Acta Biochimica Polonica

Vol. 46 No. 3/1999

739–751

QUARTERLY

Review

Isoenzymes of *N*-acetyl-β-hexosaminidase*

Krzysztof Zwierz[1✉], Anna Zalewska[1] and Walentyna Zoch-Zwierz[2]

[1]*Department of Pharmaceutical Biochemistry, Institute of Chemistry and*
[2]*I Department of Pediatrics, Medical Academy in Białystok, Poland*

Received: 08 March, 1999; accepted: 20 July, 1999

Key words: *N*-acetyl-β-hexosaminidase, isoenzymes

Biological significance, structure and posttranslational processing of *N*-acetyl-β-hexosaminidase isoenzymes are described. Clinical application of *N*-acetyl-β-hexosaminidase is also reviewed.

BIOLOGICAL SIGNIFICANCE OF *N*-ACETYL-β-HEXOSAMINIDASE

N-Acetyl-β-hexosaminidase (EC 3.2.1.52) releases *N*-acetylglucosamine and *N*-acetylgalactosamine (Fig. 1.) from the non-reducing end of oligosaccharide chains of glycoproteins, glycolipids and glycosaminoglycans [1]. Glycosaminoglycans are crucial substrates for *N*-acetyl-β-hexosaminidase [2]. Lack of glycosaminoglycan storage observed in Tay-Sachs and Sandhoff's diseases is due to the presence of hexosaminidase in amounts that are small but sufficient to prevent accumulation of glycosaminoglycans. To prevent accumulation of G_{M2} ganglioside in Tay-Sachs or gangliosides and oligosaccharides in Sandhoff's diseases, bigger amounts of enzyme are necessary [3]. Additionally, hexosaminidase promotes mitogenesis in airway smooth muscle *via* the airway smooth muscle mannose receptor [4]. *N*-Acetyl-β-hexosaminidase might be involved in modification of sugar chains of sperm membranes [5], and it has been postulated to be required for fertilization in ascidians [6] and mammals [7]. *N*-Acetyl-β-hexosaminidase released from fertilized egg cortical granules blocks poly-

*The paper was presented at the XXXIV Annual Meeting of the Polish Biochemical Society, 1998, Białystok, Poland.

✉Krzysztof Zwierz, Department of Pharmaceutical Biochemistry, Medical Academy in Białystok, Adama Mickiewicza 2a; 15-222 Białystok, Poland; tel.: (48 85) 742 2021 ext. 2252, 2236 or (48 85) 742 2059; e-mail: kzwie@amb.ac.bialystok.pl

Abbreviations: ER, endoplasmic reticulum; IL, interleukin; GalNAc, *N*-acetylgalactosamine; NeuAc-*N*-acetylneuraminic acid

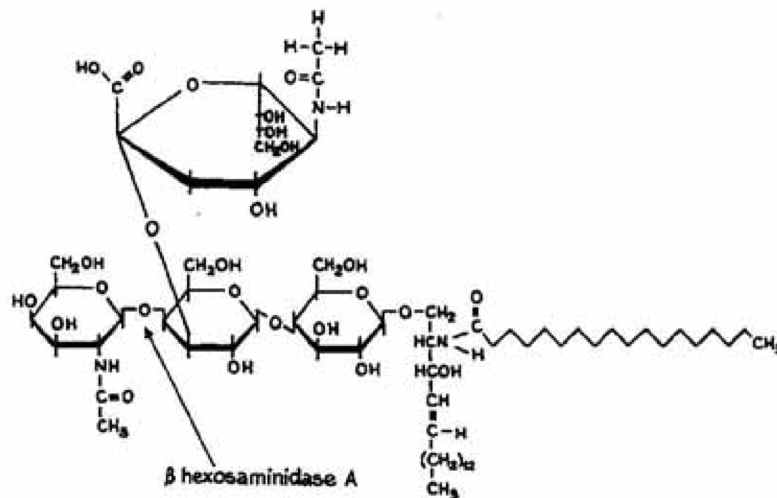

Figure 1. Reaction catalyzed by *N*-acetyl-β-hexosaminidase.

spermy by releasing the *N*-acetylglucosamine residue from a specific zona pellucida glycoprotein ZP3 necessary for binding sperm to egg coat [8].

N-ACETYL-β-HEXOSAMINIDASE GENES

Main isoenzymes of *N*-acetyl-β-hexosaminidase are composed of two polypeptide chains designated α and β [1, 9, 10] which are products of gene duplication [11]. In humans, the gene encoding pre-pro α-subunit is located in chromosome 15q23-q24 and the gene encoding pre-pro β-subunit is located in chromosome 5q13 [1, 10, 12]. Both genes have a similar length (35–40 kDa) and structure. Each has 13 introns and 12 of them interrupt coding regions (about 1.6 kb long) at analogous positions [10]. The segments essential for expression of subunit α reside within a 40 bp region, between 100 bp and 60 bp upstream of the ATG initiation codon, and for β-subunit reside within a 60 bp region between 150 bp and 90 bp upstream of the ATG codon [13]. The homology between the two pre-pro-subunits is the highest in the middle section, intermediary at the C terminus and the lowest at the *N*-terminus [12] (Fig. 2). The sequence of formed pre-pro α- and pre-pro β-subunits is in 60% nearly identical [10]. The sequence of mouse α and β-subunit is in 51% and 72% identical with that of human α- and β-subunits, respectively [10]. It seems that the common ancester gene could be similar to the *Dictyostelium discoideum* *N*-acetyl-β-hexosaminidase gene, which encodes only one polypeptide and is in over 30% identical with human α and β *N*-acetyl-β-hexosaminidase genes [10]. This may suggest that human *N*-acetyl-β-hexosaminidase genes have some conserved sequences of an ancester gene essential for proper folding and development of the catalytic activity of the resulting polypeptide chains [10].

