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# EFFECTS OF LEAD ACETATE SALT ON THE HISTOLOGICAL STRUCTURE OF SOME ORGANS IN BROILERS

(With 19 Figures)

By

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(Received at 13/8/2003)

## تأثير ملح أسيتات الرصاص علي التركيب الهستولوجي لبعض أعضاء الدجاج البدارى

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استخدم في هذا البحث ١٠٠ كتكوت عمر أسبوعين. قسمت هذه الكتاكيت إلي ٤ مجموعات ، المجموعة الأولي اعتبرت المجموعة الضابطة وقد غذيت علي عليقه متزنة فقط ، أما باقي المجموعات فقد غذيت علي عليقه متزنة مضاف إليها أملاح اسيتات الرصاص بنسبة ٥٠ ، ١٠٠ ، ٢٠٠ ملجم / كجم عليقه علي التوالي عند عمر ٥٠ يوم وزنت هذه الكتاكيت وذبحت ثم أخذت عينات من كل من الكبد والكلي والمعدة الأولي للفحص الهستولوجي وقد وجد فجوات في خلايا الكبد في المجاميع المعاملة بالإضافة إلي انه في المجموعة الرابعة قد فقدت بعض الخلايا الشكل الطبيعي لها والبعض الآخر قد تكثرت . كما وجد تجمعات ليمفاوية في الكبد أما في الكلي فقد وجد فجوات في الخلايا المبطنة للأنابيب العليا كما أن الحرف الموجب لمعامل شيف قد نقص وبعض الخلايا قد فقدت الشكل الطبيعي لها. وفي المعدة الأولي قد ظهرت كتل خلوية . القطاعات التي صبغت بالروديزونات فقد أظهرت تفاعلات قليلة في كبد المجموعة الرابعة فقط.

## SUMMARY

One hundred (two weeks old) hubberd chicken were divided into four groups; the first group considered as a control, the other groups received balanced ration contained 50,100, and 200mg lead acetate/kg ration respectively. On reaching 50 days old the birds were weighed and slaughtered.Specimens of liver, kidney and proventriculus were prepared for histological studies.The histological investigations revealed that there were lead dose-dependant vacuolation in hepatocytes. In the fourth group some hepatocytes lost their normal architectures and others were severly damaged (karyolysis and cytolysis). The Kupffer cells contained PAS

and acid fucshin positive(+ve) granules. Also there were lymphoid aggregations scattered in the liver. In the kidney, the cells lining the proximal tubules in the treated groups were more vacuolated than control, in addition in the fourth group the PAS +ve lumenal border was decreased and some cells lost their normal histological features. In the proventriculus capsulated cellular masses were observed in the lamina propria of few birds. Sections stained with Rhodizonate showed weak reaction in the liver of the fourth group and no reaction in other tissues.

***Key words:*** *Lead acetate, histology, broilers, liver, kidney.*

## INTRODUCTION

One of the important reasons of the environmental pollution is the unrecognizable sources of heavy metals. Lead or lead compounds could be highly toxic when eaten or inhaled. It is environmental contaminates which is present in almost all living organisms and is non essential for them. It is one of the common causes of poisoning in farm animals. Lead poisonign are on record due to environmental pollution (Aronson, 1978, Burows and Borchard, 1982 and Kwatra, *et al.*, 1986).

Lead appears as trace metal in virtually all foods and beverages, although it is not essential to nutrition. (Lewis *et al.*, 1990).

In Egypt the real impact of the problem of heavy metals are still insufficient in the field of poultary management, therefore the aim of this worke is to study the changes wich occur in the histological structure of some organs of broiler chickens treated with different levels of lead acetate salt from two weeks of age till seven weeks of age.

## MATERIALS and METHODS

One hundred (two weeks old) hubbard chicken were purchased from the farm of the Faculty of Agriculture, Kafr El Sheikh. The birds were divided into four groups (each group formed of 25 birds).

