# COMPARISON OF MOUSE-PROTECTIVE TESTS AND CHICK-PROTECTIVE TESTS OF OVER 600 CHEMICALS AGAINST PASTEURELLA MULTOCIDA

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McKenzie and co-workers (1948) have reported on the results of screening a number of chemicals by *in vitro* methods for possible activity against gram-negative bacteria including *Pasteurella multocida*. The present report concerns itself with the testing of selected chemicals for possible *in vivo* activity against *P. multocida*. Both mice and chicks were used as test animals. Mice were used in testing 650 chemicals against *P. multocida*, type 2 and baby chicks were used in testing 624 chemicals against *P. multocida*, type 1.

The use of chicks was suggested by the fact that Fowl Cholera caused by *P. multocida*, is a disease of considerable economic importance to the poultry industry. In our tests we have used a pure strain of New Hampshire Red chicks furnished when one day old by a local hatchery. The scope of our experiments was such that we used 800 to 1000 chicks a week for five months. The chicks were maintained in electrically-heated Jamesway brooders subdivided by tin sections to afford compartments for groups of 10 to 30 birds. Chicks make attractive test-animals because they are of usable size and weight (30–40 grams) when only one day old, and their upright posture greatly simplifies the technical maneuvers required in making injections.

The diseases of large animals caused by *P. multocida* include: swine plague, shipping fever of cattle, and hemorrhagic septicemia of sheep and horses. Two different strains of *P. multocida* were employed in our tests. They were identified serologically as type 1 and type 2 by the method of Little and Lyon (1943). The strain (type 1) used in chicks was isolated from the disease, fowl cholera, and the strain (type 2) used in mice, from the disease, hemorrhagic septicemia of bison. The strains were carried on blood agar. Inoculum for animals was grown in yeast extract broth and standardized by photoelectric determinations of density.

In our mouse-protective tests, 18–20 gram mice were infected by peritoneum with 0.5 ml of a $10^{-4}$ dilution of six-hour broth culture. An hour later, the mice received doses of 0.5 mg of chemicals by peritoneum; treatment consisted of a series of six injections at four hour intervals. Doses of 0.2 mg were used for retests. Untreated mice died in 20–30 hours. For positive controls, 2-sulfanilamidopyrimidine, was used and in these experiments we obtained 95 per cent protection.

In our chick-protective tests, we used 1–3 day old birds. The chicks were infected intraperitoneally with 0.5 ml of a $10^{-2}$ dilution of six-hour broth culture. An hour later, the chicks received doses of 0.5 mg of chemicals intraäbdominally,

treatment consisted of a series of three injections of 2.0 mg doses at intervals of three hours. Two sets of positive controls were used, one group treated with 2-sulfanilamido-5-chloropyrimidine, and the other treated with 2-sulfanilamido-4,6-dimethyl pyrimidine and, these compounds consistently protected well in these tests. All untreated chicks died.

