9233

Phosphorus Components in the Blood of Normal and Rachitic Infants.

HARRY BAKWIN, OSCAR BODANSKY AND REUBEN TURNER.

From the Children's Medical Service and Department of Pathology, Bellevue Hospital, and the Department of Pediatrics, New York University Medical College, New York.

The inorganic phosphorus of the blood in rickets has been studied at great length. In recent years comparable attempts[1-5] have been made to determine the acid soluble organic phosphorus compounds. These investigations have been handicapped by the fact that only adenosinetriphosphate[6] and diphospho-l-glyceric acid[7] have been isolated from blood and that such isolations do not as yet permit a quantitative estimation to be based on them. Consequently, indirect methods for the estimation of the organic phosphorus compounds in blood have been attempted, based upon the comparison of the rate and extent of the acid and phosphatase hydrolysis of blood filtrates and of such compounds as have been shown or are believed to exist in the blood. In view of the growing evidence, especially that recently submitted by Kerr and Daoud[8] and by Warweg and Stearns,[9] that organic phosphorus entities, calculated on the basis of such work, probably represent definite organic phosphorus compounds, a series of values are presented for groups of normal and rachitic children.

1. Fraction hydrolysable in 1 N acid (sulphuric or hydrochloric) in 10 minutes at 100°. Under these conditions, diphospho-l-glycerate and hexosediphosphate are hydrolyzed to a negligible extent.[9] Adenosine triphosphate from muscle[10, 11] or rabbit's blood[6] yields

1 Zucker, T. F., and Gutman, M., PROC. SOC. EXP. BIOL. AND MED., 1923, **20**, 372.

2 Kay, H. D., and Robison, R., *Biochem. J.*, 1924, **18**, 755.

3 Barrenscheen, H. K., and Vasarhelyi, B., *Biochem. Z.*, 1931, **230**, 330.

4 Bakwin, H., Bodansky, O., and Turner, R., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1238.

5 Stearns, E., and Warweg, G., *Am. J. Dis. Child.*, 1935, **49**, 79.

6 Fiske, C. H., *Proc. Nat. Acad. Sci.*, 1934, **29**, 25.

7 Greenwald, I., *J. Biol. Chem.*, 1925, **63**, 339.

8 Kerr, S. E., and Daoud, L., *J. Biol. Chem.*, 1935, **109**, 301.

9 Warweg, G., and Stearns, E., *J. Biol. Chem.*, 1936, **115**, 567.

10 Fiske, C. H., and Subbarow, Y., *Science*, 1929, **70**, 381.

11 Unpublished data. The specimen of adenosinetriphosphate was kindly supplied by Dr. Cyrus H. Fiske.

two-thirds of its phosphorus. The probability is that the phosphorus liberated from a trichloracetic acid filtrate represents two-thirds of the adenosine triphosphate phosphorus present.

2. Fraction not hydrolysable by bone phosphatase at pH of about 9.0 in a 4% concentration of trichloracetic ion. Under these conditions bone phosphatase does not hydrolyse diphospho-l-glycerate[12] but hydrolyses adenosinetriphosphate and sodium hexosediphosphate, the former to about 80%, the latter completely.[11] In addition, the concentration of this non-hydrolysable fraction parallels the amounts of diphospho-l-glycerate which Greenwald[7] obtained from different species. The phosphorus not liberated under these conditions probably represents, therefore, to within about 5% of the absolute value, the phosphorus from diphospho-l-glyceric acid.

Blood specimens were drawn between 3 and 5 hours after the morning meal. One volume of blood was added without the addition of an anticoagulant to 4 volumes of 10% trichloracetic acid and filtered within 10 minutes. Inorganic phosphorus, total and acid soluble phosphorus were determined by the method of Fiske and Subbarow. The readily acid hydrolysable phosphorus was determined by adding 2 cc. of trichloracetic acid filtrate to 2 cc.. of 2 N HCl, heating in a water bath at 100° for 10 minutes,[3, 4] and noting the inorganic phosphorus liberated. Values for concentration in the red blood cells were calculated.

Fraction Hydrolysable by Bone Phosphatase. To 5 cc. of the trichloracetic acid filtrate 2 cc. of 1 N NaOH and 2 cc. of 10% sodium diethylbarbiturate were added. One cc. of phosphatase extract[13] was added and the pH adjusted to 8.8 to 9.0. The mixtures were allowed to stand at room temperature with toluene until equilibrium had been reached as indicated by the constancy of the inorganic phosphorus readings (1 to 2 weeks).

