

groups of the polyols on the membrane lipoproteins⁴ cannot be ruled out. Deviations from this relationship are found for inositol and glucosamine, which, however, differ from the other polyols used in that the former bears only secondary hydroxyl groups and the latter has an additional even more polar group.

The fact that glycerol fails to protect the lysosomal structures, at least at the concentrations tested, may be due to its ability to penetrate rapidly into a lipoprotein membrane⁵. In fact, the tonicity of a solution for a membrane-limited organelle is determined by the degree of permeability of the membrane to the solute molecule. On the other hand, it is well known that sucrose, the most common polyol used to stabilize intracellular structures, does penetrate into a lipoprotein membrane, e.g. that of the mitochondrion⁶. One may then assume that also the other polyols penetrate into a lipoprotein membrane, their 'lipid-solubility' being the main factor responsible for the rate of their diffusion across the membrane⁷. If this is the case, an alternative explanation of our data would then be that the number and the type of polar groups in the polyol molecules would regulate the rate of penetration of the different solutes into the lysosome membrane and consequently determine, for each polyol, the concentration at which the highest protection of the lysosomal structures occurs⁸.

Résumé. L'effet protecteur de la glucose, de la saccharose et de la raffinose et d'autres composés hydroxylés (glycérol, glucosamine et inositol) sur les lysosomes de cœur de bœuf a été étudié en suivant la variation de la latence catalytique des hydrolases acides (β -glucuronidase, ribonucléase et β -galactosidase). La plus faible activité catalytique libre (le maximum de protection sur la membrane lysosomale) a été observée à différentes concentrations des polyoles, la plus basse étant celle de la raffinose, la plus élevée celle de la glucose et entre les deux celle de la saccharose, de la glucosamine et de l'inositol. Le glycérol ne donne aucune protection dans les conditions expérimentales indiquées dans l'article.

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⁴ A. L. LEHNINGER, J. Biochem., Tokyo 49, 553 (1961).

⁵ W. C. WERKHEISER and W. BARTLEY, Biochem. J. 66, 79 (1957).

⁷ H. TEDESCHI and D. L. HARRIS, Archs Biochem. Biophys. 58, 52 (1955).

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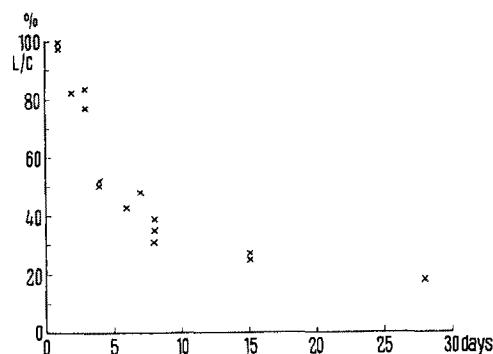
Secretory Responses and Choline Acetylase of the Rat's Submaxillary Gland After Duct Ligation

Ligation of the duct of the rat's submaxillary gland causes a marked glandular atrophy¹. Histologically the decrease in the size of the gland has been found to correspond to an atrophy of the acini while the tubules are less affected². In the present investigation, the secretory function of the duct-ligated submaxillary gland of rats was determined by measuring the secretory responses to sialagogue drugs. This seemed to be of particular interest since the tubules are very likely of great importance for the production of saliva (see OHLIN³). In addition, the effect of duct ligation on the parasympathetic neurones of the gland was studied by estimation of the activity of choline acetylase. The acetylcholine-synthesizing enzyme is localized in the cholinergic fibres of salivary glands⁴.

28 female rats weighing about 200 g were used. The right submaxillary duct was ligatured in all rats. In 15 rats the secretory responses to sialagogue agents were determined and in 13 the activity of choline acetylase.

To study the secretory responses, the rats were anaesthetized with chloralose (100 mg/kg) i.v. after preliminary ether. The submaxillary ducts were cannulated with fine glass cannulae giving about 100 drops out of 1 ml of distilled water. The amount of saliva secreted appearing at the tip of the cannula was noted after i.v. injections of 20 μ g/kg adrenalin, 10 μ g/kg methacholine, and pilocarpine given in increasing doses of from 50–1000 μ g/kg every 30–60 sec until the maximal secretory rate was reached. The secretory responses were studied 1 day to 4 weeks (see Figure) after the duct was ligatured. At the end of the experiments, the position of the ligature was controlled and the submaxillary glands were dissected, carefully cleaned and weighed.

The activity of choline acetylase in the submaxillary gland was determined 1 week after duct ligation, as previously described⁴. The enzyme activity was estimated in 2 groups of rats; 1 group contained pooled glands from 6 animals and the other from 7. The activity is expressed as total activity, μ g acetylcholine/h/gland, and concentration, μ g acetylcholine/h/g acetone powder.



The weight of the rat's submaxillary gland after duct ligation (= L) in % of that of the control gland (= C) at different time intervals.

¹ L. C. JUNQUEIRA and M. RABINOVITCH, Texas Rep. Biol. Med. 12, 94 (1954).

² S. M. STANDISH and W. G. SHAFER, J. dent. Res. 36, 866 (1957).

³ P. OHLIN, Acta Univ. lund. II, 23, 1 (1966).

⁴ I. NORDENFELT, Q. Jl exp. Physiol. 48, 67 (1963).

