HEPATIC CHANGES

HISTOLOGICAL OBSERVATIONS

Control Rats

Histological examination of the liver tissue of control rats revealed completely normal liver structure during the whole period of study. The main structural component is polygonal hepatocytic cells (parenchymal cells) which were grouped in cords that were connected to form a structural unit (lobule). The hepatocyte cords surround a central vein in the middle of the lobule and were traversed by a network of blood sinusoids that converged towards the central vein. Sinusoids are irregularly dilated vessels composed only of a discontinuous layer of fenestrated endothelial cells. The sinusoids also contain phagocytic cells of the mononuclear type known as Von Kupffer cells (fig. 1). The portal spaces are present at the corners of lobules and are occupied by the portal triads, each containing a venule, an arteriole and a bile duct embedded in a scanty of fibrous tissue as illustrated in fig. (2).

Treated rats

Macroscopically, the liver showed the same changes during the whole study. The liver appeared enlarged with dark red in colour, soft consistency and smooth on cut section. Gradually, the liver became heavier, large and dark brown in colour.

Rats treated with 1/8 LD_{50} $CdCl_2$

Liver sections prepared from rats treated with 1/8 LD_{50} for 10 days have shown various degenerative changes (fig.3). In this figure, mild hydropic degeneration which apparent being mainly symptomized by marked loss of the uniformity and regularity of the liver plates. The peripheral areas of the hepatic lobules appeared to be more injured than

the pericentral ones. Also, in the same figure the central vein appeared more dilated and congested with red blood cells. Inflammatory cells including lymphocytes and histocytes as illustrated in figure (4) infiltrated the portal tract. In addition, there was a few signet ring appearance nuclei (pyknotic nuclei).

Similar changes were observed in the materials examined on the 20 days following $CdCl_2$ treatment, but these alterations were more pronounced. The central vein was markedly dilated and congested with RBCs. Also, there were marked increase in the number of inflammatory cells, which invaded the portal tracts and sinusoids. More pyknotic nuclei and binucleated cells were distincted especially around the central areas of lobules (fig. 5).

Specimens inspected 30 days following treatment showed more cellular degeneration as shown in figure (6,7 &8). There were marked pronounced cytoplasmic granularity as well as hydropic degeneration. Nuclei of peripheral hepatocytes revealed marked increase in their size than pericentral cells, also many nuclei showed karyorrhexis and completely disappearing of others indicating and advanced degree of karyolysis. Also we observed, in these figures, marked nuclear pyknosis and increase in the number of Von Kupffer cells. The central vein showed moderate congestion and dilation. In addition, figure (6) revealed congestion and dilation of the central vein.

Figure (9), obtained from materials examined 45 days following administration with the same dose revealed that, the portal area severely infiltrated by mononuclear inflammatory cells which invaded the portal tract and sinusoids, also there were marked dilation and congestion of the radical of the portal vein, beside mild fibrosis was also noticed in the same figure.

On the other hand, the degenerative changes were still observed in the same tissue appeared as disturbing of the normal configuration of the lobules in some areas of the tissue as illustrated in figure (10). There were severe nuclear changes as; karyorrhexis, karyolysis, single cell necrosis and focal areas of necrosis.

A partial recovery involving incomplete restoration of the normality of the liver structure was achieved by the 1/8 LD_{50} -treated rats examined 30 days following abstainance of Cd-treatment. This restoration was evidenced by marked increase of the mitotic figures in the liver parenchyma as well as increase in the phagocytes (Von Kuppfer cells hyperplasia) which engulfed the necrotized tissue fig. (11). Pyknosis, karyorrhexis, karyolysis and single cell necrosis of many hepatocytes has been still observed in the same figure. Beside the previous changes, the central vein and all the blood vessels are still dilated and congested as well as infiltration of the portal tract with the inflammatory cells.

Rats treated with 1/4 LD_{50} $CdCl_2$

Comparing with, those rats treated with the 1/8 LD_{50}, the histopathological alterations in the liver tissue were early and markedly observed in the others administered with 1/4 LD_{50} $CdCl_2$.The liver tissues prepared from rats treated with this high dose for 10 days revealed marked cytoplasmic granularity of some hepatocytes while others underwent hydropic degeneration. Von Kupffer cells markedly increased than the control tissue fig (12). In the same figure, the central vein was markedly dilated and congested. Fig (13) showed also marked deformation of the hepatocytes, invasion of the portal tract with mononuclear inflammatory cells as well as congestion of the radicals of the portal vein.

After completion of 20 days following 1/4 LD_{50} $CdCl_2$ administration, the boundaries of the hepatocytes were weakly distinguished, and most of

such cells exhibited marked cytoplasmic granularity in most of the peripheral and pericentral cells as illustrated in fig (14& 15). Many nuclei of these cells revealed marked variety in their size, while others showed pyknosis, karyorrhexis and karyolysis. Also the same figure demonstrated severe infiltration of the portal tract with an enormous number of inflammatory cell. Figure (16) revealed pronounced dilation of the central vein as well as it has been congested and surrounded by inflammatory cells. Also there was an obvious increase in the pyknotic and karyolysed nuclei, beside Kupffer cells hyperplasia.

