

# A BLOOD TYPING OF HUMAN SKULL FRAGMENTS FROM THE PLEISTOCENE<sup>1</sup>

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The Midland Skull, found in 1953 in western Texas, is possibly the oldest dated specimen of man yet discovered in the New World. Wendorf, Krieger and Albritton in their report ('55) on the find, assign it to a period definitely earlier than the occupation of that area by Folsom Man. At Lubbock, Texas, 150 miles to the north of Midland, the Folsom level has been established by radiocarbon dating as approximately 10,000 years old. Although a dating of Midland material from the level containing the human skeleton was dated as  $7100 \pm 1000$  years old, the paucity of the sample has left the latter date open to question, especially since Midland human materials were found beneath a Folsom level.

From their analysis of all relevant data, Wendorf and his colleagues conclude that Midland Man lived prior to the last advance of the Wisconsin Glaciation. If so, probably he substantially preceded all other human remains in this hemisphere.

In June, 1955, Dr. Fred Wendorf supplied to the authors 20-odd fragments of Midland Man bones for blood grouping. Tests, however, were postponed until early February of 1956, which interval gave the authors an opportunity to complete, to their satisfaction, improvements and standardizations of techniques aimed at insuring more reliable results.

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Procedures were based on the work of Candela ('36, '37, '39, '40), Boyd ('34, '37, '39), and Matson ('34, '36), who, among others, successfully determined the blood types of bone, mummy tissue, and body fluids by means of the absorption (inhibition) of agglutinins. However, there has existed no hitherto practicable method of distinguishing the presence of the O antigen from an absence of A and B. Such absence of antigen might be occasioned by various agents, such as chemical action, leaching, or deterioration due to extreme antiquity. The solution of problems relating to the typing of old populations, i.e. the American Indian, which exhibit a high frequency of the O blood type, has been especially handicapped as a consequence.

Since the announcement of Boyd and Reguera ('49), and Boyd and Shapleigh ('54) of the lectins in *Ulex europaeus* seeds this laboratory has been exploring the possibilities of their use in absorption tests. While *Ulex* extracts have been quickly adopted for determinations of the Secretor factor in saliva, they have not been used, to the knowledge of the authors, for bone testing outside of this laboratory. Techniques involving the use of anti-O lectins of *Ulex* were accordingly, explored.

The use of absorbed cattle sera (beef anti-H) was also attempted, but because of its wide latitude of anti-H reaction, was found to be inconclusive and much inferior to *Ulex* extract.

Secondly, test procedures were assessed for accuracy by employing bone material of known A, B, AB, and O blood types. Autopsy material aged under controlled conditions, some specimens for a year, some for several, were used in establishing the reliability of testing procedures. Bone samples from 16th and 17th century Philippine cave sites, from the collection of the University of Michigan Museum of Anthropology, were also tested and clear reactions were obtained comparable to those given by known type bone.

Thirdly, standardized and equalized stock anti-A and anti-B sera were prepared, as well as *Ulex* extracts, and sealed and frozen in amounts suitable for individual tests.

The absorption test, as described by Candela ('37, '39) and Boyd ('37, '39), was used. This test involves the introduction of pulverized tissue or bone sample into antisera of known and identical titer, which is allowed to stand over a period of 24-48 hours. Any blood group antigen present in the sample will, as a consequence of specific antigen-antibody reaction, lower the titer of its appropriate antibody agglutinins, or even remove them altogether. Known erythrocytes, subsequently added to the centrifuged, clear supernatant antisera, act as indicators to reveal the presence or absence of agglutinins, and, accordingly, the corresponding antigen in the original sample.

All three antisera (anti-A, anti-B, and anti-H) were employed at three different titers each, namely, 32, 64, and 132. Titer in antisera is determined by means of progressively doubled dilutions with physiological saline solution, thus: 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:132. Titer indicates the highest dilution (weakest solution) capable of still yielding an observable agglutination reaction with appropriate known red cells. A serum with a titer of 32 would, accordingly, be twice as strong as one of 16.

The three titers used were not chosen arbitrarily. A preliminary test-run using anti-A, anti-B, and anti-O antisera at a titer of 16 showed all three tubes cleared of agglutinins by non-specific absorption. Therefore the antisera were of necessity used at higher titer in order to demonstrate differential absorption.

Four of the largest bone fragments, identified as portions of the skull, provided sufficient material for testing. These were finely ground together in a mortar and carefully mixed to insure uniform samples.

