The increase of glycogen concentration as a previous biochemical event for DNA synthesis 18 h after IPR stimulation cannot be applied as a general mechanism to all major salivary glands. In fact, evident hyperplasia in the P and practically none in the SL gland is promoted by IPR, and for both glands the glycogen concentration did not increase above the control values. On the other hand, DNA synthesis, hyperplasia and increased glycogen concentration can be correlated in the SM gland. In our opinion, the relationship between increased glycogen concentration and DNA synthesis is dependent upon the specific salivary gland metabolism.

It has been reported that oxidative phosphorylation in the P gland represents the major, if not sole source of high energy phosphate[8]. Hence, an increased glycogen concentration after IPR stimulation should not be the main energy source for DNA synthesis and acinar hyperplasia in this gland. Cellular division without previous glycogen accumulation has been also reported in hepatomas and liver regeneration[9]. On the other and, biochemical data showed that SM gland obtains energy through the Embden-Meyerhof[10] pathway and mainly in acinar cells[11] where the hyperplasia does occur. In this investigation the higher glycogen concentration in the SM gland agrees with this concept.

In conclusion, an increased glycogen concentration previous to DNA synthesis and further hyperplasia by a single IPR stimulation is a specific event of the SM acinar cells and not as had been reported[6] of all salivary glands.

Zusammenfassung. Das Synthese-Verhältnis des durch Isoproterenol stimulierten DNA zur Glykogenkonzentration an Ohrspeicheldrüse, Unterkieferdrüse und Unterzungendrüse der Maus wurde untersucht. Der Unterschied der Ergebnisse der drei untersuchten Drüsen hängt von der Art ihres Metabolismus ab.

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Activities of Membrane-Bound and Soluble Catechol-*O*-methyltransferase in Premature and Mature Erythrocytes from Rats[1]

The finding of an adenyl cyclase activity stimulated by β-sympathomimetics in red blood cells from rats[2] prompted investigations by Horst et al.[3] on catecholamine-inactivating enzymes in these cells; as a result of these studies, it was shown for the first time that catechol-*O*-methyltransferase (COMT)-activity is present in erythrocytes. From work of Assicot and Bohuon[4] it became evident that rat erythrocytes contain two different COMTs, a membrane-bound and a soluble enzyme, which differ with regard to substrate affinities, pH optima and thermostabilities; recently it has been shown that both enzymes differ with respect to their inhibition kinetics by tropolone also[5].

It was revealed by work from our laboratory[6–10] that the adrenergic β-receptor-adenyl cyclase system of rat erythrocytes is exclusively localized in premature red cells, i.e. reticulocytes. We therefore investigated whether or not COMT activities would show a similar distribution pattern between these easily discriminable maturation stages of rat erythrocytes.

Methods. Male Wistar rats (150–200 g) were treated with i.m. doses of 60 mg/kg acetyl-phenylhydrazine on 3 consecutive days and exsanguinated on the 7th day after the 1st injection. Reticulocyte-rich (40% reticulocytes; treated rats) and reticulocyte-poor blood (2–3% reticulocytes; controls) from about 20 rats was pooled and filtered through cotton wool[11]. The cells were washed 4 times, ghosts were prepared by hypotonic haemolysis and 5 subsequent washings[12] and investigated immediately. The $15{,}000 \times g$ supernates of the haemolysates were lyophilized and also used as an enzyme source. COMT activities were determined according to Griffiths and Linklater[13] with the pH optima adjusted after Assicot and Bohuon[4]. Protein was determined according to

Catechol-*O*-methyltransferase activities in ghost preparations and $15{,}000 \times g$ supernates from reticulocyte-poor and reticulocyte-rich erythrocyte suspensions from rats

	Reticulocytes (%)	COMT activity (nmoles/h/mg) Ghost preparations ($n = 8$)	$15{,}000 \times g$ supernates ($n = 7$)
Reticulocyte-poor	2.5 ± 0.5	0.85 ± 0.10	0.81 ± 0.13
Reticulocyte-rich	40.2 ± 1.6	4.51 ± 0.84[a]	1.13 ± 0.21

Activities are given per mg protein (ghost preparations) and per mg dry weight ($15{,}000 \times g$ supernates) resp.; substrate concentrations (^{3}H-adrenaline) were in the range of the apparent K_m values experimentally determined. [a] $p < 0.001$.

