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It has been stated, chiefly, if not exclusively, on the authority of Continental observers,[1] that the proportion of phosphoric acid in the blood is increased in certain diseases and diminished in others. These results have been obtained by operating on large quantities of blood by methods which, on account of their intricacy and the length of time required for their execution, are altogether precluded as general clinical methods. The method of estimating phosphoric acid now to be described is suggested as an easy, expeditious, and sufficiently accurate method for comparative purposes. It has the additional advantage from a clinical point of view of requiring for its operation such minute quantities of blood as can easily be spared by the feeblest patient. It is colorimetric in principle and depends on the greenish-yellow tint which even very minute traces of phosphoric acid strike with a solution of ammonium molybdate in hydrochloric acid. The ordinary solution of ammonium molybdate in nitric acid, which is used in the chemical laboratory for the separation of phosphoric acid from acid solutions, may be used for the present purpose, but the latter throws down a precipitate much more readily than the hydrochloric acid solution. As the formation of a precipitate naturally vitiates the result the former solution is to be preferred. The colour reaction is accelerated and intensified by the application of heat. In the cold some little time is required for its development. The method may be so arranged that the determinations may be made in warm solutions. As the molybdate solution, however, by itself takes on a very slight colouration on heating I prefer when very minute quantities of phosphoric acid are to be determined to use cold solutions alone. In the cold the colour produced by one part of phosphoric anhydride (P_2O_5) is distinctly perceptible in 3,000,000 parts

[1] Freund: Wiener Medicinische Wochenschrift, Band xxxvii., pp. 10, 40, 1887; Moraczewski: Zeitschrift für Physiologische Chemie, Band xxii., pp. 121–136, 1897; Virchow's Archiv, cxlvi., pp. 424–452, 1896.

of water. After considerable search I have found no reference to the application of ammonium molybdate to the colorimetric estimation of phosphoric acid, so that both the method and the particular application of it to the determination of phosphoric acid in minute quantities of blood appear to be new. The same reagent, it should be noted, strikes a similar colour with arsenic and silicic acids in solution. The former might possibly be present in blood in certain cases, and where present would be likely to interfere with the reaction. Silicic acid is not present in blood, but both it and arsenic must be absent from all reagents used. Ordinary tap-water, owing to the presence of silicic acid,[2] takes a colour with the reagent which is often many times the intensity of the colourisation obtained in these determinations. The use of distilled water is therefore a necessary condition of the experiment.

For the application of the method the following apparatus and reagents are required :—

1. Where the determinations are to be made on *measured* quantities of blood a capillary pipette for measuring small but definite quantities, such as 50 cubic millimetres, is needed. Such pipettes may be obtained from Hawksley of Oxford-street graded at every 10 cubic millimetres up to 50 cubic millimetres. Where a chemical balance is at hand small *weighed* quantities of blood are to be preferred, as correction for specific gravity is thus obviated. The capillary pipette is in that case unnecessary. The same remark applies to the determination of iron in blood by the method described by me in a former paper.[3]

2. The small platinum capsule described in that paper is also necessary, also a forceps for holding the same. In this case it need not be platinum-tipped.

3. A pipette capable of taking up and delivering one cubic centimetre of fluid.

4. Two small well-matched beakers, No. 000, of about three-quarters of an ounce capacity[4] will be found suitable

[2] From the relatively greater intensity of the colour produced by silicic acid I am of opinion that the reagent is likely to prove useful for the determination of silicic acid in potable waters without concentration, and after the separation of the silicic acid *secundum artem* for the estimation of the phosphoric acid in limited quantities, say, 25 cubic centimetres of the same water.

[3] THE LANCET, Jan. 22nd, 1898, p. 219.

[4] No. 77 of Becker and Co.'s catalogue.

for blood determinations. Beakers have the advantage of permitting the application of heat quickly and without risk of fracture. Where larger proportions of phosphoric acid (e g., in urine) are to be determined by this method somewhat larger beakers—up to No. 0 of the same set—may be used with advantage.

