Fig. II presents graphically the data obtained from following the rate of disappearance of alcohol alone and following the administration of a 10% solution of sodium pyruvate intravenously in 0.5 g per kilogram doses. It will be noted that 3 doses of sodium pyruvate intravenously over a period of 4 to 5 hours had no influence on the rate of metabolism of ethyl alcohol. The results shown in these two graphs are typical of those obtained with similar studies on 4 other dogs.

It is suggested that the results of Westerfeld, Stotz and Berg, which appear to indicate that pyruvate increases the rate of metabolism of ethyl alcohol, are due to the fact that absorption of alcohol from the stomach was not complete during the control period as was evidenced by the unusually low values for blood alcohol; and that following pyruvate administration, which may have retarded further absorption of alcohol by its hypertonicity, the true normal rate of alcohol disappearance was obtained. This is supported by the fact that the rate of disappearance of alcohol reported by these authors following pyruvate is almost identical with the rate of disappearance we have observed in our controls—*i.e.*, about 20 mg % per hour.

It is believed from our experience that the high levels of blood pyruvate reported by Westerfeld, Stotz, and Berg following sodium pyruvate by stomach tube may have been due to the struggling and other activity of the dogs. The low levels of blood pyruvate which they reported after ingestion of alcohol may, therefore, have been due to the decreased activity of the dogs as a result of the sedative action of alcohol and not to the increased utilization of the pyruvate in the metabolism of the alcohol as is thought by these workers.

Conclusions. Studies on 6 dogs show that the administration of sodium pyruvate by stomach tube or intravenously has no influence on the rate of metabolism of ethyl alcohol.

14372

The Action of Antibiotics on Organisms Producing Gas Gangrene.

CLARA M. MCKEE, DOROTHY M. HAMRE, AND GEOFFREY RAKE.
From the Squibb Institute for Medical Research, New Brunswick, N.J.

The bacteriostatic action of penicillin on the gas gangrene anaerobes and its efficacy in the treatment of experimental gas gangrene have been reported.[1-7] The present paper compares aspergillic acid[8-10] with other antibiotic agents and sulfonamides *in vitro* on *Cl. perfringens, Cl. septicum, Cl. oedematiens*, and a strain of *Cl. perfringens* made fast to sulfonamides by repeated transfer in broth containing sulfathiazole, and *in vivo* on *Cl. perfringens* infection in mice.

The medium used for the antibiotic tests *in vitro* contained 2% bacto tryptose, 0.2% glucose, 0.5% sodium chloride, 0.1% disodium phosphate, and 0.1% sodium thioglycollate; pH 7.4 to 7.6. Paraffin-vaseline seals were used on all cultures and

1 Chain, E., Florey, H. W., Gardner, A. D., Heatley, N. G., Jennings, M. A., Orr-Ewing, J., and Sanders, A. G., *Lancet*, 1940, **239**, 226.

2 Abraham, E. P., Chain, E., Gardner, A. D., Fletcher, C. M., Heatley, N. G., Jennings, M. A., and Florey, H. W., *Lancet*, 1941, **241**, 177.

3 Hobby, G. L., Meyer, K., and Chaffee, E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 277.

4 Florey, H. W., and Jennings, M. A., *Brit. J. Exp. Path.*, 1942, **23**, 120.

5 McIntosh, J., and Selbie, F. R., *Lancet*, 1942, **243**, 750.

6 McIntosh, J., and Selbie, F. R., *Lancet*, 1943, **244**, 793.

7 Hae, L. R., and Hubert, A. G., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 61.

8 White, E. C., *Science*, 1940, **92**, 127.

9 White, E. C., and Hill, J. H., *J. Bact.*, 1943, **45**, 433.

10 Jones, H., Rake, G., and Hamre, D. M., *J. Bact.*, 1943, **45**, 461.

TABLE I.
Activity *in vitro* of Some Antibiotic Agents and Sulfonamides Against the Gas Gangrene Anaerobes.
Figures given are the smallest amounts completely inhibiting growth.