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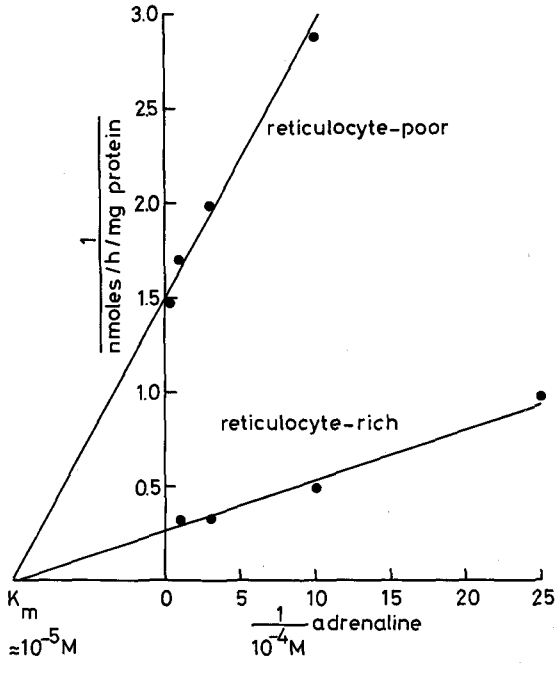
RESCH et al.[14]; reticulocytes were counted in blood smears stained with brilliant cresyl blue.

Results. The results obtained are compiled in the Table. When COMT activities were determined in the 15,000 × *g* supernates of the haemolysates, a slight but insignificant increase of enzyme activity was seen in reticulocyte-rich relative to reticulocyte-poor preparations. In ghost preparations, however, marked differences were observed: COMT activities in membrane fractions from reticulocyterich blood were about 5 times higher than those found in reticulocyte-poor preparations. From these results it is obvious that in contrast to the soluble enzyme, membrane-bound COMT is preferentially localized in the reticulocytes.

As can be seen from the Figure, despite the 5-fold difference in the maximum reaction velocities of COMT activities between reticulocyte-poor and reticulocyte-rich ghost preparations, the apparent K_m values for the substrate adrenaline were identical. This indicates that the COMT activities determined in reticulocyte-poor and reticulocyte-rich ghost preparations come from identical enzymes.

Discussion. Our investigations have revealed that COMT activities in cytoplasmic membranes and cytosol of rat erythrocytes come from two enzymes which are different not only with respect to the criteria previously established[4,5] but also regarding their relative activities in the course of red cell maturation. While soluble COMT activity seems to be evenly distributed throughout the whole erythrocyte population, membrane-bound COMT obviously decreases considerably during the process of erythrocyte maturation. Similar phenomena have been reported for a variety of other enzymes present in red cells[15–18] and also for the adrenergic β-receptor-effector system[8]; the components of the latter (β-sympathomimetically stimulated adenyl cyclase activity, cyclic AMP-phosphodiesterase and cyclic AMP-dependent protein kinase activities) have been shown to be located, at least preferentially, in the reticulocytes. Moreover, recent studies have revealed that considerable monoamine oxidase activity is present in reticulocyte mitochondria[19]. From these results and from those described above, it may tentatively be concluded that, besides binding to blood plasma proteins[20], soluble COMT in the erythrocytes provides an inactivation mechanism for circulating catecholamines, whereas membrane-bound COMT and also monoamine oxidase activities in red cells may be considered to be functionally associated with the adrenergic β-receptor-effector system of the reticulocytes.



Dependence of catechol-*O*-methyltransferase activities in ghost preparations from reticulocyte-poor and reticulocyte-rich erythrocyte suspensions from rats on the substrate (^{3}H-adrenaline) concentrations (Lineweaver-Burk plot). Note that despite a 6-fold higher maximum reaction velocity in reticulocyte-rich preparations, identical apparent K_m values are obtained.

Zusammenfassung. Membranpräparate aus retikulozytenreichem Rattenblut, erzeugt durch Behandlung der Tiere mit Acetyl-Phenylhydrazin, zeigen eine 5–6mal höhere Catechol-*O*-Methyltransferaseaktivität als retikulozytenarme Präparate aus dem Blut unbehandelter Kontrolltiere. Die Aktivität der zytoplasmatischen COMT in den beiden Erythrozytensuspensionen unterscheiden sich nicht signifikant. Offenbar kommt es im Verlauf der Erythrozytenreifung zu einer Abnahme der membranständigen, nicht jedoch der löslichen COMT-Aktivität der roten Blutzellen.

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The Effect of Skim Milk on Plasma Cholesterol in Rats

In experiments dealing with the control of cholesterolemia, we observed that skim milk lowered the levels of plasma cholesterol in rats.

Four timed-pregnant female Sprague-Dawley rats were kept on Purina® rat chow and water. 15 days after delivery, skim milk replaced water on a strictly random basis in 2 of the cages housing the mothers and their offspring. The litters were weaned at 21 days, separated according to sex, and continued either on skim milk or tap water as before. In the litters on skim milk, skim milk was added to the ground chow (1 ml/0.7 g); in the other litters, the chow was mixed with water. At 43 and