POST-TRANSLATIONAL PROCESSING OF *N*-ACETYL-β-HEXOSAMINIDASE ISOENZYMES

The protein moiety of *N*-acetyl-β-hexosaminidase is synthesized in rough endoplasmatic reticulum as pre-propolypeptide α and pre-propolypeptide β composed of 529 and 556 amino acids, respectively [1] (Fig. 2). It has been reported, that synthesis of *N*-acetyl-β-hexosaminidase isoenzymes increases 2–4-fold as with differentiation of human intestinal Caco 2 cells [14]. Pre-propolypeptide chains of *N*-acetyl-β-hexosaminidase during translocation through aqueous pores of endo-

```
α  -MTSSRLWFSLLLAAAFAGRATA-                                          22
β  -MELCGLGLPRPPMLLALLLATLLAAMLALLTQVALVVQVAEA-                       42

α  ------------- (LWPWPQNFQTSDQRYVLYPNNFQFQYDVSSAAQPGCSVLDEAFQRY     68
β  ARAPSVS-(AKPGPALWPLPLSVKMTPNLLHLAPENFYISHSPNSTAGPSCTLLEEAFRRY     101

α  RDLLFG) SGSWPRPYLTGKRH (TLEKNVLVVSVVTPGCNQLPTLESVENYTLTINDDQCL    126
β  HGYIFG) FYKWHHEPAEFQAK (TQVQQLLVSITLQSECDAFPNISSDESYTLLVKEPVAV   159

α  LLSET VWGALRGLETFSQLVWKSAEGTFFINKTEIEDFPRFPHRGLLLDTSRHYLPLSS     185
β  LKANR VWGALRGLETFSQLVYQDSYGTFTINESTIIDSPRFSHRGILIDTSRHYLPVKI     218

α  ILDTLDVMAYNKLNVFHWHLVDDPSFPYESFTFPELMRKGSYNPVTHIYTAQDVKEVIEY     245
β  ILKTLDAMAFNKFNVLHWHIVDDQSFPYQSITFPELSNKGSYSLS-HVYTPNDVRMVIEY     277

α  ARLRGIRVLAEFDTPGHTLSWGPGIPGLLTPCYS GSEP SCTFCPVNPSLNNTYEFMS     302
β  ARLRGIRVLPEFDTPGHTLSWGFGQKDLLTPCYS) RQNK (LDSFGPINPTLNTTYSFLT   334

α  TFFLEVSSVFPDFYLHLGGDEVDFTCWKSNPEIQDFMRKKGFGEDFKQLESFYIQTLLDI     362
β  TFFKEISEVFPDQFIHLGGDEVEFKCWESNPKIQDFMRQKGFGTDFKKLESFYIQKVLDI     394

α  VSSYGKGYVVWQEVFDNKVKIQPDTIIQVWREDIPVNYMKELELVTKAGFRALLSAPWYL     422
β  IATINKGSIVWQEVFDDKVKLAPGTIVEVWK-DSA--YPEELSRVTASGFPVILSAPWYL     451

α  NRISYGPDWKDFYVVEPLAFEGTPEQKALVIGGEACMWGEYVDNTNLVPRLWPRAGAVAE     482
β  DLISYGQDWRKYYKVEPLDFGGTQKQKQLFIGGEACLWGEYVDATNLTPRLWPRASAVGE     511

α  RLWSNKLTSDLTFAYERLSHFRCELLRRGVQAQPLNVGFCEQEFEQT)                  529
β  RLWSSKDVRDMDDAYDRLTRHRCRMVERGIAAQPLYAGYCNHENM)                    556
```

Figure 2. Amino-acid sequences of α- and β-subunits in human *N*-acetyl-β-hexosaminidase A.

The signal sequences and sequences removed during maturation are crossed out. The mature α and β chains are enclosed in brackets. The area of α-subunits responsible for the ability to bind specifically negatively charged substrates is underlined. The amino acids involved in creation of active site are in larger capital letters.

plasmic reticulum (ER) membrane from cytoplasm to endoplasmatic reticulum, undergo modifications including N-glycosylation (Fig. 3), folding assisted by chaperones (grp78 = glucose-regulated protein = BiP = immunoglobulin binding protein, grp170, grp94, grp72, grp58, calnexin = ER membrane-bond lectin, and calreticulin = soluble ER lectin) [15], and formation of disulfide bonds [16] stimulated by Ero1p [17]. It continues posttrans-

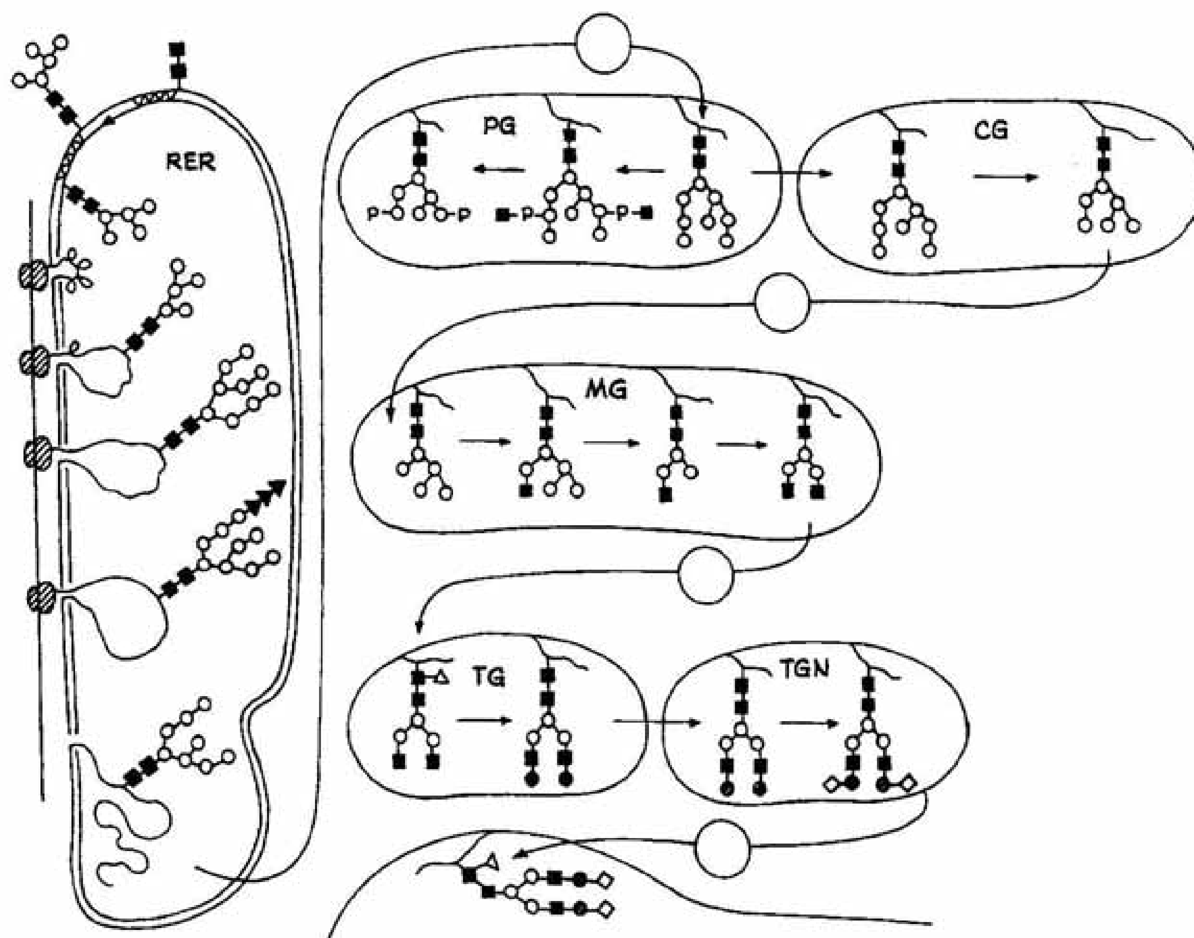

Figure 3. Processing of oligosaccharide chains of *N*-acetyl-β-hexosaminidase.

