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PYRROLOQUINOLINE QUINONE (PQQ)'UN FARE BÖBREK, KARACİĞER VE TESTİSİNE OLAN ETKİSİNİN MORFOLOJİK OLARAK İNCELENMESİ.

MORPHOLOGICAL OBSERVATIONS ON THE EFFECT OF PYRROLOQUINOLINE QUINONE (PQQ) ON MOUSE KIDNEY, LIVER AND TESTES

A. O. ELSADDER* – T. ERBENGİ ** – S. ARBAK **

SUMMARY

To investigate the effect of pyrroloquinoline quinone (PQQ) (2,7,9 - tricarboxyl - 1 H -pyrrolo [2,3 -f] quinoline - 4,5 dione) in mouse kidney, liver and testes, mice were divided into 3 groups, as control, Na HCO_3 and PQQ groups.11.5 mg / kg sodium bicarbonate and 11.5 mg / kg PQQ were injected for 4 days to Na HCO_3 and PQQ groups mice. Accordingto our morphological observations, there were no serious alterations which revealed a toxic effect of PQQ on kidney, liver and testes. Also, ultrastructural features of glomeruli and proximal tubules in kidney tissue demonstrated that PQQ was not a toxic agent.

Key words : Pyrroloquinoline quinone, liver, kidney, testes.

ÖZET

Pyrroloquinoline quinone'un (PQQ) (2, 7, 9, - tricarboxy 1- 1 H -pyrrolo (2,3 -f) quinoline - 4,5 dione) fare böbrek, karaciğer ve testisine olan etkisini incelemek amacı ile, biri kontrol, diğerleri Na HCO3 ve PQQ grupları olmak üzere 3 grup oluşturuldu. Na HCO3 ve PQQ grubu farelere, 4 gün süresince 11.5 mg / kg Na HCO3 ve 11.5 mg /kg PQQ enjeksiyonu yapıldı. Morfolojik gözlemlerimizde karaciğer, böbrek ve testiste PQQ'un toksik etkisini ortaya koyabilecek önemli bir değişikliğe rastlanılmadı. PQQ grubunda, böbrek dokusu glomerulus ve proksimal tubulusları ince yapısı, PQQ ' un toksik bir madde olmadığını vurgulamakta idi.

Anahtar Kelimeler : Pyrroloquinoline quinone, Karaciğer, Böbrek, Testis.

* Specialist in Histology and Embryology, Amman / JORDAN.
** Department of Histology and Embryology, Faculty of Medicine, Marmara University, 81010 Haydarpaşa - ISTANBUL / TÜRKİYE.

INTRODUCTION

Recently, pyrroloquinoline quinone (PQQ), acting as a new co-enzyme was mentioned in many experimental studies with its growth stimulating effect (1,2,3,4,5).

PQQ (2,7,9 - tricarboxyl - 1H - pyrrolo [2,3 -f] quinoline -4,5 dione) (fig. 1) is a compound having a pyrrole ring fused to a quinoline ring with an o -quinone group in it. Representatives of this group are found among the bacterial, NAD (P) - independent, periplasmic dehydrogenases. PQQ is known with a high redox capacity. It is resistant to rays and acids (1). PQQ is found in culture media of gram - positive and gram - negative bacteria (1). Although any presence of free PQQ is revealed at eucaryotic organism, some enzymes covalently bound to PQQ are mentioned (1,2).

It was also reported, both in animals and plants, as an agent affecting the growth rate, energy generation and survival in bacteria (6). Its therapeutical effects on cataract (4) and asthma (7) are another characteristics of PQQ.

While PQQ is known with those positive effects, its negative effects such as nephrotoxicity and haematuria were declared in other study (8).

To investigate further effects of PQQ on mammalian tissues, we want to study these effects on mouse kidney, liver and testis. Then, we aim to postulate its positive and negative results on these tissues.

