

and at equilibrium at pH 7.0; the ratio of citrate: cisaconitate: isocitrate becomes 90:4:6. Thus, for the active catabolism of citrate, a very high level of isocitrate dehydrogenase is required to remove the substrate isocitrate which may otherwise be converted to citrate by the reversible aconitase reaction. If the activity of isocitrate dehydrogenase decreases, citrate will accumulate. When citrate is present in higher amounts in mitochondria, it may come out in the cytosol and be degraded to acetyl CoA and oxaloacetate, thus providing acetyl CoA for lipid and carotenogenesis. A low isocitrate dehydrogenase activity of mitochondria from light-grown culture may thus play a significant role in increasing the lipid and carotenoids in light-grown cultures.

Isocitrate lyase activity was found to be lower in light as compared to dark-grown culture, suggesting that the glyoxylate bypass may be operating at higher rate in the dark as compared to the light. If this is the case, under these circumstances, acetyl CoA may be channeled to the glyoxylate bypass and hence may be less available for lipid and carotenogenesis in a dark-grown culture. These are some of the factors which may be playing an important role in light-mediated lipid synthesis and carotenogenesis in *N. crassa*.

The significant role of proteases in cellular regulation is well documented^{21,22}. We have also observed significant changes in the intracellular acidic, neutral and alkaline proteases in light and dark grown *N. crassa*. The activities of all these proteases were found to be much higher in light-grown cultures (table 1). Govind et al. have earlier suggested a role of protease in increasing the levels of carotenoids in *Blakeslea trispora*²³. Whether proteases control carotenogenesis in *N. crassa* is not known at present, though the possibility cannot be ruled out. Proteases may also contribute to the turnover rates of number of enzymes. It is not easy to correlate enzyme activities and the levels of proteases. Further, it is difficult to assume the sequence of events leading to light-mediated control mechanisms in the expression of various enzyme activities. However, attempts have not been made in the present studies to understand the light-mediated control mechanisms in enzyme activities or increases in lipid or carotenogenesis. The present investigation demonstrates the significant changes in the activity of a number of enzymes of carbohydrate metabolism which are considerably influenced when *N. crassa* is grown in light rather than in the dark.

- 1 Whittingham, C.P., in: Chemistry and Biochemistry of Plant Pigments, p.624. Ed. T.W. Goodwin. Academic Press, London 1976.
- 2 Batra, P.P., in: Photophysiology, vol.6, p.47. Ed. A.C. Giess. Academic Press, New York 1971.
- 3 Weeks, O.B., Saleh, F.K., Winahadikusumah, M., and Berry, R.A., Pure appl. Chem. 35 (1973) 63.
- 4 Rau, W., Pure appl. Chem. 47 (1976) 237.
- 5 Rau, W., in: The Blue Light Syndrome, p.283. Ed. H. Senger. Springer, Berlin 1980.
- 6 Harding, R.W., and Shropshire, W., Ann. Rev. Plant Physiol. 31 (1980) 217.
- 7 Savant, S., Parikh, N., and Chhatpar, H.S., Experientia 38 (1982) 310.
- 8 Bragdon, T.H., J. biol. Chem. 190 (1951) 513.
- 9 Davies, B.H., in: Chemistry and Biochemistry of Plant Pigments, p.489. Ed. T.W. Goodwin. Academic Press, New York 1965.
- 10 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. 193 (1951) 265.
- 11 Ochoa, S., Meth. Enzym. 1 (1955) 699.
- 12 Ochoa, S., Meth. Enzym. 1 (1955) 735.
- 13 Kornberg, H., and Horecker, B.L., Meth. Enzym. 1 (1955) 323.
- 14 Jagannathan, V., Singh, K., and Damodaran, M., Biochem. J. 63 (1956) 94.
- 15 Dixon, G.H., and Kornberg, H.C., Biochem. J. 72 (1959) 3.
- 16 Ong, P.S., and Gaucher, G.M., Can. J. Microbiol. 19 (1973) 129.
- 17 Johnson, J.H., Reed, B.C., and Rilling, H.C., J. biol. Chem. 249 (1974) 402.
- 18 Cerff, R., and Klopstech, K., Proc. natl Acad. Sci. USA 79 (1982) 7624.
- 19 Barran, L.R., Daoust, J.Y., Labelle, J.L., Martin, D.G., and Schneider, H., Biochem. biophys. Res. Commun. 56 (1974) 522.
- 20 Rau, W., Lindemann, I., and Rau-Haund, A., Planta 80 (1968) 309.
- 21 Goldberg, A.L., and St. John, A.C., A. Rev. Biochem. 45 (1976) 747.
- 22 Martegani, E., and Alberghina, L., J. biol. Chem. 254 (1979) 7047.
- 23 Govind, N.S., Mehta, B., Sharma, M., and Modi, V.V., Phytochemistry 20 (1981) 2483.