■, *N*-acetylglucosamine; ○, mannose; ⦶, galactose; ▲, glucose; Δ, fucose; ◇, *N*-acetylneuraminic acid; ∿∿, dolichol; RER, rough endoplasmic reticulum; PG, pre-Golgi compartment; CG, cis-Golgi compartment; MG, medial-Golgi compartment; TG, trans-Golgi compartment; TGN, trans-Golgi-network.

lationally by formation of additional disulfide bonds and sometimes in assembly of oligomers [16]. In normal conditions, catalytically inactive α (less stable) and β (more stable) subunits of *N*-acetyl-β-hexosaminidase are synthesized in approximately equal amounts, but they dimerize at different rates [10]. Homodimers $\alpha\alpha$ (isoenzyme S) are unstable and the majority of them are retained in ER and degraded [12]. Homodimerization $\beta\beta$ that creates the most stable isoenzyme B is favored by rapid cell proliferation [18]. Heterodimerization $\alpha\beta$ that creates isoenzyme A (less stable than isoenzyme B) is preceded by accumulation of properly folded phosphorylated subunit α. It is believed that high concentration of α-subunit in ER forces synthesis of heterodimer $\alpha\beta$, by mass action [19]. The presence of subunit β in isoenzyme A greatly increases the stability of subunit α in heterodimer, facilitates the transport of subunit α from ER, and changes some kinetics properties of the α-subunit active site [12]. Signal sequences (22 amino acids of subunit α and 42 amino acids of subunit β) (Fig. 2) are removed after translocation. Pre-pro-subunits that do not fold and dimerize properly to near native conformation, as in the case Val-

192-Leu mutation in α-pre-pro-subunit, are retained in ER and degraded [12, 20, 21]. Mutation of Pro-504 to Ser in β-subunit decreases the level of heterodimer transport out of the ER by approximately 45% [22]. Asp β208 and probably Asp β290 are very important in the initial folding and/or dimer formation of the pre-prosubunit β [20].

Properly folded α and β pro-subunits are transported along microtubules, by vesicular transport by microtubule directed motor-complex of dynein/dynactin [23] or kinesin [24], to pre-Golgi (Salvage) compartment [19, 23]. In the pre-Golgi compartment, some mannose residues of N-linked oligosaccharides of both properly folded subunits are initially phosphorylated [1, 19, 25]. Substitution of tryptophan at position 474 by cysteine, in subunit α, prevents both phosphorylation and secretion of subunit α [26]. Phosphorylation creates mannose-6-phosphate recognition markers which direct glycoprotein to lysosomes [1], and protect high-mannose type oligosaccharides from further processing in the Golgi comparment, which results in their conversion into sialic acid-containing complex oligosaccharides [19].

From pre-Golgi compartment isoenzymes of *N*-acetyl-β-hexosaminidase pass through the cis-Golgi → medial-Golgi → trans-Golgi → trans-Golgi network [19]. During intracellular transport lysosomal enzymes are subjected to posttranslational modifications which include glycosidic (Fig. 3) and proteolytic processing [1, 19] as well as final phosphorylation [25]. Mannose-6-phosphate receptor transmembrane proteins in the trans-Golgi network are present. They recognize mannose-6-phosphate residue on N-linked oligosaccharides of lysosomal enzymes and pro-insulin-like growth factor II, which bind to two distinct binding sites on the same receptor. Overexpression of pro-insulin-like growth factor II increases the secretion of lysosomal enzymes without affecting the rate of their synthesis, possibly by affecting the re-uptake mechanism [27]. Isoenzymes of *N*-acetyl- β-hexosaminidase, are transported from late endosome to lysosome [28], where precursor subunits are proteolytically processed to their mature forms [11, 29]. The mannose-6-phosphate receptors shuttle back and forth between trans-Golgi network and late endosome in clatrin coated transport vesicles [28]. Not all of the cargo that is tagged for delivery to lysosomes gets to its proper destination [19, 28]. It seems that some lysosomal enzymes (and among them isoenzymes of *N*-acetyl-β-hexosaminidase) are transported *via* a default pathway to the cell surface. At the cell surface these enzymes may be recaptured by mannose-6-phosphate receptors which take a detour to the plasma membrane. Lysosomal enzymes escaped to plasma membrane are returned by detoured mannose-6-phosphate receptors to lysosomes through late endosomes [28]. Catalytically active *N*-acetyl-β-hexosaminidase is formed in lysosomes [29].

SECRETION OF *N*-ACETYL-β-HEXOSAMINIDASE ISOENZYMES

A part of soluble lysosomal enzymes is delivered from lysosomes to the cell surface [30] and some of their molecules are not returned to lysosomes and are secreted from the cell surface into the extracellular fluid [19, 28]. Another route of secretion of lysosomal enzymes may lead from lysosome through late endosome and Golgi compartment to cell surface and extracellular fluid [31]. It has been reported, that microtubules play a major role in stimulated *N*-acetyl-β-hexosaminidase secretion, and it has been suggested a contributory role for microfilaments [32]. The release of *N*-acetyl-β-hexosaminidase from mast cells was induced by the IgE mediated increase in intracellular Ca^{2+} [33, 34], regulated by Ca^{2+} store-operated Ca^{2+} channels [35], inhibited by antiallergic [36, 37] and stimulated by immunomodulatory [38] drugs and antigens [37]. *N*-Acetyl-β-hexosaminidase release is regulated by a small Ras-related GTP-binding

protein Rho p21 [33] (Rho proteins control the polymerization of actin into filaments and govern the organization of body filaments into specific types of structures [28]). C5a, the late component of complement, stimulates Ca^{2+} mobilization and releases hexosaminidase from dibutyryl cAMP activated cells [39]. Cytokines are involved in secretion of *N*-acetyl-β-hexosaminidase in lacrimal gland acinar cells. Chronic treatment of these cells with a combination of IL-1α and IL-1β decreased carbachol-stimulated *N*-acetyl-β-hexosaminidase secretion by 80% [40]. IL-10 synergizes with IL-4 and IL-13 in inhibiting lysosomal enzyme secretion by human monocytes. IL-13 reduces mainly the precursor form, IL-4 and IL-10 reduce both precursor and mature forms [41]. From 5 to 50% of the newly synthesized precursors are secreted from cultured cells. Most cells secrete about 5% of the total enzyme content per day [42]. In a human hepatoma cell line (HepG2 cells), the cumulative release of extracellular activity corresponds to about 3-10% of intracellular activity. Isoenzyme B constitutes 33% of intracellular and 20% of extracellular enzyme activity [43].