After thirty days of 1/4 LD_{50} $CdCl_2$ administration, the liver sections prepared showed an intensive deterioration of hepatic tissues, as illustrated in fig. (17,18,19&20). The hepatocytes underwent severe cytoplasmic granularity and disappearing of their boundaries. Most of their nuclei had marginated chromatin inclusions, which adhesived to the nuclear membrane, as well as other nuclei revealed pyknosis, Karyorrhexis or Karyolysis. Many cells appeared necrotized and the aggregates of these cells form focal areas of necrosis which observed in most of the liver tissue. Figure (20) revealed an intensive inflammation manifested by a tremendous increase of the inflammatory cells and many narcotized hepatocytes which scattered around the central vein. The wall of this vein underwent a pronounced hyalinization, while its lumen markedly congested. There was also an obvious increase of the Von Kupffer cells (hyperplasia). Figure (17) showed a pronounced dilation of the radical of the portal vein which congested also with blood cells, adding to infiltration of the portal tract with numerous inflammatory cells. . Also, fig.(17& 18) revealed a marked proliferation of the bile ductule referring to propability of bile duct cancer incidence.

After completion 45 days following treatment with same dose, the liver tissue showed an intensive degeneration of their parenchymal cells. The

large number of hepatocytes have been severely necrotized which, observed representing the largest necrotic areas comparing to the animals of all the studied groups as shown in fig. (21). Other hepatocytes (unnecrotic) had a marked variation in their nuclear size, chromatin distribution and prominence of their nucleoli. Also many pericentral hepatocytes showed marked regenerative changes manifested by binucleation, fig. (22). An intensive dilation and congestion of the central vein, which filled with a large number of mononuclear inflammatory cells, also observed in the hepatic tissue of this group.

In addition, fig. (23) revealed a pronounced necrotic granulomatous reaction, which observed in different regions of the hepatic tissue. Interstitial fibrosis is a marked change, which also clearly observed in this group as illustrated in fig. (24). Also, fig. (25) revealed an obvious coagulation combined with inflammatory cells and marked liver fibrosis.

On the other hand, the materials inspected 30 days following abstainance of 1/4 LD_{50} $CdCl_2$ treatment showing a slight development and restoration of the normal liver architecture. The regenerative signs as illustrated in fig. (26) were manifested by a pronounced increase of the number of binucleated cells and the phagocytic Von Kupffer cells which remove the necrotic debris in the hepatic tissue as well as approximately restoring of the normal structure of the liver plates in different parts of the tissue. Fig. (27) revealed abnormal recovery distincted by atypical hepatic cells (dysplasia) and abnormal mitotic figures predicting probability of liver hepatoma. The same figures revealed that many hepatocytes are still undergo pyknosis, karyorrhexis, karyolysis as well as single cell necrosis.

Plate (I) Material fixed in buffered neutral formalin and stained with Haematoxylin and Eosin (Clayden, 1971).

Fig. (1): - Liver section of a control rat shows normal central area, normal hepatocytes (arrow), Von Kupffer cells (K), endothelial cells (E), normal sinusoids (S) and central vein (CV). x 400.

Fig. (2):- Liver section of a control rat shows the portal area which includes the bile ductule (B), radical of the portal vein (V) and portal artery (A). x 400.

Fig. (3): - Liver section of a rat treated day after day with1/8 LD_{50} of $CdCl_2$ for 10 days, shows mild hydropic degeneration (H) and congestion (C) of the central vein. x 400.

