thiobarbituric acid showed much the same picture as the dialkyl-4-thiobarbituric acids except that the twitching action lasted for a longer period of time.

The 5-ethyl-5-isoamyl-2,4-dithiobarbituric acid, administered to 15 rats, showed the most promising indications. Two of the rats showed signs of very mild twitching. Upon intravenous injection of 20–30 mg./kg., rabbits lost the righting reflex instantly and in about 12 minutes. The placing reactions were recovered in 15 to 20 minutes. No subsequent ill effects or convulsant actions were observed. Death was caused by injection of 80 mg./kg. A few experiments on cats showed the same effect as those on rabbits.

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Influence of Streptomycin on Type b *Haemophilus influenzae* [1]

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Waksman and associates (*3, 5–7*) and others (*2, 4*) have reported the efficient antibacterial action of streptomycin toward a number of Gram-negative bacilli. These results suggested that this antibiotic might be active against Type b *H. influenzae*. Although specific rabbit serum and sulfadiazine have been highly successful in the treatment of influenzal meningitis in infants and children (*1*), it was thought that streptomycin, if effective, would offer advantages over this therapy; further, when used in conjunction with serum and chemotherapy it might reduce the present fatality rate.

An attempt was made to devise a simple, reliable procedure which, by determining *in vitro* the sensitivity of a given strain of *H. influenzae* to streptomycin,[2] might predict the influence of this antibiotic on infections caused by it in humans.

The sensitivity of a number of strains of Type b *H. influenzae* was determined under conditions which revealed the influence of size of inoculum, physical state of medium (Levinthal broth or agar), and duration of incubation period during exposure to streptomycin. The results indicate that the sensitivity of a given strain can be assayed reliably by inoculating a series of Levinthal agar plates containing varying concentrations of streptomycin with a 2-mm. loop of a culture grown for six hours on Levinthal agar or broth. The lowest concentration of the drug which prevents visible growth after 48 hours of incubation is designated as the minimal effective concentration (MEC).

Twenty-two cultures, isolated prior to treatment from patients with severe Type b *H. influenzae* infections, were examined for MEC. When the Levinthal broth culture, grown for six hours, was used as the inoculum, all strains were sensitive to concentrations of approximately 3 units or less/cc. When the loop of culture used for seeding the test plate was obtained from the growth on Levinthal agar after six hours of incubation, the MEC of seven of the strains was 7.5–10 units/cc. These results suggest that Type b *H. influenzae* is among those organisms which are highly sensitive to streptomycin.

Early in the study there was isolated from a meningitis patient who had been under treatment with streptomycin for two weeks a strain of Type b *H. influenzae* capable of growth on Levinthal agar containing 525 units of the antibiotic/cc. The same organism cultivated from the spinal fluid before treatment showed a MEC of 2.5 units/cc. Demonstration of a decrease in the MEC of resistant strains following subculture in the absence of streptomycin emphasizes the need for immediate preservation of the organism by drying and sealing under vacuum if its sensitivity to streptomycin at the time of isolation from the patient is to be appraised reliably at a later date.

This experience led to examination of development of resistance *in vitro*. The progress of adaptation of 16 sensitive strains was studied by subculturing at from 24- to 48-hour intervals the growth on the Levinthal agar plate containing the highest concentration of streptomycin to another series of plates of the same medium with the same and increasing concentrations of the antibiotic. Within one to three weeks seven strains had acquired the ability to grow in the presence of 525 units/cc. Six required four weeks to reach this degree of resistance. Three strains failed to grow in concentrations above 157 units/cc. after an adaptation period of four weeks.

These experiments provided a group of resistant strains which, together with the culture made resistant during treatment of a patient, could be used for study of correlation between MEC *in vitro* and the minimal dose required for protection in mice. Mouse protection tests determined for eight different strains the minimum effective dose (MED), *i.e.* the smallest single intraperitoneal dose of streptomycin which

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[2] The streptomycin was supplied by E. R. Squibb & Sons. The dried powder contained approximately 300 (2) units/mg.

would protect 50 per cent of the mice against 20,000,000 organisms. This infecting dose represents 100,000–1,000,000 LD_{50}.