ACTIVE SITE OF *N*-ACETYL-β-HEXOSAMINIDASE ISOENZYMES

In tissues and body fluids, isoenzymes A($\alpha\beta$), B ($\beta\beta$), S ($\alpha\alpha$), P, I [9, 44] and C have been detected [45]. Their α and β subunits contain own active sites, which differ in substrate specificity and thermal stability however, dimerization is required for formation the active site [12]. The active sites of both α and β subunits (as dimers) can hydrolyse *N*-acetylhexosamines from neutral substrates, but only subunit α can hydrolyse negatively charged substrates, such as β-linked glucosamine-6-sulfate in glycosaminoglycans, or *N*-acetylgalactosamine in G_{M2} ganglioside (Fig. 1). The α-subunit has a binding site for the complex formed by G_{M2} ganglioside with G_{M2} activator protein that recognizes *N*-acetylneuraminic acid on G_{M2} [46]. Both the G_{M2} activator protein and the domain of β subunit which contains amino acids 225-556, are necessary for hydrolysis of *N*-acetylgalactosamine from G_{M2} ganglioside [11, 12] and Pro β504 Ser substitution affects the ability of isoenzyme A to hydrolyse natural, but not artificial, substrates [22]. The carboxy terminal section of each subunit is probably involved in subunit-subunit interaction [12]. There are suggestions that activator protein may also act to reduce hydrogen binding between the acetamido NH of the terminal GalNAc residue and the carboxyl group of NeuAc, freeing the GalNAc residue for hydrolysis [47]. The area of α-subunit responsible for decreased thermal stability and the ability to bind specifically to negatively charged substrates is localized in position 132-283 [12] (Fig. 2). The isoenzyme S has a limited catalytic activity, is unstable [48] and shares with isoenzyme A the ability to remove *N*-acetylglucosamine-6-sulfate, depends on unusual property of α subunit [10]. The existence of two types of active sites has been described in isoenzyme A, and of only one type of active site in isoenzyme B [9]. There is evidence that α and β subunits have in their catalytic sites one or more acidic residues, presumably located in the region of overall homology which are invariant within deduced primary sequences of many species. Asp-163 from α- and Asp-196 of the β-subunit meet such conditions for the catalytic protonated acidic residue. Less conserved Aspβ240 and probably Asp β290 residues are important in binding 4-methylumbelliferyl-β-*N*-acetylglucosamine [20]. Tse *et al.* [20] suggest a mechanism of action for *N*-acetyl-β-hexosaminidase similar to that of lysozyme, where two acidic amino acids, one protonated and other unprotonated, participate in a single or double-displacement reaction. In the case of β-subunit, the protonated group may reside in Asp-196, and unprotonated acid may be replaced by an unidentified active basic group, which re-

moves a proton from the acetamido group of the bound substrate [20]. Arg-178 in subunit α and Arg-211 in subunit β [47] meet such demands. In the β-subunit, Glu-355 was identified at the substrate binding site, by photoaffinity labeling [49] and site direct mutagenesis [50], but the relation between this residue and the active site has to be proven [12]. Site direct mutagenesis, homology studies, modeling and studies with suicide substrates suggest that Gluα323 and Aspα258 are active site residues and that Gluα323 is involved in catalysis [51].

DIFFERENTIATION OF *N*-ACETYL-β-HEXOSAMINIDASE ISOENZYMES

Isoenzymes A and S are heat labile whereas isoenzymes B and P are heat stable [9]. This possibly depends on alanine-543, as substitution of Alaβ543 by Thr is associated with thermolability of isoenzyme B [52]. Isoenzyme P which has been detected in liver, placenta [53] and serum during pregnancy, is heat stable and contains only subunit β [9]. Higher sialic acid content in isoenzyme P is the only difference between serum isoenzyme B and P. At present there is no method available to distinguish isoenzyme P from isoenzyme I [9]. Isoenzyme C has been detected in human brain [54, 55], placenta [56], liver, lung [55] and kidneys [45]. Isoenzyme C from renal tissue is located in cytoplasm, it has the pH optimum at 6.5, does not bind to ConA-Sepharose, migrates faster than isoezyme A to anode during cellulose acetate electrophoresis, has lower affinity to sodium-cresol sulfonphtaleinyl *N*-acetyl-β-D-glucosaminide (K_m = 1.16 mmol/l) than isoenzymes A and B with K_m = 0.18 and 0.22, respectively [45], and is identical with the major residual *N*-acetyl-β-hexosaminidase activity in fibroblasts from Sandhoff's disease (deficiency of isoenzyme A and B) [54]. Isoenzyme C isolated from human placenta, which has similar properties to isoenzyme C isolated from renal tissue [56], bears no immunological relationship to isoenzymes A and B. Isoenzyme C activity was detected in white blood cells from patients with Sandhoff's disease and Tay-Sachs disease (deficiency of isoenzyme A) [54]. Isoenzyme C activity is significantly increased in the neoplastic renal tissues in comparison to normal tissue [45]. Higher proportions of isoenzyme C in relation to isoenzymes A and B in human embryonic tissues, and in chick embryo fibroblasts suggest that isoenzyme C is a fetal enzyme [45]. There are suggestions that isoenzyme C is totally different from other isoenzymes and has a locus on DNA separate from that for isoenzyme A and B [57]. No information is available on biosynthesis and possible subunit structure of isoenzyme C.

Isoenzymes of *N*-acetyl-β-hexosaminidase are cleared from plasma primarily by non parenchymal liver cells (endothelial cells of the sinusoids and Kupfer cells) by a specific recognition system on the cell surface [58].

