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Characterization of β-D-*N*-Acetylhexosaminidase Isoenzymes in Man-Chinese Hamster Somatic Cell Hybrids

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INTRODUCTION

Several forms of β-D-*N*-acetylhexosaminidase (E.C.3.2.1.30) have been demonstrated in different human tissues [1–3]. Four isoenzymes, characterized by immunological and biochemical techniques [4–8], have been designated β-D-*N*-acetylhexosaminidase A, B, C and S, further to be referred to as hex A, hex B, hex C, and hex S.

Deficient hex A activity and an increased activity of hex B is found in G_{M2} gangliosidosis type 1 or Tay-Sachs disease, whereas G_{M2} gangliosidosis type 2 or Sandhoff disease is associated with a deficiency of both hex A and hex B [9–12].

Biochemical, immunological, and genetical studies suggest a structural relationship between hex A and hex B [1, 5, 10, 13–19]. Robinson and Carroll [19] and Desnick et al. [10] suggested that both isoenzymes are composed of multiple subunits, one of which is common to both forms. Two subunit models have been suggested: (1) the three locus model [10]: hex A = $(\alpha\beta)_n$, hex B = $(\beta\gamma)_n$; and (2) the two locus model: hex A = $(\alpha\beta)_n$, hex B = $(\beta\beta)_n$. Tay-Sachs disease can be explained by a defective α subunit specific for hex A and Sandhoff disease, by a defective common β subunit. Recently, strong evidence in favor of the two locus model has been presented by Beutler and Kuhl [20] in in vitro enzyme hybridization studies.

Studies of the segregation of the hexosaminidase markers in man-rodent somatic cell hybrids can discriminate between the different subunit theories. The results however are contradictory. Lalley et al. [21] found that hex A was never expressed in these hybrids in the absence of hex B, which fits the two locus model. Gilbert et al. [15] and van Someren and Beyersbergen van Henegouwen [14], however, reported an independent loss of hex A and hex B in cell hybrids, which favors the three locus model.

These conflicting results may be due to a misinterpretation of the electrophoretic patterns obtained from hybrid lines, if one relies solely on electrophoretic mobility. The formation of heteropolymeric molecules, especially forms with nearly the same electrophoretic mobility as hex A, might confuse the interpretation of the segregation

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results. Therefore, a further characterization of the hexosaminidase isoenzymes in hybrid lines is needed. It is possible to obtain information about the presence of possible heteropolymeric molecules in hybrid cell lines by using specific antisera against human and Chinese hamster hexosaminidase.

In the present study, a series of man-Chinese hamster hybrids were investigated using an anti-Chinese hamster hexosaminidase serum, a specific anti-human hex A serum, and an anti-human hex B serum. It was found that a hex A-like enzyme present in the hybrid lines consists of both Chinese hamster and specific human hex A moieties. The expression of human hex A was found to be dependent on the presence of hex B, and it was shown that a gene, coding for a specific hex A subunit is syntenic with the mannosephosphate isomerase (*MPI*) and pyruvate kinase (*PK-3*) loci, assigned to chromosome 15.

MATERIALS AND METHODS

Purification of Chinese Hamster Hexosaminidase

Chinese hamster hexosaminidase was purified 290 times from pooled homogenates of liver and kidney. The 20,000 *g* supernatant was applied on a ConA sepharose column, equilibrated with 10 mM sodium-phosphate buffer, pH 7.0, containing 0.1 mM $CaCl_2$, 0.1 mM $MnSO_4$, and 0.5 M NaCl. Hexosaminidase was eluted with 1 M α-methylglucoside in the same buffer solution; pooled fractions were dialyzed and applied to a second affinity column. *N*-acetylglucosamine, bound to CN-Br activated sepharose as described by Lotan et al. [22] was used as affinity ligand. Hexosaminidase was eluted with 10 mM *N*-acetylglucosamine and 0.2 M NaCl in 10 mM sodium-phosphate buffer, pH 6.0. Pooled fractions, containing hexosaminidase were lyophilized and used to raise antisera.

Antisera

Antisera against purified human hex A, hex B, and Chinese hamster hexosaminidase were raised in New Zealand white rabbits by a slight modification of the method described by Carroll and Robinson [23]. Specific human hex A antiserum was prepared by absorption of anti-hex A serum with human liver hex B, according to Bartholomew and Rattazzi [24].

