Glycogen and phosphorylase in Klinefelter's syndrome: a histochemical study

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Summary. The behaviour of glycogen and phosphorylase in Klinefelter's syndrome has been studied using histochemical techniques.

Material and methods. Biopsy specimens of testicular tissue have been studied in 4 adults with azoospermia, increased plasma gonadotropins, positive sex chromatin test and 47,XXY karyotype. PAS staining was carried out in all fragments for the histochemical demonstration of glycogen while some fragments were fixed in Bouin's solution for histological examinations. Control sections of PAS reaction were studied after treatment with α-amylase (Sigma Chemical Co). 1 fragment from each patient was frozen at -70 °C on dry ice and absolute alcohol. 16-µm sections cut on the crystostat were incubated at 37 °C for 2 h in a medium containing 0.1 M acetate buffer pH 5.6, glucose-1-phosphate and AMP for the histochemical demonstration of phosporylase according to the technique of Tacheuki and Kuriaki[1] and subsequently stained with PAS.

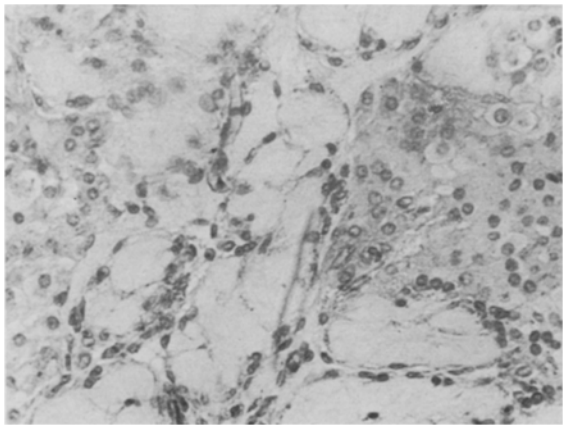

Fig. 1. Testicular biopsy: tubules appear atrophic and hyalinized; PAS reaction for glycogen is negative in the tubular wall. PAS staining, × 200.

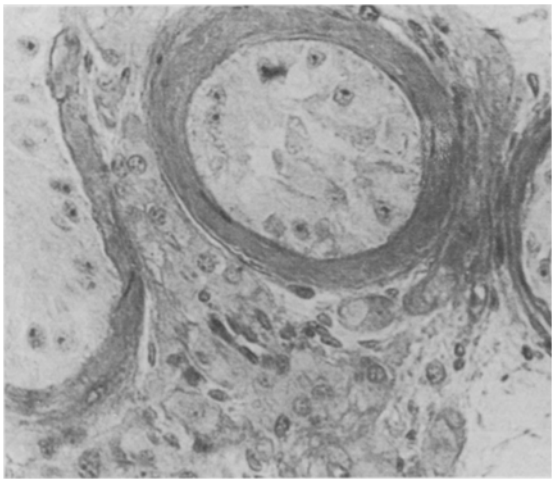

Fig. 2. Abundant Leydig cells, arranged in clumps, are visible in the interstitial tissue. Some tubules with only Sertoli cells and negative PAS reaction for glycogen are also seen. PAS staining, × 200.

Results. Light microscopy revealed the presence of tubular sclerohyalinosis in varying degree (figures 1 and 2). Most of the tubules were completely atrophic and were represented by shrunken hyalinized 'ghosts'. Very occasionally tubules with some degree of spermatogenesis were found. In the interstitial tissue, the Leydig cells were particularly abundant and were often arranged in clumps (figure 2). PAS staining revealed poor Sertoli glycogen in some tubules, whereas glycogen was lacking in the tubule wall. Similarly phosphorylase reaction was weakly positive within the tubule, whilst no enzymatic activity was revealed in the tubule wall.

