HISTOPATHOLOGICAL CHANGE OF THE TESTIS IN ADULT MICE AFTER IRRADIATION OF LASER TREATMENT

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(Received 28 december 2017 ,Accepted 4 January 2018)

Keywords: testis, Spermatogenesis,Mice.

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ABSTRACT

In the current study demonstrated the role of irradiation with stimulate the cells on division and accelerate of spermatogenesis in mice testis following low –level laser therapy and anti-inflammatory process.

Twenty -eight healthy male and female mice .They were maintained in individual cages with free access to water and a pellet balanced commercial diet for mice . All animals have been weighing by using sensitive balance and the mean body of mice (25+_5 gm) .

The stages of Q-S Switch Nd:YAG laser set-up with 632 nm 10 ns of pulse duration and 10 Hz of frequency to get a dose or energy density were : 1-set a single pulse on Q-Switch Nd:YAG laser using control panel with repetition rate of 10 Hz .

Mice were irradiated to testes a 670 nm with intensity 4 J/cm2 /day .for seven days .But mice of control group without exposure any laser.

RESULTS

The laser treated group revealed increase cell division of spermatogenesis and but microhemorrhages occurring in some regions of the interstitial space . So this results revealed that there were such finding as an atrophy of the seminiferous tubules ,dis arrangement of Sertoli cells, generation of giant multinucleated bodies.The irradiation with 632nm 4 J/cm2 following irradiation with He-Ne ,on the testis there were normal appearances of the seminiferous epithelium and interstitial tissues.

Conclusion: exposure of the testis in mice stimulate the cell to the division and repair tissue by short wave length of irradiation of laser .

INTRODUCTION

Lasers are classified into high and low power. The first is generally applied for the removal, cutting and . Coagulating of tissues, while the low-power ones are more commonly applied in the processes of tissue repair, such as muscle, joint, nerve, bone and skin injuries . The photobiological effects of laser radiation can be conventionally divided into short and long term.(1,2,3,4). The responses in the short term are those in which the effect can be observed in a few seconds or minutes after irradiation. The effects observed in the long term are those that occur hours or even days after the end of irradiation and usually involve new cell biosynthesis, especially in the proliferative phase of inflammation .(5,6).

Men with a low T level presented with such symptoms as decreased sex drive, impotence, fatigue, loss of lean muscles, fat accumulation and weight gain, an increased risk fat accumulation and weight gain, an increased risk of osteoporosis and bone fractures and an increased risk of cardiovascular disease (7) .

Although, the influence of low-level laser can be observed in the behavior of lymphocyte ,increasing their proliferation and activation ; on macrophages, increasing phagocytosis; and on fibroblasts, increasing the secretion of growth factors and enhancing the uptake of both fibrin as collagen. In addition, it contributes to increase the motility of epithelial

cells, the amount of granulation tissue and may reduce the synthesis of inflammatory mediators (8 ,9,10 ,11) .

The most commonly used laser is the Helium- Neon (He-Ne) laser of wavelength 632.8 nm. In vitro, this laser showed a modifying response of cells to ionizing radiation such as ɣ-ray(12) ,X-ray(13) and ultraviolet (UV) light(14). But a protective properties was found to be more significant when laser radiation was performed prior exposure to ionizing radiation(15). Parenteral methods are not so convenient as oral administration, which include syringe injections or transdermal applications such as gels, cream and patches. There is no denying their effectiveness. Following syringe injections of T enanthate and T cypionate, however, serum T level is abruptly increased or decreased. They are also known to cause side effects such as pain, edema or jaundice.(16). Laser was found by. Maiman, TH in 1960 and has developed rapidly

in the various disciplines of medicine, especially in dermatology. In the field of skin diseases, laser developed into laser surgery and laser cosmetic (18).

One frequent-used type of laser is Q-Switch Nd: YAG laser with 1064 nm of wavelength and 10 ns pulse duration. The laser has a short time period of modulation, high peak power and perfect coagulation properties on the tissue, even though a thermal effect occurred when approaching the visible light spectrum in the form of securities ablation (17). The mechanism of laser interaction on tissue is divided into five namely, photochemical, photothermal, photoablation, plasma production and photo disruption. The affected magnitudes are nergy density include, flounce, power density, and pulsed peak power.

