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CYTOLOGICAL AND CYTOCHEMICAL STUDIES ON THE EFFECT OF ERYTHROMYCIN ON THE SPINAL CORD OF RABBIT

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Abstract

Cytochemical analysis of the normal rabbit spinal cord reveals the presence of high concentration of proteins, RNA and carbohydrates. The mitochondria exhibit a strong reaction in the control neurons. An extensive Golgi complex is found in the nerve cell confirming its role in synthesis of the neurotransmitter substances. After oral administration of the antibiotic "erythromycin ethylsuccinate"(EES), at a dose level of 100 and 250 mg/kg.b.w., a reduction in the normal cytological structure of mitochondria and Golgi apparatus, was observed at the high dose.

Cytochemical evidence on the spinal cord of treated rabbits with erythromycin revealed an abnormal reduction of the amount of basophilic substance (Nissl bodies) in some cells, decreased in both amounts and staining properties of mucopolysaccharide and protein in the treated neurons which were obviously concomitant with the dose level.

Introduction

Erythromycin is a macrolide that acts by inhibiting the translocation reaction during protein synthesis. It is bounded to the 50S subunit of 70S ribosomes of susceptible microorganisms (Washington and Wilson, 1985). The presence of peptidyl-RNA on ribosomes decreases, at high dose, erythromycin can freeze polyribosommes in vivo (Chinali *et al.* 1988 b).

Erythromycin diminishes absorption of intestinal neutral amino acid (Navarrow *et al.*, 1992) and D- galactose sugar (Navarrow *et al.*, 1993) by inhibiting the Na+ - dependent transport located in the brush border of the enterocyte. Carbohydrates were found to undergo certain alterations accompanying pathological circumstances (El-Beih *et al.*, 1992). Also, it has been foun that ototoxicity is associated with the administration of erythromycin, which characterized by bilateral high frequency sensorineural hearing loss (Brummett, 1993). Hiller *et al.*, (1990) reported that 200mg of erythromycin in children of 8 years old caused unconsciousness.

It is known that erythromycin base is able to penetrate biological membranes and accumulate in many eucaryotic cell types, mostly concentrating in cytosol and lysosomes and small amount is accumulated in the fractions followed the order mitochondria > nuclei > microsomes (Villa *et al.*, 1988). Erythromycin diffuses readily into intracellular fluids. All tissues except the brain contain higher concentrations than in the blood, and the drug persists for some time in the tissues after it is no longer demonstrable in the circulation. The antibiotic diffuses into pleural and peritoneal fluids. Cerebrospinal fluid levels in individuals with normal meninges are 1/8 to 1/64 those in the plasma when EES is giving orally and are somewhat higher after parental therapy. In the presence of meningeal inflammation, the concentration of the blood in the spinal fluid is frequently high enough to eradicate the pneumococcus and the staphylococcus (Goodman and Gilman 1985).

A review on the subject of the effect of erythromycin on normal spinal cord shows that although a huge number of publications appeared, the majority is pharmacological and physiological interpretations. Very little attention has been paid to the cytological and cytochemical changes following erythromycin treatment. Al-Rawi (2001) studied the histological and ultrastructural changes induced by erythromycin in the spinal cord of rabbits.

Therefore, the present work was planned aiming at examination of the possible responses of mammalian spinal cord regarding their contents of essential cytological and cytochemical components, namely mitochondria, Golgi apparatus, basophilic substances, total proteins and general charbohydrates.

Material and Methods

Experiments were designed to study the effect of erythromycin ethylsuccinate (EES) on the spinal cord of immature female New Zealand white rabbits (*Oryctolagus cuniculus*). The present study was the performed on 18 female rabbits aged about 3-4 months old and weighting approximately 1.5-2.2 kg. The animals were housed in the usual stainless steel cages with wire mash floors over absorbent paper and were maintained under a control environment. The fresh commercial preparation of erythromycin ethylsuccinate (Abbott Laboratories,USA) was used in this study. Rabbits were divided into three groups of 6 animals in each. The experimental animals were given different doses of erythromycin orally for 21 days:

Group (1): Animals were given 150mg/kg b.w./day.