The first group was considered as control received balanced ration only. The second group received balanced ration contained 50mg lead acetate /kg ration. The third group received balanced ration contained 100mg lead acetate/kg ration. The fourth group received balanced ration contained 200mg lead acetate/kg ration. Ingredients composition of balanced ration were shown in the following table:

| Ingredients | % |
|---|---|
| Yellow corn | 65% |
| Soya bean | 25% |
| Concentrate | 10% |
| **Total protein** | **21%** |

On reaching 50 days old, the birds were weighed and slaughtered, specimens from different areas of liver, kidney and proventriculus were immediately obtained and fixed in Boun's solution. After fixation, the specimens were processed for paraffin sections and cut at 5-6 um were stained with Hematoxylin and Eosin stain, Crossman's trichrome stain, Periodic acid Schiff's reagent and Rhodizonate method for detection of lead in the tissues. (Bancroft and Stevens, 1990).

## RESULTS

The mean body weights of the treated broilers were lowered than that of control. The effects of different concentration of lead salts on body weights of broilers are shown in the following table;

| Group | Lead mg/kg ration | Body weight Kg |
|---|---|---|
| First group (Control) | 0 | 1841.2 |
| Second group | 50 | 1521.5 |
| Third group | 100 | 1487.5 |
| Fourth group | 200 | 1473.1 |

**Histological investigations:**

**1- Liver:**

The liver of the treated groups (2, 3 and 4) showed marked changes in hepatocytes, blood sinusoids and Kupffer cells.

The heptocytes appeared larger and contained more vacuolated cytoplasm than control (Figs. 1, 2 & 3.). In addition the hepatocytes in the fourth group were more vacuolated and ballooned with pyknotic nuclei (lost their normal architecture) (Fig. 3), and also severely damaged hepatocytes were observed (karyolysis and cytolysis) (Fig. 4). In sections stained with PAS, the hepatocytes of the treated groups (2&3) showed increasing in PAS+ve reactions (Fig. 5), while those in the fourth group the PAS +ve reactions were reduced (Figs. 3 & 4).

The blood sinusoids in groups (2 & 3) became enlarged and engorged with blood (Figs. 5 & 6), while in group 4, the blood sinusoids were obliterated due to enlargement of hepatocytes (Fig. 3).

The Kupffer cells of the treated groups contained phagocytized PAS and acid fucshin+ve granules (Figs. 6 & 7).

In addition to the a forementioned changes, the liver of the treated groups contained lymphoid aggregations of different sizes scattered in the liver (Fig. 2). There were lead dose-dependant increase in these lymphoid aggregations.

In sections stained with Rhodizonate, weak reactions were noticed as large red granules (may be in Kupffer cells) in the fourth group (Figs. 8 & 9), while in the other groups, the reactions could not be demonstrated.

**2-Kidney:**

The more noticeable changes in the kidney of the treated groups were found in the cells of the nephron tubules specially the proximal convoluted tubules where their cells became enlarged and contained more vacuolated cytoplasm than control (Figs. 10 & 11). In addition, the cells lining the proximal tubules in kidney of the fourth group appeared more vacuolated and more enlarged, some cells lost their normal histological features and the PAS+ve luminal border was decreased (Fig. 12). Also, the blood vessels were engorged with blood (Fig. 13).

In sections stained with Rhodizonate, the reactions could not be demonstrated in the all groups.

**3- Proventriculus:**

In the proventriculus of the treated groups, there were capsulated cellular masses of different shapes and sizes observed in the lamina propria of few birds. These cell masses showed high mitotic figures (Figs. 14, 15 & 16).

The secretory vesicles present in the lumen of the glands of the treated groups were decreased in their amounts than in control (Figs. 17 & 18). Also, there were increasing in lymphocytic aggregations in the lamina propria and between the glands (Fig. 19).

In sections stained with Rhodizonate the reactions could not be demonstrated in the all groups.

