The single stage coupling of trypsin to the pre-activated dextran matrix is complete within 24 h at 5 °C, as shown by the absence of free enzyme on gel chromatography. The resultant graft is stable at room temperature in aqueous solutions of neutral pH.

The Figure shows pH-rate profiles for the hydrolysis of BAPNA catalyzed by free and bound trypsin at ionic strengths of 0.17 and 1.17. The pH optimum is displaced by approximately 1 pH unit towards more alkaline values by conjugation. A similar shift towards higher pH values has been observed previously for trypsin-polyelectrolyte derivatives and in that case was attributed to the neighbouring carrier bound charged groups[7]. For this system such an explanation is inadequate. Oxidation of polysaccarides by sodium metaperiodate, under the above conditions terminates at the aldehyde level of oxidation, and the pH shift is not reduced by the large change in ionic strength[8].

The resultant pH-activity profile after treatment of trypsin with excess acetaldehyde was determined in order to separate the effects due to the matrix from those due to chemical modification of the protein molecule (Figure). In agreement with LABOUESSE and GERVAIS[9] a small alkaline shift was observed, but smaller than that caused by the polyaldehyde dextran.

The matrix had no effect, within experimental error, on the substrate dependant kinetics of trypsin. Both free and attached enzyme obeyed the Michaelis-Menten equation for the hydrolysis of BAPNA over the range $0.1–1.27 \times 10^{-3}$ *M*; the parameters determined at each pH optimum are shown in Table I.

Immobilization imparts increased resistance to autolysis (Table II). Trypsin loses 98% of its activy within 72 h at 30 °C pH 7.0, but somewhat less (86%) in the presence of 0.2 *M* calcium chloride. By contrast the conjugate retains 42% of its initial activity even after 34 days under the same conditions; calcium ions also have a small stabilizing effect on the conjugate, Table I (care was taken to avoid bacterial contamination).

This reduced autolytic capability of the carrier bound enzyme is the result of two main factors. By analogy with the usual reaction products of aldehydes with proteins, the expected sites of reaction would be to the E-amino groups of lysine so preventing the latter from binding to the active sites of other trypsin molecules. In addition, the presence of the attached dextran will sterically hinder the mutual approach of trypsin molecules. This effect is probably also responsible for the decreased degradation of casein by the conjugate which is only 4% of that of the native enzyme.

As a requisite to further elucidation of the observed kinetic properties of the conjugate, its structure is under investigation.

Table I. Apparent Michaelis constants for trypsin catalyzed hydrolysis of BAPNA in 0.1 *M* veronal buffers, plus 0.02 *M* calcium chloride, 30 °C

	$K_{(app)}$ ($\times 10^4$ *M*) pH 8.0	pH 8.85
Trypsin	7.40	5.80
PADT	5.60	5.95

Table II. Percentage retention of tryptic activity towards BAPNA, at 30 °C in 0.1 *M* citrate buffer pH 7.0 (protein concentration, 1 mg/ml).

	3 days	34 days
Trypsin	2	0
Trypsin plus 0.02 *M* $CaCl_2$	14	0
PADT	46	42
PADT plus 0.02 *M* $CaCl_2$	53	55

Zusammenfassung. Durch kovalente Bindung an Polyaldehyd-dextran wird Trypsin immobilisiert. Die polymerische Matrix stabilisiert das Enzym und bewirkt eine pH-Erhöhung der maximalen Aktivität, beeinflusst aber den Michaelis-Menten Parameter für die Hydrolyse des Substrates BAPNA nicht.

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Alteration of Erythrocyte ($Na^+ + K^+$)-ATPase by Replacement of Cholesterol by Desmosterol in the Membrane

It was shown by us that treatment of rats with an inhibitor of desmosterol reductase, i.e. 20.25-diazacholesterol, results in an increased specific ($Na^+ + K^+$)-ATPase activity in membranes of different tissues. Such an effect was shown for the sarcolemma of skeletal and cardiac muscle[1,2] and erythrocyte ghosts[3,4]. Preliminary studies[5] suggest that the alteration in ($Na^+ + K^+$)-ATPase activity is due to the replacement of cholesterol by demosterol in the membranes of animals treated with this substance.

Male Wistar rats were daily treated with 10 mg 20.25-diazacholesterol dihydrochloride in 0.2 ml of water given by an oesophageal cannula for a period of 8 weeks. Control animals were subjected to the same procedure using 0.2 ml of water only. Blood was drawn from one treated and one control animal into heparinized syringes by aortic puncture of the anesthetized animal. Plasma and red

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blood cells (RBC) were separated, RBC were washed 3 times in icecold 0.9% NaCl. Aliquots of the RBC of the treated animal were recombined with plasma of the same animal as well as with plasma of the control animal, and vice versa. Samples were kept at room temperature for about 16 h, then plasma and RBC were separated again and erythrocyte ghosts were prepared as described earlier[3]. At every step, and from every sample, an aliquot was removed for sterol analysis[1] and determination of ATPase activity. ATPase activity was determined by measuring Pi liberation from ATP (for detail see ref.[3]). Protein determination was performed according to LOWRY et al.[6].