CLINICAL IMPORTANCE OF DETERMINATION *N*-ACETYL-β-HEXOSAMINIDASE ISOENZYMES

Until recently, determinations of isoenzymes *N*-acetyl-β-hexosaminidase were mainly used in clinical diagnosis Tay-Sachs and Sandhoff diseases [1, 59]. Now however, numerous papers report determination of these isoenzymes in other pathological states [59]. In rat liver, the activity of isoenzymes A and B is under hormonal regulation. Injection of tyroxine to suckling or adult rats increases the activity of isoenzyme A in liver, whereas cortisone injected to suckling rats decreases the activity of both isoenzymes in liver [60]. Determination of *N*-acetyl-β-hexosaminidase isoenzymes is used in detection of damage to liver cells. Among liver cells, hepatocytes and macrophages are specially rich in *N*-acetyl-β-hexosaminidase activity [61]. Isoenzyme B

constitutes 35–40% of total activity in liver biopsies and serum of the same person [61]. The increase in liver enzyme activity is related to an increase in autophagocytosis [61]. There is no correlation between the activity of isoenzymes in liver and serum, which may be explained by different rates of their synthesis and release into serum [61]. Determination of serum isoenzyme B is a sensitive and inexpensive test for alcohol abuse [58, 62, 63] and alcohol abstinency during the detoxication period [64]. A relationship between the activity of isoenzymes A and B and activity of other liver enzymes, as well as their relationship to the heredity and risk factors for atherosclerosis has been reported [65]. Increased activity of the isoenzyme P in serum is observed in liver disease [66], chronic alcoholism [66] and pregnancy [66]. A decrease in total activity of *N*-acetyl-β-hexosaminidase in lymphocytes and monocytes of peripheral blood in exacerbated multiple sclerosis was accompanied by an increase in the content of isoenzyme B in the plasma membrane fraction [67]. A significantly increased activity of isoenzyme A and insignificantly of isoenzyme B, is observed in blood serum of smokers, and this increase correlates with risk factors for artherosclerosis [68]. In kidneys, *N*-acetyl-β-hexosaminidase is distributed along the whole nephron with the highest activity in lysosomes of proximal straight tubules [69]. Isoenzyme A is a part of soluble intralysosomal compartment and is secreted in urine by exocytosis. The intralysosomal and membrane bound isoenzyme B is released in urine together with disrupted lysosomal membranes. Thus the urinary activity of isoenzyme A reflects the secretory activity of tubular cells (functional isoenzymuria), and isoenzyme B reflects the breakdown of tubular cells (lesional type isoenzymuria) [70]. Determination of total activity of *N*-acetyl-β-hexosaminidase in urine is used in early monitoring of human renal transplantation [71]. Although determination of isoenzyme B in urine does not provide significant additional information for the early diagnosis of rejection after transplantation, it may reflect a selective tubular disorder [71]. An increase in isoenzyme B excretion in urine reflects damage to structure and activity of proximal renal tubules caused by fever [72], renal contrasts [73], hypertension [74], upper urinary tract infection [75], toxic cytostatics [76], or metals [70]. In serum of diabetics with vascular complications the proportion of isoenzyme B to A is significantly lower than in normal controls, whereas in urine the proportion of isoenzyme B is significantly greater, than in controls [77]. Isoenzymes of *N*-acetyl-β-hexosaminidase have also been evaluated in neoplastic tissues. Resurgence of fetal isoenzymes of *N*-acetyl-β-hexosaminidase in rat hepatoma has been reported [78]. Human colonic carcinomas and human uterine cervical carcinoma have higher activity of isoenzyme B, whereas in normal colonic mucosa and normal human uterine cervical tissue, the activity of isoenzyme A is higher [79, 80]. Isoenzymes from tumor extracts of ovarian adenocarcinoma are more active and labile to heat, as well as to acidic pH, in comparison to normal tissue [81]. Isoenzyme B from cervical carcinoma exhibits some characteristic variations in pH, and temperature sensitivity, substrate concentration optima and isoelectric points in comparison to control [80]. Increase an activity of both isoenzymes is observed in sera of cancer patients. The increase in the activity of isoenzyme B is predominant over that of isoenzyme A [82]. Prevalence of isoenzyme A and A-like intermediate forms characterize leukemic cells of myeloid origin, whereas greater amounts of isoenzyme B and B-like intermediate forms characterize leukemic cells of lymphoid origin [83].

REFERENCES

1. Zwierz, K., Juszkiewicz, J., Arciuch, L. & Gindzieński, A. (1992) *N*-Acetyl-β-D-hexosaminidase – enzyme Tay-Sachs's and Sandhoff's diseases. *Post. Bioch.* **38**, 127–132 (in Polish).

2. Suzuki, K., Sango, K., Proia, R.L. & Langman, C. (1997) Mice deficient of all forms of lysosomal β-hexosaminidase show mucopolysaccharidosis-like pathology. *J. Neuropathol. Exp. Neurol.* **56**, 693–703.

3. Sango, K., McDonals, M.P., Crawley, J.N., Mack, M.L., Tifft, C.J., Skop, E., Starr, C.M., Hoffmann, A., Sandhoff, K., Suzuki, K. & Proia, R.L. (1996) Mice lacking both subunits of lysosomal β-hexosaminidase display gangliosidosis and mucopolysaccharidosis. *Nature Genet.* **14**, 348–352.

4. Lew, D.B., Brown, E.R., Dempsey, B.K., Wright, H.M. & Malik, K.U. (1997) Contribution of PKC to β-hexosaminidase induced airway smooth muscle proliferation. *Am. J. Physiol.* **272**, L 639–643.

5. Raczek, S., Yeung, C.H., Hasilik, A., Robenek, H., Herstle, L., Schulze, H. & Cooper, T.G. (1995) Immunocytochemical localization of some lysosomal hydrolases, their presence in luminal fluid and their directional secretion by human epididymal cells in culture. *Cell Tissue Res.* **280**, 415–425.

6. Godknecht, A. & Honegger, T.G. (1991) Isolation, characterization and localization of a sperm-bound *N*-acetylglucosaminidase that is indispensable for fertilization in the ascidian *Phallusia mammillata*. *Dev. Biol.* **143**, 398–407.

7. Hermo, L., Adamali, H.I., Mahuran, D., Gravel, R.A. & Traser, J.M. (1997) β-Hexosaminidase immunolocalization and α- and β-subunit expression in the rat testis and epidymis. *Mol. Reprod. Dev.* **46**, 227–242.

8. Miller, D., Gong, X., Decker, G. & Shur, B.D. (1993) Egg cortical granule *N*-acetylglucosaminidase is required for the mouse zona pellucida block to polyspermy. *J. Cell Biol.* **123**, 1431–1440.

9. Perez, L.F. & Tutor, J.C. (1998) Assay of β-*N*-acetylhexosaminidase isoenzymes in different biological specimens by means of differentiation of their activation energies. *Clin. Chem.* **44**, 226–231.

