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THE DETERMINATION OF SMALL QUANTITIES OF FLUORINE IN DICALCIUM PHOSPHATE.*

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INTRODUCTION.

The physiological effect of fluorine has been the subject of numerous studies in recent years. It has been shown by experiments upon animals that fairly large doses have a definite retarding influence upon growth. This was established by the use of rock-phosphate, containing 3-4% fluorine, to supply a mineral supplement in the diet of farm animals. The animals receiving this supplement did not flourish as did animals receiving calcium-phosphorus supplements in other forms, and the undesirable effects were traced to the presence of fluorine.

In smaller quantities, fluorine is found to affect the teeth. McClure and Mitchell (1), in studies made upon rats, found that in spite of earlier evidence to the contrary (2) there was no difference between soluble and insoluble fluorides in this respect. DeEds and Thomas (3) in a study of the effect of various fluorides and fluosilicates upon the teeth of male white rats, reported that the solubility of the fluorine compound was not a factor in its toxic effect, and that amounts of 0.5-1.0 mg. of fluorine per day per Kg. body weight can produce definite signs of toxicity. Smith and Leverton (4) also concluded, from experiments upon rats, that the solubility of the fluoride bears no relation to the amount which will cause initial tooth damage. Smith (5, 6, 7) in a study of the effect of drinking water upon children during the formation of the permanent teeth found that mottled teeth were always associated with the use of water containing more than 0.9 parts per million of fluorine.

Dicalcium phosphate has been found to be a valuable dietary supplement in doses of 3-5 Gm. daily. The phosphoric acid from which it is manufactured is derived from fluorine-bearing phosphatic rock, and a method for the determination of fluorine in the dicalcium phosphate is necessary, since traces of fluorine may persist through the manufacturing operations, and remain as calcium fluoride.

Various methods of determining fluorine in relation to phosphatic materials have been studied by Reynolds (8) who recommends the procedure of Willard and Winter (9, 10). This is based upon the distillation of the sample with 60% perchloric acid, the fluorine being volatilized in the form of hydrofluosilicic acid. It is determined in the distillate by titration with thorium nitrate solution, using a sodium alizarin sulphonate-zirconium nitrate lake as indicator. The method is rapid and accurate and can be used without recourse to special apparatus. In a

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later paper (11) Reynolds showed that phosphoric acid could not be used in place of perchloric acid for the isolation of the fluorine, for the presence of phosphoric acid in the distillate introduced serious errors into the results. He further showed that in the distillation of large samples of insoluble phosphates enough phosphoric acid appeared in the distillate to affect the results. He recommended applying a correction for this phosphate or eliminating it by neutralizing the distillate, evaporating it to small bulk and repeating the distillation with perchloric acid.

EXPERIMENTAL.

Our early studies were directed to the application of Reynolds' modification of the Willard and Winter method. The amounts of fluorine present in good quality in dicalcium phosphate are of the order of 0.005% (50 parts per million) or less. The distillation of a 10-Gm. sample of such material gave a final solution to be titrated which was equivalent to less than 1.0 cc. of the thorium nitrate solution. The use of more dilute thorium nitrate solution did not lead to greater accuracy owing to the indefinite end-point produced. In addition, the method required two distillations, two evaporations from 150 cc. to 5 cc. and various transfers from one vessel to another. Not only was there the possibility of loss of fluorine, but considerable time was required. A better method of determining the fluorine in the distillate was therefore sought.

Colorimetric methods for the determination of fluorine have recently been described by Sanchis (12) and by Wichman and Dahle (13). Of these two, that of Sanchis offered the most promise as it is stated by the author to be unaffected by traces of phosphate (up to 5 parts per million). The method, which was originally designed for the determination of fluorine in drinking water, was successfully applied to the distillates obtained by the Willard and Winter method. It consists of measuring the fading of a zirconium alizarin lake produced by the unknown fluorine distillate by comparison with the fading produced by known amounts of fluorine. Our procedure follows:

I. ISOLATION OF THE FLUORINE.

Ten grams of the sample are placed in the distillation flask (to be described below) together with 15-20 glass beads and 20 cc. of distilled water. Forty cc. of 60% perchloric acid are then added through the dropping funnel and the contents of the flask distilled. The temperature rises from 110° C. to 135° C. and is then maintained between 135° and 150° by addition of water through the dropping funnel. Two hundred cc. of distillate are collected and the volume of the distillate made up to exactly 250 cc. with distilled water. It is advisable to remove the beads after three or four distillations. Their surfaces may be revived by boiling for a short time with hydrochloric acid.