Choline acetylase activity of the submaxillary gland of the rat 1 week after duct ligation on one side

No. of glands pooled	Enzyme activity in μg ACh/h/g acetone powder		Enzyme activity in μg ACh/h/whole gland		Gland weight (mg)		Ligated/contralateral, %
	Ligated	Contralateral	Ligated	Contralateral	Ligated	Contralateral	
7	600	300	10.2	11.5	97 \pm 4.3	169 \pm 9.7	57 \pm 2.8
6	620	260	8.6	9.1	80 \pm 4.2	158 \pm 6.4	51 \pm 3.6

The weight of the submaxillary gland was found to be markedly decreased after duct ligation (Figure), in accordance with previous results^{2,5}.

The secretory response to 20 μg adrenalin/kg was about 1½ drops of saliva from control glands. It was gradually decreased within the first 6 days after the duct was ligatured and later no secretion could be seen. Similarly, the secretory response to 10 μg methacholine/kg was decreased to zero during the first 3 days after the operation, while control glands produced about 1½ drops. From control glands a maximal flow rate to pilocarpine given repeatedly was reached after a total dose of 1–5 mg pilocarpine/kg; the maximal flow rate was about 4 drops/min. The secretory response to pilocarpine was markedly reduced after duct ligation; usually less than 1 drop of saliva was produced in 10–15 min.

1 week after duct ligation the total activity of choline acetylase was found to be similar in ligatured and control glands. On the other hand, the enzyme concentration was markedly increased, from about 300–600 μg acetylcholine/h/g acetone powder, corresponding to the pronounced decrease in gland weight (Table).

The size of salivary glands is to a large extent dependent on the secretory activity⁶. The atrophy of the rat's submaxillary gland after duct ligation agrees with this suggestion. It seems obvious, however, that not only the secretory inactivity of the ligatured glands is responsible for the atrophy, since the decrease in gland weight is much more marked after duct ligation than after section of the secretory nerves⁷. The ligation atrophy of the rat's submaxillary gland does not seem to be due to changes in the parasympathetic secretory neurones, since the activity of choline acetylase was found to be unchanged after the duct was ligatured. Similarly, the sympathetic neurones of the gland are on the whole unaffected by duct ligation⁸.

A salivary gland after duct ligation has been called a 'resting gland'⁵. The present results indicate that, within the first week after duct ligation, the secretory responses of the rat's submaxillary gland to sialagogue drugs deteriorate markedly; later only very small amounts of secretion can be elicited by intense stimulation with pilocarpine, which evokes a lively flow of saliva from control glands. Thus, the ability of the glandular cells to secrete is almost completely lost after duct ligation, though the tubules, which are supposed to be of great importance for the production of saliva (see OHLIN³), remain histologically more or less unaffected².

Zusammenfassung. Abbinden des Ausführungsganges der Submaxillarisdrüse der Ratte führt zu ihrer Gewichtsabnahme. Nach i.v. Gaben von Methacholin und Adrenalin verschwindet die Sekretion innerhalb von 4–6 Tagen nach der Operation, während bei Pilocarpin noch eine sehr verminderte Speichelsekretion persistiert. Die spezifische Fähigkeit der sekretorischen Zellen war zusehends verringert. Die Gesamtaktivität der Cholin-Acetylase blieb ähnlich derjenigen der Kontrolldrüsen, was für eine unveränderte Funktion des Parasympathikus spricht.

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22nd September 1966.*

⁵ L. C. U. JUNQUEIRA, *Expl Cell Res.* 2, 327 (1951).

⁶ H. D. HALL and C. A. SCHNEVER, *Proc. Soc. exp. Biol. Med.* 117, 789 (1964).

⁷ P. OHLIN and C. PEREC, *Q. Jl exp. Physiol.* 51, 196 (1966).

⁸ N.-E. ANDÉN, K.-A. NORBERG and L. OLSON, *Acta physiol. scand.* 66, 501 (1966).

Chromosomes of Some Squirrels (Mammalia – Sciuridae) From the Genera *Sciurus* and *Glaucomys*

The family Sciuridae contains the subfamilies Sciurinae and Petauristinae¹. North American Sciurinae include the tribes Sciurini (genus *Sciurus*, *Microsciurus*, *Syntheosciurus*, *Guerlinguetus*), *Tamiasciurini* (genus *Tamiasciurus*) and *Marmotini* (genus *Marmota*, *Spermophilus*, *Ammospermophilus*, *Cynomys*, *Eutamias*, *Tamias*)². Among the Petauristinae, only the genus *Glaucomys* is found in North America. Another classification included *Tamiasciurus* within the tribe Sciurini and raised *Tamias* and *Eutamias* to tribal rank, *Tamiini*³.

Chromosome analysis has been applied successfully to taxonomic study of many genera of Sciuridae including *Spermophilus*⁴, *Ammospermophilus*⁵, *Tamias* and *Eutamias*⁶, *Marmota*⁷, *Cynomys*⁸ and *Tamiasciurus*^{5,9}. Because considerable interspecific and intraspecific variation was present in most taxa, chromosomes were of greater systematic value at these rather than suprageneric levels.

The present investigation describes chromosomes from 4 species of *Sciurus* and the 2 species which comprise *Glaucomys*, thereby completing a karyological survey of North American Sciuridae.

The following specimens were studied: *Sciurus carolinensis carolinensis* GMELIN, Florida, Lake County, 1 male;