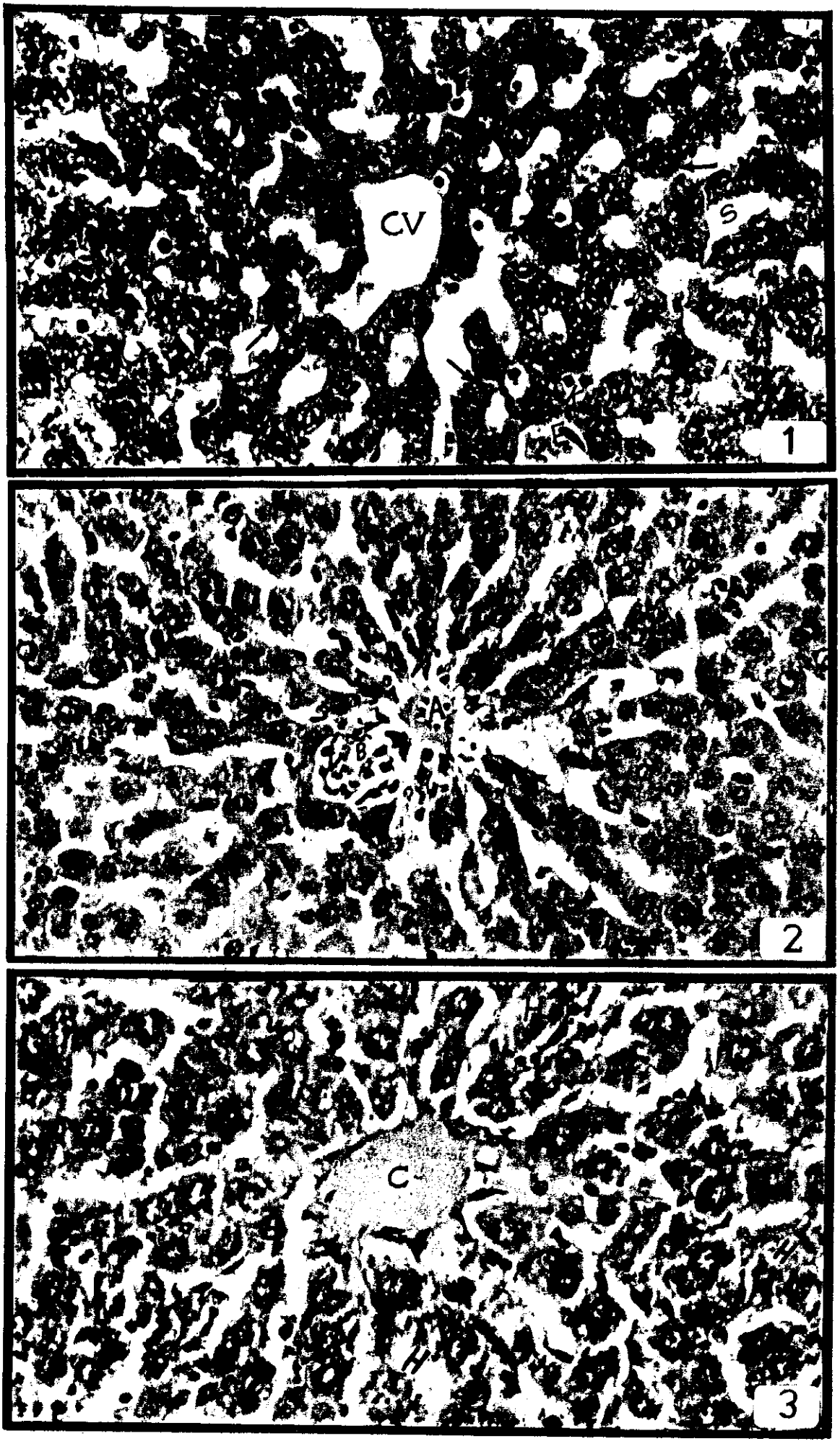
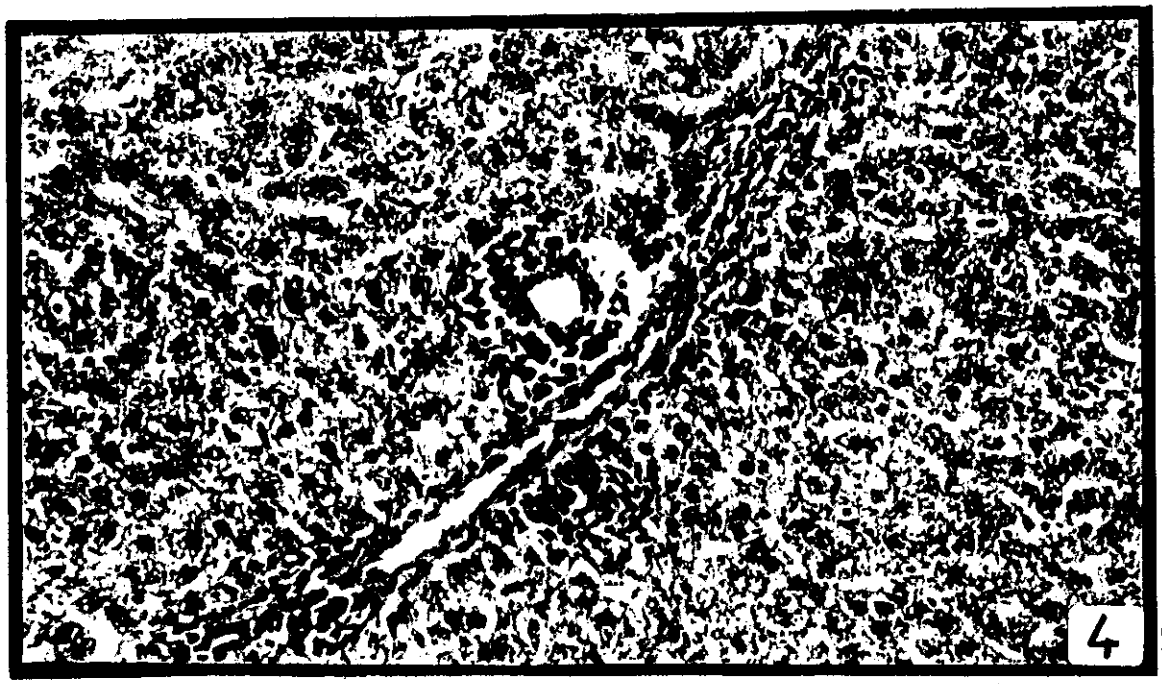