Cell Lines

Normal fibroblasts, hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficient fibroblasts, or normal leukocytes were used as human parental cell lines in the fusion procedure. The Chinese hamster parental cell lines used were thymidine kinase (TK) or HPRT deficient fibroblasts. Details of the fusion procedure and the isolation and propagation of the hybrid cell lines have been described previously [25]. Preparation of cell lysates was carried out as described by Meera Khan [26]. The human controls used were a tetraploid epithelial kidney cell line (T-cell) [27] and a normal fibroblast cell strain.

Electrophoresis

Electrophoresis of hexosaminidase, MPI, and PK-3 was carried out on cellulose acetate gel (Cellogel, Chemetron, Milan, Italy). Hexosaminidase electrophoresis was performed in 0.05 M potassium-phosphate buffer, pH 6.6, for 1 hr at 4°C. The staining procedure for hexosaminidase was carried out according to Okada and O'Brien [11]. The electrophoresis and staining procedures of MPI and PK-3 have been published by van Someren et al. [28].

Immunoprecipitation

Lysates were incubated with antiserum overnight at 0°C. After incubation, the treated lysates were centrifuged at 35,000 *g* for 20 min. The supernatant was used for electrophoresis.

RESULTS

Electrophoresis of lysates of normal human fibroblasts reveals three bands of activity: hex B, hex A and hex C (fig. 1*a*, lane 1). In lysates of Chinese hamster fibroblasts, two bands of activity, described as hex 1 and hex 2 (fig. 1*b*, lane 1), were found.

The specificity of the antisera against Chinese hamster hexosaminidase and against human hex A and hex B was tested with lysates of human and Chinese hamster fibroblasts. The resulting electrophoretic patterns are illustrated in figure 1*a* and 1*b*, lanes 2, 3, and 4. With the concentrations used, the antiserum against Chinese hamster hex 1 does not cross-react with human hex A, hex B, and hex C (fig. 1*a*, lane 2) nor with

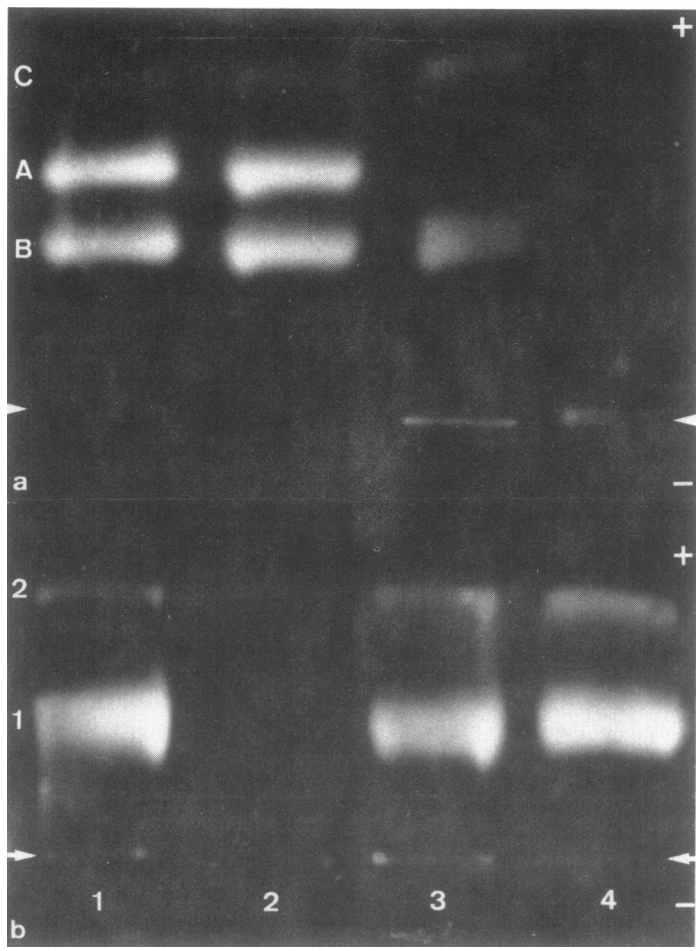

FIG. 1.—Electrophoretic patterns of human (*a*) and Chinese hamster (*b*) fibroblast lysates. *Lane 1*, A, B, C refer to hex A, hex B, and hex C; 1 and 2 to Chinese hamster hex 1 and hex 2: *lane 2*, after treatment with anti-Chinese hamster hexosaminidase serum; *lane 3*, after treatment with specific anti-human hex A serum; *lane 4*, after treatment with anti-human hex B serum. The amount of lysate used in the various lanes depended on the antibody concentrations of the antisera, explaining differences in staining intensity.

