ANNALS OF GASTROENTEROLOGY 2004, 17(4):385-389

Original article

The impact of corticosteroids on hepatocytes ultrastructure – experimental study in rats with electron microscopy

D. Kapatsoris[1], Ch. Pilichos[1], Athina Preza[2], I. Kounavis[3], C. Fotiadis[2]

SUMMARY

Background and Aim: Corticosterstoids are drugs widely used in common clinical practice.Their effect on liver morphology and function is not fully elucidated.The aim of this expenmental study is to investigate the impact of cortisol on fasted rat hepatocytes, by means of electron microscopy.

Materials and Methods: Fifty male Wistar rats weighing 130-150gr were divided into two experimental groups: Group I (N=30): Hydrocortisol 8mg/100gr i.p. and Group II (N-20): Normal saline i.p. Animals were sacrificed 30 min and 180 min after injection and liver specimens were taken. According to OsO_4 -fixation protocol, ultra fine tissue sections were obtained and stained for electron microscopy.

Results: The following findings were observed in >90% of cortisol treated animals and were significantly more frequent than in control group (P<0.05). A. At 30 min: Decondensation of chromatin fibers was noticed, whereas nuclear envelope and nucleoli remained almost intact. Endoplasmic reticulum increased in size and complexity, as well as the number of mitochondria. The number of lysosomes decreased. B. At 180 min: Chromatin remained fully decondensated. Large nucleoli were apparent within the nucleus. The external membrane of the nuclear envelope was devoid of ribosomes. Concerning all others cytoplasmic organelles, no differences were noticed between different groups.

Conclusions: 1. Even late after cortisol administration, liver parenchymal cells do not enter mitosis. Thus, a direct effect of cortisol on hepatic regeneration is highly improbable. 2. Cortisol increases protein synthetic activity of hepatocytes, by means of both chromatin decondensation and r-RNA nucleolar production. 3. Increased cell metabolism is assisted by adequate adaptation of intracellular energy-transducing systems.

[1] 1st Department of Internal medicine, Sismanoglion Hospital, Athens, Greece, [2] 3rd Surgical Department of the Athens University, Attikon Hospital, Athens, Greece, [3] Department of Cytology, Mamatsion General Hospital, Kozani, Greece

Author for correspondence:

Dr. Charalampos Pilichos, Eufroniou 34 -16121- Athens, Greece, Tel-Fax: +30 210 7224992, e-mail: hpilichos@hotmail.com

INTRODUCTION

Corticosteroids are drugs widely used in clinical practice, mainly for their immune modulating properties. Although to date the effect of corticosteroids on liver histology has been adequately studied, the variations in ultrastructure of hepatocytes are not fully elucidated. Current knowledge mainly relles on observations in adrenalectomized or fasted animals and concerns changes in the morphology of the endoplasmic reticulum and glycogen deposits.[1,2] Nevertheless, the impact of exogenously administered corticosteroids on fasted animals might be of particular interest because ultrastructural changes possibly affect liver cell functionality, in different ways.

This experimental study aims to investigate the impact of cortisol on rat hepatocytes morphology and function, by means of electron microscopy. Emphasis has been given to variations concerning particular organelles, such as nucleus, endoplasmic reticulum, mitochondria and lysosomes (Figures 1, 2, 3).

MATERIALS AND METHODS

Animals and experimental groups

Fifty male Wistar rats weighing 130-150gr were used in our experiments. They were maintained under standard conditions of temperature and lighting (25°C 12 h

