VI. THE INORGANIC PHOSPHORUS CONTENT OF THE BLOOD OF NORMAL CHILDREN

PRELIMINARY COMMUNICATION

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ATTENTION has recently been directed by Howland and Kramer [1921] to the fact that the inorganic phosphorus content of the serum is lower in rachitic than in normal children. Hess has confirmed this finding [Hess and Unger, 1922], but states [Hess and Lundagen, 1922] that in some cases in which the presence of rickets was undoubted he found no reduction. He has further called attention to the fact that a slight seasonal variation of the amount of inorganic phosphorus in the blood of healthy children occurs, the highest figures being obtained in July, and the lowest in March. György [1922] and von Meysenbug [1922] have also published figures showing the reduction of inorganic phosphorus in the blood of rachitic infants. Furthermore, considerable attention is at present directed towards the question of the possibility of producing rickets in rats by a deficiency of phosphoric acid in the diet. It seemed desirable to repeat and extend these observations, and the prevalence of rickets in the slum districts of Glasgow from which our hospital patients are drawn has been taken advantage of to prosecute such a study. This investigation is still in progress.

One of the first essentials in undertaking an investigation like the present one is, of course, a knowledge of the amounts of inorganic phosphorus to be found, and the range of physiological variation occurring in the blood of healthy children. Table I shows the results of other investigators.

Table I.

Authors	Inorganic phosphorus as mg. per 100 cc.		
	Whole blood	Plasma	Serum
Howland and Kramer [1921]	—	—	4·5 to 6·8
Tisdall [1922]	—	—	4·6 to 6·4
György [1922]	5·2	—	—
Hess and Unger [1922]	4·0 to 4·8	—	—
Von Meysenbug [1922]... ..	—	—	4·1 to 5·8
McKelleps, De Young and Bloor [1921] ...	—	1·2 to 4·4	—

The results agree fairly closely and indicate that the range of variation, if one excludes the very low figure of 1.2 given by McKelleps, De Young and Bloor, is 4.0 to 6.8 mg. per 100 cc.

In the course of a series of estimations of the phosphorus contents of the blood and serum for comparison with rachitic figures, one or two interesting points emerged and are therefore recorded in this preliminary report.

EXPERIMENTAL ERROR.

The method used throughout these experiments is that of Bell and Doisey [1920]. Marriott and Haessler's technique [1917], as well as that of Tisdall [1922] and that of Bloor [1918] take much longer to complete without giving more accurate results.

Myers and Shevsky [1921] have recently criticised the method of Bell and Doisey and state that more accurate results are obtained when the standard contains not over 0.25 % more phosphorus than the specimen to be examined. They further observed that blood sometimes requires more of the molybdic acid and quinol solution than does serum. For this reason the standards used throughout this investigation are as far as possible made up to contain a quantity of phosphorus similar to that of the blood sample to be tested, and for whole blood double the quantities of molybdic acid and quinol advocated by Bell and Doisey are used.

In order to gain an estimate of the experimental error involved in the use of the method 14 consecutive estimations on the same blood sample were carried out. In each case 5 or 10 cc. of lysed diluted dog's blood were separately treated and subjected to colorimetric comparison with a freshly made up standard. The calculated values of phosphorus per 100 cc. of the unknown are given in Table II.

Table II.

	Quantity of diluted blood used	Estimated values of P per 100 cc. of diluted blood
	cc.	mg.
1	10	1.7
2	10	1.9
3	10	2.0
4	5	1.8
5	10	2.1
6	10	2.0
7	5	1.8
8	5	1.8
9	10	2.0
10	10	1.9
11	10	1.9
12	5	1.9
13	10	2.1
14	10	1.7
Average 1.9		+0.2 -0.2

From the series of figures it is estimated that the error is plus or minus 0.2 when the average is 1.9, or roughly 10%. This is therefore taken as the margin of experimental error.

THE DISTRIBUTION OF INORGANIC PHOSPHORUS IN THE BLOOD.

