DISCUSSION

ON THE ALLEGED EFFECT OF POLARIZED LIGHT ON FILMS OF STARCH

IN SCIENCE[1] there was published last summer a note describing the observations concerning the action of polarized light reported by Miss Semmens at the Cape Town meeting of the British Association for the Advancement of Science. This note pointed to the very striking result that a beam of moonlight—known to be polarized—was able to hydrolyze (to simple sugars apparently) boiled starch carried in films on filter-paper. Her demonstration was based on the fact that objects able to cast shadows on the disks of paper left traces revealed on developing with an iodine solution. The shadows or zones not exposed to radiation were said to appear slightly deeper in color than the exposed parts.

At that moment the *Scientific Transactions* of the British Association[2] was not available to give more information as to her procedure, but from the note referred to we tried to repeat these experiments both with moonlight and with artificial illumination. Since then we have had access to the original abstract of the communication, but unfortunately it is scarcely more explanatory. It was attempted to have our technique as close to hers as could be guessed from the abstract.

Disks of filter-paper (four different makes represented by nine different types of paper as to size of grain), were treated with different solutions of starch (corn, potato and soluble starch) at concentrations ranging from 0.5 per cent. to 5 per cent. The filter-paper was thoroughly washed with distilled water (cold or boiling). These disks were used under three conditions: thoroughly wet, damp and air-dried. Some were exposed on a glass plate, the shadow-casting objects being made of brass, steel, bakelite and blocks of glass covered with black paper. Care was taken to mark with suitable points the corners of the blocks. In a few cases, also, the whole procedure was carried out in a Petri dish. We irradiated the objects perpendicularly to the plane of the paper, taking the trouble to reorient the carrier of the disks every fifteen to thirty minutes when moonlight was used. We checked also the fact that the moonlight was polarized. The artificial light used was a thousand watt bulb whose rays were passed through a large Nicol prism; heat filters were placed between lamp and prism; the spectrum of the light extended from 420 mμ to 700 mμ. Some controls in the dark and in ordinary light of the same intensity as the polarized light were run at the same time. The intensity of illumination varied from 57 m. c. to 550 m. c.; the duration of irradiation ranged from fifteen minutes to six hours. The development of the disks was done with different concentrations of Lugol's solution diluted so as to give a color ranging from barely visible to deep blue-black coloration.

Our results were striking in their consistency: in no circumstances could the author or persons called to judge the differences in colors (and all these were familiar with the iodine starch reaction) detect any difference that could be related to the supposed phenomenon.

If Miss Semmens's contention is correct, there must have been present in her procedure some as yet unrecorded feature. It is at least apparent, however, that the alleged effect can not be of general occurrence.

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INCREASED ACCURACY IN THE DETERMINATION OF CARBONATES IN SOIL

IT has been known for a long time that in the determination of carbonate in soils carbon dioxide is frequently obtained from some soil constituent other than carbonate minerals, and that the amount so obtained is likely to be greater if the soil is unusually rich in organic matter and comparatively strong hydrochloric acid at a high temperature is employed for decomposing carbonates. With many soils the error from this cause is by no means negligible when 1:10 hydrochloric acid at room temperature or even 1:50 acid at 30° C. *in vacuo* is used. The source of carbon dioxide is commonly considered to be the soil organic matter, but no detailed explanation of the reaction involved has come to the writer's attention.

Considerable evidence has been secured indicating that this error is due to oxidation of organic matter to carbon dioxide, as the result of reaction with manganese dioxide native to the soil and the added acid. Not only is the amount of carbon dioxide obtained from sources other than carbonates increased by addition of powdered manganese dioxide to the soil prior to acid treatment, but also determinations of the active manganese dioxide in the soil by measurement of oxygen evolved from soil and 15 per cent. hydrogen peroxide indicate for similar soils a relation between this active manganese and the carbon dioxide obtained by acid treatment.

Addition of ferrous chloride to the acid reduces carbon dioxide from this source to a very small amount when 1:50 hydrochloric acid at about 30° C.

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[1] SCIENCE, 70: xiv, August 30, 1929.