Table 1 records the MEC and MED for the eight cultures, four of which were sensitive and four resistant according to *in vitro* tests. Strains 1, 2, 3, and 4 were isolated from patients before treatment with streptomycin and had never been exposed to this drug *in vitro*. These same strains, after acquiring varying degrees of resistance to streptomycin according to *in vitro* assay (1a in a patient and 2a, 3a, and 4a *in vitro*), required correspondingly higher MED in mice. In fact, we have not been able to demonstrate that it is possible to protect mice against strains capable of *in vitro* growth at 525 units/cc. and above. Strain 1b, produced by cultivating 1a in Levinthal broth in the absence of streptomycin, is shown to be sensitive *in vivo* and *in vitro*. It seems clear that high correlation exists between the MEC *in vitro* and the MED necessary to protect mice against 15,000,000–20,000,000 organisms under the circumstances of the experiments. The results indicate that the simple *in vitro* test recommended should suffice for assaying the sensitivity of strains from patients to be treated with streptomycin.

TABLE 1

RELATION BETWEEN MEC* ON LEVINTHAL AGAR AND MED† SUFFICIENT TO PROTECT MICE AGAINST 20,000,000 ORGANISMS PER MOUSE

Strain No.	Sensitive Strains MEC	Sensitive Strains MED	Resistant Strains MEC	Resistant Strains MED
1	1.1	39.0		
1a			525.0	> 630.0
1b	13.0	39.0		
2	1.6	19.5		
2a			73.0	315.0
3	1.1	19.5		
3a			> 525.0	> 1,575.0
4	0.8	19.5		
4a			1,078.0	> 1,575.0

* Minimum effective concentration *in vitro* units/cc. of culture media.
† Minimum effective dose units per mouse required for protection of 50 per cent of animals.

The results of earlier studies of the protective capacities of serum and sulfadiazine against lethal Type b *H. influenzae* infections in mice have been published (*1*). Experiments were designed to compare the protective power of streptomycin alone and in conjunction with sulfadiazine with that obtained by specific serum along with sulfadiazine. The results are tabulated along with those reported previously.

Protective Agent	*Maximum LD_{50} Protection*
Sulfadiazine	10,000
Specific rabbit serum	10,000–100,000
Sulfadiazine and serum	1,000,000
Streptomycin	100,000,000
Streptomycin and sulfadiazine	100,000,000

It is seen that the protective capacity of streptomycin alone equals that obtained by combining its action with sulfadiazine. This degree of protection by streptomycin was exhibited in four experiments in which two different strains of Type b *H. influenzae* were tested. Protection beyond 100,000,000 LD_{50} has not been explored, but the data show clearly that streptomycin alone is more effective against massive *H. influenzae* infections in mice than serum used in conjunction with sulfadiazine.

The foregoing observations have led us to treat 10 influenzal meningitis patients with streptomycin alone. The results, which will appear elsewhere, have been encouraging.

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Urinary Recovery of Penicillin After Oral Administration With Antacids and Buffers

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The importance of neutralizing gastric acidity for the therapeutic success of orally administered penicillin has been unquestionably established (*1*). Early studies on penicillin absorption and excretion have proved that most of the orally administered penicillin is destroyed by stomach acidity (*1, 9*). The presence of small amounts of penicillin in the urine and in the blood after oral administration indicated a partial penicillin absorption from the intestines; moreover, a corresponding increase in both urine and blood penicillin levels has been produced by the oral administration of larger doses of this antibiotic (*9*).

These observations suggested that oral therapy of penicillin is possible, once sufficient penicillin intake has been assured and its destruction by gastric acidity eliminated. The extent to which the gastric acidity is neutralized determines the efficacy of using a particular buffer or antacid for the absorption of penicillin. Several antacids (*10*), buffers (*10*), and oils (*4, 6*) in fluid form or in gelatin capsules have been used to fulfill this purpose. The results reported in the literature are somewhat confusing and conflicting (*2, 3, 5, 8*). In most instances the investigations were carried out by comparing the responses of sev-