10. Neufeld, E.F. (1989) Natural history and inherited disorders of a lysosomal enzyme β-hexosaminidase. *J. Biol. Chem.* **264**, 10927–10930.

11. Pennybacker, M., Liessem, B., Moczall, H., Tifft, C.J., Sandhoff, K. & Proia, R.L. (1996) Identification of domains in human hexosaminidase that determine substrate specificity. *J. Biol. Chem.* **271**, 17377–17382.

12. Tse, R., Wu, Y.J., Vavougios, G., Hou, Y., Hinek, A. & Mahuran, D.J. (1996) Identification of functional domains within the α and β subunits of β-hexosaminidase A through expression of α-β fusion proteins. *Biochemistry* **35**, 10894–10903.

13. Norflus, F., Yamanaka, S. & Proia, R.L. (1996) Promoters for the human β-hexosaminidase genes HEX A, and HEX B. *DNA Cell Biol.* **15**, 89–97.

14. Zhang, Y., Wick, D.A., Haas, A.L., Seetharan, B. & Dahms, N.M. (1995) Regulation of lysosomal and ubiquitin degradative pathways in differentiating human intestinal Caco-2 cells. *Biochim. Biophys. Acta* **1267**, 15–24.

15. Kuznetsov, G. & Nigam, S.K. (1998) Folding of secretory membrane proteins. *N. Engl. J. Med.* **339**, 1688–1695.

16. Tatu, U. & Helenius, A. (1997) Interaction between newly synthesized glycoproteins, calnexin and a network of resident chaperones in the endoplasmic reticulum. *J. Cell. Biol.* **136**, 555–565.

17. Pollard, M.G., Travers, K.J. & Weissmann, J.S. (1998) Ero 1p: A novel and ubiquitous protein with an essential role in oxidative protein folding in the endoplasmic reticulum. *Mol. Cell* **1**, 171–182.

18. Emiliani, C., Locci, P., Becchetti, E., Marinucci, L., Bellachioma, G. & Orlacchio, A. (1991) Increase in intermediate forms of

β-N-acetylhexosaminidase during rat liver development and regeneration. *Int. J. Biochem.* **23**, 215–219.

19. Mahuran, D. (1995) β-Hexosaminidase: Biosynthesis and processing of the normal enzyme and identification of mutations causing Jewish Tay-Sachs disease. *Clin. Biochem.* **28**, 101–106.

20. Tse, R., Vavourgios, G., Hou, Y. & Mahuran, D.J. (1996) Identification of an active acidic residue in the catalytic site of β-hexosaminidase. *Biochemistry* **35**, 7599–7607.

21. Hou, Y., Vavourgios, G., Hinek, A., Wu, K.K., Hechtman, P., Kaplan, F. & Mahuran, D.J. (1996) The Val 192 Leu mutation in the α-subunit of β-hexosaminidase A is not associated with B 1 variant form of Tay-Sachs disease. *Am. J. Hum. Genet.* **59**, 52–58.

22. Hou, Y., McInnes, B., Hinek, A., Karpati, G. & Mahuran, D. (1998) A Pro 504 → Ser substitution in β-subunit of β-hexosaminidase A inhibits α-subunit hydrolysis of G_{M2} ganglioside resulting in chronic Sandhoff disease. *J. Biol. Chem.* **273**, 21386–21392.

23. Presley, J.F., Cole, N.B., Schroer, T.A., Hirschberg, K., Zaal, K.J.M. & Lippincott-Schwartz, J. (1997) ER- to -Golgi transport visualized in living cells. *Nature* **389**, 81–85.

24. Hamm Alvarez, S.F., DaCosta, S., Yang, T., Wei, X., Gierow, J.P. & Mircheff, A.K. (1997) Cholinergic stimulation of lacrimal cells promotes redistribution of membrane associated kinesin and secretory protein, β-hexosaminidase, and increases kinesin motor activity. *Exp. Eye Res.* **64**, 141–156.

25. Lazzarino, D.A. & Gabel, C.A. (1988) Biosynthesis of the mannose-6-phosphate recognition marker in transport impaired mouse lymphoma cell. *J. Biol. Chem.* **263**, 10118–10126.

26. Petroulakis, E., Cao, Z., Clarke, J.T., Mahuran, D.J., Lee, G. & Tiggs Raine, B. (1998) W 474 C amino acid substitution affects early processing of the α-subunit of β-hexosaminidase A and is associated with G (M2) gangliosidosis. *Hum. Genet.* **11**, 432–442.

27. Hoeflich, A., Wolf, E., Braulke, T., Koepf, G., Kessler, U., Brem, G., Rascher, W., Blum, W. & Kiess, W. (1995) Does the overexpression of pro-insulin-like growth factor-II in transfected human embryonic kidney fibroblasts increase the secretion of lysosomal enzymes. *Eur. J. Biochem.* **232**, 172–178.

28. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. & Watson, J.D. (1994) *Molecular Biology of the Cell;* pp. 610–615, Garland, New York, London.

29. Casciola-Rosen, L.A. & Hubbard, A.L. (1991) Hydrolases in intracellular compartments of rat liver cells. Evidence for selective activation and/or delivery. *J. Biol. Chem.* **266**, 4341–4347.

30. Lemansky, P., Brix, K. & Herzog, V. (1998) Iodination of mature cathepsin D in thymocytes as an indicator for its transport to the cell surface. *Eur. J. Cell Biol.* **76**, 53–62.

31. Miączyńska, M. (1997) Rab/Ypt type of GTPases and their escort proteins (REP) in the regulation of membrane transport. *Post. Bioch.* **43**, 231–237 (in Polish).

32. DaCosta, S.R., Yarber, F.A., Zhang, L., Sonee, M. & Hamm Alvarez, S.F. (1998) Microtubules facilitate the stimulated secretion of lacrimal acinar cells. *J. Cell Sci.* **111**, 1267–1276.

33. Yonei, S.G., Oishi, K. & Uchida, M.K (1995) Regulation of exocytosis by the small GTP-binding protein Rho in rat basophilic leukemia (RBL 2H 3) cells. *Gen. Pharmacol.* **26**, 1583–1589.

34. Sudo, N., Tanaka, K., Koga, Y., Okumura, Y., Kubo, C. & Nomoto, K. (1996) Extracellular ATP activates mast cells *via* a mechanism that is different from the activation induced by the cross-linking of Fc receptors. *J. Immunol.* **156**, 3970–3979.

35. Fischer, M.J., Paulussen, J.J., Tollenaere, J.P., deMol, N.J. & Janssen, L.H. (1998) Structure-activity relationship of astemizole derivatives for inhibition of store operated Ca^{2+} channels and exocytosis. *Eur. J. Pharmacol.* **350**, 353-361.