Anti-A and anti-B sera were employed in the dilutions as specified. Anti-O was prepared from *Ulex europaeus* in stronger concentration than that employed on saliva in the Secretor

test. Preparation consisted of the following: 25 gm of seed were finely ground in 500 cm<sup>3</sup> of physiological saline in a Waring blender. The mixture was then alternately frozen and thawed, and stirred for 24 hours under refrigeration in order to obtain maximum extraction. The extract was cleared by prolonged centrifugation, methiolate added to a 1:5000 concentration as preservative, and the solution frozen in vials. Extract so prepared will exhibit sufficient concentration to tolerate dilution even when used at the high titer required in the following tests.

#### RESULTS

Readings of agglutination were taken at the end of a 30-minute interval, and again at the hour. Readings were as follows:

	32 TITER	64 TITER	132 TITER
<i>30 minutes:</i>			
Anti-A serum	—	—	±
Anti-B serum	—	+	+++
Anti-O serum	—	±	++
<i>One hour:</i>			
Anti-A serum	—	—	+
Anti-B serum	±	++	++++
Anti-O serum	—	++	+++

It may be noted that non-specific reduction in titer removed almost all agglutinins of the 32 titer antisera. In observing the clear differentiation in the removal of anti-A agglutinin in the 64 and 132 titer tubes, in contrast to the remaining anti-B and anti-O agglutinins, the authors felt justified in assuming a tentative type A for the specimen submitted.

However, two further questions presented themselves: (1) could non-specific absorption conceivably be greater in the anti-A antiserum than anti-B or anti-O, and (2) could the effect of soil contamination influence the results of the typing?

In attempting to solve the first problem, a series of dilutions employing the same antisera used in the bone typing

were set up, and samples of kaolin and benzonite, a diatomaceous earth, were added in the same amounts as the ground bone previously tested. Kaolin proved to absorb non-specifically by approximately one tube, that is, sera of titers of 64 were lowered to titers of 32. Benzonite, however, effected a more profound non-specific absorption, titers of 64 being lowered to 4, a total of four tubes cleared of agglutinins.

Significantly, however, the three agglutinins were equally affected, with no different degrees of non-specific absorption observable. We conclude from this that non-specific absorption, while probably a function of particle size and composition, does not account for the differential absorption which was observed in the bone testing.

The second problem involved the testing of sand samples sent to the laboratory by Doctor Wendorf. These included samples from the 5 deposits of sand distinguishing the Scharbauer Site: white sand, which formed the firm floor of the blowout in which the human bones were found; the gray calcareous sand constituting the matrix in which the remains were embedded; the red Judkins sand overlying the gray, and exposed along the bottoms and sides of the blowout; the tan sand of the Monahans formation; and the superficial loose sand.

Sand samples were added in equal amounts to serial dilutions of the standard antisera; results again showed no differential absorption between anti-A, anti-B, and anti-O antisera, although non-specific absorption lowered all titers approximately two tubes. We conclude from this that the nature of the materials surrounding the human bone, while exhibiting some non-specific absorption, do not account for the A reaction observed.

A bone found in the same deposit with the human remains, and identified as that of a rabbit, did not cause a differential reaction when the same techniques used in typing the skull were employed. This test offered further evidence that the A type antigens were not intrusive from the sand, nor mixed with other animal remains. That the specimen was indeed

human was indicated beyond doubt by the use of anti-human immune serum over which was layered an extract of the specimen. A distinct precipitin reaction was thus obtained comparing closely to the human control, in contrast to controls of dilute cow and buffalo sera.

Attempts to type the Midland Man fossil by means of a saline extract of ground bone in order to avoid the problem of non-specific absorption proved futile, however, extracts being too weak in antigenic strength to effect observable absorption. In the event of a larger sample, which would permit extraction and concentration by lyophilization, the authors feel that such a procedure might be utilized.

While the absorption reaction on anti-A serum reported here can be taken as presumptive evidence of A antigens, it is nevertheless important to establish the presence of complex organic material in the bone sample. In the report by Wendorf et al. ('55) on the Midland Discovery, Worman and Waskins (table 7) state that the organic content of the bone to be close to 1%, suggesting the presence of sufficient organic material to make testing for antigen residues entirely feasible. In addition to this, we undertook to test the bone sample for the presence of bound amino acids, since Abelson ('54) has shown that ancient bone, some going back to the Devonian, can be made to yield amino acids.

Acid hydrolysis of a small quantity (0.5 cm<sup>3</sup>) of washed bone released appreciable amounts of amino acids (aspartic acid, glutamic acid, glycine, alanine, valine, and leucine) as revealed by dimensional paper chromatography. Thus complex organic compounds are shown to be still present in the sample.

In conclusion, the Midland Man find is very probably type A. As tests were performed to ascertain that the sample is human and does contain complex organic material, as well as eliminating the possibility of differential non-specific absorption or contamination from soil or associated fauna, the authors feel that the blood typing is as reliable as can be achieved by known methods.

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