The aim of this study to demonstrated effect of LLLT on testis and revealed histopathological change occurring in the tissue treatment with laser irradiation .

MATERIALS AND METHODS

The Experimental Animals : Twenty -eight healthy male mice .They were maintained in individual cages with free access to water and a pellet balanced commercial diet for mice . They were obtained from the animal house of the biology department –college of education for pure sciences /Ti-Qar university .The mice were put in the plastic cages breeding with metal lids and brush the cage with sawdust, environmental condition controlled at the constant photoperiod (12 hours day/12 hours night), ventilation temperature ranged between (20-24 c).Animal subdivided into three group : first group(7 animals) no treated with irradiation, Second group (7 animals) treated with Q-S Switch Nd:YAG laser irradiation and third group (7 animals) treated with He-Ne laser and UVC irradiation .

Body weight : All animals have been weighing by using sensitive balance and the mean body of mice (25+_5 gm) .

Laser Irradiation : The stages of Q-S Switch Nd:YAG laser set-up with 632 nm 1o ns of pulse duration and 10 Hz of frequency to get a dose or energy density were:1-set a single pulse on Q-Switch Nd:YAG laser using control panel with repetition rate of 10 Hz .(17) .

He-Ne laser and UVC Irradiation: Mice were irradiated to testes a 670 nm with intensity 4 J/cm2 /.for seven days .But mice of control group without exposure any laser. (30) .

Animals treatment : Seven days after treatment, seven randomly-chosen animals in each group were anesthetized with ether to remove the ipsilateral testis and epididymis of one side (left or right, alternately chosen) and one random side of the seminal vesicles. After further 5 days, the organs were removed from the other animals in the same way. On removal, the organs were immersion-fixed in Bouin's fluid, and then, approximately 30 h later, stored in 70% ethanol for a few days. Then, the testis and epididymis were separated and the organs were weighed using an electronic balance (accuracy 0.1 mg). The organ volumes were calculated by dividing the weights by a density of 0.93 g/mL. The density measured after fixation in Bouin's fluid followed by dehydration or storage in 70% ethanol was consistently found to be approximately 0.93 for the testis (28, 29) or epididymis (unpublished data), and the testicular tissue volume obtained after fixation in the fixative and then storage in 70% ethanol (28).

Light Microscope study: For histological study , several samples of testis were fixed in formalin 10% for 24 hours and washed up in tap water for 4-5 hours and then moved the samples to many histological technique as followed(Dehydration ,Clearing, Infiltration, Embedding, Cutting, Staining and mounting .(29) .Viewed at perfect magnification of light microscope (Olympus) with camera Canon .

RESULTS AND DISCUSSION

The results of the current study have been shown that the testis of control group is surrounded by thin capsule which does not give off septa.figure(1). They are showing many seminiferous tubules containing the spermatogonia, primary spermatocyte, secondary spermatocyte ,spermatids and spermatozoa. Figure(2). Histological examination showed that there were no change In the cellular morphology following irradiation with a 670 nm , but microhemorrhages occurring in some regions of the interstitial space Figure(3) following those with an 632 nm one .All these results agree with (19) they are describe the laser irradiation on rats.

Our results revealed that there were such finding as an atrophy of the seminiferous tubules , dis arrangement of Sertoli cells, generation of giant multinucleated bodies Figure(4) .This observation was similar with (20).

The irradiation with 670 nm 360 J/cm2 following irradiation with He-Ne ,on the testis there were normal appearances of the seminiferous epithelium and interstitial tissues figure(5).This results disagree with Some others which describe the low level laser irradiation(670 nm) on Wister rats that in the same areas ,the sections examined under polarized light showed a predominance of greenish-yellow fibers with irregular birefringence and the sections examined on post surgery days 5,granulation tissue with intense angiogenesis and an increased mononuclear (round) cellular infiltrate with many macrophage and lymphocyte were observed. These authors also noted however that the seminiferous epithelium and interstitial tissues were irregularly arranged and elongated tubules following the irradiation with 830 nm. Previous studies have shown that laser irradiation of lower doses have positive and negative bio stimulatory effects on various endocrine glands such as thyroid gland sand pancreatic islets (21,22).

