XCII. THE USE OF SODIUM FLUORIDE AS A BLOOD ANTICOAGULANT IN BLOOD PHOSPHORUS DETERMINATIONS.

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(Received January 29th, 1935.)

FOR estimating the acid-soluble phosphorus fractions of the blood—especially the inorganic blood phosphorus—we possess an excellent colorimetric technique of a simple character; in capable hands the experimental error should not be more than 3 %.

The real difficulty in blood phosphorus estimations is that the several acid-soluble fractions are extremely unstable. The organic acid-soluble compounds are rapidly hydrolysed in shed blood; when blood is allowed to stand without separating the red blood cells from the plasma a rapid and great increase of inorganic phosphorus results. But even if the red blood corpuscles are separated immediately after the withdrawal of the blood an increase in the plasma inorganic phosphorus is to be expected, as blood plasma contains an amount of ester-phosphorus corresponding to 0.2–0.5 mg. P per 100 ml. of blood; in some cases even 1.0–2.0 mg. P per 100 ml. of plasma can be found without any appreciable haemolysis. If blood becomes more alkaline ester synthesis is possible from inorganic phosphorus, resulting in a fall of the inorganic phosphorus content; this is liable to happen if blood is strongly shaken to defibrinate it: much CO_2 is lost and the blood becomes more alkaline.

The hydrolysis of ester-phosphorus takes place both in serum and in plasma of blood to which oxalate, citrate, hirudin or heparin is added as anticoagulant¹, as Table I shows.

Table I. *Inorganic blood phosphorus in mg. per 100 ml. of blood.*

	Oxalate		Citrate		Hirudin	
Immediate determination	3.26	2.86	3.29	3.20	4.10	3.13
After 1 hour	3.45	3.10	—	3.33	4.20	—
After 5 hours	—	3.28	3.73	3.60	—	3.42

Blood standing at room temperature.

It is obvious, and this fact is well known to all workers on phosphorus metabolism, that only determinations done in blood immediately after the blood has been collected have any value. In scientific work this is no great objection, but in clinical work this fact offers a great difficulty. Many physicians are unable to do an analysis immediately after the withdrawal of the blood, and in many cases it is impossible even to keep the blood at freezing temperature because it must be sent to a laboratory somewhere else. Many determinations which physicians out of town ask us to do give useless results for this reason.

¹ Heparin is unsuitable for blood in which phosphorus must be determined as it contains phosphorus.

The impression is often held that there is no longer any risk of an undue increase of inorganic blood phosphorus if blood or plasma has been precipitated with trichloroacetic acid, and that the filtrate obtained in this way can be kept for some time, as the strong acid should prevent all phosphatase action. This is not true: the same hydrolysis will occur in the acid protein-free filtrate. Table II illustrates this fact; the figures are taken from experiments of Byrom and of myself [Byrom, 1931].

Table II. *Inorganic phosphorus in protein-free filtrate after precipitation with 20 % trichloroacetic acid (mg. per 100 ml. of blood).*

Byrom:	Room temperature	38°
0 hours after precipitation	4.89	4.89
5 " "	5.06	—
24 " "	5.62	6.50
48 " "	5.82	7.10
Burkens:		
0 hours after precipitation	2.80	2.80
5 " "	3.58	3.71
24 " "	3.85	4.70

In blood, serum, plasma or protein-free filtrates, estimations give quite unreliable results after a few hours. Yet this difficulty can be avoided quite easily by using sodium fluoride to prevent the clotting of the blood instead of the ordinary anticoagulants.

Sodium fluoride has a double action on the blood: it prevents clotting when added in the same concentration as that usually employed with oxalate; and it prevents all phosphatase action. The result is that blood, when treated with sodium fluoride, can be kept for days without any hydrolysis or synthesis of ester-phosphorus occurring. The amount of inorganic phosphorus of blood or plasma remains entirely unchanged, as do the relative amounts of the several acid-soluble fractions. This is even the case if the blood is laked.¹

10 mg. of sodium fluoride as powder were added per 5 ml. of blood.

