thesized compounds in which both sulfonamides and antibiotics are incorporated in the same molecule, it is entirely conceivable that comparisons will be made between fairly low molecular weight substances and those, like proteins, having weights perhaps 100 or more times as great. While it may be expedient to use milligrams as a standard of reference, chemically this is unsound.

Since one of the primary goals of pharmacology and chemotherapy is the discovery of the relationship of chemical structure to pharmacological action, a chemical terminology will expedite chemical reasoning. It is an arduous task to survey the literature and to convert milligrams to molar concentrations in studying the physicochemical relationship of structure to biological activity. The addition, in papers, of one column of molar concentrations complementing the milligram data would be not only considerably time-saving to other investigators but enlightening as well.

It is suggested, therefore, that when comparative values and the biological activities of chemotherapeutic substances are reported, a molar or micromolar concentration (cf. the excellent tables of Hjort, *et al.*, *2, 6*; and DeGraff, *et al., 1*) be used as a standard.

References

1. DeGraff, A. C., Paff, G. H., and Lehman, R. A. *J. Pharm. exp. Therap.*, 1941, **72**, 211.
2. Hjort, A. M., deBeer, E. J., and Fassett, D. W. *J. Pharm. exp. Therap.*, 1940, **68**, 69.
3. Latven, A. R., and Molitor, H. *J. Pharm. exp. Therap.*, 1939, **65**, 89.
4. Litchfield, J. T., Jr., White, H. J., and Marshall, E. K., Jr. *J. Pharm. exp. Therap.*, 1939, **67**, 437.
5. Northey, E. H. *Chem. Rev.*, 1940, **27**, 85.
6. Paff, G. H., Lehman, R. A., and Halperin, J. P. *Proc. Soc. exp. Biol. Med.*, 1945, **58**, 323.
7. Robinson, H. J., and Molitor, H. *J. Pharm. exp. Therap.*, 1942, **74**, 75.
8. Walker, H. A., and Van Dyke, H. B. *J. Pharm. exp. Therap.*, 1941, **71**, 138.
9. White, H. J., Bratton, A. C., Litchfield, J. T., Jr., and Marshall, E. K., Jr. *J. Pharm. exp. Therap.*, 1941, **72**, 112.

4-Thio- and 2,4-Dithiobarbituric Acid Derivatives

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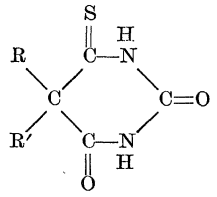
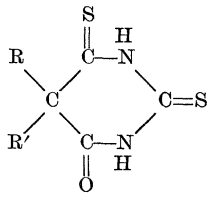
Until recently no substituted barbituric acids were known in which more than one of the oxygen atoms were replaced by sulfur, although the preparation of 2,4,6-trithiobarbituric acid by the action of potassium hydrosulfide on 2,4,6-trichloropyrimidine had been described (*2*). Henze and Smith (*5*) reported the preparation of 5,5-diethyl-2,4,6-trithiobarbiturate and 5-ethyl-5-phenyl-2,4,6-trithiobarbiturate, stating that these possessed no hypnotic properties. In 1944 Carrington (*3*) prepared the 2-thio, 2,4-dithio and 2,4,6-trithio derivatives of a series of barbiturates, using those barbiturates that are well-known hypnotics. No pharmacology was reported.

Using hydrogen sulfide under pressure, we have prepared several 4-thiobarbiturates (I) and 2,4-dithiobarbiturates (II) from the corresponding imino compound.



(I)



(II)

The imino group in other organic compounds has been replaced by sulfur. Carbon disulfide was used by Hofmann (*7*) and Hobrecker (*6*) to prepare diphenylthiourea from diphenylguanidine. Thioamides were prepared by Bernthsen (*1*), using amidines and hydrogen sulfide. Imido esters were converted to esters of thioncarborylic acid by Matsui (*8*) in 1908. Many iminobarbituric acid derivatives (*4*) are known, and it was thought that the imino group in these might respond in a like manner.

A few grams of a dialkyl-4-iminobarbituric acid or a dialkyl-4-imino-2-thiobarbituric acid were mixed with several hundred cubic centimeters of absolute ethyl alcohol that had been saturated previously with hydrogen sulfide at 10 pounds pressure. This was quickly placed in a bomb and heated at 150° C. for 12 hours. The product was isolated by evaporation of the alcohol, dissolving the residue in 5 per cent sodium hydroxide, filtering, making slightly acid with hydrochloric acid, and precipitating the thiobarbiturate.

In the 5,5-dialkyl-4-thiobarbituric acid series, only two compounds have been tested pharmacologically: 5,5-diethyl-4-thiobarbituric acid and 5-ethyl-5-isopropyl-4-thiobarbituric acid. After intraperitoneal administration, both of these compounds showed a marked depressant action, causing anesthesia with a rapid onset and short duration when administered to rats or rabbits. This depressant action was accompanied, however, by a stimulating or convulsant action which caused the muscles of the test animals to twitch slightly during the early stage of anesthesia.

In the 5,5-dialkyl-2,4-dithiobarbituric acid series, four compounds have been tested pharmacologically by intraperitoneal administration to rats. The 5,5-diethyl-2,4-dithiobarbituric acid, 5-ethyl-5-n-butyl-2,4-dithiobarbituric acid, and 5-ethyl-5-isopropyl-2,4-di-

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.

References

1. Bernthsen, August. *Ber. dtsch. chem. Ges.*, 1877, **10**, 1240.
2. Buttner, Ernst. *Ber. dtsch. chem. Ges.*, 1903, **36**, 2234.
3. Carrington, H. C. *J. chem. Soc. Lond.*, 1944, 124.
4. Conrad, M. *Ann. Chem.*, 1905, **340**, 310.
5. Henze, H. R., and Smith, P. E. *J. Amer. chem. Soc.*, 1943, **65**, 1090.
6. Hobrecker, F. *Ber. dtsch. chem. Ges.*, 1869, **2**, 689.
7. Hofmann, A. W. *Ber. dtsch. chem. Ges.*, 1869, **2**, 460.
8. Matsui, M. *Mem. Coll. Sci. Eng., Kyoto Univ.*, 1908, **1**, 285.

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

[1] The work reported in this communication was supported by grants from the Commonwealth Fund.

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