A biphasic dose response has been frequently observed where laser irradiation of lower doses have a much better effect in stimulating and repairing tissues as compared with that of high doses.(18). This infact agree with our study.

On the other hand the sections examined were revealed that use the (LLLT) in mice may be stimulated increase spermatids to form spermatozoa in seminiferous tubules in this study ,this results is similar to study (23) which describe effect of low –level laser irradiation on rats that exposure of bone to (LLLT) , 5 days stimulate osteoblast to repair of bone and synthesis of fibers and increase matrix in bon tissue.

To confirm the effectiveness of the laser treatments, various types of laser should be tested. This should also be followed by the establishment of a safe, solid protocol depending on the wavelength, power intensity and an ability of the different types of lasers to penetrate the tissue, further studies are also warranted to examine the mechanisms by which the laser treatments affect serum T level as compared with the conventional types of testosterone replacement therapy. In addition, it should also be

thoroughly examined for whether the LLLT has any adverse effect on the testis. (24).This results may be agreed with our results.

So, changes in sperm head morphology may be an applicable screen for radiation exposure doses effect on germ cells and events of late phase of spermiogenesis. This He-Ne laser toxic properties was reported by (25)on sperm head morphology of mice .This in fact agree with ours study , and also by (26) on lymphocyte cells survival and it`s DNA damage.

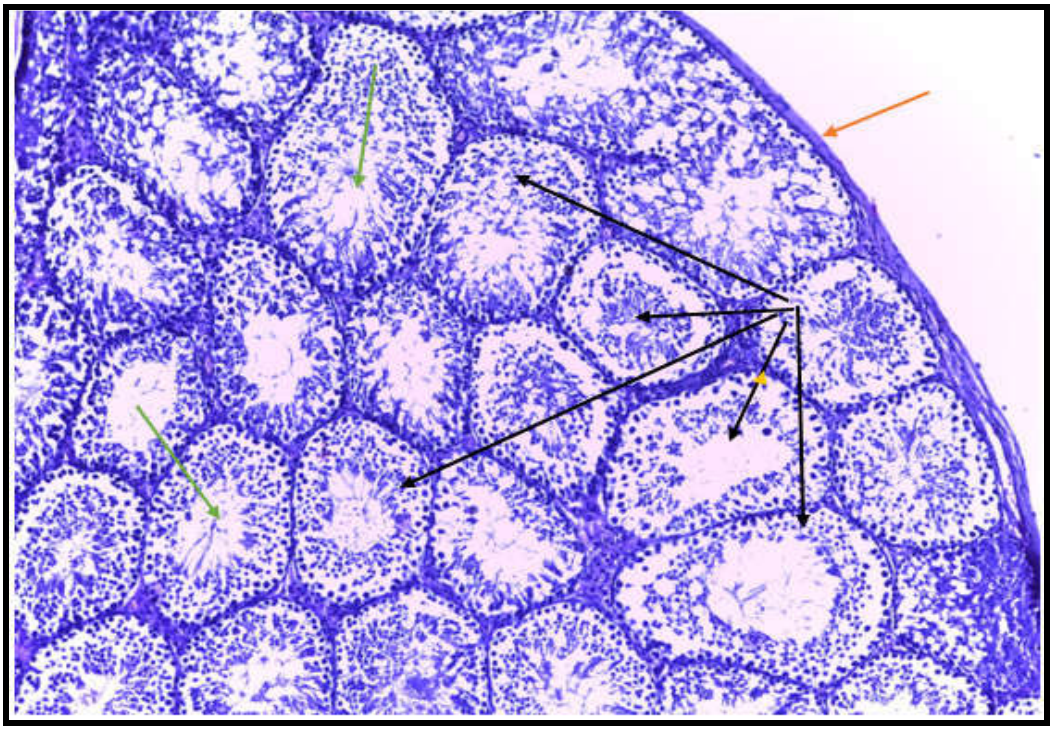
CONCLUSIONS

In conclusion, the present study demonstrated that laser therapy revealed an histological potential, stimulating cells activity and increasing mitosis for many cells . These data highlight the huge potential of the use of laser therapy to improve cells metabolism. Further long-term studies should be developed to provide additional information concerning the late stages of the laser stimulation on spermatogenesis and division spermatogonia to synthesis many of spermatozoa in testis .