## DISSCUSION

The present study revealed that the liver of the treated groups showed marked changes in hepatocytes,sinusoids and Kupffer cells. The

hepatocytes became larger and contained vacuolated cytoplasm, this vaccuolation was lead dose dependant, so in the fourth group some cells became more vacuolated and ballooned with pyknotic nuclei, other cells lost their normal architectures and damaged hepatocytes were observed.Yossef *et al.* (1995) found the same results in broilers after 17 days of lead administration to one day old Lohman chicks .The blood sinusoids were more dilated and congested with blood in treated groups than in control. This vascular dilatation and congestion results in stasis of blood,dificient oxygenation and compression of blood vessels, thus lead to atrophic liver cells (Elwi, 1963; Hodeghem and Miller, 1987), this may explain presence of severely damaged cells in the liver of the fourth group. Also Bakalli *et al.*, 1994,may explain this damage when they detected that l ead has inhibition effect on the Delta-aminolevulinic acid dehydratase enzyme (which participating in heme synthesis) in blood and other tissue.

In sections stained with Rhodizonate, weak reactions were noticed as large red granules in the Kupffer cells in the liver of fourth group which fed 200 mg lead /kg ration,while in other groups the reaction could not be seen.This results were in agreement with Stanchev *et al.*, 1989 and Bakalli *et al.*, 1994 where they detected that the concentration of lead in blood ,liver,kidney,femur and gluteus muscle increased with increasing dietary lead,also, Willet *et al..*,1994 could explain this results when they revealed that large quantities of lead were not removed by the kidneys so that ,the majority of the absorbed lead remained in the bodies.

The liver of the treated groups showed lymphoid aggregations of different sizes allover the liver,this aggregations were increased with increasing of lead in ration Youssef *et al.*, 1995 showed a dose dependant lymphocytic depletion in bursa of Fabricius and spleen.

In kidneys, the more noticeable changes of the treated groups were found in the cells of the nephron tubules specially in the proximal convoluted tubules where their cells were enlarged and contained vacuolated cytoplasm .In addition the cells of proximal tubules in the fourth group were more enlarged and more vacuolated, some cells lost their histological pattern and PAS +ve brush border was decreased Youssef *et al.*, 1995 showed renal damage in broilers after 17 days of lead administration .In sections stained with Rhodizonate the reaction could not be demonstrared in all groups. Streit and Nagel, 1993 revealed that lead was not concentrated in the kidneys or liver of adult and young

bats, while Kutkat *et al.*; 1997 revealed that lead was more precipitated in livers and kidneys of chickens.

In the provenriculs of the treated groups there were capsulated cellular masses of different shapes and sizes were observed in lamina propria in few indviduals.These cell masses showed high mitotic Fig.ures .Jan, 2000 found that compounds of lead and cadmium are well known carcinogens to experimental animals and humans.

**In conclusions:** from our study we can conclude that lead has effect on the liver and kidneys tissues, where it causes changes in the normal histological features of hepatocytes in liver and cells lining the proximal tubules in kidney. Also it causes the appearance of capsulated cellular masses in the lamina propria of the proventeiculus.. The lead could be demonstrated in tissues of livers after administration of high concentrations of lead using Rhodizonate method.

## ACKNOWLEDGMENTS

I am grateful to stuff members of Physiology and Biochemistry Department, Vet. Med. Tanta Uni. For their help.

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## FIGURES

**Fig. 1:** Section in liver of control group showing the PAS reactions in hepatocytes Kupffer cells(arrows).(PAS-reagent ;x:400).

**Fig. 2:** Section in liver of broilers fed balanced ration contained 50mg lead acetate/kg ration showing,vaccuolations in hepatocytes (arrow),lymphoid aggregations(arrow head), and the PAS +ve reaction.(PAS-reagent;x:400).

**Fig. 3:** Section in liver of broilers fed balanced ration contained 200mg lead acetate/kg ration showing vaccuolation of hepatocytes(arrows),pyknotic nuclei(arrow heads).(PAS-reagent; x:400).

**Fig. 4:** Section in liver of broilers fed balanced ration contained 200mg lead acetate /kg ration showing damaged hepatocytes(arrow).(PAS-reagent;x:400).

**Fig. 5:** Section in liver of broilers fed balanced ration contained 100mg lead acetate /kg ration showing large vacuoles (arrow), PAS+ve granules in hepatocytes (arrow heads). (PAS-reagent; x:1000).

