HISTOPATHOLOGICAL STLUDY 0F RECTAL MUCOSA IN ACUTE DIARRHOEA

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INTRODUCTION

Acute diarrhoea is a major public health problem especially in tropical countries. However, the related aetiology is controversial. While Maiya et al.,(1975) and Black et al.,(1980) attributed the cause of acute diarrhoea to the prevalence of bacterial enteric pathogens in such countries, Mata et al,(1965) roported frequent cases that revealed the presence of such bacterial enteric pathogens in asymptomatic people.

At the same time, little is known about the rectal biopsy findings in acute diarrhoea. Therefore, the aim of this study is to describe the histopathological findings of rectal mucosa in patients with acute diarrhoea in an attempt to correlate the histological and clinical features hoping to determine the extent to which information obtained from rectal biopsy might influnce the diagnosis or management of these patients.

METERIAL AND METHODS

The material of the present study comprised 25 patients complaining from acute diarrhoea (defined as 3 or more loose stools per day for 6 days or less). Their ages ranged from 20 to 55 years. None of the patients had taken antibiotics in the preceding period.

All patients were subjected to the following investigations :

I) Assessment of the clinical se-

verity of illness taking into account the duration of diarrhoea, number and volume of stool, extent of dehydration, vomiting, toxicity, blood pressure...

2) Microbiological Investigations:

Faecal specimens were collected from the patients with diarhoea in sterile screw copped bottles "Bijo bottles and transported immediately to the microbiology department to be examined.

Cultivation and isolation :

The foecal specimens were cultured on the following media.

Mac-Conkey Agar, salmonella and shigella agar and thio sulfate-citrate - Bile salts - surose (TCBS) agar. They were inoculated into selenite-F broth and alkaline peptone water and subcultured on to the solid media used above after incubation at 37°C overnight. (Cruickshank et al. 1975).

The isolated colonies were identified in systematic manner including cultural characters and colony morphology, micro-scopical films, biochemical activities using the (Enterotube) II (Roche) for identification of the isolated strains and sero typing using the agglutinating Coli test sera to identify the enteropathogenic strains of E.coli (Behringwerke).

3) Sigmoidoscopy :

For the presence or absence of oedema, visible vascular pattern and of contact bleeding with or without ulceration. The appearances were graded as normal or abnormal.

4) Rectal biopsy :

This was taken before treatment with Chevalier Jackson forceps at approximately 8-11cm. from the anal margin. The biopsies were fixed in 10% neutral formaline. After routine processing and embedding in paraffin, sections were cut and stained with Haematoxylin and Eosin for histopathological examination.

RESULTS

I) Clinical Severity :

3 patients were judged to have a mild illness, 6 were moderately ill and 16 had a severe illness.

2) Microbiological Investigations:

9 patients revealed enteropathogens. These were considered to have infective diarrhoea. The remaining stool cultures were negative.

It has been noticed that there was no correlation between the microbiological investigation and the clinical severity of the illness.

3) Sigmoidoscopy :

3 cases showed ulceration.

4) Rectal biopsies :

Normal appearance of the mucosal acini with or without minimal laminal oedema were present in 10 patients. The remaining cases revealed mucosal ulceration and or erosion (present in 5 cases). Mixed infiltrate of acute and chronic inflammatory cells in superficial lamina propria was noticed. stricking alterations in some blood vessels in the lamina propria were congestion with stagnation of red blood cells and fibrin strands in the lumen suggesting intravrascular coagulation. Platelets aggregatlons was prominant in many of these vessels. The endothelial lining of these blood vessels was markedly swollen and in many areas the endothelium was denuded. Free haemorrhage into the lamina propria was present. These vascular changes occurred in patients with infective diarrhoea and in patients with negative Stool Cultures. Also, there was no correlation between these vascular changes and mucosal ulcerations detected at sigmoidoscopy or in the biopsy. On the other hand, good correlation was present between vascular changes and the clinical severity of illness, as they were present in one patient with mild illness, 4 out of 6 moderately ill patients and in 12 out of 16 severely ill patients.

DISCUSSION

The infomations about the histological appearance in acute diarrhoeas are still too limited to provide a basis for use of rectal biopsy in diagnosing such conditions, (Moroon and Dawson, 1972 and Day et al., 1978).