CV
S
1
A
B
2
C
3

Plate (II)- Material fixed in buffered neutral formalin and stained with Haematoxylin and Eosin (Clayden, 1971).

Fig. (4):-Liver section of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 20 days shows invasion of the portal tract with a large number of inflammatory cells (I) as well as mild hydropic degeneration(H) and cytoplasmic granularity of most of the hepatic cells (G). x 250

Fig. (5):- Liver section of a rat treated day after day with1/8 LD_{50} of $CdCl_2$ for 20 days shows marked hydropic degeneration (H), nuclear pyknosis (arrows), sinusoidal obliteration (S O) and infiltration of the portal tract with inflammatory cells (I). x 400

Fig. (6):-Liver section of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 30 days shows marked variation of the nuclear size of the hepatic cells, karyorrhexis (R), Karyolysis (L) and nuclear pyknosis (P). Congestion of the central vein as well as marked increase of the number of Von Kupffer cells (arrow) was also observed. x 400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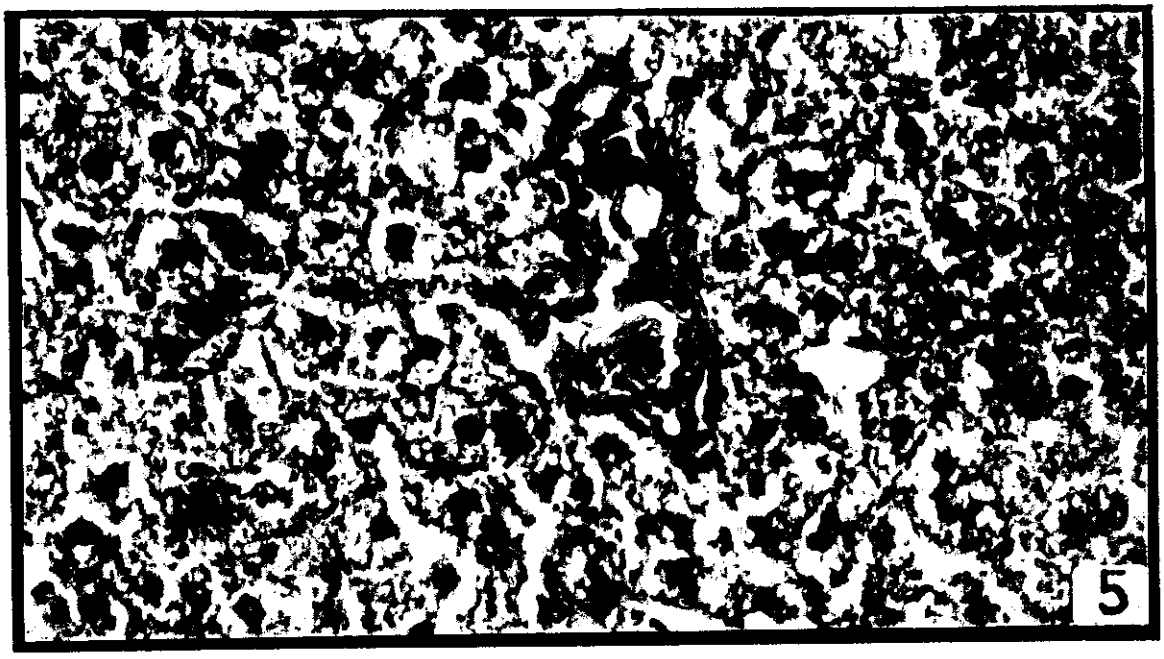
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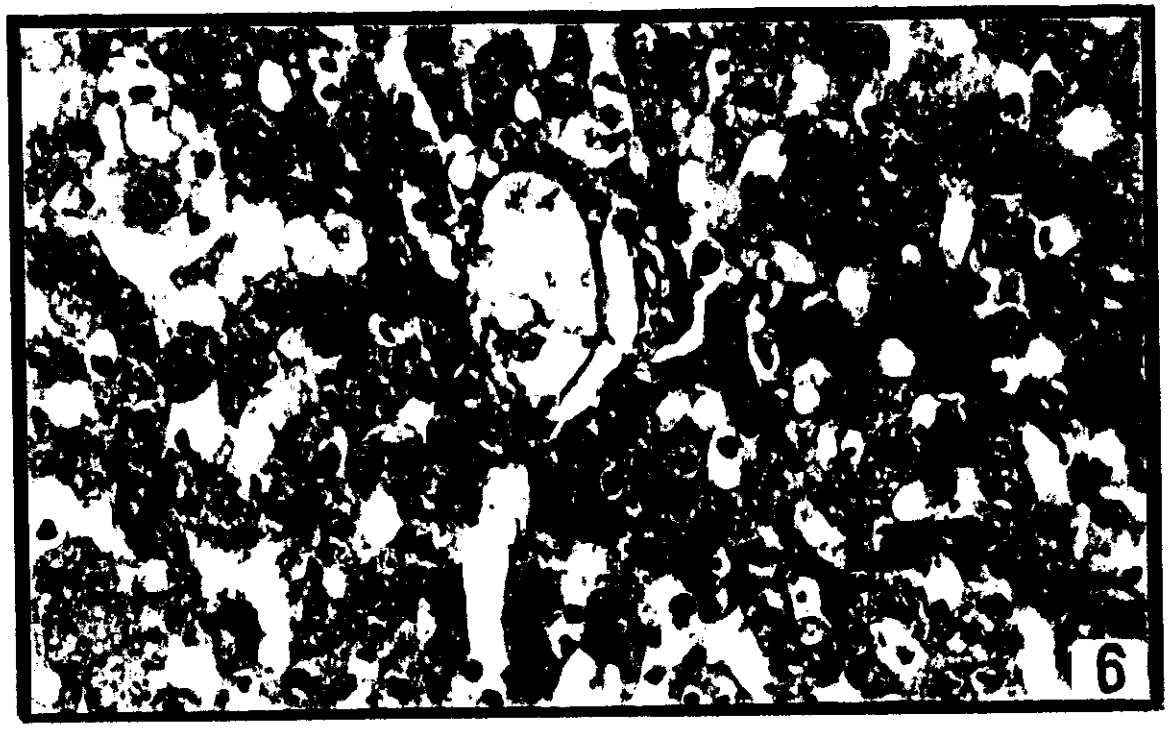
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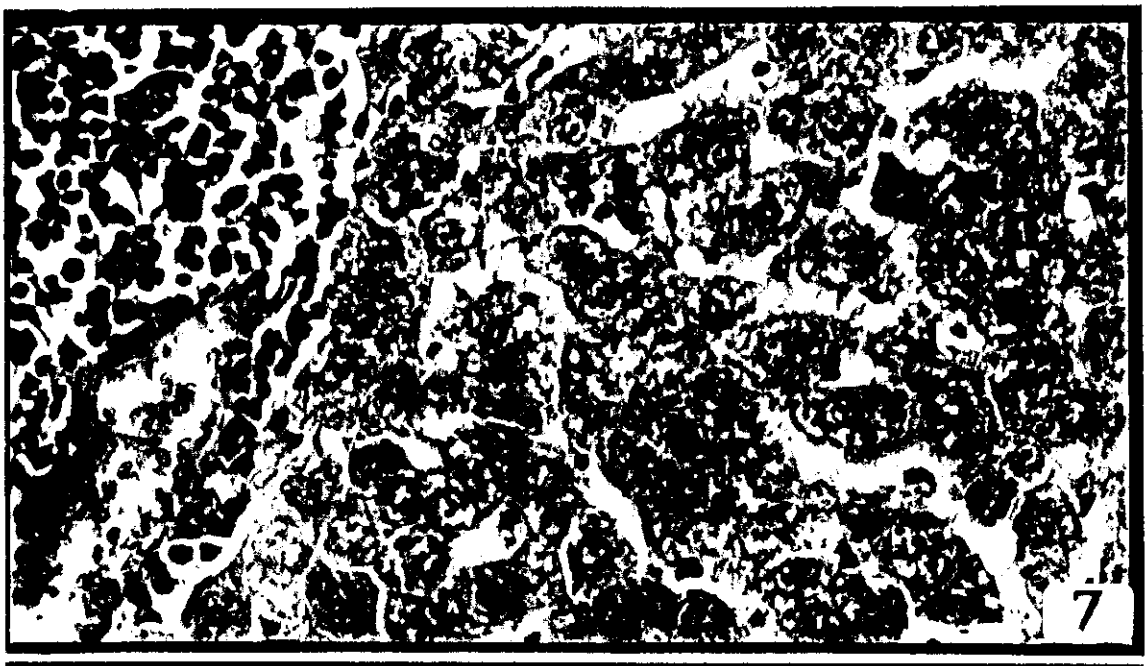
Plate (III) - Material fixed in buffered neutral formalin and stained with Haematoxylin and Eosin (Clayden, 1971).

