

*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.

FRANCES KRASNOW AND A. S. ROSEN.

*From the Laboratory of Biological Chemistry of Columbia University at the College of Physicians and Surgeons.*

Interest in the *lecithin* content of blood in syphilis necessitated an investigation of the various procedures for the estimation of phosphorus<sup>1-8</sup> or more specifically, lipin phosphorus.<sup>2-6, 9</sup> It was found that "test tube" oxidations by  $\text{H}_2\text{SO}_4$  and  $\text{HNO}_3$  had to be discarded. The danger of bumping with the small amount of reagents used became a source of great concern for micro-determinations.<sup>2-5, 7, 8, 11</sup> This led to the use of  $\text{H}_2\text{SO}_4$  and  $\text{H}_2\text{O}_2$  (suggestions of Baumann<sup>11</sup> and Briggs<sup>3</sup>). 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  $\text{H}_2\text{O}_2$  employed.<sup>6</sup>

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

---

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

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

<sup>3</sup> Briggs, A. B., *J. Biol. Chem.*, 1924, lix, 255.

<sup>4</sup> Greenwald, I., *J. Biol. Chem.*, 1915, xxi, 29.

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

<sup>6</sup> Krasnow, F., and Rosen, A. S., *Proc. Soc. Exp. Biol. and Med.*, 1928, xxv, 352.

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

<sup>8</sup> Tisdall, F. F., *J. Biol. Chem.*, 1922, 1, 329.

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

<sup>10</sup> Whitehorn, J. C., *J. Biol. Chem.*, 1924, lxii, 133.

<sup>11</sup> 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  $\text{HNO}_3$  (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.  $\text{H}_2\text{SO}_4$  (sp. gr. 1.84), 5 cc.  $\text{KClO}_3$  (saturated solution) and 4 cc. of a mixture of saturated  $\text{KNO}_3$  and a saturated solution of  $\text{NaNO}_3$  (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,<sup>8</sup> 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.  $\text{NaOH}$  (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.  $\text{K}_4\text{FeCN}_6$  solution (20%) and 2.5 cc.  $\text{HCl}$  (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  $\text{HCl}$ . 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.