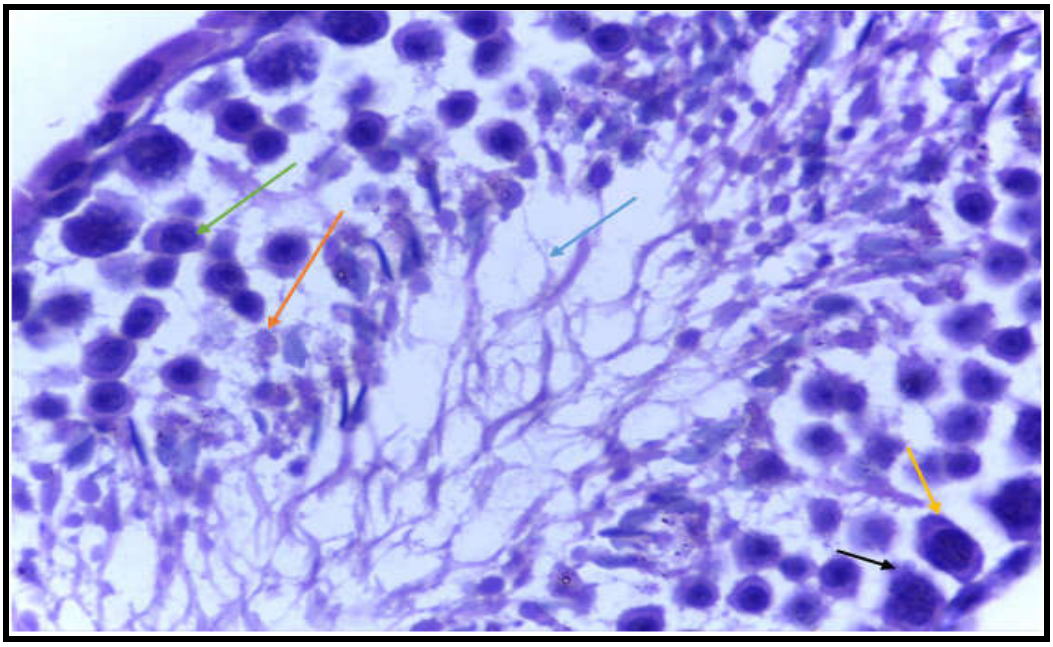
RECOMMENDATIONS

1-We are suggesting to study of low lower laser therapy in many type of the birds and showing stimulate of the cells division ,such as spermatogenesis in testis.

2-Study of aggregation and effect of short wave length on the many of Aves organs.



Figure(1).Cross section of testis in mice showing normal seminiferous tubules (black arrow), capsule (orange arrow)sperms (green arrow) (H&Esatin,100 X).



Figure(2).Cross section of testis revealed , spermatogonia (black arrow),primary spermatocyte (green arrow),secondary spermatocyte(orange arrow),spermatids (pink –orange arrow) spermatozoa(blue arrow). (H&Estain,1000x).

