feeding of the basal ration does not enter into the following calculations, the value is recorded for reference purposes. The calories stored as liver glycogen at each of the 4-hr. periods at which determinations were made, were calculated from the glycogen values found. By formulating a suitable equation for calculating the theoretical calories stored as glycogen (based upon actual values found in the control animals) by animals of different weight in the control and vitamin injected groups, and comparing this calculated value with that found in the injected groups, a ratio of the effectiveness of the vitamin was readily determined, with unity assigned to the values found in the controls. The utility of this ratio calculated for the 4-, 8- and 12-hr. periods (Table I) is primarily one of convenience for evaluating the data presented in graphical form and also serves as a numerical index of the relative effectiveness of the particular factors in contributing to the liver glycogen levels found under the prevailing experimental conditions. Such a numerical expression likewise emphasizes the significance and complementing role of these vitamins and their interrelated functions which contribute to maintenance of normal metabolism and efficiency of food utilization.

SUMMARY

1. Impoverishment of body stores of thiamin, riboflavin, vitamin B_6 and pantothenic acid resulting from inadequate dietary intake causes lowered liver glycogen levels indicating inefficient food utilization, deficient reserve energy and an unbalanced metabolism.

2. Animals depleted of any one of these factors but supplied with the others showed a prompt and characteristic response in elevation of the liver glycogen level following administration of the missing factor.

3. Liver glycogen patterns obtained over a 24-hr. period following controlled forced feeding of unit amounts of food and vitamin administration were characteristically different for each of the vitamin entities studied.

4. The data do not disclose a specific glycogenic or glycogen hydrolyzing role for any of the factors. Each undoubtedly complements the others in maintaining a balanced state of metabolism.

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Inhibiting Effect of Various Compounds on Curative Action of Sulfanilamide and Sulfapyridine in Infected Mice*

By George W. Raiziss, M. Severac and J. C. Moetsch

Lockwood (1, 2), Stamp (3), Green (4) and Fleming (5) contributed to the study of substances inhibiting the bacteriostatic effect of sulfanilamide. Woods and Fildes (6, 7) found that *p*-aminobenzoic acid present in yeast inhibits the bacteriostatic effect of sulfanilamide. Selbie (8) showed that *p*-aminobenzoic acid administered orally inhibits the therapeutic effect of sulfanilamide in mice infected with *Streptococcus hæmolyticus*.

EXPERIMENTAL

We studied the inhibiting effect *in vivo* of *p*-aminobenzoic acid on sulfanilamide and sulfapyridine administered to infected mice. Closely related products of *p*-aminobenzoic acid and other chemical compounds were included in this investigation.[1] Some mice were infected intraperitoneally with pneumococcus type II and others with *Streptococcus haemolyticus*. Groups of 5 mice used for the studies on streptococcus were infected intraperitoneally with 200 minimum lethal doses of *Streptococcus haemolyticus*, strain C-203, of which the average minimum lethal dose was 0.5 cc. of 1:10,000,000 dilution of broth culture. Treatment with sulfanilamide or sulfapyridine was given by mouth in a dose of 10 mg. immediately after infection, and once daily for the following two days. Ten milligrams of *p*-aminobenzoic acid or other compound was administered by mouth one hour after each treatment.

* From the Dermatological Research Laboratories, Philadelphia, Pa., Division of Abbott Laboratories, North Chicago, Ill.

[1] While we were engaged in this research, McCarty (9) reported that *p*-aminobenzoic acid is capable of destroying the curative effect of sulfapyridine for type I pneumococcic infection in mice.

