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Study the Pathological Effects of the Combination of Estrogen and Progesterone Hormones on Some Organs Experimentally Induced in Mice

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Summary

The aim of this study was design to investigate the pathological changes for one month after therapeutic and toxic doses of subcutaneous injection of estrogen and progesterone combination hormones in mice, on the target organs testis and epidydimus in males and uterus and ovary in females. As well as the effects on non-target organ of Brain, liver spleen, intestine, stomach, kidney and lung in both sexes. The results showed sever pathological changes in male's testis and epidydimus and in females, uterus and the ovary. It is characterized by some pathological changes in toxic group less severity than in the therapeutic group.

Also, in non-target organs brain and spleen of toxic group of males and females showed some pathological changes while therapeutic group almost appear normal. The liver and kidney were affected in both groups (therapeutic and Toxic) in males and females. Other organs like intestine stomach, Lung doesn't showed any change in both groups.

Keyword:-estrogen, progesterone, toxic, therapeutic.

دراسة التاثيرات المرضيه المستحدثه تجريبياً لخليط هرموني الاستروجين والبروجسترون على بعض الاعضاء في الفئران

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الخلاصة

هدفت هذه الدراسة الى التحري عن التغيرات النسجيه المرضية الناجمة عن حقن هرموني الاستروجين والبروجسترون في الفئران لمدة شهرتحت الجلد، تم أخذ الخصي والبربخ في الذكور والارحام والمبايض في الاناث وكذالك تاثيرها على الدماغ والكبد والطحال والامعاء والكلى و المعدة والرئة في كلا الجنسين . نتائج التجربة تشيرالى وجود تغيرات شديدة في المجموعة السمية, اما في المجموعة العلاجية كانت اقل حدة. اما الاعضاء الاخرى الغير مستهدفة كالدماغ، والطحال، والكبد، والرئة، والمعدة ،والامعاء، والكلى، فقد وجدت التجربة وجود تغيرات مرضية في الدماغ، والطحال في المجموعة السمية في كلا الجنسين، الامر الذي لم يلاحظ في المجموعة العلاجية. اما الاعضاء الاخرى كالكبد، والكلية فقد وجدا متأثرين في كلا المجموعتين، وفي كلا الجنسين. بالنسبة للأعضاء الاخرى كالمعدة، والامعاء، والرئة فلم تظهر اية تغييرات نسجية.

مفاتيح البحث: استروجين, بروجسترون, الفئران.

Introduction

Steroid hormones are divided into five groups according to receptor which they bind, glurocorticoid, mineral corticoid, androgen, estrogen and progesterone. Steroid hormone help in control of the inflammation, immune function, salt, water and balance and development of sexual characteristic and ability to withstand illness and injury. These steroid hormones are generally synthesized from the cholesterol in the gonads and adrenal gland. These hormones are lipid soluble and they can pass through receptor (1).

Estrogen is steroid hormone produced in the ovary (2) and its known as hormone of female, its essential for development of accessory genital organ and

secondary sexual characteristic (3,4). The synthesis and secretion of estrogen stimulated by follicle stimulating hormone FSH which is in turn controlled by the hypothalamus gonad tropic releasing hormone (GnRH), high level of estrogen suppress the release of GnRH providing negative feedback control of hormone(2).

Progesterone is sex steroid hormone which is conjugated with estrogen to regulate the function of accessory sex organ during an ovarian cycle (5). It's secreted mainly from the corpus luteum in the ovary and placenta (3). Also was produced from the cortex of the adrenal gland in both male and female (6).

Smaller amount of progesterone are also produced in the testes and glial cells of the brain in both sexes (7). Progesterone receptors are located in the uterus and mammary gland in female (8). These receptors are found in the testis and prostate in male (9).

Progesterone prepare the uterus for the implantation of fertilized ovum and promote the secretary changes in the endmoetrium (3), and stimulate the endometrial gland to enlarge and increase secretion of water, salt and glycoprotein for the nutrition of embryo (10 and 11). On the other hand prevent the contraction of uterus during gestation by blocking the oxytocin receptor and still quiescent during the period of pregnancy (10) and suppress the immunity in order to prevent the rejection against embryo (12).

