CCX. THE EFFECT OF HYDROGEN ION CONCENTRATION UPON THE PRECIPITATION OF CERTAIN BASIC SUBSTANCES BY PHOSPHOTUNGSTIC ACID.

BY RUDOLPH ALBERT PETERS.

From the Department of Biochemistry, Oxford.

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THOUGH it has been known in a general way that acidity affects the precipitation of basic substances by phosphotungstic acid, it is not usually realised that the chemical constitution of the base has a marked influence upon the p_H at which precipitation occurs. The observations recorded below have been applied already in the concentration of vitamin B₁ [Kinnersley and Peters, 1930], and experiments are in progress to ascertain how far the method can be generalised¹.

EXPERIMENTAL.

Phosphotungstic acid (Mercks' puriss.) 10 % is carefully brought with 20 % NaOH to approximately p_H 6.0. The reagent should not be made actually alkaline during this process. The substance for test in a concentration of at least 1.0 mg. per cc., is brought to p_H 7-8, and 1-2 drops (1/20 cc.) of the phosphotungstate are added. If there is no opalescence, $N/100-N/10$ H₂SO₄ is cautiously added from a micro-burette until a faint opalescence appears. Such opalescence is an indication of commencing precipitation, and upon standing for some hours precipitation at the indicated p_H becomes complete. No precipitation appears to take place upon standing unless the opalescent stage has first been reached.

The p_H at which the precipitate first appears differs markedly for different bases. For instance, whereas histamine first precipitates at p_H 7.4-7.8, ammonium salts do not precipitate until the solution has a p_H of 1-2. Bases therefore which precipitate towards the more alkaline reactions can be freed from ammonium salts by this treatment. The only exception to the rule that these precipitates tend to dissolve in alkaline solution and to reappear in acid is guanidine, with which the precipitation over the range p_H 3-10 takes place in the opposite sense.

Table I shows the results obtained by this method for several bases.

The estimations of p_H were made by a colorimetric method and cannot be relied upon to more than ± 0.15 p_H .

¹ A preliminary account appeared in *Chem. and Ind. (1930) Proc. Biochem. Soc.* p. 517.

Table I. p_H and precipitation of bases.

Compound*	Conc. mg. per 1.00 cc.	p_H
1. Histamine	5.0	7.8
2. Aminomethylglyoxaline	3.5	6.8
3. 4 (or 5)-Glyoxaline methyl ethyl carbinol ...	3.3	4.8†
4. Glyoxaline-4 (or 5)-formaldehyde	3.9	3.8
5. 4 (or 5)-Methylglyoxaline	3.7	3.8
6. Arginine (nitrate)	2.3	3.7
7. 4-Hydroxy-2-6-dimethylpyrimidine	5.2	3.6
8. Lysine (picrate)	5.1	3.3
9. <i>dl</i> - α -Hydroxy- β -glyoxaline-4 (or 5)-propionic acid	3.8	3.2
10. 2-Hydroxy-4-6-dimethylpyrimidine	5.2	3.2
11. Glyoxaline-4 (or 5)-acetic acid	4.1	3.0
12. Choline	4.6	2.9
13. 2-Methylglyoxaline	2.6	2.9
14. Creatinine	5.0	2.9
15. Histidine	5.0	2.8
16. Ergothioneine	—	2.5
17. 2-Thiol-4 (or 5)-amino-methylglyoxaline ...	4.7	2.2

The p_H recorded is that for the appearance of the first opalescence.

* Compounds 3, 7, 10, 16, 17 were kindly supplied by Dr J. M. Gulland, compounds 5, 13 by Prof. Pyman, and 9 and 11 by Prof. Barger. The remaining compounds were from commercial sources.

† p_H somewhat doubtful. A sample of the platinum salt kindly supplied by Dr Gulland precipitated at p_H 2.8 in conc. 4 mg./cc.

Certain tentative observations can be made. The unsubstituted glyoxaline compounds appear to precipitate at about p_H 3.0, and this point is not much affected by combination with acetic or hydroxypropionic acid. The aldehyde group appears to change the point of precipitation to the alkaline side, as does a long side chain. The base histamine with a 2-carbon chain attached begins to precipitate actually at an alkaline p_H , and aminomethylglyoxaline at a slightly less alkaline reaction. The introduction of —SH seems to alter the precipitation point markedly to the acid side of p_H 3.0, which is the point at which the simple glyoxaline compounds precipitate.

Guanidine compounds really show two points of precipitation, one alkaline and one acid, which vary according to the degree of substitution of the compound. If a guanidine solution of p_H 5.0 approx. is treated with phospho-

Table II. *Guanidine compounds.*

Substance	Conc. mg. per cc.	p_H	Notes
Guanidine sulphate	5.0	7.0	Precipitate not redissolved by alkali; redissolves upon increasing acidity to p_H 4.0, but is reprecipitated in 5 % sulphuric acid
Aminoguanidine	15	8.5	Redissolved by excess alkali; precipitated by strong sulphuric acid
<i>asym</i> -Dimethylguanidine	5	Nil	Not precipitated at all by alkali, but precipitated in 5 % sulphuric acid
Methylguanidine	5	8.5	Faint precipitate, redissolved by alkali, precipitated by 5 % H_2SO_4
Arginine nitrate	5	7.0	Redissolved by alkali, further precipitate appears on the acid side at p_H 3.7

The p_H given in this table refers to the point at which a precipitate first appears upon passing from a more acid to a less acid reaction.

tungstic acid, no precipitate appears. Addition of alkali to p_H 7.0 induces precipitation, as also does acidification to p_H 1.0. Table II embodies the observations made.

It seems to be clear that for the alkaline precipitation the NH_2 group must be intact. Introduction of methyl or NH_2 reduces the tendency to precipitate, and dimethyl suppresses it. Arginine shows a faint tendency to precipitate. It is to be noted that the two precipitation reactions for arginine appear to be quite distinct.

The relation between the concentration of the base and the p_H at which precipitates first appear has been studied more fully for creatinine, guanidine, and less fully in the case of histidine and histamine. The curves in Fig. 1

Fig. 1. p_H and phosphotungstate precipitation.

show the results obtained. For histamine, below 1 mg. per cc. most of the compounds become too soluble to obtain results. For histidine and creatinine a variation of concentration from 1 to 10 mg. per cc. changes the p_H for precipitation from 2.5 to 3.5 approx. With creatinine the curve rises somewhat more steeply. A variation in concentration of six times only affects the p_H of precipitation by some 0.8 p_H . For neutralised guanidine sulphate it will be seen that the curve for alkaline precipitation rises much less steeply.

It would be unwise to draw conclusions as to the nature of these precipitates until more work has been done, but since they do not tend to appear until some of the compounds are neutralised, it would seem that the bases are precipitating as salt complexes with the phosphotungstates. Some evidence has been obtained that in mixtures of bases there is a shift in the positions of these precipitation points. It is likely that these results explain certain

difficulties which have appeared in the decomposition of phosphotungstic acid precipitates. It would clearly be very difficult to decompose a guanidine phosphotungstate of this nature by baryta. In most cases re-resolution of the phosphotungstates mentioned in Table I is to be obtained by taking to a point approximately 2.0 p_H units more alkaline than that of precipitation.

SUMMARY.

The hydrogen ion concentration at which phosphotungstate precipitates of certain bases appear depends to a large extent upon the chemical constitution of the base. With most of the bases studied the precipitation appears upon passing from a more alkaline to a less alkaline solution; with guanidine compounds, this is reversed.

REFERENCE.

Kinnersley and Peters (1930). *Biochem. J.* **24**, 1856.