setts boys may reflect true differences in the populations sampled. The simplest explanation that fits our data is that of a steady increase in outer fat in both sexes, with a temporary interruption in the male during the period of steroid hormone differentiation.

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Dissolution of Interlayers from Intergradient Soil Clays after Preheating at 400°C

Abstract. Dehydroxylated interlayers have been removed from chlorite-vermiculite-montmorillonite intergrades by boiling the preheated sample in 0.5*N* NaOH for 2.5 minutes. Elements extracted can be conveniently analyzed spectrophotometrically. A convenient method of estimating the amount of elements present in interlayer positions and preparing the sample for analysis for expanding and nonexpanding mineral components is thus provided.

An intergradient chlorite-vermiculite-montmorillonite is one of a series of layer silicates having heat stability of the 14-A spacing, cation exchange capacity, and specific surface intermediate between those of the nonexpanding mineral chlorite and the two expansible and collapsible minerals, vermiculite and montmorillonite. Layer silicates with properties intergradient between those of chlorite and expanding layer silicates have been identified in several soils in widely separated areas of the United States (*1, 2*) and in England (*3*).

The intergradient properties of such clays, relative to properties of the typical minerals, have confounded qualitative identification and prevented quantitative determination of each of the component clay minerals in soils. Also, the aluminum generally present in intergradient clays of soils actively affects chemical reactions, particularly those of soil acidity and liming, phosphorus fixation, and potassium fixation.

It has been found that the elements which give the intergradient properties can be dissolved from between the layers after dehydroxylation of the interlayers of preheating the dry, loose, hydrogen-saturated clay at 400°C for 4 hours. The dissolution is accomplished by boiling the preheated clay sample in 0.5*N* NaOH (at a ratio of 40 ml of NaOH to 40 mg of clay) for 2½ minutes, which is the differential dissolution procedure of Hashimoto and Jackson (*4*). The Al and Si dissolved by the treatment are immediately determined colorimetrically (*5*). The iron oxide precipitated during the NaOH treatment is removed by the dithionite-citrate-bicarbonate method (*6; 7*, p. 57). Removal of allophane from the clay by the same dissolution procedure applied to a sample with only 110°C preheating (*4*) permits calculation of the Al that is made soluble by the 400°C preheating.

Elements dissolved from the samples preheated at 110° and 300°C had a small effect on the intergradient properties, as is shown by some increase in intensity of the 18-A peak (Fig. 1) at the expense of the 14-A peak. However, the NaOH treatment of a different sample preheated at 400°C resulted in a marked increase in the amount of material which expanded to 18-A with Mg saturation and glycerol solvation and in the amount which collapsed to 10-A on K saturation and heating at 300°C in x-ray diffraction preparations (*7*, p. 184).

A small amount (about 0.6 percent) more alumina removed after preheating at 400°C than after preheating at 300°C is responsible for the marked difference in the 14-to-10-A collapse in the x-ray preparation (Fig. 1). This aluminous interlayer which impaired 14-to-10-A collapse on heating at 300°C is stable to higher temperatures than is free $Al(OH)_3$ as gibbsite, but to lower temperatures than complete $Al(OH)_3$ interlayers in chlorite. This is interpreted to indicate the presence in the intergrade of islands of positively charged hydroxy aluminum which bridge between potentially collapsible (and expansible) silicate layers. The percentage of alumina removed, when compared with that in complete gibbsite-like interlayers in aluminous chlorite, indicates that the islands in the intergrade are of limited extent.