Materials and Methods

Thirty six mice from both sexes (18 males and 18 females) were used. The animals were diet locally and water was *adlibittum* a long the period of experiment 30 days. Estrogen hormone (Vetastrol by Abuaihan Pharmaceutical Co, Iran), 10 ml each ml contain 2 mg Oesteradiol benzoate, and progesterone (Vetagesterone, Abuaihan Pharmaceutical Co, Iran), 10 ml, each ml contain 25 mg progesterone were used in this experiment. The animals were divided into three equal groups as the following:-
Therapeutic group: - which contain 12 animals 6 males and 6 females which were received 16 mg estrogen and 20 mg progesterone (13). Toxic group: - which contain 12 animals 6 males and 6 females which were received 32mg estrogen and 40 mg of progesterone (13). Control group: which contain 12 animals 6 males and 6 females which are treated with sun flower oil only in the same manner.

Progesterone was prepared for injection after diluted with sun flower oil for subcutaneous injection (13) all animals were subcutaneous injection daily for one month. Animals were sacrificed by ether inhalation and post mortum examination was done for all the organs. The histopathological sample of 1-2 cm were taken from the lesion kept in formalin 10% for fixation processed routinely staining (Hematoxyllin and eosin stain) and all the lesion were be recorded.

Results and Discussion

The animals were sacrificed after one month of injection and histopathological examination of organs mention above recorded that: - The testis of male of toxic group showed loss of spermatogonia (Fig 1). While testis of therapeutic group were showed less severity represented by incomplete spermatogenesis with presence of multinucleated spermatid giant cell in the lumen of semineferrous tubules (Fig 2). The epidydimus of toxic group showed empty of sperms (Fig 3). The uterus of toxic group showed hyperplasia of epithelial lining cells of endimetrium with papillary projection extend to the lumen of the uterus (Fig 4).

Also other section showed eosinophilic homogenous substances appear in the lumen of endometrial gland (Fig5). While in the theruptuic group the lesion showed less severity characterized by moderat hyperplasia of epithelia and presence of protinicious exudates with poly morphonuclear cell and mononuclear cell in their lumen. (Fig 6).

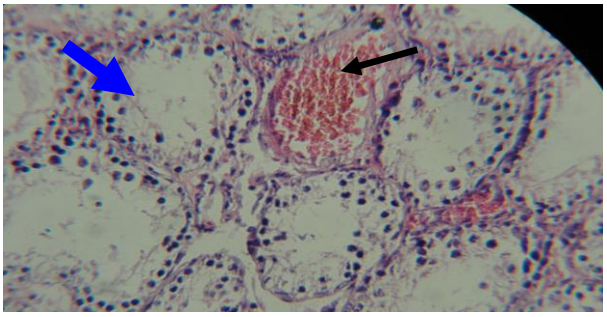
The ovary of toxic group showed increase number of primary and secondary follical with congestion of stromal blood vessels (Fig 7).

Other non target organ like brain, the toxic group showed focal glyosis in both male and female (Fig. 8) and prineuronal odema in other area (Fig 9). While the therapeutic group showed no clear pathological changes.

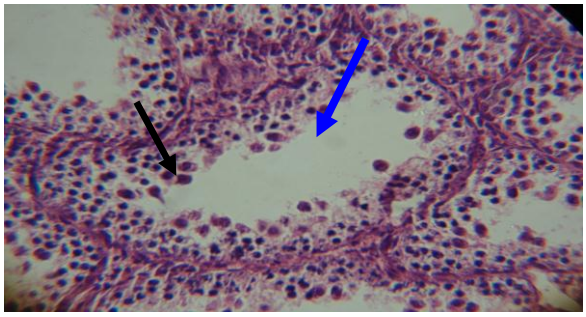
Spleen of toxic group of both male and female showed deplesion of white pulpe with increase number of megakaryocyte (Fig 10).While therapeutic group the spleen appear normal.

Liver of toxic group showed mononuclear cell aggregation in the portal area around bile duct and congestion of blood vesselse with necrotic area by pyknotic of nuclei or dispear (Fig 11). Therapeutic group: liver showed mononuclear cell aggregation in the portal area around bile duct with congestion of blood vessels (Fig 12).

