

Exocrine Pancreas under the Effect of Glucocorticoids: Histological and Morphometry Study

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Abstract

- Background** The histological changes obtained from the effect of glucocorticoids on exocrine pancreas is well known but little is known about the morphometrical changes on the acinar cells and ducts which are associated with the histological findings.
- Objective** To reveal these morphometrical changes which are associated with the histological changes occurred on the exocrine pancreas by the use of different doses and durations of dexamethasone.
- Methods** Healthy female rabbits were used and divided to six groups. The first four groups regarded as the treated groups, they received different doses and different durations of dexamethasone sodium phosphate. The fifth and sixth group considered as the control group. The pancreas obtained from these animals were processed to make a paraffin blocks, the slides obtained were stained by H&E stain, and Masson's acid Fuchin Aniline blue Trichrome stain (MT). Diameter of serous acini and intercalated ducts were measured, height of acinar cells and intercalated duct cells and counting of serous acini number in a unit area were estimated.
- Results** Histologically, by H&E, degenerative changes of serous acinar cells were evident which were dose and duration related. By MT stain there was decrease in collagen fibers in the interlobular spaces. Morphometrically, the diameter of serous acini, height of acinar cell and glandular density were decreased when the dose and duration of treatment were increased.
- Conclusion** There was increase in exhausted acinar cells as compared to metabolically active cells when the dose and duration of dexamethasone sodium phosphate treatment were increased.
- Key words** Exocrine pancreas, glucocorticoids and morphometry.

Introduction

Many previous studies showed that the glucocorticoid affects the cells of various organs of body⁽¹⁾. Glucocorticoids mediate their effects through a specific intracellular receptors present in almost all cell types including exocrine pancreatic tissues⁽²⁾. So the glucocorticoids can induce cell maturation, cell differentiation or even cell death (apoptosis)⁽³⁾. Therefore glucocorticoid acts directly on pancreatic acinar cells⁽⁴⁾. These are accomplished by their effects on increasing

amylase and trypsin activities which depend on the dose and duration of steroid treatment⁽⁵⁻⁷⁾. The histological effects of glucocorticoids on exocrine pancreas were demonstrated by some workers⁽⁸⁾ as enlarged lumina of the acini, zymogen depletion of acinar cells, and abundance of cytoplasmic vacuoles with deformation and distortion of the nucleus and cytoplasm. But others suggested that hydrocortisone- treated animals showed a higher density of zymogen granules in the acinar cells and an increased number of autophagic vacuoles in the pancreatic acinar cells⁽⁹⁾.

The glucocorticoids had a dual effects on exocrine pancreas so that glucocorticoids in appropriate doses has a stimulating effect upon the acinar cell function, whereas large doses of these drugs reduce the exocrine tissue secretory function and affecting its protein-synthesizing capacity, also the corticosteroid deficiency is accompanied with morphofunctional atrophy of the exocrine region of the pancreas⁽¹⁰⁾. In contrast; Other workers demonstrated this dual effects of glucocorticoids on the rat pancreas: inhibition and potentiation by that chronic treatment with large doses of glucocorticoid may sensitize the acinar cells and induce hypersecretion of trypsin and lipase, whereas acute treatment inhibit the secretory function of exocrine pancreas⁽¹¹⁾. Puccio *et al.*⁽¹²⁾ observed that hydrocortisone induced pancreatic hypertrophy and significantly increased enzymatic activities independent to the dose used, but with high dose, there was a diminution of both cell size and number of zymogen granules associated with increased excretion of secretory products, in contrast to that, the low dose did not modify acinar cell size and they caused a significant increase of the number of granules per cell. They concluded that hydrocortisone was an important modulator of pancreatic development in the rat by inducing stimulation of pancreatic activities associated with modifications in cell structure and components and this varied according to the dose.

The glucocorticoids are required for the maintenance of the structural integrity of the exocrine pancreas, in addition to estrogens and other factors from adrenals or testes. So that the exocrine pancreas of the castrated-adrenalectomized rats showed changes in the shape of the acini with widening of intralobular and interlobular spaces, partial depletion of zymogen granules and reduction of acinar lumen size. However, by giving glucocorticoids, the histological findings were changed and they concluded there was enlargement of lumen of the acini, increasing in zymogen granules content of acinar cells, so the glucocorticoids are

one of the important factors for the structural integrity of the exocrine pancreas⁽¹³⁾.

