

Le lesioni che portano alla distruzione di detti elementi interessano soprattutto i nuclei e consistono in fenomeni di picnosi, di cariolisi e di carioressi. Non infrequentemente i granuli cromatinici si condensano verso la periferia del corpo nucleare, limitatamente ad un tratto del contorno oppure lungo tutta la circonferenza, conferendo al nucleo rispettivamente l'aspetto ad anello con castone o anello semplice.

Il processo alterativo non appare uniformemente distribuito nelle stesse sezioni. Infatti sin dai primi giorni di trattamento accanto a tubuli gravemente compromessi se ne riconoscono numerosi altri perfettamente integri. Negli animali trattati più a lungo, per altro, il numero dei tubuli esenti o presentanti solo modeste alterazioni diventa esiguo.

Precocemente si assiste alla comparsa di cellule giganti multinucleate, alcune che sono da ritenersi derivate dagli spermatidi, altre dagli spermatociti.

Le cellule interstiziali non rivelano nè modificazioni morfologiche, nè variazioni del numero.

Nell'epididimo fino al 10° giorno si dimostra la presenza di elementi di sfaldamento, rappresentati, oltre che da spermatozoi, dagli altri stadi di maturazione della linea

seminale in preda ad alterazioni regressive e da cellule giganti multinucleate; a partire dall'11° giorno il lume appare otticamente vuoto.

Il monofluoroacetato sodico agisce dunque elettivamente sull'epitelio delle vie seminali, interessando inizialmente gli stadi intermedi e solo tardivamente gli spermatogoni. Esso si comporta pertanto come la Fluoroacetamide.

Summary. The lesions caused by sodium monofluoroacetate on the testis of the albino rat are described. They consist of regressive modifications of the seminiferous tubules which initially cause damage to the intermediate stages and only later to the spermatogonia. The action of sodium monofluoroacetate is similar to that of fluoroacetamide.

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The Effect of Denervation on Respiratory Enzymes in the Submaxillary Gland of the Rat

The activity of respiratory enzymes in salivary glands of cats and rabbits increases after denervation causing a glandular hypertrophy, e.g. sympathetic denervation of the cat's submaxillary gland, but decreases after denervation causing an atrophy, e.g. parasympathetic denervation of the cat's submaxillary gland¹. In the present investigation the activity of some respiratory enzymes, succinic dehydrogenase, cytochrome oxidase and fumarase, was studied after denervation of the submaxillary gland of rats. Histologically the gland shows atrophic changes both after parasympathetic and sympathetic denervation^{2,3}.

Litters of 4-6 female rats bred at this Institute were used; 2 rats of each litter were taken as controls. The animals, weighing about 200 g, were 4-5 months old when

studied. The right submaxillary gland was parasympathetically or sympathetically denervated. The denervations were preganglionic parasympathetic by section of the chorda-lingual nerve or postganglionic sympathetic by excision of the superior cervical ganglion. All denervations were performed under ether anaesthesia. The rats were killed 3 weeks later by cervical dislocation. The submaxillary glands were removed, cleaned, weighed and homogenized. The enzymic activity was estimated manometrically for succinic dehydrogenase and cytochrome oxidase and spectrophotometrically for fumarase as

¹ I. NORDENFELT, P. OHLIN, and B. C. R. STRÖMBLAD, *J. Physiol.* 152, 99 (1960).
² R. S. SNELL, *Z. Zellforsch. mikrosk. Anat.* 52, 686 (1960).
³ R. S. SNELL and J. R. GARRETT, *Z. Zellforsch. mikrosk. Anat.* 48, 639 (1958).

Activity of respiratory enzymes (mean ± S.E.M.) in parasympathetically or sympathetically denervated submaxillary glands of the rat and in the corresponding control glands

	No. of glands	Succinic dehydrogenase activity		Cytochrome oxidase activity		Fumarase activity	
		per gland	per g gland tissue	per gland	per g gland tissue	per gland	per g gland tissue
Control	18	615 ± 39.2	3630 ± 211	2500 ± 146	14,800 ± 760	1770 ± 81.5	10,500 ± 400
Parasympathetically denervated	17	300 ± 32.3*	2540 ± 264 ^b	1630 ± 111*	13,800 ± 880	988 ± 42.7*	8,340 ± 311*
Control	12	918 ± 24.4	5530 ± 139	3400 ± 74.0	20,600 ± 610	2680 ± 104	16,200 ± 660
Sympathetically denervated	8	879 ± 36.1	5580 ± 212	2680 ± 271 ^b	16,900 ± 1270 ^b	2520 ± 107	16,000 ± 760

* $p < 0.001$ ^b $p < 0.01$ when compared with control.

earlier described^{1,4}. The activity is expressed in units per gland and per g of glandular tissue. One unit of activity corresponds to a utilization of 1 μ l oxygen at 37°C in 30 min for succinic dehydrogenase and cytochrome oxidase, and to a change in optical density of 0.001/min at 240 m μ for fumarase.