As already described by us, erythrocytes of rats treated with 20.25-diazacholesterol show a partial replacement of cholesterol by desmosterol and an increased specific activity of ($Na^+ + K^+$)-ATPase, whereas the basic Mg^{++}-dependent ATPase is unchanged[3]. Since it is known[7] that cholesterol of erythrocyte membranes exchanges readily with the plasma cholesterol pool, we first investigated whether desmosterol behaves similarly. In our experiments, a new equilibrium was obtained after about 16 h, when incubation was performed at room temperature, whereas a demonstrable exchange did not occur at a temperature of 4 °C. Preliminary studies had also shown that simple storing of heparinized blood at room temperature does not significantly alter the transport ATPase of erythrocytes.

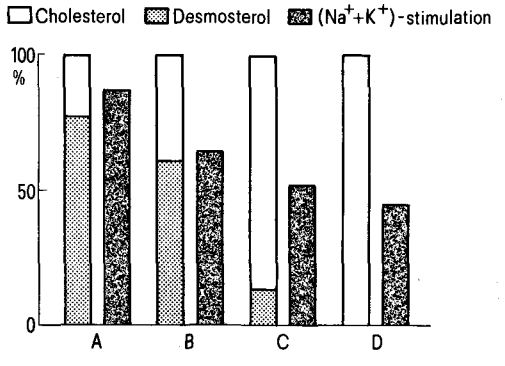

Fig. 1. Sterol composition (%) and ($Na^+ + K^+$)-induced stimulation (%) of rat erythrocyte ghosts.
A) Ghosts from RBC of a treated animal incubated with homologous plasma. B) RBC of a treated animal incubated with control plasma. C) Control RBC incubated with plasma of the treated animal. D) Control RBC incubated with control plasma. The graph represents a typical exchange – experiment.

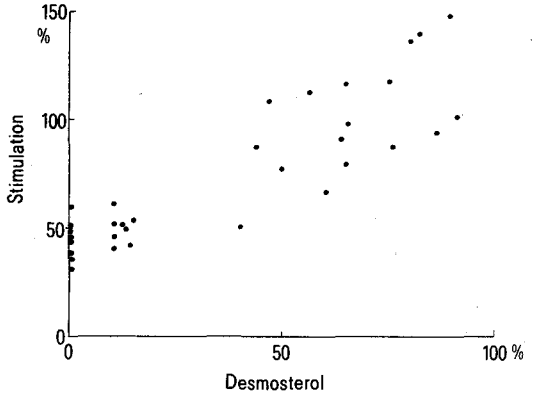

Fig. 2. Scatter plot showing the relationship of ($Na^+ + K^+$)-stimulation of the Mg^{++}-dependent ATPase of rat erythrocyte ghosts to % desmosterol of total sterols.

As can be seen from Figure 1, incubation of desmosterol containing RBC with control serum leads to a decrease of desmosterol and an increase of cholesterol percentage in the membrane. The total amount of sterols remained unchanged as compared to the controls incubated in control plasma. It also can be seen that lowering the desmosterol percentage in the RBC of the treated animal lowers the specific ($Na^+ + K^+$)-ATPase, whereas desmosterol incorporation into control RBC increases it.

Results obtained with 20.25-diazacholesterol fed rats are summarized in the Table. In all experiments performed, the tendency was the same. Incubation of RBC from treated animals with control plasma resulted in a decreased specific ($Na^+ + K^+$)-ATPase, while incubation of control RBC with desmosterol containing plasma from treated animals increased the specific ($Na^+ + K^+$)-ATPase activity.

The dependency of ATPase stimulation upon desmosterol percentage in the membranal sterol moiety can easily be seen in Figure 2. The results obtained by these in vitro experiments fit well with our findings that the specific ($Na^+ + K^+$)-ATPase in heart sarcolemma increases with increasing desmosterol content in the plasma-membrane[8]. It is suggested that the replacement of cholesterol by its natural precursor influences the ($Na^+ + K^+$)-ATPase activity by altering its surrounding environment, which might influence the particular conformation of the transport enzyme. But it cannot be decided whether the observed effect is due to the decreased cholesterol content or the presence of the desmosterol. The first possibility might be supported by the finding of BRUCKDORFER et al.[9], who showed that cholesterol depleted erythrocytes exhibit considerably altered membrane characteristics, such as an increased glycerol permeability and osmotic fragility.

