a) UMP pyrophosphorylase; b) UMP phosphatase, probably a 5′-nucleotidase (unpublished observations); c) uridine nucleosidase. Thise enzyme has been purified to homogeneity in this laboratory and studied in detail by MAGNI et al.[9]. It is inhibited by ribose and glucose-6-phosphate. d) e) Cytidine deaminase and cytosine deaminase. They have been studied and characterized by IPATA et al.[10,11]. These enzymes are inhibited by several nucleotides, and show regulatory properties. This scheme is supported by the fact that in baker's yeast, uridine phosphorylase is absent[12,4] and uridine kinase activity is very low compared to uridine nucleosidase activity[12].

Summary. Uridine 5′-monophosphate pyrophosphorylase was found to be present in baker's yeast. The enzyme preparation, purified about 30-fold, shows a strict specificity toward uracil and requires Mg^{++} for its activity.

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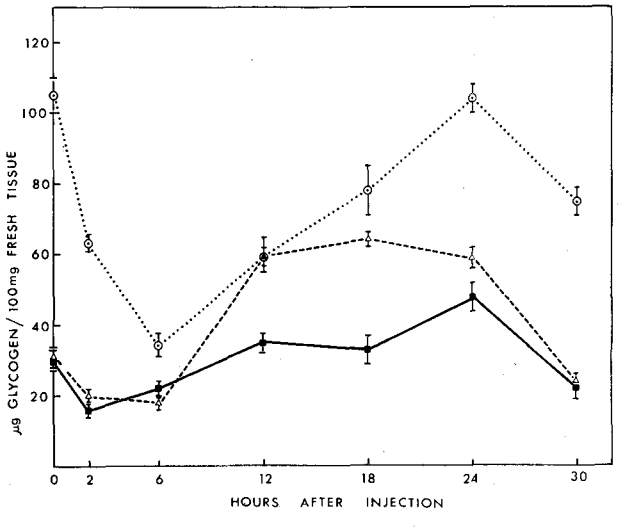
Glycogen Concentration in Isoproterenol-Stimulated Salivary Glands of Mice

The influence of isoproterenol (IPR) on the major salivary glands of rodents has been extensively studied and was recently reviewed[1]. IPR promotes glycogen breakdown through adenylate cyclase activation[2,3]. This sympathicomimetic drug was pointed out as the most efficient adenylate cyclase stimulator in the salivary gland cells[4]. Glycogenolysis in the rat submandibular gland was also observed after parasympathetic stimulation[5].

MALAMUD and BASERGA[6] reported a rapid decrease of glycogen concentration in the pooled parotid (P), submandibular (SM) and sublingual (SL) glands soon after IPR injection in mice. On the other hand, 18 h after this stimulation the glycogen concentration reached a value 5 times higher than the control one. After this peak, there was a new evident glycogenolysis process occurring simultaneously with DNA synthesis up to 30 h after the injection.

The 3 major salivary glands of the mouse, however, present different structures, metabolisms and functions. Therefore, the purpose of this investigation was to study the influence of a single IPR injection on the glycogen concentration of each gland separately.



Effects of isoproterenol on the glycogen concentration in the parotid (■ - - - - ■), submandibular (△ - - - - △) and sublingual (⊙ · · · · · ⊙) glands of mice. Values are means ± SEM. Each value is the mean of 6 to 10 different determinations.

Material and methods. A total of 140 white male adult mice (28–35 g) divided into 7 groups of 20 animals each were used. All animals were allowed regular diet and water ad libitum.

The animals were injected i.p. with a single dose of DL-isoproterenol-HCl (Sigma) solution (7 mg/30 g of body weight). The mice were killed by cervical dislocation at 0, 2, 6, 12, 18, 24 and 30 h after the injection. The sacrifice was always performed at 08.00 h.

Each salivary gland was dissected out, carefully cleaned and weighed. An amount of approximately 90, 60 and 80 mg of SM, SL and P gland respectively was used for each glycogen determination, using the method described by JOHAN and LENTINI[7].

Results and discussion. The glycogen concentration showed different features in the 3 major IPR-stimulated salivary glands. At time zero, the concentration was higher in the SL and similar in P and SM glands. No difference was observed between the uninjected control mice and the injected ones sacrificed immediately after IPR administration. 2 h after the IPR stimulation, the glycogen concentration decreased in the P gland, but still decreasing up to 6 h for the SM and SL glands. Glycogen concentration increases from 6 h after stimulation, returning to the control values in the P and SL glands. However, in the SM gland its accumulation is more evident, reaching twice the control value 18 h after the IPR injection.

Therefore, the interpretation of MALAMUD and BASERGA[6] for the increased glycogen concentration in the pooled major salivary glands must be changed. According to our results, only the SM gland presents a higher glycogen concentration relative to the control values.

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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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