MATERIALS AND METHODS

12 male mice of 40 g average body weight were used and accomodated in the same room, fed a regular diet and water ad libitum. Mice were divided into 3 groups : A) Control group (n = 4) = mice were fed only with regular diet and water; B) Sodium bicarbonate (Na HCO_3) group (n = 4) = mice were injected intraperitoneally a daily dose of 11.5 mg / kg body weight Na HCO_3 for 4 consececutive days; C) Pyrroloquinoline quinone (PQQ) group (n = 4) = PQQ dissolved in 2 % Na HCO_3 solution (11.5 mg / dl) was administered intraperitonally for 4 consecutive days at a daily dose of 11.5 mg / kg body weight.

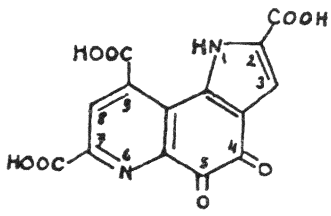

Fig. 1: Structure of PQQ.

At the 5th day of the experiment, mice were sacrified under ether anesthesia. For light microscopic investigations, kidney, liver and testes materials were fixed in Bouin's solution. Following paraffin embedding, sections were stained with Haematoxylin - Eosin and Periodic Acid - Schiff (PAS) methods and investigated at Olympus BH - 2 light microscope. For electronmicroscopical investigation, kidney material was fixed in 2.5 % phosphate - buffered glutaraldehyde and then postfixed in 1% OsO4 solution for one hour. Thin sections taken from Vestopal W blocks (400-600 A°) were contrased with uranyl acetate and lead citrate Reynold's method. Then, sections were investigated and evaluated by JEOL 100 C electronmicroscope.

RESULTS

CONTROL GROUP

KIDNEY : At light microscopical level, glomerular structure of the kidney was observed with the prominent capillary tufts and PAS + basal laminae. While proximal tubular cells were noticed with PAS + brush border, distal tubules were seen lined by cuboidal cells. At electronmicroscopical level, a normal appearance of renal corpuscle with endothelial cells, podocytes and basal lamina of Bowman's capsule were observed (fig. 2). Proximal tubular cells were quite apparent with their microvilli and basal labirynths (fig. 3).

TESTES : Testes were investigated at the light microscopical level. They were normal in appearance with seminiferous tubular germinal epithelium and Sertoli cells (fig 4).

LIVER : Liver was investigated at the light microscopical level. Apparent localization of sinusoids between hepatocytes together with the central localization of central vein within the lobulus were observed in that group (fig . 5.)

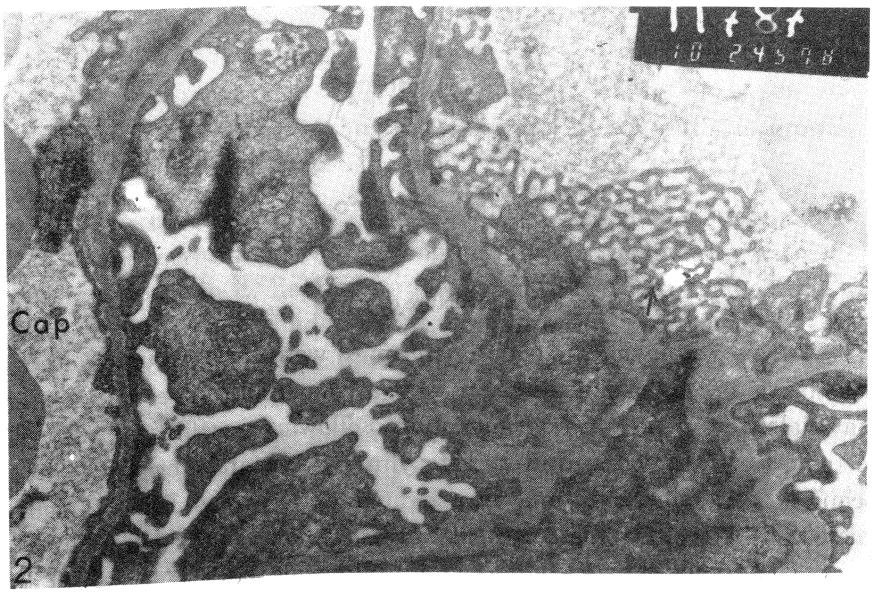

Fig. 2: Podocytes and fenestrated capillary (Cap) are observed at control group glomerulus. X20.000

