time after the cut, the after-potentials became smaller. At a healed end, after-potentials were absent or were negative in sign. The negativity increased during a train and was followed by a positive phase.

The sign of spike (positive with respect to the indifferent lead) and possibly the sign of the after-potentials and the injury currents recorded at the freshly cut end by this technique appear to be consistent with the sign of membrane resting and action potentials recorded by intracellular electrodes. The use of the technique may therefore result in less confusion than prevails when conventionally, externally obtained negative-upward records are compared with the intracellularly obtained observations. Less length of nerve than is ordinarily required is adequate with this method, and the inconvenience and deleterious effects of air and oil are eliminated (*5*, *6*).

DEXTER M. EASTON
Division of Physiology, Department of Biological Sciences, Florida State University, Tallahassee

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6. This research was supported by grant No. B-927 from the National Institutes of Health, U.S. Public Health Service.

27 June 1960

Three γ-Globulins in Normal Human Serum Revealed by Monkey Precipitins

Abstract. Precipitating antibodies specific for three normal human γ-globulins of relatively slow electrophoretic mobility were prepared in monkeys and demonstrated by immunoelectrophoresis in conjunction with absorption techniques in which two myeloma globulins were used as absorbents.

Monkeys were selected for the preparation of antibodies to human serum proteins because antibodies prepared in a more closely related species might be more discriminating for minor antigenic differences among the serum proteins than antibodies prepared in a more distantly related species (*1*). Rhesus monkeys were immunized with normal human γ-globulin prepared by cellulose ion-exchange chromatography (*2*). Doses of 5.0 to 50 mg of γ-globulin were injected subcutaneously or intramuscularly. The first dose was emulsified in complete Freund's adjuvant; subsequent doses in incomplete adjuvant or in saline were given at monthly or biweekly intervals. Sera were analyzed by immunoelectrophoresis in agar gel used in conjunction with absorption techniques as previously described (*3*).

Figure 1 is a photograph of a stained immunoelectrophoretic agar plate showing the precipitin bands which appear when a monkey antiserum (E235) against normal γ-globulin reacts with the electrophoretically separated globulins of normal human serum, myeloma serum Br, myeloma serum Ro, and a mixture of the two myeloma sera (*4*). Figure 2 is a photograph of a stained plate showing the precipitin bands which result when the same monkey antiserum (E235) is absorbed with each myeloma serum (Br and Ro). The electrophoretic patterns of Br (1:16 dilution in saline), normal human serum (undiluted), and Ro (1:16 dilution in saline) are shown superimposed on the results of double diffusion. The left trough had been filled with E235 absorbed with Br; the right trough, with E235 absorbed with Ro.

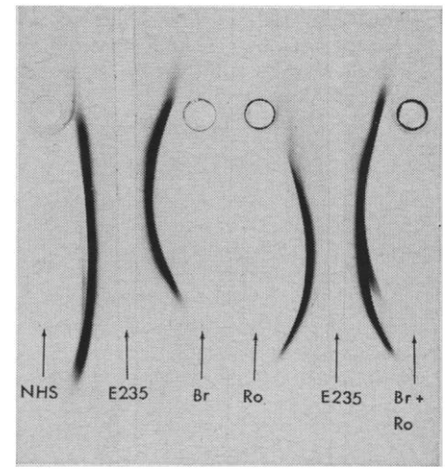

Fig. 1. The precipitin bands which appear when monkey antiserum E235 reacts with the electrophoretically separated globulins of normal human serum, myeloma serum Br, myeloma serum Ro, and a mixture of the two myeloma sera.

After E235 is absorbed by either myeloma serum (Fig. 2), the antibodies remaining no longer react with the myeloma serum used for absorption but do react with normal serum to yield a long and short precipitin band. The two long bands are asymmetrical with respect to the electrophoretically separated "slow" γ-globulins, indicating that the unabsorbed antibody in the left trough reacted with a γ-globulin (closer to the anode) of faster average mobility than the unabsorbed antibody in the right trough; these two γ-globulins are designated γ-A and γ-B, respectively (*5*). The two shorter bands are symmetrical and represent reactions of an unabsorbed antibody specific for a third γ-globulin which is designated γ-C. The precipitin reaction patterns in Fig. 2 were also obtained with γ-globulins which were considered free of macroglobulin (*2*). Thus, γ-A, γ-B, and γ-C are presumably 7S γ-globulins.

The antibody to γ-A prepared by absorption of E235 with Br was found to react also with Ro; thus, γ-A and Ro have an antigenic determinant (designated as X) in common. Antibody to γ-B prepared by absorption of E235 with Ro was found to react also with Br; thus, γ-B and Br have an antigenic determinant (designated as Z) in common. In Fig. 1, the coalescence observed when the mixture of Br and Ro react with E235 suggests the presence of antibody to an antigenic determinant (designated as Y) common to Br and Ro. That Br and Ro have an antigenic determinant in common was also indicated by other monkey antisera which were completely absorbed by either Br or Ro. Finally, the antigenic determinant on γ-C is designated as W.

Still other monkey antisera, T710 and E221, in their reactions with normal γ-globulins, yielded long, broad precipitin bands which showed splittting at the cathode or anode end, respectively, and coalescence at the anode or cathode end, respectively (*6*). Each fork of the split band could be shown to coalesce with a band formed with one of the myeloma globulins (*6*, *7*). These results suggested that γ-A and γ-B correspond in two of their antigenic determinants with the two myeloma globulins Ro and Br. Thus, γ-A and Ro have determinants X and Y, while γ-B and Br have determinants Y and Z.

Accordingly, the reaction of unabsorbed E235 and normal serum, which results in the long, broad band (Fig. 1), represents the superimposed reactions of anti-X, anti-Y, and anti-Z with γ-A (XY) and γ-B (YZ), and, as would be expected, the antibodies appear to react with a γ-globulin of intermediate mobility between γ-A and γ-B. When E235 is absorbed by Br (YZ), it should contain anti-W and anti-X (Fig. 2). Since anti-Y has been removed, the reaction of anti-X with γ-A (XY) is slightly closer to the trough than when unabsorbed E235 is used (Fig. 1), and the separation of this band from the short band due to anti-W with γ-C (W)

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*).

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*).

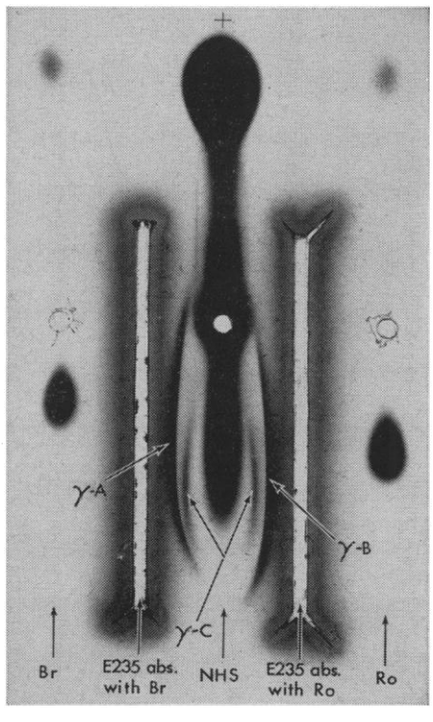

Fig. 2. The precipitin bands which appear when monkey antiserum E235, absorbed with myeloma sera Br and Ro, reacts with the electrophoretically separated γ-globulins of normal human sera. Because of endosmosis in agar gel, the electrophoretic patterns are displaced toward the cathode. At *p*H 8.6, all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

SHELDON DRAY

Laboratory of Immunology, National Institutes of Health, Bethesda, Maryland

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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