

Conclusions. Administration of a lens protein (*Beta crystallin*) by mouth following similar administration of ox-gall resulted, in rabbits, in the production of lens precipitins. Insulin similarly administered yielded negative results in 3 rabbits but gave positive shock effects in 5 out of 6 guinea pigs.

4138

A Method for Determination of Lipin Phosphorus.

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Interest in the *lecithin* content of blood in syphilis necessitated an investigation of the various procedures for the estimation of phosphorus¹⁻⁸ or more specifically, lipin phosphorus.^{2-6, 8} It was found that "test tube" oxidations by H_2SO_4 and HNO_3 had to be discarded. The danger of bumping with the small amount of reagents used became a source of great concern for micro-determinations.^{2-5, 7, 8, 11} This led to the use of H_2SO_4 and H_2O_2 (suggestions of Baumann¹¹ and Briggs³). Digestions were greatly facilitated, but certain difficulties were encountered. The figures obtained were not constant and often too high, the variations depending on the amount of H_2O_2 employed.⁶

After much manipulation, a satisfactory technique was evolved, which may be detailed as follows:

Blood (0.5 cc.) is pipetted into 10.0 cc. of an alcohol-ether mixture (3:1) contained in a 50 cc. volumetric flask, preferably fitted

¹ Bell, R. D., and Doisy, E. A., *J. Biol. Chem.*, 1920, **xliv**, 55.

² Bloor, W. R., *J. Biol. Chem.*, 1915, **xxii**, 133; 1916, **xxiv**, 447; 1915, **xxiii**, 317; 1918, **xxxvi**, 33.

³ Briggs, A. B., *J. Biol. Chem.*, 1924, **lix**, 255.

⁴ Greenwald, I., *J. Biol. Chem.*, 1915, **xxi**, 29.

⁵ Grigaut, M. A., *J. de Pharmacie et de Chimie*, 1925, 8th series, **i**, 97.

⁶ Krasnow, F., and Rosen, A. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1928, **xxv**, 352.

⁷ Pouget, I., et Chouchak, D., *Bull. Soc. chimique de Paris*, 1909, series **4**, **v**, 104.

⁸ Tisdall, F. F., *J. Biol. Chem.*, 1922, **1**, 329.

⁹ Randels, F. S., and Knudson, A., *J. Biol. Chem.*, 1922, **liii**, 53.

¹⁰ Whitehorn, J. C., *J. Biol. Chem.*, 1924, **lxii**, 133.

¹¹ Baumann, E. J., *J. Biol. Chem.*, 1924, **lix**, 667.

with a glass stopper, and brought to boiling by immersion in a water-bath. Digestion is continued for about 3 min. After cooling, alcohol-ether is added to the 50 cc. mark and the whole shaken vigorously. The filtrate (25 cc.) is transferred quantitatively to a casserol of 150 cc. capacity and evaporated to dryness over a water-bath. Then 10 cc. of HNO_3 (sp. gr. 1.42) is added and the dish rotated in such a way that the acid loosens the dried extract from the sides. 0.1 cc. H_2SO_4 (sp. gr. 1.84), 5 cc. KClO_3 (saturated solution) and 4 cc. of a mixture of saturated KNO_3 and a saturated solution of NaNO_3 (1:1) are introduced. The casserol is placed in the oven at 85-95° C. and digestion continued until the mixture becomes white. A safe interval is about 20 hours. A longer period does not matter. The white crystalline residue is dissolved in water by heating over a small free flame and transferred to a centrifuge tube (15 cc.). Including at least 2 washings, the total volume should not exceed 10 cc.

Following the Tisdall method,* the phosphate is precipitated with 1 cc. of the strychnine-molybdate reagent and stirred 3 times during 15 min. After being centrifuged for 3 minutes at high speed, the supernatant fluid is decanted and the precipitate washed twice with water, each time centrifuging one minute. 0.5 cc. NaOH (1%) is added and stirred until solution is complete. This is diluted with water to about 2.5 cc. and transferred to a 25 cc. volumetric flask fitted with a glass stopper. The centrifuge tube is washed twice with water, using 2.5 cc. each time and the washings added. 5 cc. $\text{K}_4\text{Fe(CN)}_6$ solution (20%) and 2.5 cc. HCl (sp. gr. 1.18) are introduced. The mixture is allowed to stand for 10 to 12 minutes and made up to volume with water. Readings are made immediately against a phosphate standard, prepared as directed by Tisdall. The formula for calculating the lipin phosphorus, P, in 100 cc. of whole blood is: $P = 20/R \times 5$ where 20 is the reading of the standard and R the reading of the unknown. To obtain the corresponding *lecithin* content, the value of P is multiplied by 25.

Tests have shown that the blood may be kept over night in the ice-chest and the procedure outlined above may be conveniently interrupted at any point except after the addition of the concentrated HCl. Continued study is indicating that only slight modifications will be necessary to enable us to apply this technique to the determination of total phosphorus in blood and other biological fluids.