0014-4754/84/121382-03\$1.50 + 0.20/0

© Birkhäuser Verlag Basel, 1984

Autoradiographic investigation of cell proliferation in the adrenal cortex of castrated female rats under the influence of oestradiol

H. Ueberberg, H.-G. Muff and G. Trieb

Department of Experimental Pathology, Dr Karl Thomae GmbH, D-7950 Biberach/Riss (Federal Republic of Germany), 6 April 1983

Summary. Ovariectomy and subsequent treatment with ovarian hormone produces a temporary increase in DNA-synthesizing cells in the zona glomerulosa of the adrenal cortex.

Key words. Rat, female, castrated; oestradiol; ovariectomy; cell proliferation; adrenal cortex, proliferation; DNA-synthesizing cells.

The kinetics of proliferation in the adrenal cortex has already been the subject of numerous investigations¹⁻⁷. In female animals it has been observed that there is a fluctuation in the number of DNA-synthesizing cells related to the oestrous cycle⁷. The aim of this study was to investigate the kinetics of proliferation in the adrenal cortex of castrated female rats which received ovarian hormone.

Material and methods. The investigation was performed in 88 female rats of the Chbb: THOM (SPF) strain. Ovariectomy was performed under Sodium Evipan (1 ml/kg b.wt) anesthesia

in all animals when they were 60 days old. Five oestradiol (Ovocyclin = Depotoestradiol, Ciba, Basle) treated animals and 3 control animals treated with saline were investigated at each point in time as indicated in figure 1. Exactly 4 weeks after ovariectomy the animals in the treated group received a single i.m. injection of 2.5 mcg/g b.wt oestradiol.

The animals in the control group were given 0.3 ml of a physiological saline solution. 1 h before sacrifice the animals received a single i.v. injection of 1 µCi/g b.wt of ³H-thymidine. Immediately following sacrifice the uteri and adrenal glands

were removed and their wet weight determined. The adrenal glands were fixed in Bouin's solution for 24 h. Subsequently they were embedded in methacrylate. The histological sections which were then prepared were 4 µm thick and were stripped with AR 10 Kodak film. The exposure lasted 30 days. For the analysis of the histoautoradiograms 3000 cells were randomly selected from each of the 3 cortical zones (z. glomerulosa, z. fasciculata, z. reticularis) from both adrenal glands. The ³H-thymidine labelled cell nuclei and mitoses were identified. The results obtained formed the basis for the calculation of the ³H-

index (number of labelled nuclei per 1000 nuclei) and the mitotic rate (number of mitoses per 1000 nuclei).

Results. The effect of oestradiol administered to a castrated female rat can be seen from the state of the uterus⁸⁻¹⁰. Like these authors we observed a continuous increase in the weight of the uterus (fig. 1). After 14 h the weight of the uteri in the treated group was significantly greater than that of the control group. This increase had not yet reached a maximum by the time scheduled for ending the investigation, namely 122 h. In contrast, the ovarian hormone had no influence whatsoever on the weight of the adrenal glands.

In this study the action of oestradiol lasted between 3 and 122 h. Particular attention was given to the investigation of the kinetics of proliferation in the zones of the adrenal cortex following ovariectomy and a single administration of ovarian hormone in relation to time. An unequivocal result was observed only in the zona glomerulosa. About 14 h after the administration of oestradiol an increase in the DNA synthesizing cells began to take place in this zone and this reached a maximum at 38 h. Thereafter a decrease in the DNA synthesis occurred. At the end of the investigation the values in the treated animals were the same as those obtained in the controls (fig. 2). An almost comparable influence was observed on the rate of mitosis in the cells of the zona glomerulosa. No significant changes in the ³H-Index or in the Mitotic Rate were found in the zonae fasciculata and reticularis.