Group (2): Animals were given 300mg/kg b.w./day.

Another group of animals of similar age and weight were used as control and received no treatment.

Animals were dissected after 21 days, their spinal cords were removed and portions of it were fixed in several fixatives including Aoyama's fluid, Regaud's fluid, Carnoy's fluid and 5% neutral formalin. The tissue was then embedded in parrafin wax and sectioned (2 mμ thick). For detectionn of mitochondria, sections were stained with Heidenhain's iron haematoxylin. The Golgi apparatus was demonstrated by impregnating the material with silver using Aoyama's technique (cf. Gatenby and Beams 1950). RNA basophilia was demonstrated by the Borret's methylene blue method. The periodic acid Schiff's (PAS) method was used for mucopolysaccharides. Total proteins were demonstrated by mercury bromophenol blue (Pearse 1985).

Results

Cytological results:

Mitochondria:

The perikaryon of control neuron contains numerous mitochondria interspersed throughout the Nissl substance. The mitochondria have the form of granules of different size, short rods and filaments distributed in the perikaryon (Fig.1).

Examination of neurons of rabbits in group 1 showed that the mitochondria were within the normal control limits.

Cytological examination of neurons of group2 showed decrease in mitochondria reaction while certain areas of the cytoplasm given the appearance of dense accumulations of large mitochondria with the form of roads and spheroids, displaying various degrees of mitochondrial degeneration (Fig. 2).

Golgi apparatus:

In the control group, Golgi apparatus normally appeared in the form of large perinuclear masses, strongly positive Aoyama- silver nitrate reaction (Fig. 3).

There was, however, a marked decrease in the quantity of argynto masses displayed by faint stained perinuclear masses which was observed in group 1 (Fig. 4) and in group 2 (Fig. 5).

Cytochemical results:

Basophilic substances:

Sections from spinal cord manifested a rather strong reactivity for Nissl bodies (RNA) basophilic substances with Borret's methylene blue, indicating their richness in these inclusions (Fig. 6). The nucleolus was

strongly basophilic i.e. major portion of RNA was concentrated in the nucleolus.

In rabbits of group 1 the concentration of the basophilic substance in most neurons was more or less similar to normal (Fig. 7, Table 1).

In rabbits of group 2, peripheral concentration of RNA was noted in the enlarged neurons and neuroglia cells, while their nuclei were appeared nearly transparent (Fig. 8).

Total proteins:

Using mercury bromophenol blue staining (Hg-BPB), it was found that the bodies of neurons of control rabbits were characterized by a high concentration of total protein (Fig. 9). The reaction in the cytoplasm, appeared in the form of fine homogeneously distributed protein granules.

The amount of protein in the neurons of the treated rabbits in group 1 decreased more than in the control and protein appeared in aggregated granules in the cytoplasm (Fig. 10). This decrease was more pronounced in group 2 (Fig. 11).

General carbohydrates:

Carbohydrates were generally high in the spinal cord of control neurons. The cytoplasm gave a strong positive diffuse reaction with PAS, indicating the presence of mucopolysaccharides (Fig. 12).

In the neuron cytoplasm of group 1, the amount of diffuse mucopolysaccarides was more or less similar to that described in the control neurons (Table 1).

After EES treatment in group 2 there was a decrease in the amount of mucopolysaccharides (Fig. 13).

Discussion

Cytochemistry is rarely applied in Pharmacology and Toxicology. McGinty *et al.* (1973) stated that cytochemical methods could facilitate the diagnosis of functional imbalance, which cannot be identified by morphological criteria.

