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Communication

Isoenzymes of *N*-acetyl-β-hexosaminidase in complicated pregnancy[○★]

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The activity of *N*-acetyl-β-hexosaminidase was found to be significantly higher in the placentas collected after delivery from women in puerperium with symptoms of prolonged pregnancy or complicated by EPH gestosis, than in placentas from normal pregnancy. Isoelectrofocusing of placenta homogenates showed the presence of isoenzymes A, P and B of *N*-acetyl-β-hexosaminidase. Different isoenzyme A patterns in homogenates were observed in placentas obtained from normal and prolonged pregnancies and in those complicated by EPH gestosis.

N-Acetyl-β-hexosaminidase (HEX) releases *N*-acetylhexosamine residues from the non-reducing terminus of oligosaccharide chains [1]. The activity of HEX has been detected in kidney, spleen, liver, gastric mucosa, walls of blood vessels, cerebral cortex, neoplastic tissues and in the placenta of animals and humans [2]. An increase in HEX activity in the blood and urine is recognized as an indicator of damage to the renal tubules with normal glomerular function [3, 4]. A consistent increase of HEX activity in serum has been reported during pregnancy [5]. Differences in the activity of HEX observed in serum during pregnancy may be indicators of degradation of trophoblast and placenta [6]. The pathologi-

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Abbreviations: HEX, *N*-acetyl-β-hexosaminidase; EPH, edema, proteinuria, hypertension.

cal course of early pregnancy may be correlated with symptoms of imminent abortion [6].

Different isoenzymes of HEX have been found in tissues and body fluids. Isoenzymes A, B and I were detected in human plasma [7]. Isoenzyme P, derived mainly from the placenta, increases in serum during pregnancy and in some liver diseases [8, 9]. Isoenzymes A, B, I1, I2, P, C and S were detected in the serum of pregnant and non-pregnant women [10]. An increase in the activity of all HEX isoenzymes [5, 10], and particularly that of isoenzyme P, synthesized and excreted by the placenta [11] have been observed in the serum of pregnant women.

MATERIALS AND METHODS

Materials. Women in labour were divided into three groups:

1. Control (n = 46) women with a normal course of pregnancy and delivery. Delivery begins with spontaneous contraction activity of the uterus between the 38th–41st weeks of pregnancy.

2. Prolonged pregnancy (n = 30). Pregnancy lasting more than 42 weeks or delivery at normal time but with clinical signs of biologically prolonged pregnancy (placental insufficiency, maceration and shedding of the epidermis of the newborn etc.).

3. EPH gestosis (n = 24). Full term pregnancy with symptoms of EPH gestosis (edema, proteinuria, hypertension). Based on the gestosis index, clinical classification of EPH gestosis depends on the intensity of these symptoms during labour. (In the gestosis index, the intensity of EPH gestosis symptoms – edema, proteinuria and hypertension – are evaluated by a number of points and the total score gives the classification of the gestosis). The women in our groups were classified as having mild and middle forms of EPH gestosis.

The placentas were collected after delivery. Samples of the placentas (3 cm × 3 cm × 3 cm) were taken from the peripheral part. Blood from the placental tissue was removed by perfusion with 0.9% NaCl to obtain a colourless perfusate and a uniform pink colour of the tissue. One gram portions of tissue were suspended in 10 ml of 0.05 M citrate buffer, pH 4.3 and homogenized in a Polytron type homogenizer. The homogenates were centrifuged at 6000 × ***g*** for 60 min at 4°C and the supernatants were collected [12]. For electrofocusing the supernatants were centrifuged in microconcentrators (Centricon 30, Amicon, 1000 × ***g*** for 2 h at 4°C). The activity of the enzyme and protein concentration were determined before and after concentration.

