19. Deposit a drop of this mixture on 2 clean glass slides and smear with smearing slide, same as ordinary blood smear.

20. Repeat 15, 16, 17, using 2 units of salt sol. instead of 1, and proceed as in 18 and 19.

21. When dry, stain these films, marked for identification, with Wright's stain.

22. Limit the ocular field by cross hair preparation (counting eye-piece).

23. Examine stained smears by high power, and if satisfactory—

24. Examine under oil immersion.

25. Count the bacteria and the red corpuscles in successive fields until 500 red blood cells have been counted.

26. Determine the number of bacteria per cc. by the following equation: R. B. C. : Bacteria :: 5000 million : X.

27. Decide upon the dose per cc. and the total quantity of vaccine to be supplied, and from this determine the quantity of undiluted emulsion to be put into the dispensing bottle (vaccine bottle).

28. After the one hour has elapsed (step 11), cut tube and pipette off the quantity desired, which put into dispensing bottle.

29. With a sterile pipette remove about 1/10 cc. of emulsion, which put into a tube of melted dextrose agar, held at about 45° C. Make a shake culture.

30. Re-seal tube.

31. Add the necessary quantity sterile salt sol. to the emulsion in the dispensing bottle, thoroughly mix.

32. Remove about 1/10 cc. from bottle and put into melted dextrose agar (29).

33. Mark tube step 29 "emulsion," and tube step 32 "bottle."

34. Put into the completed vaccine 0.25 percent trikresol.

35. Put sterile rubber cap on dispensing bottle.

36. Paraffin and label.

This, gentlemen, completes the making of the vaccine, excepting the delivering and collecting of fee.

Again I want to impress upon you the necessity of extreme cleanliness and the need of using nothing but sterile appliances. Furthermore, the vaccine should not be released until 24 hours incubation of the tubes (steps 29 and 32) prove the absence of living micro-organisms.

I trust that I have succeeded in demonstrating to you that the making of autogenous vaccines is not as difficult a proposition as would appear at first glance and that the average pharmacist, with a little training, can prepare these vaccines successfully to the benefit of the patient, the satisfaction of the physician, the enrichment of himself and the credit of his profession.

THE MANUFACTURE AND ASSAY OF HYPOPHOSPHOROUS ACID.

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This acid was introduced into the United States Pharmacopoeia of 1890, and retained in the eighth revision, largely because of its value as a preservative of pharmaceutical preparations containing iodides, which are liable to decomposition by exposure to light and air.

It does not require a very long memory to recall the difficulties experienced in keeping these preparations, and the losses sustained by pharmacists as a result of failure to prevent oxidation or decomposition.

In 1888, Mr. John Devine conducted a series of experiments to determine the value of hypophosphorous acid as a reducing agent in the preservation of ferrous compounds, and the minimum quantity required for the purpose.

He embodied the results of his work in a paper read before this Section at the 1889 meeting.

Its use for this purpose met with more or less objection at that time, but now has official sanction.

Various processes have been suggested for the preparation of hypophosphorous acid, but it seems that purity of product did not receive much consideration, if one may judge by the results obtained by some, if not most, of the methods given.

As is often the case, the most direct method, and the best from the standpoint of product obtained, is not the one which lends itself most readily to easy and speedy manipulation; the method referred to is that by which barium hypophosphite is prepared by boiling a solution of barium hydroxide with phosphorus and afterward decomposing the barium hypophosphite with the exact equivalent of sulphuric acid. The method is direct, the by-products few and easily eliminated, and with a reasonable amount of care, the product is of a high degree of purity. But prolonged boiling is required, the method is slow and tedious and, because of limited solubility of barium hydroxide, a large volume of water is required in proportion to the amount of final product. This method is suitable only for operations on a rather large scale.

Another method given in the Dispensatories, and also mentioned in several standard works on chemistry, is the decomposition of calcium hypophosphite, in solution, by means of oxalic acid, and the equation illustrating the reaction is usually given:

$$Ca(H_2PO_2)_2 + H_2C_2O_4 = Ca\,C_2O_4 + 2\,H_3PO_2.$$

This is a good example of a large class of equations which do not tell the whole truth; it works out to a nicety on paper and to all appearances it is only necessary to filter out the calcium oxalate and have hypophosphorous acid.

Here is where the utility of the U. S. P. ammonia test is shown: neutralize a portion of the acid, made in this way, with ammonia, and a precipitate appears at once.

Calcium oxalate is insoluble in water but is soluble to a considerable extent in hypophosphorous acid, consequently the product in this case is hypophosphorous acid saturated with calcium oxalate.

This was pointed out in 1887 by Mr. George Lunau (Phar. Jour. and Trans., Mch 19, 1887), and in view of the poisonous character of the impurity the method should have been abandoned long ago, and mention of it either dropped from chemical text books or attention directed to the true character of the products of the reaction.

In the first issue of the National Formulary in 1888 a formula is given for the preparation of hypophosphorous acid by decomposing potassium hypophos-

phite with tartaric acid in presence of dilute alcohol and water and the formula was retained in the second and third editions.