Discussion. The presence of glycogen and phosphorylase has recently been demonstrated in the cytoplasm of the Sertoli cells and at the level of the smooth muscle cells of the tubule wall of the normal human testis[1-4]. The absence of glycogen and phosphorylase in the smooth muscle cells in Klinefelter's syndrome was therefore of particular interest. Several investigations have been performed on these cells, and it appears that from a functional point of view they are related to the cyclic contractility of the seminiferous tubule[1-7]. It is also well known that these cells (also called myoid) are one of the most important anatomical elements of the 'blood-testis permeability barrier' regulating the entrance of substances and hormones from the vascular stream to the seminiferous tubule[8-14]. Recently studies on the cellular localization of gonadotropins demonstrated that these cells, like the Leydig cells, are another site of activity of LH gonadotropins[15-17]. It has therefore been hypothesized that the gonadotropins may influence the function of the permeability barrier.

It is also well known, however, that histological lesions in Klinefelter's syndrome commence during puberty with the formation of tubular sclerohyalinosis. Ultrastructural studies[17] have shown that this thickening is secondary to an accumulation of hyaline material and collagen fibres which in turn is secondary to an abnormal behaviour of the smooth muscle fibres of the tubular wall. The failure in the present histochemical studies to demonstrate glycogen and phosphorylase in the myoid cells of the tubule wall thus appears, indirectly, to confirm its pathogenesis. It is therefore tempting to suggest that the thickening of the tubule wall in Klinefelter's syndrome is indicative of a functional deviation, primarily genetic in a fibrillogenetic evolution, of the smooth muscle cells revealed during puberty by gonadotropin stimulation.

1 T. Tacheuki and H. Kuriaki, J. Histochem. Cytochem. *3*, 153 (1955).
2 A. Fabbrini, M. Re and G. Spera, Experientia *24*, 789 (1968).
3 C. Conti and A. Fabbrini, Gli ipogonadismi maschili. Ed. L. Pozzi. Roma, 1968.
4 A. Fabbrini, M. Re and C. Conti, J. Endocr. *43*, 499 (1969).
5 E.C. Roosen-Runge, Anat. Rec. *109*, 413 (1951).
6 M.H. Ross and I.R. Long, Science *153*, 1271 (1966).
7 A.E. Castro, A. Alonso and R.E. Mancini, J. Endocr. *52*, 129 (1972).
8 M. Dym and D.W. Fawcett, Anat. Rec. *161*, 301 (1970).
9 D.W. Fawcett, V.L. Leak and P.M. Heidger, J. Reprod. Fert., suppl. *10*, 105 (1970).

10 D.W. Fawcett, in: Male fertility and sterility, vol. 5, pag. 13. Ed. R.E. Mancini and L. Martini. Academic Press, London-New York 1974.
11 C.J. Flickinger and D.W. Fawcett, Anat. Rec. *158*, 207 (1967).
12 B.P. Setchell, J.K. Voglmayr and G.M.H. Waites, J. Physiol. *200*, 73 (1969).
13 B.P. Setchell, in: The testis, vol. 1, p. 101. Ed. A.D. Johnson, W.R. Gomes and N.L. Vandemark. Academic Press, New York-London 1970.
14 B.P. Setchell, in: Male fertility and sterility, vol. 5, p. 37. Ed. R.E. Mancini and L. Martini. Academic Press, London-New York 1974.
15 R.E. Mancini, A.E. Castro and A.C. Seiguer, J. Histochem. Cytochem. *15*, 516 (1967).
16 A.E. Castro, A.C. Seiguer and R.E. Mancini, Proc. Soc. exp. Biol. Med. *133*, 582 (1970).
17 M. Re, G. Frajese, G. Spera and M. Savioli, Progr. med. *31*, 415 (1975).

Lack of synaptic reorganization in inner plexiform layer (IPL) of retina following ganglion cell degeneration[1]

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Summary. Ganglion cell degeneration in the rat retina following optic nerve lesion does not induce the formation of new synapses even after 1 year of postoperative recovery.

The retina is an attractive area for studies of synaptic plasticity due to its isolation and highly organized structure. However, while other investigators have reported retinal plasticity following either light deprivation or light damage[2-4], Chernenko and West[5] were unable to replicate these results when extensive controls were used. The purpose of this study was to determine whether the retinal inner plexiform layer (IPL) would exhibit synaptic reorganization more convincingly under the more extreme conditions which have demonstrated plasticity in other areas of the nervous system.