Determination of* N*-acetyl-β-hexosaminidase activity. Determination of *N*-acetyl-β-hexosaminidase activity was performed by the Chatterjee *et al.* method [13] according to Zwierz *et al.* [14]. To 50 μl of diluted placental supernatant (to obtain A_{410} 0.2–0.8 of liberated *p*-nitrophenol), 150 μl of substrate (*p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide, Sigma) and 250 μl of phosphate/citrate buffer, pH 4.7, were added and the mixture was incubated for 60 min at 37°C. The enzymatic reaction was terminated by the addition of 1 ml of borate buffer at pH 9.8. The liberated *p*-nitrophenol was determined colorimetrically at 410 nm. Protein was determined by the method of Lowry *et al.* [15] with bovine serum albumin as a standard.

Electrofocusing of* N*-acetyl-β-hexosaminidase. Isoenzymes of *N*-acetyl-β-hexosaminidase were electrofocused in Multiphor II (Pharmacia Biotech), with a power supply EPS 3500 (Pharmacia Biotech), as follows: to a Whatman 3 paper strip (0.5 cm × 1 cm) lying on the surface of polyacrylamide gel (7.5%) with ampholine pH 3.5–9.0 (2%), 24 cm × 11 cm and 1 mm thick, 20 μl of concentrated supernatants (40–80 μg of protein) and a broad pI calibration kit (Pharmacia Biotech) were applied. The cathode buffer was 0.2%

ethanolamine and the anode one 0.04% H_2SO_4 [9]. Electrofocusing was performed for 2 h at 4°C, maintained by a Multitemp thermostat (Pharmacia Biotech). After separation, enzymatic activities were detected by immersing the gels in a mixture containing 1.5 mg of substrate (α-naphtyl-AS-BI-*N*-acetyl-β-glucosaminide, Sigma) and Fast Garnet (Fluka), and incubating at room temperature for 18 h [16]. The stained gels were immersed in 10% acetic acid to interrupt the enzymatic reaction, fix the resulting colour complex and destain the background. For storage, the gels were sealed in polyethylene foil in a mixture of 10% acetic acid, 10% methanol and 10% glycerol. The stored gels were dried in a Gel Dryer (Kucharczyk t.e., Poland) and densitometrically evaluated using a Gel Documentation System 5000 (UVP, England) and a computer program. The activity of a given HEX isoenzyme in a particular isoelectrophoretic run was calculated from the total HEX activity determined colorimetrically in the concentrated supernatant and from the densitometric data.

RESULTS

It was found that the specific activity of *N*-acetyl-β-hexosaminidase in unconcentrated homogenates of placentas from prolonged pregnancies was 4.37±1.01 mkat/kg protein (Table 1), and in placentas obtained from pregnancies complicated with EPH gestosis the value was 4.85±1.52 mkat/kg protein. These values were significantly higher ($P < 0.01$) than the specific activity of the enzyme in the homogenates of placentas from normal pregnancies (control group) where the specific activity was only 3.52±1.21 mkat/kg protein.

To obtain distinct and uniform colour bands after electrofocusing, the supernatants were concentrated and preliminary purification was performed by centrifugation in Centricon 30 concentrators. We found an increase in the specific activity of HEX after concentration of the supernatants (HEX isoenzymes have a molecular mass of about 100 kDa and concentration in Centricon 30 removes proteins of a molecular mass below 30 kDa). The specific activity of HEX in concentrated supernatants from placentas from normal pregnancies was 7.07±2.83 mkat/kg protein, from placentas from prolonged pregnancies 5.51±1.56 mkat/kg protein and from placentas obtained from pregnancies complicated by EPH gestosis – 7.86±3.15 mkat/kg protein. The electrofocusing of supernatants from homogenates of placentas on polyacrylamide gels revealed the presence of three isoforms of *N*-acetyl-β-hexosaminidase (Fig. 1), which were identified as A, P and B, according to Hayase & Kritchevsky [9]. The greatest intensity of the bands was observed in pregnancy complicated by EPH gestosis. The number of bands in the region of isoform A differed in the homogenates from the placentas from normal pregnancies (3 bands), prolonged (2 bands) ones, and pregnancies complicated by

Table 1. Specific activity of *N*-acetyl-β-hexosaminidase in homogenates obtained from placentas from normal pregnancies, prolonged ones and complicated by EPH gestosis

	Specific activity of HEX (±S.D.) in homogenates of placentas [mkat* /kg protein]	
	Before concentration	After concentration
Control	3.52 ± 1.21	7.07 ± 2.83
EPH gestosis	4.85 ± 1.52*	7.68 ± 3.15
Prolonged pregnancy	4.37 ± 1.01*	5.51 ± 1.56

*$P < 0.01$, Significant differences in comparison with the control group; *mkat = millikatals.