In the present study, inflammatory cells were detected in the lamina propria of the rectal mucosa, a finding

which of unknown significance as there are no adequate studies of normal control subjects . The apparent increase in the number of chronic inflammatory cells in the lamina propria of rectal mucosa may merely represent normal variation in mucosal cell populations Morson and Dawson, 1972). An alternative interpretation was given by Dronfield et al. (1974). They claimed that this finding may be pathological and may represent a significant inflammatory rosponse.

The present study demonstrated the presence of vascular endothelial changes. These were in the form of swelling and focal atrophy of the endothelial cells. Some of these vessels were occluded by thrombi, A finding consistent with that reported by Hjort and Rappaport, (1965). These vascular changes are due to bacterial endotoxin and immunological reaction consuming complement. This excplanation is strongly cofirmed by an experimental induction of similar changes (Called LSR) by an intravenous provocative dose of bacterial endotoxin administered in rabbits sensitised by intradermal injection of a small dose of endotoxin given 24 hours earlier (Taichmall, 1971). The 2nd explanation is that these changes were initiated by the increased peristalsis and intraluminal pressure. These could introduce the bacterial pathogens from the lumen of the rectum mechanically into the lamina propria and their lysis causes release of endotoxin in the close vicinity of blood vessels, with production of these changes (Sundsmo and Fair, 1983).

A third esplanation could be that the initiation of diarrhoea is dependent on destabilization of the epitheliolumi-nal interface. This facilitates the entery of bacterial pathogen to the lamina propria. Alternat.vely this epitheliolu-minal interface destabilization Increases the absorption of free endotoxins from the lumen which may result in the vascular lesions, (Minnie et al. 1985),

Whether destabilization of the epi-thelioluminal interface is a causative factor in the pathogenesis of diarrhae or not. It needs further study to

investigate such possibility.

All these explanations need the presence of bacterial pathogens or their toxins. However, in the present study these vascular losions were present with equal frequency in patients with bacterial pathogens and in whom with no detactable bacterial pathogen therefore, we attribute the vascular ohanges in acute diarrhea to a merely traumatic effect produced by the increased peristalsis, This is confirmed by the prevalence of the vascular changes in patients with more serious clinical illness as detected in the present study. Thereforoe vascular lesions are the results of diarrhea and not a cause.

The present study demonatrated the presence of free haemorrhage into the lamina propria in the rectal mucosa. we suggest that this finding is due to the endothelial cells damage.

From the above mentioned data we concluded that since the epithelioluminal interface of the gastrointestinal tract is a primary barrier between the internal and external environment, its integrity is essential for the maintenance of health. In acute diarrhea this barrier is affected eithor primarily or secondarily.

Factors which control the stability of this barrier are not known and their understanding may be a key for plaining effective means to manage and prevent diarrhae.

Summary

Acute diarrhoea is a major public health problem, however the related aetiology is controversial. This study was done upon 25 patients complaining of acute diarrhoea, their age ranges from 20 - 55 years, all were males. All patient were subjected to clinical microbiological and sigmoidoscopic study. Rectal biopsy was taken from every patient and subjected to histopathological examination.

The most important change was the vascular lesion which occur in all patients with +ve and -ve stool culture.

These changes include endothelial cell swelling , focal atrophy and some vessels were occluded by microthrombi. These changes may be due to destabilization of the epitheliolumenal membrane which normally acts as a barrier between the internal and external environment of the gut. Our conclusion is that the underlying cause of acute diarrhea is at least partly primary or secondary affection of epitheliolumenal barrier.

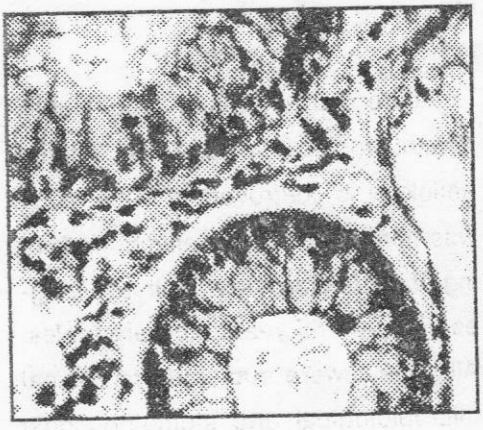

Fig. I : Showing normal glands with relative increase of inflammatory cells in the lamina propria. The capillary shows endothelial cell hyperplasia & starting microthrembi formation (H X & E. X 200).

Fig. 2 : Microthrombi in the capillary showing endothelial cell swelling. The thrombi formed of fibren,threads' platelets & R.B.C s (H X & E. X 200).

