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CURRENT PROBLEMS IN RESEARCH

Blood Groups of the Ancient Dead

Paleoserology has provided a new tool for the anthropological study of gene flow in the past.

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The blood groups of the dead have been of interest primarily in medicolegal fields. Landsteiner and Richter had already, in 1903, surveyed the possible use of blood-group data in forensic medicine (*1*); it is probable that the first application, in this field, of knowledge of the reactions of the blood was by Lattes in 1916, when he presented evidence on blood stains before an Italian court (*2*). The techniques first developed allowed distinction to be made only between human and nonhuman blood, but with subsequent improvements, and the discovery of groups other than the ABO antigens, the value of such evidence has increased enormously, and much further research has been done.

The anthropological applications of statistics on blood groups of the living are well known, and such statistics have been put to widely increasing use since the original discovery of the Hirszfelds in 1919 (*3*). The pattern of frequencies varies considerably from one population to another, and deductions on racial origins and degrees of affinity have been shown to be valuable; indeed, in some respects they are superior to the metrical characters of the early anthropologists, because they appear to be less subject to the fluctuations of environment. It is natural that interest should have extended to work on historical and archeological material, in an attempt to compare the distribution of blood groups among the living with that among their ancestors. The techniques used owe much both to medicolegal research and to the interest of biochemists in the occurrence of blood-group substances in body tissues and fluids.

The first application of these techniques was by William and Lyle Boyd (*4*). In 1933 they published a note on blood grouping by means of preserved muscle. This was followed by work on dried muscle and saliva, and then on Egyptian and American mummified tissues. Their choice of technique would appear to have been influenced by the work of Lattes on stains, and the method was highly successful on muscle, but its application to bone did not satisfy them, and it was left to Candela to develop methods suitable for cancellous bone, and to show that bone could give comparable results. The work of the latter in establishing that the blood groups may be identified in bone (*5*) is of wide significance to the anthropologist. Bones are relatively more abundant than preserved flesh, and survive under more varied conditions and to a greater age.

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These workers were attempting to identify the A and B antigens and also to show the presence of M and N. Matson (*6*) contributed experiments on the use of bovine anti-O sera on saliva, on muscle obtained at autopsy, and on mummified muscle. The idea of extending his work to bone does not appear to have occurred to him, in spite of the very encouraging results he obtained on other tissues. Recent authors, such as Thieme, Otten, and Sutton (*7*) and Laughlin (*8*), have applied these methods to particular problems and have attempted the solution of some of the difficulties outlined by the earlier workers. Recent use of paleoserological methods has been made in an attempt to distinguish between the rival skulls of Swedenborg (*9*). The attempt, although unsuccessful because of the occurrence of the same antigen in both skulls, shows an interesting use of these techniques in detective anthropology.

In an early paper the Boyds discussed the medicolegal applications of the blood grouping of tissues (*10*). The greater range of the possible applications of such blood grouping is well illustrated by the extensive interest felt amongst archeologists, historians, and anthropologists today. Anthropologists trained in paleoserological techniques are now working in four laboratories, two in the United States and two in Europe. For innovation these laboratories still rely heavily on the interest and cooperation of forensic medicine, but their basic technique continues to be that of the earlier workers already cited.

Technique

In their paper "Blood grouping in forensic medicine" (*10*), the Boyds describe the technique which, in its broader outlines, has become the principal tool of the paleoserologist. This is based on the standard inhibition test. It was made possible by the discovery that the blood-group substances are not confined to the red blood corpuscles but may be found extensively and

sometimes in greater concentration throughout the organs and body fluids. It is possible to demonstrate the presence of group-specific substances in, for example, saliva, by testing its power to inhibit the action of its corresponding agglutinins on fresh red cells in the standard red cell-serum agglutination test. Similarly, the Boyds showed that dried and ground muscle could be mixed with antisera of known group and could inhibit agglutination in later tests with red cells. At first, the initial mixture of tissue and serum was allowed an inhibition time, at room temperature, of 1 hour. Subsequent tests showed that a period of at least 24 hours, in the refrigerator, gave more satisfactory results. Too long a period of inhibition can sometimes produce nonspecific absorption.