The findings in normal and rachitic children are compared in Table I. The acid insoluble phosphorus is unchanged. In infants with rickets the acid soluble phosphorus is reduced. The inorganic phosphate, the readily acid hydrolysable phosphorus,[4] and the component not hydrolysable by bone phosphatase all participate in this reduction. The differences between the 2 groups for both whole blood and red blood cells are statistically significant.

The mean value for the readily acid hydrolysable phosphorus obtained in this study (4.9 mg. P per 100 cc. for normal infants) is in the same range as the values obtained in adults by Barrenscheen and Vasarhelyi (5.7 mg. per 100 cc.) and Kerr and Daoud

12 Bodansky, O., and Bakwin, H., *J. Biol. Chem.*, 1934, **104**, 747.

13 Bakwin, H., and Bodansky, O., *J. Biol. Chem.*, 1933, **101**, 641.

TABLE I.
Phosphorus Components in the Whole Blood and Red Blood Cells of Normal and Rachitic Infants.

	Whole Blood						Red Blood Cells					
	Non-Rachitic			Rachitic			Non-Rachitic			Rachitic		
	No. of cases	Mean and P.E.* mg. %	Standard Deviation mg. %	No. of cases	Mean and P.E.* mg. %	S.D. mg. %	No. of cases	Mean and P.E.* mg. %	Standard Deviation mg. %	No. of cases	Mean and P.E.* mg. %	S.D. mg. %
Total Phosphorus	41	38.8±.58	5.5	32	34.6±.42	3.5	—					
Acid Insoluble	41	12.5	—	32	12.2	—	—					
" Soluble	56	26.3±.43	4.8	35	22.4±.32	2.8	35	67.5±1.18	10.3	30	60.0±1.01	8.2
Inorganic†	56	4.4±.06	0.7	37	2.5±.10	0.9	34	2.4±0.15	1.3	32	0.9±0.13	1.1
Organic	54	22.1±.28	3.0	34	20.0±.30	2.6	35	64.7±1.11	9.7	34	58.4±0.77	6.6
Readily Acid Hydrolysable†	55	4.9±.07	0.8	36	4.0±.09	0.8	45	14.0±0.28	2.8	36	11.8±0.24	2.1
Not Hydrolysable by Bone Phosphatase	36	14.0±.31	2.8	26	11.9±.35	2.6	35	40.6±1.06	9.3	26	33.9±0.89	6.7
Hematocrit %	47	35.0±.58	5.9	36	34.0±.46	4.1						
		Serum Inorganic Phosphate										
	39	5.3±.09	0.8	33	3.2±.12	1.0						

*P.E. means probable error.
†These groups include values previously reported.[4]

(5.4 mg. P per 100 cc.). Warweg and Stearns obtained a mean value of 2.4 mg. P for this fraction.

The fraction not hydrolysable by bone phosphatase, probably diphosphoglycerate, is 63.4% of the ester phosphorus in the normal and 64.6% in the rachitic group; these values agree well with the "phosphoglycerate" fraction as determined by Warweg and Stearns (mean, 68% of the ester phosphorus in adult man).

The residual phosphorus, after the readily acid hydrolysable and the "phosphoglycerate" phosphorus are subtracted from the ester phosphorus, is considered by Warweg and Stearns to represent phosphorus of hexosephosphate nature. Their estimate for this fraction is 20.6% of the ester phosphorus. They do not consider Fiske's isolation of adenosinetriphosphate from rabbit's blood and his findings that two-thirds of this phosphorus is readily hydrolysed. Kerr and Daoud[8] found that the ratio of the readily hydrolysable phosphorus to the nucleotide N was the same in the trichloracetic acid filtrate of human blood as in adenosinetriphosphate, indicating that the latter compound was present. Since the value given by Warweg and Stearns for the readily acid hydrolysable phosphorus seems too low, and since they do not consider the probability that it is a portion of the adenosinetriphosphate molecule, it would appear that their estimate for the hexosephosphate fraction is too high. According to our calculations, the residual phosphorus, probably hexosephosphate, is about 5% of the ester phosphorus; this value is much more in accord with the figure of Kerr and Daoud and the estimates of Mai[14] and Lawaczek.[15]

Summary. 1. The phosphorus of the blood was fractionated by a combination of acid and phosphatase hydrolysis and the results compared in normal and rachitic infants. 2. In infants with rickets there is a reduction in the acid soluble phosphorus of the blood which is made up of decreases in the inorganic phosphate, the readily acid hydrolysable phosphorus, and the fraction not hydrolysable by bone phosphatase under stated conditions. 3. The chemical nature of these fractions is discussed.

14 Mai, H., *Z. Kinderheilk.*, 1928, **45**, 653.

15 Lawaczek, H., *Biochem. L.*, 1924, **145**, 351.