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دراسة هستوباثولوجيه للغشاء المخاطى للمستقيم فى حالات الاسهال الحاد

الغرض من هذه الدراسة معرفة التغيرات الهستوباثولوجيه فى القولون فى حالات الاسهال الحاد وذلك لقلة المعلومات الباثولوجيه فى مثل هذه الحالات .

اجريت هذه الدراسة على ٢٥ مريضا اعمارهم مابين ٢٠ - ٥٥ سنه وكلهم من الذكور .

وقد تعرض كل منهم للفحص الاكلينيكى والميكروبيولوجى ثم اخذت عينه بالمنظار الشرجى لكل منهم . وقد تم فحص كل الحالات هستوباثولوجيا ووجد تغيرات من اهمها زيادة عدد الخلايا الالتهابيه بالغشاء المخاطى للقولون .

وكانت التغيرات فى الشعيرات الدمويه من اهم التغيرات التى حدثت حيث تورمت بعض الخلايا المبطنه للشعيرات الدمويه وضمر البعض الاخر كما تكونت جلطات ميكروسكوبيه صغيره بهذه الشعيرات وقد كان سبب هذه التغيرات هو وجود الاندوتوكسين الخاص بالبكتريا والذى دخل الى الغشاء المخاطى عن طريق عدم ثبات الغشاء المخاطى .

وقد ثبت من الدراسة أن الغشاء (Epith elioluminal) فى الجهاز الهضمى يعد الحاجز الاولى بين البيئة الخارجية والداخلية. كما أن عدم ثبات هذا الغشاء هى المسئولية عن الاسهال سواء تأثر تأثراً أولياً أو ثانوياً وان الاسهال ينتج عن عدم ثبات هذا الغشاء سواء تأثر تأثراً أولياً أو ثانوياً.

However, this finding is contradictory to that is observed by Mustafa (1990) who reported that snake venom depresses the immune system and causes lysis of the lymphocytes in both rats and humans.

The hearts of the Cerastes cerastes envenomated guinea pigs showed swollen cardiac muscle fibers with cloudy cytoplasm with 0.5 ug/gm body weight of venom . Dilated congested blood vessels and paravascular round cell infiltration were added with the dose of 1 ug/gm body weight of Cerastes cerastes venom These cardiac changes coincided with the results of Unkovic - Cvetkavic et al. (1983), who mentioned that the cardiac muscle fibres contained disrupted contractile elements and nuclei were rarely seen indicating parenchymal degeneration of the myocardium. Also he observed great dist ance between fibrils that indicates oedema of the cardiac fibres. In addition, loss of transverse striation in focal areas of the swollen muscle fibers with haemorrhages in between were observed with 1.5 and 2 ug/gm body weight of venom. These results were in agreement with the observations of Tu and Homma (1970). After repeated administration of small doses of Cerastes Cerastes venom, mild swollen muscle fibers with loss of muscle striation and fatty infiltration of the cardiac muscle together with round cells were seen.

Petkovic et al. (1979) Stated that the degenerative changes may be responsible for the arrest of the heart . In the present investigation , it is being assumed that a very toxic cardiotoxin is contained in the venom of Cerastes cerastes which is responsible for all these pathological changes.

The results of the present study revealed no histo pathological changes in the brain in either administration of single different doses or repeated small doses of the venom. This is coincided with the results of Gitter et al. (1962) and is attributed to the fact that the snake venom is devoid of neurotoxins (Labib et al.,1979).

Timperly (1968) reported that alkaline phosphatase is an essential

enzyme for transport process across membranes so, it occurs frequently at the sites of active transport such as blood capillaries and sinusoids as well as cellular plasma membranes.

The Present work demonstrated that the activity of this enzyme was slightly increased with a small dose (0.5 ug/gm body weight) of Cerastes cerastes venom. This finding may be denoting augmented transfer across membranes as an attempt from the enzyme to spare the cells from toxic induced damage.

With increasing doses of Cerastes cerastes venom, alkaline phosphatase activity decreased in all organs except the brain which remained unchanged. Mohamed et al. (1975) reported that inhibition of alkaline phosphatase activity may be due to structural changes in the organs with altration of the sites of location. Moreover we speculate that this decrease may be due to direct toxicity of the venom. The observed decreased activity in the liver starting in the periphleral hepatocytes may be attributed to the distribution of hepatic blood supply.

