is more distinct. Similarly, when E235 is absorbed by Ro (XY), the reaction of anti-Z with γ-B (YZ) is distinctly separate from the short band. Finally, when E235 is absorbed by a mixture of Br (YZ) and Ro (XY), only the short band corresponding to γ-C (W) appears; no antibodies to γ-A and γ-B remained. That γ-C has no antigenic determinants in common with γ-A or γ-B was also suggested by the fact that the precipitin band due to γ-C would not coalesce with a band formed by either myeloma globulin (*6*, *7*). It is of interest to point out that the faster myeloma globulin Br (Fig. 1) corresponds in its antigenic determinants to the slower normal γ-globulin, γ-B (Fig. 2). However, this relationship is reversed with selected myeloma globulins which are of slower mobility than Ro and have the same two antigenic determinants as Br and γ-B (*8*).

None of three horse or three rabbit antisera when investigated in the same manner could distinguish between the antigenic properties of Br and Ro nor between γ-A and γ-B (*9*). One of the horse antisera (Blue Boy) had antibodies for a γ-globulin which appeared to correspond to γ-C. The γ-globulin found by Goodman with chicken antisera also appears to correspond to γ-C (*10*). It will be of interest to compare γ-A, γ-B, and γ-C to the γ-globulins, G_1 and G_2, found by Oudin with rabbit antibodies (*11*).

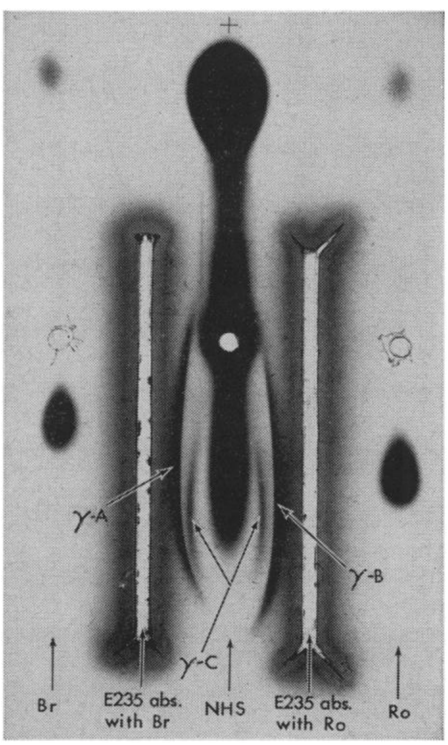

Fig. 2. The precipitin bands which appear when monkey antiserum E235, absorbed with myeloma sera Br and Ro, reacts with the electrophoretically separated γ-globulins of normal human sera. Because of endosmosis in agar gel, the electrophoretic patterns are displaced toward the cathode. At *p*H 8.6, all the proteins in serum are negatively charged and move toward the anode under the influence of an electric field.

The availability of precipitating antibodies specific for three normal human γ-globulins should facilitate many studies of considerable interest concerning these γ-globulins, such as: quantitative estimation in serum and other body fluids (*11*, *12*); fractionation and purification (*2*, *13*); chemical structure, most particularly in the analysis of fragments resulting from enzyme digestion (*14*); antibody properties, in infectious diseases and diseases of supposed immunologic etiology (*15*); cytological localization by fluorescent antibody (*16*); and possible genetic differences (*17*). Of immediate clinical interest, the quantitative estimation of these γ-globulins in serum should be useful for early diagnosis and study of diseases which involve qualitative and quantitative changes in the γ-globulins, such as in multiple myeloma (*8*).

The finding of three "slow" γ-globulins with monkey antibodies, instead of the one usually found with horse or rabbit antibodies, focuses renewed attention on the classical principle of "immunologic perspective" for immunochemical investigations (*1*). As stated by Boyd, "it would be desirable, whenever possible, to use as the antibody-producing animal a species not too distantly related to the group whose relationships we wish to study, instead of using rabbits for all such experiments" (*1*).

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25 July 1960

Three-Dimensional X-ray Reflections from Anthracite and Meta-Anthracite

Abstract. Careful analysis of x-ray scattering intensities of demineralized meta-anthracites and high-rank anthracites formed during the Pennsylvanian geological period has revealed the presence of three-dimensional (hkl) reflections of graphite, demonstrating unequivocally that coals graphitize with metamorphism. Graphitization has been observed also with a coal formed before the Cambrian period, much earlier than most coals. A significant degree of graphitization occurs by coalification when the graphite-like layers attain a size of 25 to 30 angstroms as compared to 100 A or more by the heat treatment of amorphous carbons.