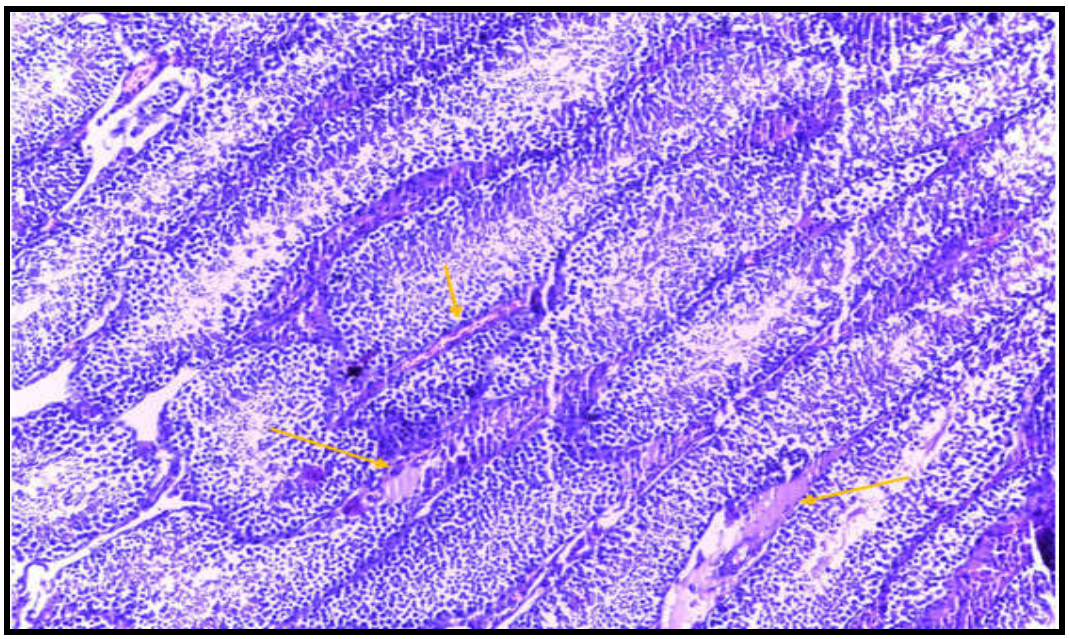
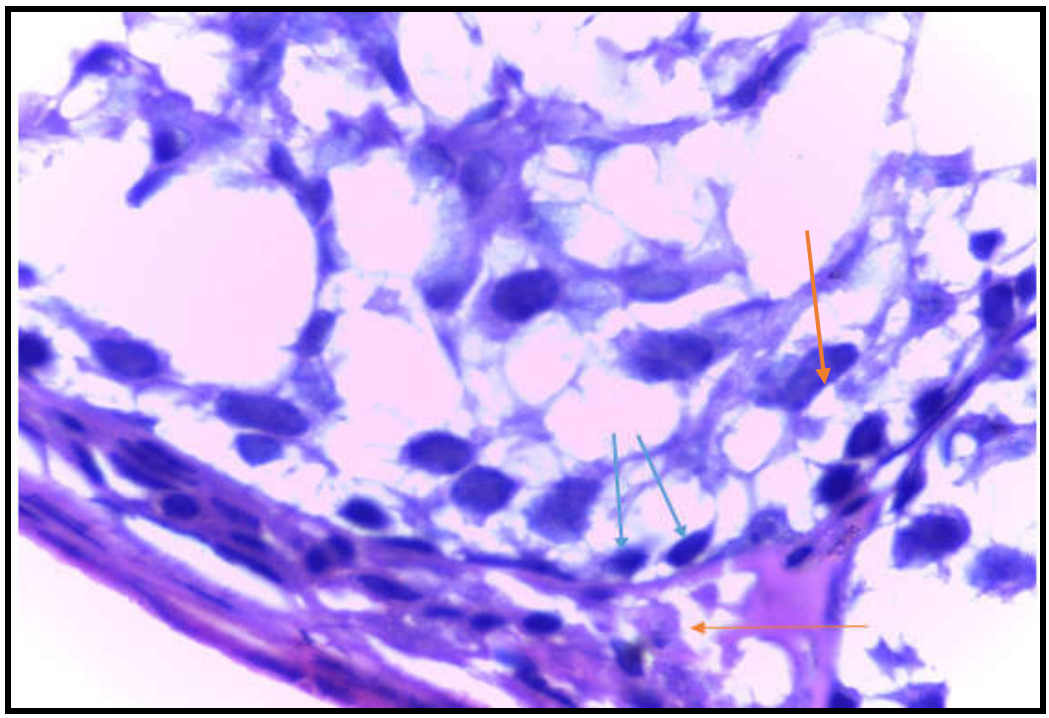
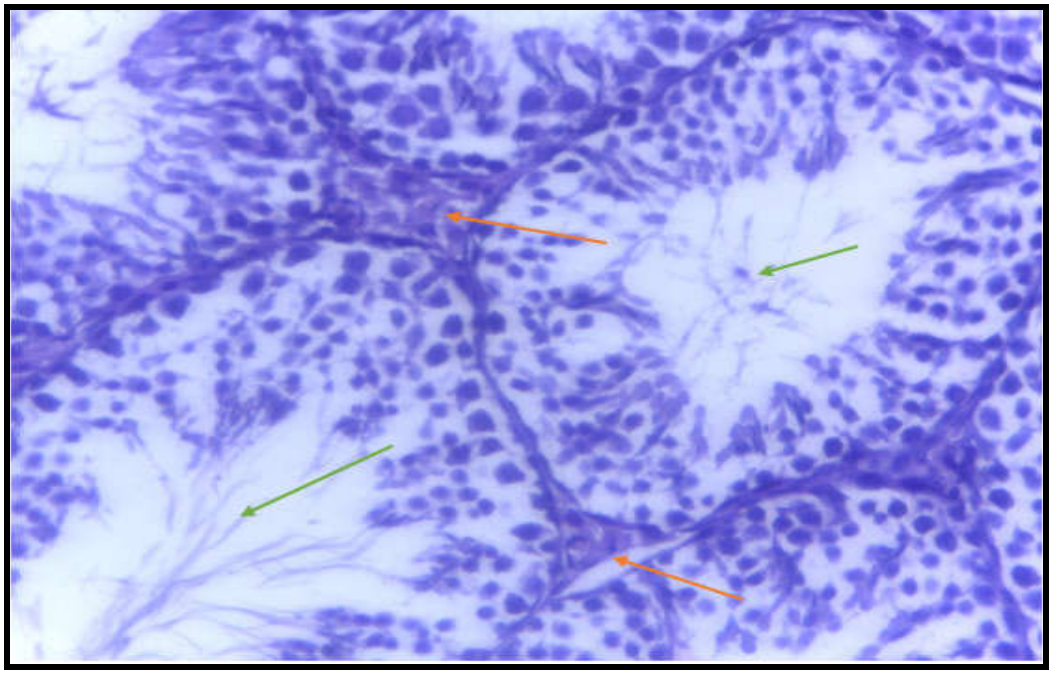