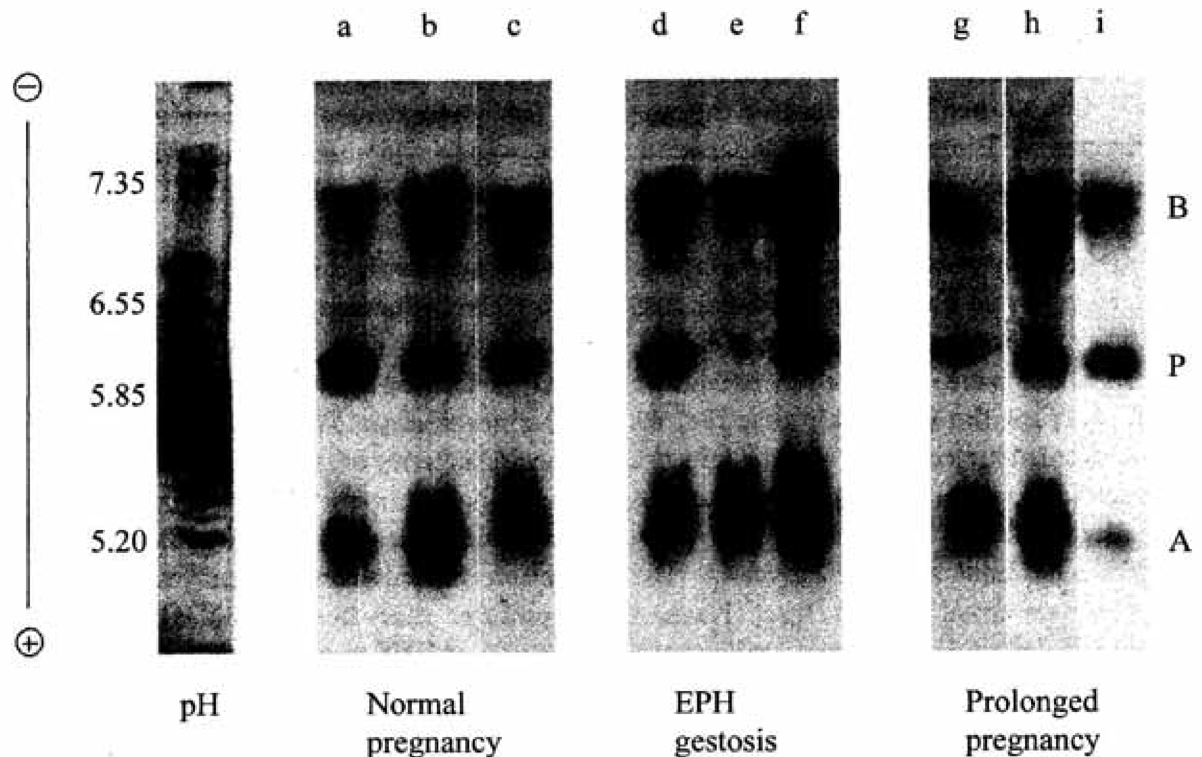


Figure 1. Isoelectrofocusing of isoenzymes of *N*-acetyl-β-hexosaminidase in concentrated supernatants of the placental homogenates of 3 normal pregnancies, 3 prolonged ones and 3 complicated by EPH gestosis.

The protein contents in μg/20 μl of concentrated placental supernatants applied to the IEF gel were: a) 58.8; b) 75.6; c) 57.6; d) 40.8; e) 67.2; f) 63.6; g) 48.0; h) 51.5; i) 54.2.