36. Paulussen, J.J., Fischer, M.J., Roelofsen, E.P., Horbach, D.A., deMol, N.J. & Janssen, L.H. (1996) Oxatomide and derivatives as inhibitors of mediator release from a mast cell model. Structure activity relationship. *Arzneimittelforschung* **46**, 496-501.

37. Fischer, M.J., Paulussen, J.J., Horbach, D.A., Roelofsen, E.P., van Miltenburg, J.C., deMol, N.J. & Janssen, L.H. (1995) Inhibition of mediator release in RBL-2-H-3 cells by some H 1-antagonist derived anti-allergic drugs: Relation to lipophilicity and membrane effects. *Inflamm. Res.* **44**, 92-97.

38. Lüllmann Rauch, R., Pods, R. & Von Witzendorff, B. (1995) Tilorone-induced lysosomal storage of sulphated glycosaminoglycans can be separated from tilorone enhancement of lysosomal enzyme secretion. *Biochem. Pharmacol.* **49**, 1223-1233.

39. Kaneko, Y., Okada, N., Baranyi, L., Azuma, T. & Okada, H. (1995) Antagonistic peptides against human anaphylotoxin C5a. *Immunology* **86**, 149-154.

40. Lambert, R.W. (1998) Do cytokines have a role in the regulation of lacrimal gland acinar cell ion transport and protein secretion. *Adv. Exp. Biol. Med.* **438**, 499-503.

41. Lügering, N., Kucharzik, T., Stein, H., Winde, G., Lügering, A., Hasilik, A., Domschke, W. & Stoll, R. (1998) Il-10 synergizes with IL-4 and Il-13 in inhibiting lysosomal enzyme secretion by human monocytes and lamina propria mononuclear cells from patients with inflammatory bowel disease. *Dig. Dis. Sci.* **43**, 706-714.

42. Skudlarek, M.D., Novak, E.K. & Swank, R.T. (1984) Processing of lysosomal enzymes in macrophages and kidney; in *Lysosomes in Biology and Pathology* (Dingle, I.T., Dean, R.T. & Sly, W.S., eds.) **7**, pp. 17-44.

43. Hultberg, B., Floren, C.H., Isaksson, A. & Jensen, E. (1995) Liver disease and serum hexosaminidase levels. Studies in a human hepatoma cell-line (Hep.G2 cells). *Liver* **15**, 99-103.

44. Gosiewska, A., Rutkowska, J., Szelengowicz, M., Najmoła, I., Gindzieński, A. & Zwierz, K. (1986-87) Purification and properties of *N*-acetyl-β-D-glucosaminidase from human gastric mucous membrane. *Roczn. Akad. Med. Białystok* **31-32**, 29-38.

45. Yoshida, K.-I. (1994) Demonstration and some properties of *N*-acetyl-β-D-hexosaminidase (HEX) C isoenzyme in human renal tissues: Relative increase in HEX C activity in renal cell carcinoma. *Clin. Chim. Acta* **226**, 55-65.

46. Li, S.C., Wu, Y.Y., Sugiyama, E., Taki, T., Kasama, T., Castellato, R., Sonnino, S. & Li, Y.T. (1995) Specific recognition of *N*-acetylneuraminic acid in G_{M2} epitope by human G_{M2} activator protein. *J. Biol. Chem.* **270**, 24246-24251.

47. Hou, Y., Tse, R. & Mahuran, D.J. (1996) Direct determination of the substrate specificity of the α-active site in heterodimeric β-hexosaminidase A. *Biochemistry* **35**, 3963-3969.

48. Geiger, B., Arnon, R. & Sandhoff, K. (1977) Immunochemical and biochemical investigation of hexosaminidase S. *Am. J. Hum. Genet.* **29**, 508-522.

49. Liessem, B., Glombitza, G.J., Knoll, F., Lehman, J., Kellermann, J., Lottspeich, F. & Sandhoff, K. (1995) Photoaffinity labeling of human lysosomal β-hexosaminidase B. Identification of Glu-355 at the substrate site. *J. Biol. Chem.* **270**, 23693-23699.

50. Pennybacker, M., Schuette, C.G., Liessem, B., Hepbildikler, S.T., Kopetka, J.A., Ellis, M.R., Meyrowitz, R., Sandhoff, K. & Proia, R.L. (1997) Evidence for the involvement of Glu-

355 in the catalytic action of β-hexosaminidase B. *J. Biol. Chem.* **272**, 8002–8006.

51. Fernandes, M.J., Yew, S., Leclerc, D., Henrissat, B., Vorgias, C.E., Gravel, R.A., Hechtman, P. & Kaplan, F. (1997) Identification of candidate active site residues in lysosomal β-hexosaminidase A. *J. Biol. Chem.* **272**, 814–820.

52. DeGasperi, R., Gama Sosa, M.A., Grebner, E.E., Mansfield, D., Battistini, S., Sartorato, E.L., Raghavan, S.S., Davis, J.G. & Kolodny, E.H. (1995) Substitution of alanine 543 with threonine residue at the carboxy terminal end of the β-chain is associated with thermolabile hexosaminidase B in a Jewish family of oriental ancestry. *Biochem. Mol. Med.* **56**, 31–36.

53. Arciuch, L.P., Arciszewski, K., Zwierz, K., Bielecki, D.A. & Południewski, G. (1998) Isoenzymes of placental *N*-acetyl-β-hexosaminidase in pregnancy complicated by EPH gestosis. *Proc. XXXIV Meeting of the Polish Biochemical Society, Białystok*, G-20, p. 116 (in Polish).

54. Besley, G.T.N. & Broadhead, D.M. (1976) Studies on human *N*-acetyl-β-D-hexosaminidase C separated from neonatal brain. *Biochem. J.* **155**, 205–208.

55. Poenaru, L. & Dreyfus, J.C. (1973) Electrophoretic study of hexosaminidases: Hexosaminidase C. *Clin. Chim. Acta* **43**, 439–446.

56. Penton, E., Poenaru, L. & Dreyfus, J.C. (1975) Hexosaminidase C in Tay-Sachs and Sandhoff disease. *Biochim. Biophys. Acta* **391**,162–169.

57. Swallow, D.M., Evans, L., Sala, E.N. & Harris, H. (1976) Characterization and tissue distribution of *N*-acetylhexosaminidase C: Suggestive evidence for a separate hexosaminidase locus. *Ann. Hum. Genet.* **40**, 55–66.

