References

1. ALLEN, B. M. *Biol. Bull.*, 1917, **32**, 117.
2. BLOUNT, R. F. *J. exp. Zool.*, 1932, **63**, 113.
3. *Ibid.*, 1945, **100**, 79.
4. BURCH, A. B. *Univ. Calif. Pub. Zool.*, 1946, **51**, 185.
5. SMITH, P. E. *Anat. Rec.*, 1916, **11**, 57.
6. TAYLOR, A. C. and KOLLROS, J. J. *Anat. Rec.*, 1946, **94**, 7.

A Very Water-soluble Riboflavin Derivative

George B. Stone

Technical Service Department, Chas. Pfizer & Co., Inc., Brooklyn, New York

For the past ten years a not inconsiderable amount of investigation has been carried out in an effort to increase the water solubility of riboflavin (vitamin B_2) either by the use of solubilizers or by the preparation of soluble derivatives. Despite some thirty or more references and patents, representing several hundred solubilizers or soluble derivatives, few, if any, are of practical significance for pharmaceutical application. Riboflavin is not only sparingly soluble in water, but in almost every other solvent. It is, however, relatively soluble in concentrated sulfuric acid. Investigation of this significant solubility in concentrated sulfuric acid led to the isolation of a very water-soluble riboflavin derivative.

The compound was prepared by dissolving 50 g of riboflavin, little by little, in 200 ml of concentrated sulfuric acid with vigorous stirring, while the temperature was maintained at 40°–50° C. Mixing was continued for 1–2 hr until the mixture was homogeneous and it was then quenched by pouring it over 1 kg of cracked ice. The resulting solution was neutralized with slurried calcium hydroxide to a pH of 6.5, with the temperature being maintained below 70° C. The precipitated gypsum was filtered off, washed with hot water, and then repulped with hot water, filtered, and again washed. All washings were added to the original filtrate. Assay by the fluorometric method indicated that the original 50 g of riboflavin was present in this solution. The solution was concentrated under vacuum to a volume of less than 500 ml and filtered to remove further gypsum precipitated during concentration. The filtrate was then freeze-dried to yield 114 g of a fluffy yellow-orange powder, which assayed fluorometrically 57.2% riboflavin, equivalent to a yield of 100% based on the weight of the riboflavin employed originally.

The compound is stable in air and nonhygroscopic. It is very soluble in water, and aqueous solutions containing 10% wt/vol of riboflavin have been prepared—a solubility 1,000 times greater than that of riboflavin U.S.P. It is soluble in methanol and slightly soluble in ethanol, and it decreases in solubility with the higher alcohols. It is soluble in glycerine, propylene glycol, and pyridine; slightly soluble in acetone, glacial acetic acid, and chloroform; insoluble in benzene, ether, ethyl acetate, methylethylketone, and carbon tetrachloride. Aqueous solutions are heat-stable at 15 psi for 120 min in the pH range from 1.0 to 6.5.

Preliminary chemical analysis seems to indicate that the compound may be represented by the empirical formula, $C_{17}H_{18}N_4O_{15}S_2Ca$, inasmuch as the compound contains calcium and sulfur, a portion of the sulfur being present as sulfate.

Fluorometric assay of the material yields a value of 57.2% riboflavin. The absorption spectrum is identical with riboflavin U.S.P., having the same maxima and minima, but proportionately displaced because of the lesser riboflavin content. Paper chromatographic absorption analysis indicates the material is a pure compound, much more water-soluble than riboflavin U.S.P.

Microbiological assay by the U.S.P. XIII revision method, which includes a preliminary hydrolysis at 15 psi for 30 min, yielded a value of 33.0% riboflavin. Omission of the preliminary hydrolysis gave a value of 1.5% riboflavin, whereas increase of the time of hydrolysis to 120 min gave a value of 42.2% riboflavin.

Biological assay for riboflavin by a standard rat growth method employing a basal vitamin B complex-free diet, supplemented with those members of the B complex other than riboflavin, indicated that the riboflavin potency for the rat is almost nil. There were indications of a slight antivitamin activity of this compound.

Further investigation of this material is anticipated.

A Correction for Linkage in the Computation of Number of Gene Differences

Everett R. Dempster and Leon A. Snyder[1]

Genetics Division, University of California, Berkeley

The Castle-Wright formula (*2*) for estimating the number of gene pairs differentiating two strains with respect to some quantitative character is based on the increased variance of the F_2 as compared to the parental variance. A number of postulates on which this derivation is based (*1, 5, 6, 7*) may be listed as follows: (1) The parents are homozygous. (2) All the plus alleles differentiating the two strains with respect to the character considered are in one parent and all the minus alleles in the other. (3) All gene differences affecting the character have equal effects. (4) The effects of different allelic substitutions are additive. (5) There is no linkage. Deviations from postulates 2 to 5, with the possible exception of special epistatic effects (postulate 4) will always increase the F_2 variance, and therefore, since the latter appears in the denominator of the expression for gene number, will bias the estimate toward lower values. Since actual situations are usually at variance with most of the postulates listed, the expression in general leads to minimum rather than unbiased estimates of the number of gene differences.

Some modifications have been devised for relaxing the postulates or otherwise extending the applicability of

[1] Present address: Department of Plant Sciences, University of Oklahoma, Norman, Oklahoma.