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Comparison of Inorganic Phosphate Contents of Serum, Fluoride Plasma and Native Plasma.†

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In the course of work on the clearances of various urinary constituents (White and Monaghan[1]) we noted a difference between the inorganic phosphate values of serum and of fluoride plasma, where 0.25 to 0.3% NaF was used as anticoagulant. A few weeks later Gaebler[2] reported that 4.2% NaF inhibited or abolished the color production with the Benedict-Theis[3] or the Fiske-Subarrow[4] method and that this interference could be avoided by adding $AlCl_3$. Gaebler gives no data on the extent to which 1% NaF, the concentration which he used as anticoagulant, influences color production in the phosphate methods. Since, however, the lower phosphate content of fluoride plasma as compared with serum corresponded roughly with the extent to which the total nitrogen of the plasma was less than that of serum, it might be inferred that 1% NaF interferes little and that the lower phosphate content of the plasma is due in the main to its dilution by the osmotic effect of the fluoride.

* P represents a preliminary, C a complete manuscript.

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1 White, H. L., and Monaghan, B., *Am. J. Physiol.*, 1933, **104**, 412.

2 Gaebler, O. H., *J. Biol. Chem.*, 1932, **99**, 99.

3 Benedict, S. R., and Theis, R. C., *J. Biol. Chem.*, 1924, **61**, 63.

4 Fiske, C. H., and Subarrow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

His results may have been somewhat complicated by the facts that the serum lacked fibrinogen nitrogen and that 1% NaF caused some hemolysis. Gaebler also reported that in 7 cases out of 8 serum separated after the blood had stood 30 minutes at about 25° showed a higher inorganic phosphate content than did native plasma separated immediately, while oxalate plasma always showed a lower phosphate content than native plasma. He suggests that serum is less suitable than native plasma for inorganic phosphate determinations.

We have (1) investigated the effect upon the color production of the addition of 0.3, 0.6, 0.9, and 1% NaF to known phosphate solutions and of 0.3% NaF to serum, (2) compared the inorganic P (Benedict-Theis method) and total N (macro Kjeldahl) contents of 0.3% fluoride plasma and of native plasma, both separated immediately, and in another set of experiments added hematocrit readings, substituting heparin or hirudin for native plasma, and (3) compared the inorganic P contents of serum and of native plasma, both separated immediately.

In the first case 5 cc. of phosphate standard containing 0.05 mg. P was compared with the same standard plus 3, 6, 9, and 10 mg. NaF. With the standard set at 20. the standard plus 3 mg. NaF read in duplicate 20.0 and 20.1, standard plus 6 mg. NaF 20.0 and 19.8, standard plus 9 mg. NaF 20.3 and 20.3, and standard plus 10 m. NaF 20.4 and 20.7. A dog serum gave in duplicate 4.14 and 4.14 mg. P per 100 cc. while with 0.3% added NaF it gave 4.06 and 4.15 mg. P per 100 cc.; a beef serum (in the refrigerator several days and showing considerable hemolysis had 7.82 and 7.70 mg. P per 100 cc. while with 0.3% NaF added it had 7.70 and 7.70 mg. per 100 cc. It is evident that the inhibiting effect of NaF is just beginning to manifest itself at a concentration of 0.9 or 1%.

To carry out the second and third comparisons referred to in the second paragraph we anesthetized dogs with sodium barbital and put a paraffined cannula into either a femoral or a carotid artery. At each experiment 3 blood samples were drawn within a few seconds. After washing out dead space blood one sample was received into a paraffined tube, one into a plain tube and one (15 cc.) into a tube containing 45 mg. NaF. All 3 were in the centrifuge in less than 2 minutes after being drawn and were centrifuged at 3000 r.p.m. for 5 minutes. With reasonable care in paraffining no difficulty was experienced in getting native plasma entirely free from coagulation. No hemolysis occurred in any samples of serum, fluoride plasma or native plasma. Phosphate determinations in

duplicate were carried out by the Benedict-Theis method on all 3 samples immediately after centrifugation; in the last 7 experiments total N determinations were also carried out on native and fluoride plasma, 2 cc. being used for a macro Kjeldahl. Not all of the N determinations reported in Table I were made in duplicate; the N figures given are the averages of duplicates when these were run.

TABLE I
Mg. per 100 cc. average of duplicate determinations.

Exp. No.	Serum P	Native Plasma P	Fluoride Plasma P	Native Plasma N	Fluoride Plasma N	Native P / Fluoride P	Native N / Fluoride N
1	5.4	5.6	3.7			1.51	
2	5.5	5.7	4.7			1.21	
3	3.8	3.7	3.3			1.12	
4	3.9	3.9	lost				
5	5.6	5.6	5.1			1.10	
6	6.0	5.8	5.4			1.07	
7	3.9	3.8	3.5	905	820	1.09	1.10
8	2.5	2.5	2.2	995	897	1.12	1.11
9	4.6	4.5	4.1	998	903	1.10	1.11
10	3.2	3.3	2.9	965	885	1.13	1.09
11	3.4	3.3	3.1	928	883	1.06	1.05
12	4.7	4.6	4.2	938	874	1.10	1.08
13	5.0	5.0	4.4	945	823	1.14	1.15

It is seen in Table 1 that the phosphate content of 0.3% fluoride plasma is always lower than that of native plasma. In the last 7 experiments the phosphate differences correspond rather closely to the total nitrogen differences. Since we have shown that 0.3% NaF does not affect color production the most probable interpretation is that the lower phosphate value for fluoride plasma is due in most cases to dilution by the osmotic effect of the added fluoride. In 2 experiments, however, (1 and 2) and particularly in experiment 1, the P content of the fluoride plasma was so far below that of native plasma that it seemed improbable of explanation on the sole basis of osmotic dilution. It appeared that in the experiments of Table I the lower P content of fluoride plasma could usually but not always be accounted for by osmotic dilution.