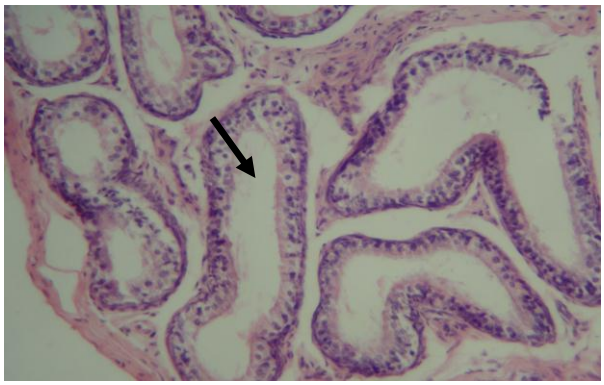
Kidney of toxic group showed hemorrhage of cortical renal tubules (Fig 13). Kidney of therapeutic group showed perivascular mononuclear cell aggregation (Fig 14).



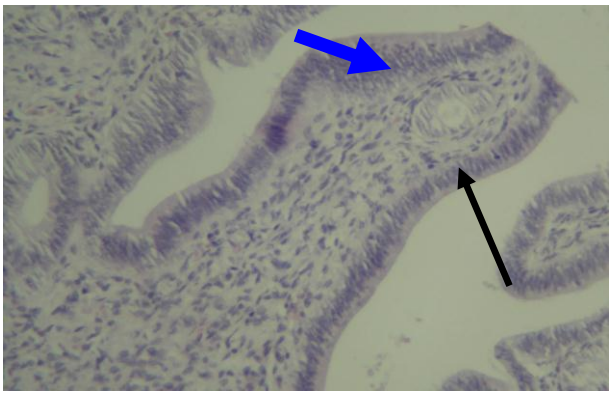
Figure(1): Histopathological section of testis of mice in toxic group treated with (estrogen and progesterone) showed testicular degeneration, and absence of spermatogonia (➡) and congestion of blood vessels(➡) after one month (H&E) 40x.



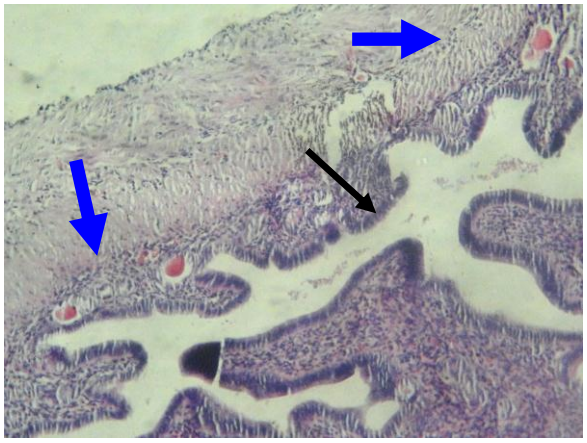
Figure(2): Histopathological section of testis of mice in therapeutic group treated with (estrogen and progesterone)showed incomplete spermatogenesis(➡) with presence of multinucleated giant cell in the seminiferous tubules(➡) after one month (H&E)40x.



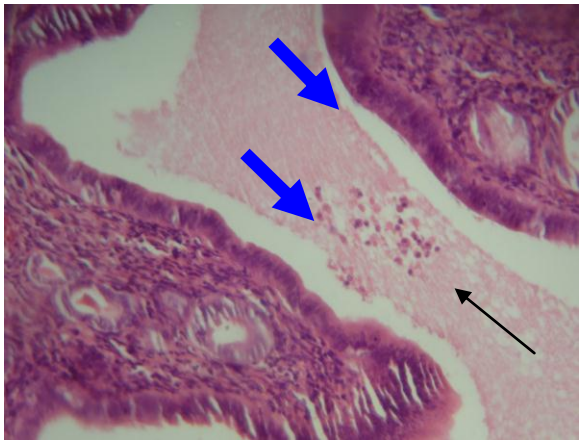
Figure(3): Histopathological section of epididymus of mice in toxic group treated with (estrogen and progesterone) appear testicular degeneration with empty and vaculation of epithelial lining cell(➡) after one month(H&E) 40x.