Fig. (7):-Liver section of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 30 days shows blocking of the portal tract with a large number of inflammatory cells (I) as well as congestion of the radical of the portal vein (C). Nuclear pyknosis (P), cyoplasmic granularity (G) and obliteration of the sinusoids (S O) are also noticed. X 400

Fig. (8):- Liver section of a rat treated day after day with1/8 LD_{50} of $CdCl_2$ for 30 days shows marked cytoplasmic granularity (G), nuclear pyknosis (P) and hydropic degeneration (H). Marked infiltration with the inflammatory cells (I) are also obviously observed. x 400

Fig. (9):- Liver section of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 45 days shows marked dilation of the radical of the portal vein (D), infiltration of the portal tract with the inflammatory cells (I) mild fibrosis (F) and single cell necrosis (N). x 400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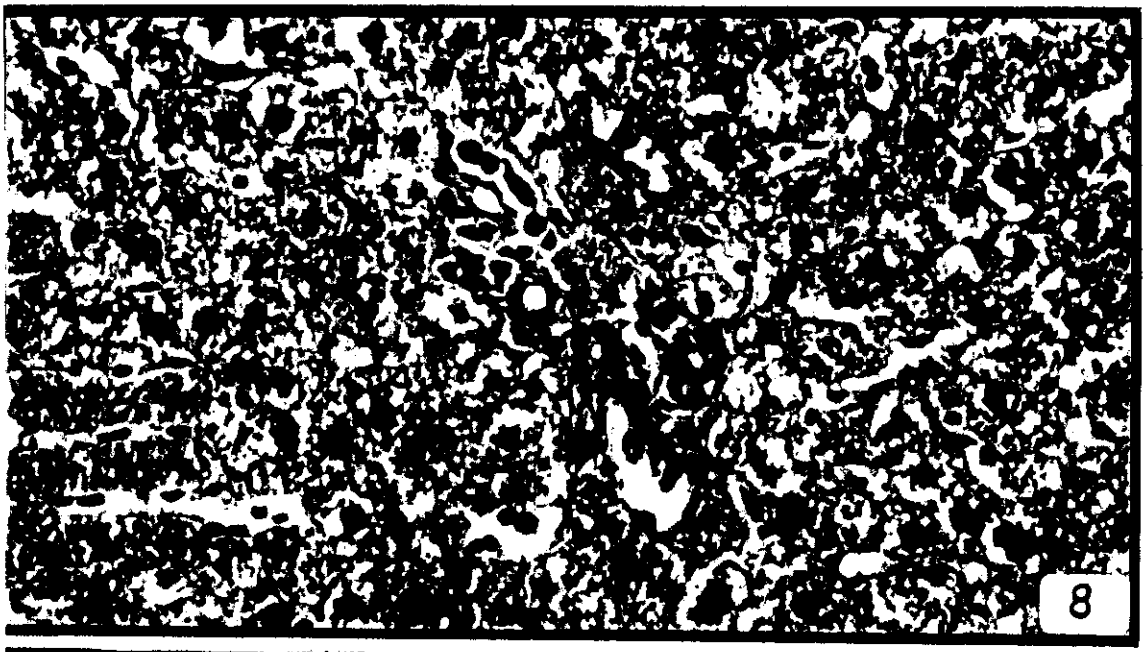
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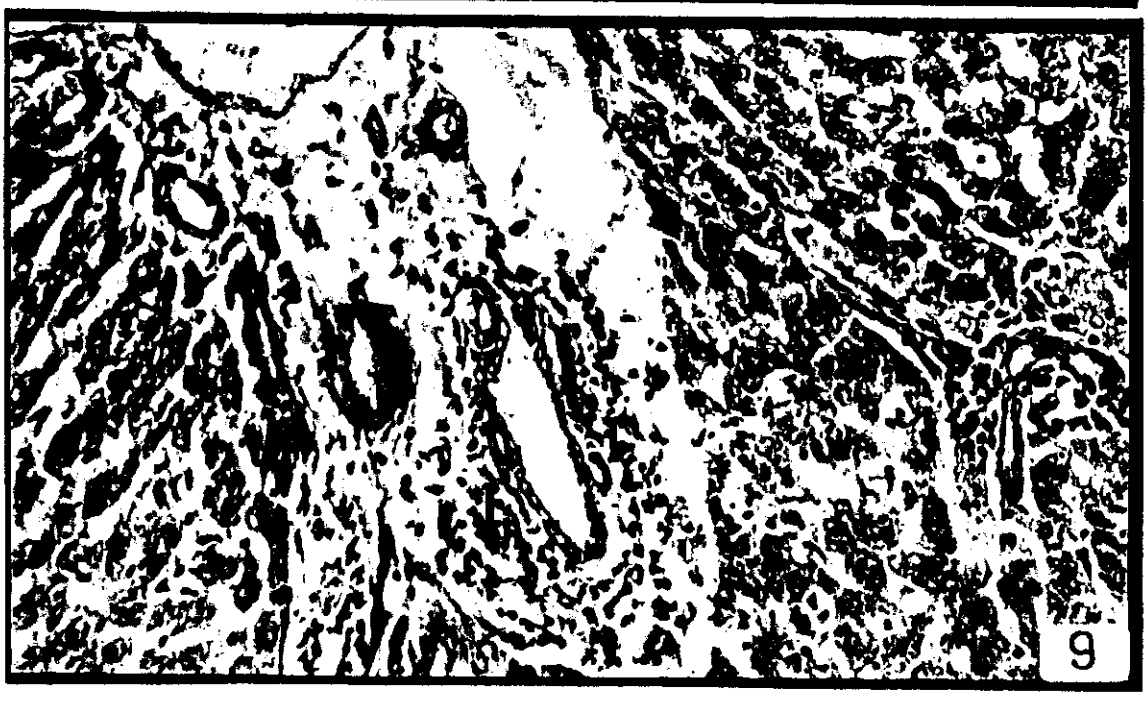
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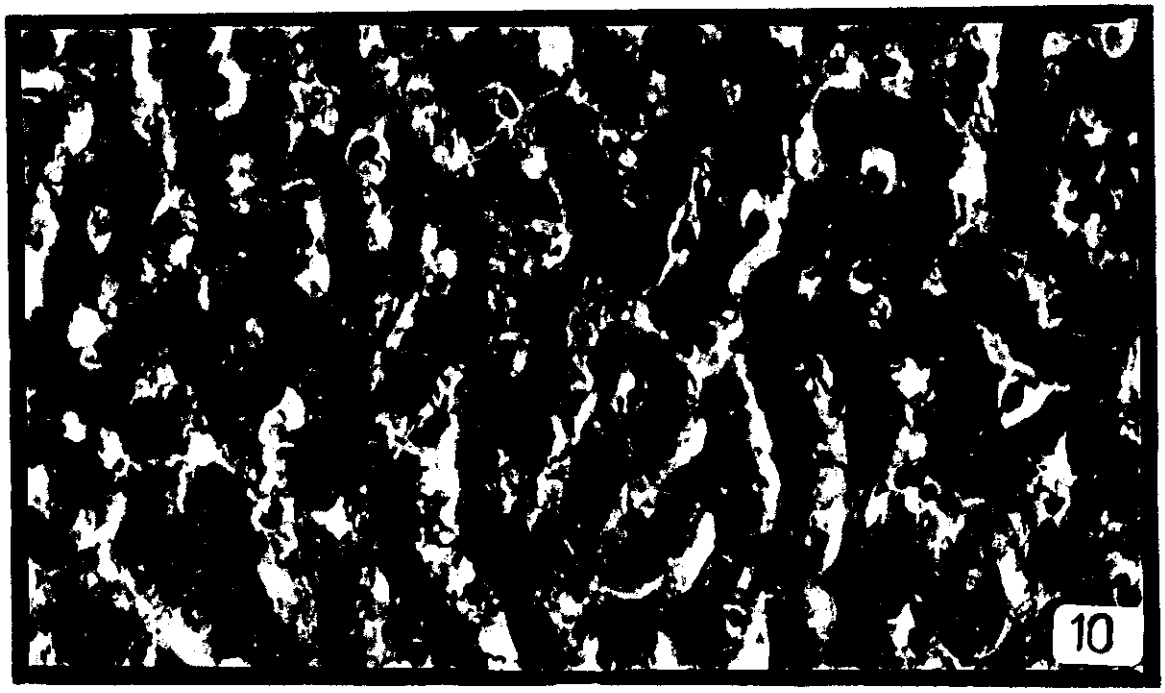
Plate (IV) -Material fixed in buffered neutral formalin and stained with Haematoxylin and Eosin (Clayden, 1971).