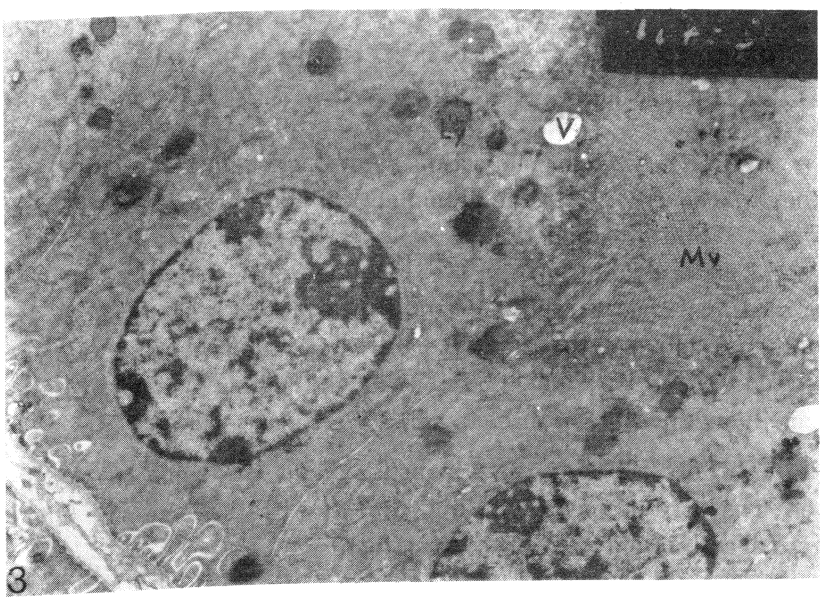

Fig. 3: Microvilli (Mv), pinocytotic vesicles (V) and osmium - dense lysosomes (Ly) are seen at renal tubules of control group mouse kidney. X10.000.

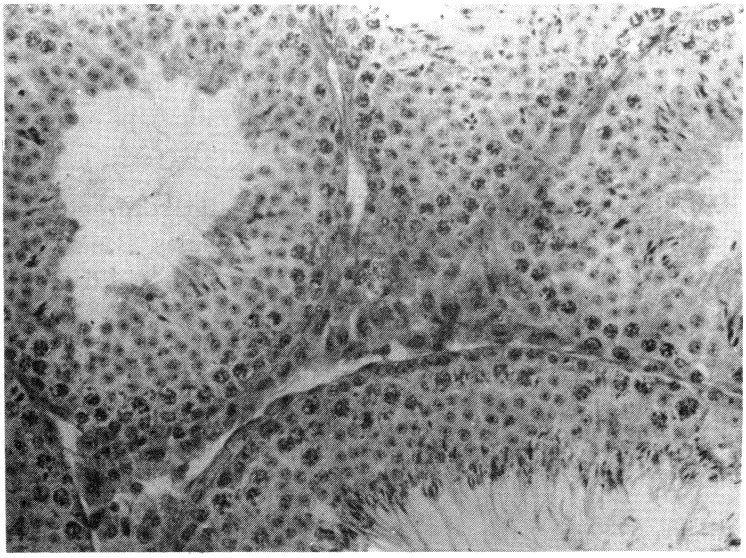

Fig.4 : Control group photomicrograph indicates normal spermatogenesis and spermiohistogenesis at seminiferous tubules of testis. PAS (Periodic acid - schiff) reaction. X290.

