containing 1 g of Hyflo Super Cel[1] for each 1.5 mg of enterotoxic material, complete adsorption of the enterotoxin was achieved. Elution was obtained by washing the column with 10 ml of citrate-phosphate buffer solution of pH 7.8 and 0.12 ionic strength for each gram of adsorbent. The procedure was performed at 5° C. The eluate was dialyzed, centrifuged, and lyophilized. Even though the activity of enterotoxin cannot be determined quantitatively, the results (Table 1) do indicate a ten- to twentyfold purification by the chromatographic procedure.

The material obtained by this method contained very little carbohydrate (probably less than 5%). It gave a positive ninhydrin reaction and a color reaction equivalent to that given by proteins with the method of Lowry *et al.* (*6*). Examination by the Oudin immunological technique (*7*) showed the presence of at least four antigens, of which only two were concentrated by the chromatographing. Preliminary electrophoretic examination showed at least two substances to be present, one of which was toxic and one nontoxic. The toxic material contained about 80% of the nitrogen.

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[1] A diatomaceous silica obtained from Johns-Manville.

A Simplified Method for the Determination of Chromic Oxide (Cr_2O_3) when Used as an Index Substance[1]

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Schürch *et al.* (*1*) have pointed out the advantages of using chromic oxide as an index substance for the determination of digestion coefficients. They have described a colorimetric method using sodium peroxide fusion for the determination of chromic oxide. One disadvantage of this method is that when the final solution is determined colorimetrically it does not follow the Beer-Lambert law.

Carroll and co-workers (*2*) have used the chromic oxide technique for the determination of the site of the nitrogen absorption in rats. With a rapid, simple, accurate method for the determination of chromic oxide (Cr_2O_3) its usefulness as a reference substance can be extended.

[1] Published with permission of the director of the North Dakota Agricultural Experiment Station.

During the past several years, perchloric-sulfuric acid digestion with molybdenum as a catalyst has been used for the determination of phosphorus (*3, 4*). This method has been successfully applied to the determination of chromic oxide.

Preparation of oxidizing reagent: Dissolve 10 g sodium molybdate in 150 ml distilled water. Add slowly 150 ml concentrated sulfuric acid. Cool. Add 200 ml perchloric acid (70–72%) and mix thoroughly.

Analytical procedure: Transfer 100–500 mg sample containing 1–5% Cr_2O_3 which has been ground through a 40-mesh sieve to a dry 100-ml Kjeldahl flask, calibrated to 110 or 100 ml. Add 5 ml of the oxidizing reagent in such a manner that it will wash any adhering particles down the side of the flask. Heat flask over microburner which has had the holes in the asbestos board enlarged so as to allow more heat to come in contact with the flask. Oxidation will begin in 1 or 2 min. Allow the sample to digest until a clear digestion mixture is obtained. In the oxidation of some samples, black particles adhere to the neck and sides of the flask. In these cases turn flask 180°, allowing the samples to digest 2 or 3 min longer. Turn off the burner and add 2 ml perchloric acid (70–72%) to the digestion mixture and then reheat. Cool slightly and add 50 ml distilled water. Cool to room temperature and make up to volume in the calibrated Kjeldahl flask, and let flask stand a few minutes to allow silica to settle. Transfer solution gently from the Kjeldahl flask to a colorimetric tube, and read (440 μ filter) against distilled water set at 100.

Prepare a standard curve by oxidizing a known amount of chromic oxide (Cr_2O_3) which is to be used as a reference substance. Dilute contents to a definite volume with distilled water. Take different aliquots of this solution and dilute with distilled water to give different concentrations of chromic oxide within 10–120 μg/ml when the Evelyn Photoelectric Colorimeter is used.

Different amounts of chromic acid were oxidized and made up to a definite volume and read in an Evelyn Photoelectric Colorimeter. It was found that the Beer-Lambert law was applicable when the transmission was more than 30%. This was also true when the oxidized Cr_2O_3 solution was diluted to different known concentrations with distilled water.

The method was tested further by oxidizing different-sized samples of feces or feeds containing chromic oxide (Cr_2O_3). The equivalent feces or feed contents were plotted against the galvanometer reading on a semilogarithmic 1-cycle graph paper. A straight line was obtained.

This method is rapid, simple to manipulate, and accurate. A set of six samples can be oxidized within 10 min.

It might be added here that several thousand samples have been oxidized in this manner under the direction of the senior author for the determination of phosphorus. No explosions have resulted. One precaution is adhered to, and that is never to oxidize more than a 500 mg sample.

