the shale, it does not follow that the same relationships hold universally.

A thin black shale layer in the Spergen limestone 5½ miles north of Ste. Genevieve, Mo. (*3*), is radioactive and apparently was the source of the constituents that gave rise to a carnotite film on limestone below. The shale was found by spectrographic analysis to contain uranium and vanadium.

Shale visibly barren of carnotite was disaggregated and separated into size fractions as follows: coarse fraction > 4 μ (settling equiv to spheres); medium fraction, 1–4 μ; fine fraction, 1–0.2 μ; and superfine fraction < 0.2 μ (*4*). The coarse and medium fractions contained illite, quartz, and calcite, and the finer fractions contained only illite, as shown by x-ray powder diffraction patterns. Carbon was concentrated progressively in the finest fractions as shown by darker colors, and by more pronounced exothermic reaction in differential thermal analysis.

The uranium content of the fractions was tested by fusing .05 g of a 1:1 mixture of shale and a standard kaolinite, with 2 g sodium fluoride, and by comparing the fluorescence of the fusion under the ultraviolet light (Mineralight) with a standard arbitrary scale of shale-kaolinite mixtures. The medium and fine fractions of the shale showed unit fluorescence, the coarse fraction fluoresced equivalent to a one-half shale mixture, and the superfine fraction fluoresced equivalent to a one-tenth shale mixture. Hence, the shale fraction richest in carbon gave the weakest fluorescence, from which it is concluded that the uranium is concentrated in the clay and not in the carbon fractions.

Leaching experiments were conducted to determine the ease of removal of uranium. Two g shale was stirred and allowed to stand in 50 ml solvent for 24 hr. After filtering, the filtrates were evaporated to 15 ml, and 10 drops of each was fused with 2 g sodium fluoride. Hydrochloric, nitric, and sulfuric acids, aqua regia, and dilute sodium carbonate were effective solvents, but hot and cold water, ethyl alcohol, carbon disulfide, and carbon tetrachloride did not extract enough uranium to produce fluorescence. Normal propyl alcohol dissolved a small amount, which fluoresced weakly.

Attempt to exchange the uranium cationically was made by mixing 10 g pulverized shale with 1 liter 0.1 *N* KCl and with 1 liter 0.5 *N* $BaCl_2$. Cations of barium and potassium were chosen for exchange because of their relatively large sizes, which are comparable to the uranium and UO_2 ions. After thorough shaking, the suspensions were allowed to stand for 24 hr and then filtered. The filtrates and the clay residues were tested for uranium by the fluorescence methods described above. The filtrates, however, showed no fluorescence, and the clay residues four fifths the intensity expressed as unity; hence, it is concluded that the uranium is held by the clay minerals in a condition not easily exchangeable.

In the absence of further data on behavior of the uranium in the clay it is perhaps best to forego unsupported speculation as to where the uranium resides. Apparently it is with the clay, not primarily with the carbon, and is not easily exchangeable as a cation.

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Chromatography in the Purification of Staphylococcal Enterotoxin

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Several methods have been employed for the purification of staphylococcal enterotoxin (*1*), but none has been entirely satisfactory. In an attempt to purify the enterotoxin chromatographically we have found diatomaceous silica to be a satisfactory adsorbent. Other investigators have used this material successfully for the purification of a variety of substances (*2–5*).

The enterotoxin samples were prepared for chromatographing by precipitation from saturated $(NH_4)_2SO_4$ solutions or by precipitation from solution at pH 3.5 (*1*). When these samples were dissolved in citrate-phosphate buffer solution of pH 6.35 and 0.02 ionic strength and passed through a column

TABLE 1

Enterotoxin Adsorption on Hyflo Super Cel

Expt No.	Treatment of crude enterotoxin	Equivalent amount fed* (ml)	Mg fed	Mg N fed	Monkey feeding results† (No. positive/No. fed)
1	$(NH_4)_2SO_4$ precipitation	200	7.4	0.670	5/30
1a	$(NH_4)_2SO_4$ precipitation, adsorption on Hyflo Super Cel	200	0.172	0.009	0/6
		1000	0.860	0.045	5/6
2	Dried	40	416.8	37.7	1/6
		200	2084.0	188.4	4/6
2a	pH 3.5 precipitation, adsorption on Hyflo Super Cel	200	0.260	0.020	2/6
		400	0.520	0.040	6/6

* Volume of crude toxin solution used to obtain the amount of material fed.

† Assay for the presence of enterotoxin was made by feeding the samples in solution to young rhesus monkeys by stomach tube. Vomiting within 5 hr was accepted as a positive reaction for enterotoxin.

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.