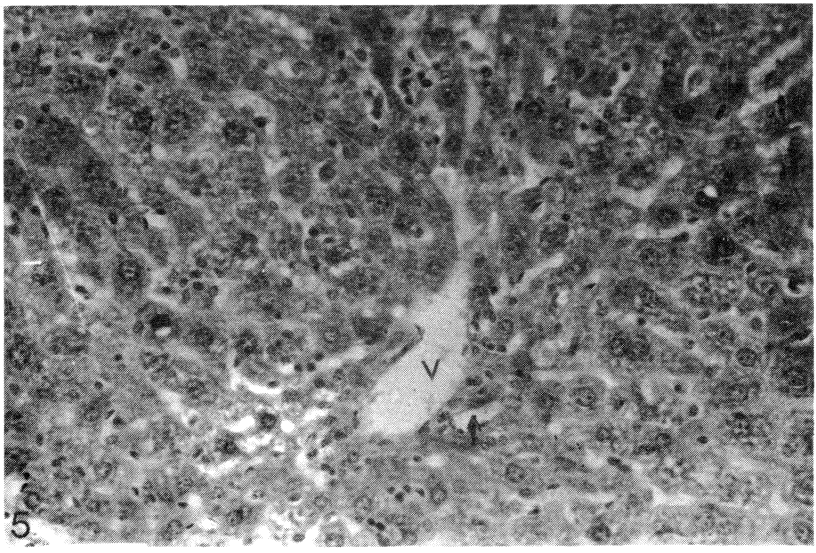

Fig. 5 : Classical appearance of liver lobule with vena centralis (V) hepatocytes and sinusoids (arrow) is observed at the control group mouse liver. Haematoxylin - Eosin staining. X290.

$Na\ HCO_3$ GROUP AND PQQ GROUP

Light and electronmicroscopical findings for both Na HCO3 group and PQQ group were similar. So, the following evaluations are related to both of these groups.

KIDNEY : Light microscopical investigations revealed normal morphology of glomeruli, proximal and distal tubules. Basal lamina of nephrons and microvilli of proximal tubules were PAS - positive (fig. 6).

At electronmicroscopical level, in glomerulus, slightly swollen endothelial cells and an increase in fenestrata stucture of them were quite interesting (fig. 7). Proximal tubular cells represented an increase in lysosomes, pinocytotic vesicles and vacuoles together with a regular arrangement of microvilli (fig. 8).

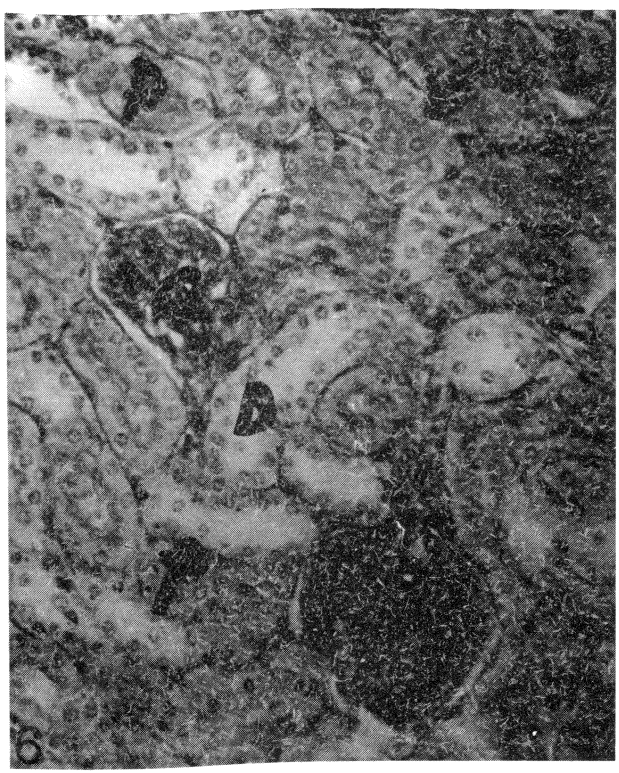

Fig.6 : PQQ group photomicrograph reveals normal kidney cortex morphology with glomeruli (G) and tubules. Brush border of proximal tubules (P) and all basal laminae (arrow) were PAS - positive. D : distal tubule. PAS (Periodic Acid - Schiff) reaction. X290.