Preheating the clay at 500°C destroyed the kaolinite, as is shown by the loss of the 7.2- and 3.57-A x-ray diffraction peaks (Fig. 1). The possibility that the 7.2-A peak is indicative of chlorite is ruled out by the absence of 14- and 4.6–4.8-A peaks (*8*) in the 400°C/K/300°C sample (Fig. 1) and by the fact that soil chlorites which lose their 7-A peak and reinforce their 14-A peak on

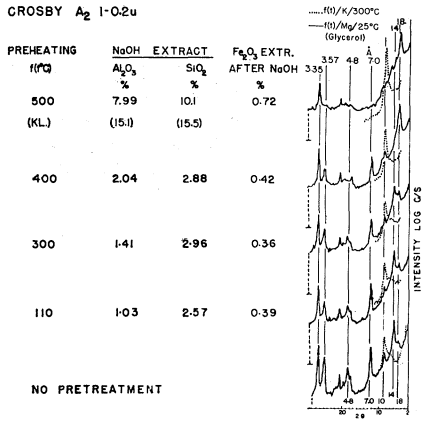

Fig. 1. Elements extracted and x-ray diffraction curves before and after NaOH extraction of the 1–0.2 μ fraction of Crosby soil, A_2 horizon, with various temperatures of preheating, $f(t°C)$, K or Mg saturation, and glycerol solvation followed by 25° or 300°C heating of slides for x-ray diffraction.

500°C preheating are not appreciably dissolved by subsequent NaOH boiling (*9*). The differences (Fig. 1) between the alumina and silica extracted by the same NaOH extraction procedure (*4*) after the 400° and 500°C preheatings allocate to 15.1 and 15.5 percent kaolinite based on the two elements, respectively. Considerable increase in intensity of the 10-A peak (Fig. 1, 500°C/K/300°C versus 400°C/K/300°C) may be due to increase in ordering produced by the removal of a small amount more interlayer aluminum after preheating at 500°C than was removed after preheating at 400°C. Thus the alumina allocated to kaolinite may be slightly high despite the agreement between the allocations to kaolinite of alumina and silica. A little excess silica must be allocated to a small amount of nontronite destroyed, as is shown by the small increase in iron oxides released by 500°C preheating over those released by 400°C preheating; this would allow for a small amount more alumina from the intergrade made soluble by the 500°C preheating.

The dehydroxylation-NaOH method of dissolving interlayers from layer silicate intergrades was found to be more effective, more rapid, and far more convenient for analysis of the elements extracted than the citrate (*2*) or fluoride (*10*) methods previously available. This method was tested on intergradient clays from Tatum soil (Virginia) and Cookeville soil (Kentucky) and was found to give results comparable to the above results with the Crosby soil clay (*11*).

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Color Reaction for Certain Amino Acids, Amines, and Proteins

Abstract. Proteins, certain amino acids, and amines undergo a potentially useful color reaction. The reaction involves the apparent formation of pyrroles when the compounds are allowed to react with acetonylacetone. The pyrroles yield colored complexes on coupling with *p*-dimethylaminobenzaldehyde. This report describes the specificity and possible uses of this reaction in colorimetric measurements and in paper chromatographic detection of these compounds.

In experiments measuring hexosamine by the Elson-Morgan reaction (*1*) in various crude extracts, it was found that substitution of acetonylacetone for acetylacetone gave values much higher than expected on the basis of the indole HCl (*2*) or standard Elson-Morgan (*1*) methods. Preliminary results (*3*) indicated that this was not due to a greater color yield per unit of hexosamine, for, with a standard glucosamine sample, the use of acetonylacetone resulted in less color (about one-half) than use of acetylacetone. This suggested the possibility that the acetonylacetone reacted with a wide variety of amino compounds to form pyrroles (*4*) which were subsequently available for coupling with *p*-dimethylaminobenzaldehyde.