Alkaline phosphatase activity remain unchanged in the brain confirms the absence of histopathological changes in the present study and both are attributed to deficiency of the venom from neurotoxin, (Labib et al., 1979).

It has been shown that in the present study administration of antivenom after the venom markedly minimized the toxic induced changes in different organs. A finding which is consistent with that of Rosenfeld, (1971) and Reid, (1972) who stated that in snake bite poisoning , specific antivenom is the most important therapeutic agent to neutralize the venom.

Summary and Conclusion :

This work is an attempt towards a better and clear understanding of the effect of snake venom (Cerastes cerastes) on the different tissues of the experimental animals. Assessment of the results occurring after the use of antivenom in the

treatment of envenomation was done.

The study includes the following groups :

Group I :

To demonstrate the effect of single different doses (0.5, 1, I.5 and 2 ug/gm body weight) of Cerastes cerastes venom. The different organs taken (Livers, kidneys, spleens, lungs and heart) showed marked histopathological and histochemical (alkaline phosphatase) changes which their degree being proportional according to the dose given. On the other hand, the brain was the least sensitive of all the organs examined.

Group II :

To demonstrate the effect of repeated small doses (0.5 ug/gm, body weight) of Cerastes cerastes venom every other day for six doses on the different tissues of guineapigs . The same changes reported with group I were noticed but to a lesser extent.

Group III :

To demonstrate the effect of polyvalent antivenom after the venom. The toxic - induced changes were markedly reduced.

It is to be concluded from this study that single different doses of Cerastes cerastes venom have both histopathological and histochemical effects on different tissues and the degree of these effects being proportional to the dose given. Repeated small doses have a lesser effect.

It is recommended that the appropriate antivenom should be given to prevent or reduce the damaging effect of the venom.

Table (1) : Show the death rate in different groups .

Group of Animal	No. of Animals	No. of Death	Percentage
Group I :			
1st subgroup.	10	-	-
2nd subgroup.	10	-	-
3rd subgroup.	10	2	20%
4th subgroup.	10	7	70%
Group II :	10	-	
Group III :	10	-	

Table (2) : Histochemical (Alkaline Phosphatase) Effect of Single Different And Small Repeated Doses of Cerastes Cerastes Venom.

Experimental Organ	Control	Single Doses				Repeated Doses
		0.5 Ug/g	1 Ug/g	1.5 Ug/g	2 Ug/g	
* Kidney :						
- Cortex :						
. Glomeruli .	-	+	+	-	-	-
. Tubules .	+++	+++	+++	++	+	++
- Medulla.	-	-	-	-	-	-
* Liver :						
- Central hepatocytes.	-	+	+	-	-	+
- Peripheral hepatocytes	-	+	-	-	-	-
* Heart.	+	++	+	+	+	++
* Lung .	+	++	+	+	+	+++
* Spleen :						
- Lymphoid follicles.	+	++	+	+	-	+
- Red pulp .	+++	+++	++	++	+	++
* Brain .	++	++	++	++	++	++

\- : Negative.
\+ : Mild activity.
++ : Moderate activity.
+++ : Strong activity.

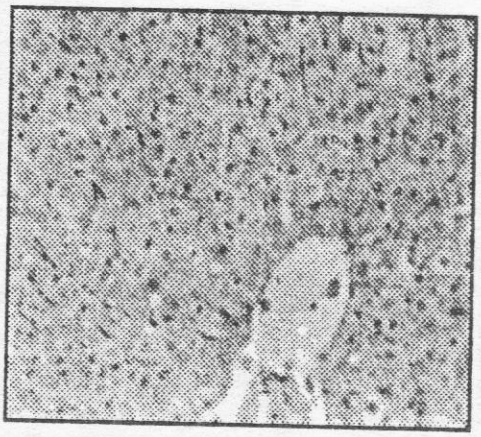

Fig. 1 : Section of liver of guinea pig received 0.5 ug/um. of Cerastes Cerastes (C. C.) venom, showing granularity and vacuolization of the hepatocytes with dilatation of the central vein (Hx. & E. x 4o).