An interpretation of the published results of inorganic phosphorus estimations in normal children is, as in the case of calcium, rendered somewhat difficult in view of the fact that in some cases serum, in others plasma, and in still others whole blood has been used, since our knowledge of the distribution of the inorganic salts is at best very incomplete.

Howland and Kramer [1921] pointed out that a definite increase in the phosphorus of the serum results if the latter is allowed to stand in contact with the clot at room temperature for any length of time. This introduces a grave source of error in serum estimations and renders any investigation into the distribution of the inorganic phosphorus between corpuscles and plasma particularly difficult. The error which may result from this change is illustrated by two different series of blood and serum estimations in normal children. In every case the whole blood was immediately shaken up in 15 cc. of distilled water, 5 cc. of trichloroacetic acid added and the flask vigorously shaken and allowed to stand ten minutes before filtering off the protein. In the first series of cases no time limit was set for the separation of the serum from the clot though all were ready for filtration within three hours of the collection of the blood. The blood samples of the second series were subjected to 40 minutes' centrifugalisation immediately after venipuncture and the serum was then quickly poured off. The results are collected in Tables III and IV.

Table III. Series 1.

Blood P estimated immediately and serum P estimated after standing from 1½ to 3 hours in contact with the clot.

Number	Age (years)	Sex	Inorganic P of whole blood mg. per 100 cc.	Inorganic P of serum mg. per 100 cc.
1	8	Male	5.1	6.1
2	8	"	5.4	7.2
3	7	"	—	6.0
4	9	"	5.1	5.5
5	10	"	4.8	6.0
6	8	"	4.0	4.8
7	12	"	—	6.8
8	13	"	4.8	6.5
			Average 4.86	Average 6.1

Range of variation 4.0 to 5.4 Range of variation 4.8 to 7.2

Table IV. Series 2.

Inorganic P of whole blood estimated immediately. Serum in every case separated from clot within 40 minutes of venipuncture.

Number	Age (years)	Sex	Inorganic P of whole blood	Inorganic P of serum
1	3	Female	—	4.6
2	5	Male	4.2	4.2
3	6	"	4.0	4.2
4	6	"	4.8	5.1
5	7	"	4.2	4.4
6	9	"	5.1	5.4
7	10	"	4.8	4.7
8	11	"	5.1	5.1
			Average 4.6	Average 4.7

Range of variation 4.0 to 5.1 Range of variation 4.2 to 5.4

It is quite evident that all the serum results of Series 1 are higher than those of Series 2 and since the estimations were carried out within a few days of each other and the same standard solutions employed, one can only conclude that the difference must be ascribed to the greater length of time taken to separate the serum in the first series.

The fact that the serum phosphorus is so variable, therefore, seems to render it unsuitable for comparative estimations, particularly since we know little of the changes which may be taking place during the 30 minutes which must of necessity elapse before any quantity of serum can be separated off.

Denis and von Meysenbug [1922] have criticised the use of plasma on the ground that they found citrate and oxalate to be a source of considerable error in the use of the Bell-Doisey method.

From a consideration of these points it appears that whole blood is most suitable for use in estimating the inorganic phosphorus, for though a definite increase also occurs in the lysed blood on standing each specimen can be subjected to exactly the same procedure immediately it is withdrawn from the vein.

A study of the figures given in Table IV leaves no doubt that the whole blood if used at once gives exactly the same results as serum which has been separated from the clot within 40 minutes. This leads one to suppose that the corpuscles contain exactly the same amount of inorganic phosphorus as the serum, or that, if the corpuscles are richer in this element than the serum or plasmas, as suggested by Bloor [1918], a very rapid change in the distribution of the blood phosphates must take place during the process of clotting. As a standard for comparison with the rachitic figures the collected results for the whole blood of all the normal children available are grouped in Table V.

Table V.