TABLE 1

*Chemicals Found Active Against* Pasteurella multocida *in Both Mice and Chicks*

| CHEMICAL NAME | RESULTS OF MOUSE TESTS | | | RESULTS OF CHICK TESTS | | |
|---|---|---|---|---|---|---|
| | *P. multocida*, TYPE 2 HEMORRHAGIC SEPTICEMIA | | | *P. Multocida*, TYPE 1 FOWL CHOLERA | | |
| | Total Tests | Total Mice | Per cent Protected | Total Tests | Total Chicks | Per cent Protected |
| 2-Sulfanilamidopyrimidine | 11 | 110 | 100 | 2 | 20 | 95 |
| 2-Sulfanilamido-4-methylpyrimidine | 4 | 40 | 90 | 2 | 20 | 90 |
| 2-Sulfanilamido-4,6-dimethylpyrimidine | 12 | 95 | 98 | 5 | 40 | 86 |
| 2-Sulfanilamido-4,5,6-trimethylpyrimidine | 2 | 20 | 75 | 1 | 10 | 60 |
| 2-Sulfanilamido-5-bromopyrimidine | 3 | 30 | 77 | 2 | 20 | 100 |
| 2-Sulfanilamido-5-chloropyrimidine | 3 | 25 | 72 | 4 | 40 | 100 |
| 2-Sulfanilamido-4-methoxypyrimidine | 2 | 20 | 50 | 1 | 10 | 80 |
| 2-Sulfanilamido-4-ethoxypyrimidine | 3 | 25 | 28 | 1 | 10 | 30 |
| 2-Sulfanilamido-5-chloro-4-methoxypyrimidine | 2 | 20 | 0 | 1 | 10 | 80 |
| 2-Chloro-$N^1$-(2-pyrimidyl)-sulfanilamide | 2 | 20 | 70 | 1 | 10 | 50 |
| 2-Sulfanilamidopyrazine | 3 | 30 | 90 | 1 | 10 | 90 |
| 2-Sulfanilamido-5-chloropyrazine | 2 | 20 | 75 | 1 | 10 | 90 |
| 2-Sulfanilamido-6-chloropyrazine | 4 | 40 | 82 | 1 | 10 | 90 |
| 5-Sulfanilamido-2-bromopyridine | 2 | 20 | 80 | 1 | 10 | 70 |
| 5-Sulfanilamido-2-chloropyridine | 2 | 20 | 90 | 1 | 10 | 80 |
| 2-Sulfanilamido-5-nitropyridine | 3 | 30 | 70 | 1 | 10 | 90 |
| 2-Sulfanilamidothiazole | 3 | 30 | 16 | 2 | 20 | 70 |
| 2-Sulfanilamido-4-tertiary butylthiazole | 6 | 55 | 100 | 2 | 20 | 50 |
| 2-Sulfanilamido-benzothiazole | 2 | 20 | 50 | 1 | 10 | 60 |
| $N^1$-(2,5-Dimethylbenzoyl)-sulfanilamide | 3 | 25 | 25 | 1 | 10 | 40 |
| $N^1$-(3,4-Dimethylbenzoyl)-sulfanilamide | 4 | 35 | 48 | 1 | 10 | 40 |

Amounts of Chemicals given were as follows:

Mouse Tests: A series of six injections of 0.5 mg doses (Retests: 0.2 mg doses) at intervals of four hours.

Chick Tests: A series of five injections of 1.0 mg doses at intervals of three hours.

Amounts of culture given were as follows:

Mouse Tests: 0.5 ml of 10–4 dilution of 6 hour broth, in peritoneum, one hour before treatment.

Chick Tests: 0.3 ml of whole 6 hour broth, by mouth, one hour before treatment.

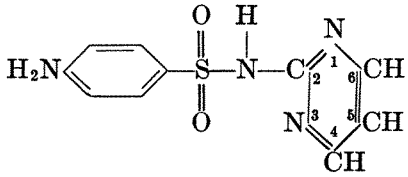
Since the results of the mouse-protective tests indicated that a group of sulfonamides would protect animals infected with *P. multocida* intraäbdominally, in the latter experiment it was thought desirable to test the ability of these same sulfonamides to protect chicks infected by mouth. In these tests, the chicks were infected by mouth with 0.3 ml of whole six-hour broth culture. Oral

inoculation caused death in 20–30 hours. Mortality was nearly, but not always 100 per cent. Treatment consisted of a series of five injections of 1.0 mg doses of sulfonamide at intervals of three hours. The results of these tests paralleled the results of the mouse-protective tests, as shown in table 1.