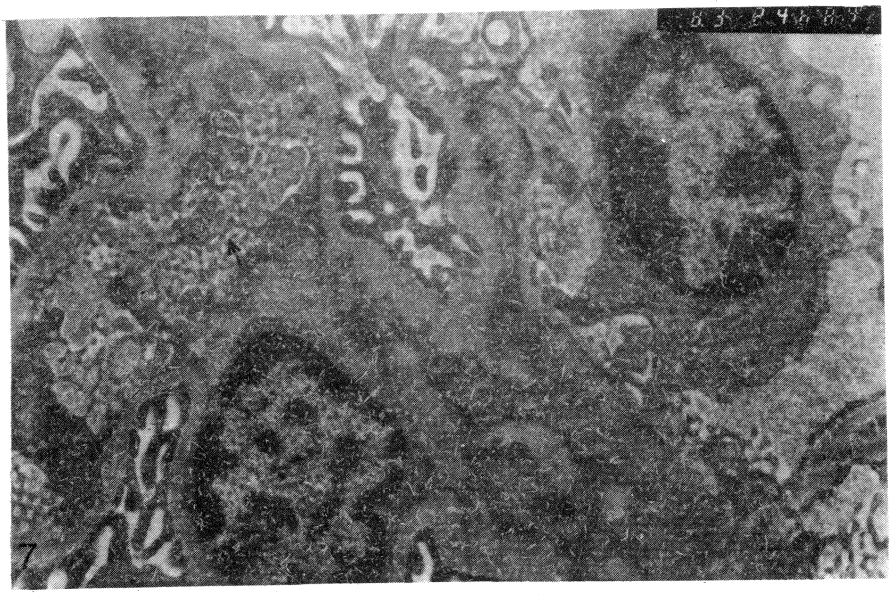

Fig. 7 : PQQ group kidney glomerulus structure reveals a well - developed fenestration at the capillary endothelium. X16.000

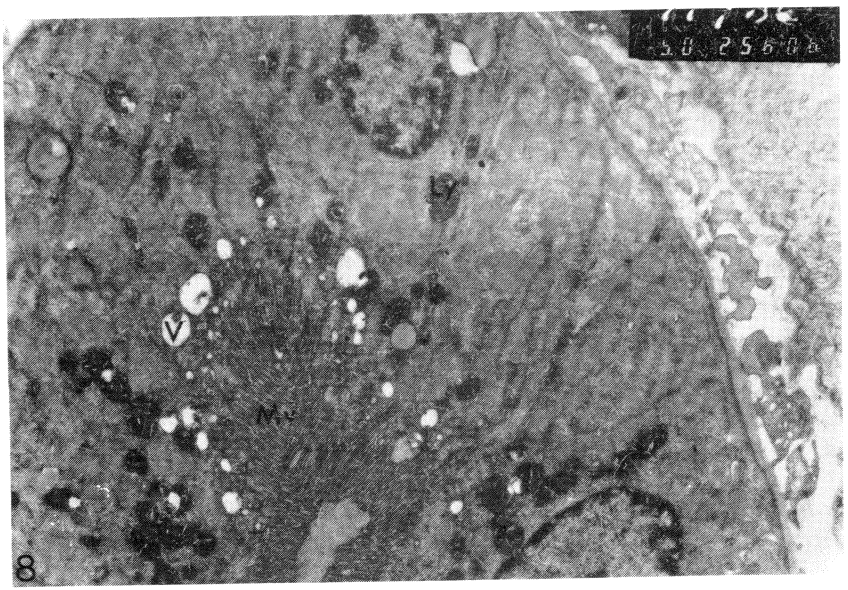

Fig. 8 : At the proximal tubules of PQQ group kidney, apical microvilli (Mv) pinocytotic vesicles and vacuoles (V) together with lysosomes (Ly) are seen. X10.000.

TESTES : At light microscopial level, seminiferous tubular germinal epithelial cells seemed to be similar to those of the control group (fig. 9).