In the present study, erythromycin was found to cause many cytological changes include decrease in the mitochondrial amount as well as in Golgi apparatus and this decrease which is dose dependent. In this concern, Villa *et al.* (1988) reported that erythromycin induced a defect in the inner mitochondrial membrane and caused contamination for all fractions in hepatocyte culture with approximately 3 and 12% of lysosomes and mitochondria. Jeffrey *et al.* (1987) determined the cytotoxicity in the perfused rat liver with various concentrations of erythromycin, and found that it increased the number of secondary lysosomes and caused enzyme leakage with presence of degenerating cells with swollen mitochondria.

Golgi apparatus is an organelle of importance in neurons as it synthesises neurotransmitter substances or their precursors in the perikaryon from where they are transported along the axon to the synapse to be released when appropriately stimulated (Burkitt et al., 1993). Yousif and Al-Rawi (2000 a & c) found a decrease in stainability of Golgi apparatus in epithelial cells of EES treated rabbit jejunum and cardiac muscle fibers. Similar results were obtained in the mouse liver after treatment with EES by Al-Rawi (2000).

RNA is a universal constituent of all living cells, and has a role in protein synthesis (Casperson, 1964). Investigations have shown a correlation between RNA concentration and protein with changes associated in tissue damage. Rodgers *et al.*(1990) reported that erythromycin acts as an inhibitor of protein and DNA synthesis, it possessed irregular membranes which were partially or fully lysed. Chinali *et al.* (1988 b) indicated that premature release of peptidy- tRNA accounts for only part of the inhibitory action of erythromycin in cell-free system. This inhibition of erythromycin was partial and remained constant during the amino-acid polymerization reaction. This could be due to a rapid and irreversible inhibition of a fraction of translating ribosomes.

In degeneration of the central nervous tissue in different pathological conditions, Mackey *et al.* (1964) and Barron *et al.* (1967) reported about freeing of ribosomes into the cytoplasm either as free granules or as clusters. The present results confirm these findings that RNA basophilic substances in the neurons treated with high dose of EES was reduced and scattered in the perkaryon. Moreover total protein was reduced in EES-treated cells. In agreement with this result, Sidransky *et al.* (1986) suggested a rapid disaggregation of polyribosomes associated with inhibition of protein synthesis.

Mandal *et al.* (1982) recorded that the inhibition of intra-mitochondrial protein synthesis by some antibiotics produced a decrease in protein and RNA content. Turska *et al.* (1977) declared that the inability of mitochondrial translation by antibiotic chloramphenicol, causes inhibition of incorporation of proteins into the mitochondrial structure.

EES markedly stimulated a release of peptidyl-tRNA from the ribosomes, producing a 50% inhibition of amino-acid incorporation into total peptides and a 95% repression of the synthesis of long peptide chain (Chinali et al., 1988a). A premature release of peptdyl-tRNA from translating ribosomes was proposed as the mechanism responsible for the inhibitory action of erythromycin on protein synthesis. These findings are in line with those of Chinali et al. (1988b) and Carvalho et al. (1995) who demonstrated the higher inhibition of EES produced by in vivo than in vitro,

a transient inactivation of ribosomes which is expected to produce an inhibition of polypeptide synthesis that increases with the reaction rate.

Another conspicuous cytochemical alternation recorded in the present experiments included the decrease amount of mucopolysuccharides in the neurons of the treated rabbits.

These findings obviously confirm the results achieved by Al-Rawi (1998) who demonstrated a marked decrease in mucopolysuccharides in the small intestine (Yousif and Al-Rawi, 2000b) and cardiac muscle fibers (Yousif and Al-Rawi, 2000d) on EES treated rabbits. On the other hand, EES with different molecular structures has been described as an inhibitor sugar and amino acid transport (Washington and Wilson, 1985; Navarro *et al.*, 1993). Fever *et al.* (1966) attributed glycogen depletion to the increase lysosomal activity under such pathological consequences. Glycogen depletion progressed hand in hand with elevated glucose-6- phosphatase activity leading to marked glycogenolysis (Abdel Raheem *et al.*, 1987).

