## The sickling phenomenon as a basis for legal exclusion of paternity

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ONE of the laboratory procedures frequently requested by the courts is the application of blood-group genetics to problems of identity. The most common of these problems is that of exclusion of paternity. This procedure requires the determination of the blood groups of the accused man, the mother, and the child or children. These data are then reviewed to determine whether or not any of the accepted laws of heredity are violated by assuming that the defendant is indeed the father of the child. Obviously if the data do not fit into recognized theories of inheritance the defendant can be excluded from consideration. Any such exclusion is predicated on the assumption that the plaintiff is really the biological mother of the child.

The evidence supplied by blood tests can be used only to exclude, and not to prove paternity. In many jurisdictions, laws have been passed empowering the courts to order blood-grouping tests in cases in which paternity or related problems may be at issue. The law in the State of Ohio was passed in 1939, but even before that time there were several instances in which the court ordered blood tests at the request of a defendant in bastardy trial.<sup>1</sup>

The Ohio Code provides that men who are defendants in bastardy trials can request the court to order blood-group tests of the mother, the child, and the defendant for the purpose of determining whether or not it is possible to exclude paternity by such tests. Such exclusions as are demonstrated are admissible as evidence, but failures to exclude are not admissible as evidence.<sup>2</sup>

The American Medical Association Committee has defined allowable blood groups as the ABO, MN, and Rh blood antigen systems.<sup>3</sup> Further studies have established the usefulness of the Kell antigen and the Duffy (Fy<sup>a</sup>) antigen.<sup>4</sup> There is as yet no statutory authorization for the application of other polymorphic characteristics. Unfortunately, the wording of the law is such that the courts have correctly interpreted the admissible blood tests as being determination of blood groups only.

This report is a review of our own experience in demonstrating paternity exclusions by the use of the appearance of the sickle cell (S)

hemoglobin in a child when it is absent from the blood of both the mother and the putative father. This characteristic has been selected because it is seen most frequently in Negroes, a group of people who are particularly disadvantaged by the statistical probabilities of exclusion by means of blood-group antigens as compared with the Caucasian population.<sup>5</sup>

The application of blood group genetics to paternity exclusion studies in the Negro race has resulted in a great number of exclusions. Our data from 200 such studies indicate that an exclusion rate of about 13 percent can be expected from the cases referred to us by the Juvenile Court of Cuyahoga County and by the individual lawyers. This means, in effect, that since we have a theoretic exclusion possibility of 55 percent, utilizing the blood-group systems ABO, MN, Rh, Kell, and Duffy, then only half of the innocent men are excluded by blood-grouping tests. The difference between the 55 percent possibility and the 13 percent actually observed is accounted for by the fact that the theoretic percentage is based on the supposition that the men involved are actually innocent, while the cases referred to us by the courts include many men who are not in actuality innocent. Indeed, in many instances, it might be observed that the biological fact that permits a man to be excluded is due to good fortune rather than to moral behavior on his part.

Several factors serve to make the theoretic exclusion rate among the Negroes compare unfavorably with that in Caucasian populations. In order to demonstrate exclusions, it is necessary to have a broad spectrum of genetic characteristics that might occur in the individuals studied. Obviously, if a population is homogeneous, there will be a great number of individuals with the same particular blood-group combinations. One is less likely to be able to exclude an accused man when all that can be deduced from the serologic data is that the father could or could not be a man having similar blood-group antigens as are found in the blood of the accused. Thus, more chances for exclusions are found in the relatively heterogeneous Caucasian group and fewer opportunities for exclusion occur in the relatively homogeneous Negro group.6 It is for this reason that it seems reasonable to search for other polymorphic characteristics in the Negro race in an attempt to see whether or not such characteristics might be of help in increasing the percentage of exclusions in such studies. One of the obvious polymorphisms that occur almost exclusively among Negroes is the occurrence of the gene responsible for S hemoglobin. The inheritance of this characteristic is believed to occur so that the gene expresses itself in the homozygous state as the disease sickle cell anemia, and in the heterozygous state as the relatively innocuous sickle cell trait.