LIVER : Light microscopical observations revealed normal histological structure of liver lobules, hepatocytes and sinusoids (fig. 10).

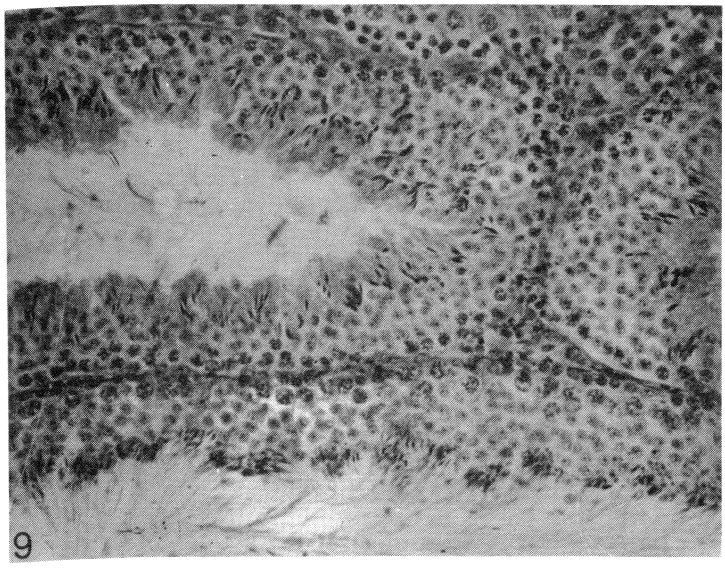

Fig.9 :Different stages of spermatogenesis and spermiohistogenesis are observed on the seminiferous tubular germinal epithelium in PQQ group. Haematoxylin - Eosin staining. X290.

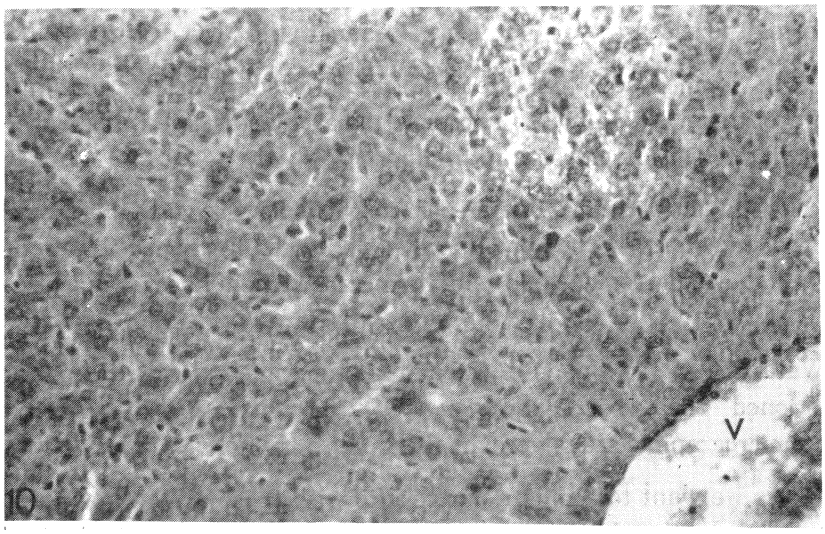

Fig. 10 : Any degenerative finding is observed at the PQQ group mouse liver. V : vena centralis. Haematoxylin - Eosin staining. X290.

DISCUSSION

As a newly discovered coenzyme, PQQ is still discussed with its stimulatory effect and is thought to be improved as a new pharmacological substance (1,3). But, some new preliminary studies researching its toxic effects are going on (8).

To control toxic effects of PQQ mentioned in study of Watanabe et al (8), we use the same dose of PQQ at the same period of time. Thus, we obtained different findings than those of Watanabe et al (8). Furthermore, not only kidney proximal tubular cells, another organs related to metabolic and reproductive activity, such as liver and testes, were investigated and evaluated in our study.

