such a way that its translocatability is ordinarily not conspicuous in intact plants. Yet by properly combining the effects of exfloration and defoliation with photoperiodic illumination, it is possible to demonstrate not only stimulus transfer, but flower inception in normally vegetative regions.

The vegetative stimulus of long-day also appears to exert an inhibitive influence upon stimulus transfer because, in intact plants flowering is restricted to regions receiving short-day treatment, whereas in the defoliated plants (4a and 5b), on the other hand, there is clear cut evidence of transfer. In fact, both the flowering stimulus of short-day and its inhibition in long-day exhibited a direct quantitative relationship to the amount of foliage. The largest number of flowers on defoliated parts appeared on those plants with the greatest number of leaves under short-day illumination. Contrariwise, impedance of transfer varied directly with the number and size achieved by leaves before their removal from defoliated parts.

The postulation of a specific florigenic inductor derives further support from the recent conclusive determination that carbohydrate-nitrogen relations in the soybean are not, as hitherto frequently assumed, the direct causative factors of flowering.[6] Formation of flower primordia precedes the shift in nutrient balance characteristically associated with the transition from the vegetative to the reproductive phase. It seems safe to predict the early isolation and identification of this florigenic agent, a discovery which may provide an explanation for the paradox of its formation in some species under long-day and in others under short-day conditions.

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A Stable Metaphosphate Preparation for Use as a Protein Precipitant.*

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That metaphosphoric acid causes essentially quantitative precipitation of proteins, in pH ranges acid to the isoelectric point of

[6] Murneek, A. E., *Mo. Agric. Exp. Sta. Res. Bul.*, 1937, **268**, 84.

* Contribution from O. S. A. Sprague Memorial Institute, University of Chicago, and Division of Agr. Biochemistry, University of Minnesota. Paper No. 1550, Journal Series, Minnesota Agricultural Experiment Station.

the protein, has long been known. Its use as a means of removal of the protein from blood serum preliminary to analysis for other components of the blood has been studied by Folin[1] and by Hiller and Van Slyke.[2] Due to its instability in aqueous solution and to the difficulty of obtaining a uniform commercial reagent, metaphosphoric acid has lost favor as a standard protein precipitant, although its action closely simulates that of the more expensive trichloracetic acid commonly used (in 10% solution).

The object of this paper is to describe a method of preparation and manner of use of metaphosphoric acid which will largely eliminate the objections which have tended to prevent its use as a routine protein precipitant.

Metaphosphoric acid is commonly prepared by heating the ortho-acid to drive off one mole of water per mole of ortho phosphoric acid, or by the addition of one mole of water to one mole of P_2O_5. The acid so formed is a strong acid (pKa = ca. 1.5) and may be highly polymerized. When placed in solution in water, it is hydrolysed rapidly back to the ortho-acid. The commercial meta acid, obtained as sticks of glacial metaphosphoric acid, always contains sodium, usually containing approximately one atom of Na per 2 atoms of P. It is sometimes given the formula, $NaPO_3 . HPO_3$. Titration curves made on solutions of such products, however, show that all of the acid which is present is in the ortho form. The remainder of the phosphorus is normally present as sodium meta phosphate. When a solution of the "glacial" acid is boiled, all the meta phosphate present is changed quickly to ortho, the same reaction being accomplished at room temperature in the course of 2-3 months. The hydrogen ion is the catalyst for this hydrolysis of the meta to the ortho form, and its action is enormously intensified by heat. If the solution is brought to a pH of 7 shortly after preparation, the meta phosphate is stabilized and will be hydrolyzed only very slowly even at the boiling temperature of the solution (as determined by titration of the second hydrogen of the ortho phosphate present).

The method of preparation and use of meta phosphoric acid given below is based upon the evidence that, in solution, meta phosphoric acid itself is very unstable while the sodium salt of meta phosphoric acid, especially in a solution made somewhat alkaline with Na_2CO_3 or NaOH, is stable.

The method recommended for the preparation of the stable Na

[1] Folin, O., *J. Biol. Chem.*, 1919, **39**, 259.

[2] Hiller, A., and Van Slyke, D. D., *J. Biol. Chem.*, 1922, **53**, 253.

metaphosphate is that of heating $NaH_2PO_4 . H_2O$ to drive off 2 molecules of the H_2O. When this is done, it is found that at a relatively low temperature, the one water of crystallization is eliminated and that by the time the temperature has reached 400° C. a second water has been completely lost. At this point, the preparation, which has the formula $(NaPO_3)_x$, has the appearance of a white amorphous powder soluble in water. This salt shows all of the precipitations characteristic of pyrophosphates, but has the ability to precipitate protein in acid solution (which is a characteristic test for meta phosphate to distinguish it from pyrophosphate and orthophosphate). If now, the white powder $(NaPO_3)_x$ is further heated to about 700°C. it melts and forms a glass upon cooling. Solutions of this form of meta phosphate have a greater power to precipitate protein (do so in lower concentrations) than do those obtained from the amorphous powder prepared at 400°C. Conductivity-concentration curves and freezing point depressions measurements made with solutions of these 2 forms of Na metaphosphate show[3] that the white powdery Na metaphosphate acts as a uni-trivalent strong electrolyte, having a formula $Na_3(PO_3)_3$, while the glass form acts as a weak electrolyte so highly polymerized that, except at very high dilutions, only a part of the Na is ionized. The valence of the complex polymerized ion is much higher than 3. No formula can be given for this form of Na meta phosphate, at present. Both of these forms are relatively stable in water solution and retain their power to precipitate protein (in acid solution) over a period of months after standing at room temperature. However, hydrolysis occurs slowly and it is possible that depolymerization proceeds also over that period of time. The latter phenomenon is being further studied, but it seems to be a rather slow process, if it occurs at all separate from the process of hydrolysis. Due, perhaps, to traces of NaH_2PO_4 which will remain even after long periods of heating the melted salt, the pH of aqueous solutions of these preparations of Na metaphosphate is of the order of 5.5. If a small amount of Na_2CO_3 or NaOH is added to the solutions of these salts, bringing the pH to 8 or 9, the stability of the metaphosphate, at ordinary temperatures, is practically permanent. This is illustrated in Table I, where the stability, at the boiling temperature, is measured at the various pH values.