**Fig. 6:** Section in liver of broilers fed balanced ration contained 50mg lead acetate /kg ration showing dilated blood sinusoids (arrows), Kupffer cells contained PAS+ve granules(tow arrows).(PAS-reaagent;x:1000).

**Fig. 7:** Section in liver of broilers fed balanced ration contained 100mg lead acetate /kg ration showing acid fucshin+ve granules in Kupffer cells(arrows).

**Fig. 8 & 9:** Section in liver of broilers fed balanced ration contained 200mg lead acetate /kg ration showing lead inside the tissue (arrows).

**8:** (Rhodizonate with counter stain(light green);x:100)

**9:** (Rhodizonate without counter stain;x:1000).

**Fig. 10:** Section in kidney of control group showing the PAS+ve luminal border.(PAS-reagent;x:400).

**Fig. 11:** Section in kidney of broilers fed balanced ration contained 100mg lead acetate /kg ration showing vacuolation in cytoplasm of cells lined the proximal tubules. (PAS-reagent; x:1000).

**Fig. 12:** Section in kidney of broilers fed balanced ration contained 200mg lead acetate /kg ration showing more vacuolated cytoplasm of cells lined the proximal tubules (arrows), decreasing of PAS+ve luminal border (two arrows), pyknotic nuclei (arrow heads).(PAS-reagent;x:1000).

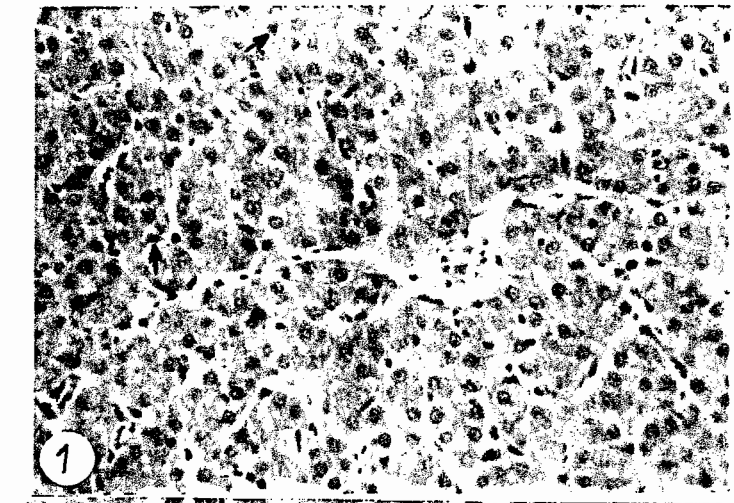
**Fig. 13:** Section in kidney of broilers fed balanced ration contained 50mg lead acetate /kg ration showing that the blood vessels were engorged with blood. (arrow) (PAS-reagent;x:100).

**Fig. 14, 15 & 16:** Sections in proventriculus of broilers fed balanced ration contained 50mg lead acetate /kg ration showing cellular masses in the lamina propria (arrows), mitotic Fig.ures (two arrows). (H&E stain; 14,x:100 and 15&16,x:400).

**Fig. 17:** Section in proventriculus of control group showing the secretory vesicles in the lumen of glands (arrow).(PAS-reagent;x:400).

**Fig. 18:** Section in proventriculus of broilers fed balanced ration contained 200mg lead acetate /kg ration showing decreasing in the amount of secretory vesicles. (PAS-reagent; x;400).