5. A solution of ammonium molybdate in hydrochloric acid. About five grammes of the salt are dissolved in a few cubic centimetres of strong hydrochloric acid and made up to 250 cubic centimetres with distilled water.

6. A solution of carbonate of soda and ammonium nitrate. This must be free from silicic acid. In making the determinations tabulated below the solution used was prepared by placing metallic sodium in contact with distilled water. Five or six pieces of sodium, each of about the size of a pea, are thrown one after the other on the surface of about 100 cubic centimetres of distilled water and the resulting solution of caustic soda saturated by passing into it washed carbonic acid. About two grammes of pure ammonium nitrate are then dissolved in it and the solution is placed in a Schuster's dropping bulb for use.

7. Pure hydrochloric acid 1 to 1.

8. A standard solution of sodium ammonium phosphate ($Na(NH_4)HPO_4+4H_2O$). This salt can easily be obtained in a pure state and 2·944 grammes are weighed out and dissolved in a litre of distilled water. One cubic centimetre of this solution = 0·001 gramme of phosphoric anhydride P_2O_5. For use five cubic centimetres of this solution are made up to 100 cubic centimetres when one cubic centimetre of the latter solution = 0·00005 gramme of P_2O_5.

A determination is made as follows. 50 cubic millimetres of blood taken from a finger-prick with the usual precautions are placed in the platinum capsule, or if a weighed quantity is to be operated on it is placed directly in the capsule, the capsule with the blood in it weighed, and the exact weight of the capsule being known the weight of the blood taken is also known. A couple of drops of the sodium carbonate and ammonium nitrate solution from the Schuster's dropping bulb are dropped on the blood. The capsule is then seized with the forceps and its contents first slowly evaporated over a Bunsen flame and when quite dry gently incinerated with the precautions already described in the case of the iron determinations. Caution is specially indicated when deflagration of the ammonium nitrate commences, so

that none of the contents may be lost by too forcible combustion. When this is safely over the capsule is heated to bright redness till every trace of organic carbon has entirely disappeared. The capsule is then allowed to cool. A couple of drops of hydrochloric acid are then added, but so as to avoid any risk of loss by effervescence, and the capsule is again gently warmed till perfect solution of the contents is effected. They are then carefully washed into one of the beakers (A) with warm water from a small wash bottle. One cubic centimetre of the molybdate solution is added to this beaker and a like quantity to the other beaker (B) and both are made up to the same level with warm water from the wash bottle. In case of any possible contamination of the reagents with silicic or arsenic acids—and it is to be remembered that with time they take up traces of silicic acid from the containing bottles—two drops each of the hydrochloric acid and soda carbonate solutions may also be added to beaker B. Both beakers are now placed on a sheet of white paper spread on a cast-iron plate which is supported on a quadrupod and which has previously been heated and continues to be kept heated by a Bunsen burner placed beneath it. The wash water may be conveniently warmed by standing the flask on the same plate. The whole arrangement must be placed in a good light which equally illuminates both beakers. Beaker A will be found to show an amount of colouration proportional to the amount of phosphoric acid present in the specimen of blood under examination and this degree of colouration has now to be matched by gradually adding in a few drops at a time the standard solution of sodium ammonium phosphate from a measuring burette to beaker B, a minute being allowed after each addition for the full development of the colour. The tints are compared by looking down rather than through the beakers on to the white ground below. The number of cubic centimetres or fractions of one cubic centimetre required to produce equal tints is the measure of the quantity of phosphoric acid present. I find that the ordinary stoppered 50 cubic centimetre burette delivers at each drop as nearly as may be 0·05 of 1 cubic centimetre, so that if the number of drops necessary to give equal tints with the phosphoric acid in 50 cubic millimetres be divided by two we get the result in parts per 10,000 parts of blood. Thus if 19 drops of the solution have been required to give equal tints that specimen contains 9·5 of P_2O_5 in 10,000 parts of blood. If a weighed quantity has been taken a simple calculation is necessary. Thus, suppose 0·093 gramme has been taken and