One must be sure that the adding of sodium fluoride does not have more influence on the development of the colour in the colorimetric analysis than has oxalate. Phosphorus estimations made simultaneously in serum and in plasma of blood after adding oxalate or fluoride give identical results (Table III).

Table III. *Inorganic phosphorus in serum and plasma of blood to which oxalate or fluoride had been added (mg. per 100 ml.).*

Serum	2.92	3.65	3.08
Oxalate-plasma	2.95	3.60	3.15
Fluoride-plasma	2.90	3.60	3.15

Table IV shows that keeping the plasma for hours at 38° causes no increase in the amount of inorganic phosphorus if sodium fluoride has been added. A protein-free acid filtrate of blood can also be kept. Of course evaporation must be prevented.

After addition of sodium fluoride as a blood anticoagulant, blood or plasma can be despatched without running any risk of obtaining faulty results. If it is advisable to perform the estimation on serum, sodium fluoride can be added

¹ If laked blood is kept for hours at 38° ultimately ester hydrolysis occurred.

after the separation of the serum. Blood, plasma or serum can be kept in this way till ultimately (after several days) the growth of bacteria in some cases will cause decrease of the amount of inorganic phosphorus.

Table IV. *Inorganic blood phosphorus (mg. per 100 ml. of plasma).*

Fluoride-plasma:

Immediate determination	3.52	3.20	2.78	2.68	4.89	3.20
After 5 hours at room temperature	3.54	3.21	—	—	—	—
After 48 hours at room temperature	—	—	—	2.73	4.97	—
After 16 hours at 38°	—	—	2.83	—	4.97	3.15

Protein-free filtrate of fluoride-plasma:

Immediate determination	3.50	2.85	4.95	—	—	—
After 5 hours at room temperature	3.50	—	4.95	—	—	—
After 16 hours at 38°	—	2.80	4.95	—	—	—

Sodium fluoride is not very soluble in water and therefore it is impossible to prepare a concentrated (*e.g.* 20 %) solution. It is necessary to add the sodium fluoride as powder: 10 mg. per 5 ml. of blood. A little more or less makes no difference. Very large amounts of sodium fluoride prevent all development of colour in the colorimetric phosphorus analysis, exactly as is the case with high concentrations of oxalate or citrate. A 2 % solution of sodium fluoride in distilled water can also be used: 1 part to 9 parts of blood. In that case there arises some difficulty in calculating the dilution of the blood. If the analysis is performed in whole blood the dilution is 1:10 and the amount found must be multiplied by 10/9; if the estimation is made in plasma it is necessary to reckon with the fact that only the blood plasma is diluted. Actually it would be necessary in cases where a 2 % sodium fluoride solution is used instead of the powder to determine the relative volume of the plasma before and after the adding of the sodium fluoride. However, I found that if the blood had a normal or nearly normal ratio of the relative volumes of corpuscles and plasma the addition of one-tenth part of a 2 % solution of sodium fluoride results in a dilution of the plasma of 14–16 %. By multiplying the value found by 100/85 the real amount is estimated fairly accurately.

SUMMARY.

On standing an increase in inorganic blood phosphorus is found in serum or in plasma of blood to which oxalate, citrate or hirudin has been added to prevent clotting; in the acid protein-free filtrate of blood, plasma or serum the same increase occurs. Adding sodium fluoride prevents clotting of the blood and also all phosphatase action. Ester-phosphorus hydrolysis and synthesis do not occur and correct values for inorganic phosphorus are ensured, even when the blood or plasma is kept for hours or days at room temperature or at 38°. When the correct amount of sodium fluoride (10 mg. per 5 ml. of blood) is used no influence is found on the colour development in the analysis.

REFERENCE.

- Byrom (1931). The clinical significance of blood phosphorus. Clinical interpretation of aids to diagnosis. (London.)