A study of the specificity (*3*) toward a number of compounds was undertaken. Amino acids, sugars, organic acids, purines, pyrimidines, amines (primary, secondary, and tertiary), and many miscellaneous compounds were tested. None of the sugars glucose, galactose, fucose, and rhamnose, at concentrations as high as 10 mg/ml, gave appreciable color. Citric acid at the same level gave no color. The following purines, pyrimidines, amino acids, amines, and miscellaneous compounds, at 0.5 to 2 mg/ml levels (or saturation for those of low solubility), gave little or no color: xanthine, allantoin, adenine, creatine, guanine, hemin, uracil, urea, ribonucleic acid, triethanolamine, choline chloride, diphenylamine, aspartic acid, leucine, phenylalanine, glutamic acid, proline, cystine, methionine, isoleucine, valine, tyrosine, and tryptophan. In Table 1 are given the relative light absorptions at 530 mμ, of the compounds reacting positively. The color from compounds giving positive results was red, with the exception of aniline, which gave a very intense yellow color. The following proteins in concentrations as low as 1 mg/ml also gave fairly intense red complexes: gelatin, egg albumin, lysozyme, and gastric mucin. It is evident that the sensitivity varied widely, being particularly high with certain amino acids, amines, and proteins.

The positive reaction found for NH_4OH (Table 1) is quite interesting and is included to indicate that precautions must be taken to exclude it; exclusion is also necessary when Ninhydrin is used (*5*).

The conclusion that the color developed with the amino acids, amines, and proteins was due to a coupling of *p*-dimethylaminobenzaldehyde with some product (perhaps pyrrole) formed by a reaction with acetonylacetone (but not with acetylacetone) is supported by the following control determinations. Direct treatment of the compounds with *p*-dimethylaminobenzaldehyde without prior acetonylacetone treatment gave no color other than a light violet with gastric mucin and a very slight yellow with tryptophan, citrulline, and urea. Treatment with acetonylacetone without heat gave similar results. Treatment with *p*-dimethylaminobenzaldehyde preceded by a heating period in the presence of Na_2CO_3 but without acetonylacetone also gave the same results. Use of the standard Elson-Morgan method with acetylacetone on the same compounds yielded only the typical red color with glucosamine and a slight yellow with tryptophan, citrulline, and urea.

The potential usefulness of this reaction stems from the following. First, the absorption spectra of representative materials—egg albumin, gastric mucin, lysozyme, lysine, arginine, glycine, tryptamine, histamine, and ethanolamine—all show peaks at 530 mμ. The light absorption at this wavelength is linear with concentration for the amino acids below 0.1 to 0.2 mg/ml, for the proteins below 1 to 2 mg/ml, and for the amines below 0.05 to 0.1 mg/ml.

Table 1. Relative color intensities of compounds reacting positively in the acetonylacetone test.

Compound	Optical density/mg at 530 mμ
Alanine	0.374
Histidine	0.406
Arginine	0.720
Lysine	1.10
Threonine	0.865
Glycine	2.00
Serine	0.285
Ornithine	1.70
Tyramine	1.60
Citrulline	0.595
NH_4OH	0.950
Ethanolamine	1.70
Ethylenediamine	1.90
Ethylamine	1.90
Tryptamine	1.70
Histamine	1.70
Glucosamine	1.70

Second, the method may be useful in the measurement of protein. A comparison of this method with the Biuret method (*6*) in the measurement of protein is shown in Table 2. It is evident that the variation between individual proteins with the acetonylacetone method is no greater than it is with the Biuret method and that it would be much less than with methods based on the measurement of a single amino acid (or types of amino acids such as those with 280 mμ absorption, or reactions specific for tryptophan or tyrosine). The sensitivity of the acetonylacetone method is about 10 times greater than that of the Biuret method, as is shown in Table 2. The sensitivity is, however, less than that with the Folin phenol method (*7*) by a factor of 5 to 10.

Third, the method may be useful as a dip reagent in paper chromatography. The most suitable method tried (*8*) resulted in pink to lavender colors with four lambda spots on filter paper of the

Table 2. Comparison of the specificity and sensitivity of the biuret and acetonylacetone methods in the measurement of proteins.

Protein	Optical density/mg at 530 mμ	
	Biuret	Acetonylacetone
Gelatin	0.032	0.375
Egg albumin	0.036	0.265
Lysozyme	0.045	0.406
Gastric mucin	0.039	0.318