Number	Date 1922	Sex	Age	State of health and nutrition	Inorganic P of whole blood in mg. per 100 cc.
1	July	Male	9 mths.	Normal	5.2
2	"	Female	5 yrs.	"	4.2
3	"	Male	6 "	"	4.0
4	"	"	6 "	"	4.8
5	"	"	7 "	"	4.2
6	"	"	8 "	"	5.1
7	"	"	8 "	"	4.0
8	Aug.	"	9 mths.	"	6.6
9	"	"	9 yrs.	"	5.1
10	"	"	9 "	"	5.1
11	"	"	10 "	"	4.8
12	"	"	10 "	"	4.8
13	"	"	11 "	"	5.1
14	"	"	12 "	"	5.3
15	"	"	13 "	"	4.8
16	Oct.	"	15 mths.	"	4.1
17	"	"	14 "	"	4.8
18	Nov.	"	8 "	"	5.0
19	"	"	8 "	"	6.6
20	"	"	14 "	"	6.2
21	Dec.	"	3 "	"	5.5
22	"	Female	2½ yrs.	(Convalescent pneumonia)	4.0
23	"	Male	3 mths.	Normal	4.7

Average = 4.9

Range of variation 4.0 to 6.6

The results in Table V show that the average normal phosphorus content of the blood in children is 4.9 mg. per 100 cc. The range of variation allowed for by a probable experimental error of 10 % is 4.4 to 5.4, whereas the actual range is 4.0 to 6.6. The variation in the normal amount of inorganic phosphorus in the blood is therefore not entirely due to experimental error and no figures between 4.0 and 6.6 mg. per 100 cc. can be regarded as abnormal. Children under two years undoubtedly give slightly higher results than those over three years, while in two adults whose blood has been examined the results are still lower than those obtained from the older children. That is to say, the inorganic phosphorus of the blood appears to fall with increase in age, as pointed out by Howland and Kramer, but the seasonal variation described by Hess and Lundagen [1922] has not been apparent from the figures so far available.

EFFECT OF FEEDING ON THE NORMAL BLOOD PHOSPHORUS CONTENT.

In the case of the younger children some were breast fed and some bottle fed on different diets, but there appeared to be no connection between the type of feeding and the phosphorus values obtained. The question as to whether an increase in the inorganic phosphorus may result from the recent consumption of phosphorus-rich diet is, however, one which must be raised, since it seems not improbable that the slight variations obtained may be due to differences in the stage of digestion. For the purpose of throwing light on this problem the following experiment is recorded. In the course of a parathyroidectomy investigation carried out by Professor Noël Paton and Mr Watson, whom I have to thank for permission to publish this result, the latter took from the jugular vein of a dog samples of blood, (a) after a period of fasting, and (b) at increasing intervals after the ingestion of a quantity of lean meat.

The results of the experiment are tabulated below.

Table VI.

	Time	Remarks	Inorganic P of whole blood in mg. per 100 cc.
Exp. 1	9.30 a.m.	After 15 hours' fasting	2.8 %
	11.30 a.m.	" 17 " " "	2.9
	2.30 p.m.	" 20 " " "	3.0
	6.30 p.m.	" 24 " " "	2.8
Exp. 2	1 p.m.	No food for 6 hours. Given 200 g. lean meat	—
	2 p.m.	1 hour after food	2.97
	3 p.m.	2 hrs. " "	3.17
	4 p.m.	3 hours " "	2.97

The above figures show that the amount of inorganic phosphorus in the blood is uninfluenced by fasting for 24 hours and by the recent ingestion of a quantity of food rich in phosphorus.

CONCLUSIONS.

1. The range of experimental error in the Bell-Doisey method of estimating the inorganic phosphorus of the whole blood was found to be not more than plus or minus 10 %.

2. The inorganic P content of the whole blood of 24 normal children between 3 months and 13 years of age ranges between 4.0 and 6.6 mg. per 100 cc. Average 4.9.

3. Blood and serum give the same results provided the blood protein is precipitated immediately and the serum is separated from the clot within 40 minutes.

4. The use of serum which has been allowed to stand in contact with the clot for longer periods may be a source of considerable error. It is therefore better to use the whole blood.

5. Twenty-four hours' fasting and the recent ingestion of a diet rich in phosphorus failed to produce any demonstrable variation in the inorganic blood phosphorus content of a normal dog.

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