and subjected to 3 cycles of freezing and thawing. Virus yield was measured by plaque-forming technique.

Results. Plaque inhibitory effect obtained



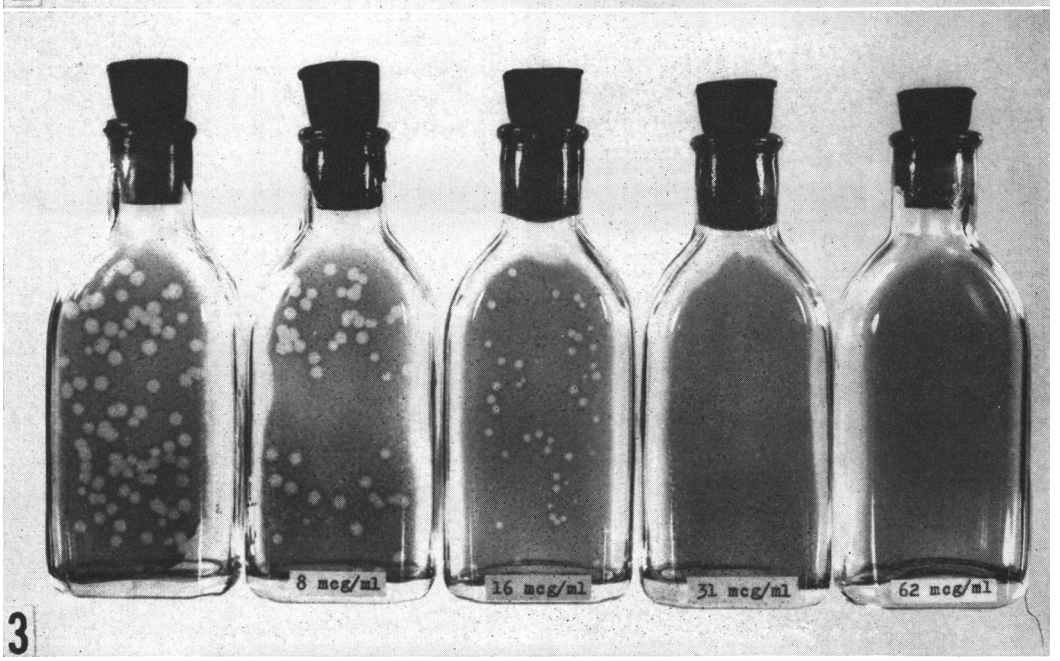


FIG. 2. Plaque inhibitory effect of FFB on VSV by the drug-in-agar disc assay.
FIG. 3. Plaque inhibitory effect of FFB on VSV by the drug-in-agar overlay assay.

TABLE I. Inhibitory Effect of FFB on Plaque Formation of Myxoviruses Obtained by Dilution Assay.

Dose of FFB (μg/ml)	NDV				WSN				VSV			
	No. of plaques		Size (mm) of plaques		No. of plaques		Size (mm) of plaques		No. of plaques		Size (mm) of plaques	
	72 hr	120 hr	72 hr	120 hr	72 hr	120 hr	72 hr	120 hr	72 hr	120 hr	72 hr	120 hr
62*	0	0			0	0			0	0		
31	0	0			0	0			0	0		
16	138	154	1	<1	0	83		<1	28	34	2	3
8	159	166	1–2	1–2	84	112	2	1–3	33	33	3–4	5–6
0	160	176	3	3	85	138	2–3	2–4	44	44	5	7

* Minimum toxic dose to chick embryo cells.

by the drug-in-agar disc assay. For this work, NDV, WSN and VSV infections in chick embryo cells were used. When the agar disc containing FFB was placed on the agar overlay in different concentrations, the results shown in Fig. 1 were obtained. When a disc containing 2 mg/ml of the compound was used for NDV, a plaque-free zone 42 mm in diameter was observed, whereas the cytotoxic zone was 40 mm. With 500 μg/ml of the compound a plaque-free zone, 28 mm in diameter, was obtained, without any toxic zone. Such a complete inhibition with no cytotoxicity by FFB was always obtained in a reproducible manner and the diameter of each plaque-free zone was not reduced during a 10-day observation period. The plaque inhibitory effect on VSV is shown in Fig. 2. Results on either WSN or VSV were almost the same as those in Fig. 1.

Plaque inhibitory effect by the drug-in-agar overlay. To obtain the critical concentration necessary for virus inhibition, the compound was dissolved in maintenance medium in 2-fold dilutions in the agar overlay. The results obtained with NDV, WSN and VSV are given in Table I. A slight cytotoxic effect of FFB was observed at 62 μg/ml, but at 31 μg/ml chick embryo cells appeared normal and plaque development by NDV was completely inhibited. As shown in Table I and Fig. 3, the compound contained in agar medium in concentrations of 16 and 8 μg/ml presented definitely smaller plaques in size than those of the untreated control at 72 or 120 hours after virus, whereas the number of plaques was not significantly reduced. With WSN and VSV, almost the same results were obtained.

