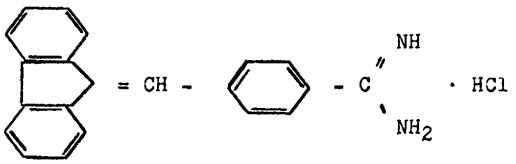
MER-27, a Suppressant of Non-Bacterial Pneumonia in Mice. (24672)

KATHERINE A. LUDWIG, ISAAC RUCHMAN AND F. JOSEPH MURRAY
Dept. of Microbiology, Wm. S. Merrell Co., Cincinnati, O.

Suppression of non-bacterial pneumonia in mice has been accomplished by use of Xerosin, a crude bacterial filtrate(1). This extract arrested lung consolidation caused by viruses (1-4) and *E. coli* endotoxin(5). In our research program we uncovered a synthetic compound, MER-27, which also arrests lung consolidation caused by viruses or endotoxin.

Materials and methods. MER-27 is chemically 9-(*p*-guanylbenzal)-fluorene hydrochloride.



The drug is insoluble in water and suspensions were made with the aid of a small amount of Triton.* *Xerosin.* This was prepared from culture filtrates of *Achromobacter xerosis* n. sp. and was kindly supplied by Dr. Vincent Groupé. Desired concentrations were prepared in water. *Induction of Pneumonia.* a. *Influenza (PR8) Virus.* For viral inoculation 0.05 ml of 10^{-4} dilution of frozen mouse lung suspension containing 10,000 ID_{50} were instilled intranasally into mice under light ether anesthesia. Three days later the mice were killed and at autopsy the degree of consolidation determined, according to the method of Horsfall(6). Values of 0-4+ were given to each lung. Average lesion score of treated mice/average lesion score of untreated controls x 100 yielded the index. Values of 50 or less indicated activity. The lower the figure the greater the activity. b. *E. coli Endotoxin (LPS).* Mice were inoculated intranasally as above with 0.1 mg of LPS† and degree of consolidation determined at 3 days. *Swiss mice* of either sex weighing between 10 and 12 g were used for virus inoculation and those weighing between 18 and 20 g were used for LPS inoculation. *Treatment.* Unless mentioned otherwise, aqueous suspensions of drug were administered subcutaneously once a day for 3 days. One hour after first dose, mice received either the influenza virus or LPS. Animals were sacrificed on third day after infection and degree of consolidation compared with that of untreated controls.

Results. Effects of MER-27 on Lung Consolidation Induced by Influenza Virus. a. *Consolidation.* Results in Table I show that at maximum tolerated dose of 50 mg/kg for MER-27 and 100 mg/kg of Xerosin both compounds were equally effective in suppressing pneumonia.

b. *Effectiveness of MER-27 by Various Routes of Inoculation.* Having established that MER-27 was active in suppressing viral pneumonia, the next experiments were concerned with dose-effect relations and route of inoculation (Table II). Confirmation was readily obtained that subcutaneous treatment of mice with MER-27 arrested development of pneumonia. Inhibition was demonstrated with daily doses of 20 mg/kg or more, with borderline activity at 10 mg/kg. Activity was exhibited by the peritoneal route only when the maximum tolerated dose of 20 mg/kg was given. The finding that MER-27 was effective by oral route was of interest. A dose of 100 mg/kg was active and at the 50 mg/kg level there was still some activity. Xerosin was not active orally.

c. *Failure of MER-27 to Influence Viral Multiplication.* It has already been shown that Xerosin interferes with production of pulmonary consolidation without having any effect on multiplication of the virus. Experiments indicated that MER-27 acts in a similar manner. Mice were infected intranasally with serial 10-fold dilutions of virus prepared in phosphate buffer with pH 7.2. One group of mice was treated with MER-27 (40 mg/kg subcutaneously) prior to infec-

* Winthrop Labs. WR-1339.

† *E. coli* lipopolysaccharide from Difco Labs.

TABLE I. Effects of MER-27 and Xerosin on Influenzal Pneumonia in Mice.

Drug	mg/kg*	Degree of lung consolidation†																Avg score	Index‡
MER-27	50	3	2	2	1	1	1	1	1	0	0	0	0	0	0	0	0	.7	26
Controls		4	4	4	3	3	3	3	3	3	3	2	2	2	2	1	1	2.6	
Xerosin	100	2	1	1	1	1	1	1	1	0	0	0	0					.8	26
Controls		4	3	3	3	3	3	3	2	2	2	2	1					3.1	

* Daily for 3 days subcut.
† 0-4+ indicates degree of lung involvement.
‡ Index = Avg treated/avg control × 100.

tion, followed by MER-27 inoculations at 24 hours and again at 48 hours. Another group was untreated. Results of titrations in treated and untreated groups showed MER-27 was without effect on the LD_{50} titers of challenged mice. A more definitive study of comparative titrations in treated and untreated mice was instituted. Serial dilutions of virus were instilled intranasally as before. Treated mice were given 5 doses of drug subcutaneously according to following schedule: First dose one hour prior to infection, subsequent doses 24, 30, 48 and 54 hours later. MER-27 at dosage levels employed exerted little effect on LD_{50} titers which were recorded daily. Additionally, at conclusion of test, surviving mice were sacrificed at ninth or eleventh day and degree of lung consolidation calculated to yield ID_{50} (Infective Dose) titers. Again significant differences were not observed, thus indicating that without any effect on virus multiplication MER-27 could still substantially interfere with lung consolidation.

