fraction after alkaline hydrolysis. It is not an organic base of the type described as occurring in cod-liver oil. It is not cholesterol, but similar to cholesterol in its solubilities. The suggestion is made that it may be a sterol related to cholesterol or a cholesterol derivative. The fatty acids of cod-liver oil are entirely inactive in curing rickets.

78 (1825)

The distribution of inorganic phosphate of the blood between plasma and cells.

By T. F. ZUCKER and MARGARET B. GUTMAN.

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

Since Greenwald's work on the organic acid soluble phosphorus our knowledge of the various phosphorus compounds in the blood is steadily increasing and acquiring significance. The determination of the inorganic blood phosphate, however, has been very questionable, particularly in corpuscles, due to the ease with which the organic acid soluble phosphate is hydrolyzed. Any method which requires considerable time or in which the phosphate has to be precipitated, or in which the red cells are washed or otherwise manipulated, comes very gravely under suspicion of having allowed a significant amount of hydrolysis to take place.

When working only with plasma these precautions are not so necessary. Bloor's[1] figures for inorganic phosphate in the corpuscles are admittedly high. A method very well suited to estimation of inorganic phosphate is that of Bell and Doisy[2] in which the color of the blue reduction product of phosphomolybdic acid is measured as in Folin's uric acid and phenol determintion, the limiting factor, however, being the phosphate.

Our results have been briefly as follows:

When working rapidly with the Bell and Doisy method, the inorganic phosphate in the plasma and the whole blood is the same within the limit of error of the method. The few exceptions to this were traced to improper handling of the blood or too long a time elapsing before the determination. Even here the whole

[1] Bloor, *Jour. Biol. Chem.*, 1918, xxxvi, 49.

[2] Bell and Doisy, *Jour. Biol. Chem.*, 1920, xliv, 55.

blood gave figures much lower than Bloor's. This shows that the phosphate ion in its relation to cells and plasma behaves differently from all the other ions studied in this respect. The chloride, for instance, is never present in cells and plasma in the same concentration. This exceptional role of the phosphate ion is, however, not so surprising when we consider the organic acid soluble phosphate, in a sense the counterpart of the chloride combined with protein.

TABLE I.

DISTRIBUTION OF INORGANIC PHOSPHATE BETWEEN WHOLE BLOOD AND PLASMA.

Adults.			Children.		
Subject.	Whole Blood.	Plasma.	Subject.	Whole Blood.	Plasma.
P.G. 1.....	3.5	3.4	R.M. 1.....	3.0	3.01
P.G. 2.....	3.52	3.52	R.M. 2.....	3.02	2.78
G.M. 1.....	4.0	3.78	M.E.....	4.0	4.01
G.M. 2.....	3.98	3.94	H.R.....	4.61	4.63
J.G.....	3.1	2.8	D.B.....	4.17	4.26
E.G.....	3.38	3.36	R.M.....	4.10	4.08
T.Z.....	3.51	3.50	F.B.....	3.75	3.49
			M.L.....	3.16	3.18
			A.A.....	4.17	4.12
			A.S.....	4.25	4.35

A conclusion of practical import is that when inorganic phosphate is to be determined in blood, it is immaterial whether it is done on whole blood or plasma, providing it is done immediately by the method of Bell and Doisy.

If the colorimetric reading is made within half an hour after the blood is drawn, whole blood is no higher than plasma, but if an hour elapses the whole blood is higher by about 0.5 mg.; after several hours the difference is a milligram or more.

It seems reasonable enough to assume that if at the time the blood is drawn the phosphate ion is always present in equal concentration inside and outside of the cell, it is freely diffusible into and out of the cell when the blood circulates. Iverson [1] has shown that phosphate in organic acid soluble form can accumulate in the corpuscle at a good rate if inorganic phosphate is injected or added to blood. We would, therefore, expect that the phosphate formed by hydrolysis would diffuse out of the cells into

[1] Iverson, *Biochem. Zeit.*, 1921, cxiv, 297.

the plasma. This seems not to be the case in the blood after it is taken from the body.

TABLE II.

TO ILLUSTRATE EFFECT OF TIME AFTER DRAWING BLOOD ON THE INORGANIC PHOSPHATE.

	Within ½ Hr.	Within 1 Hr.	After 5 Hrs.	After 20 Hrs.
I. Plasma.....		2.4	2.5	
Blood.....	2.5	2.8	3.3	
II. Plasma.....		2.55		2.53
Blood.....	2.6	3.08		4.02

These results lead to the following conception of the rôle of inorganic and organic acid soluble phosphorus in the blood: The red cell is totally permeable to the phosphate ion, *i.e.*, no "osmotic influences" control the distribution of the phosphate ion inside and outside of the cell. Phosphate ions can be taken up by the cell and stored as organic acid soluble phosphate (Iverson). This organic phosphate is hydrolyzed very easily when there is need for phosphate ion in the plasma, similar to the liver glycogen yielding blood sugar. The diffusion of the phosphate out of the cell, however (at least in vitro), is slower than its rate of formation by hydrolysis. To substantiate this view we will still have to show under what conditions inorganic phosphate can diffuse out of the cell. We have no indication so far that phosphate distribution is influenced by the CO_2 tension. We are now collecting data on this point, as well as on the whole subject from the point of view of the acid soluble phosphate.

79 (1826)

Observations on the inorganic phosphate of blood in experimental rickets of rats.

By **M. B. GUTMAN** and **V. KNEELAND FRANZ** (by invitation).

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

The work of Howland and Kramer[1] on the level of the inorganic phosphate in the blood in human rickets, and some con-

[1] Howland, J., and Kramer, B., *Amer. Jour. Dis. of Child.*, 1921, xxii, 105.