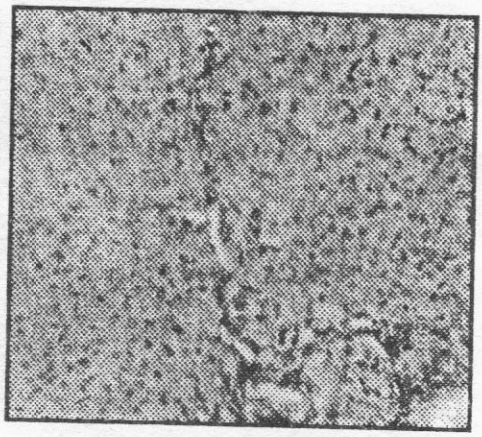

Fig. 3 : Sction of liver of guinea pig received repeated small doses of C. C. Venom, showing proliferation of the bile ducts in the portal tracts with round cells infiltration (Hx. & E. x 40).

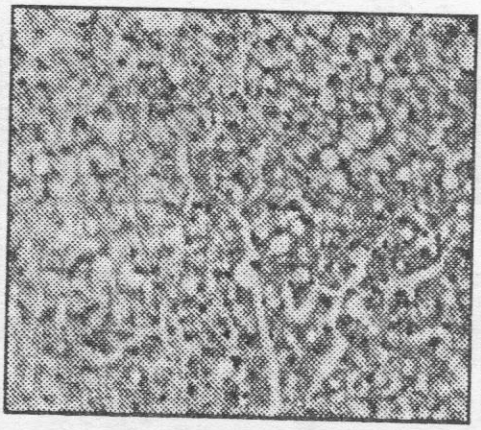

Fig. 2 : Section of Liver of guinea pig received 1.5 ug/gm. of (C. C.) venom, showing fatty change (Hx. & E. x 40).

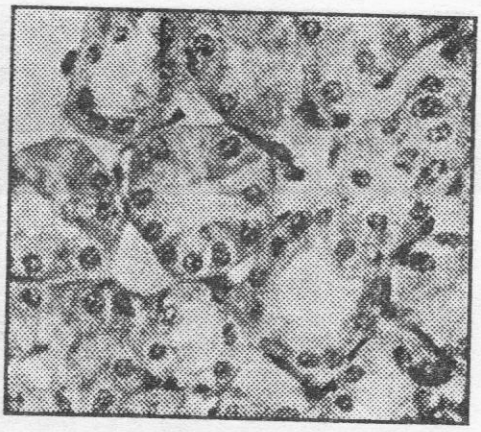

Fig. 4 : Section of kidney of guinea pig received 0.5 ug /gm. of (C. C.) venom, showing Cloudy swelling of the proximal convoluted tubules with cytoplasmic vacuolization (Hx. & E. x 40)

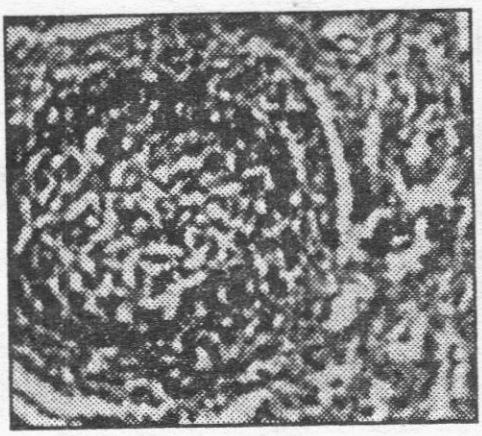

Fig. 5 : Section of spleen of' guinea pig received 1 ug/gm. of C. C. venom, showing hyperplasia of the lymphoid follicles with prominent germinal centre (Hx. & E. x 100).

Fig. 7 : Section of heart of guinea pig received 1.5 ug./gm. 0f C. C. venom, showing congestion of the blood vessels with haemorrhage in between the cardiac muscle fibers (Hx. & E x 40).

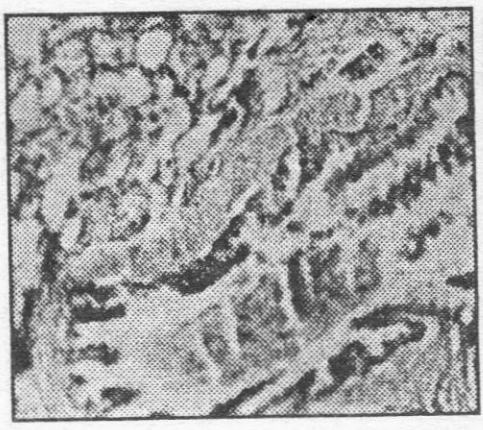

Fig. 6 : Section of lung of guinea pig received 1.5 ug/gm. of C. C. Venom, showing haemorrhage in the interstitial tissue (Hx. & E. 40).