EPH gestosis (4 bands). The differences in the isoenzyme patterns of the placentas of women with EPH gestosis were related to the values of the gestosis index; a more intensive saturation of the bands occurred in cases with a higher gestosis index (with dominant symptoms of hypertension and proteinuria).

The isoelectric points of HEX isoenzymes, evaluated by the broad pI calibration kit, were: isoenzyme A – pH 5.2, isoenzyme B – pH 7.3 and isoenzyme P – pH 6.0 [9, 17].

After isoelectrofocusing and densitometric evaluation of the separated bands, in the homogenates of placentas from normal pregnancies the following were found: isoenzyme A – 51.0% (3.60 mkat/kg protein), B – 28.5% (2.02 mkat/kg protein), and P – 20.5% (1.45 mkat/kg protein). In placentas of pregnancies complicated by EPH gestosis: A – 34.7% (2.66 mkat/kg protein), B – 37.7% (2.90 mkat/kg protein), and P – 27.6% (2.12 mkat/kg protein) and in those of prolonged pregnancies: A – 40.8% (2.25 mkat/kg protein), B – 23.4% (1.29 mkat/kg protein), and P – 35.8% (1.97 mkat/kg protein) (Fig. 2).

Isoenzyme A dominated in the homogenates of placentas from normal and prolonged pregnancy, 50% and 41% of the total activity, respectively. The highest activity of isoenzyme B was observed in the homogenates of placentas with EPH gestosis. Isoenzyme P constituted the highest percentage of the total activity in the homogenates of placentas from prolonged pregnancies (Fig. 3).

DISCUSSION

In clinical practice the detection of fetus risk is in some cases very difficult. The methods applied to date are neither sufficiently sensitive nor specific. Dysfunction of the placenta

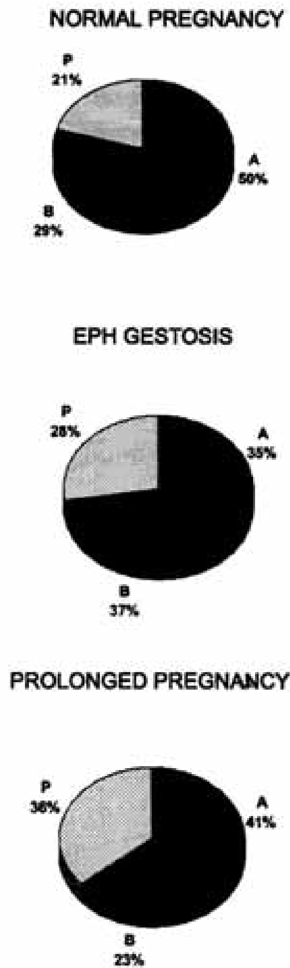
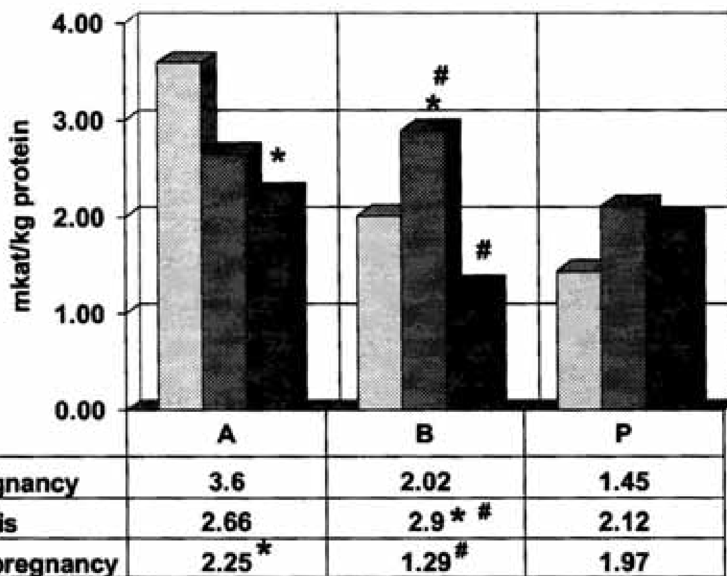