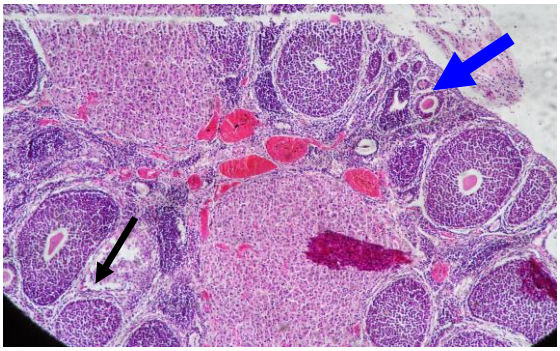
Figure(4): Histopathological section of uterus of mice in toxic group treated with (estrogen and progesterone) showed hyperplasia of epithelial lining cell of endometrium(➡) with papillary projection extend to the lumen(➡)(H&E) 40x



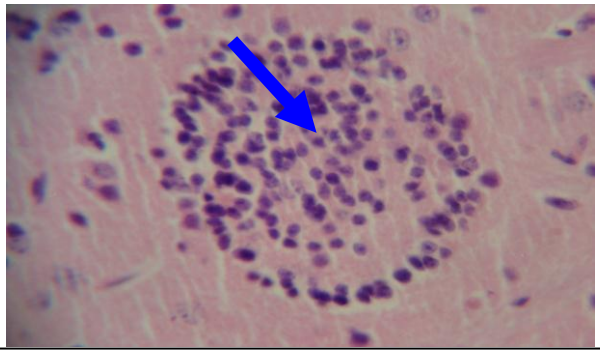
Figure(5): Histopathological section of uterus of mice in toxic group treated with (estrogen&progesterone)showed osinophilic haemogenous substances appear in the lumen of endometrial gland(➡) with papillary projection extend to the lumen (➡) (H&E) 20x.



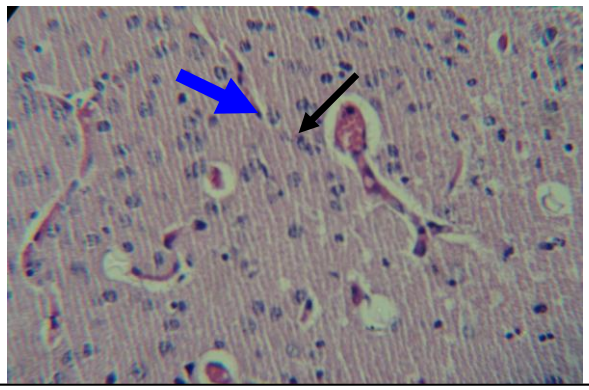
Figure(6): Histopathological section of uterus of mice in theraputic group treated with (estrogen and progesterone)showed protinicious exudate with polymorpho and mononuclear cell in their lumen and sub epithelial layer(➡) with moderate hyperplasia of epithelial lining of endometrium(➡) (H&E) 40x.



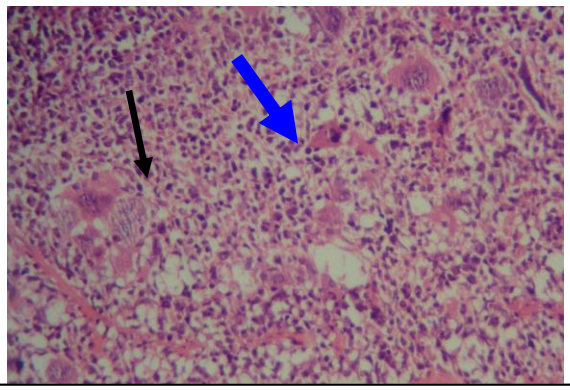
Figure(7):Histopathological section of ovary of mice in toxic group treated with (estrogen and progesterone) showed increase number of primary (➡) and secondary follical(➡) after one month .(H&E)40x.



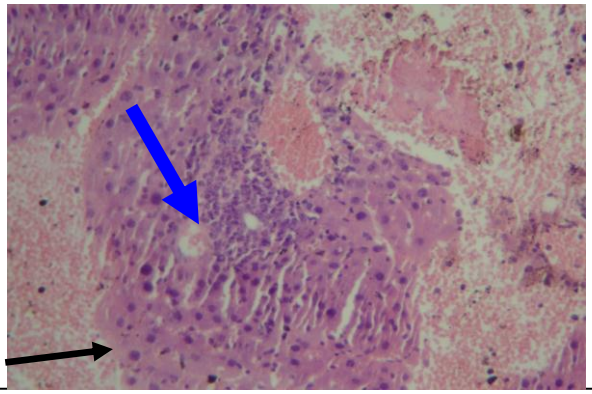
Figure(8):Histopathological section of brain of mice in toxic group treated with (estrogen and progesterone) showed focal glyosis (➡).(H&E)40x.