The use of both naturally occurring and immune antisera was advocated very early, as a means of establishing controls on the reactions obtained. The number of antisera used is often governed by the amount of material available; ideally, no fewer than three of each specificity are used. This is important in that it cannot be guaranteed that all tissue samples will react uniformly with the entire range of antisera in the panel. Morgan (*11*) has drawn attention to the presence of "many different resultant macromolecules . . . in different individuals belonging to the same ABO group, for example group A, depending on the subgroup and Lewis genotype of the individual. Thus, one would not expect the secretions of all group A individuals to yield products which are identical in properties and chemical constitution." In this connection it is also advisable to obtain regular supplies of the same antisera from individuals, and to be thoroughly familiar with the behavior of these antisera. Pooled sera are not usually used; it is generally considered more valuable to deduce results from the reactions of a panel of individual sera. Type O serum was popular with some workers at first, but was later discarded because of the unequal strength of the anti-A and anti-B components. Dodd (*12*) has also shown that there is often a linkage between the two agglutinins, which results in lowering of the titer of both where genuine inhibition has affected only one. It is surprising, in view of the evidence against the use of this serum, to find it being advocated by Harley in 1944 (*13*). Unless the sera used show an approximately equal absorption power and avidity, they will not provide adequate comparisons on the inhibitory powers of any two tissue samples and will throw little light on the amount of blood-group substance present. While this point may have only minor medicolegal significance, it is of great interest to the anthropologist comparing survival rates of group-specific substances according to age and environment.

The titer of the antisera is determined throughout with the use of fresh 1-percent suspensions of A_2 and B cells from the same donors. The use of A_2 cells is recommended because they give the more sensitive reading of neutralization of the anti-A component, rather than of the anti-A_1 component (*14*). Buffered saline, containing 1 percent sodium azide as a preservative, is used as a diluent throughout.

After inhibition, which is usually carried out in 10- by 75-mm tubes, the samples are centrifuged to throw down tissue particles, and the supernatant is removed. This is then titrated in order to determine, by comparison with its previous titer, whether its strength has been affected by contact with the tissue sample. The Boyds suggested a titer of four tubes before absorption, and held that a satisfactory standard of inhibition would be the removal of agglutinins over these four tubes. In practice in this laboratory, a titer of three tubes is considered to be satisfactory, especially with weakly reacting samples. The Boyds noted the unreliability of a single test. Our practice is to repeat the test at least three times on any given sample with any one serum, in order to obtain a confirmation of the inhibition. Fresh material, or that containing a high proportion of organic matter, will show some absorption at the seventh or eighth test.

The quantity of tissue used in proportion to a given volume of serum is never a constant for a variety of samples. Various workers advocate the use of 0.5 gram or more. Boyd and Boyd (*15*) used only 0.08 gram of ground muscle to 0.3 milliliter of serum. The quantity of material available will often be the deciding factor. In this laboratory it is usually found convenient to begin tests with 0.2 gram of tissue to 0.4 milliliter of antiserum, and to make adjustment in further tests in accordance with the results obtained. This proportion is usually satisfactory, except in the case of very weakly reacting tissue samples. A volumetric technique is used for titration, the drop technique being regarded as less accurate (*14*).

Other Methods

Other methods have been used in an attempt to establish the presence of group-specific substances in tissues. Matson made use of a simple alcohol extraction on material of known group but discarded the method because of hemolytic effects. Since his paper appeared, extraction techniques have been perfected by biochemists seeking to purify and analyze the components of the blood-group substances (see *16*). These methods require relatively large quantities of a tissue or a body fluid containing a high concentration of group-specific substance. For the purposes of paleoserology, however, a highly purified product is not essential, and this field offers much promise if a technique can be evolved from this basis which is both simple and efficient for our needs. Margery Gray investigated this subject in 1952 (*17*). While her results were not conclusive, her work represents a first step in an interesting direction.

Other techniques present possibilities worthy of examination. In particular, modifications of the precipitin and Coombs (antiglobulin) tests would appear to be suitable for application to aged human tissue. Stains up to 60 years old have been identified as human by the precipitin test. This can provide an invaluable preliminary to typing for particular antigens. This technique, because it is valid for all tissue proteins, can also be applied successfully to extracts of bones. Work on the Coombs technique has been published by Allison and Morton (*18*) and by Ruffie and Ducos (*19*). Ducos (*20*) has shown the possibility of identifying a wide number of antigens in blood stains, for forensic purposes. His work should be followed up in paleoserology in order to discover the extent of its application to aged stains and tissues.