Substance tested	*Cl. perfringens* W*	*Cl. perfringens* ST fast	*Cl. septicum* *	*Cl. oedematiens* *
Penicillin M837-A 500 O.U./mg				
O.U/ml	0.15	0.135	0.075	0.025
μg/ml	0.3	0.27	0.15	0.05
Aspergillic Acid 6+15 256 R.U./mg				
R.U./mg†	10.2	7.6	10.2	5.1
μg/ml	40	30	40	20
Tyrothricin				
μg/ml	0.05		0.01	
Gliotoxin				
μg/ml	100			
Sulfathiazole				
μg/ml	25	>250		
Sulfadiazine				
μg/ml	7	>185		
Sulfanilamide				
μg/ml	150	>250		
Homosulfanilamide				
μg/ml	16	5		

* We are indebted to Drs. G. B. Reed and J. H. Orr for these cultures.

† R.U./mg were determined by comparison of the activity *in vitro* of this preparation with a standard preparation.[10]

tests with *Cl. oedematiens* and *Cl. septicum*.

In setting up the tests, decreasing amounts of broth solutions of the different antibiotic substances were placed in a series of 13 x 100 mm tubes, broth added to bring the volume in each tube to 3.8 ml, and the tubes placed in boiling water for 15 minutes. After cooling, 0.2 ml of the proper culture dilution to contain 5000-50,000 organisms was added to each tube as inoculum. In all tests control tubes containing no antibiotic substance were included. The tubes were incubated for 16-18 hours at 37°C and the smallest amount of antibiotic substance inhibiting growth, as indicated by lack of turbidity, was taken as the end-point. Penicillin and gliotoxin were added to the tubes after boiling, because both are unstable at high temperatures.

For the sulfonamide tests *in vitro*, freshly prepared MacLeod's medium,[11-12] containing 0.1% sodium thioglycollate and free from *p*-amino benzoic acid, was used. The sulfonamides were dissolved in M/15 phosphate buffer and sterilized by autoclaving. The technic of the tests was the same as above except that boiling was omitted. *Cl. perfringens* W and a sulfonamide-fast strain of *Cl. perfringens* were the only anaerobes which uniformly grew well in this medium. The results of these tests are given in Table I.

In repeated tests with tyrothricin, the minimum inhibiting concentrations varied from 0.6 to 0.01 μg per ml, indicating that this agent has about the same activity *in vitro* as the penicillin preparation tested.

Owing to the different types of media used, the sulfonamide tests and the antibiotic tests are not strictly comparable. The susceptibility of the sulfathiazole-fast strain of *Cl. perfringens* to the bacteriostatic action of the antibiotic agents was to be expected since it has been shown that sulfonamide-fast pneumococci are inhibited *in vitro* by penicillin.[13-14] Also of interest were the results of preliminary experiments with homosulfanilamide (4-amino methyl benzol sulfonamide),* which differs

11 MacLeod, C. M., *J. Exp. Med.*, 1940, **72**, 217.

12 MacLeod, C. M., and Mirick, G. S., *J. Bact.*, 1942, **44**, 277.

13 Powell, H. M., and Jamieson, W. A., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 387.

14 McKee, C. M., and Rake, G., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 275.

* This compound appears in the German literature under the name Marfanil. (Schreus, H. T., and Brauns, A., *Klin. Woch.*, 1941, **20**, 1233.)

from the other sulfonamides in not being inhibited by *p*-amino benzoic acid.

Albino mice weighing 20-23 g were infected with 0.05 ml of a 10^{-1} dilution of a 6-hour culture of *Cl. perfringens* W given intramuscularly in the thigh. The dilution was made in broth containing $CaCl_2$ so that each mouse received 5 mg of calcium chloride with the inoculum. This 6-hour culture, undiluted, contained 2 to 5 M.L.D.s of toxin per ml as determined by the lecithin test.† The mice were treated by intramuscular injection of 0.1 ml of the substance to be tested, given in the same area in the thigh as the infecting dose, either immediately following infection or one hour later.