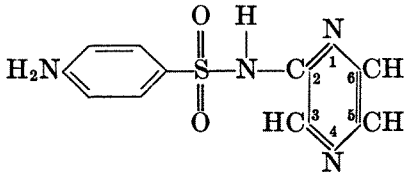
The chemicals were weighed in such amounts that doses of 0.5, 1.0, and 2.0 mg were obtained in 0.5 ml volumes which was the standard volume injected into the peritoneal cavity of the animals. Distilled water was used as the diluent where possible. All of the sulfonamides dissolved readily in N/100 sodium hydroxide. A few highly insoluble chemicals formed suspensions suitable for injections.

## EXPERIMENTAL RESULTS

Mouse-protective tests have been used to determine possible *in vivo* activity for a total of 650 chemicals listed in the results of *in vitro* tests against *Pasteurella multocida* in table 3 of McKenzie's report (1948). Chick-protective tests have been used with 624 of the chemicals. The chemicals found active in both tests are shown in table 1. They are all recently developed sulfonamides. Nine of



2—Sulfanilamidopyrimidine



2—Sulfanilamidopyrazine

the active compounds are derivatives of 2-sulfanilamidopyrimidine; two are derivatives of 2-sulfanilamidopyrazine; two are derivatives of 5-sulfanilamidopyridine; one is a derivative of 2-sulfanilamido pyridine; two are derivatives of 2-sulfanilamidothiazole; and two are derivatives of benzoyl sulfanilamide. The chemicals showing superior activity were 2-sulfanilamidopyrimidine, 2-sulfanilamidopyrazine, and their heterocyclic substituted chloro-, bromo-, and methyl compounds. The compounds listed in table 3 showed similar activity in both mice and chicks, although the infections were caused by two serologically different types of *P. multocida*.

The following four sulfonamides were found active in mouse-protective tests: 2-sulfanilamido-5-bromo-4,6-dimethylpyrimidine (100% of 10 mice); 2-sulfanilamido-5-bromo-4-aminopyrimidine (100% of 10 mice); 2-sulfanilamido-3-

chloropyrazine (80% of 10 mice); and 2-sulfanilamido-5-aminopyridine (55% of 20 mice). Since they were available in small quantities, they were not tested in chicks. The total number of sulfonamides found active against *P. multocida* was twenty-five.

None of the 650 chemicals other than the sulfonamides showed activity *in vivo*. The fact that only certain sulfonamides were found active against *P. multocida* explains the once common belief that sulfonamides are ineffective against infections caused by gram negative organisms. The twenty-five sulfonamides listed in this report because of their activity against *P. multocida* were selected from tests of four nuclear substituted sulfonamides in mice and three in chicks; thirty-four $N^1$ substituted, acylic sulfonamides in mice and thirty in chicks; fifty-four $N^1$ substituted, isocyclic sulfonamides in mice and forty-nine in chicks; forty-four $N^1$ substituted, heterocyclic sulfonamides in mice and forty-two in chicks; four $N^4$ substituted sulfonamides in mice and three in chicks; seven $N^1$, $N^4$ substituted sulfonamides in mice and seven in chicks; eight sulfanilyl-derivatives in mice and eight in chicks. All of these compounds are listed in table 3 of McKenzie's report (1948).

The results of McKenzie's tests of 650 compounds for *in vitro* activity against *P. multocida* show that this organism is sensitive to many guanidines, phenols, sulfones, organic acids and dyes, as well as to the sulfonamides listed here. It is of interest that none of these compounds showed *in vivo* activity in the absence of *in vitro* activity and that of the compounds showing *in vitro* activity only the above mentioned sulfonamides were protective in animals.

## SUMMARY AND CONCLUSIONS

Mouse-protective tests and chick-protective tests have been used to determine possible *in vivo* activity for over 600 chemicals tested for *in vitro* activity against *P. multocida* by McKenzie and co-workers (1948). None of the chemicals other than the sulfonamides showed *in vivo* activity against *Pasteurella multocida*. The chemicals showing superior activity were 2-sulfanilamidopyrimidine, 2-sulfanilamidopyrazine and their chloro-, bromo-, and methyl derivatives.

## REFERENCES

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