**Fig. 19:** Section in proventriculus of broilers fed balanced ration contained 200mg lead acetate/kg ration showing the lymphoid aggregations. (arrows). (PAS-reagent; x:40).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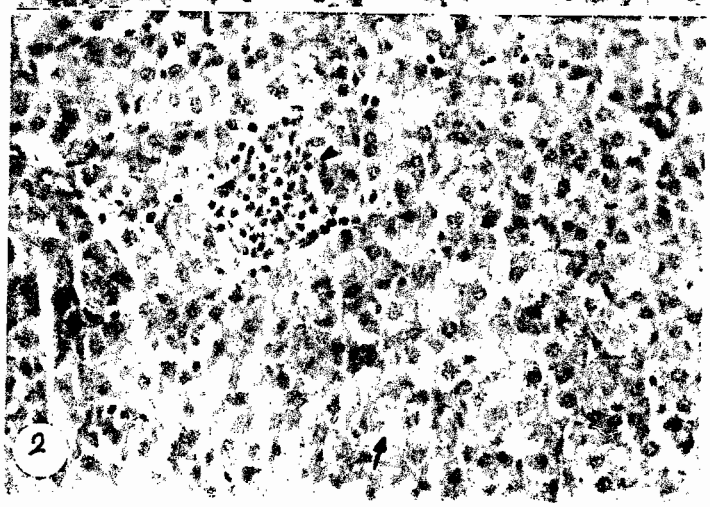
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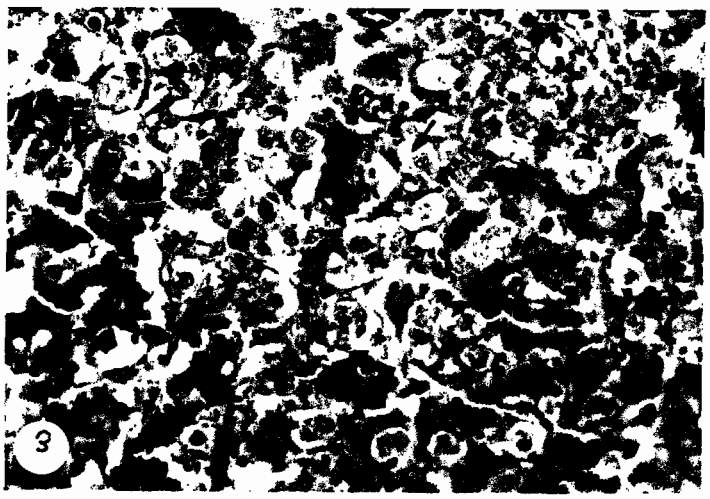
