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Distribution of phosphorus in the blood.

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It is well known that when blood is allowed to stand after it has been drawn, its content of inorganic phosphate will gradually increase to 1 mgm. or more above the figure which is obtained when the determination is carried out immediately. This indicates that the organic substances containing phosphoric acid which occur in the blood, particularly in the red cells, in connection with which Greenwald has introduced the name "acid soluble phosphorus" are slowly hydrolyzed to yield inorganic phosphate. This hydrolysis, however, is incomplete even when aided by boiling with dilute acid. This finding would seem to indicate that there must be two types of organic phosphoric acid combination in the blood,—one of which is easily hydrolyzed and the other one not. To investigate this further, we have carried out phosphorus determinations by Tisdall's micromethod on the protein-free filtrate of blood, obtained by means of trichloracetic acid. We determined the inorganic phosphate immediately and again after boiling the filtrate for two hours. The total phosphorus in the filtrate (acid soluble phosphorus) was also determined. The results are shown in the accompanying chart. This shows that a constant amount of phosphoric acid is split off by boiling, approximately 10 mgm. of phosphorus being thus obtained in human blood and 13 mgm. in rat's blood. This then leaves for the organic hydrolysable phosphoric acid in the human 6-7 mg. and in the rat 5-7 mg. If we subtract the figure obtained after boiling from the total acid soluble, we obtain the figure representing the amount of phosphoric acid contained in organic combination which is not hydrolysed under the conditions stated. We have, therefore, evidence of three forms of phosphoric acid which will pass into aqueous filtrates on coagulation of the protein.

It is evident that the above needs further substantiation. In the first place, we must give evidence that the determination of

inorganic phosphate really represents all inorganic phosphate present, for it might be thought that the above results are merely a simulation of what we claim, due to limitations of the method used. We, therefore, added to a sample of blood, sodium phosphate equivalent to 1 mgm. of P per 100 c.c. of blood. The table shows the results. We find a complete recovery both in the inorganic phosphate and the determinations after boiling within the limit of error of the method.

To demonstrate the completeness of hydrolysis of the hydrolysable substance, we have done further experiments in which hydrochloric acid or nitric acid were added to the filtrate before boiling. The trichloracetic acid is probably all decomposed before the end of the two hours boiling. Either of these acids when added in amounts to produce a concentration of about N/4 will somewhat interfere with an accurate determination by the Tisdall method, so that after two hours boiling lower results are obtained. However, when we continued the boiling for four hours and then applied the Tisdall method, the results were identical with those obtained on boiling with only trichloracetic acid present in the filtrate. (11.1 mgm. with HNO_3 against 11.3 without HNO_3 on beef blood.)

It might still be objected that the micromethod is not applicable. We have, therefore, repeated the experiments on a larger scale, with 200 c.c. of blood filtrate using for the determinations of phosphorus the well-recognized method of preliminary precipitation with ammonium molybdate, precipitation and reprecipitation with magnesia mixture, ending with gravimetric determinations as magnesium pyrophosphate. The small amount of phosphorus which results even from the 200 c.c. of filtrate does not allow an accurate quantitative determination, but it clearly shows that boiling with acid yields a relatively constant figure distinctly higher than the inorganic and distinctly not equal to the total phosphorus of the filtrate.

The only objection still remaining, is the possibility of incomplete precipitation in blood filtrates by all molybdic acid reagents and a partial removal of an inhibiting substance by boiling with acid. A determination of the phosphate obtained by precipitating the blood filtrate directly with magnesia mixture (heated, and allowed to stand over night) gives values which are incorrect, due to impurities in the precipitate. We have, however,

DISTRIBUTION OF PHOSPHORIC ACID IN BLOOD

	Inorganic mg.	After boiling mg.	Total acid soluble mg.	Total Organic		Organic hydrolysable		Organic non-hydrolysable		Remarks
				mg.	Per cent.	mg.	Per cent.	mg.	Per cent.	
Human.....	2.65	9.40	19.3	16.65	86.4	6.75	34.9	9.9	51.3	March
	2.80	10.50	20.0	17.2	86.0	7.70	38.0	9.5	47.5	June
	2.55	9.30	19.0	16.45	86.8	6.75	35.3	9.7	51.0	December
Rat, normal.....	7.6	13.5	19.5	11.9	61.1	5.9	30.2	6.0	30.8	Flour diet containing 20 per cent. dry milk.
	6.66	13.4	20.0	13.4	67.0	6.74	33.7	6.6	33.0	Flour diet containing 20 per cent. dry milk and 2 per cent. Na_2CO_3
	5.4	13.0	20.6	15.2	73.8	7.6	36.9	7.6	37.0	Flour diet with 5 per cent. dry milk.

RECOVERY OF ADDED PHOSPHATE

Blood, human ..	2.65 2.76	11.05 11.1 11.2
Same blood + 1 mg. P per 100 c.c.	3.60 3.56	12.1 12.3 12.05

determined that, after this procedure which causes hydrolysis, a considerable quantity of phosphorus is present in the filtrate. This amount agrees closely with that of the fraction which we have termed "non-hydrolysable phosphate."

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Further observations on the chemistry of cod liver oil.

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It has been stated previously[1] that the constituent of cod liver oil which influences the mineral metabolism, when cod liver oil is used in treating rickets, is contained in the unsaponifiable fraction of the oil. Further attempts towards isolating the active material have yielded the following results. A good yield of crude product can be obtained by directly extracting cod liver oil with 95 per cent. alcohol. This mixture of fatty acids, a small amount of oil, and other substances, is saponified with sodium hydroxide, and when the calcium soaps are precipitated from an aqueous solution, the unsaponifiable material including the active substance, is precipitated with the soap. From this calcium soap, acetone will extract the active material. In this manner we have obtained preparations of the active material which after a dilution of 1:1000, are as active as the original cod liver oil. The chemical nature of the substance has not yet been determined, but we believe that we are approaching its actual isolation.

With regard to the properties of the active material thus obtained, we can say that it is not toxic in doses of more than 50 times the curative dose. A single large dose in our experiments brought about healing at the same rate as a succession of small doses. The purified active material is entirely free from fat soluble A as shown by the fact that it will not cure xerophthalmia when a subsequent treatment with butter fat does cure the condition.

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[1] Zucker, Pappenheimer and Barnett, PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 167.