days. Each dose is somewhat higher than the single narcotic dose given by Sollmann and Hanzlik,[1] but no narcosis was produced. However, the series was small, consisting of 3 animals in each group. Nevertheless, it seems justifiable to conclude that there is no significant difference in the two products as regards their effects presumably on the optic nerve in the rabbit.

Conclusions. 1. There is no difference in toxicity between synthetic and natural methanol when injected intraperitoneally into the white mouse. 2. Both the synthetic and natural methanol in equal amounts produce apparent blindness in rabbits.

It appears that the pathology of methyl alcohol poisoning is quite well established. The object of the experiments as submitted was to compare the toxicity of the synthetic product with that of the natural methanol. In acute poisoning with methyl alcohol frequently no gross pathological changes are observed; the characteristic changes in the retina appear only somewhat late and subsequent to optic atrophy.

5520

Determination of Plasma Phosphatase.

AARON BODANSKY.
With the Technical Assistance of Lois Hallman and R. Bonoff.
From the Laboratory Division of the Hospital for Joint Diseases, New York City.

The significance of phosphatase in bone physiology was suggested by Robison and his coworkers. Increased plasma phosphatase was recently reported by Kay in *ostitis fibrosa, ostitis deformans,* active rickets and *osteomalacia.* Kay also reported a method for the determination of plasma phosphatase.[1] In an experimental study of plasma phosphatase in *ostitis fibrosa,* which we produced in young guinea pigs and dogs by prolonged treatment with parathormone[2, 3, 4, 5] we found Kay's method of determination of plasma

[1] Sollmann and Hanzlik, Experimental Pharmacology, Philadelphia, 1928, 270.

[1] Kay, H. D., *J. Biol. Chem.*, 1930, **89**, 235, 249. In the latter paper Kay also reviews the previous work of Robison and associates.

[2] Bodansky, A., Blair, J. E., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 708; *J. Biol. Chem.*, 1930, **88**, 629.

[3] Jaffe, H. L., Bodansky, A., and Blair, J. E., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 710; *Arch. Pathol.*, 1931, **11**, 207.

phosphatase unsatisfactory. We have avoided the disadvantages of Kay's method by the use of an M/10 solution of sodium diethyl-barbiturate (veronal) as a buffer.* When it is added to plasma in the proportions which we employ, the resulting mixture has a pH of about 8.6. This reaction is not changed after the addition of the substrate—a solution of sodium beta-glycerophosphate (Boots Drug Company, Nottingham, England) containing about 5 mg. of phosphorus per cc.† ‡ Nor does the pH change perceptibly during the action of the phosphatase, even when continued for 72 hours.

Our procedure follows: Two cc. of twice centrifuged plasma are sufficient. To each cc. of plasma are added 7 cc. of water and 2 cc. of the veronal solution. A 10 cc. aliquot containing 1 cc. of plasma is withdrawn into a glass stoppered test tube and 1 cc. of the glycerophosphate solution is added. After a single inversion of the tube it is placed in the water bath at 37°. (No preservative is necessary, the toluene added with the glycerophosphate being sufficient.) After 2 hours the tube is rapidly cooled, and 5 cc. of 10% trichloracetic acid are added. About 14 cc. of filtrate are obtained. The total inorganic phosphate is determined in duplicate or triplicate aliquots by a modification of the Kuttner-Cohen method.[6] The plasma phosphorus is deducted and the phosphatase activity is calculated in terms of units (mg. of inorganic phosphorus liberated in 1 hour) per 100 cc. of plasma.

A control determination of plasma phosphorus may be made upon an aliquot of the plasma-veronal mixture (without glycerophosphate), after keeping it in the water bath for 2 hours. However, this treatment affects the phosphorus values so little (although a slight rise is consistent) that a plasma phosphorus determination on an aliquot not so treated or on a sample of the original plasma is permissible.

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[4] Jaffe, H. L., and Bodansky, A., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 795; *J. Exp. Med.*, 1930, **52**, 669.

[5] Bodansky, A., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 797; *J. Exp. Med.*, 1931, **53**, No. 5 (May).

* L. Michaelis recommended the use of veronal as a buffer within the range of pH 6.8 to 9.6 (*J. Biol. Chem.*, 1930, **87**, 33). Levene and Dillon used a veronal-HCl mixture (pH 8.6) to buffer the hydrolysis of nucleic acid by intestinal phosphatase (*J. Biol. Chem.*, 1930, **88**, 753).

† We prefer a fresh solution, although it keeps well when preserved with a drop of toluene in the refrigerator.

‡ We are under obligation to Dr. H. D. Kay for our first supply of sodium beta-glycerophosphate.

[6] Bodansky, A., Hallman, L., and Bonoff, R., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 762.

The advantages of our technique are as follows: (1) The volume of plasma required is small, the details of the test are simple and conveniently carried out. (2) The pH of the plasma-veronal-glycerophosphate mixture remains practically constant at about 8.6. (3) The hydrolysis of the glycerophosphate is about twice as rapid as in Kay's procedure. (4) The reaction is retarded so little during the first 2 hours that the *initial* velocity may be calculated in terms of mg. of phosphorus liberated per cc. per hour without substantial error. (5) The phosphorus liberated by 1 cc. in 2 hours is in all cases ample for several aliquot determinations. (6) Plasma phosphatase tests of the same sample or of samples secured from the same subject 24 hours apart check very well; samples obtained at greater intervals may show differences, probably attributable to physiological variability.

With the above technique as a basis, empirical factors may be determined which allow the use of 0.2 cc. of plasma for a phosphatase determination with an accuracy of ±10% when larger quantities of plasma are not available.

5521

Deviation from the "Beer Law" in the Kuttner-Cohen Method for Determination of Phosphorus.

AARON BODANSKY, LOIS HALLMAN AND RISSEL BONOFF.

From the Laboratory Division of the Hospital for Joint Diseases, New York City.

Recently, when determinations of inorganic phosphate in mixtures containing sodium glycerophosphate (used as a substrate in plasma phosphatase determinations) were required, the use of heat involved in the Benedict-Theis method rendered it obviously unsuitable. The many advantages of the Kuttner-Cohen method,[1] with the modifications later suggested by Kuttner and Lichtenstein[2] and Raymond and Levene,[3] recommended themselves to us. We found this method capable of yielding very accurate results (within ±2% of the known quantities), after correction for the considerable deviations from the "Beer law". These deviations were determined by a large number of analyses, charts were constructed and tables compiled for more convenient reading.

[1] Kuttner, T., and Cohen, H. R., *J. Biol. Chem.*, 1927, **75**, 517.

[2] Kuttner, T., and Lichtenstein, L., *J. Biol. Chem.*, 1930, **86**, 671.

[3] Raymond, A. L., and Levene, P. A., *J. Biol. Chem.*, 1928, **79**, 628.