In the second series of 6 experiments hematocrit readings were added to the total N and inorganic P determinations. Since paraffined tubes could not be used for hematocrit readings, heparin or hirudin plasma was substituted for native plasma. In the first experiment this was compared with 0.3% NaF plasma, in the second, third, and fourth with both 0.3% NaF plasma and with plasma from blood to which had been added 0.418% NaCl (isotonic with 0.3% NaF) and in the fifth and sixth with both 0.3% NaF plasma

and plasma from blood to which had been added 0.876% potassium oxalate (isotonic with 0.3% NaF). The same amount of hirudin (0.2 mg. per cc.) or of heparin (0.2 mg. per cc.) was added to all the samples, including the fluoride, chloride and oxalate bloods. Hematocrit readings, total N by macro Kjeldahl and inorganic P determinations were done in duplicate on all samples; the figures are the averages of these duplicates.

TABLE II

Ex. No.	Added	% plasma	Total N mg. per 100 cc.	Inorganic P mg. per 100 cc.	% plasma 2 / plasma 1	N 1 / N 2	P 1 / P 2	% plasma 3 / plasma 1	N 1 / N 3	P 1 / P 3
1	hirudin (1)	63.7	992	4.7	1.09	1.06	1.27			
	fluoride (2)	69.4	937	3.7						
2	heparin (1)	63.2	938	7.6	1.11	1.08	1.21	1.10	1.06	1.10
	fluoride (2)	70.0	866	6.3						
	chloride (3)	69.4	883	6.9						
3	heparin (1)	63.7	913	7.0	1.11	1.03	1.13	1.10	1.12	1.06
	fluoride (2)	70.4	884	6.2						
	chloride (3)	69.8	813	6.6						
4	hirudin (1)	65.0	1056	5.8	1.09	1.09	1.11	1.09	1.11	1.09
	fluoride (2)	70.5	965	5.2						
	chloride (3)	70.9	950	5.3						
5	hirudin (1)	62.4	1039	6.7	1.08	1.10	1.13	1.12	1.13	1.24
	fluoride (2)	67.4	946	5.9						
	oxalate (3)	69.7	919	5.4						
6	hirudin (1)	64.6	1041	7.0	1.07	1.12	1.09	1.12	1.13	1.25
	fluoride (2)	69.3	928	6.4						
	oxalate (3)	72.3	922	5.6						

The results given in Table II show that the degree of dilution of the plasma as indicated by the total N figures usually corresponds with that shown by the hematocrit readings. In 7 cases out of 11 the drop in the P content of plasma from blood to which a salt has been added corresponds fairly closely to the degree of dilution of the plasma. In 4 cases the drop in P content is definitely greater than can be accounted for by dilution; 2 of these cases are with fluoride plasma and 2 with oxalate plasma. Neither fluoride nor oxalate in the concentrations used influences the color production of the P method. The findings of Table II constitute an extension and confirmation of those in Table I, showing that the drop in P content of plasma when an anticoagulant salt is used is usually but not always accounted for in the main by the dilution effect. In the cases where the drop in plasma P is greater than can be accounted for by dilution the absolute amount of inorganic P in the plasma has apparently decreased; the mechanism is not clear. In the 3 cases where a salt not an anticoagulant (NaCl) was used the drop in

plasma P corresponds rather well with the degree of dilution; whether this would hold consistently in a large series we cannot say.

We have found, as did Gaebler for heparin, that both heparin and hirudin may transmit some material to a trichloracetic filtrate which causes difficulty in matching colors. This difficulty appears to a varying extent in a series of plasmas compared with a given lot of heparin or hirudin but is in no case so great as to introduce any large error. We obtained no hemolysis with 0.3% NaF, very slight with 0.876% potassium oxalate and definite hemolysis with 0.418% NaCl.

Table I also shows, contrary to Gaebler's observations, that there is no significant difference between the inorganic P content of serum and of native plasma. This may be due to our having centrifuged both blood samples immediately, while Gaebler let his serum bloods stand for 30 minutes at about 25° before centrifugation.

Summary. A concentration of 0.3% NaF does not influence the color production with the Benedict-Theis method for inorganic phosphates; an inhibiting effect is beginning to manifest itself with 1% NaF.

Plasma obtained by adding 0.3% NaF to blood shows a lower content of inorganic P than does native plasma or hirudin plasma. This difference is usually but not always accounted for by the osmotic dilution due to the added salt, the extent of dilution being measured by total N and hematocrit findings. In the cases where the drop in plasma P is greater than can be accounted for by dilution, the absolute amount of inorganic P in the plasma is apparently decreased.

The inorganic P content of serum is the same as that of native plasma.