In order to clarify these results, further studies are in progress to determine whether the changes observed are the result of a direct effect following the administration of ovarian hormone or whether they are exerted via the anterior lobe of the pituitary gland.

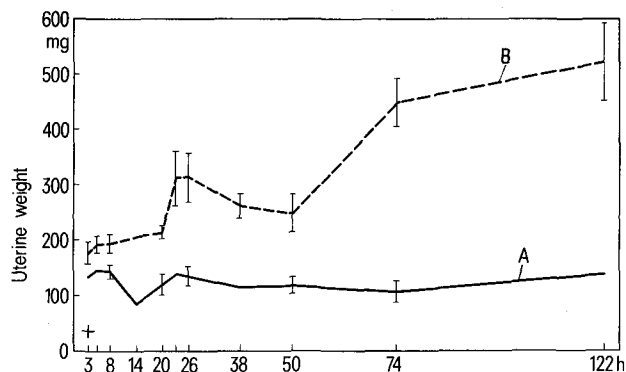


Figure 1. Time dependent increase in uterine weight; A, control group; B, oestradiol group.

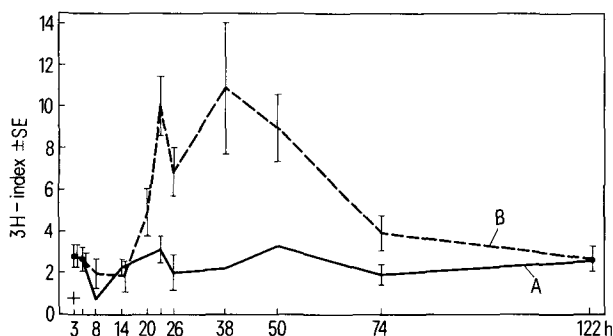


Figure 2. DNA-synthesis in the cells of the zona glomerulosa of the adrenal gland of female rats after ovariectomy and application of oestradiol; A, control group; B, oestradiol group

- 1 Machemer, R., and Oehlert, W., *Endokrinologie* 46 (1964) 77.
- 2 Stöcker, E., Kabus, K., and Dhom, G., *Z. Zellforsch.* 65 (1965) 206.
- 3 Stöcker, E., Kabus, K., and Dhom, G., *Z. Zellforsch.* 72 (1966) 1.
- 4 Hunt, T.E., and Hund, E.A., *Anat. Rec.* 156 (1966) 361.
- 5 Ueberberg, H., Stöcker, E., Städtler, F., *Virchows Arch. B Zellpath.* 6 (1970) 97.
- 6 Pappritz, G., Trieb, G., and Dhom, G., *Z. Zellforsch.* 126 (1972) 421.
- 7 Pappritz, G., Keazor, H., and Ueberberg, H., *Cell Tissue Res.* 184 (1977) 269.
- 8 Craudall, D., *Anat. Rec.* 72 (1938) 195.
- 9 Barks, O.L., and Overholser, M.D., *Anat. Rec.* 70 (1938) 401.
- 10 Nemetschek-Gansler, H., *Virchows Arch. path. Anat. Physiol.* 343 (1968) 197.

0014-4754/84/121384-02\$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1984

Ultrastructural changes in uterine myometrium of mice with experimentally-induced adenomyosis¹

T. Mori, Y. Ohta and H. Nagasawa

Zoological Institute, Faculty of Science, University of Tokyo, Bunkyo-ku, Tokyo (Japan), Department of Biology, School of General Education, Tottori University, Koyama, Tottori (Japan), and Experimental Animal Research Laboratory, Meiji University, Tama-ku, Kawasaki (Japan), 1 December 1983

Summary. Ectopic pituitary transplantation induced a high incidence of adenomyosis in SHN mice. Early signs of the development of adenomyosis were the penetration of stromal connective tissue into myometrium followed by uterine gland invasion. Associated with these changes, the inner layer of myometrium showed the involution of smooth muscle cells and distended intercellular spaces.

Key words. Mouse, uterine myometrium; uterine myometrium, mouse; adenomyosis; ectopic pituitary transplantation; prolactin.