the fastmoving Chinese hamster hex 2 band. The specific anti-human hex A serum does not remove hex B and hex C, whereas hex A disappeared (fig. 1*a*, lane 3). The anti-human hex B serum reacts with both hex A and hex B but not with hex C (fig. 1*a*, lane 4). No reaction of specific anti-human hex A and anti-human hex B sera with the Chinese hamster hexosaminidases was observed (fig. 1*b*, lane 3 and 4). These results were confirmed with double immunodiffusion in agarose gels [29] and by antigen-antibody titrations in various combinations of antigens and antisera.

Four types of hybrid lines could be distinguished by electrophoresis of different man-Chinese hamster cell hybrids (fig. 2). They are indicated as hex A′+/hex B+, hex A′+/hex B−, hex A′−/hex B+, hex A′−/hex B−. The band of activity with a mobility comparable to hex A is called hex A′.

Lysates prepared from these four different types of hybrid clones were treated with the various antisera, and the resulting isoenzyme patterns of hexosaminidase were studied by electrophoresis. The results obtained with a hex A′+/hex B− hybrid are shown in figure 3. After treatment with anti-Chinese hamster hex 1 serum, both the Chinese hamster hex 1 and the hex A′ band disappear. The specific anti-human hex A serum removes the hex A′ activity, whereas no reaction with Chinese hamster hexosaminidase was observed. Anti-human hex B serum does not change the electrophoretic pattern. Nine hex A′+/hex B− hybrid clones from five independent fusion experiments were tested in this way; they invariably showed the same immunological characteristics.

Eight hex A′+/hex B+ hybrid cell lines from independent fusion experiments were also studied with antisera (fig. 4). After treatment of the lysates with anti-Chinese hamster hex 1 serum, the hex 1 band disappears along with part of the activity of the hex A′ band (fig. 4, lane 3). Various concentrations of the anti-Chinese hamster hex 1 serum were used, but a complete removal of the activity on the hex A′ position could not be attained. The activity of hex B does not seem to be affected. With specific anti-human hex A serum, all the activity is removed at the hex A′ position, whereas hex B and

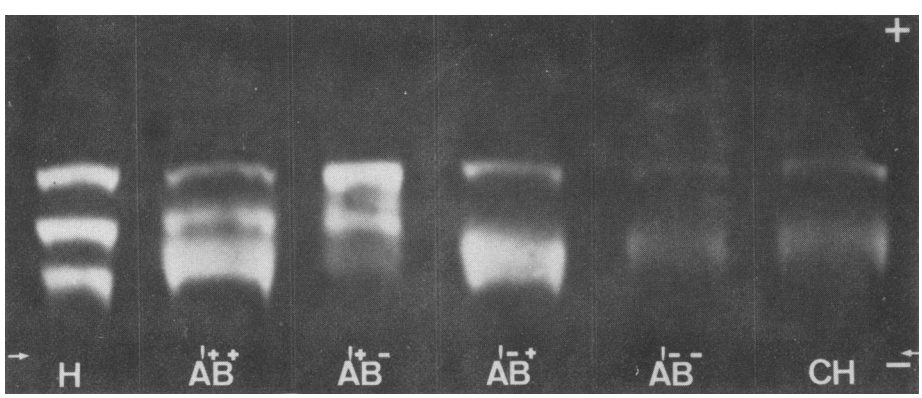

FIG. 2.—Hexosaminidase electrophoretic patterns. *From right to left*: H = human T-cell (Electrophoretic mobility of hex isoenzymes of T-cell and human fibroblast are comparable; the only difference is the intensity of the fastest moving band.) A′+/B+ = hybrid clone with activities at hex A′ and hex B position; A′+/B− = hybrid clone with activity at hex A′ position; A′−/B+ = hybrid clone with activity at hex B position; A′−/B− = hybrid clone having lost the activities at hex A′ and hex B position.

