Summarizing, these data indicate that only the pH and the presence of the local anesthetics in the medium of the main incubation play a role for the glucose transport and not the preincubation of the erythrocytes. Therefore, erythrocytes not preincubated with local anesthetics were used in our further investigations.

We then studied the affinity of the local anesthetics to the transport system of glycose at the pH-range between 7 and 9. The dissociation constants (K_i) at pH 7, 7.5, 8, 8.5 and 9 were determined and the log K_i were plotted against the pH-values (figure 1). The derivations of the obtained curves dlog K_i/dpH against pH yield straight lines (some of them shown in figure 2), which is evidence that the relation between log K_i and pH is in accordance with a quadratic function; its graphic presentation is a parable.

From the equation of the derived parables: $dy/dx = 2ax + b$ and the parables $ax^2 + bx + c = y$ (where x = the pH of the medium and y = the corresponding log K_i) we calculated the parameters a, b and c and the vertex (this is the pH-value where log K_i is minimum, table 2).

One will find that the increase of the parameter 'a' also results in an increase of '$-$ b' and 'c'. This roughly agrees with a gradual decrease of the pH of the vertex. The smaller is 'a', i.e. the smaller the slope of dy/dx (the smaller the influence of the pH of the medium on log K_i), the higher are the pH-values of the vertex (table 2); it thus indicates those pH-values where the affinity of the local anesthetics to the transport system becomes maximal, and at further increase of the pH begins to decrease.

According to the equation $pH = pK + \log L/L^+$, we calculated the relation of the uncharged (L) and the charged (L^+) form of the local anesthetics in the vertex. (The pK-values of some local anesthetics are communicated in the literature[11]). In table 2, it can be found that the relation of the 2 forms varies in individual local anesthetics. This implies that the largest effect of the local anesthetics on the glucose transport in erythrocytes is dependent on a specific relation of the uncharged to the charged form of each local anesthetic. This also might be considered an indication to the question of which of both forms of the local anesthetics are effective in other biological processes.

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The hydrolysis of some L-amino acid-p-nitranilides with the normal and pregnant's serum aminopeptidases

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Summary. From 4 serum aminopeptidases (2 of pregnant and 2 of nonpregnant women's sera), the placental lysosomal (mol. wt 320,000) splits Lys-NAp only. B-Cys-NAp is hydrolyzed from the both placental enzymes, i.e. lysosomal and microsomal (mol. wt 145.000) AP. Ala-NAp is split by both nonpregnant serum AP more readily than Leu-NAp.

Aminopeptidase (AP), present in women's serum and hydrolyzing L-leucine-β-naphtylamide, L-leucine-p-nitranilide, L-cystine-di-β-naphtylamide or S-benzyl-L-cysteine-p-nitranilide, rises during pregnancy. Its level in the serum may be used as criterion for the function of placenta. This placental AP was separated from AP which is present in nonpregnant women's serum[1]. 2 placental isoenzymes (CAP_1 and CAP_2) were found in the serum[2,3]. Mizutani et al.[4] differentiated the placental AP from the nonplacental one on the basis of resistence to L-methionine-inhibition and sensitivity to heat. We compared the hydrolysis of some substrates which were derived from L-amino acid-p-nitranilides, using the normal and pregnant women's serum, NaCl-eluate from placenta and 2 fractions of the pregnant serum from Sephadex G-200 column.

When obtained, the serum and placenta samples were immediately frozen until used. The aryl-amidase activity was followed as 'reaction rate' using p-nitranilides of L-leucine (Leu-NAp), L-lysine (Lys-NAp). L-alanine (Ala-NAp), S-benzyl-L-cysteine (B-Cys-NAp) and L-phenylalanine (Phe-NAp) as substrates. The free p-nitraniline was measured at 405 nm (Vitatron) and the units were calculated in the usual manner. Comparing some arylamidases activity in the pregnant and nonpregnant serum and in placenta, we followed the possibility of determining the placental AP directly by means of a suitable substrate and to differentiate the placental AP from the nonplacental one. The unproportional hydrolysis of Leu-, Ala-, Lys- and B-Cys-p-nitranilides by pregnant and normal serum indicated that more than one AP split these substrates, as reported by some authors for L-leucyl-β-naphthylamide as substrate[2-4].

In the serum or nonpregnant women, we failed to find (in our conditions of 25 °C/1 min 0.1 ml serum pH 7.2) any activity splitting Lys-NAp. At the beginning of pregnancy, Lys-arylamidase appears in the serum, and when the placenta is formed, the activity increase up to maximum values (figure 1). This Lys-AP activity is inactivated by heating and is practically insensitive to L-methionine inhibition. By molecular sieving on Sephadex G-200 column, it displayed a single peak in the high molecular fraction (figure 2). The optimum pH in the phosphate buffers was found to be 7.2, the activity is completely inhibited by 1.10 phenantroline (0.01 M); an addition of $CoCl_2$ (in

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concentration 1.10^{-4} M) to the serum enhances this activity. Lys-NAp is one of the substrates split by the so-called aminopeptidase-B which occurs in the tissues, human fetal liver[5], amniotic fluid[6] and in human uterus wall[7]. It is not present in rat serum[8] while human serum has only a weak, probably red-cell derived, ability to split this substrate. AP-B may convert kinin-10 (kallidin) to kinin-9 (bradykinin).