Tube dilution tests. The viruses sensitive to the compound in tissue culture tubes so far tested are NDV, WSN, VSV, and type 1 poliovirus. In tube cultures, 2000 μg/ml of the compound definitely showed cytotoxicity to such cells as chick embryo, HeLa and monkey kidney during the first 20 hours, whereas no toxicity was observed with 1000 μg/ml. As shown in Fig. 4, it is evident that 1000 μg/ml of FFB had the inhibitory effect on the growth of VSV in chick embryo cells, reducing the virus yield per tube to 1/20 of the control. When the fact that the number of infectious centers is not reduced in the presence of the compound (Table I) is taken into consideration, the observation may lead to a conclusion that virus yield per cell was reduced to 1/20 of the control. The degree of susceptibility of the viruses to the compound varied. VSV and WSN were more susceptible to the compound than NDV in the same chick

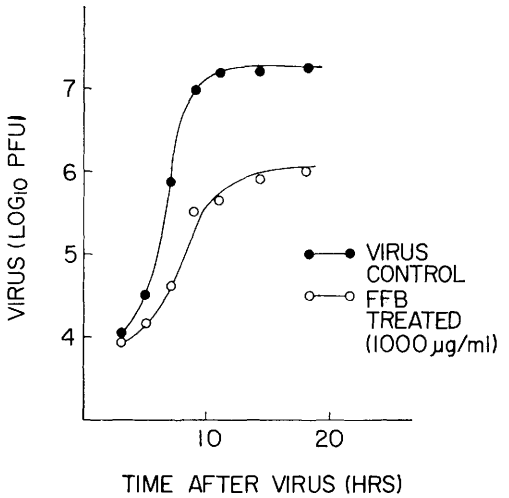


FIG. 4. Inhibitory activity of FFB *in vitro** against multiplication of VSV.
* Chick embryo cells.

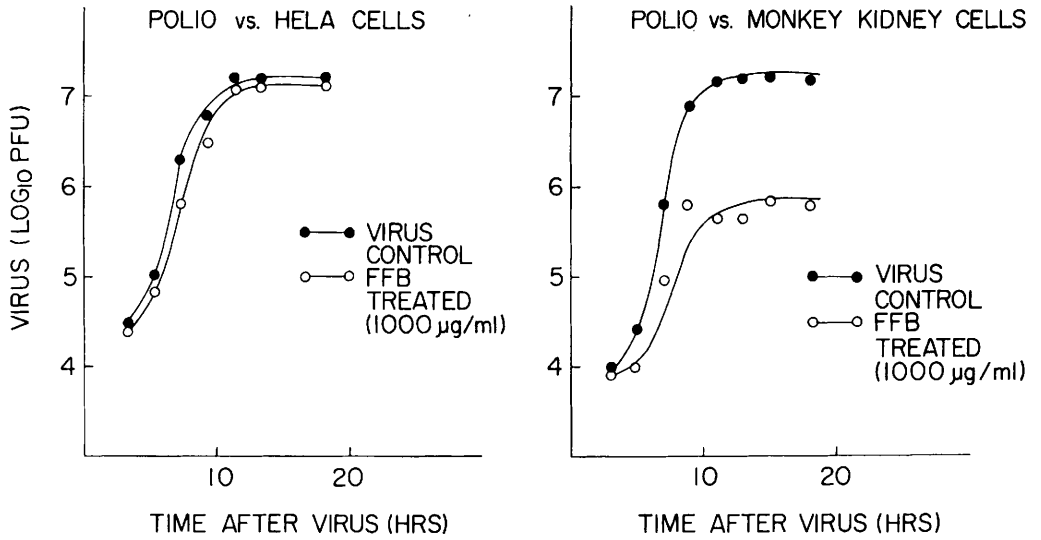


FIG. 5. Inhibitory activity of FFB *in vitro* against multiplication of poliovirus.

embryo cells, and a therapeutic index, 8, was obtained for VSV.

With type 1 poliovirus, two kinds of host cells such as monkey kidney and the S_3 clone of HeLa cells were infected. Virus yield was measured by plaque forming technique using the S_3 clone of HeLa cells. Fig. 5 shows the plaque inhibitory activity of the compound against poliovirus in different host cells. There was a big difference in sensitivity of poliovirus to the compound when different host cells were used, *i.e.*, in monkey kidney cells the virus was more sensitive to the compound than in HeLa cells. In this test system no cytotoxicity was observed during the first 20 hours even with 1000 μg/ml of FFB in contrast to the toxic effect of 62 μg/ml under agar. However, the same chemotherapeutic index was obtained.

To explain the difference in both cytotoxic and effective antiviral levels of the compound in different test systems the effect of Bacto-agar on the cytotoxicity of FFB was examined in chick embryo cells. Three-day-old cell monolayers in prescription bottles were covered with 5 ml of agar overlay containing various concentrations of the compound. After a 48-hour incubation, the cytotoxic levels were determined by neutral red staining. As shown in Table II, even 500 μg/ml of the compound showed no toxicity in chick embryo cells covered with YLE or LE medium containing 0.25% or less of Bacto-agar, whereas cytotoxicity was observed with 125 μg/ml in YLE medium containing 1% or 0.5% Bacto-agar, and with 500 μg/ml in LE medium.

Discussion. N^1-furfurylbiguanide hydrochloride attracted attention because of its marked inhibitory effect at the concentration of 100 μg/ml on the plaque forming of NDV in chick embryo cells by diffusion assay. This ability of the compound prompted further testing of its antiviral activity against other selected viruses which could be titrated in a quantitative manner, such as vesicular stomatitis virus and the WSN strain of influenza A virus. These two viruses, particularly VSV, eventually were shown to be more sensitive to the compound than NDV by diffusion assay using the same chick embryo cells.

By drug-in-agar overlay using chick embryo cells, plaque inhibitory activity of the

TABLE II. Effect of Bacto-Agar on Appearance of Cytotoxicity by FFB in Chick Embryo Cells.

Medium	Dose of FFB (μg/ml)	Agar concentration in medium (%) 1.0	.5	.25	.12	0
YLE	500	C.T.*	C.T.	—	—	—
	125	C.T.	C.T.	—	—	—
	0	—	—	—	—	—
LE	500	C.T.	C.T.	—	—	—
	0	—	—	—	—	—

* C.T. = cytotoxic.

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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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

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