The conclusion which may be drawn is that the macrolide antibiotic, erythromycin exerts an excitatory effect on the cytochemical component RNA, protein and total carbohydrates of rabbit spinal cord.

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Table (1): Cytological and Cytochemical analysis of the spinal cord of normal and erythromycin treated rabbits.

Group	Cytological analysis		Cytochemical analysis				
	Mitochondria	Golgi apparatus	Borret's methelene blue		PAS- reaction	Bromophenol blue	
	Cytoplasm	Cytoplasm	Cytoplasm	nucleus	Cytoplasm	cytoplasm	nucleus
Control	+++	+++	++++	++	+++	+++	++
Group1	+++	++	+++	+	+++	++	+
Group2	++	+	++	+	++	+	+

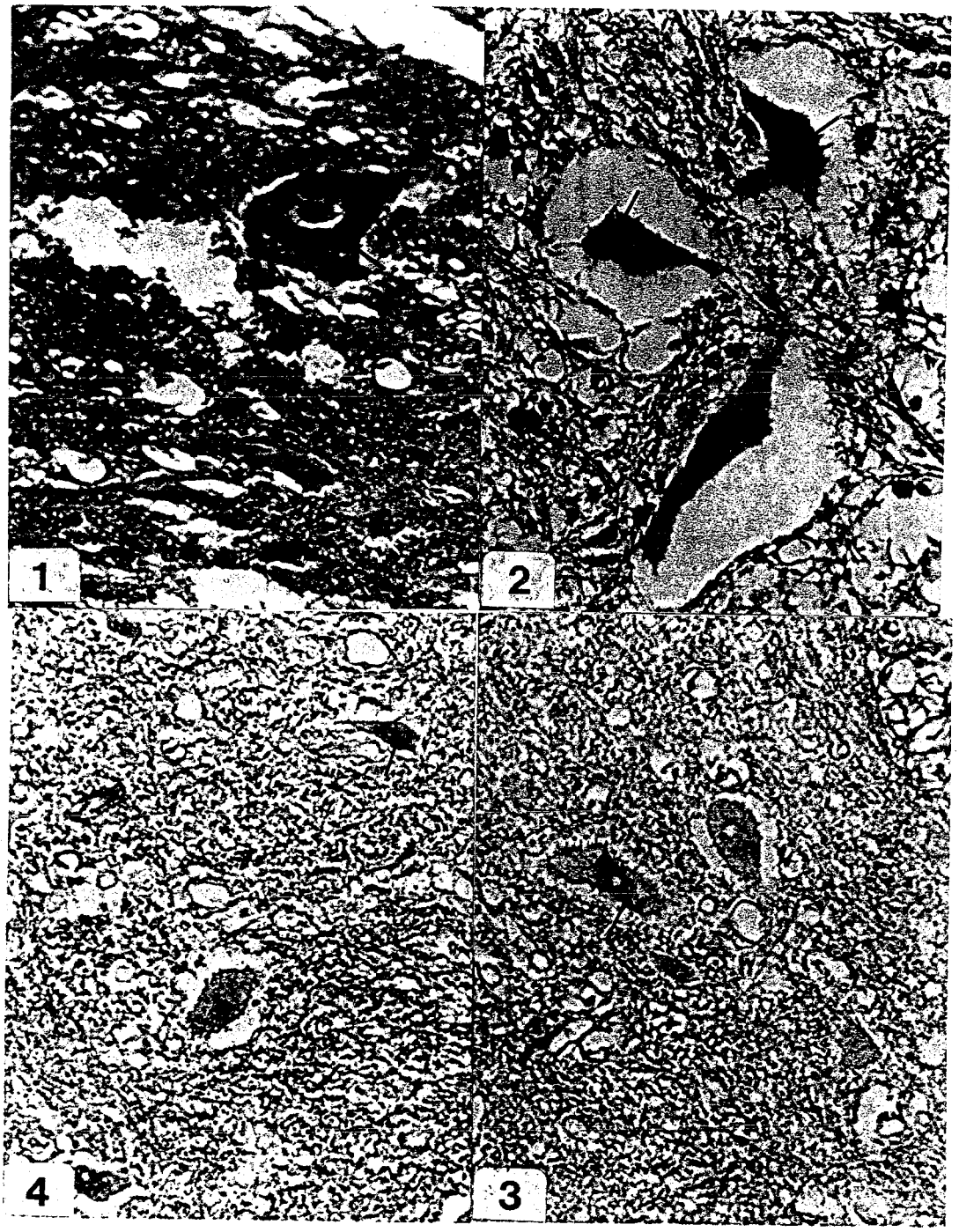
List of Figures

Fig. (10): T.S. EES-treated spinal cord (group 1), showing slight decrease in protein content and appeared in aggregated granules in the cytoplasm of neuron (Hg-BPB, x 400).

Fig. (11): T.S. EES-treated spinal cord (group 2). Note pronounced decrease of total protein more than in the control of the neurons (Hg-BPB, x 400).

Fig. (12): T.S. control spinal cord. The cytoplasm gave a strong positive diffuse reaction, indicating the presence of high mucopolysaccharides content in neurons and axons (arrows) (PAS-reaction, x 400).