The results of the experiments *in vivo* are given in Table II (immediate treatment) and Table III (delayed treatment). Since it is difficult to be sure that the drug is being given in exactly the same place as the infecting dose irregular results, such as appear in Table II, might be expected. Without the use of large numbers of animals it would not be possible to determine 50% protecting dose of any substance.

TABLE II.
Cl. perfringens Infection in Mice Treated Locally, at the Time of Infection, with Sulfonamides or Antibiotic Agents.

No. of mice	Treatment	Lived	Died
5	Sesame Oil	0	5
5	15 O.U. (0.1 mg) Penicillin*	5	0
5	1 mg Aspergillic Acid*	4	1
5	1 " Tyrothricin*	0	5
5	1 " Gliotoxin*	0	5
10	None	0	10
10	0.5 mg Aspergillic Acid	10	0
10	5 O.U. (0.033 mg) Penicillin	5	5
10	2.5 mg Sodium Sulfadiazine	10	0
10	None	0	10
10	0.2 mg Aspergillic Acid	3	7
10	0.1 " " "	2	8
10	None	0	10
10	0.25 mg Sodium Sulfadiazine	10	0
10	0.05 " " "	5	5
10	None	1	9
10	0.1 mg Sulfadiazine	10	0
10	0.05 " Sodium Sulfadiazine	7	3

* Suspended in 0.1 ml of sterile sesame oil. In other experiments solutions were used.

† We are indebted to Dr. W. L. Koerber of the Biological Laboratories of E. R. Squibb & Sons for the toxin assay. (Koerber, W. L., and Alture-Werber, A., *J. Immun.*, 1942, **45**, 223.)

TABLE III.
Cl. perfringens Infection in Mice Treated Locally, One Hour After Infection, with Sulfonamides, Antibiotic Agents, or Antitoxin.

No. of mice	Treatment	Lived	Died
10	None	0	10
10	10 O.U. (0.101 mg) Penicillin	10	0
10	5 O.U. (0.05 ") "	10	0
10	2 mg Sodium Sulfadiazine	9	1
10	0.2 " " "	10	0
10	10 Units Antitoxin	10	0
10	1 " "	8	2
10	None	0	10
10	2.5 O.U. (0.025 mg) Penicillin	10	0
10	0.5 O.U. (0.005 ") "	7	3
10	1.0 mg Sodium Sulfadiazine	9	1
10	0.05 " " "	1	9
10	1 Unit Antitoxin	7	3
10	0.1 " "	0	10

Experiments with guinea pigs infected via wounds by the method of Reed and Orr[15] were not successful due to the frequency of intercurrent infections and to the toxicity for guinea pigs of repeated doses of penicillin.[16]

Summary. 1. The antibiotic substances and sulfonamides arranged in the order of decreasing bacteriostatic properties *in vitro* for the anaerobes of the gas gangrene group are: Tyrothricin, penicillin, sulfadiazine, sulfathiazole, aspergillic acid, gliotoxin, and sulfanilamide.

2. Penicillin and aspergillic acid were active *in vitro* against a sulfathiazole-fast strain of *Cl. perfringens*.

3. When given locally to mice infected intramuscularly with *Cl. perfringens*, penicillin and sodium sulfadiazine were more effective than aspergillic acid. Gliotoxin and tyrothricin were not effective in doses of 1 mg suspended in sesame oil.

[15] Reed, G. B., and Orr, J. H., *War Med.*, 1942, **2**, 59.

[16] Hamre, D. M., Rake, G., McKee, C. M., and MacPhillamy, H. B., *Am. J. Med. Sci.*, 1943, **206**, 642.