that 1·6 cubic centimetres are required to give equal tints we have—

0·093 gramme blood contains 0·00005 × 1·6 grammes P_2O_5
or 0·093 ,, ,, ,, 0·00008 ,, ,,
,, 93 grammes ,, ,, 0·08 ,, ,,
,, 9300 ,, ,, ,, 8 ,, ,,
,, 10,000 ,, ,, ,, $\frac{8 \times 10{,}000}{9300} = 8{\cdot}6$

Below are tabulated a series of determinations which have been made by this method. These give as nearly as may

Amount of Phosphoric Acid in Blood as Obtained by the Method Described.

No. of specimen.	Date.	Age	Sex.	Quantity of blood taken.	Phosphoric anhydride (P_2O_5) (parts in 10,000) found.	Result calculated as phosphorus in 10,000 parts.
				Cubic millimetres.		
1	10/7/98	42	M.	50	9·5	4·15
2	11/7/98	—	—	50	9·0	3·93
3	12/7/98	37	M.	50	6·0	2·62
4	13/7/98	37	F.	50	6·5	2·84
5	14/7/98	33	M.	50	8·0	3·50
6	16/4/99	43	M.	50	9·7	4·24*
7	,,	22	F	50	6·0	2·62
				Grammes.		
8	30/4/99	22	M.	4·327	7·96	3·48†
9	11/6/99	2(	M.	0·045	5·56	2·43
10	12/6/99	34	M.	0·038	13·12	5·73‡
11	,,	21	M.	0·0755	6·62	2·89
12	,,	32	M.	0·0845	8·31	3·64
13	4/7/99	18	M.	0·093	8·6	3·76
—	—	—	—	Average	8·067	3·52

* Strength of test solution had sensibly increased from evaporation in the interval. † A gravimetric determination throughout. ‡ An exceedingly florid subject.

Note.—Nos. 1, 2, and 6 are the same individual. The results of Column 7 are found from Nos. of Column 6 by multiplying these by $\frac{62}{142} = 0{\cdot}437$.

be 8 in 10,000 parts of blood as the average for healthy blood. This is probably very near the truth. These determinations, as will be seen, have been made partly on measured and partly on weighed quantities of blood. The former are subject to a slight correction for specific gravity. The latter, as already indicated, are to be preferred, but in such cases a delicate balance is a requisite. No. 8 of the series is a gravimetric determination and was made in general confirmation of the grade of the quantities obtained by the present method.

The method now described is applicable to the determination of phosphoric acid in other substances, whether organic or inorganic. The following among other results have been obtained on samples of urine. In each case a preliminary incineration as with blood was made. The result in each case has also been checked by determinations by other methods.

No. of specimen.	Specific gravity.	Character.	Quantity taken.	Percentage.	Method.
1	1028	Of lactation.	0·1235 gramme.	0·263	Colorimetric.
		"	10 cubic centimetres.	0·272	Volumetric.
		"	10 cubic centimetres.	0·270	Gravimetric.
2	1011	Nephritic.	0·489 gramme.	0·0542	Colorimetric.
			10 cubic centimetres.	0·0625	Volumetric.
3	10085	Of nervous excitement.	0·319 gramme.	0·0329	Colorimetric.
			10 cubic centimetres.	0·034	Volumetric.

A sample of cow's milk gave by this method: first determination, 0·2655 gramme being taken, 0·213 per cent.; a second determination, 0·0705 gramme being taken, 0·219 per cent. A determination of the serum of the blister of a burn gave 2·6 in 10,000 parts, 0·098 gramme being the quantity taken.

After a year's experience I am of opinion that the method just described yields satisfactory results and in addition to its being applicable as a clinical method for the determination of phosphoric acid in blood it is also likely to prove useful in many other cases where minute quantities of phosphoric acid have to be estimated.