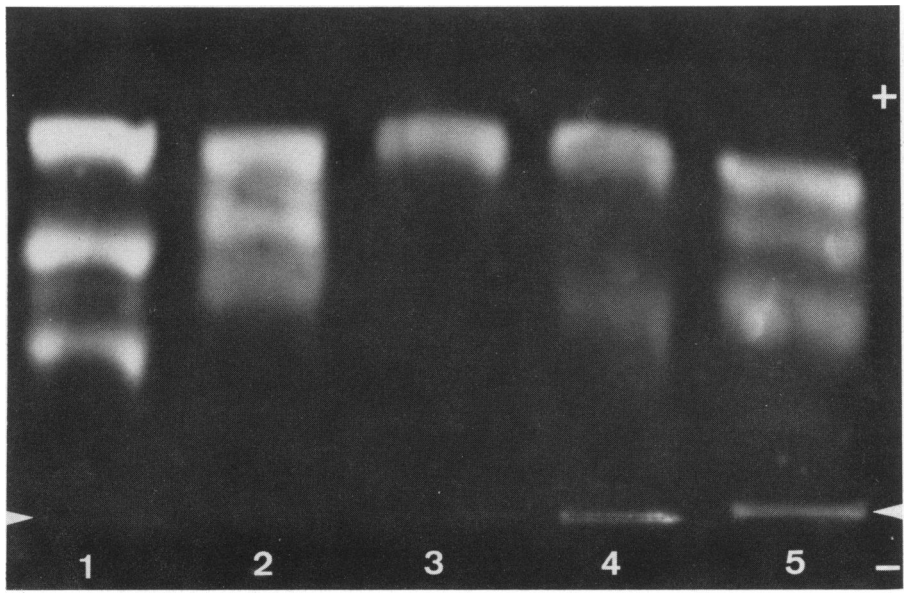

FIG. 3.—Electrophoretic patterns of a hex A′+/hex B− hybrid, following treatment with different antisera. *Lane 1*, human T-cell; *lane 2*, hybrid without antiserum treatment; *lane 3*, hybrid following treatment with the anti-Chinese hamster hexosaminidase serum; *lane 4*, hybrid following treatment with the specific anti-human hex A serum; *lane 5*, hybrid following treatment with the anti-human hex B serum.

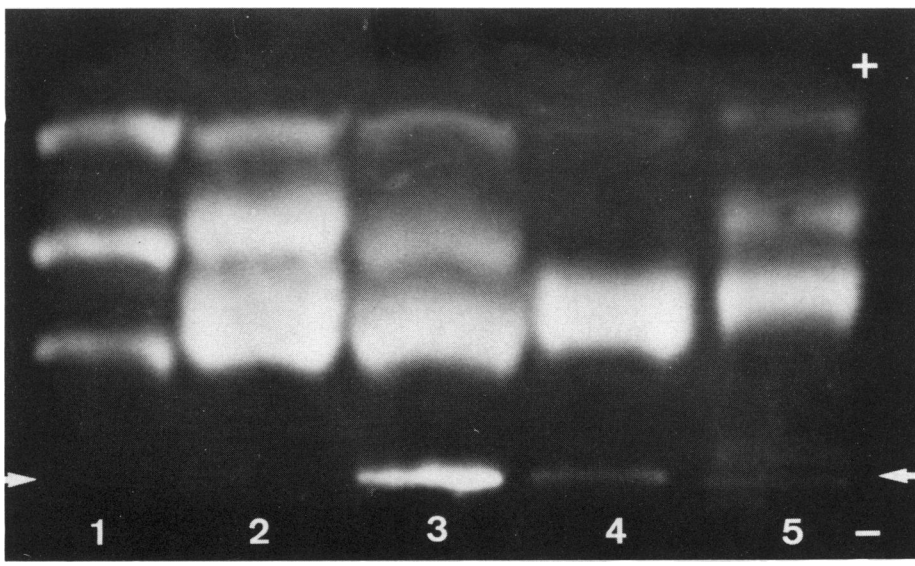

FIG. 4.—Electrophoretic patterns of a hex A′+/hex B+ hybrid, following treatment with different antisera. *Lane 1*, human T-cell; *lane 2*, hybrid without antiserum treatment; *lane 3*, hybrid following treatment with the anti-Chinese hamster hexosaminidase serum; *lane 4*, hybrid following treatment with the specific anti-hex A serum; *lane 5*, hybrid following treatment with the anti-hex B serum.