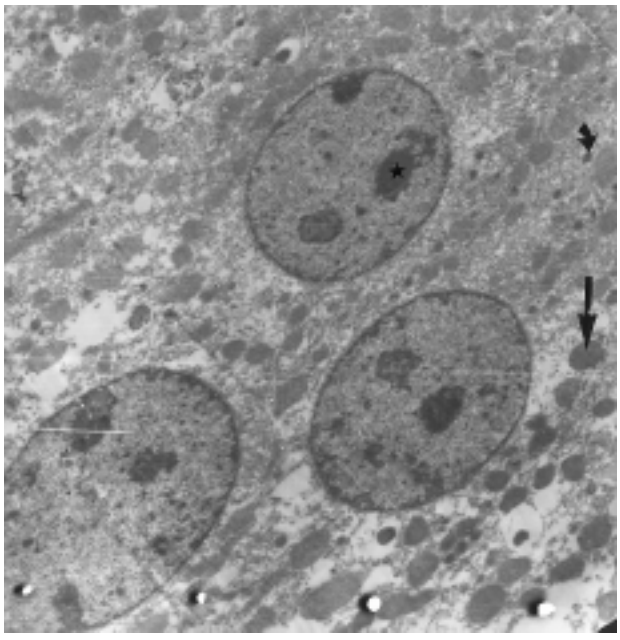

Figure 1. Control group: Three nucleus with nucleoli (*) mitochondria (arrow) and lysosomes (small arrow) x 20.000.

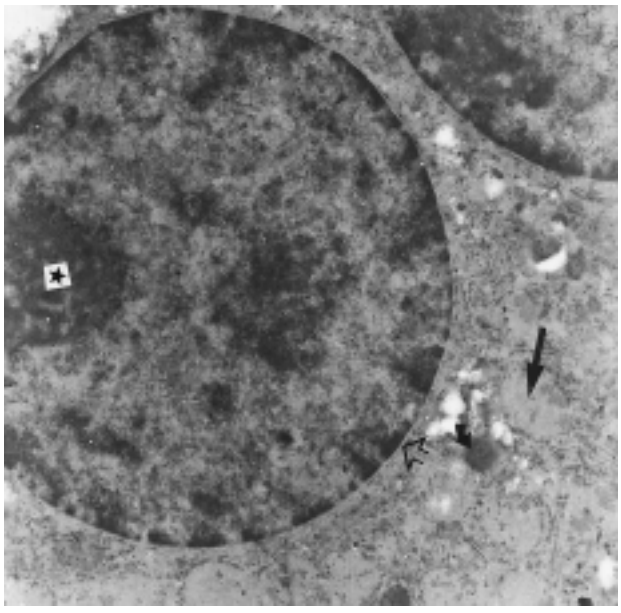

Figure 2. Control group: Two nucleus with nucleoli (*) mitichondria (arrow), lysosomes (small arrow) and nuclear membrane (small linear arrow) x 35.000.

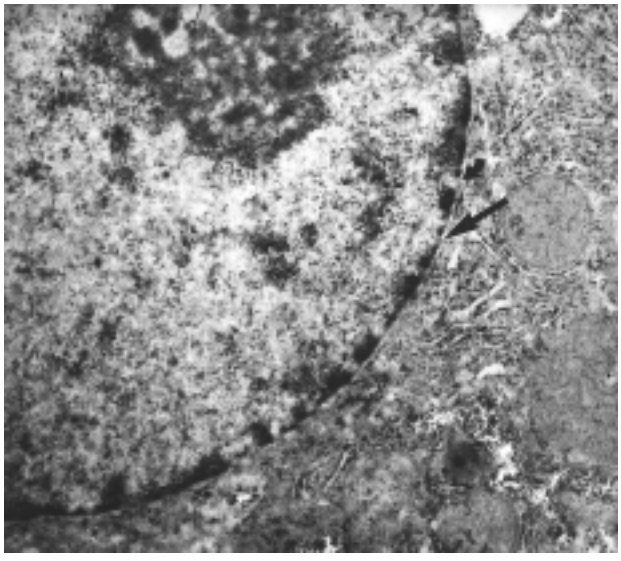

Figure 3. Control group: Normal morphology of mitochondria, endoplasmic reticulum, nuclear membrane (arrow) chromatin and nucleoli x 35.000.

daily respectively), had free access to water and were fasted during 48 hours in order to achieve complete glucogen depletion.

Accordingly to their treatment, animals were devided into two experimental groups: **Group I** (N=30): Hydrocortisol 8mg/100gr of body weight intraperitoneally, and **Group II** (N=20): Controls, which were given 2 ml of normal saline intraperitoneally. The selection of the dose of 8 mg/kg of body weight was chosen at random, so, based on the results, we would be able to continue our study with a variety of dosages and observe the impact on liver tissue. Under general anesthesia (ketamine and midazolame), the rats were sacrificed at 30 and 180 min. At this timing we believed we would have findings from the study of the hepatic tissue due to rapid activity of the corticosteroids of the intraperitoneal infusion.