This study aims to evaluate the effect of dexamethasone on the rabbit's exocrine pancreas histologically and morphometrically.

Methods

Histology:

Healthy White New Zealand female rabbits weighing between 1000-1250 g were used and kept in separated plastic cages and fed *ad-libitum*. The animals were divided into five groups, seven animals in each. The first group was treated daily for 10 days with (0.5 mg/kg b.w.) intramuscular injection of dexamethasone sodium phosphate (ZMC import- export GmbH Germany) in the thigh muscle. The second group treated with (1.5 mg/kg b.w.) of the same reagent for 10 days, third group received (0.5 mg/kg b.w.) of dexamthasone for 15 days, the fourth one treated with (1.5 mg/kg b.w.) of dexamethasone sodium phosphate for 15 days, the fifth group considered as control animals (1), they received equal amounts of 0.9% saline solution as intramuscular injections for 10 days. Sixth group of control animals (2) also were received intramuscular injections of 0.9% saline solution for 15 days.

Twenty-four hours after the last injection, the animals were anaesthetized with chloroform. After dissection of the abdomen, the pancreas were removed and the glands were fixed in 10% formaline solution for 24 hrs., dehydrated, cleared, embedded in paraffin and the blocks obtained were sectioned and stained by Haematoxylline and Eosin stain (H&E), and Masson's acid Fuchin Aniline blue Trichrome stain (MT).

Staining methods and techniques were done on the basis of Luna⁽¹⁴⁾.

Morphometry:

On the stained sections, the mean diameter of the serous acini and intercalated ducts with the height of both acinar cells and intercalated duct cells, and the number of the serous acini in a unit area were determined using the Visopan

Projection Microscope. In determination of these parameters a 500 X magnification, which is achieved with the 40/0.65 objective, was used, each division of the measuring ruler of the microscope corresponds to a length of $2 \mu\text{m} = 0.002 \text{ mm}$. the unit area in counting of cells was an area of 0.0144 mm^2 . After completion of measurement operation, statistical analysis was done between treated groups and the control one using unpaired T-test ⁽¹⁵⁾.

Results

Histological changes:

The histological findings of all the treated animals showed preservation of the exocrine pancreatic architecture, however the histological changes which were detected differed from one animal to another and even from one pancreatic lobule to another within the same animal.

The following histological changes were observed in both H&E and Masson's Trichrome (MT) sections in the treated groups as compared to control one (Figures 1 and 2).

1. Distortion in the arrangement of the acinar cells and ill defined cellular outlines in many areas of the gland, although they were preserved in other areas (Figure 3).
2. There were vacuoles in the cytoplasm of the acinar cells in many areas which were sometimes very large and differ in their size and location (Figure 3).
3. Deformed nucleus and cytoplasm of acinar cells were seen (Figure 3).
4. Evidence of zymogen depletion was observed in many areas, although some areas still preserved little zymogen granules (Figure 3).
5. In most acinar cells, there were irregular nuclei, but in some cells a rounded nuclei with prominent nucleoli were also observed (Figure 3).
6. There were proliferated duct cells in many ductal structures (Figure 4). These changes were seen in all treated groups and started to become to more extent as the dose and duration of treatment were increased starting from the first to the fourth group.

7. Shrinkage of the acinar cells with deformed and pyknotic nuclei (Figure 5). These features start to appear from the second group and become more evident in the third and fourth group i.e. as the dose and duration of treatment were increased.
8. Masson's Trichrome sections shows that the collagen fibers in the interlobular stroma of exocrine pancreas of treated animals were much less than that of control group (Figures 2 and 6).

