activity is due to an infinitesimal trace of the missing alkali element, the second being that it is due to some unstable isotope of the commoner alkali metal. Without going into details, it may be stated that each alternative has, at present, more experimental facts in its disfavor than in its favor, and a third entirely novel explanation is by no means excluded. The present method appears to furnish some hope of advancing our solution of this question for if, by migration of a potassium or rubidium salt, it should be found that the radioactivity was concentrating in the very front or in the very rear of the section, then the isotopic explanation would presumably fall into the discard and further investigation might very conceivably justify the announcement of the discovery of the missing element.

The experiments thus far completed on potassium have not given any final results. It is true that no noticeable concentration of the radio-activity in either the front or the rear has been obtained, but this can quite plausibly be ascribed to the fact that the mobility of the unknown alkali metal ion is substantially the same in aqueous solution as that of potassium ion. The heavier members of the alkali metal group, indeed, all have ionic mobilities in water which are substantially identical within the limits of experimental error. In methyl alcohol solution, however, it has been shown that the mobilities differ very markedly and consequently it may be expected that the addition of some methyl alcohol to the aqueous gel will stagger the values sufficiently to enable a separation to be secured if any unknown alkali metal is present. This point is being tested experimentally at the present time.

Finally, the possibility is being investigated of the applicability of the method to the separation of organic isomers of various types. More complex biochemical problems, such as the concentration or isolation of specific proteins or even of vitamins from natural sources, are probably also open to attack by the ionic migration method, but the experimental technique in such cases has not yet been worked out in detail.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

DETERMINING SOIL MOISTURE RAPIDLY AND ACCURATELY BY METHYL ALCOHOL

IN a former communication (this Journal, April 5, 1927) the use of alcohol was proposed as a basis for a very rapid means of determining the moisture content of soils and possibly of other materials. The form of alcohol that was then suggested was ethyl alcohol. In order to ascertain whether there are other liquids that would be more satisfactory than ethyl alcohol, an investigation has been conducted in which a large number of liquids have been examined. It has been discovered that of all the liquids studied, methyl alcohol seems to be the most satisfactory, as it is the most powerful dehydrating agent. Indeed, this form of alcohol seems to be able to replace or reduce the moisture content of soils down to practically the absolutely dry basis, as will be readily seen from the data below.

	Percentage of water recovered from water added to oven dry soils. Per cent.
Sand	100.05
Loam	100.03
Clay	99.99
Muck	99.01
Silica gel	99.30

The directions for executing a moisture determination by methyl alcohol are the same as those already published (this Journal, April 15, 1927) for ethyl alcohol. There are five points in the procedure, however, that one must always pay special attention to. These are first, the soil must be stirred with a strong rod and reduced to the particle state so the alcohol can come into intimate contact with the entire soil mass. Second, the liquid must be always filtered. Third, great care must be taken to prevent evaporation. The latter can be mainly accomplished by keeping the funnel covered during filtering. Fourth, the temperature of the liquids should always be recorded and reduced to the same basis. And fifth, in calibrating the hydrometer, the specific gravity of the absolute alcohol should be taken under controlled temperature. Allowing the alcohol to stand in running tap-water, to attain the temperature of the latter is sufficient.

It is advisable to use absolute methyl alcohol.

In case of soils containing more than 50 per cent. of moisture, such as muck and peats, only about 10 grams of soil should be used to 50 cc of alcohol.

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A CONVENIENT METHOD OF MEASURING QUANTITIES OF CHLOROPLAST PIGMENTS

ALTHOUGH the photosynthetic mechanism in the leaves of plants has long attracted the attention of

workers in science, the relation between quantities of chloroplast pigments and growth has scarcely been touched. Apparently, the chief difficulty has been the lack of a rapid and fairly accurate method which did not require a great outlay of chemical equipment.