Treatment of liver tissue

The whole of the hepatic tissue was prepared for observation under the electron and simple optical microscope. From this tissue random samples were selected for electron and simple microscopy. Both periportal and perivenous hepatocytes were included in our sample. Fixation for 4 hrs in cacodylate-buffered glutaraldehyde a 0-4˚ C was followed by washing for 24 hrs in a cacodylate/sucrose wash and then by post fixation with osmium tetroxide, dehydration in ethanol 50% for 10 min, 70% for 20 min in 2% acetic uranyl, 85% for 10 min 95% for 10 min, 100% in 10 min and 100% in 10 in room temperature, and embedment in Epon 812. Thin sections were cut on Sorvall MT-1 Porter-Blum ultramicrotome with diamond knives and subsequently examined in a Philips EM 300 electron microscope, at an accelerating voltage of 80kV. Additionally, a slice from each liver lobe was

fixed in 10%buffered formalin for routine histological .examination. Paraffin sections were stained with hematoxylin-eosin and by the periodic acid-Schiff technique. An experienced cytologist, who was unaware of clinical features, examined tissue specimens and cell micrographs.

DISCUSSION

In previous studies, it has been well established that liver parenchyma is a tissue-target for corticosteroids. The hormones seem to bind to a chromatin receptor resulting in an increase of the cellular metabolic activity, which is dependent on the dosage, the timing after cortisol administration and the animal model.[3,4]

Our work clearly demonstrates an induction of hepatocyte functionality early (30 min) after hydrocortisol administration, as expressed by the morphology of decondensated chromatin within the nucleus. The increase in nucleoli volume seems related to an increased r-RNA (external nuclear membranes are devoid of ribosomes, which form aggregates -polyribosomes- in the cisternae of the rough endoplasmic reticulum) and subsequently protein synthesis, requiring energy production by an adequate number of mitochondria. Thus a cellular adaptation is made in order to support the increased cellular functionality. However, even late after cortisol administration, liver parenchymal cells do not enter mitosis. Thus, a direct effect of cortisol on hepatic regeneration is highly improbable. The observation that, with a simple optical microscope no substantial microscopical alterations were recognized leads us to believe that in the chosen times, dosages and route of administration of corticosteroids only ultramicroscopic alterations of the hepatic tissue can be recognized.

Our results are in corcondance, but appear in a briefer tome spectrum (30 min), with the original previous work of Nash et al, who, in a similar animal model, observed, 1 hour after radiolabelled hydrocortisol administration, a significant increase in perichromatin fibers, and of Young et al, who reported a twofold increase in m-RNA synthesis.

The results we obtained from this study mainly refer to protein synthesis of the liver parenchyma cells. However, as other investigators have found that in other cell populations corticosteroids suppress protein synthetic activity in a notable proportion,[8,9] we may conclude a differential action of corticosteroids in different tissues, which should be further elucidated.

RESULTS

The study of the paraffin sections after the hematoxylin-eosin dye of both experimental groups after 30 as

