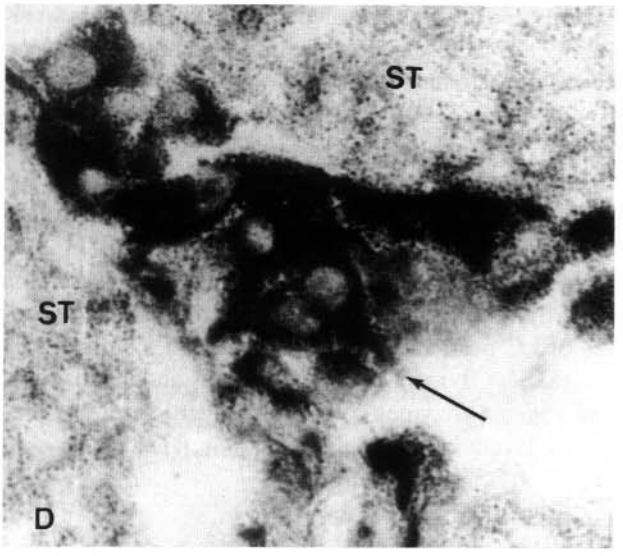


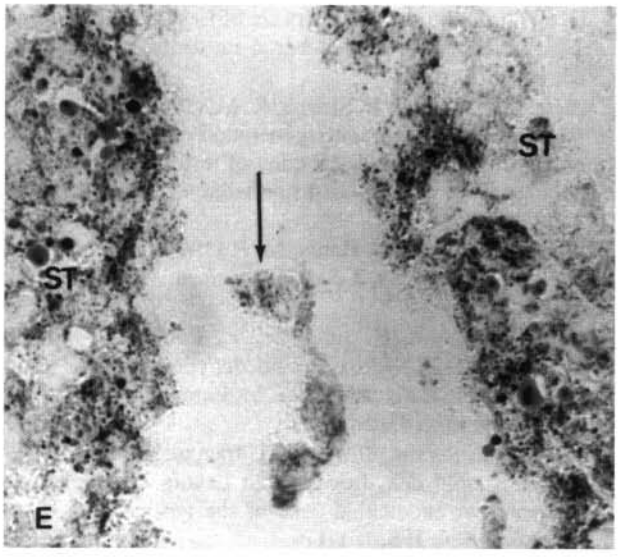


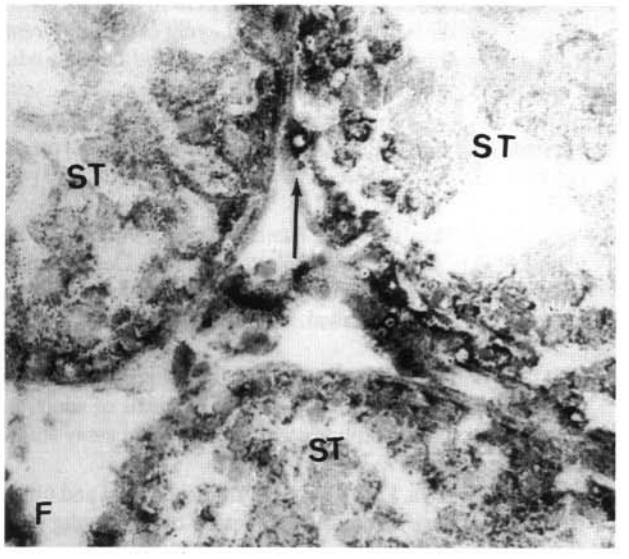


*Fig. 1.* Histochemical visualization of 3β-HSD and NADH2-diaphorase activity in the LCs on a frozen section of the rat testis. A. Control rat testis. A strong activity of 3β-HSD was found in the cytoplasm of mature adult-type LCs in the interstitium (arrow). Magnification 400x. B. By 21 days after EDS treatment, the presence of 3β-HSD activity was seen in the cytoplasm of newly formed cells corresponding to immature LCs (arrow). Magnification 600x. C. By 30 days after EDS treatment the 3β-HSD activity was confined to the cytoplasm of newly formed mature LCs (arrow). Magnification 400x. D. Control rat testis. Strong enzyme activity of NADH2-diaphorase was evident in the cytoplasm of mature LCs (arrow) and weaker staining was observed in seminiferous tubules (ST). Magnification 600x. E-F. A weak NADH2-diaphorase activity can be seen in single newly formed LCs after 21 and 30 days, respectively (arrow), as well as in the seminiferous tubules (ST). Magnification 200x, 400x.

activity for 3β-HSD, which is one of the most relevant steroidogenic markers for LC function. The developmental changes in histochemical staining for NADH2-diaphorase revealed the same pattern as for 3β-HSD, which suggested a close inter-relationship between this enzyme and NAD as an essential cofactor involved in the steroidogenic cycle.

Following EDS treatment, single morphologically recognizable LCs reappeared in the testis after about two weeks. The only steroidogenic enzyme to show an increase by day 14 post EDS was 3β-HSD (O'Shaughnessy and Murphy, 1991). Based on the biochemical data on enzyme activity the authors suggested that 3β-HSD was the first enzyme expressed during LC differentiation, although it is not clear whether this occured before or after the cells acquired specific morphological characteristics of LCs. However, new findings by Ariyaratne et al. (2000) provide evidence for first immunoreactivity for 3β-HSD, cyt P-450scc and P450c17 on the 11$^{th}$ postnatal day in elongated precursor mesenchyme-like cells. These results are in contrast to the enzyme histochemical data of Haider and Servos (1998), who established the first 3β-HSD active cells on day 13 postpartum. An explanation of this discrepancy might be that on the 11$^{th}$ day, progenitor LCs produce the enzyme protein (visualized by immunocytochemistry), but its enzyme activity appears two or three days later, as established by the enzyme histochemical technique. In this respect, our findings about the first 3β-HSD enzyme activity on the 14$^{th}$ day after EDS treatment and the strong staining intensity of this enzyme on day 21 post EDS and onwards coincide with the differentiation of progenitor LCs via immature type toward mature LCs acquiring highest capacity for production of testosterone (Ge et al., 1996). Therefore, the increase in steroidogenic enzyme activity occurs in tandem with the development of cellular organelles (mentioned above) responsible for steroidogenesis. Moreover, regeneration of LCs was reflected by elevated serum testosterone levels, which reached the normal range (Sharpe, 1994; Teerds, 1996). Our data