58. Kärkkäinen, P., Poikolainen, K. & Salaspuro, M. (1990) Serum β-hexosaminidase as a marker of heavy drinking. *Alcoholism: Clin. Exp. Res.* **14**, 187–190.

59. Ostrowska, L., Zwierz, K., Koniusz, Z. & Gindzieński, A. (1993) Function, properties and clinical significance of *N*-acetyl-β-hexosaminidase. *Post. Hig. Med. Dośw.* **47**, 67–79 (in Polish).

60. Oberkotter, L.V., Kern, R. & Koldovsky, O. (1980) Effects of cortisone and thyroxine on suckling rat liver *N*-acetyl-β-glucosaminidase isozymes. Comparison with adult reactivity. *Biochim. Biophys. Acta* **630**, 279–286.

61. Elsafi, M.E., Hultberg, B., Isaksson, A., Hägerstrand, J., Prytz, H. & Stenram, U. (1994) Lysosomes and human liver disease: A biochemical and immunohistochemical study of β-hexosaminidase. *Eur. J. Clin. Chem. Clin. Biochem.* **32**, 669–673.

62. Hultberg, B., Isaksson, A., Berglund, M. & Moberg, A.-L. (1991) Serum β-hexosaminidase isoenzyme: A sensitive marker for alcohol abuse. *Alcoholism: Clin. Exp. Res.* **15**, 549–552.

63. Wehr, H., Czartoryska, B. & Milewski, B. (1995) Serum β-hexosaminidase activity in hepatic diseases of alcoholic and non-alcoholic origin. *Wiad. Lek.* **48**, 91–95 (in Polish).

64. Hultberg, B., Isaksson, A., Berglund, M. & Alling, C. (1995) Increases and time-course variations in β-hexosaminidase isoenzyme B and carbohydrate-deficient transferrin in serum from alcoholics are similar. *Alcoholism: Clin. Exp. Res.* **19**, 452–456.

65. Hultberg, B., Isaksson, A., Lindgren, A., Israelsson, B. & Brattström, L. (1996) Plasma β-hexosaminidase isoenzymes A and B in patients with cerebral infarction. *Clin. Chim. Acta* **224**, 35–44.

66. Hultberg, B. & Isaksson, A. (1983) Isoenzyme pattern of serum β-hexosaminidase in liver disease, alcohol intoxication and pregnancy. *Enzyme* **30**, 166–171.

67. Orlacchio, A., Martino, S., Sarchielli, P., Gallai, V. & Emiliani, C. (1998) β-*N*-Acetylhexosaminidase in peripheral blood lympho-

cytes and monocytes in the different forms and stages of multiple sclerosis. *J. Neurochem.* **71**, 1168–1176.

68. Hultberg, B., Isaksson, A., Nilsson, J.A. & Fidgarde,. F. (1994) Serum β-hexosaminidase isoenzymes are related to risk factors for atherosclerosis in a large population of postmenopausal women. *Clin. Chim. Acta* **227**, 59–68.

69. Schmidt, H., Mall, A. & Bockhorn, H. (1986) Catalytic activity of alkaline phosphatase and *N*-acetyl-β-hexosaminidase in human cortical segments: Heterogenous changes in acute renal failure and acute rejection following kidney allotransplantation. *J. Clin. Chem. Clin. Biochem.* **24**, 961–970.

70. Bernard, A., Thielemans, N., Roels, H. & Lauwerys, R. (1995) Association between NAG-B and cadmium in urine with no evidence of a threshold. *Occup. Environ. Med.* **52**, 177–180.

71. Bourbouze, R., Gluckman, J.-C., Frantz, P., Paraire, M., Baumann, F.Ch., Luciani, J., Percheron, F. & Legrain, M. (1985) Early monitoring of human renal transplantations by *N*-acetyl-β-D-glucosaminidase isoenzyme activities in urine. *Clin. Chim. Acta* **149**, 185–195.

72. Biernacka, A. & Zoch-Zwierz, W. (1997) "Proximal tubular proteinuria" in children with fever caused by respiratory tract infections. *Ped. Pol.* **72**, 343–351 (in Polish).

73. Winiecka, W. & Zoch-Zwierz, W. (1991) An evaluation of the nephrotic action of Uropolin on children with nycturia. *Przegl. Lek.* **48**, 218–220 (in Polish).

74. Zoch-Zwierz, W., Winiecka, W., Tomaszewska, B. & Wierciński, R. (1992) Assessment of *N*-acetyl-beta-D-glucosaminidase (NAG) and its isoenzymes in urine of children with essential and secondary hypertension. *Wiad. Lek.* **45**, 498–502 (in Polish).

75. Zoch-Zwierz, W. (1990) The activity of *N*-acetyl-beta-D-glucosaminidase (NAG) and that of its isoenzymes in urine of children with acute urinary tract infection. *Ped. Pol.* **65**, 43–47 (in Polish).

76. Tomaszewska, B. & Zoch-Zwierz, W.M. (1995) Renal tubular toxicity of cytostatics in children with acute lymphoblastic leukemia. *Ped. Pol.* **70**, 915–920 (in Polish).

77. Severini, G., Alibertti, L.M. & DiGirolamo, M. (1988) *N*-Acetyl-β-glucosaminidase isoenzymes in serum and urine in patients with *Diabetes mellitus*. *Clin. Chem.* **34**, 2430–2432.

78. Weber, A., Poenaru, L., Lafarge, Ch. & Schapira, F. (1973) Modification of hexosaminidase isozymes in rat hepatoma. *Cancer Res.* **33**, 1925–1930.

79. Brattain, M.G., Kimball, P.M. & Pretlow, T.G. (1977) β-Hexosaminidase isoenzymes in human colonic carcinoma. *Cancer Res.* **37**, 731–735.

80. Bhuvarahamurthy, V. & Govindsamy, S. (1996) Purification and characterization of hexosaminidase from human uterine cervical carcinoma. *Gynecol. Oncol.* **60**, 188–196.

81. Chatterjee, S.K., Chowdhury, K., Bhattarya, M. & Barlow, J.L. (1982) β-Hexosaminidase activities and isoenzymes in normal human ovary and ovarian carcinoma. *Cancer* **49**, 128–135.

82. Lo, C.H. & Kritchevsky, D. (1978) Human serum hexosaminidases: Elevated B form isoenzyme in cancer patients. *J. Med.* **9**, 313–336.

83. Martino, S., Emiliani, C., Tabilio, A., Falzetti, F., Stirling, J.L. & Orlacchio, A. (1997) Distribution of active α- and β-subunits of β-hexosaminidase as a function of leukemic cell types. *Biochim. Biophys. Acta* **1335**, 5–15.