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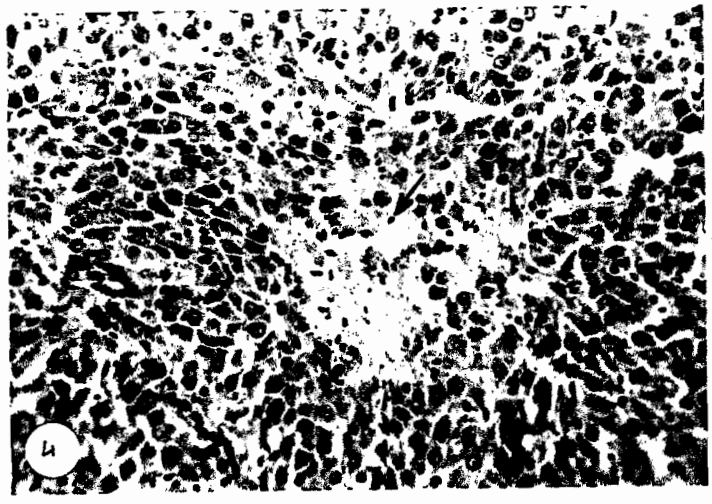
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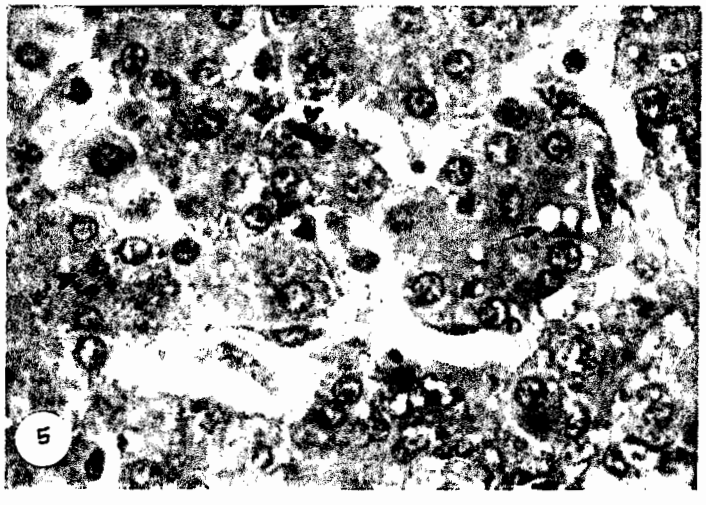
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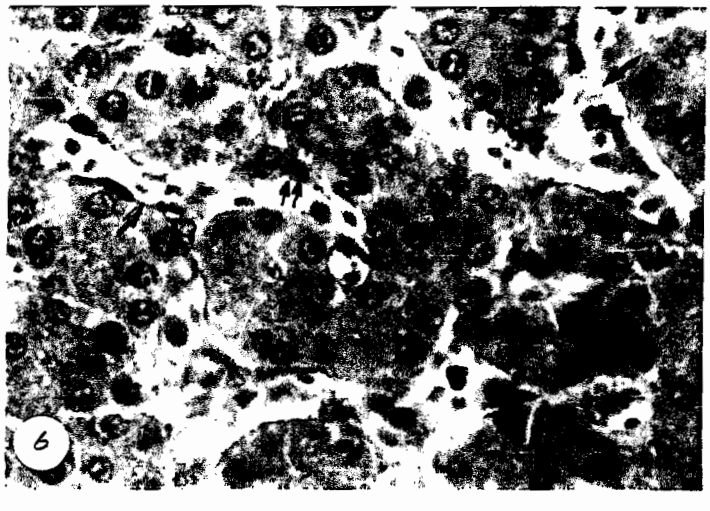
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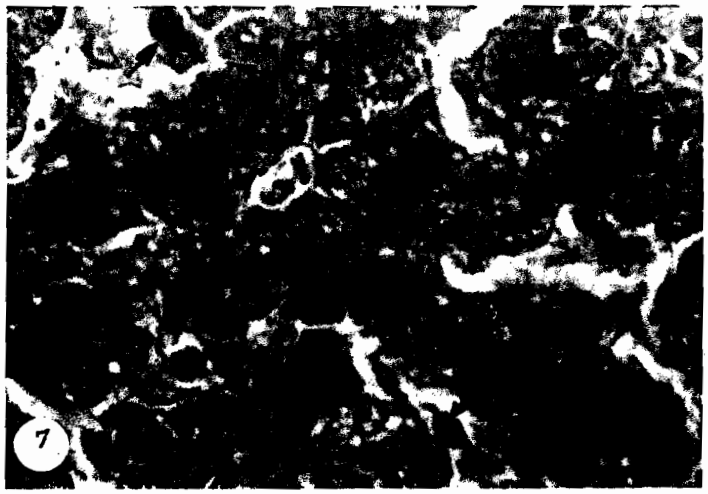
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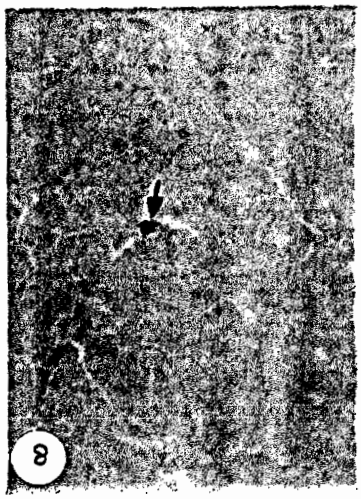