Effects of MER-27 on Lung Consolidation

TABLE II. Effects of MER-27 on Influenzal Mouse Lung Consolidation.

Route	Dose,* mg/kg	Avg score Treated	Control	Index
Subcut.	5	2.0	2.8	71
	10	1.6	"	57
	20	.9	"	33
	40	.5	2.4	21
	50	.8	2.5	32
Intraper.	2.5	2.4	2.5	96
	5	2.0	"	80
	10	1.5	"	60
	20	1.3	"	52
Oral	12.5	2.0	2.3	87
	25	2.1	"	91
	50	1.3	"	57
	100	1.0	"	43

* Daily for 3 days.

Induced by LPS. Groupé has shown that LPS administered intranasally produces in mice an inflammatory response in lungs similar to that seen after influenza virus inoculation(5). This type of response was modified by treatment with Xerosin. Results of tests in Table III show that MER-27 suppressed

TABLE III. Effect of MER-27 on Chemically (LPS*) Induced Pneumonia in Mice.

Treatment†		Avg score‡	Index
MER-27	50 mg/kg	.6	25
Controls		2.4	
MER-27	100 "	.4	19
Controls		2.1	

* LPS—10 mg/kg intranasally.
† Treatment—MER-27 subcut. for 3 days.
‡ Score—determined on 3rd day.

pneumonia induced by LPS.

Discussion. Suppression of viral lung lesions in mice has been demonstrated following administration of Xerosin, a crude bacterial filtrate(1). Further work showed that Xerosin modified lung lesions which were induced by inoculation of certain chemicals(4,5). Because of its action in suppressing edema, hemorrhage and cellular infiltration in lungs, Xerosin was properly classified as an anti-inflammatory agent. In its action Xerosin closely resembled Cortisone. A comparison between Xerosin and Cortisone showed that although both compounds were effective anti-inflammatory agents, certain differences emerged(5). Xerosin was able to suppress virally and chemically induced pneumonia; Cortisone was effective only against chemically induced pneumonia. Xerosin could modify influenza - precipitated neurotoxicity, whereas Cortisone could not. Another difference is the known ability of Cortisone to enhance infection, whereas Xerosin was unable to do so.

In a search for a chemically defined compound with Xerosin-like activity various synthetics with anti-inflammatory activity were evaluated. MER-27 showed most promise and was extensively investigated. Its activity in suppressing pneumonia more closely paralleled that of Xerosin. However, certain advantages over Xerosin stood out: 1. MER-27 was effective by oral route; Xerosin was not. 2. Xerosin introduced into lungs caused pneumonia which could be arrested by MER-27 or Xerosin itself. MER-27 was unable to cause lung consolidation. 3. MER-27 was a synthetic compound. A recent report(5) that a derived pyrimido-pyrimidine was active in suppressing pneumonia was not borne out in our tests. At most, this compound showed only a borderline activity which according to our criteria would have been regarded as negative.

Our results demonstrate ability of MER-27 to suppress non-bacterial pneumonia in mice. Other activities of this compound will be reported.

Summary. An organic synthetic compound, MER-27, suppresses pneumonia in mice induced by influenza virus when given parenterally or orally. The compound has no effect on viral multiplication. MER-27 also suppresses pneumonia induced by *Escherichia coli* endotoxin. MER-27 does not enhance viral infection and therefore closely resembles Xerosin in its action.

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Nitrous Oxide Solubility in Fetal and Uterine Tissues in Human Pregnancy.* (24673)

N. S. ASSALI AND M. ROSS
Dept. of Obstetrics and Gynecology, University of California Medical Center, Los Angeles

The formula $\left(CBf = \frac{V_u S}{\int_{t_0}^{t} (A-V)\,dt} \right)$ devised by Kety and his co-workers(1) to calculate cerebral blood flow contains in its numerator a factor (S) which refers to the ratio of N^2O dissolved per g of brain to that dissolved per cc of blood at a constant N^2O tension and at 37°. In adopting this formula for measurement of uterine blood flow in human pregnancy(2), the factor S was taken empirically as equal to unity, a value which had been found experimentally for the brain. Others(3) have determined this factor on homogenates of a whole fetus but the age and condition of the fetus were not given and the values for each type of tissue were not known. Because of the variety of tissues contained in a pregnant uterus, it was deemed essential to determine this factor for each individual fetal tissue as well as for the myometrium and placenta. In this way, not only the N^2O technic for measuring blood flow to the pregnant uterus would be better substantiated but also solubility of N^2O in the various fetal tissues would be known.

Method. Samples of fetal tissues varying in weight from 2 to 10 g were obtained immediately after death from one 5 months immature fetus (therapeutic abortion), one 8 months premature fetus and one full term fetus. The latter 2 fetuses died shortly after delivery for no obvious reasons. The tissues studied are listed in Table I. Samples of myometrial tissue were obtained from patients undergoing cesarean section and placental tissues were collected from spontaneously delivered pregnancies. Each sample weighed accurately, homogenized, equilibrated

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