II. DETERMINATION OF FLUORINE IN THE DISTILLATE.

Since the Sanchis method is only accurate for quantities of less than 0.3 mg. fluorine the procedure is modified according to the fluorine content of the original sample.

The reagents required are:

- (a) Standard sodium fluoride, 1 cc. = 0.01 mg. fluorine.
- (b) Three-normal hydrochloric acid.
- (c) Three-normal sulphuric acid.
- (d) Indicator solution, prepared as follows:
 1. 0.17 Gm. sodium alizarin sulphonate is dissolved in 100 cc. distilled water.
 2. 0.87 Gm. crystalline zirconium nitrate is dissolved in 100 cc. distilled water.

Equal volumes of 1 and 2 are mixed and allowed to stand over night, and the resulting solution diluted with four volumes of distilled water.

(a) *Fluorine Content above 0.003% (30 Parts per Million) or Entirely Unknown.*—A small aliquot of the distillate, say 10 cc., is withdrawn and the amount of 0.1 normal sodium hydroxide required for exact neutralization determined by titration using phenol phthalein as indicator. Three aliquots, 10, 25 and 50 cc., are then placed in 250-cc. flasks and the amounts of 0.1 normal sodium hydroxide required to neutralize them added. The volumes are then made up to 95 cc. and 2-cc. portions, accurately measured, of three-normal hydrochloric acid, three-normal sulphuric acid and indicator solution, are added. The flasks are heated rapidly to the boiling point and then cooled in a dark cupboard over night. At the same time and with the same three-normal hydrochloric acid, three-normal sulphuric acid and indicator solution a number of standards are made up from the sodium fluoride solution covering the range 0 to 0.3 mg. fluorine (0 to 30 cc. of the solution). Two-cc. intervals are usually sufficient, but for great accuracy 1-cc. intervals may be used. The volumes are made up to 95 cc., the reagents added and the boiling carried out exactly as for the unknown aliquots.

After standing over night the colors of the unknowns are matched with the standards and the fluorine content of the sample calculated. The use of three aliquots for an unknown is usually sufficient to give at least one of them within the range 0-0.3 mg. fluorine. If, however, the fluorine content is known to be within certain limits, say 0.005-0.008%, obviously only one aliquot need be taken and a smaller number of standard samples used.

(b) *Fluorine Content below 0.003% (30 Parts per Million).*—The distillate is collected and made up to 250 cc. as before and the small aliquot titrated with 0.1 normal sodium hydroxide. The whole remaining distillate is then neutralized and evaporated to 90 cc. This is then treated with three-normal hydrochloric acid, three-normal sulphuric acid and indicator solution as before and compared with a set of standard solutions. This procedure permits the color comparison to be made at a higher point in the scale 0-0.3 mg. fluorine, where the matching is easier, the red colors at the lower end being less easy to distinguish. In calculating the fluorine content of the sample allowance must be made here for the small aliquot titrated with 0.1 normal sodium hydroxide.

The above procedure was applied to a number of mixtures of a pure dicalcium phosphate with known amounts of fluorine, and found to give satisfactory results. The pure dicalcium phosphate was prepared from recrystallized specimens of sodium phosphate and calcium bromide.

TABLE I.—RESULTS OF ASSAYS ON 10-GM. SAMPLES OF DICALCIUM PHOSPHATE.

	Mg. Fluorine Added.	Total Fluorine Found—Mg.	Added Fluorine Found.
Dicalcium phosphate	..	0.06	..
Dicalcium phosphate + sodium fluoride	0.10	0.15	0.09
Dicalcium phosphate + sodium fluoride	0.20	0.25	0.19
Dicalcium phosphate + calcium fluoride*	0.25	0.31	0.25

* The addition of the calcium fluoride was accomplished by placing 0.5 Gm. pure lime in the distilling flask and adding a solution of sodium fluoride containing the desired quantity of fluorine. The mixture was boiled to insure complete reaction and the 10 Gm. of pure dicalcium phosphate added. The distillation and determination were then carried out as described above.

DISCUSSION OF RESULTS.

Although the Willard and Winter distillation may be carried out without special apparatus, there is danger that excessive amounts of phosphoric acid may be carried over into the distillate by entrainment. This is especially the case with the large samples used here, and would either lead to serious errors, or necessitate a second distillation, as recommended by Reynolds (11). It was found in practice that the

special distillation flask shown in the sketch minimized phosphoric acid entrainment and had other advantages.