Willstätter and his coworkers devised a simple method of extracting and purifying these pigments, and estimated the quantities colorimetrically. They used solutions of potassium dichromate as color standards for carotin and xanthophyll, having previously evaluated the standards in terms of the pigment in question. The chlorophylls (a plus b) were estimated quantitatively by saponifying the chlorophylls with methyl alcoholic potash to form chlorophyllins. These solutions of chlorophyllins were taken up in water and then measured colorimetrically, using as standards, solutions made up from a known quantity of pure chlorophyll which had been similarly transformed to chlorophyllins. These colorimetric methods are not then measured colorimetrically, using as standards are not chemically stable and (2) because the tint of potassium dichromate solutions is not identical with that of carotinoid solutions, therefore, giving variable results.

Willstätter's method of extraction and separation of the chloroplast pigments has been given in detail by Stiles.[1] Recently Dr. F. M. Schertz, of the United States Department of Agriculture, has modified Willstätter's method, and kindly made the revised method available to the writer for certain investigations on the relations between chloroplast pigments and growth in maize. Dr. Schertz's method, which is simple and adequate for the study of problems of this nature, is now in the press.

The quantitative estimation of the pigments after extraction and separation has also been investigated by Schertz.[2, 3]. He finds the spectrophotometric analysis of solutions a more accurate method than the use of Lovibond slides in a colorimeter. However, the spectrophotometer is an expensive instrument and available to a very limited number of workers.

Since early in 1925 the writer has been using a method of estimating chloroplast pigments in solution which has given consistently good results, is simple, inexpensive and within the reach of the ordinary research laboratory. The amounts of the respective pigments are determined by comparing with artificial color standards of identical tint making use of a Duboscq colorimeter.

The chlorophylls (a plus b) were obtained in the form of aqueous solutions of the chlorophyllins (a plus b which are green by transmitted light. A color standard which matches the tint of the mixture of chlorophyllins obtained from maize was prepared by making 0.3 cc of a one half per cent. aqueous solution of Malachite Green and 11.2 cc of a one half per cent. aqueous solution of Napthol Yellow (Martius yellow) upto 5,500 cc with distilled water. The concentration of color in this standard is the equivalent of 10.708 milligrams of pure chlorophyll (supplied by Dr. Schertz) saponified to chlorophyllins and diluted with water to make 1 liter.

The carotin color standard was made by adding 3.4 cc of a one half per cent. aqueous solution of Napthol Yellow and 0.5 cc of a one half per cent. aqueous solution of Orange G. crystals to 1 liter of distilled water. The tint of this standard is identical with that of pure carotin dissolved in petrol ether and is the equivalent in concentration with carotin solutions containing 1.890 mgms of carotin per liter of solvent.

The xanthophyll color standard was made by adding 2.8 cc of a one half per cent. aqueous solution of napthol yellow to 1 liter of distilled water. The tint is identical with that of pure xanthophyll dissolved in petrol ether and is the equivalent in concentration of 1.537 mgms of xanthophyll per liter of solvent.

Evaluation of the carotinoids was accomplished by making readings of solutions of the pigments in the colorimeter in terms of their respective standards, and also making readings of the solutions in the König-Marten's spectrophotometer at the Bureau of Standards at Washington through the courtesy of that bureau and with the assistance of Dr. Schertz.

There appear to be several advantages in using these artificial color standards for quantitative estimation of the chloroplast pigments: (1) The materials and equipment are inexpensive and are therefore within the reach of a large number of workers in science. (2) The method is fairly accurate, because the tint of the standards is practically identical with that of the pigment solutions, and delicate readings may be made in the colorimeter by varying the volume of the solutions in question. (3) The method is rapid; readings may be made as soon as purification is complete, thus eliminating the large errors caused by decomposition of the pigments in manipulation. (4) The one half per cent. aqueous solutions of the dyes keep for many months (20 by test) without deterioration. 10 grams of each of the dyes, solutions of which have been evaluated in terms of the pigments, should be sufficient for a period of years.

[1] Stiles, Walter, "Photosynthesis. The assimilation of carbon by green plants," London, 1925.

[2] Schertz, F. M., "The quantitative determination of carotin by means of the spectrophotometer and the colorimeter," *Jour. Agr. Research* 26 (1923), p. 383.