X-ray diagrams of most amorphous carbons and coals contain three or more diffuse bands in the angular positions of the (001) and the two-dimensional (hk) reflections of graphite. With the development of the theory of diffraction in random layer lattices by Warren (*1*) it became possible to analyze the x-ray patterns of amorphous carbons and coals in terms of randomly stacked graphite-like layers (aromatic molecules) (*2*). Such analyses readily yield the size of the layers and the height of the stacks.

When carbons are heated at high temperatures, the dimensions of the parallel layer groups increase; the layer size increases more rapidly and attains a larger ultimate value than the height of the stack (*3*). With more extensive heat treatment, some layers assume positions that are oriented with neighboring layers. This three-dimensional orientation, manifested by modulations of the (hk) reflections, is identical with that of graphite and hence is termed graphitization (*4*). Franklin and Warren observed the onset of graphitization whenever the layer size approached a value of about 80 to 100 A.

High-rank anthracites and meta-anthracites constitute the highest ranks in the classification series of coals (*5*). If coals ultimately become graphite (this view is often expressed) the highest rank coals should exhibit three-dimensional reflections of graphite. In Fig. 1 are shown the diffraction patterns of Ceylon graphite and a meta-anthracite from Newport County, Rhode Island. This coal was formed during the Pennsylvanian geological period. A demineralized sample containing 0.5 percent ash had the following composition by weight percent on a moisture- and ash-free basis: 97.9 C, 0.21 H, 1.7 O, and 0.2 N. The x-ray data were obtained on an XRD-5 diffractometer, with CuKα radiation. The recorded intensities were corrected for polarization.

As seen in Fig. 1, the meta-anthracite gave rise to all the observed three-dimensional reflections of graphite, that is, (101), (102), (103), (112), (201), (114), (203), (116). The (120) and (121) bands remained unresolved. The average layer diameter calculated from the line broadening of the (110) reflections was about 3000 A, and the average height of the stack calculated from the line broadening of the (002) reflections was 250 A. X-ray reflections of this and other meta-anthracites studied (*6*) unequivocally demonstrate

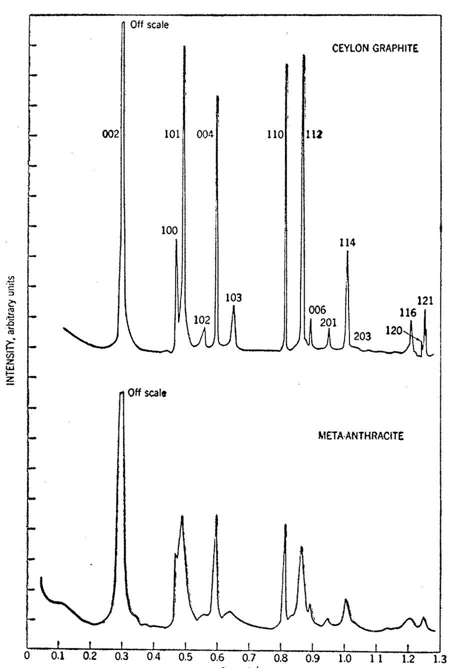

Fig. 1. X-ray scattering intensities of Ceylon graphite and meta-anthracite from Newport County, Rhode Island.

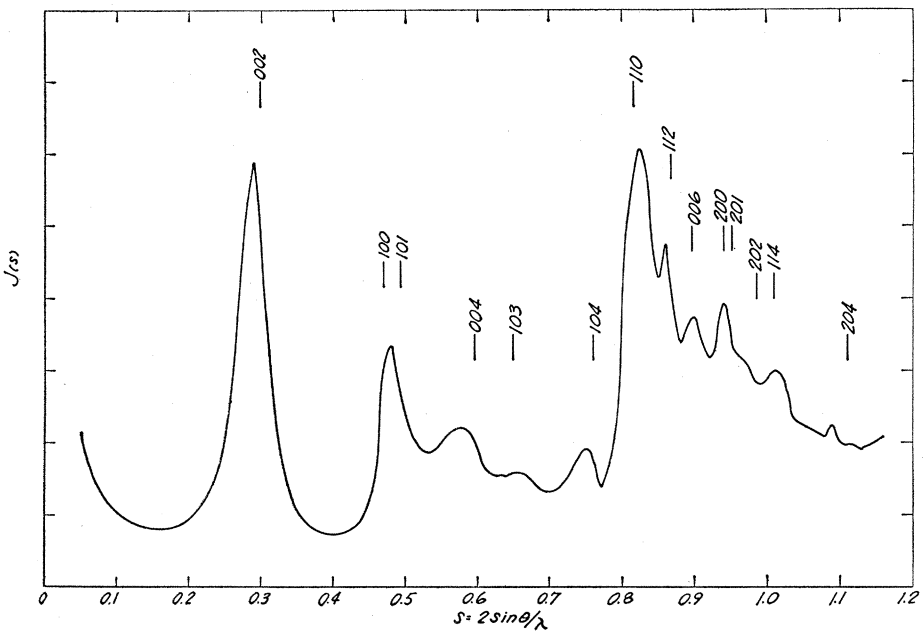

Fig. 2. X-ray scattering intensities of pre-Cambrian anthracite from Iron County, Michigan, showing modulation of the (11) band.

the presence of well-developed three-dimensional crystallinity of graphite.

