and lysine, whereas in the F_{act} hydrolysate we found lysine, asparaginic acid, glycine, serine, glutamic acid, threonine, alanine, phenylalamine, leucine and isoleucine, tryptophan, methionine and valine. The protein nature of the factors is also confirmed by their extremely high lability: even at 4° they lose most of their activity in several h; these factors were then incubated at 60 °C for 1 h, consequently their biological activity disappeared completely in each case (figure 1 shows that catalase activity after administration of F_{inh} subjected to heat inactivation practically coincides with control curve 1). It can been seen that the catalase activity was falling after treatment of F_{inh} with trypsin, too (figure 1, curve 4). Moreover, the densitograph of the inhibiting factor changed, too (figure 2C). In all probability, it is caused by the destruction of the factor by trypsin. These data seem to establish the destructive effect of trypsin on F_{inh}, which shows, in turn, the protein nature of the latter. The high concentration of the SH-groups per g protein is also worthy of note (table).

Thus, the substances isolated from rat liver cell cytoplasm are proteins and are biologically active preparations, which either stimulate or suppress the rate of catalase synthesis in liver.

The pH-dependence of glucose transport inhibition by local anesthetics in human erythrocytes[1]

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Summary. Local anesthetics vary in inhibition of glucose transport in human erythrocytes at different pH-values in the incubation media.

Diverse biological reactions are influenced by local anesthetics[2–4]; among such processes, the glucose transport in human erythrocytes is inhibited by different kinetics, depending on the local anesthetics applied[5]. The molecular mechanism of their action is not yet clarified; neither it is known whether local anesthetics are effective in the charged or uncharged form[3,6]. In this study, the influence of pH (i.e. of the relation between the charged and the uncharged form) on the inhibition of the glucose transport by local anesthetics is investigated.

Material and methods. Human blood was collected in ACD solution (11 g sodium-citrate, 35 g glucose, 4 g citric acid with aqua bidest. ad 1000 ml). The erythrocytes were preloaded by 4 washings with isotonic NaCl solution, containing 200 mM glucose. The further preincubation of the cells is described in the various experiments: 150 µl of these preloaded cells were incubated for 5 sec at 20 °C in 10 ml phosphat-buffer of the desired pH with 0.038 mM C^{14}-glucose and local anesthetics of the concentrations as indicated in figure 1. The further procedure used for the incubation and the analytical methods were described in previous papers[7,8]. The inhibition constants were calculated from the equation:

$$K_i = \frac{[I]}{\frac{v_0}{v_i} - 1} \quad \text{(under the condition } [S] \ll K_m)^9,$$

where v_0 = velocity of the non-inhibited glucose uptake
v_i = velocity of the inhibited glucose uptake
[S] = glucose concentration in the medium
[I] = concentration of the localanesthetic
K_m = Michaelis constant of the carrier-glucose complex

The relative inhibition 'i' in percent was calculated from

$$i = \left(1 - \frac{v_i}{v_0}\right) 100.$$

The derivation of the parables was carried out by a graphic method using a mirror ruler[10].

Results and discussion. As described previously, relative inhibition of glucose transport by local anesthetics at pH 4 was smaller compared with that found at higher pH-values[5]. Inefficiency of the charged forms, increasing with the decrease of pH-value, might be due to their

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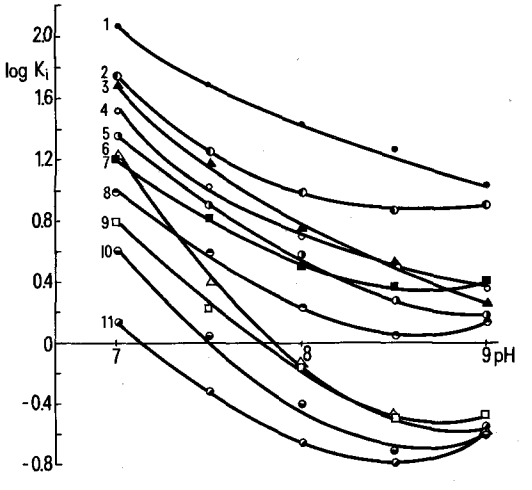

Fig. 1. The logarithmic dependence of the dissociation constants(K_i) of the local anesthetics on the pH. Erythrocytes were preloaded with 200 mM glucose and incubated for 5 sec at 20 °C in phosphat-buffer at pH 7.9, containing C^{14}-glucose and the local anesthetics in the indicated concentrations. *1* 0.5 mM brufacaine, *2* 15 mM mepivacaine, *3* 12 mM procaine, *4* 8 mM lidocaine, *5* 6 mM hostacaine, *6* 4.5 mM ultracaine, *7* 1.5 mM tetracaine, *8* 3 mM baycaine, *9* 1.6 mM butacaine, *10* 1.2 mM oxybuprocaine, *11* 0.35 mM dibucaine. (The concentrations used are the same as in our previous paper[5].)