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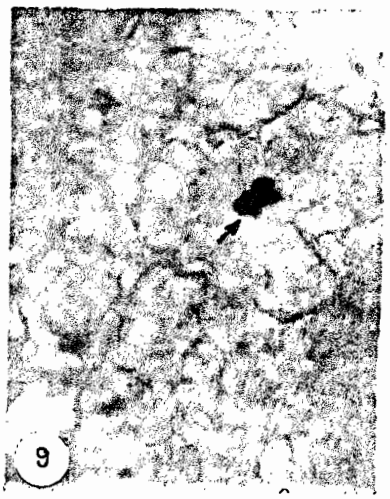
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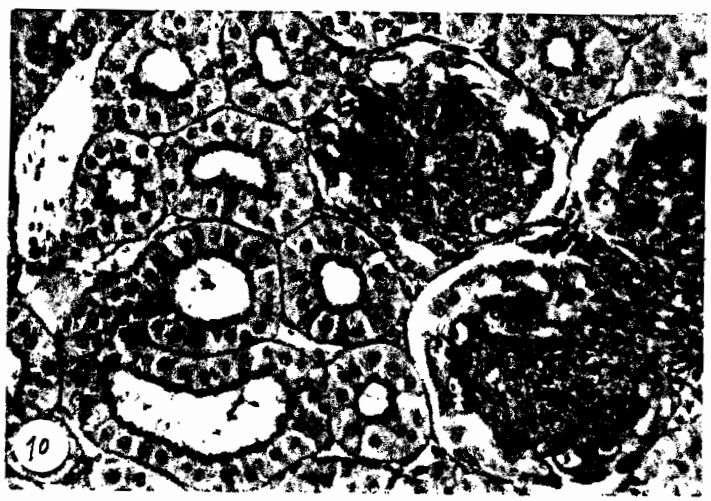
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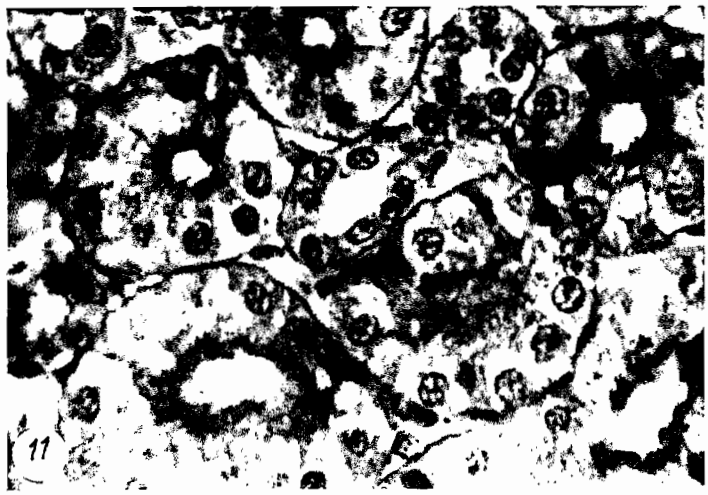
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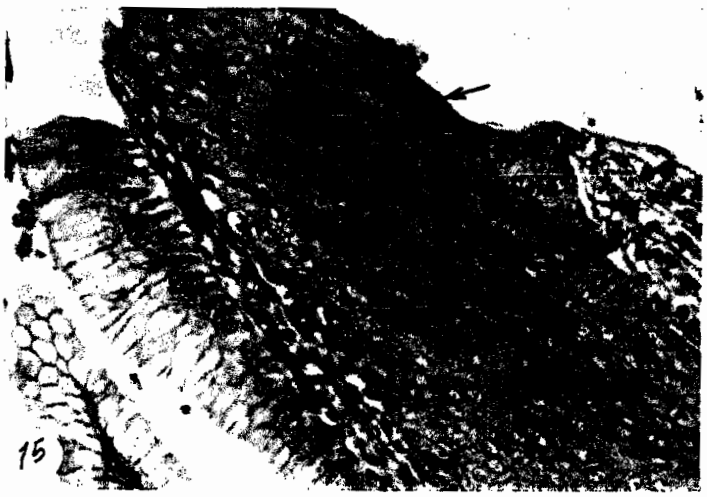
