This experiment showed that the decrease of hyaluronidase activity was mimicked by a decrease in hyaluronate concentration soon after the chicks hatched and that high levels of hyaluronidase activity exist in embryonic brain concurrently with high concentrations of hyaluronate. Seventeen-day embryos had a hyaluronate content of 6.9 μg per milligram of lipid-free tissue (dry weight) while chick brain 23 days old (3 days after the chicks hatched) and also brain from chicks 7 weeks after hatching contained 2.2 μg per milligram of lipid-free tissue (dry weight. The drop in hyaluronate may explain the decrease in hydration discussed in the previous section, since high hyaluronate concentrations are often associated with a high degree of hydration (*13*). Bondareff and Pysh (*14*) observed that the volume of extracellular space decreases during early development of the rat brain.

The parallel presence of both high concentrations of hyaluronate and high hyaluronidase activity during early development of brain presents a situation different from that encountered in the systems studied previously. In the regenerating newt limb (*1*) and the chick embryo cornea (*2*), limb, and vertebrae (*3*) the developmental sequences can be separated into a morphogenetic phase during which cells accumulate by proliferation and migration to a suitable location and a phase of overt differentiation [for example, see (*6*) and (*15*)]. The former phase is characterized by hyaluronate production, the latter by hyaluronidase activity. In the developing brain of the chick a series of migrations and differentiation continue throughout embryonic life (*16*). It is important to note that most, if not all, of the neuronal migrations have stopped in the chick at the time of hatching (*17*), while neuronal migration in mammalian species continue after birth (*18*). Thus in the chick coincident high concentrations of hyaluronate and high hyaluronidase activity correspond to an overlapping series of neuronal migrations and differentiations which give rise to the complex layers of brain cells. It is not yet known whether the enzyme and substrate are compartmentally separated in relation to cells that are differentiated as opposed to migrating or proliferating, or whether rapid turnover of hyaluronate is a uniform characteristic of the embryonic brain.

In our studies of the developmental sequences of skeletal and corneal development we have suggested that thyroxine may be involved in the control of hyaluronate concentrations and hyaluronidase activity (*5, 6*). This is of particular interest here since the effects of hypothyroidism in brain development are known to include a failure of the proper timing of neuronal migrations (external granule cells to internal granule cells) and a failure of the proper differentiation of other neurons (for example, Purkinje cells) (*19*).

JON R. POLANSKY
BRYAN P. TOOLE
JEROME GROSS

Developmental Biology Laboratory, Departments of Medicine and *Biological Chemistry, Harvard Medical School* and *Massachusetts General Hospital, Boston 02114*

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HL-A Antigens in Mummified Pre-Columbian Tissues

Abstract. *Tissue extracts of pre-Columbian mummies, from 500 to 2000 years old, were found to inhibit specific antibodies to HL-A. Two-thirds of the specimens tested gave positive results. Patterns of reactions obtained with different antiserums detecting the same antigen were concordant and consistent with known relations between HL-A antigens. The distribution of antigens found was similar to that observed in present-day descendants of the ancient populations studied. Although artifacts due to contaminating substances could have occurred, the reactions resembled in many respects those of HL-A antigens rather than those of nonspecific cross-reacting inhibitors. Development of a technique for HL-A typing of mummified remains may open new possibilities for anthropologic studies.*

Determination of the ABO blood groups has been used extensively in anthropology. It provides a way of identifying and characterizing populations on the basis of genes. The possibility of typing mummified remains for ABO blood groups was realized in 1937 (*1*). Since then, blood groups have been studied in mummified tissues from Egypt, the Aleutian Islands, the southwestern United States, and South America (*1, 2*).

The HL-A system of histocompatibility antigens is emerging as one of the most polymorphic genetic systems of man and has been recognized as a powerful tool for anthropologic studies (*3*). The HL-A system comprises two genetic loci, LA and Four, each having 15 or more alleles. New alleles are still being identified, especially in non-European populations.

In the study reported here HL-A antigens were determined in mummified tissues by using an antibody inhibition technique. Thirty-three specimes were used (Table 1). They were obtained through the courtesy of several anthropologists who documented their origins and cultural affiliations (*4*).

The tissues consisted of fragments of skin from various parts of the body or scalp. Extracts were prepared by shredding dry tissue in a VirTis homogenizer

and pressing through a stainless steel sieve to obtain a fine powder. Two-hundred milligrams of each powder were extracted twice in 2 ml of saline, with constant stirring, at 37°C, for 30 minutes. The two extracts were combined and dialyzed against water at 4°C, and the protein concentration was determined (*5*) with a commercial protein standard (Lab-trol, Dade, Miami, Florida). Each sample was then freeze-dried and dissolved in fresh rabbit serum to a concentration of 8 mg/ml; from this working solutions containing 2, 4, and 6 mg/ml were made for testing.