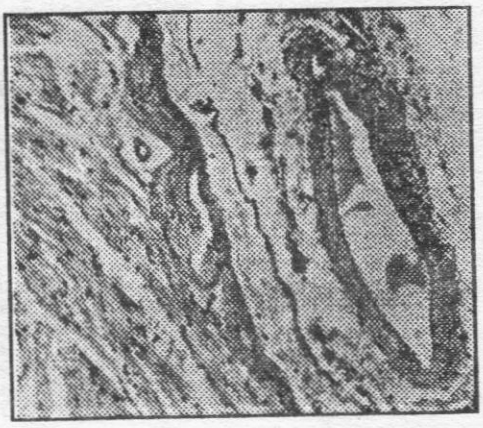

Fig. 8 : Section of heart of guinea pig received repeated small doses of C. c. venom showing paravascular round cell infil tration (Hx. & E. x 40).

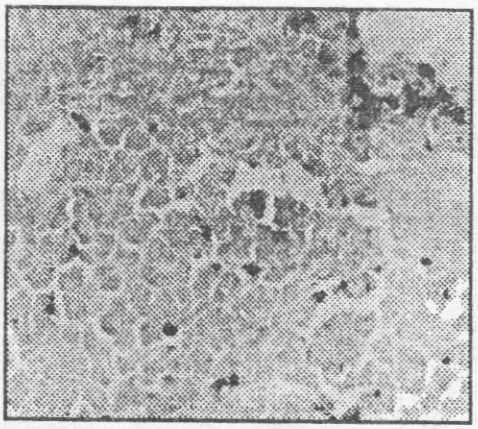

Fig. 9 : Section of liver of control guinea pig, showing negative alkaline phosphatase activity in both perihperal and central hepatocytes (Gomori's method x 150).

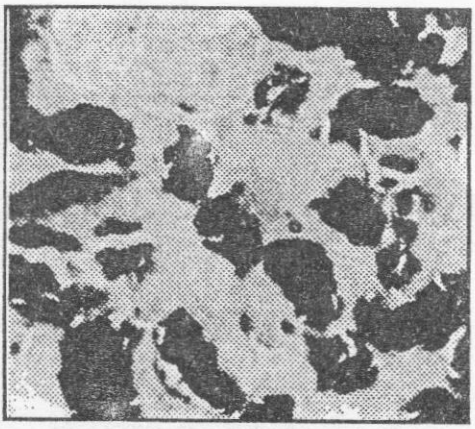

Fig. 11 : Section of kidney of control guinea pig, showing strong (+++) alkaline phosphatase activity in the tubular cells (Gomori 's method x 150).

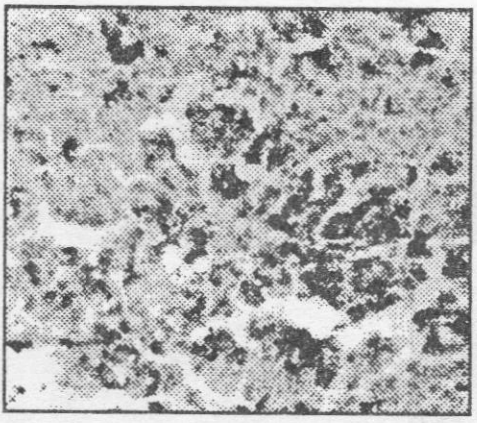

Fig. 10 : Section of liver of guinea pig received 1 ug/gm. of C.C. venom, showing negative (-ve) alkaline phosphatase activity in peripheral hepatocytes and mild (+) activity in central ones (Gomori 's method x 150).

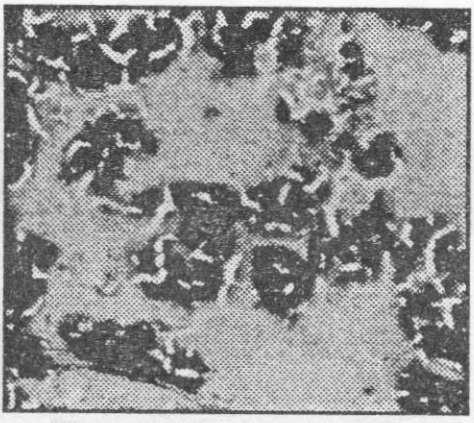

Fig. 12 : Section of kidney of guinea pig received 1.5 ug/gm. of C. C. venom, showing moderate (++) alkaline phosphatase activity (Gomori's method x 150).