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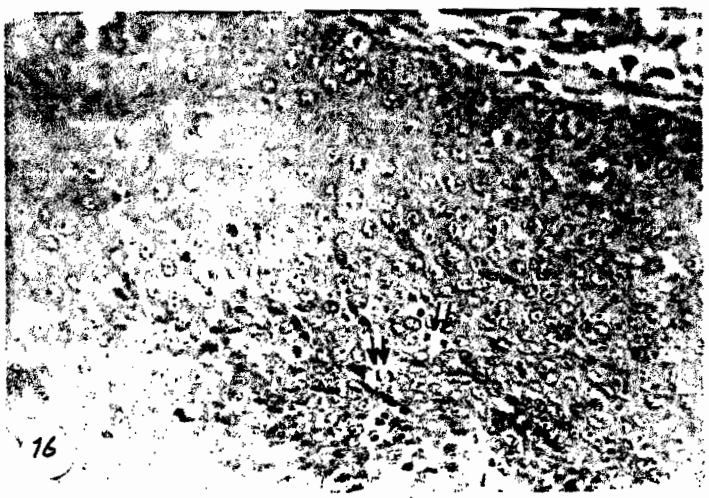
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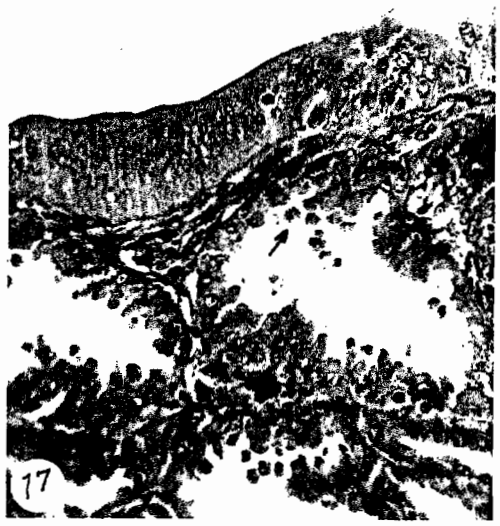
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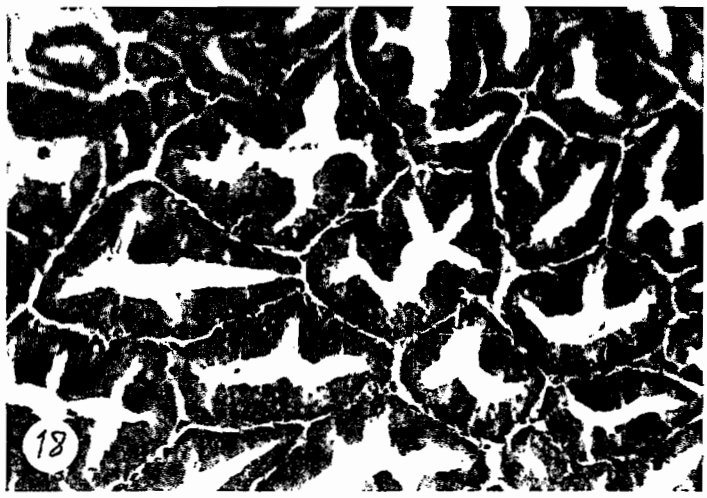
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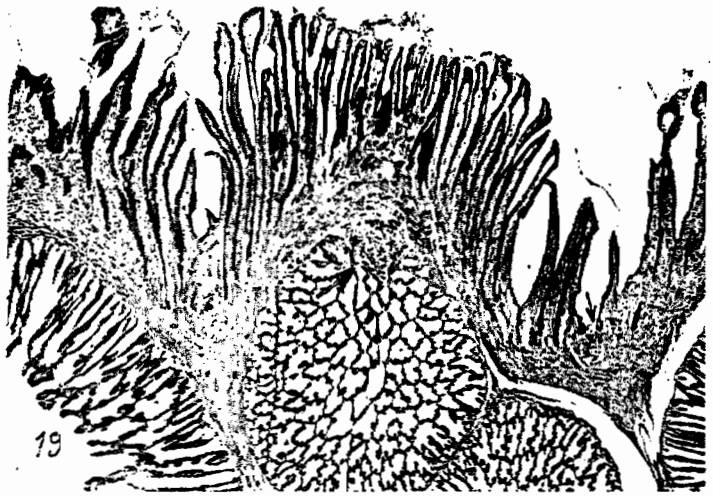
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