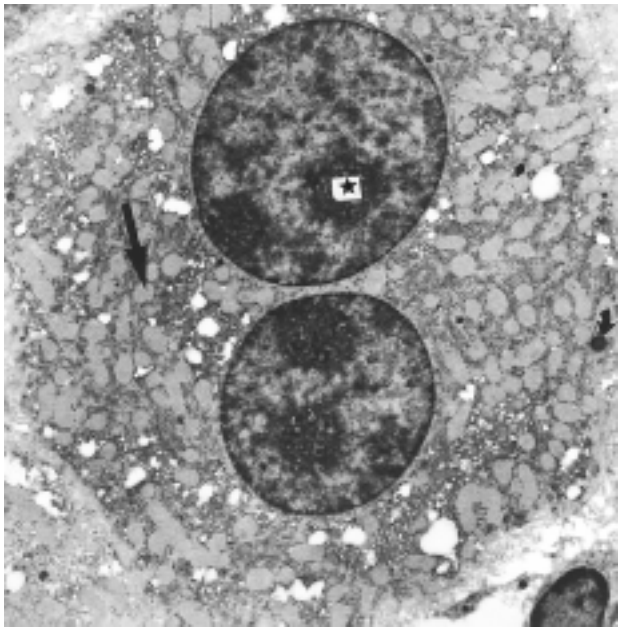

Figure 4. 30 min following cortisol administration: Two nucleus with nucleoli (*), mitochondria (arrow) and lysosomes (small arrow) x 20.000.

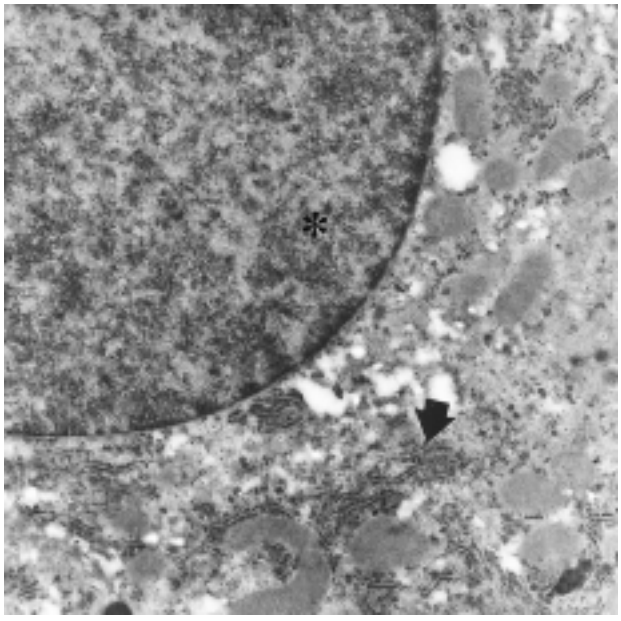

Figure 5. 30 min following cortisol administration diffusion of chromatin within the nucleus (*). In cytoplasm mitochonria and increased granular endoplasmic reticulum x 35.000.

well as 180 min showed no substantial microscopical alterations and/or deviations from normal.

A. At 30 min: A significant decondensation of chromatin fibers was noticed in cortisol treated hepatocytes, whereas the nuclear envelope and the nucleoli remained almost intact (Figure 4). Endoplasmic reticulum increased in size and complexity (Figure 5), arranged in parallel array of cisternae well as the number of mitochondria. The number of lysosomes slightly decreased (Figure 6). No other alterations were observed.

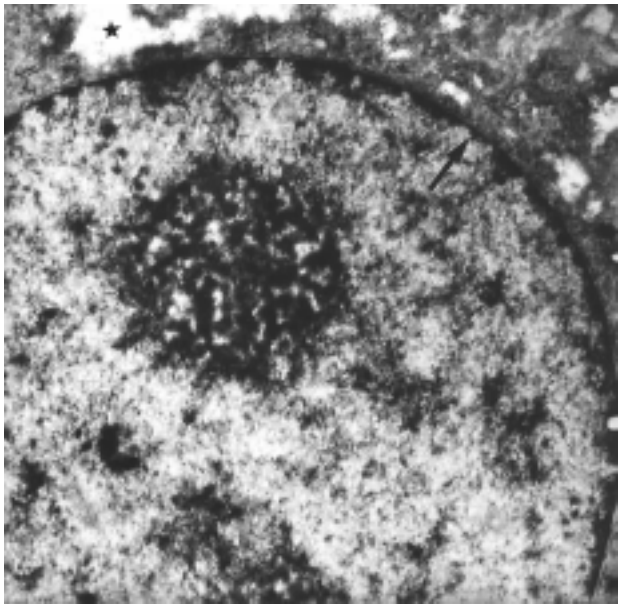

Figure 6. 30 min following cortisol administration nucleolus, nuclear membrane and empty cytoplasmic spaces x 40.000.