[3] Schertz, F. M., "The quantitative determination of xanthophyll by means of the spectrophotometer and the colorimeter," *Jour. Agr. Research* 30 (1925), p. 253.

Different lots of the same dyes may vary in purity, and therefore each new lot should be evaluated in terms of solutions of pigments of known concentrations. The color standards fade slowly when exposed to light and should be made up fresh from the one half per cent. aqueous solutions every few weeks. The one half per cent. solutions should be discarded if they become turbid or if sediments appear.

Full details regarding the preparation and use of these color standards, as well as the results of the studies on relations between chloroplast pigments and growth of maize, will be published in the near future.

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SPECIAL ARTICLES

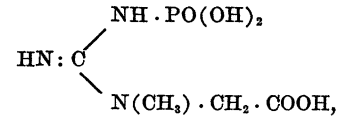
THE ISOLATION AND FUNCTION OF PHOSPHOCREATINE

I. ISOLATION

IN a previous communication[1] we have offered indirect proof of the presence in voluntary muscle of a compound containing one molecule each of creatine and phosphoric acid. The amount of this substance in muscle is considerable (0.4 to 0.5 per cent.); in fact it (and not free creatine) is the principal "extractive" as long as the muscle has not been stimulated or otherwise disturbed. During muscular contraction the compound undergoes hydrolysis, and the same change occurs outside the body under the influence of an enzyme in the muscle or of acid, whereas resynthesis takes place when fatigued muscle is permitted to recover.

At the time of our first report, the leading evidence for the existence of a creatine-phosphoric acid compound depended upon its separation from free creatine by precipitation with copper in very slightly alkaline solution. Under all the conditions enumerated above the precipitate so formed contains creatine and a peculiarly unstable form of phosphoric acid in equimolecular proportions, excepting when—in consequence of stimulation or some other cause—complete hydrolysis of the substance has occurred, and then the copper precipitate is free from both the named constituents. In view of the quantitative nature of the evidence, and of the variety of conditions under which the test has been applied, a different explanation of the results described is hardly possible, and we felt no hesitation therefore in stating that such a compound actually exists. The precise nature of the substance, however—in particular the question whether it contains anything besides creatine and phosphoric acid—can hardly be settled with certainty except by its isolation in the pure state.

Within a few days of the publication of the paper mentioned, we succeeded in isolating a barium salt in crystalline form, but the method of preparation was unsatisfactory and the yields were very poor. After many variations of the original procedure had been tried, the conclusion was finally forced upon us that the use of barium for this purpose is successful only when preceded by a series of preliminary separations (with other metals) in the course of which a large amount of material is lost. By using calcium in place of barium, however, most of the phosphocreatine in protein-free muscle filtrates can readily be separated, in a crystalline condition, from all the other organic phosphoric acid compounds present, but in order to remove these impurities without hydrolysis of the desired product it must be crystallized from alkaline solution. Under these circumstances the result is not a single substance. It contains both secondary and tertiary salts, and (partly because carbonate is present) the carbon content is too high. To obtain the pure secondary salt, having the composition required by theory, special measures must be taken, for this salt in aqueous solution is acid and therefore unstable. The final product crystallizes in spherulites, and has the composition $C_4H_8O_5N_3PCa.4H_2O$. The most probable structure of the new substance is hence the following:



and its most characteristic chemical property, *viz.*, marked instability in acid solution, is in fact characteristic also of the few other known compounds which contain the group $-NH.PO(OH)_2$. This is the first substance containing phosphorus attached to nitrogen to be isolated from natural sources, and the instability of the phosphamic group marks it as one of considerable biological importance, as will be seen in the next section. The details of preparation will be published elsewhere.

II. FUNCTION

In spite of much investigation, the function of creatine in muscle has remained as much a mystery as it was at the time of the discovery of this substance 97 years ago. Having found that most of the creatine in normal resting muscle is combined with phosphoric acid, and that the compound is destroyed during contraction at a rate which rivals that of glycogenolysis and lactic acid production, we naturally anticipated

[1] C. H. Fiske and Y. Subbarow, SCIENCE, Vol. 65 (403)—1927.