Figure 2. Distribution of *N*-acetyl-β-hexosaminidase isoenzymes in concentrated supernatants of the homogenates of placentas from normal pregnancies (n = 8), complicated by EPH gestosis (n = 8) and prolonged (n = 8) ones.

observed in pregnancies prolonged or complicated by EPH gestosis is accompanied by changes in metabolism [18], particularly by an increase in catabolic processes, brought about by lysosomal enzymes including *N*-acetyl-β-hexosaminidase [11, 19, 20].

We found HEX activity to be the lowest in the placentas from normal pregnancies (3.52 mkat/kg protein). In the placentas from prolonged pregnancies the HEX activity was significantly higher (4.37 mkat/kg protein). In the group of women with symptoms of EPH gestosis the HEX activity was also significantly higher (4.85 mkat/kg protein) than in normal pregnancy.

Soga *et al.* [21] observed a high activity of HEX in the placental tissue and decidual membrane near the end of pregnancy in comparison with the previous weeks of pregnancy. This may be related to the influence of released lysosomal enzymes on fibers of collagen and elastin in the region of uterine cervix, which represent one of the exponents of preparation of the lower region of the genital tract to labour. Other investigators observed the same relationship, in respect of increased HEX activity [19]. They suggested that the major importance of this mechanism is in preparation for labour. Lysosomal enzymes released from the placenta [20, 22] may change the structure of the lower genital tract



	A	B	P
Normal pregnancy	3.6	2.02	1.45
EPH gestosis	2.66	2.9* #	2.12
Prolonged pregnancy	2.25*	1.29#	1.97

Figure 3. Specific activity of *N*-acetyl-β-hexosaminidase isoenzymes in concentrated supernatants of the homogenates of placentas:

▒, normal pregnancy (n=8); ■, pregnancy complicated by EPH gestosis (n=8); ■, prolonged pregnancy (n=8). *Statistical significance in comparison with control group $P < 0.05$. #) Statistical significance in comparison with groups of complicated pregnancies $P < 0.1$.

making it more susceptible to stretching by changing the proportion of elastin and collagen fibers to the muscles. The increase in the activity of the lysosomal enzymes may influence the internal remodeling of the placental structures before labour [23].

The finding of increased activity of HEX in the placentas of women with prolonged pregnancy, which confirms our previous report [24], is probably connected with a longer term rebuilding the lower segment of the genital tract, accompanied by lack of normal contractory activity [25]. This is not true in the case of placenta derived from pregnancy complicated by EPH gestosis. In this case the observed increase in HEX activity is not connected with prolonged pregnancy. Rather, the increase in the activity of catabolic enzymes is connected with processes taking place in the placental tissue [26, 27]. Lysosomal exoglycosidases are typical catabolic enzymes and their activity increases in tissues undergoing intensified remodeling. In prolonged pregnancy, frequently complicated by prolonged ischaemia of the fetus, the natural degenerative processes are considerably intensified, and they can be detected microscopically and macroscopically [28]. Placental insufficiency has been observed in pregnancy complicated by EPH gestosis [29], but the microscopic changes were different in character than in prolonged pregnancy. In the area of chorionic villi edema, a thickening of the barrier between the blood of the mother and fetus, and embolisms and infarcts in the chorionic villi were found [28]. These processes may be related to the increase in the activity of the lysosomal enzymes in the placenta.

The finding of no significant differences in the isoelectrofocusing patterns of HEX isoenzymes in normal placentas, compared with placentas from prolonged and complicated pregnancy by EPH gestosis is somewhat surprising. However, the patterns obtained are in agreement with the data obtained by other investigators [9, 10], who observed the presence of isoenzymes A, B and P in the human placenta and demonstrated heterogeneity of each fraction. The irregularities observed in the fractions of isoenzymes of HEX in the pathologies investigated may be explained by different courses of degradation of placental tissue in these pathologies, reflected by the different microscopical pictures.

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