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.