A modified cytotoxicity inhibition test was used (*6*), employing antiserums against HL-A 1, HL-A 2, HL-A 3, HL-A 9, HL-A 10, W28, and SAL of the first series, and HL-A 5, HL-A 7, HL-A 8, HL-A 12, W5, W10, and W27 of the second series of the HL-A system (*7*). Serum SAL, which was prepared by planned immunization between two volunteer subjects in Peru (*8*), detects a new allele of the LA locus. Each serum was titrated to determine the optimal dilution, which usually was the highest dilution killing 100 percent of the target cells. Target lymphocytes for cytotoxicity testing were obtained from typed reference donors. Lymphocytes were prepared by flotation on Ficoll-Hypaque (*9*) and were frozen at liquid nitrogen temperature (*10*) in small portions. Cytotoxicity tests were read by the fluorochromasia technique (*11*).

Inhibition of the cytotoxic antibodies resulted in fluorescein-labeled lymphocytes remaining visible at the end of incubation and was expressed as percentage of a control lymphocyte preparation incubated without antibody. A mummy extract was scored positive when at least 50 percent inhibition was observed with the highest concentration of antigen used. All determinations were performed at least twice, and in most cases two or more typing serums were used to determine an HL-A specificity. The variation between duplicate inhibition tests was less than 25 percent. Tests with dirt from an area where tissues were obtained, as well as powder extracted from the wrappings of three positive specimens, produced no inhibition. Controls consisted of target lymphocytes incubated (i) with antiserum without mummy antigen, (ii) with antiserum and mummy antigen but without complement, (iii) with complement alone, and (iv) with antigen alone.

Table 1. Origin, location, time periods, and detection of HL-A antigens in mummified pre-Columbian tissues.

Origin	Location	Time period	Tested (*N*)	Positive (*N*)
Pacatnamu (Chimu)	Peru	A.D. 800–1200	11	4
Toyopa Cave	Mexico	A.D. 900–1200	3	3
Ancón	Peru	A.D. 1200–1400	3	3
Huaca Malena	Peru	A.D. 1200–1400	2	2
Corpus	Peru	A.D. 1200–1400	10	8
Paracas	Peru	600–100 B.C.	4	2
Total			33	22

In addition, standard inhibition with lymphocytes known to contain the HL-A antigen being tested for were included with each experiment.

Positive results for at least one antigen were obtained in 22 of 33 specimens tested for 14 HL-A antigens (67 percent). In all groups, originating from different sites, some extracts were reactive (Table 1). Specimens from Paracas, which are the oldest tested, showed two positive samples out of four. The Pacatnamu specimens, which showed the lowest frequency of positive tests (4 in 11), were obtained from a looted cemetery where the tissues had been exposed. Most of the specimens from the other sites were excavated and retrieved in their original wrappings. The Paracas samples were preserved in a museum cabinet. Those from Corpus and Huaca Malena were unwrapped a short time before testing.

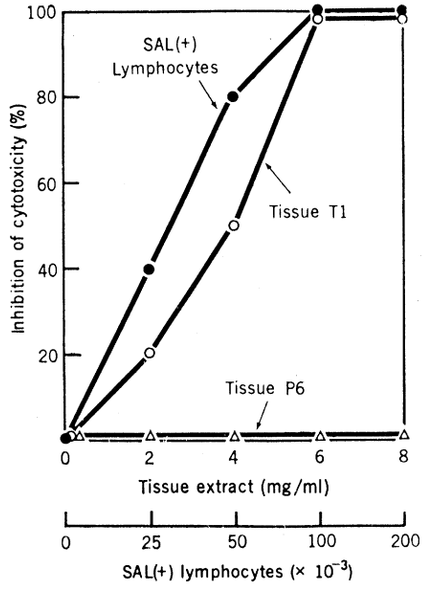

Fig. 1. Results of inhibition test. Cytotoxic serum SAL was used undiluted and killed 100 percent of target lymphocytes. The results are based on inhibition of this reaction. (Closed circles) SAL(+) lymphocytes produced complete inhibition when 100×10^3 or 200×10^3 cells were used; (open circles) tissue extract T1 showed complete inhibition at protein concentrations of 6 and 8 mg/ml; (triangles) tissue P6 did not inhibit serum SAL at concentrations tested.

With few exceptions one to four HL-A antigens were found in each individual typed. In each case multiple antiserums for the same specificity produced concordant results. Where more than four antigens were found for one specimen the results could be explained by known cross-reactivity of HL-A antigens (*12*). Thus, the presence of HL-A 2 and HL-A 9 in specimens T2, T1S, and P9 most likely represents a single antigen reacting with both HL-A 2 and HL-A 9 serums. The same is true of HL-A 2 and W28 (P8, T3, P9, and P2), HL-A 5 and W5 (T2, T3, T4, and C2), HL-A 7 and W27 or W10 (T1S and T2S). Such cross-reactions among HL-A antigens usually cannot be separated by absorption (*13*), although certain quantitative differences have been described (*14*).