Tables 1a and b. Glucose uptake in preincubated erythrocytes with or without phosphat-buffer in the presence or in the absence of localanaesthetics in a medium-containing procaine, tetracaine and lidocaine at pH 7.5 (a) and at pH 5.5 (b)

Table 1a

	Medium at pH 7.5 with	Glucose uptake $\left(\frac{\mu moles \cdot 10^3}{ml\ ery \cdot sec}\right)$	Relative** inhibition (%)
Erythrocytes* preincubated with NaCl/gluc.	0	36.0	–
	Procaine	20.6	43
	Tetracaine	23.0	36
	Lidocaine	21.1	42
Erythrocytes* preincubated with NaCl/gluc. containing procaine	0	36.0	–
	Procaine	21.0	42
Erythrocytes* preincubated with NaCl/gluc. containing tetracaine	0	36.0	–
	Tetracaine	22.4	36
Erythrocytes* preincubated with NaCl/gluc. containing lidocaine	0	35.4	–
	Lidocaine	19.6	45

Table 1b

	Medium at pH 5.5 with	Glucose uptake $\left(\frac{\mu moles \cdot 10^3}{ml\ ery \cdot sec}\right)$	Relative** inhibition (%)
Erythrocytes* preincubated with phosphat-buffer/gluc. at pH 5.5	0	21.1	–
	Procaine	21.6	0
	Tetracaine	24.0	0***
	Lidocaine	20.4	0
Erythrocytes* preincubated with phosphat-buffer/gluc. at pH 5.5, containing procaine	0	18.6	–
	Procaine	19.6	0
Erythrocytes* preincubated with phosphat-buffer/gluc. at pH 5.5, containing tetracaine	0	20.3	–
	Tetracaine	23.6	0***
Erythrocytes* preincubated with phosphat-buffer/gluc. at pH 5.5, containing lidocaine	0	20.6	–
	Lidocaine	20.6	0

*Erythrocytes were preloaded with 200 mM glucose in 0.9% NaCl (see 'material and methods'). **Relative inhibition = i (see 'material and methods'). ***This actually was an activation of about 15%. The concentration of procaine was 12 mM, of tetracaine 1.5 mM and of lidocaine 8 mM.

slower penetration into the membrane. We wanted to find out whether or not this assumption was true. For this reason, we preincubated erythrocytes in 0.9% NaCl or at pH 5.5 for 30 min with the respective local anesthetics (representing a member of 3 different types of inhibition[5]). Glucose uptake was measured at pH 5.5 or 7.5 during incubation of 5 sec in a medium which also contained the corresponding local anesthetics. In tables 1a and 1b, it is seen that at pH 5.5 the non-inhibited glucose uptake in erythrocytes, not preincubated with local anesthetics, is much smaller (about 40%) than at pH 7.5. This is in accordance with our previous results[7]. Furthermore, the relative inhibition of glucose uptake at pH 7.5 is only dependent on the presence of the local anesthetic in the medium, but not from the preincubation of the erythrocytes with local anesthetics before the main incubation (table 1a). The results at pH 5.5 are similar; here the preincubation with procaine leads to a small inhibition, in comparison with the erythrocytes not preincubated with procaine. Tetracaine in the medium results in a small activation of the glucose uptake, in comparison with the incubation without tetracaine in the medium.

Table 2. Parameters of the parables, representing the relation between log K_i and pH and the quotient of the uncharged to the charged form of local anesthetics in the vertex

Local anesthetic*	a	–b	c	pH of the vertex	$\frac{L^{**}}{L^+}$
Brufacaine	0.08	1.8	10.5	11.2	–
Procaine	0.17	3.5	17.5	10.0	138
Lidocaine	0.20	3.8	18.0	9.4	219
Hostacaine	0.25	4.7	21.5	9.2	98
Mepivacaine	0.28	4.9	22.1	8.7	30
Ultracaine	0.32	5.5	23.9	8.6	–
Baycaine	0.34	5.9	25.9	8.7	–
Oxybuprocaine	0.39	6.8	29.4	8.8	9
Butacaine	0.42	7.4	32.0	8.8	4
Dibucaine	0.44	7.4	31.0	8.4	2
Tetracaine	0.46	8.3	36.6	8.9	26

*Formulas see Lacko et al.[5]. **L = local anesthetic uncharged; L^+ = local anesthetic charged.

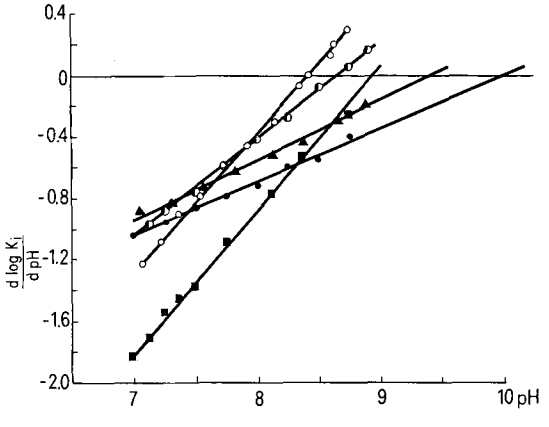

Fig. 2. Straight lines obtained by derivation of some parables from figure 1. ○, Dibucaine, ×, ultracaine, ■, tetracaine, ▲, lidocaine, ●, procaine. The intersection of the straight lines with the abscissa was calculated.

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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