Fig. (13): T.S. EES-treated spinal cord (group 2). Note the decrease in amount of mucopolysaccharides in the cytoplasm of the neurons (PAS-reaction, x 400).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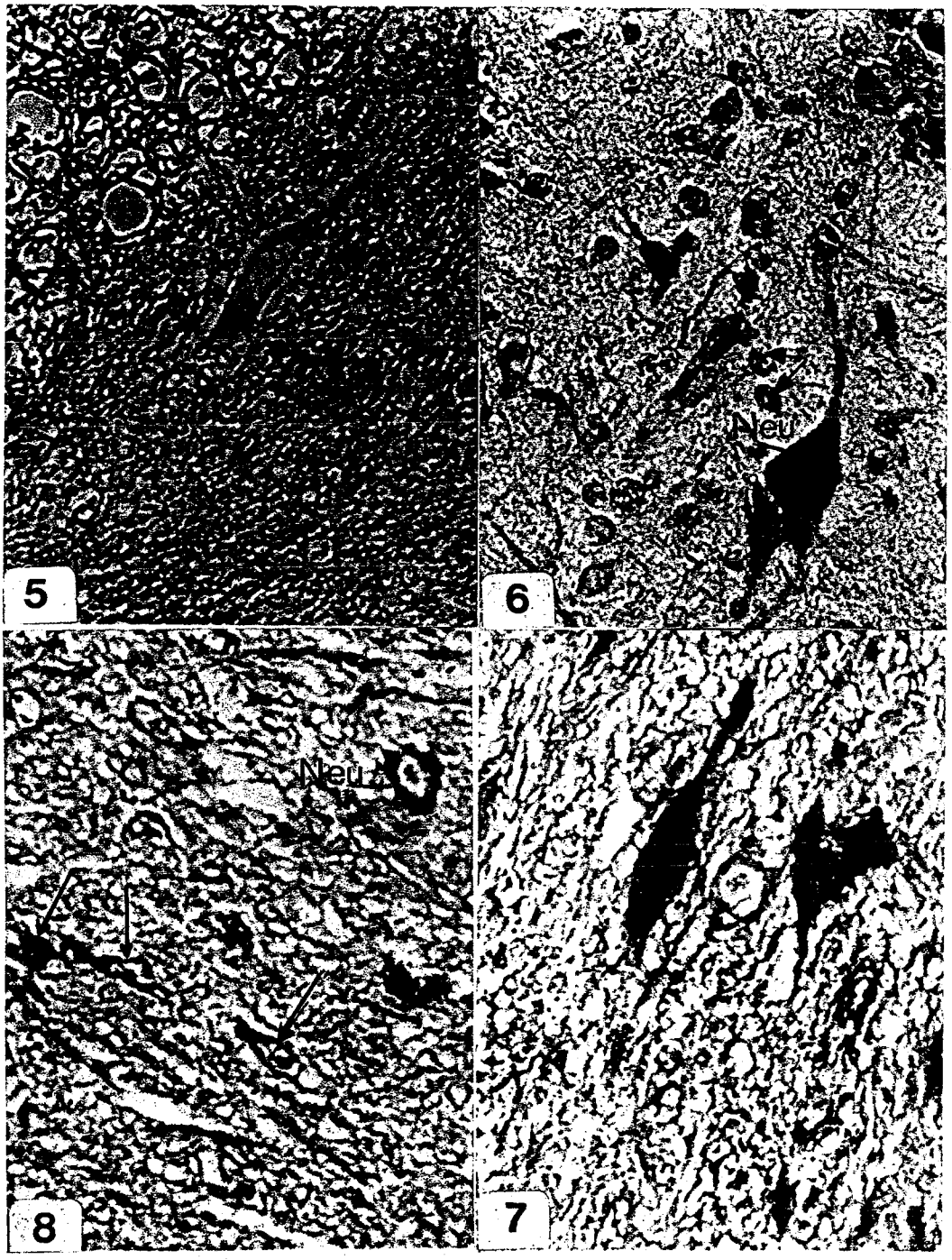
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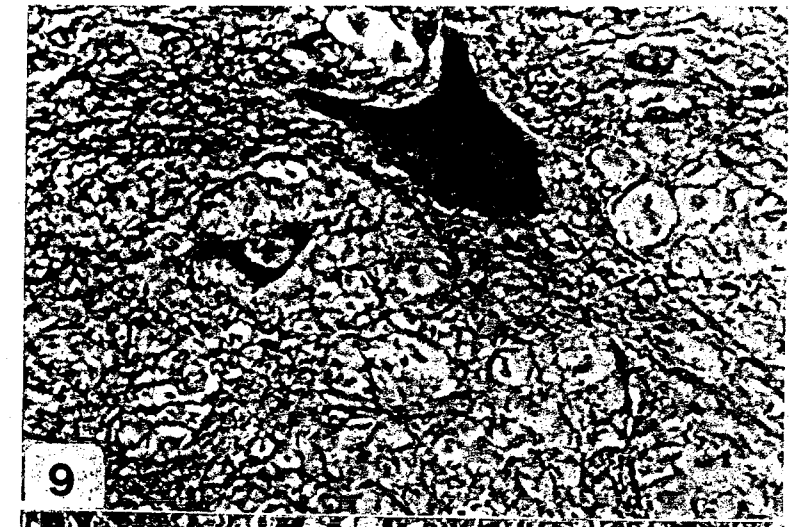