The similarity between the antigen frequencies in mummified remains and those of present-day populations in North America and South America is striking (Table 2). Particularly interesting in these populations compared with Europeans is the similar increased frequency of HL-A 2, HL-A 9, W28, and W5, and the absence of HL-A 1, HL-A 3, HL-A 10, HL-A 7, HL-A 8, and HL-A 12. The pre-Columbian population, mostly from Peru, was more similar to the Quechuas and Ixils than to the Papagos of North America in regard to W28 and W27. The higher frequency of HL-A 5 has been reported in the Aymara Indians of Chile (Table 2).

Typical results of an inhibition experiment are shown in Fig. 1. Serum SAL, used in this test, was completely inhibited by 100×10^3 SAL-positive lymphocytes. Similarly, complete inhibition was observed with 6 mg/ml of tissue extract T1, a specimen from

Chimu (A.D. 800 to 1200). This mummy extract produced 50 percent inhibition at a concentration of 4 mg/ml and about 20 percent inhibition at 2 mg/ml. A second specimen (P6, Ancón, A.D. 1200 to 1400) had no inhibitory effect even at the highest concentration tested.

The possibility that these inhibition reactions could have been due to contaminating substances has to be considered. It is surprising that HL-A antigens would be preserved after 2000 years. Glycoproteins, polysaccharides, and lipopolysaccharides, as well as streptococcal M1 protein, have been reported to inhibit antibodies to HL-A (*15*). However, these inhibitory reactions were not as strong as inhibition by solubilized HL-A antigens and usually affected some antiserums to an HL-A specificity and not others. Furthermore, cross-reactions of the type seen with HL-A antigens were not observed with any consistency (*14*). The serologic results reported here appear to be different in that 2 to 4 mg of tissue protein per milliliter produced more than 50 percent inhibition; concordant results were observed with different antibodies detecting the same HL-A specificity; and cross-reactions typical of HL-A serology were present in most cases. Also striking was the similarity of the inhibition curves obtained with mummy extracts and with fresh lymphocytes, as shown in Fig. 1.

The tests for antigens of the HL-A system were positive in only two-thirds of the specimens. In some of these only one or two antigens were found instead of four. In most cases this was probably the result of loss of antigenicity due to denaturation. The fact that more antigens were found in specimens preserved under better conditions supports this view. However, this study was limited to 14 antigens, and some specimens may have contained antigens which were not tested.

As in the studies of ABO blood group antigens (*16*), a similarity in HL-A antigens has been found between prehistoric and present-day American Indian populations. The prehistoric samples were mostly from inhabitants of the coastal desert regions of Peru. Their HL-A profile (Table 2), which matched that of American Indians in general (*17–20*), resembled that of the Ixils of Guatemala and of the Quechuas of the Peruvian highlands with respect to W28 and W27 and that of the Aymaras of Chile in regard to HL-A 5. The antigen frequencies given are based on a small sample which was not homogeneous in origin. To arrive at more accurate figures additional work will be needed; this should be possible in areas such as the coast of Peru, where well-preserved mummified specimens are available for such investigations.

Table 2. Frequency of HL-A antigens in American Indian populations. The (unpublished) results for the Caucasian population were obtained at the Dallas Tissue Typing Laboratory by P.S. The data for present-day American Indian populations are from the following sources: Papago Indians of North America, Perkins *et al.* (*17*); Guatemalan Ixils, Corley *et al.* (*18*); Quechua Indians of Peru, Tittor *et al.* (*19*); and Aymara Indians of Chile, van der Does *et al.* (*20*). Abbreviation: N.T., not tested.

Antigen	Frequency (%) in					
	Caucasian population ($N = 100$)	American Indian populations				
		Present-day				Pre-Columbian ($N = 22$)
		Papago ($N = 101$)	Ixil ($N = 79$)	Quechua ($N = 90$)	Aymara ($N = 37$)	
			First series			
HL-A 1	38	0	4	2	3	0
HL-A 2	51	75	65	86	70	60
HL-A 3	27	1	0	1	6	0
HL-A 9	21	58	35	38	24	23
HL-A 10	7	2	0	0	0	0
HL-A 11	11	1	14	1	0	N.T.
W28	5	0	40	21	8	32
W19	13	21	32	16	66	N.T.
SAL	7	N.T.	N.T.	N.T.	N.T.	45
			Second series			
HL-A 5	19	9	18	8	35	36
HL-A 7	32	2	0	7	0	4
HL-A 8	27	1	0	1	0	0
HL-A 12	28	3	5	3	0	4
HL-A 13	4	0	0	1	0	N.T.
W5	3	29	49	58	3	23
W10	14	18	27	13	30	19
W14	4	0	1	3	0	N.T.
W16	18	33	38	20	19	N.T.
W22	1	0	0	1	0	N.T.
W27	7	12	0	1	0	5

PETER STASTNY
Department of Internal Medicine, University of Texas Health Science Center, Dallas 75235, and *Dallas Veterans Administration Hospital, Dallas 75235*

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