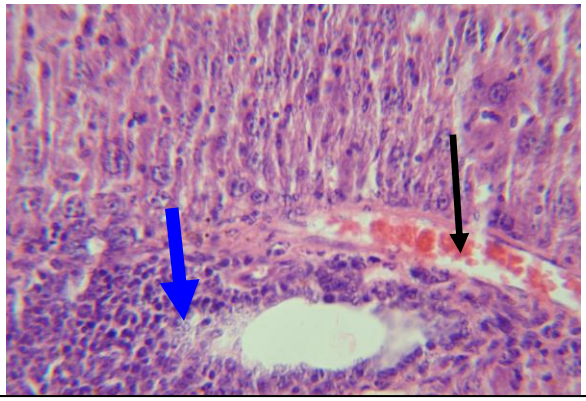
Figure(9): Histopathological section of brain of mice in toxic group treated with (estrogen and progesterone) showed privascular odema( ) with congestion ( ) after one month (H&E) 40x.



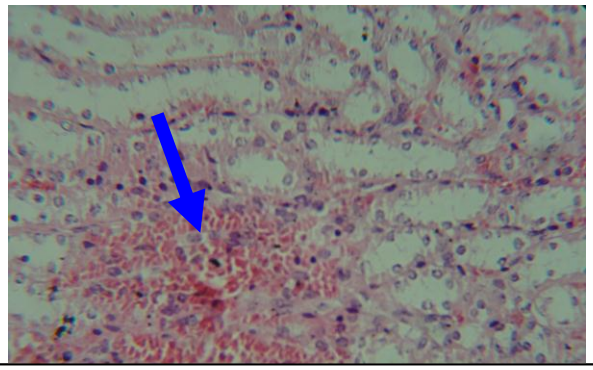
Figure(10): Histopathological section of spleen of mice in toxic group treated with (estrogen and progesterone) showed depletion of white pulpe ( )with increase number of megakaryocyte( ) after one month (H&E) 40x.



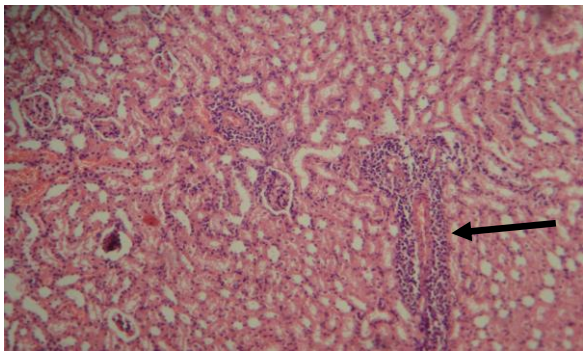
Figure(11): Histopathological section of liver of mice in toxic group treated with (estrogen and progesterone) after one month showed mononuclear cell aggregation in the portal area around bile duct( ) with necrotic area by pyknotic of nuclei or disappear( ) after one month (H&E) 40x.



Figure(12):Histopathological section of liver of mice in thereputic group treated with (estrogen and progesterone) showed mononuclear cell aggregation in the portal area around bile duct( ) with congestion of blood vessel( ) after one month (H&E) 40x.



Figure(13): Histopathological section of kidney of mice in toxic group treated with (estrogen and progesterone) showed haemorhagic of cortical renal tubule(➡) after one month (H&E) 40x.



Figure(14): Histopathological section of kidney of mice in theraputic group treated with (estrongen and progesterone) showed perivascular mononuclear cell aggregation after (➡)one month. (H&E) 40x.

The actions of steroid hormone are thought to be mediated through their binding to steroid receptor (14). Which is divided into two types α and β receptor (15). Present study are focus on the pathological effect of both hormones on the target and non – target organ on both male and female in mice.

