Technical Papers

On Reporting the Comparative Values and the Biological Activities of Chemotherapeutic Agents

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The existence of many chemotherapeutic agents, among which the sulfonamides and antibiotics are at present the most prominent, presents the problem of determining the comparative therapeutic values of these compounds.

It is customary to compare bacteriological and pharmacological activity, especially among the sulfonamides and the antibiotics, on the basis of the milligram or milligram per cent concentration appearing in the blood or other fluids.

Often, however, there are considerable differences in the molecular weights of substances tested for therapeutic activity (*5*). It should be obvious that in making comparisons of biological activity the use of the milligram standard favors those compounds with the lower molecular weights.

Litchfield, *et al.* (*4*) stated that discrepancies in the comparisons made by different workers of the effectiveness of different drugs point to the need for a reliable quantitative method for assessing the activity of such compounds. They suggested that, since the concentration of a drug in the blood and tissues is the determining factor in therapy, a quantitative evaluation of effectiveness should be based on blood concentration. They added, however, that comparative values in reference to a suitable standard offer the most reliable measure of effectiveness.

It is proposed that these refinements be carried one step further. Although most pharmacologists are aware of the importance of considering molecular weights and molar concentrations in comparing the activities of compounds, only a few record these aspects in the literature. Moreover, often where comments are made concerning molar concentrations, they are not sufficiently extensive. The several references which are cited to show the absence of molecular standards for comparison have been chosen more or less at random from the current literature, are assumed to be representative of current practice, and are not to be construed as either direct or implied adverse criticism of the authors.

That misinterpretations, although frequently not serious, do result from nonmolar evaluation becomes evident from a study of the following comparisons:

(1) White, *et al.* (*9*) studied the qualitative antibacterial activity, both *in vitro* and *in vivo,* of 126 compounds against the B-hemolytic streptococcus in mice. "The final concentration of drug in each case was 10 ± 1 mg. per cent. *In vivo* tests were carried out by the drug-diet procedure." From their tables, compounds Nos. 20, 49, 82, and 106 have the same relative qualitative *in vitro* activity (++), although Nos. 20 and 106 are in saturated solutions having a concentration of less than 10 mg. per cent. The *in vivo* activity is also essentially the same for these four compounds. When these derivatives are compared on a molar standard, the activity ratio becomes as follows: No. 49—1, No. 106—3.2, No. 82—4.0, and No. 20—4.4.

(2) Latven and Molitor (*3*) have investigated the intravenous toxicity of eight organic solvents. From their data, given in cubic centimeters, the LD_{50}'s in grams and in moles have been calculated and are as follows:

	LD_{50} in grams	Order of increasing toxicity	LD_{50} in moles	Order of increasing toxicity
Al (70%)	3.56	6	0.077	4
Al (90%)	4.44	4	0.097	2
C	4.41	5	0.033	7
D	2.71	8	0.015	8
Eg	3.35	7	0.054	5
El	0.62	9	0.005	9
Gl.	7.55	2	0.082	3
Pg	8.30	1	0.109	1
Trig.	7.23	3	0.048	6

While the difference in the order of toxicity is not striking, there are differences in five of the eight compounds. It is not too unlikely that, had longer chain compounds of the same series been included, the discrepancies would have been greater.

(3) In the determination of therapeutic ratios, the toxicities of the compounds investigated are important. Walker and Van Dyke (*8*) have shown that, molecularly, sulfanilamide has an acute toxicity (LD_{50}) of about 13.6 millimoles (or 2.314 grams/kilo body weight when injected as a single subcutaneous dose in Swiss mice. From data in a report by Robinson and Molitor (*7*) the approximate acute LD_{50} of gramicidin injected intravenously into white mice is 3.0 mg./kg. (or 0.002 millimole/kg.). For the sake of the example, let us assume that, intravenously, the LD_{50} of sulfanilamide for the Swiss mice would have been only one-half that of the subcutaneous dose. Compared to sulfanilamide, therefore, gramicidin is 386 times as toxic, milligram for milligram. Mole for mole, however, gramicidin is 2,900 times as toxic.

Since it is likely that there will eventually be syn-

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

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

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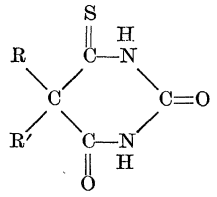
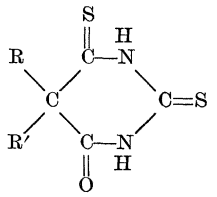
Until recently no substituted barbituric acids were known in which more than one of the oxygen atoms were replaced by sulfur, although the preparation of 2,4,6-trithiobarbituric acid by the action of potassium hydrosulfide on 2,4,6-trichloropyrimidine had been described (*2*). Henze and Smith (*5*) reported 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-