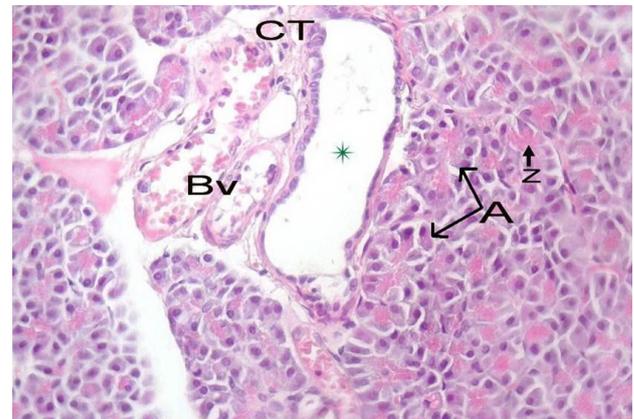


Figure 1. Light micrograph of the control rabbit exocrine pancreas showing the serous acini (A) containing zymogen granules (Z) and the interlobular duct (*), blood vessels (Bv) and connective tissue (CT). (H&E X 400)

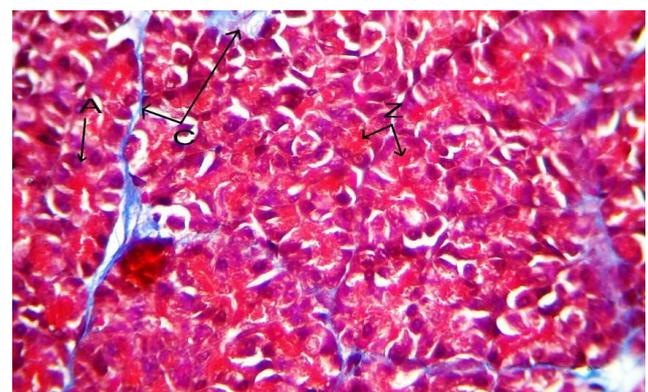


Figure 2. Light micrograph of the control rabbit exocrine pancreas showing the serous acini (A) containing zymogen granules (Z) and the collagen fibers separating the lobules (C). (MT X 400)

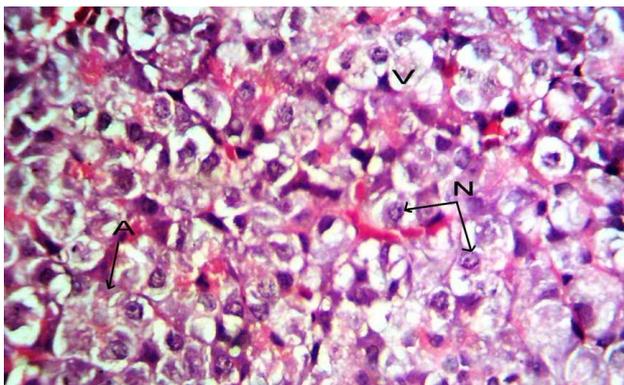


Figure 3. Light micrograph of the treated rabbit exocrine pancreas (group 1, 2, 3 and 4) showing distortion in the arrangement of acinar cells (A) with vacuolation (V) and depleted zymogen, deformed nuclei with appearance of prominent nucleolus in some areas (N). (H & E X 600)

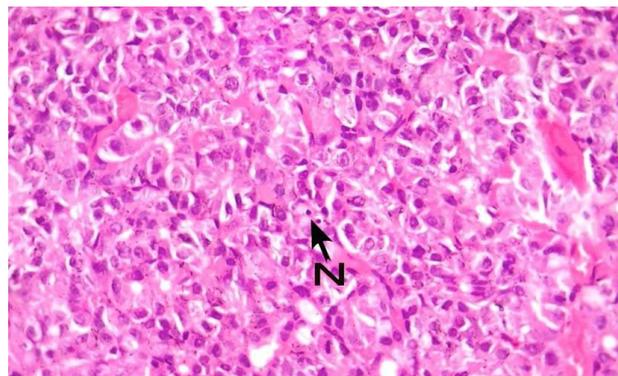


Figure 5. Light micrograph of treated rabbit exocrine pancreas (group 2, 3 and 4) showing pyknotic nuclei (N) with shrinkage of their acinar cell. (H & E X 400)