LADBROOK et al.[10] have suggested that cholesterol, by influencing the mobility of the phospholipid hydrocarbon chains, acts as some kind of a regulator on membrane fluidity, reducing the mobility of hydrocarbon chains in the fluid state and having an opposite effect on the gel state. On the other hand, membrane fluidity also greatly depends on the grade of unsaturation of the fatty acid moiety[11], and SMITH[12] has shown that specific ($Na^+ + K^+$)-ATPase is affected by an alteration of the amount of double bonds in membranal phospholipids. As changes in the fatty acid composition in our experiments do not occur, it is tempting to suggest that the double bond in the side chain of demosterol is responsible for an altered membrane fluidity.

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$(Na^+ + K^+)$-ATPase stimulation (%) and % desmosterol of total sterols of erythrocyte ghosts ($n = 8$)

	A		B	C		D
Stimulation of Mg^{++}-ATPase by Na^+ and K^+ (%)	113.1 (87.7–149.5)		92.3 (64.6–118.8)	50.2 (44.9–54.1)		44.5 (39.5–49.1)
P		< 0.005			< 0.001	
Desmosterol of total sterols (%)	75.4 (57–90)		61.6 (43–75)	12.8 (11–15)		— —
P		< 0.005				

For the meaning of ABC and D see legend of Figure 1. P was determined by using the paired Student t-test.

And the maintenance of a fluid-like environment due to its lipid constituents is the prerequisite for an optimal function of transport enzymes, as could be shown by spin label studies[13,14].

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Summary. Cholesterol of red blood cells (RBC) is readily exchanged by desmosterol and vice versa. The resulting alteration in the sterol composition influences the specific $(Na^+ + K^+)$-ATPase activity. It is suggested that this effect is due to an altered membrane fluidity.

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Monoamine Oxidase Activity in *Helix pomatia*

It has been reported that although monoamine oxidase (MAO) exists in molluscan nervous tissue, it is not able to metabolize a tryptamine or serotonin substrate[1]. However, a number of other experiments[2–7] have shown both 5-hydroxytryptamine (5-HT) and dopamine (DA) to be slightly metabolized by presumably MAO, though the activity is very low. It was therefore decided to analyze the distribution and nature of MAO in the snail *Helix pomatia* in order to establish the functional significance of the enzyme in the nervous system.

Methods and materials. Tissues were dissected from active snails into ice cold snail saline[8], weighed and then homogenized in ice cold 0.15 *M* KCl, giving 10–100 mg tissue/ml. Aliquots were taken for protein assay[9]. MAO activity was analyzed according to Wurtman and Axelrod[10] with minor variations. The reaction mixture had a total volume of 60 µl consisting of 20 µl homogenate, 20 µl phosphate buffer (pH 7.4, 0.5 *M*), and 20 µl ^{14}C-tryptamine solution (47 mCi/mmole; 0.05 µCi diluted with unlabelled tryptamine to give 12 nmole/20 µl in 0.01 *N* HCl). The mixture was incubated at 37 °C for 60 min, stopped by the addition of 70 µl 2 *N* HCl and 100 µl therefrom twice extracted with 2 ml toluene. The toluene was separated from the aqueous phase by centrifugation, added to 10 ml scintillation fluid and counted in a Packard Liquid Scintillation Counter. 2 to 3 determinations were made from each tissue homogenate. Blank values were obtained as zero-time reactions. The activity was corrected for the efficiency of the extraction procedure and expressed as nmole tryptamine metabolized/g tissue/min and nmole tryptamine metabolized/g protein/min.

In several cases, ^{3}H-5-HT (17.3 Ci/mmole, 0.2 µCi diluted with unlabelled 5-HT to give 6 nmole/20 µl):, ^{3}H-DA (7.2 Ci/mmole, 0.1 µCi diluted to give 6 nmole/20 µl) or ^{14}C-tryptamine (47 mCi/mmole, 0.1 µCi diluted to give 6 nmole/20 µl) was used as substrate and the resulting products extracted into 2 ml of ethylacetate/benzene (1:1 by volume).

To test drug effects, dissected suboesophageal ganglia were partially desheated and incubated for 60 min at 20 °C in snail saline containing various concentrations of drugs together with 0.1 mg/ml ascorbic acid. The ganglia were then blotted dry and their MAO activity was analyzed.

Results and discussion. A great variation of MAO activity occurred in the different tissues (see Table), being highest in the liver (62.11 nmole/g protein/min) and absent in the albumen gland, flagella and radula retractor muscle. In the nervous tissue, the MAO activity (nmole/g protein/min) in the buccal ganglion was 26.81, while in the supraoesophageal and the suboesophageal ganglia it was 16.86 and 13.34 respectively. This is less than 1% of that reported for vertebrate nervous tissue[11], but is similar to that found in other molluscs[12].

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