The target organ of male represented by the testis in toxic group, the organ showed absence of spermatogenesis, this process result called azoospermia which mean complete absence of sperms. This result was in agreement with (16 and17) whom reach azoospermia after administration with estrogen also. This result was agreed with (18and 19). Which return this result to the effect of high dose of progesterone. They reported that the inhibition of the spermatogenesis may be result from the inhibition of LH and FSH hormones responsible for the activity of sertoli cell leads to suppression of spermatogenesis (azoospermia). Other group (therapeutic group) showed incomplet spermatogenesis with presence of spermatid multinucleated gaint cell, these cell indicating that there is testicular degeneration occurs in the semineferrous tubules of testis. These cells occur after spermatocyte degeneration which appears to be secondary change resulting from distrupt sertoli cell to germ cell association these result was in agreement with (20).

Epidydimus of the toxic group appear empty of sperms, this result occurred may be due to the high doses of both hormones.

Other target organ of the female of toxic group represented by the uterus, the lesion showed hyperplasia of epithelial lining cell of endometrium with papillary projection extend to the lumen of the uterus, this lesion consider preneoplastic change as many author think like (21), who consider the proliferative lesion of uterus epithelial like hyperplasia are preneoplastic (22). The effect estrogen may be occur as a result of progesterone due to high dose of this hormone and the papillary projection of endometrial epithelia lining cell with increase number of endometrial gland. These lesion said to be the classical response to progesterone (23) and progesterone cause increase dilatation of endometrial gland (16). These feature might be attributed to the high number of progesterone receptor present on the endometrial lining to which progesterone bind after diffusion through cell membrance and then bind to progesterone response element in the nucleus resulting in activation of mRNA transcription allowing increase division rate (24and25).

Proliferative phenomena have been assignees as uterine preneoplastic changes are lesion as some scientist (21and 22) mentioned.

In the therapeutic group which has less severity characterized by moderate hyperplasia of epithelid lining cell restricted to the superficial layer of the endometrium epithilia as (23) mention. Koss, (26) consider this lesion as a classical response of this organ to the stimulation caused by estrogen due to high number of estrogen receptor in it.

The ovary of toxic group showed increase number of primary and secondary follicles but never reach the maturation also no ovulation will occure. This occure due to elevated levels of estrogen also effect these growing follical inducing cystic dilatation of them. These result are strongly similar to the result of (16,26 and 27). In addition to that (28) which found that the follicular cyst in ovary of rats of their studies which were receiving environmental estrogen also they got complete absence of corpus luteum. While (29) thought that these follicals produces further estrogen and cause endometrial hyperplasia.

Other non- target organ like brain of toxic group of both male and female showed focal glyosis and perineuronal and perivascular odema. This is return to the presence of progesterone receptor in the glial cell (7). The high dose of progesterone in this group cause proliferation of glial cell (15). They consider progesterone receptor is gene transcription, also the presence of perineuronal odema may be a result from high dose of progesterone. Other author refers to the same thing they refer to the present of estrogen which induced in sizable quantities in the brain (30). While the brain of therapeutic group appear normal without any change.

The spleen of toxic group of male and female showed depletion of white pulpe with increase number of megakargocyte, this result agree with (31and 32). Who suggest that progesterone and estrogen cause inhibition cellular immunity by prolong exposure to both hormones (11). Revealed that progesterone cause depression of lymphoid tissue. Similarly to (33) which found that progesterone inhibit the activity of lymphocyte and monocyte.

The liver of toxic group of both sexes showed mononucleur of poly morphonculer cell aggregation in the portal area around bile duct with congestion of blood vessles with necrotic area characterized by pyknotic of nuclei or dispear while in therapeutic group the lesion showed mononuclear cell aggregation in portal area around bile duct with vacculation of hepatocyte, this occur duo to increase of fat level in the blood(lipidemia)duo to metabolism disturbance of fat and due to formation of lipoprotein in blood circulation (34). While the aggregation of inflammatory cell around the central vein return to the effect of progesterone which cause increase proliferation of leukocyte (35). While necrosis may occur duo to high dose (toxic effect) of both hormones.

The kidney of toxic group of male and female showed Hemorrhagic of cortical renal tubules, this result from high dose of both estrogen and progesterone. While in the therapeutic group the kidney showed preivascular mononuclear cell aggregation this associated with the stimulation of the steroid hormone (estrogen and progesterone).

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