A point of interest was to find whether coals of a different origin also developed graphitic structure. A coal from Iron County, Michigan, that is believed to have been formed before the Cambrian geological period, much earlier than most coals, was studied. Demineralized samples of this coal, containing 0.1 percent ash and 0.7 percent moisture, had the following composition by weight percent on a moisture- and ash-free basis: 93.4 C, 0.84 H, 1.2 N, 4.3 O, and 0.3 S. The coal can be classified as an anthracite (*5*). The recorded x-ray intensities were corrected for air scattering, polarization, and absorption, and were converted into atomic units by a trial-and-error procedure by matching the unmodulated portion (low-angle side) of the (11) band with the Warren equations. The results are shown in Fig. 2.

As shown in Fig. 2, the pre-Cambrian coal gave rise to many three-dimensional (hkl) reflections, indicating a significant degree of graphitization. Especially to be noted is the very pronounced modulation of the (11) band. The positions of the graphite reflections are marked in the figure for purposes of comparison. The (103), (104), (112), and (114) peaks are distinct; the (200) and (201) peaks are unresolved; the (202) peak appears as a shoulder; and the (204) peak is displaced somewhat. It is evident that the modulation of the (11) band preceded that of the (10) band; the modulation of the (11) band has resulted in resolution of the (110) and (112) peaks, whereas the (10) band remains unresolved. In the diffraction patterns of the meta-anthracites, most of the peaks are distinctly resolved (see Fig. 1).

Of particular interest is the size of the layers in the anthracite samples showing the beginning of the modulations of the (11) reflections. The layer diameter L_a was calculated by using $L_a = (a_2 - a_1)/\pi(s_2 - s_1)$, where s is defined from $s = 2 \sin \theta/\lambda$, θ being the Bragg angle and λ the wavelength of x-radiation; a_2 and a_1 are functions determined from the theoretical profile of a two-dimensional lattice reflection derived by Warren; subscripts 2 and 1 refer to the angular positions of the intensity curve where the intensity is one-half of the peak intensity. Values of L_a of the pre-Cambrian coal calculated from the (11) and (10) bands were 31.2 and 38.4 A, respectively. It is probable that closer agreement could have been obtained had the curve been demodulated. An estimate of 39.5 A for the layer size was obtained from the hydrogen-to-carbon ratio of the coal by assuming a pericondensed ring system.

A high-rank anthracite from Providence County, Rhode Island, having an ultimate composition, by weight percent, of 95.2 C, 0.89 H, 1.8 O, 0.3 N, and 1.8 S, had an x-ray pattern (not shown) very similar to that of the pre-Cambrian coal—that is, it showed the modulation of the (11) band. L_a as determined from the (11) band was only 26 A.

X-ray diagrams of these coals showed unequivocally that coals graphitize with metamorphism regardless of origin. Moreover, a significant degree of graphitization occurs by natural process when the layers attain a size of about 26 A, as compared to 100 A or more by the heat treatment of amorphous carbons.

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5 August 1960

Effects of Supernumerary Chromosomes on Production of Pigment in Haplopappus gracilis

Abstract. One of the two types of supernumerary chromosomes found in *Haplopappus gracilis* effects pigment production in the achene walls. When one, two, and four supernumerary chromosomes were added to the basic complement, a corresponding increase in the amount of one type of pigment was found to occur. No overlapping of the effects on pigment production was observed among different numbers of supernumeraries or between the supernumeraries and normal chromosome complement.

Supernumerary chromosomes have been reported for many plant and animal species (*1*). They have been found to be devoid of genes in the usual sense, but in certain species a reduction in vigor, fertility, and sexual maturity can be attributed to their presence (*2*). Some of these effects are correlated with increased numbers of supernumeraries (*3*). However, attempts to relate a definite phenotypic effect with a certain supernumerary chromosome have been unsuccessful thus far. A possible exception may obtain in *Plantago*, in which one extra chromosome has been found to be associated with male sterility (*4*).