[2] See *Scient. Transact.*, Brit. Assoc. Adv. Sci., Meeting South Africa, p. 12 and 70, 1929.

is employed for decomposing soil carbonates. With considerably stronger acid or at a higher temperature there is a slight error, but by the use of 1:10 acid with ferrous chloride at the temperature noted it is possible to recover very accurately all carbon dioxide from the most resistant dolomite mixed with soil. With some soils it has been noted that the results secured by use of the stronger acid are more accurate than those with a weaker reagent and longer time of action. It is evident that time of action as well as concentration of acid and temperature is a factor, and that oxidation is probably not the only reaction involved, although it may be the most important.

The decomposition of soil carbonates should therefore be effected at the lowest temperature and with the most dilute acid that can be used, consistent with recovery of all carbon dioxide from resistant carbonates in a short time. By the use of a special reagent to prevent oxidizing action the accuracy of the determination is greatly increased. It is planned to publish at an early date a detailed description of an apparatus and procedure for the determination of soil carbonates, including the above and other improvements in technique, whereby the determination can be conducted with the utmost certainty of correct results in a shorter time and with less trouble than is possible with any method previously described.

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THE BEHAVIOR OF SUDAN III WHEN FED WITH CARBOHYDRATE

THE significance of the use of Sudan III in the study of fat metabolism is based on the observations of several investigators,[1] who have shown that this fat-soluble dye when mixed with a fat or oil is readily absorbed from the alimentary tract and is deposited in adipose tissue. The writer has found, in the course of some experiments on fat metabolism, that not only does a stained fat result in deposits of colored fat in the body, but cornstarch plus Sudan III will give the same results in white rats, six to eight weeks old or older, after four days of feeding only cornstarch and a large amount of dye. Similar results were obtained when the extraneous fat occurring in cornstarch was removed and this extracted cornstarch plus Sudan III was fed. Since it was thought possible that the fatty acids attached to a complex carbohydrate in corn α amylose might be responsible for the transport of the dye, other carbohydrates, such as sucrose, lactose and dextrin, were each fed separately with Sudan III. Each animal was allowed only distilled water from 5 P. M. one day until 8 A. M. the next day. The dye, one gram in eighty grams of the carbohydrate, was mixed with the dry material and fed without the addition of any other foodstuff. Five well-matched lots of five animals each were run on the following carbohydrates, cornstarch, rice-starch, dextrin, lactose and sucrose. In each case the dye was deposited especially in subcutaneous fat and the fat around the intestines and the testes. Exceptions were noted, however, in very young rats that became emaciated on this diet, and on autopsy, after four or five days, almost no fat was seen in the body.

Negative results occurred after feeding only two days, perhaps because the animals had not yet taken enough of the dye to show color. On the third day faint pink fat was noted in those animals autopsied, and on the fourth and fifth days unmistakable coloring of adipose tissue was found.

During the feeding of these animals results were noted that have been recorded by other writers, that the urine became pink in a day or two and that the feces were very dark red when first excreted and later became black and hard.

It has been claimed that Sudan III clings to the fat or fatty substance of the food with which it is fed and that it must be borne in mind that fat is necessary for the transport of the dye. In the present experiments, the question as to whether or not the dye fed with carbohydrate attaches itself to fat in its course through the body or whether fat is synthesized from the carbohydrate fed and deposited as colored fat can not be answered now.

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ANTHOCYANIN AS AN INDICATOR

IN connection with the article by O. B. Pratt and H. O. Swartout on "Fruit and Vegetable Pigments as Indicators" (SCIENCE, May 9, 1930, p. 486), I would like to call attention to an article by Professor W. J. Gies and myself, published in 1918 (*Proc. Soc. Exp. Biol. and Medicine,* 16, 8 (1918)) on plant pigments, in which the suggestion was then made that anthocyanin (obtained from the tulip, crimson king, etc.) could be used as an indicator. In fact, the properties of this anthocyanin were compared with phenolphthalein and it was shown that the range of the former varied from pH 5.3–9.2.

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[1] Gage and Gage, "Sudan III Deposited in the Egg and Transmitted to the Chick," SCIENCE, 28: 494, 1908; "The Coloration of Milk in Lactating Animals and the Staining of Growing Adipose Tissue in Suckling Young," *Anat. Rec.*, 3: 203, 1909; Mendel and Daniels, "The Behavior of Fat-soluble Dyes and Stained Fat in the Animal Organism," *Jour. Biol. Chem.*, 13: 71, 1912–13.