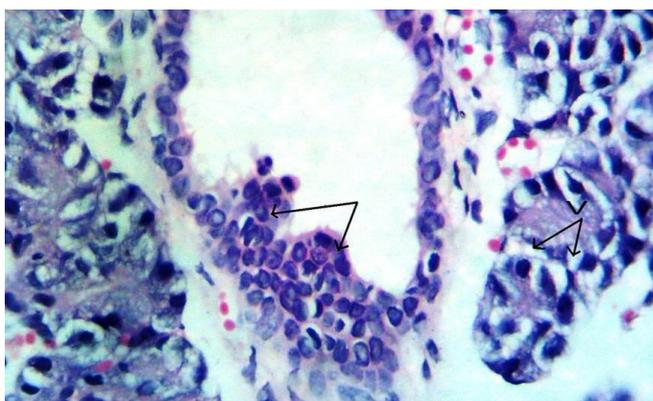


Figure 4. Light micrograph of treated rabbit excretory duct (group 1, 2, 3 and 4) showing proliferated duct cells (→), serous acini with vacuolation (V). (H & E X 600)

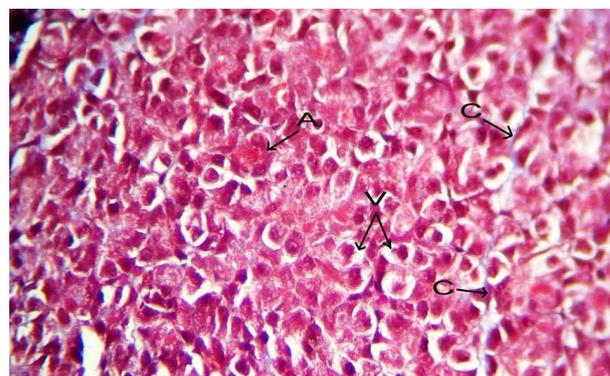


Figure 6. Light micrograph of the treated rabbit exocrine pancreas (group 1, 2, 3 and 4) showing distortion in the arrangement of acinar cells (A), vacuolation (V), deformed cytoplasm and nuclei, depleted zymogen associated with decrease in collagen fibers in between the lobules (C). (MT X 400)

Morphometrical changes:

The following changes were seen and presented in table 1 as numerical data:

1. The diameter of serous acini started to decrease as the dose and duration of dexamethasone treatment were increased and, that change appeared statistically significant in all treated groups.
2. The height of acinar cells also decreased starting from the first treated group to the

fourth one (as the dose and duration of treatment increased).

3. The diameter of intercalated ducts remained near the control value.
4. The height of intercalated duct cells also was not changed.
5. The number of serous acini in a unit area (glandular density) was decreased as the dose and duration of dexamethasone treatment were increased.

Table 1. Morphometrical analysis of exocrine pancreas

Groups	Serous acini		Intercalated ducts		Glandular density (no. of acini)
	Diameter	Height	Diameter	Height	
first group (10 low)	25.23 ± 0.9*	15.5 ± 0.7*	20.3 ± 1.3	5.1 ± 0.3	22.6 ± 0.6
Second group (10 high)	19.45 ± 0.9**	12.0 ± 0.7**	20.1 ± 0.9	5.1 ± 0.3	19.3 ± 0.5**
Third group (15 low)	21.46 ± 0.6**	13.44 ± 0.8**	20.2 ± 1.2	5.2 ± 0.3	20.0 ± 0.5**
Fourth group (15 high)	16.71 ± 0.7**	9.63 ± 0.6**	20.1 ± 0.6	5.1 ± 0.3	17.2 ± 0.7**
Fifth group (Control(1))	29.25 ± 1.2	18.38 ± 1.1	20.5 ± 1.6	5.2 ± 0.3	23.6 ± 0.8
Sixth group (control(2))	29.20 ± 1.1	18.30 ± 1.1	20.4 ± 1.7	5.3 ± 0.4	23.5 ± 0.9

* Significant difference from control at $P \leq 0.05$, ** at $P \leq 0.001$

Discussion

Two types of cellular responses to glucocorticoid were observed. Some cells showed an increase in their metabolic activity as judged by the rounded nuclei with enlarged prominent nucleoli and zymogen depletion. At the same time in these metabolically active cells, there was a beginning of degenerative processes at the cellular level. These degenerative changes seen, indicated that, the cells ceased the protein synthesis and are in the process of becoming exhausted or non functional and therefore led to increase cellular turn over, which improved morphometrically by decrease in the diameter of serous acini and decrease in the height of acinar cells since the reduction in the zymogen production (decrease protein synthesis and loss of zymogen granules) which occurred made the apical area of acinar cells depleted from zymogen and shrinkage occur, therefore, decreased the height of cells and in turn the diameter of acini⁽¹⁶⁻¹⁸⁾. These findings were in agreement with the results obtained by Finkelbrand *et al*⁽⁸⁾ who divided these cells into two types, dark cells mean the metabolically active cells and the light cells, which are the cells, ceased their protein synthesis and become exhausted.