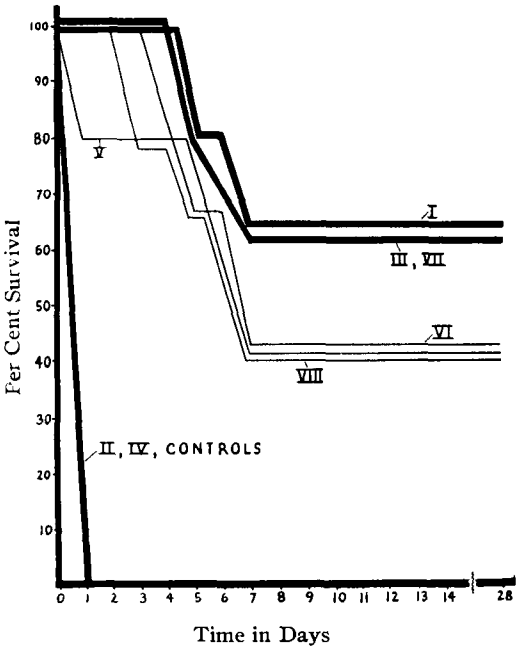

Fig. 1.—Inhibiting Effect of Various Chemical Compounds on Sulfanilamide and Sulfapyridine in *Streptococcus hæmolyticus* Infection in Mice.[1]

(I) Sulfanilamide, (II) sulfanilamide + *p*-aminobenzoic acid, (III) sulfapyridine, (IV) sulfapyridine + *p*-aminobenzoic acid, (V) sulfapyridine + *o*-aminobenzoic acid, (VI) sulfapyridine + *p*-aminobenzenesulfonic acid, (VII) sulfapyridine + thiamin chloride and (VIII) sulfapyridine + *p*-hydroxybenzoic acid.

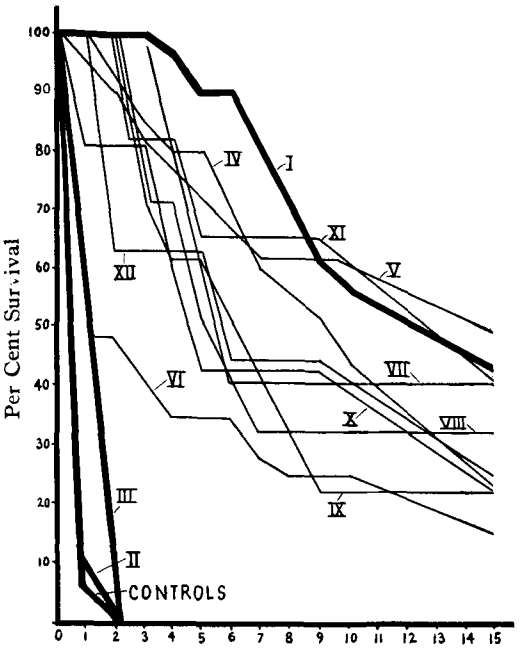

Fig. 2.—Inhibiting Effect of Various Chemical Compounds on Sulfapyridine in Type II Pneumococci Infection in Mice.

(I) Sulfapyridine, (II) sulfapyridine + *p*-aminobenzoic acid, (III) sulfapyridine + *p*-aminobenzamide, (IV) sulfapyridine + *p*-hydroxybenzoic acid, (V) sulfapyridine + *p*-aminobenzenesulfonic acid, (VI) sulfapyridine + *o*-aminobenzoic acid, (VII) sulfapyridine + glycocoll,[2] (VIII) sulfapyridine + 5-acetylaminosalicylic acid, (IX) sulfapyridine + 5-aminosalicylic acid, (X) sulfapyridine + nicotinic acid, (XI) sulfapyridine + thiamin chloride and (XII) sulfapyridine + cevitamic acid.

We found that 100% of streptococcus-infected mice treated with sulfanilamide alone survived for four days and 60% for twenty-eight days, while all mice treated with sulfanilamide plus *p*-aminobenzoic acid died twenty-four hours after treatment. The same was true with sulfapyridine and *p*-aminobenzoic acid. However, other compounds given simultaneously with sulfapyridine seemed to have had slight inhibiting effect.

For the pneumococcic tests, mice were infected intraperitoneally with 10 minimum lethal doses of type II pneumococcus of which the average minimum lethal dose was 0.5 cc. of 1 : 10,000,000 dilution of broth culture. These mice received sulfapyridine orally in doses of 10 mg. three times daily at 9 a. m., 5 p. m. and 12 m. for five days, at 9 a. m. and 5 p. m. on the sixth day, and at 9 a. m. on the seventh day—a maximum of eighteen treatments. Ten milligrams of *p*-aminobenzoic acid or other compounds was given by mouth one hour after each treatment with sulfapyridine.