Figure (3) . Cross section of testis revealed microhemorrhages in some region between seminiferous tubules (orange arrows) . (H&E stain,400 X)



Figure(4).Cross section of testis mice showing generation of giant multinucleated(blue arrow) dis arrangement of Sertoli cells(orange arrow),(H&E stain, 1000 X).



Figure(5).Cross section in testis of mice showing normal seminiferous tubules Leydig cell(orange arrow), sperms (green arrow).(H&E stain,400 X).

التغيرات النسجية المرضية لخصى الفئران البالغة بعد التشعيع بعلاج الليزر

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الخلاصة

أوضحت الدراسة الحالية دور الإشعاع في تحفيز الخلايا على الانقسام وتسريع عملية تكوين النطف في خصى الفئران متبوعة بعلاج الليزر منخفض المستوى والمراحل التي تعمل ضد الالتهاب

ثمانية وعشرون من ذكور الفئران تم تقسيمها في أقفاص مجهزة بالماء والغذاء للفئران . جميع الحيوانات وزنت بالميزان الحساس ووجد لها معدل وزن ٢٥+-٥ غرام .إشعاع الليزر :مرحلة التشعيع بالليزر Q-s switch :Nd: YAG.مع طول موجي ٦٣٢ نانومتر ١٠ ns من دورات التحفيز لغرض حصول كثافة عالية من التردد مستخدما نبضة تحفيز واحدة . ليزر الهيليوم-نيون مع UVC إشعاع :الفئران شععت في الخصى ب ٦٧٠ نانومتر وبقدرة ٤ جول لكل سنتمتر مربع في اليوم . لمدة سبعة ايّام لكن بقيت فئران مجموعة السيطرة بدون التعرض للإشعاع . النتائج :المجاميع المعاملة بعلاج الليزر أظهرت بان هنالك تسريع في انقسام الخلايا المكونة للنطف لكن هنالك نزف قليل في بعض المناطق في النسيج الخلالي . كذلك أوضحت النتائج هنالك عدم انتظام في شكل النبيبات المنوية وعدم انتظام في خلايا سرتولي وكذلك تضاعف في حجم انوية الخلايا . التشعيع ب ٦٣٢ نانومتر و ٤ جول لكل سنتمر مربع متبوع بإشعاع ليزر الهيليوم نيون في الخصى كان هنالك تغير في

طلائية النبيبات المنوية والنسيج الخلالي .الخلاصة : تعرض خصى الفئران الى الليزر يحفز الخلايا للانقسام وكذلك إصلاح الأنسجة التالفة بواسطة الإشعاعات ذات الأطوال الموجية المنخفظة من الليزر.

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