The results were dose and time dependent, i.e. when the period of treatment was elongated and the dose administered was increased, the exhausted cells or non-functional cells were increased and the metabolically active cells decreased and even rarely seen, as well as morphometrically there was a decrease in the glandular density (number of serous acini in unit area) which confirmed that the exhausted cells

were increased and the active cells decreased as the dose and duration of dexamethasone given increased.

The depleted zymogen granules observed in this study were due to excretion of the secretory products of the cells by the effect of glucocorticoids⁽¹²⁾. These findings would emphasize the explanation that the glucocorticoid increases the secretion of enzymes in the pancreatic acinar cells^(6,19). However, the chronic use of glucocorticoid produces blockade on enzyme excretion⁽²⁰⁾. The results also suggested that the zymogen depletion as well as the other signs of acinar cell stimulation might be accompanied by an increased amylase activity. However, when the acinar cells became exhausted and ceased protein synthesis, a decrease in zymogen granule contents of acinar cells may be occurred associated with a decrease in the height of these cells^(8,16,17,21).

The appearance of nuclei with prominent nucleoli was indicative of increased metabolic activity of acinar cells⁽⁸⁾. But in other cells of group 2, 3 and 4, the pyknotic nuclei appeared with shrinkage of acinar cells occurred associated with decrease diameter of serous acini and low height of acinar cells. These changes in the nuclei were indicative of degenerative changes⁽²²⁾.

The presence of vacuoles that differed in their size and locations in the cytoplasm of the treated acinar cells was due to local cellular degeneration^(23,24).

The irregular cell outlines observed were indicated the degenerative changes occurred in the acinar cells which would end by cell lysis⁽⁸⁾.

In general, the degenerated cytoplasm of acinar cells was seen in this study also described by many workers^(22,25).

Glucocorticoids stimulate the synthesis of pancreas-specific proteins, most probably via a cytoplasmic-nuclear receptor system^(26,27). These corticosteroids appear to either promote specific RNA accumulation or enhance the transcription of RNAs from gene loci intimately related with pancreas-specific proteins^(4,28-32). Thus, glucocorticoids seem to play an important role in the maintenance of a proper level of exocrine enzymes in the differentiating embryonic pancreas⁽³³⁻³⁵⁾ as well as in the mature gland⁽¹³⁾. It should, however, be emphasized that only very low concentrations of corticosteroids (10^{-8} - 10^{-6} M) are needed for their regulatory effect in the intact animal⁽⁸⁾. In contrast, higher concentrations more than that level of these hormones appear to adversely affect the synthetic activity of the pancreatic acinar cells which occurred in this study.

Wellmann and Volk⁽²⁵⁾ describe the proliferated duct cells observed in this study, it is one of the mechanisms involved in the neogenesis of B cells of pancreas⁽³⁶⁾. Non significant changes were obtained by the measuring of the diameter of intercalated ducts and the height of duct cells since these structures have nothing to do with the synthesis of proteins which was suppressed in the acinar cells of this study⁽¹⁸⁾.

In the present study, there were changes in the intensity of staining with Masson Trichrome of the connective tissue because there was a decrease in collagen fibers in the interlobular spaces. This is because the glucocorticoids are regarded as a fibroblast growth inhibitor factor (anti-fibrotic agent)^(37,38).

So the glucocorticoids are important hormones for maintaining the structural integrity of the exocrine pancreas⁽¹³⁾, however, the effects of these glucocorticoids on the exocrine pancreatic structure and function are dose and duration dependent⁽³⁹⁾. Therefore, it is important to notice that an appropriate dose of glucocorticoid renders a stimulating effect upon the acinar cell function, whereas large doses of the glucocorticoid reduce the exocrine tissue secretory function and affect its protein-synthesizing capacity^(10,40).

In conclusion, acinar cells were shifted from metabolically active cells to exhausted cells (non-functional) and degenerative changes appeared as the dose and duration of treatment of glucocorticoid increased more than that needed by the body.