The *p*-aminobenzoic acid completely inhibited the therapeutic effect of sulfapyridine; all of the 20 animals died after two days. Five mice which were given sulfapyridine plus *p*-aminobenzamide died also after two days—the latter drug probably hydrolyzes in the animal body into *p*-aminobenzoic acid. *p*-Hydroxybenzoic acid (25 mice) and *p*-aminobenzenesulfonic acid (25 mice) did not substantially interfere with the curative effect. *o*-Aminobenzoic acid (15 mice) has partially inhibited sulfapyridine. Glycocoll interferes slightly with the therapeutic effect. 5-Amino- and 5-acetylaminosalicylic acid (10 mice each) showed only a moderate inhibiting action. While nicotinic acid, also cevitamic acid, showed some inhibition, thiamin chloride did not interfere to any appreciable degree with the effect of sulfapyridine, all tested in groups of 5 mice.

[1] Ten milligrams of sulfanilamide or sulfapyridine was given orally immediately after infection, and once daily for the following two days. Ten milligrams of *p*-aminobenzoic acid or other compound was given orally one hour after each treatment.

[2] Glycocoll = aminoacetic acid.

SUMMARY

The therapeutic effect of sulfapyridine by mouth on mice infected with pneumococcus type II or *Streptococcus hæmolyticus* was completely annihilated when *p*-aminobenzoic acid was administered simultaneously. The

isomeric *o*-aminobenzoic acid proved to be much less inhibiting. The replacement of the amino group by the hydroxyl, the carboxylic acid by the sulfonic acid group, and the introduction of a third substituent into *p*-aminobenzoic acid resulted in a substantial destruction of the inhibitory effect. Nicotinic acid, cevitamic acid and even to a lesser degree thiamin chloride have only a slight inhibitory effect on sulfapyridine.

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A Note on the Determination of Arsenic in Organic Arsenical Compounds*

By F. B. Rodman† and Harold N. Wright‡

Numerous methods of analysis have been advocated for the determination of the arsenic content of organic arsenical compounds. Foremost among these are the gravimetric method of Treadwell and Hall (1) and the volumetric method of Lehmann (2). The Treadwell–Hall gravimetric method has been recognized as having a comparatively high degree of accuracy, but is laborious and time consuming. The Lehmann volumetric method was adopted as the official method of assay for arsphenamine and neoarsphenamine in U. S. P. X (1926) and has remained the official method of assay since that time. While the Lehmann method has been considered satisfactory for the assay of arsphenamine and neoarsphenamine it has not been considered satisfactory for the analysis of some of the more refractory arsenicals such as arsanilic acid and sodium cacodylate.

Myers and DuMez (3) made a comparative study of several methods of analysis for the assay of arsphenamine and neoarsphenamine. They assayed a number of specimens of these drugs by four methods, namely (*a*) the Treadwell–Hall gravimetric method, (*b*) Lehmann's, (*c*) Ewins' (4), and (*d*) Gaebel's (5) titration methods. These workers concluded that the Treadwell–Hall and Lehmann methods gave nearly identical results, while the results obtained by Ewins' or Gaebel's methods were invariably low.

Wright, *et al.* (6), in a study of the crystalloid and colloid fractions of arsphenamine and neoarsphenamine, found it impossible to employ the official Lehmann volumetric method in this work since "the oxidation with potassium permanganate and sulfuric acid employed in this method is apparently inadequate to cause the complete breakdown of 'aged' solutions of the arsphenamines, which become decidely refractory to oxidation and almost invariably give low results with the Lehmann method." This was found to be particularly true with the colloidal fractions. Robertson (7) also has concluded that the Lehmann method, while useful in routine assays, is not satisfactory for research purposes.

EXPERIMENTAL

In connection with an investigation of the comparative distribution and retention of the crystalloid and colloid fractions of the arsphenamines to be reported elsewhere (8) a series of comparative analyses of the arsenic content of the sample of neoarsphenamine employed in this study was made by both the Treadwell–Hall and Lehmann methods.

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† Department of Physiology and Pharmacology, University of Alberta Medical School, Edmonton, Alta., Canada.
‡ Department of Pharmacology, University of Minnesota Medical School, Minneapolis, Minn.