Fig. 13 : Section of kidney of guinea pig received 2 ug/gm. of C.C. venom, showing mild (+) alkaline phosphatase activity (Gomori's method x 150)

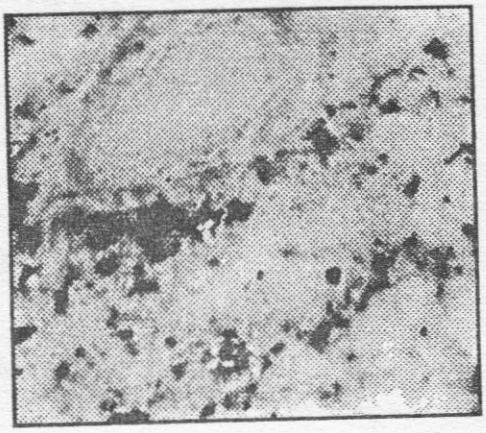

Fig. 15 : Section of lung of guinea pig, received 0.5 ug / gm. of C. C. venom, showing moderate (++) alkaline phosphatase activity (Gomori's method x 100).

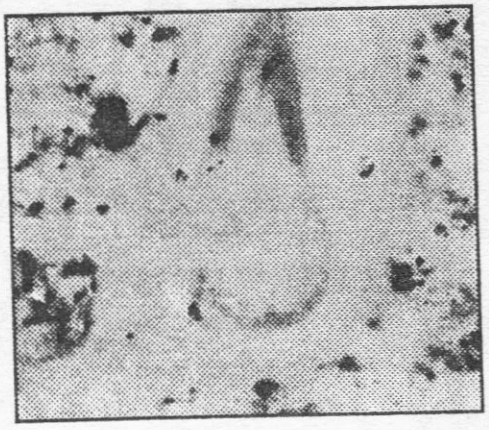

Fig. 14 : Section of lung of control guinea pig, showing mild alkaline phosphatase activity (Gomori 's method x 150)

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التأثيرات الهستوباثولوجيه والهستوكيميائيه لسم الثعبان مع تقييم للمصل المضاد لسم الثعبان فى حيوانات التجارب

المشتركون فى البحث :

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ملخص البحث :

أجرى هذا البحث لدراسة التأثير الضار الناتج عن اعطاء سم الحيه المقرنه عن طريق الحقن فى حيوانات التجارب (الخنازير الغينيه) على انسجة الجسم المختلفة (الكبد - الكلى - الطحال - الرئه - القلب - والمخ) وقد اشتمل البحث على المجموعات الرئيسية الاتيه :

المجموعة الاولى :

اجريت تجارب هذه المجموعة لمعرفة تأثير الجرعه الواحده المتزايدة من سم الحيه المقرنه وهى ٥ر ، ٥ ر ١ ، ٢ ميكروجرام / جرام من وزن الجسم عن طريق الحقن البريتونى والتى فحصت بعد اربعة ساعات من اخذ الجرعه .

وقد شوهدت تغيرات هستوباثولوجيه وهستوكيميائية (الفوسفاتيز القلوى) واضحه فى الاعضاء المختلفة ماعدا المخ الذى كان اقلهم حساسية لسم الحيه المقرنه .

المجموعة الثانية :

اجريت هذه المجموعة لمعرفة تأثير الجرعة الصغيرة المتكرره من سم الحيه المقرنه وهى ٥ ر ميكروجرام / جرام من وزن الجسم عن طريق الحقن البريتونى مره كل يومين لستة جرعات. على الانسجه المختلفة والتى فحصت اليوم التالى من آخذ آخر جرعه .

وقد لوحظت تغيرات اقل من المجموعة الاولى .

المجموعة الثالثه :

اجريت تجارب هذه المجموعه لمعرفة التأثير من اعطاء المصل المضاد لسم الثعبان بعد جرعه السم مباشرة وقد لوحظ نقص واضح فى التغيرات السابقة .

ومن هذا البحث نستنتج أن سم الحيه له تأثير هستوباثولوجى وهستوكيميائى على الانسجه المختلفة وهذا التأثير يتزايد كلما زادت جرعة السم وهذا التأثير يقل بدرجة كبيرة اذا اعطى المصل المضاد للسم على وجه السرعه .