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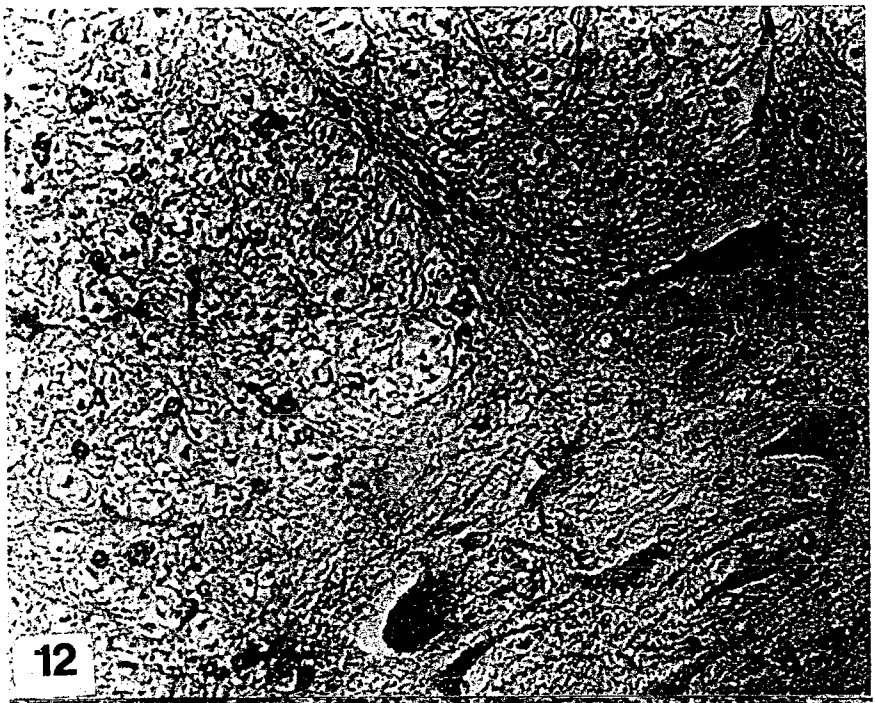
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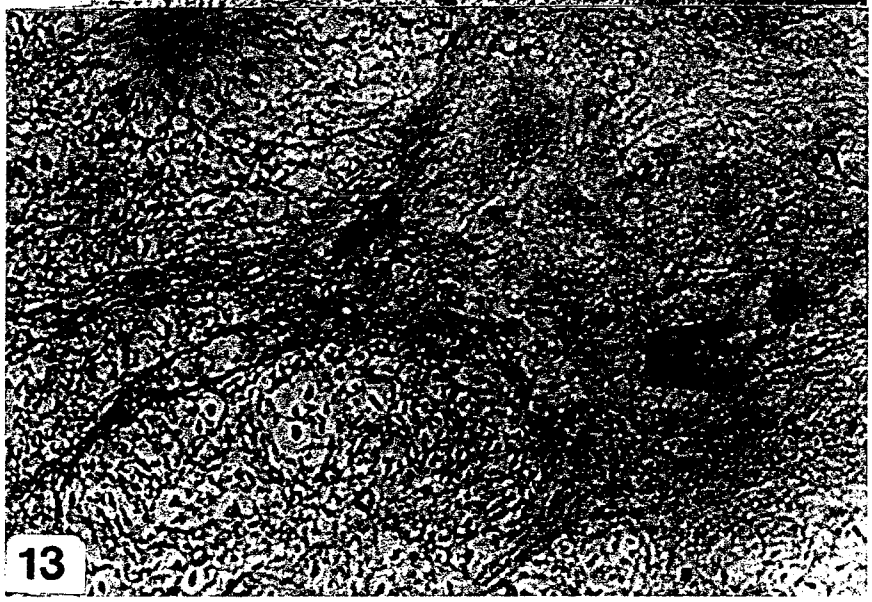
13

الملخص العربى

دراسات خلوية وكيميائية خلوية على تأثير عقار الإرثروميسين على النخاع الشوكي للأرنب

ميســـاء الراوي

قسم العلوم ــ كلية التربية للبنات ــ مكة المكرمة

أجريت هذه الدراسة لغرض معرفة التأثيرات الجانبية التي تحدثـــها الجرعـــات المعطاة عن طريق الفم من المضاد الحيوي الإرثروميسين لمـــدة ٢١ يومـــاً علـــى النخاع الشوكي للأرانب النيوزيلندى الأبيض. أحدث المضاد الحيـــوي نقصـــاً فـــي محتوى كلٍ من الميتوكوندريا وجهاز جولجي. أظهرت التفاعلات الكيميائية الخلويـــة تغير ونقص في المادة القاعدية في سيتوبلازم الخلايا العصبية ونقص في محتـــوى البروتين بها، كما نتج نقص في كمية السكريات المخاطية العديدة.

والجدير بالذكر أنه كانت هناك علاقة وثيقة بين مدى هذه التغـــيرات المرضيـــة وبين مقدار الجرعة المعطاة من هذا العقار.