The amount of hydrolysis was measured by titrating the second hydrogen of the ortho phosphate present at the end of the time of boiling the solutions. The solution containing equimolar quanti-

[3] Briggs, D. R., paper in preparation.

TABLE I.
% of Hydrolysis of Sodium Metaphosphate (glass) in 2% Solution After Boiling the Solutions at Various Initial pH Values for Specified Lengths of Time.

Solution	Time of Boiling, hr.	Approximate % Hydrolyzed
Freshly dissolved Na metaphosphate	0	0.5
" " " " (pH = 5.5)	1	10.0
Na metaphosphate and NaOH to pH = 7	1	2.0
" " " " " pH = 9	1	0.6
" " " " " pH = 10	1	0.5
1 mole Na metaphosphate + 1 mole H_3PO_4 pH = 2	½	100.0

ties of the Na metaphosphate and ortho phosphoric acid corresponds to a solution of commercial glacial metaphosphoric acid.

A method of preparing sodium meta phosphate (glass) is as follows: Pure $NaH_2PO_4 . H_2O$ is heated, carefully at first to prevent spattering, and then strongly in a muffle furnace or over a gas flame until the whole of the salt is melted into a clear melt. It should be maintained at this temperature for perhaps an hour to insure complete expulsion of water. The heating may be done in a porcelain crucible. Slight pitting of the glass covering of the crucible will occur, however, and a platinum crucible is better. The melt, when cooled to the point where it will just flow should be poured on a platinum surface or removed from the crucible in drops on a platinum wire. (Other metal surfaces may be used if traces of salts of these metals are no detriment in subsequent use of the meta phosphate.) After cooling, it will easily separate from the metal and can be kept dry indefinitely in this form. A 10% solution may be made up and sufficient NaOH or Na_2CO_3 added to bring the pH of the solution to 8 or 9. This may be kept at room temperature. It will maintain its original precipitating efficiency for at least several months.

When using this solution to precipitate proteins an amount of metaphosphate solution equivalent to 0.2 gm. of sodium metaphosphate per gm. of protein constitutes an adequate excess. Thus to 100 cc. of blood serum containing approximately 8 gm. of protein 16 cc. of 10% solution would be added. Sufficient acid, HCl or HAc, is then added to bring the solution to a pH of 2.5 or 3.0, under which conditions the protein will be quantitatively removed as a flocculent white precipitate.

As a protein precipitant, metaphosphoric acid has an advantage over trichloracetic acid in that it is so much cheaper. Also, such high concentrations of precipitant are not necessary to cause complete removal of the protein from solution when metaphosphoric

acid is used. It can be completely removed by dialysis against water at pH of 7 or 8. It does not precipitate amino acids and is no stronger a precipitant for polypeptides than is trichloracetic. The above method of preparation and use eliminates its usual disadvantage of instability and variability in precipitating power.

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Determination of Iodine in 5 cc. of Blood.

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Four and one-half inches of ⅜″ Visking sausage casing is moistened at one end and tied to a frame. Five cc. of blood is run into the open end from a syringe or pipette. The open end is tied and the string attached to the other side of the frame, stretching the casing as much as it will bear, and it is dried 40 min. at 100° in an oven. It is then placed in a screw-feed stoking device with detachable water-cooled platinum tip and burned with 120 cc. of oxygen per minute in a platinum combustion tube heated by gas. The screw feed is advanced 1″ in 20 min. by motor. The combustion products are led through a pyrex absorber containing 3 sintered glass discs and 25 mg. of sodium azide in 10 cc. of water. At the end of the combustion the stoking tube is removed from the platinum tip, which is allowed gradually to heat, the gas flame is turned off, and the absorber contents and ash are transferred to a 100 cc. pyrex still, using 4 cc. N/10 NaOH and 10 cc. H_2O to rinse out. Most of the water is evaporated off while passing a small current of air to prevent bumping, and 3 cc. 6 *N* H_2SO_4 is introduced into the still. The iodine is distilled over into a 10 cc. receiver containing 1 cc. of bromine water without immersing the tip. The trap is flamed to prevent iodine remaining in drops of water in it. When SO_3 fumes appear in the still, the flame is reduced to 5 mm. and continued for 4 more minutes. After allowing the air to continue until all condensate has run down into the receiver, the tip is washed, volume made to 9 cc., and an air current blown through it in a steam bath for ½ hour to remove the bromine. Twelve and one-half mg. KI is introduced, the volume is made to 10 cc., and it is titrated with 0.001 *N* sodium thiosulfate in a Lochte-Hoover burette with divi-