Recently, Shimao et al. (5) realized the purification of factor A as PQQ and identified it for the first time as bacterial growth factor. We know that the toxic and biological effects of PQQ are not so clear. Minimal toxic dose of it together with PQQ pool in animal tissue are still unknown. Watanabe et al. (8). claimed that it was not possible to demonstrate nephrotoxicity mechanism of PQQ by relating it to the renal

histological differences revealed in experimental studies. They also reported the necessity for the studies related to the PQQ dosage.

In our study, as we could not determine a prominent nephrotoxicity, it is almost impossible to say PQQ is a real toxic agent, according to our ultrastructural findings.

Watanabe et al. (8), in their study wanted to investigate the real nephrotoxic effect of PQQ in rats. They pointed out the fast excretion of PQQ kidneys in one hour following its intraperitoneal injection.

The reason to use only a single dose of PQQ is based on the study of Watanabe et al (8) done at the similar experimental conditions. They mentioned the toxic effect of PQQ demonstrated by a single photomicrograph of kidney proximal tubule.

As we want to compare our results with those of Watanabe et al. (8). we tried to establish the same experimental conditions So, 3 groups of mouse (control, Na HCO3 and PQQ groups) were formed.

Although Watanabe et al. (8). declared the vacuolar degeneration, atrophy and necrosis at the proximal tubular cells, we could not determine any necrotic findings at the light and electronmicroscopical levels (fig. 6 and 8). We also noticed no degenerative structure at the ultrastructural examination of the glomeruli except some swellings at the capillary endothelial cells together with a developed fenestrata which were minimal physiological changes seen in normal conditions, as well (fig. 7). As we could not have any pathalogical findings at the level of podocytes, pedicels and glomerular basal lamina (fig. 7), we can conclude PQQ effect is very limited on the glomerulus structure.

Compared with the control group, Na HCO_3 group proximal tubular cells were observed with an increasing number of vacuoles and pinocytotic vesicles. That implied a prominent reabsorption in that group.

In PQQ group, a faster reabsorption was concluded by the presence of regular microvilli arrangement, active basal labirynth and an increasing number of pinocytotic vesicles (fig. 8) at the proximal tubular cells.

Many authors postulated the positive effects of PQQ by mentioning its mitotic division regulatory effect in living systems (9). Nishigori et al.

(4) demonstrated the cataract - preventive effect of PQQ in chicks. One of the positive stimulatory effect of PQQ was demonstrated in a plant study (10) in which polens taken from lilium were mixed with PQQ in a culture medium and PQQ was seen to stimulate the plant growth.

So, we can conclude that pyrroloquinoline quinone (PQQ) has no apparent toxic effect on the kidney, liver and testes in mice. We also may say that according to our light and electron microscopical findings these properties of PQQ bring more chance to the using area in the future.

REFERENCES

1. Duine, J. A., Van der Meer, R. A., et al. : *Annu. rev. Nutr.,* **10**, 297 -318 (1990).
2. Corey, E. J., Tramontano, A. : *J. Am. Chem. Soc.* **103**, 5599 - 5600 (1981).
3. Glatz, Z., Kovar, J. et al : *Biochem. J.* , **242**, 603 -606 (1982).
4. Nishigori, H., Katsumata, M., et al. : *Life Sci. Vd.,* **45**, 593 -598 (1989).
5. Shimao, M., Yamamoto, H., et al. : Agric. Biol Chem., **11** , 2873 -2876 (1984).
6. Duine, J. : *Eur. Biochem.,* **200,** 271 284 (1991).
7. Maruyama, Y., Iwayama, A., et al. : *Chem. Abstr.,* 1988; **109** , 222 - 272 (1988).
8. Watanabe, A., Hobara, N., et al. : *Hiroshim J. Med. Sci.* **38**, 49 - 51 (1989).
9. Hartmann, C. , Klinman, J. P. : *Biofactors,* **1,** 41- 49 (1988)
10. Duine, J. A. , Jongejan , J. A. : *Annu. Rev. Biochem.,* **58**, 403 - 426 (1989).

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