The large ground joint (size 15 or 20) by which the dropping funnel is attached serves as a convenient means of introducing the sample and beads and is easily washed clean by the 20 cc. of water next added. The short Vigreux column (6 inches long) and sleeve method of attaching the thermometer prevents contact between rubber and hot perchloric acid as well as entrainment. The ground joint connecting the condenser to the distillation flask may be dispensed with but it is preferable to the frequent replacement of softened rubber stoppers.

The phosphate content of a number of distillates was determined by applying the method for phosphate under ammonium nitrate in the American Chemical Society Recommended Specifications for Analytical Reagent Chemicals (14) to 50-cc. aliquots of the distillate. Only traces were found, the largest quantity measured was 0.05 mg. phosphoric acid. This quantity would be present if the 50-cc. aliquot were used, and upon dilution to 95 cc. and addition of reagents (total volume *ca.* 100 cc.) would correspond to a phosphate content of 0.5 part per million under Sanchis' conditions. In cases where the entire distillate must be used after evaporation this would

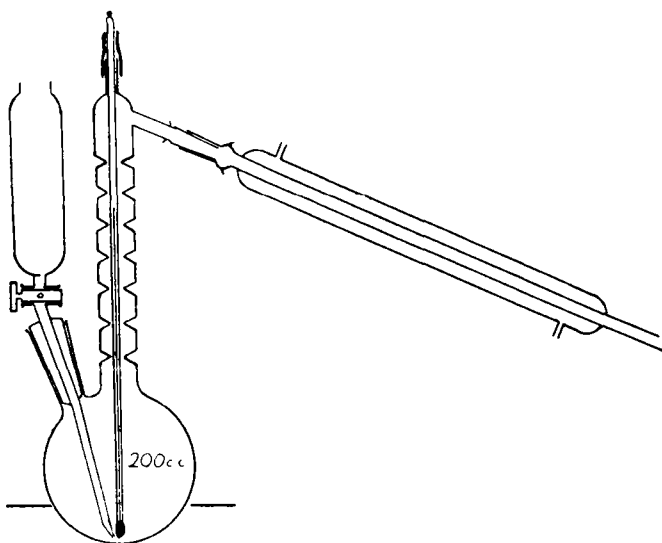


Fig. 1.

amount to the limiting value of 5 parts per million. In only one case, however, was the phosphate measurable and by not forcing the distillation too rapidly there is little likelihood of error from this source.

SUMMARY.

A method for the determination of small quantities of fluorine in dicalcium phosphate has been described. The procedure involves the isolation of the fluorine as hydrofluosilicic acid by distillation with perchloric acid and the colorimetric determination of the fluorine in the distillate by means of zirconium nitrate-sodium alizarin sulphonate. A convenient apparatus for the distillation of the large samples necessary has also been described.

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A NEW TYPE OF HYPNOTIC AMIDE. *N*-(β -KETO PROPYL)
DIETHYL ACETAMIDE. *¹

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N-(β -keto propyl) diethyl acetamide



was prepared as an example of a new type of hypnotic amide, in which it was hoped that the acetyl residue might contribute the hypnotic activity residing in acetone itself, so that the activity residing in the diethylacetyl residue would be reinforced. Actually we found that although the compound did display hypnotic action, it was of a very low potency as compared with hypnotics currently in use.

EXPERIMENTAL.

10.1 Gm. of diethylacetyl chloride, boiling point 130–140° C., were added to a solution of 12 Gm. of anhydrous pyridine in 50 cc. of anhydrous benzene. Immediately, the white crystalline molecular compound separated. To this was now added 10 Gm. of amino acetone hydrochloride prepared from chloroacetone by the well-known Gabriel synthesis.² The reaction mixture was now agitated without heating, whereupon the molecular compound of pyridine and the acid chloride soon disappeared and a yellow crystalline material separated out in its place. After three hours this appeared to be complete, but the reaction mixture was allowed to stand over night.

On the next day, the reaction mixture was diluted with ether and extracted with dilute sulphuric acid solution in order to remove the pyridine; the benzene-ether solution was dried with sodium sulphate and concentrated. Only about 1.25 Gm. of product were obtained in crystalline condition. After recrystallization from benzene the product was pure white and crystalline, melting at 96–97° C.

Nitrogen: Found, 7.91%; calculated for $\text{C}_9\text{H}_{17}\text{O}_2\text{N}$, 8.18%.

The minimum effective hypnotic dose for rats is slightly less than 1600 mg. per kilo, as determined in our Biological Research Laboratories. We gratefully acknowledge this assistance.

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² Houben and Weyl "Arbeitsmethode der organische chemie," 2nd Edition, volume 4, page 258.