Ala-NAp is hydrolyzed by the normal women's serum and serum of women in the 1st trimester of pregnancy, more readily than Leu-NAp. The activity in the whole serum during pregnancy increases up to maximum level in the 38–39th week (figure 1), whereas at the end of pregnancy the level of Ala-NAp remains below that of Leu-AP. Within the period from 22 to 28th week of pregnancy, there is a shift in the levels of Leu-AP and Ala-AP in the serum, and at the end of the term hydrolysis of Leu-NAp always exceeds that of Ala-NAp. B-Cys-NAp was proposed for the estimation of serum oxitocinase activity[9]. It is hydrolyzed by placental AP more quickly than the other substrates.

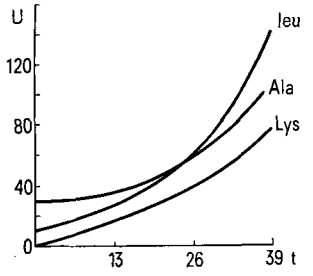

Fig. 1. Leu-AP, Ala-AP and Lys-AP activity during normal pregnancy. The activity was determined in 0.1 ml serum, 0.001 M substrate (L-leu-, L-ala- and L-lys-nitroanilides) concentration and phosphate buffer pH 7.5 for Ala-NAp, pH 7.2 for Lys-NAp. t = Term of gravidity (in weeks); U = units/litre of serum.

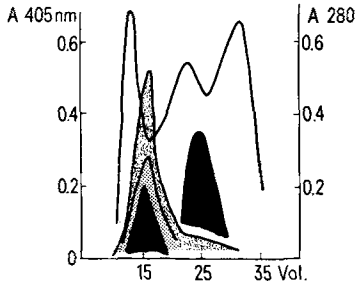

Fig. 2. Fractionation of pregnant serum (3 ml) on Sephadex G-200 (column 25 × 40 cm) in phosphate-NaCl (0.05 M–0.15 M) buffer pH 7.8 and distribution of AP activity. Areas splitting Leu-NAp ▒; Lys-NAP ▒ and Ala-NAp ■ are expressed in A_{405} nm, A280 is protein distribution (—). Vol. is volume of eluate (fraction No.).

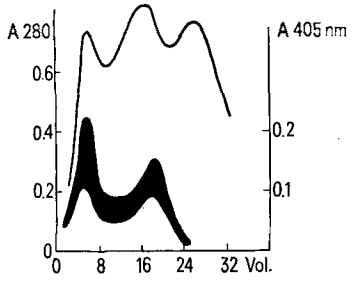

Fig. 3. Fractionation of normal women's serum (3 ml) on Sephadex G-200 (column 25 × 40 cm) in phosphate-NaCl (0.05 M–0.15 M) buffer pH 7.8 and distribution of AP activity. Areas splitting Ala-NAp ■ and Leu-NAp □ are expressed in 405 nm. Vol. is volume of eluate (fraction No.). A_{280} is protein distribution (—).

In order to characterize the aminopeptidases of pregnancy serum, we have in 2 fractions obtained on Sephadex G-200 (figure 2) followed some properties of the aminopeptidases; the substrate specificity, thermostability, sensitivity to L-methionine inhibition and inactivation of oxitocin. The 1st (high molecular) fraction split all substrates used: Lys-, Ala-, B-Cys- and Leu-NAp. Ala-AP is well inhibited by L-methionine, Lys-AP and B-Cys-AP are not sensitive to it. The activity is heat unstable (60 °C/30 min), the fraction inactivates oxitocin (established by a test on the isolated rat uterine horn), and the oxitocinase activity of this fraction is inhibited by L-methionine. The 2nd fraction splits Ala-NAp, B-Cys-NAp and Leu-NAp, and is inhibitable by L-methionine. The fraction has always the oxitocinase activity. This fraction comprises joint localization of normal AP (mol. wt 145,000) and placental microsomal AP (mol. wt 145,000). Both have similar properties, however, the nonpregnant AP does not split B-Cys-NAp. The normal nonpregnant serum splits Ala-NAp more quickly than Leu-NAp. During fractionation on Sephadex G-200, this activity is localized in 2 fractions (high and medium molecular) which become well separated (figure 3). Oya et al.[10] found in the normal nonpregnant serum aminopeptidase splitting L-leucin-β-naphtylamide in the medium molecular fraction (mol. wt 145,000) only. The activity of both fractions is inhibited by L-methionine and is relatively thermostable. In the placental eluate we have found activity which splits all 4 substrates. Further studies on characterization of serum and placental aminopeptidases are in progress.

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