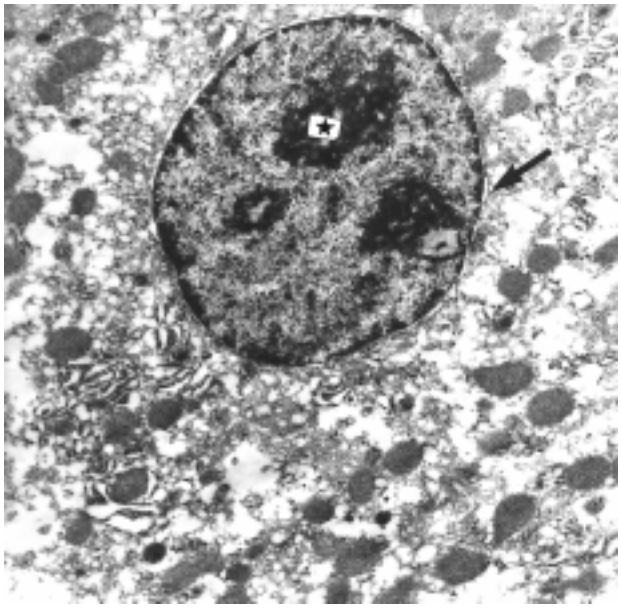

Figure 7. 180 min following cortisol administration with nucleolus (*) and nuclear membrane (arrow) x 20.000.

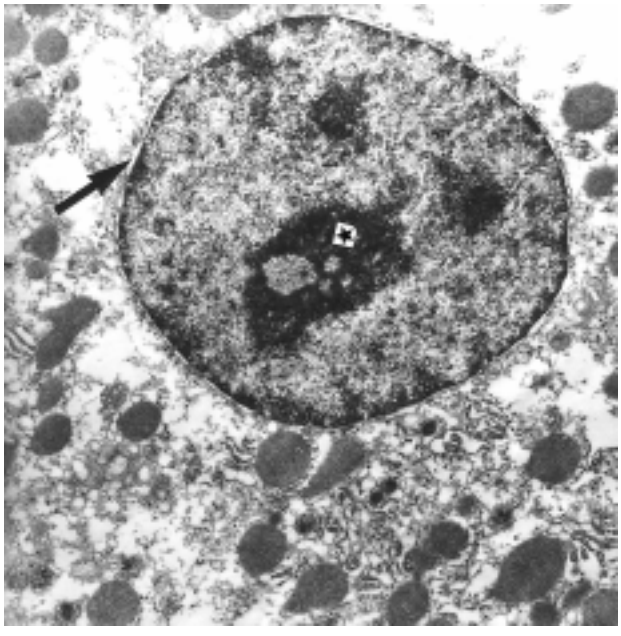

Figure 8. 180 min following cortisol administration: Nucleus with nucleolus (*) and nuclear membrane (arrow) x 20.000.

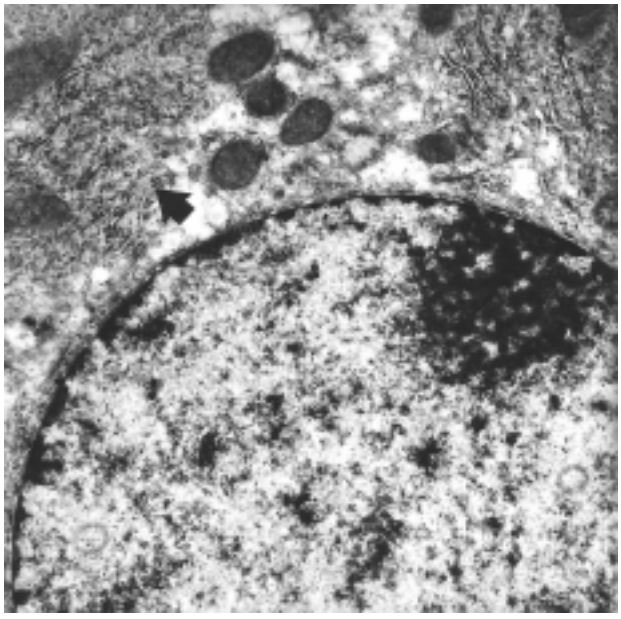

Figure 9. 180 min following cortisol administration: Chromatin, nucleoli, nuclear membrane and prominent granular endoplasmic reticulum (arrow) x 35.000.