Chinese hamster hex 1 activities remain unchanged. When treated with anti-human hex B serum, part of the activity at the hex A′ position remains, and a normal Chinese hamster activity was detected. Also in this case, various dilutions of the antiserum were tested, and always residual activity at the hex A′ position remained present. Figure 5 illustrates the typical electrophoretic patterns of a hex A′−/hex B+ hybrid after incubation with the three antisera. Seven clones were tested. Hex B could be removed only by the anti-human hex B serum. The Chinese hamster hex 1 band cross-reacts with the anti-Chinese hamster hex 1 serum. In hex A′−/hex B− hybrids, changes in the electrophoretic pattern were observed only after treatment with the anti-Chinese hamster hex 1 serum, which removes the Chinese hamster hex 1 band.

A relationship between the absence and the presence of the hex A′ band, MPI and PK-3 was established, as shown by the data from several fusion experiments (table 1). No syntenic relationship was found with 24 other enzyme markers tested.

DISCUSSION

The discrepancies in the literature between data obtained in various experiments with man-rodent somatic cell hybrids [14, 15, 21] may be due to different interpretations of the hexosaminidase electrophoretic patterns from hybrid cell lines with particular regard to the activity at the human hex A position. The characterization of the isoenzymes in the hybrid cell lines can be greatly improved by the use of anti-Chinese hamster and anti-human hexosaminidase sera, as seen in the present study.

In hex A′+/hex B− hybrid clones, the hex A′ band was shown to interact with both

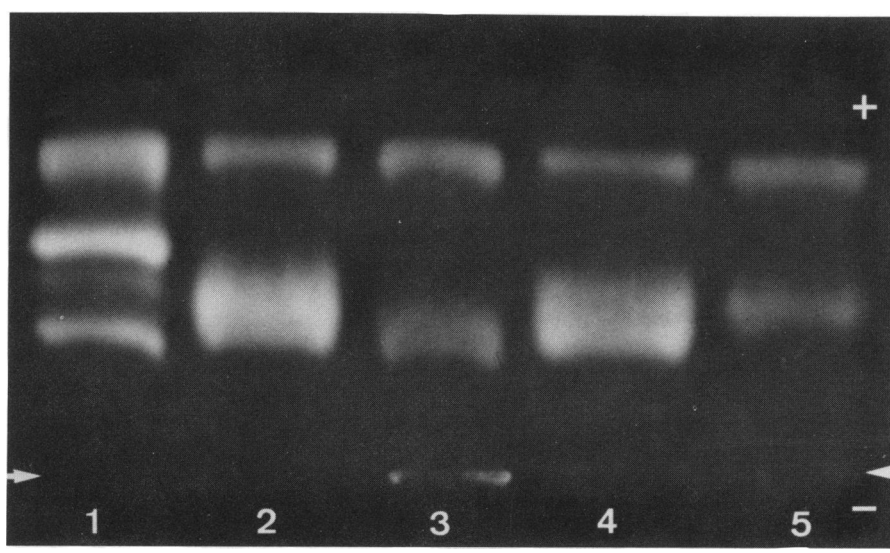

FIG. 5.—Electrophoretic patterns of a hex A′−/hex B+ hybrid, following treatment with different antisera. *Lane 1*, human T-cell. In fresh homogenates an additional band of activity can be found between hex A and hex B. This band corresponds to the intermediate forms 1_1, 1_2, as seen by DEAE cellulose chromatography [34]. *Lane 2*, hybrid without antiserum treatment; *lane 3*, hybrid following treatment with the anti-Chinese hamster hexosaminidase serum; *lane 4*, hybrid following treatment with the specific anti-hex A serum; *lane 5*, hybrid following treatment with the anti-hex B serum.