Investigations into a number of other techniques are also being carried out. Their main purpose is to examine the amount of organic matter remaining in tissue aged under varying conditions, to determine the rate of preservation of blood-group substances, and to evaluate different methods of biochemical assay in relation to these studies.

Environmental factors, first investigated by Thieme, Otten, and Wheeler (*21*), also pose a problem on which study is urgently needed. Expert advice on soil conditions and bacterial effects has made it possible to set up experiments in these fields. It will be several

years before these experiments mature. It is hoped that they will show the extent and nature of bacterial contamination. This is known to present well-defined problems, in that bacterial antigens have the power to inhibit agglutination in human sera in a manner closely similar to that of the A and B antigens. One theory on the solution of this problem is that analysis for rare sugar components of bacterial cell walls may show whether inhibition can be attributed to bacterial contaminants or not. The value of this theory has yet to be determined, but it would appear to offer at least a partial solution to the problem.

Stability of Antigens

The stability of antigens may also be influenced by soil type and *p*H, and by rain action. It must be concluded that tissues present in soil for a sufficient length of time will, depending upon the rate of decay (particularly of fats) allowed by the nature of the soil, have lost by leaching any water-soluble antigens originally present. It remains to be determined what length of time this process takes in, for instance, chalk as compared with clay soils, rainfall, drainage, and temperature all being taken into consideration. It is certain that autopsy material used for control and experimental purposes still contains quite a high proportion of blood, even after fat has been extracted. Some attempt should be made to leach this out artificially, if such material is to provide comparison with unknown burial material of any antiquity.

Bacteria have been shown to present other problems, in addition to reactions similar to those of A and B antigens. Iseki and Okada (*22*) and Iseki and Ikeda (*23*) have concerned themselves with the decomposition of A and B group-specific substances by bacteria. Watkins (*24*) has shown the presence of blood-group decomposing enzymes in a protozoan. Such action, while it deprives the mucopolysaccharide of its original specificity, appears to leave H specificity. This finding has been used to suggest that H substance acts as a precursor for the synthesis of A and B. If this is so, and enzymic degeneration of A and B allows the appearance of results closely similar to, if not identical with, those obtained with the H antigen, then the need for a positive test for group O becomes more urgent.

The difficulties in this direction become immediately apparent when one studies the effects of sera on fresh red-cell suspensions in standard laboratory diagnosis of blood group. Here, the use of unknown sera with known red cells shows positive reactions for group O, where the sera will agglutinate both A and B cells. But in the use of known (anti-A and anti-B) sera, positive reactions occur only with cells having the A or B antigen. The presence of group O cells is presumed where reactions are consistently negative. In identifying the group in tissue samples it is possible to use only this part of the technique, because of the rapid post-mortem degeneration of agglutinins. This cannot be satisfactory when the possibilities of antigen degeneration are taken into consideration. The desirability of a positive test for group O is well illustrated by the Boyds' work on remains of prehistoric American Indians (*25*); 226 individuals were typed for this series, and of these only 13 gave positive results for A or B or both. Morgan and Watkins (*26*) have shown that antisera which are preferentially active against group O cells are of two kinds, one, which they call anti-H, being inhibited by the saliva of group O secretors and the other, anti-O, not being so inhibited. In this laboratory, experiments have been carried out with these sera on tissues whose ABO (H) character has been determined ante mortem. Anti-H sera of plant and animal origin would appear to give results which differ from those of sera with an anti-O specificity. Naturally occurring human anti-O sera would appear to inhibit, clearly and potently, all group O material. The presence of agglutinins to other antigens does not appear to affect the action of the anti-O component, and thus the usefulness of this serum would appear to merit closer investigation.

Nonspecific Absorption

In typing any large series of aged human tissues, where a number of results are recorded as AB, a query must be raised. Nonspecific absorption of all sera may occur in such material. The causes are in general obscure but may sometimes be associated with particle size in bone, with mineralization, or with contamination. The presence of fat in material having a high organic content may sometimes be the cause. It would seem very probable that early attempts to group mummified tissues gave erroneous results because of the presence of embalming fluids, and the Boyds noted a number of anomalous effects in such material (*27*). Probably the best safeguard against this type of reaction is the regrouping of the sample with a series of antisera of both human and animal origin, one of which at least is not specific for either the A or B antigen. Other solutions have been suggested, mainly along the lines of substitution of a protein medium for the physiological saline in which the inhibition test is normally carried out. The use of sera of human origin for this purpose must always be regarded with suspicion, because of the occurrence of alcohol-soluble group-specific substances in the sera of all individuals, regardless of secretor status (*28*). It would be preferable, and probably of some value, to experiment with diluents of animal origin, provided that these could be shown to be devoid of substances similar to the ABO antigens.