A variety of situations have been reported in which the central nervous system will undergo synaptic reorganization. In most of these studies it has been shown that the destruction of one input may cause another input to form new synapses onto the vacated postsynaptic sites[6-8]. However, a second model, reported by Ralston and Chow[9], suggests that there may also be situations in which the removal of postsynaptic membrane may induce the presynaptic processes to form synapses on membrane previously not involved in synaptic interaction.

In the present study it was desired to determine whether following optic nerve section and consequent degeneration of ganglion cells and their dendrites, the bipolar and amacrine cells would use amacrine processes to fill the postsynaptic sites vacated by ganglion cells. The model used here, then, is that of Ralston and Chow[9] as it considers whether a presynaptic process which has lost its postsynaptic element will seek out membrane which previously had not been involved in synaptic interaction. The amacrine cells were thought to be good candidates for such postsynaptic elements as a certain proportion of their processes already take part in postsynaptic relationships in a manner similar to ganglion cell dendrites. Amacrine and ganglion cell processes are both found to be postsynaptic to other amacrine processes, and amacrine and ganglion cell processes are found to be simultaneously postsynaptic to the same bipolar cell axons in synaptic complexes (called dyads)[10].

Method. 20 Sprague-Dawley rats averaging 39 days of age at the time of operation were used. In the interest of uniformity of treatment the rats were divided into 5 groups of 4 rats each so that each group, containing 3 lesioned and 1 control animal, could be processed at the same time. Radio frequency lesions were stereotaxically placed in the optic chiasms. Following 1 year of recovery, all rats were anesthetized with sodium pentobarbital and their eyes enucleated and fixed by immersion in osmium tetroxide and glutaraldehyde as described elsewhere[5]. All retinas were embedded in Epon and serially sectioned at 50 µm for accurate isolation of the desired area[11]. Areas 1 mm nasal to the optic disc were mounted on plastic stubs and sectioned at a silver-gold thickness for electron microscopy. These sections were mounted on formvar-coated 1×2 mm slot grids, stained with uranyl acetate and lead citrate, and examined in a Philips 300 electron microscope. Following enucleation, the brains were removed to verify optic nerve degeneration. From the 20 rats, 3 experimental and 3 control retinas (from corresponding groups) were selected for further study on the basis of complete optic nerve degeneration and good fixation. Retinal mosaics extending through the entire depth of the IPL were constructed for each retina at a print magnification of $\times 21{,}600$.

Results and discussion. The experimental mosaics covered a total of 5971 µm^2 and included 726 synapses while the control mosaics covered a total of 7592 µm^2 and included 687 synapses. All 3 experimental mosaics showed even on cursory inspection a loss of ganglion cell processes in the IPL (figures 1 and 2). This coincided with a 36% decrease in IPL thickness which is probably due entirely to the loss of ganglion cell dendrites. Because of these immediately apparent differences plus a loss of almost all ganglion cell axons and somata, it was not possible to score the mosaics without knowing the condition to which each mosaic belonged. However, we believe that the results were not biased by this as we had fully expected synaptic reorganization to occur, and, as will be seen, we found no evidence of this.

An effort was made to identify all processes (both pre- and postsynaptic) which participated in synapses. Each mosaic was gone over at least 3 times by 2 scorers, often simultaneously with much discussion. We have found this technique to give a minimum of drift to criteria used in synaptic

Columnar incidences of the various categories of synaptic relationships for the control and experimental groups

	Incidence (per 1 µm wide column)		Change (%)
	Control	Experimental	
Amacrine (total)	4.445	3.860	−13.2
A/A	0.899	0.886	− 1.4
A/B	0.699	0.665	− 4.9
A/G	0.671	0.292	−56.5
A/?	2.176	2.017	− 7.3
Bipolar (total)	0.457	0.432	− 5.5
B/A-or?	0.050	0.070	+40.0
B/(G-A) or (G-?)	0.057	0.018	−68.4
B/(A-A)	0.143	0.146	+ 2.1
B/(A-?) or (?-?)	0.207	0.198	− 4.3