References

1. Davani B. Increased glucocorticoid sensitivity in pancreatic β - cells: Effects on glucose metabolism and insulin release. PhD Thesis. Kongl Carolinska Medico Chirurgiska Institutet. Sweden; 2003.
2. Matthes H, Kaiser A, Stier U, et al. Glucocorticoid receptor gene expression in the exocrine and endocrine rat pancreas. *Endocrinology*. 1994; 135(1): 476-79.
3. Longui CA. Glucocorticoid therapy: minimizing side effects. *J Pediatr (Rio J)*. 2007 Nov; 83(5 Suppl): S163-77.
4. Logsdon CD, Moessner J, Williams JA, Goldfine ID. Glucocorticoids increase amylase mRNA levels, secretory organelles, and secretion in pancreatic acinar AR42J cells. *J Cell Biol*. 1985; 100: 1200-8.
5. Manso MA, Garcia-Montero AC, Rodriguez AI, et al. Hydrocortisone induce an increase of amylase content in individual zymogen granules from rat pancreas. *J Steroid Biochem Mol Biol*. 1997; 62(5-6): 439-48.
6. Swarovsky B, Steinhilber W, Scheele GA, Kern HF. Coupled induction of exocrine proteins and intracellular compartments involved in the secretory pathway in AR4-2J cells by glucocorticoids. *Eur J Cell Biol*. 1988; 47(1): 101-11.
7. Takeuchi T, Ogawa M, Sugimura T. Effects of various hormones and adrenalectomy on the levels of amylase in rat pancreas and parotid gland. *Experientia*. 1977; 15: 33(11): 1531-2.
8. Finkelbrand S, Coleman R, Silberman M. The exocrine pancreas in triamcinolone-treated mice. *Acta Anat*. 1978; 102: 348-57.
9. Gartemann H, Zeitz M, Emde C, et al. Three-day continuous drainage of pancreatic juice in the cortisone-treated conscious rat: analysis of enzyme activities and ultrastructural changes. *Cell Tissue Res*. 1985; 242(1): 191-6.
10. Mozheiko LA. Structuro-metabolic aspects of the effect of the adrenal cortex on the pancreas. *Probl Endokrinol (Mosk)* 1983; 29(4): 81-6.
11. Otsuki M, Okabayashi Y, Ohki A, et al. Dual effects of hydrocortisone on exocrine rat pancreas. *Gastroenterology*. 1987; 93(6): 1398-403.
12. Puccio F, Chariot J, Lehy T. Influence of hydrocortisone on the development of pancreas in suckling rats. Ultrastructural morphometric and biochemical studies. *Biol Neonate* 1988; 54(1): 35-44.