This process is less objectionable than that in which oxalic acid is used because the impurity is not poisonous, but the product is very impure nevertheless, and there is an additional objection because of the expense due to alcohol lost in the operation.

In the American Journal of Pharmacy (Dec., 1908, p. 583), Dr. Gunnar Heikel proposes a method by which calcium hypophosphite in solution is decomposed by a solution of ammonium oxalate; the products of the reaction being calcium oxalate, precipitated, and ammonium hypophosphite in solution in which the calcium oxalate is completely insoluble. After filtering, the solution is boiled with barium carbonate until decomposition is complete, as shown by the absence of ammonia in the vapor.

In an experiment after this method carried out by the writer, boiling was continued for twenty hours, and at the end of that time ammonia was still being given off in appreciable volume.

Bearing in mind that the hydroxides of the alkalies and alkaline earths readily displace ammonia, a trial of barium hydroxide was made and the result was very satisfactory. On adding $Ba(OH)_2$ to a cold solution of ammonium hypophosphite, elimination of ammonia begins within a few minutes, and on warming, the reaction is accelerated and about two hours' heating on a steam or boiling water bath is sufficient to expel all the ammonia, and a solution of barium hypophosphite with some insoluble residue, remains.

This is to be filtered, and filter and residue washed with hot water until a small portion of the washings gives little or no precipitate on the addition of a few drops of dilute sulphuric acid.

The formula in detail is as follows:

Calcium Hypophosphite	350 Gm.
Water	2000 Cc.
Oxalic Acid	190 Gm.
Strong Ammonia Water	qs.
Barium Hydroxide	630 Gm.

Dissolve the calcium salt in the water by gentle heat and, while hot, add the oxalic acid. Stir a few minutes, then remove from heat and add strong ammonia water until in slight excess. Allow to stand until cold, then test a portion of the clear liquid by adding a little oxalic acid; if this causes a precipitate, more oxalic acid must be added to the solution until no further precipitate is produced, keeping the solution alkaline by the further addition of ammonia if necessary. Now filter and wash the residue with hot water. To the mixed filtrate and washings add the barium hydroxide and heat on a steam or water bath, with frequent stirring, until free from ammonia, at the same time allowing the solution to become concentrated by evaporation.

The mixture must not be allowed to evaporate to dryness, however, as that would cause decomposition with the production of the highly poisonous phosphine gas.

When free from ammonia, which may be ascertained by holding a strip of moistened red litmus paper in the ascending vapor, filter and wash the residue

with hot water. Mix the filtrate and washings and weigh, then determine accurately the percentage of barium in the solution. From this calculate the weight of standardized dilute sulphuric acid required to exactly decompose the barium compound.

Mix, heat on steam or water bath until the volume is reduced at least one-half, then allow to cool and filter. The product should be entirely free from barium and give only a faint test for sulphuric acid.

As a matter of interest, some experiments were carried out using barium dioxide to decompose the ammonium hypophosphite.

Adding the barium dioxide to a warm and fairly concentrated solution of the ammonium salt, vigorous reaction set in at once, accompanied by brisk effervescense, the development of considerable heat and the rapid elimination of ammonia.

After standing until the reaction had somewhat abated, the mixture required but a short period of heating on a steam bath to complete the decomposition.

Considering the character of the constituents of this reaction, one would expect that a portion of the hypophosphite would be oxidized to phosphate; on filtering the mixture, washing the residue, dissolving a portion in nitric acid and applying the molybdate test, a heavy yellow precipitate of ammonium phosphomolybdate was produced.

The barium dioxide was tested in the same way and shown to be free from phosphate.

No phosphate was found in the filtrate, but that was scarcely to be expected in view of the alkaline condition of the solution.

The assay of hypophosphorous acid is best conducted by the method worked out in this laboratory by Mr. Horace North and published in the American Journal of Pharmacy for April, page 147.

Put 1 cc. hypophosphorous acid in a tared, stoppered Erlenmeyer flask and weigh accurately.

Add 20 cc. of water recently boiled, to expel CO_2, and a few drops of phenolphthalein solution. Titrate with N/5 $Ba(OH)_2$ V.S. (standardized against N/5 HCl V.S.) until a permanent pink color is produced, and calculate the weight of absolute acid.

Put the flask in a water oven for one hour, then collect any precipitate that may have formed on a 7 cm. Swedish filter, washing with hot water until the filtrate no longer yields a turbidity with a few drops of dilute sulphuric acid, and ignite the filter in a platinum crucible. Deduct the ash of the filter from the residue and divide the corrected weight, in milligrams, by the weight, in grams, of absolute acid in the quantity taken.

The quotient is the barium number and it indicates the proportion of sulphuric, phosphoric or other acids whose barium salts are insoluble under the conditions of the test. If an estimate of the sulphuric acid alone is required, the residue may be treated with hydrochloric acid, washed, ignited and weighed.

In a product fit for medicinal use, the barium number should not exceed 5.

The product may now be concentrated to 50 percent or diluted to 10 percent as required.

LABORATORY OF LEHN AND FINK.