The weight of the submaxillary gland decreased from 168 ± 2.7 (30)⁵ mg to 119 ± 3.3 (17) mg after parasympathetic denervation and to 158 ± 4.0 (8) mg after sympathetic denervation.

After parasympathetic denervation the activity of all 3 enzymes decreased when it was expressed per gland (Table). The diminished activity was especially marked for succinic dehydrogenase and fumarase. The activity of these 2 enzymes was also decreased when it was calculated per g of glandular tissue. The decrease in total activity of cytochrome oxidase corresponded to the glandular atrophy after denervation since the activity was not significantly changed when it was expressed per unit weight.

After sympathetic denervation, the activity of succinic dehydrogenase and fumarase was not significantly changed. The activity of cytochrome oxidase was decreased, however, when it was calculated per gland and per unit weight (Table).

In the Table it can be observed that the activity of all 3 enzymes differed in the 2 control groups. This was due to the fact that the experiments on parasympathetic and sympathetic denervation were carried out at different times so that the rats did not belong to the same strain and further the diet was slightly changed.

It is known that the submaxillary gland of rats atrophies after denervation. Histologically atrophic changes have been observed in all the secretory cells after parasympathetic denervation², but only in the acinar cells after sympathetic denervation³. In salivary glands of different

species, it has been shown that the activity of succinic dehydrogenase is very low in the acinar cells but marked in the duct cells⁶, and that cytochrome oxidase is more evenly distributed in the different glandular cells⁷. The localization of fumarase in salivary glands is not known. In the present experiments the denervation atrophy of the rat's submaxillary gland has been found to coincide with a reduced activity of enzymes concerned with aerobic metabolism just as previously seen in salivary glands of cats and rabbits¹. The findings indicate that the concentration of succinic dehydrogenase and fumarase decreases in the atrophic cells after parasympathetic denervation and that the concentration of cytochrome oxidase is diminished in the atrophic acinar cells after sympathetic denervation. These results suggest, furthermore, that the activity of fumarase is low in the acinar cells.

Zusammenfassung. Es wurde in der denervierten Submaxillarisdrüse der Ratte die Aktivität verschiedener respiratorischer Enzyme bestimmt. Succinodehydrase und Fumarase zeigten eine verminderte Aktivität nach parasympathischer Dezentralisierung, ebenso verhielt sich Cytochrom-Oxydase nach sympathischer Denervierung.

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⁴ P. OHLIN, Quart. J. exp. Physiol. 47, 238 (1962).

⁵ Mean \pm standard error of mean (number of observations).

⁶ H. A. PADYKULA, Am. J. Anat. 91, 107 (1952).

⁷ R. M. SCHNEIDER and P. PERSON, Exp. Cell Res. 20, 627 (1960)

Betaine Excretion by the New-Born Infant¹

In a previous communication² we reported that the 'inhibition assay' principle³ had been applied to detect myoinositol in the pooled urine of new-born infants and in the urines of a number of older children with generalized aminoaciduria. We now report that similarly betaine has been found in significant quantities in the pooled urine of healthy new-born infants and in a number of older children with generalized aminoaciduria but not in the urine of healthy older children or adults.

In this procedure biological specimens were compared by placing impregnated paper discs on the surface of an agar culture medium containing a microorganism whose growth is prevented by an appropriate inhibitor. In the process of testing the urine of new-born infants in this manner, employing *Bacillus subtilis* 6051 inhibited by β -2-thienylalanine, an activity was observed similar to that produced by phenylalanine and phenylpyruvic acid. Subsequent paper chromatography of a sample of pooled new-born infants' urine clearly demonstrated that the unknown substance was neither of these two substances. Further, if spores of *B. subtilis* were used as the inoculum instead of vegetative cells, β -2-thienylalanine inhibition was not prevented by the unknown substance. Paper

chromatography and inhibition assay of urine specimens from 100 individual new-born infants showed that this activity was uniformly present. Similar investigations of individual healthy adults failed to reveal the presence of this activity. The above data led us to undertake the isolation and identification of the activity.

Preliminary purification by paper chromatography employing three different solvent systems, followed by ion-exchange chromatography led to a purified sample which did not possess the benzenoid absorption in the ultraviolet typical of phenylalanine and its metabolites. A comparison with tables of R_f values compiled by FINK⁴, indicated that the compound had properties similar to betaine. The nuclear magnetic resonance spectrum of the

¹ This investigation was supported in part by Grant B-1960 and NB-03935 from the National Institutes of Health, U.S. Public Health Service.

² J. P. SCHIRMER JR., H. TIECKELMANN, and R. GUTHRIE, Exper. 20, 602 (1964).

³ R. GUTHRIE and H. TIECKELMANN, Proc. London Conf. Sci. Study of Mental Deficiency (May and Baker Ltd., Dagenham, England 1962), p. 672.

⁴ K. FINK, R. E. CLINE, and R. M. FINK, Anal. Chem. 35, 389 (1963).