Anti-H Agglutinins

In recent years studies on plant agglutinins (lectins) have revealed the existence of antibodies to the ABO antigens, including some specific for A_2. Probably the best known of these is the extract of *Ulex europaeus*, now widely used as an anti-H agglutinin. Anti-H agglutinin from the seeds of this and other plants has been used for typing of tissues, as has also the naturally occurring anti-H agglutinin of eel serum (*29*), bovine anti-H (6- described as anti-O) (*30*), and immune rabbit serum. The use of anti-H agglutinin was proposed by Mourant (*31*) for assistance in the diagnosis of group O. The opportunity presented, to demonstrate positively the presence of a third antigen H, is of considerable importance. It is not yet known whether the H antigen degenerates at a rate different from the rates for A or B antigens; in the absence of reactions for A and B antigens and in the presence of reactions for H, the sample may be presumed to be group O. The opportunity to add this information to that provided by the ABO group gives further scope for anthropological deductions.

Anthropological Applications

It has been said that many of the criteria for the identification of the dead can be forged, although it is

almost impossible to deceive the expert. No forgery can be undertaken with the blood groups, unless we accept the interference of bacteria as criminal action! The use of statistics on the ABO groups throws light on many anthropological problems. One of the most interesting, touched on by the Boyds, is the date of introduction of these groups into America by the successive waves of migrants. Candela's data on Aleut mummies (*32*) have been used by Laughlin (*33*) in discussing the origins and racial affinities of the present population of the islands. It is possible that further studies would show relative dates for the introduction of the different ABO groups into the American continent. Group O is predominant there today, notably in South America, where work on ancient mummies and skeletons could contribute to the solution of such problems as the origins and racial affinities of Inca and pre-Inca peoples. But North America shows areas of high incidence of group A—an incidence that increases as one moves northwards. When did this gene arrive, and where did it come from? Is it possible to demonstrate the absence of group B in old Indian populations, or will its presence suggest an Asian origin, as has been postulated for the Eskimo? It is equally possible that it could be demonstrated that groups A and B had been lost in New World populations of the present day. Many fascinating problems can be posed, on the origins and the migrations of peoples all over the world. As our techniques improve and our knowledge extends, we shall add many more. It is the function and the adventure of paleoserology to solve them (*34*).

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Optics of Light Sources Moving in Refractive Media

Vavilov-Cherenkov radiation, though interesting, is but an experimental instance of a more general problem.

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For a number of years the Vavilov-Cherenkov effect appeared as but a peculiar optical phenomenon difficult to observe. Light emission was induced by using radioactive preparations, and the glow was observed visually (*1*). The weakness of the glow seemed to preclude any application of the phenomenon in physics, and this was even more true in engineering.

Peculiarities of Radiation in a Medium

Since the theory of the Vavilov-Cherenkov effect appeared (*2–4*), the phenomenon could be regarded as an instance of superlight-velocity optics. This was a singular example in this field, which seemingly was isolated from any other known physical phenomena. It was evident that in principle other manifestations of superlight-velocity optics were also possible, but their observation appeared very complicated. For example, even the first calculations indicated that if the Vavilov-Cherenkov radiation were induced not by an electric charge but, say, by the magnetic moment of an electron, it should be so weak that its experimental detection would not be feasible (*5*). It was likewise evident that it would be difficult to create conditions for observation of atoms moving at superlight velocities (*6*).

Theoretical analysis of all these problems was for a number of years of interest chiefly from the viewpoint of principle.

Progress in nuclear physics and the improvement of experimental techniques in recent years has resulted in the fact that the Vavilov-Cherenkov effect has found numerous applications in the physics of high-energy particles. A connection between this phenomenon and many other problems has also been found, as, for example, the physics of plasma, astrophysics, the problem of radio wave generation, the problem of acceleration of particles, and so on.

A broader approach to the treatment