TABLE 1

ABSENCE AND PRESENCE OF HEX A′, MPI, AND PK-3 IN PRIMARY AND SECONDARY HYBRID LINES

	MPI		PK-3	
	+	−	+	−
Hex A′:				
+	30	3	27	3
−	0	28	0	35
PK-3:				
+	33	0	. . .	. . .
−	0	29	. . .	. . .

the anti-Chinese hamster hex 1 and the specific anti-human hex A sera, whereas no cross-reaction with the anti-human hex B serum was found. This finding indicates, that in these hybrids the isoenzyme at the hex A′ position is not a normal human hex A, but a heteropolymeric molecule, containing both specific human hex A and Chinese hamster hexosaminidase moieties. The formation of heteropolymeric hexosaminidase molecules in man-Chinese hamster hybrids has been suggested before by Ropers and Schwantes [17], who supposed a heteropolymeric molecule at the hex A position to consist of Chinese hamster and human hex C subunits. In their theory, hex B and hex C are homopolymers, $(\beta\beta)_n$ and $(\alpha\alpha)_n$, respectively, whereas hex A consists of B− and C subunits $(\alpha\beta)_n$. Van Cong et al. [30] described a band of activity comparable with hex A′, which they called "hex A fast." A relationship was demonstrated between the presence and absence of "hex A fast," MPI, and hex C. Following the subunit model of Ropers [16], "hex A fast" was explained as a heteropolymeric molecule with α subunits from human hex C and β-like subunits from Chinese hamster hexosaminidase. Recently it has been shown that at the hex C position two isoenzymes (hex C and hex S) can be detected [3, 20]. In view of these studies, it is likely that the fastest moving hex band described by Van Cong is identical with hex S. Our electrophoresis system does not discriminate between hex S and hex C.

The residual activity in hex A′+/hex B+ hybrid clones that could be detected at the hex A′ position after treatment with the anti-Chinese hamster hex 1 or anti-human hex B serum indicates the presence of two different isoenzymes at the hex A′ position. Since no activity is left after treatment with the specific anti-human hex A serum, a heteropolymeric molecule is thought to be present, similar to the heteropolymeric molecule found in hex A′+/hex B− hybrid clones at the hex A′ position, which consists of human hex A and Chinese hamster hex 1 moieties. The other isoenzyme at the hex A′ position cross-reacts with specific anti-human hex A and anti-human B sera and appears to represent a normal human hex A molecule.

From these studies, we conclude that normal human hex A is present only in the presence of hex B, which is in agreement with the results of Lalley et al. [21]. A heteropolymeric isoenzyme can be present independently of human hex B. The results of our present study fits into the two locus subunit model. According to this theory, the heteropolymeric isoenzyme at the hex A′ position has been shown to possess α subunits from human hex A and β-like subunits from Chinese hamster hex 1. The formation of

other heteropolymeric molecules, in particular between the human β subunit and an α-like subunit from the Chinese hamster cannot be excluded. The resolution of our electrophoresis system in the hex 1 and the hex B region is, however, insufficient to distinguish possible heteropolymeric molecules.

Evidence for the syntenic relationship between *hex A, MPI*, and *PK-3* [14, 27, 31, 32] and the assignment of *MPI* to chromosome 15 [33] puts this synteny group on chromosome 15. Our results show a relationship between the absence or presence of the isoenzymes on the hex A′ position, and that of MPI and PK-3. With respect to the two locus subunit model, the gene coding for the α subunit of hex A is localized on chromosome 15. Previously, *hex B* has been assigned to chromosome 5 by Gilbert et al. [14]. According to this model, it should be the gene, coding for the β chain which was localized on chromosome 5.

Studies of the expression of hexosaminidases in man-Chinese hamster hybrid cell lines can be a tool for understanding the molecular structure of hexosaminidase, provided that the characterization of human, Chinese hamster, and possible heteropolymeric components does not rely solely on electrophoretic mobilities.

SUMMARY

A series of man-Chinese hamster hybrids were investigated with the use of an anti-Chinese hamster hexosaminidase serum, a specific anti-human hex A serum and an anti-human hex B serum. The expression of human hex A was found to be dependent on the presence of hex B. A heteropolymeric molecule is formed independently of hex B, which consists of Chinese hamster and specific hex A moieties. It has an electrophoretic mobility nearly identical to hex A. A relationship between the absence and presence of the heteropolymeric molecule, mannosephosphate isomerase (MPI), and pyruvate kinase (PK-3), assigned to chromosome 15, was established. With respect to the two locus subunit model, the gene coding for the α subunit, specific for hex A, has been localized on chromosome 15.

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