13. Beaudoin AR, St-Jean P, Proul XJ, Grondin G. Influence of steroids on the exocrine pancreas: presence of laminated bodies in the acinar lumen following castration and adrenalectomy. *Pancreas*. 1989; 4(2): 219-27.
14. Luna LG. *Manual of histological methods*. 3rd ed. New York: McGraw-Hill Company, 1968.
15. Harris M, Taylor G. *Medical Statistic Made Easy*, Martin Dunitz Company, London UK, 2004; p. 9,6,24.
16. Bockman DE, Boydston WK, Anderson MC. Origin of tubular complexes in human chronic pancreatitis. *Am J Surg*. 1982; 144(2): 243-9.
17. Bockman DE, Guo J, Buchler P, et al. Origin and development of the precursor lesions in experimental pancreatic cancer in rats. *Lab Invest*. 2003; 83: 853-9.
18. Zarebska A, Krolikowska-Prasal J. Ultrastructural changes in the parotid serous cells of white male rats after experimental encortone. *Folia Morphol (Warsz)*. 1992; 51 (4): 299-311.
19. Kleene R, Zdziebło J, Wege K, Kern HF. A novel zymogen granule protein (ZG29P) and the nuclear protein MTA1P are differentially expressed by alternative transcription initiation in pancreatic acinar cells of the rat. *J Cell Sci*. 1999; 112: 2539-48.
20. Pescador R, Manso MA, Rebollo AJ, deDios I. Effect of chronic administration of hydrocortisone on the induction and evolution of acute pancreatitis induced by cerulein. *Pancreas*. 1995; 11(2): 165-172.
21. Schmid RM, Kloppel G, Adler G, Wagner M. Acinar-Ductal-Carcinoma Sequence in Transforming Growth Factor- α Transgenic Mice. *Ann NY Acad Sci*. 1999; 880: 219-230.
22. Alkasiy KSN. The protection of pancreas by interleukin-2 (IL-2) from the damaging effects of hydrocortisone. *The Veterinarian* 1998; 8 (2): 65-76.
23. Ahmad MH. The effect of triamcinolone diacetate on the parotid gland of the female rats. A light and electron microscopy study. MSc Thesis. University of Mosul 1993.
24. Al-Moula AD. Histological effects of cortisol on salivary glands in female rabbits. Msc Thesis. University of Mosul 2001.
25. Wellmann KF, Volk BW. Fine structure of pancreas in cortisone-treated guinea pigs and rabbits. *Arch Pathol Lab*. 1976; 100: 334-9.
26. Grossman A, Boctor AM, Band P, Lane B. Role of steroids in secretion- modulating effect of triamcinolone and estradiol on protein synthesis and secretion from the rat exocrine pancreas. *J Steroid Biochem*, 1983; 19(2): 1069-81.
27. Vegiopoulos A, Herzig S. Glucocorticoids, metabolism and metabolic diseases. *Mol Cell Endocrinol*, 2007; 275(1-2): 43-61.
28. Estival A, Pradel P, Wicker-Planquart C, et al. Regulation of amylase and chymotrypsinogen expression by dexamethasone and caerulein in serum-free- cultured pancreatic acinar AR4-2J cells. Influence of glucose. *Biochem J*. 1991; 279: 197-201.
29. Felig P, Frohman LA. *Endocrinology and Metabolism*. 4th ed. McGraw Hill 2001, p. 421-91, 609-32,.
30. Logsdon CD, Akana SF, Meyer C, et al. Pancreatic acinar cell amylase gene expression: selective effects of adrenalectomy and corticosterone replacement. *Endocrinology*. 1987; 121(4): 1242-50.
31. Logsdon CD, Perot KJ, McDonald AR. Mechanism of glucocorticoid- induced increase in pancreatic amylase gene transcription. *J Biol Chem*. 1987; 262(32): 15765-9.
32. Mozheiko LA. Structural and functional interrelations between the liver and endocrine system of the pancreas in disorders of corticosteroid balance. *Probl Endokrinol (Mosk)*. 1985; 31(4): 68-72.
33. Lee PC, Kratz B, Kim O, et al. Expression of the amylase gene in the rat exocrine pancreas during postnatal development: effect of dexamethasone. *Biochim Biophys Acta*. 1990; 1049(3): 244-8.
34. Sasaki R, Mura M, Takeuch T, et al. Premature induction of amylase in pancreas and parotid gland of growing rats by dexamethasone. *Biochem Biophys Acta*. 1976; 428(3): 619-26.
35. Van Nest G, Raman RK, Rutter WJ. Effects of dexamethasone and 5- bromodeoxyuridine on protein synthesis and secretion during in vitro pancreatic development. *Dev Biol*. 1983; 98(2): 295-303.
36. Rosenberg L, Rafaeloff R, Clas D, et al. Induction of islet cell differentiation and new islet formation in the hamster - further support for a ductular origin. *Pancreas*. 1996; 13(1): 38-46.
37. Cutroneo KR, Sterling KM. How do glucocorticoids compare to oligo decoys as inhibitors of collagen synthesis and potential toxicity of these therapeutics? *J Cell Biochem*. 2004; 92: 6-15.
38. Saito T, Tazawa K, Yokoyama Y, et al. Surgical stress inhibits the growth of fibroblasts through the elevation of plasma cortisol concentration. *Surg Today*. 1997; 27(7): 627-31.
39. Garcia-Montero AC, Manso MA, Rodriguez AI, et al. Glucocorticoids regulate L- fucose glycoconjugates in rat pancreatic zymogen granules. *Mol Cell Endocrinol*. 1997; 133(2): 117-25.
40. Mozheiko LA. Histochemical study of enzyme activity of the exocrine and endocrine pancreas at different glucocorticoid levels in animal body. *Probl Endokrinol (Mosk)*. 1986; 32(6): 59-62.

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