B. At 180 min: Chromatin remained fully decondensated. Large nucleoli were apparent within the nucleus (Figure 7) and the external membrane of the nuclear envelope (Figure 8) was devoid of ribosomes, in cortisol treated cells. Concerning all others cytoplasmic organelles and particularly the rough endoplasmic reticulum, no differences were noticed between different groups (Figures 9, 10).

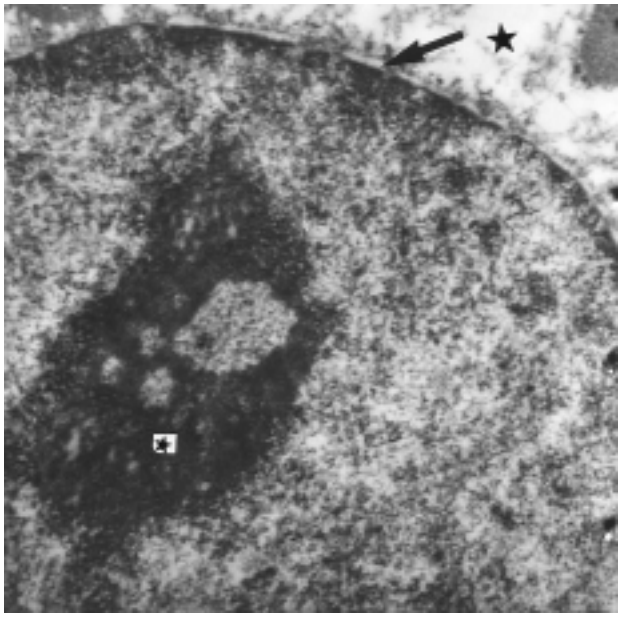

Figure 10. 180 min following cortisol administration: Nucleolus (*), nuclear membrane (arrow) and empty cytoplasmic spaces x 40.000.

REFERENCES

1. Winton DJ, Flaks B. Fine structural alterations induced in the rat hepatocyte by adrenalectomy: influence of deoxycorticosterone acetate or fasting. Exp Path 1987; 68:475-484.
2. Babcock MB, Cardell RR. Fine structure of hepatocyte from fasted and fed rats. Am J Anat 1975; 143:399-438
3. Baxter JD, Thomkin GM. Specific cytoplasmic glucocorticoid hormone receptors in hepatoma tissue culture. PNAS 1971; 68:932
4. Clark J, Peck EZ. Nuclear retention of receptor-estrogen complex and nuclear acceptor sites. Nature 1976; 260:635-6.
5. Nash E, Puvion E, Bernhard W. Perichromatin fibrils as component of rapidly labeled extranucleoral RNA. J Ultr Res 1973; 53:395-405.
6. Young H, Scolkick E, Parks W. Glucocorticoid receptor interaction and induction of murine mammary tumor virus. J Biol chem. 1975; 250:3337-3339.
7. Beck CA, Weigel NL. Effects of hormone and cellular modulators of protein phosphorylation on transcriptional activity, DNA binding and phosphorylation of human progesterone receptors. Mol Endocrinol 1992; 6:607-620.
8. Bajwa W, Birnie GD. Changes induced in rat liver polysomal polyadenylated RNAs by depletion of circulating glucocorticoids. Nucleic acid Res 1982; 10:3541-3560.
9. Abraham A, Sekeris C, et al. Inhibitory action of steroid hormones on RNA synthesis of isolated thymus nuclei. Acta Biochem Biophys 1971; 247:562-5.
10. Guerrieri F, Pellecchia G, Lopriore B, et al. Changes in ultrastructure and the occurrence of permeability transition in mitochondria during rat liver regeneration. Eur J biochem 2002; 269:3304-3312
11. Cuezva JM, Krajewska M, De Heredia ML, et al. The bioenergetic signature of cancer. Cancer Res 2002; 62:6674-6681.
12. Clauvert AM. (1983-1980) practical Methods in electron microscopy. Volumes1-8, Elsevier.