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Glyceraldehyde Phosphate Dehydrogenase of Green Plants [1,2]

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The operation of the glycolytic cycle in the metabolism of green plants has received strong experimental support from the findings of Benson and Calvin (*1*) that known intermediates of glycolysis are the principal products of short-time photosynthesis. In very short periods of photosynthesis, of the order of several seconds, most of the newly fixed $C^{14}O_2$ was found in the carboxyl of phosphoglyceric acid (*1, 2*). With increasing exposure to light, the proportion of total radioactivity fixed in the phosphoglycerate decreased, and newly fixed carbon dioxide was detected in a relatively large array of compounds, including triosephosphate, hexose diphosphate, and sucrose (*1*).

$$\text{Glyceraldehyde-3-phosphate} + H_2PO_4^- + DPN^+ \rightleftharpoons 1,3\text{-phosphoglycerate} + DPNH + H^+. \quad (1)$$

The identification of phosphoglycerate as the main early product of photosynthesis pointed to the special importance in green plants of glyceraldehyde phosphate dehydrogenase, the enzyme catalyzing the reversible reduction of phosphoglycerate to triosephosphate according to Eq. (1).[3]

Without this enzyme, the path from phosphoglycerate to carbohydrate synthesis would be blocked. However, the occurrence of glyceraldehyde phosphate dehydrogenase in green plants was recently called in question. Tewfik and Stumpf (*3*), studying the degradation of fructose diphosphate, found that this enzyme, although present in the seed, disappeared from the leaves of pea plants, and Stepka (*4*) concluded from inhibition studies of *Chlorella* with iodoacetamide that reaction 1 is not involved in sugar formation in photosynthesis.

The distribution of glyceraldehyde phosphate dehydrogenase in mature green leaves of sugar beets, spinach, sunflower, and tobacco was studied in our laboratory. Plants were grown in synthetic nutrient solutions in a greenhouse until mature leaves were formed. Immediately following the harvest, the leaves were washed in glass-distilled water and stored in a turgid condition in the cold (5° C). The midribs were removed and 60 g of leaf blades prefrozen at −35° C were blended for 30 sec with 150 ml 0.1 *M* K_2HPO_4. To the blend was added slowly, with stirring, 450 ml cold acetone (−35° C), and the slurry was filtered with suction. The filtrate was discarded, and the residue (acetone powder) was rinsed several times with cold acetone, sucked dry until the odor of acetone could no longer be detected, and dried *in vacuo* over P_2O_5. The dry acetone powder was stored at −35° C; 0.5 g acetone powder was extracted with 10 ml cold water for 10 min, centrifuged, and the residue discarded. The supernatant fluid was filtered, and the clear filtrate, designated acetone powder extract, was used as a source of the glycolytic enzymes. Similar acetone powder extracts were prepared, using either 0.05 *M* phosphate or 0.2 *M* tris (hydroxymethyl) amino methane buffer at pH 7.3. The activity of glyceraldehyde phosphate dehydrogenase was measured by a slight modification of the optical test of Warburg and Christian (*5*) in a Beckman Model DU spectrophotometer. This test is based on the increase in optical density at 340 mμ of the reaction mixture, as a result of the reduction of the pyridine nucleotide, when the reaction proceeds from left to right in accordance with Eq. (1).

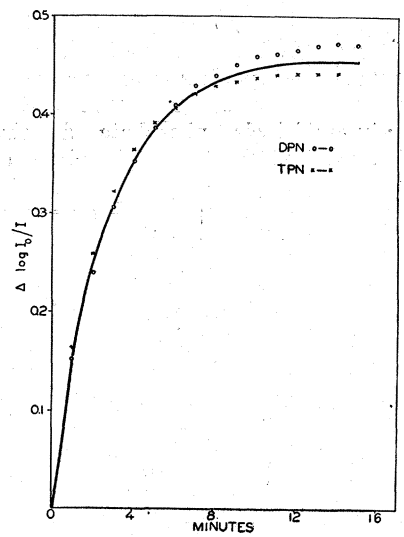

FIG. 1. Oxidation of glyceraldehyde-3-phosphate by crude leaf glyceraldehyde phosphate dehydrogenase. The reaction mixtures contained each 0.3 mg of a dioxane compound of DL-glyceraldehyde-1-bromide-3 phosphoric acid, 0.3 ml of the enzyme preparation and the following in μm: DPN, 0.214 *or* TPN, 0.218; sodium arsenate, 15; sodium fluoride, 10; tris (hydroxymethyl) amino methane buffer pH 8.2, 100; and water to 3.0 ml final volume. The reaction was started by adding the glyceraldehyde phosphate. Quartz cuvettes, $d = 10$ mm.

The acetone powder extract was found to contain aldolase, isomerase, and glyceraldehyde phosphate dehyrogenase (*6*). Glyceraldehyde phosphate dehydro-

[1] Aided by a grant from the U. S. Public Health Service.

[2] After this paper was submitted for publication, M. Gibbs reported independent observations on glyceraldehyde phosphate dehydrogenase in green plants (*Nature*, **170**, 164, [1952]).

[3] The following abbreviations will be used: DPN+—oxidized diphosphopyridine nucleotide (coenzyme I); DPNH—reduced diphosphopyridine nucleotide; TPN+—oxidized triphosphopyridine nucleotide (coenzyme II); TPNH—reduced triphosphopyridine nucleotide.