Elgin.

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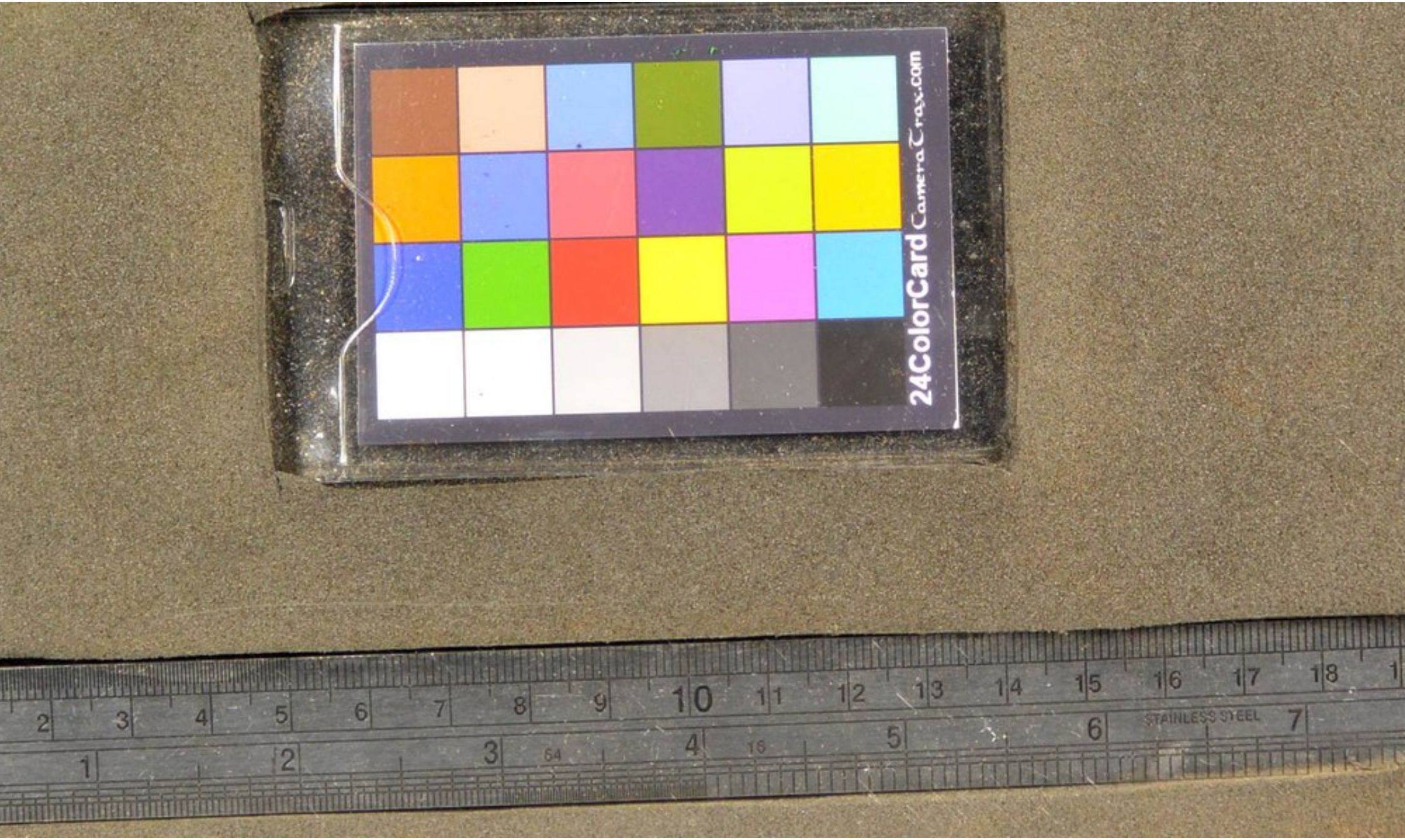
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