In a previous report of the supernumerary chromosomes in *Haplopappus gracilis* (Nutt.) Gray (*5*), it was pointed out that plants containing the larger type of supernumeraries could be distinguished by certain morphological characteristics of the leaves and stems. In addition, the achene coats of plants with supernumerary chromosomes were found to be a dark red color while the normal chromosome type was usually brown or, rarely, reddish at maturity.

The purpose of this preliminary study was to determine whether the larger type of supernumerary chromosomes exhibited an additive effect on pigment production in the achene coat as they were increased in number in the plant.

Using seed from reciprocal crosses of plants with $2n = 4 + 2$ and $2n = 4 + 3$, we grew a number of progeny in the greenhouse with normal diploids ($2n = 4$) having brown or reddish achenes. The chromosome numbers of all the plants were determined from somatic cells of immature heads. Several plants with chromosome numbers of $2n = 4 + 1$ and $2n = 4 + 2$ were obtained, but only two plants with $2n = 4 + 3$ and one with $2n = 4 + 4$ were grown to maturity. The mature achenes were harvested, dated, and stored at room temperature until used.

The pigments were extracted from ten weighed achenes in 3 ml of cold HCl in 90 percent ethyl alcohol, in a Tenbroeck all-glass tissue grinder, and the nonsoluble cell debris was removed by centrifugation. The solution containing the pigment from the brown achenes was yellow, while that from the reddish achenes and the red supernumerary fruits was varying shades of pink. Spectrophotometric analyses were carried out in a Beckman model DU spectrophotometer. Weight differences of the achenes were corrected after analysis.

The yellow pigment solutions, after dilution, yielded absorbance maxima at two points, 335–340 mμ and 270–275 mμ. The latter peak sometimes appeared only as a small shoulder in the absorbancy curve. The pink pigment solutions (undiluted) gave absorbance maxima at three points, one at 535–540 mμ, and the other two at or near the same wavelengths as the yellow after equivalent dilution. It thus appears that two and possibly three different pigments are present and that the yellow pigment occurs in the reddish diploid type as well as in plants having the supernumerary chromosomes.

In Fig. 1, the absorbance maxima of the two higher wavelengths of one sample have been plotted for each of the normal and aneuploid types. As the figure indicates, there is little or none of the pink pigment present in the brown achenes with a normal chromosome number. However, the data show a greater amount of the pink pigment in the achenes with supernumeraries than is found in the reddish type with the normal chromosome complement. In addition, there is an increase in the amount of pigment when one, two, and four supernumeraries are added to the normal complement. The plants with three supernumeraries produced less pigment than those with one and two, but it is important to note that this amount was somewhat greater than it is for the reddish diploid type and that it did not overlap with any of the other supernumeraries.

The yellow pigment occurred in greatest quantities in the brown achenes. A lesser amount was found in the reddish type, and the quantity varied in the achenes of plants with different numbers of supernumerary chromosomes. Whether there is a relationship between the general decrease in the amount of yellow pigment and the increase in red pigment in plants with up to two supernumeraries remains to be determined. A direct relationship might be expected if the two pigments were dependent upon a common precursor. An explanation is wanting also for the peculiar effect of three supernumeraries on the production of the red pigment. Nevertheless, the importance of the data presented here lies in the fact that each supernumerary chromosome exerts a definite effect upon the production of both yellow and pink pigments, and that no overlapping of the effects was found.

Genetic analysis, now in progress, should yield information on the mode of inheritance of pigment production.

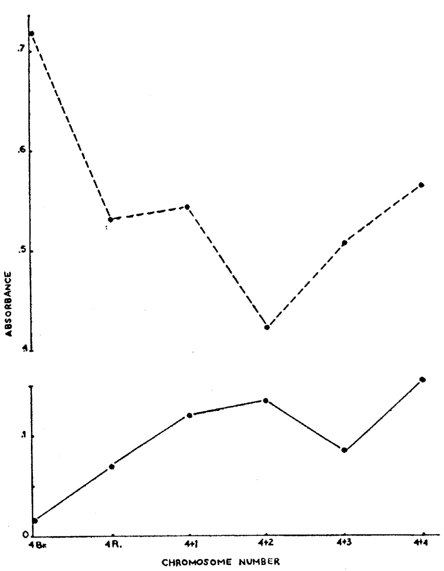

Fig. 1. Upper curve (dashed lines) shows absorbance maxima at 335–340 mμ of brown (4B) and reddish (4R) diploid achenes, and aneuploid types with one to four supernumerary chromosomes. The lower curve shows the absorbance maxima at 535–540 mμ for the same chromosome types.