Children who have the sickle cell trait must have at least one parent who is either a carrier for the sickle cell gene or who has sickle cell anemia. Thus, it is possible to establish an exclusion if the sickle cell phenomenon is demonstrable in the child's blood and is absent from the blood of both the mother and the putative father. Conversely, if the accused man has clinically proved sickle cell anemia, he cannot be the father of a child with normal A hemoglobin only. A more difficult decision might be made in the case of a child who has manifest sickle cell anemia and a mother who demonstrates the trait. Such a child could not be the offspring of a normal parent. In one instance, a man who had been clinically diagnosed as having sickle cell anemia was accused in a bastardy case. Since the child in question demonstrated the sickle cell trait, the man could not be excluded as the child's possible father.

## Test for sickling

The test for sickling is comparatively easy to do and consists merely of preparing a suspension of erythrocytes in a reducing agent such as sodium metabisulfite and observing the preparation over a 20-minute period for the appearance of the typical sickle cells. Positive results may be tested by electrophoresis of the hemoglobin, using paper strips with a barbital buffer at pH 8.6. These technics were used to confirm an exclusion on the basis of the sickle cell trait alone.<sup>7</sup>

Because of the dangers of missing the sickle cell phenomenon in a preparation it is necessary to check any presumptive exclusions electrophoretically. Our own experience indicates that the screening test does miss some cells that should be positive for sickling, either because the cells tested were older than 24 hours, or because anaerobic conditions were not satisfactory.

Figure I is an illustration of a study in which the sickle cell tests had been confirmed by electrophoretic pattern. The presence of the abnormal S hemoglobin in the erythrocyte is manifest in the two top characteristic bimodal curves of the child's blood and the known AS control blood. This can be compared to the normal A pattern of the three lower curves prepared from the blood of the mother, the defendant, and a normal A control.

A review of the last 107 paternity exclusion tests involving Negroes shows that 14 (13 percent) were excluded by conventional blood-grouping technics. The ABO system accounted for six exclusions, the MN system for two, and the Rh subtypes for six. One of the Rh exclusions was supported by the appearance of the sickle cell trait in the child, and its absence in the blood of the adults in the study. This was interpreted to indicate that the father of the child was a man other than the defendant. Further, it could be concluded that the real father had either sickle cell anemia or the sickle cell trait. In this same series, two other cases were excluded on the basis of the sickle cell trait alone. These findings seem to indicate that the detection of the sickle cell factor is less effective in excluding paternity than either the ABO or the Rh blood groups, and is about as effective as the MN system. However, the determination of the sickle cell phenomenon

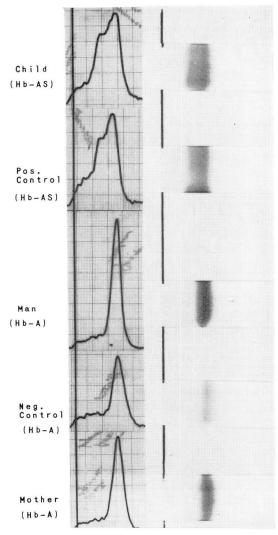


Fig. 1. Paper electrophoretic patterns of hemoglobin specimens obtained in a paternity exclusion investigation. The upper curve is that of the child, the middle curve that of the defendant, and the bottom curve that of the mother. A barbital buffer, pH 8.6 was used.

in paternity cases appears to be much more effective than the determination of the Kell or the Duffy antigens, as these produced no exclusions in this Negro group.

## Conclusions

The incorporation of sickle cell screening into paternity exclusion studies is obviously not going to exclude a great number of defendants in bastardy suits, but it will establish the innocence of a few if such tests become accepted by the courts. In our experience, three exclusions were obtained in 107 paternity studies, only one of which would have been detected by blood-grouping technics. The acceptance of the presence of abnormal hemoglobin as evidence by the courts would encourage further clinical investigations into other polymorphisms, and would increase the probability of demonstrating new bases for exclusions.

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