Fig. (10):- Liver section of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 45 days shows hyperplasia of Von Kupffer cells (H), single cell necrosis (N), changes in the nuclear size and prominent nucleoli (arrows). x 400

Fig. (11):- Liver section of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 45 days and left for 30 days after Cd abstainance, shows partial recovery of the hepatic tissue. A large number of necrotic cells (N) and pyknotic nuclei (arrows) are still observed. x 400

Fig. (12):- Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 10 days shows mild dilation (D) and congestion (C) of the central vein. Mild inflammation (I) is also observed around the central vein and there were many binucleated cells. x 400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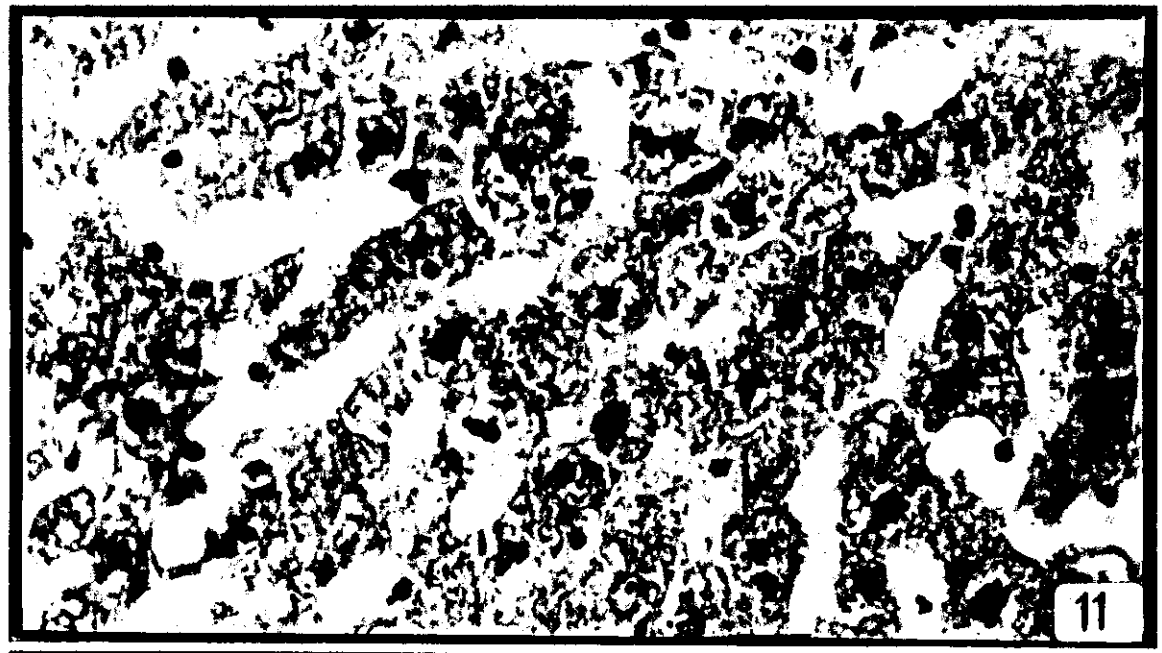
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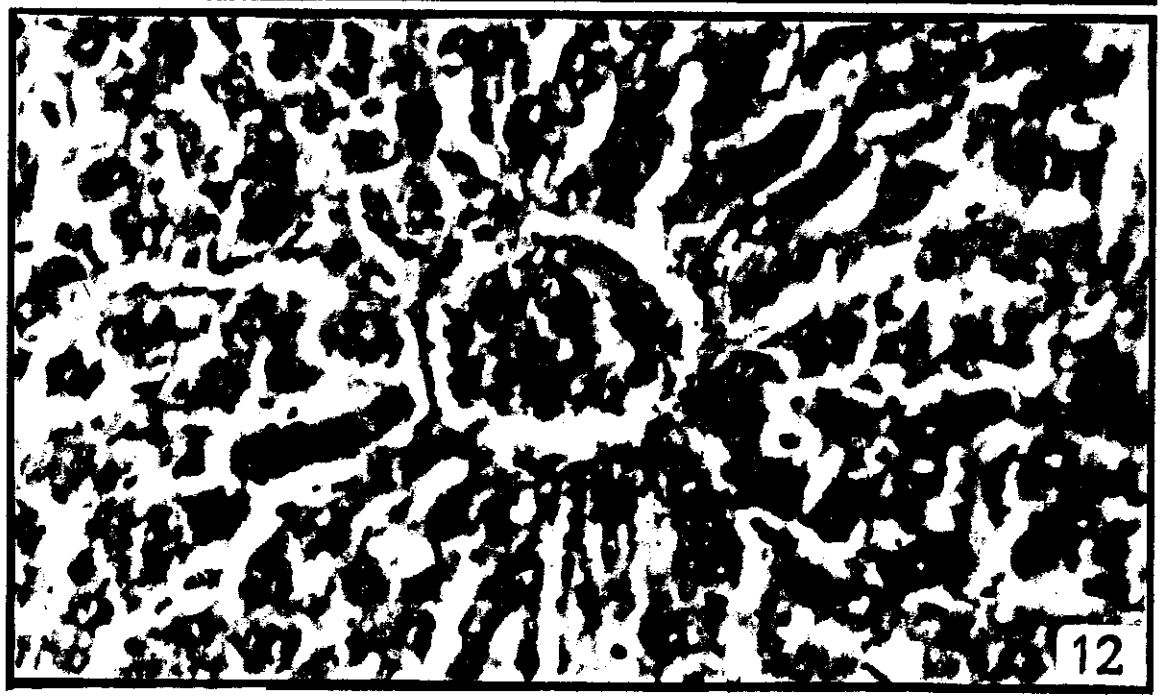
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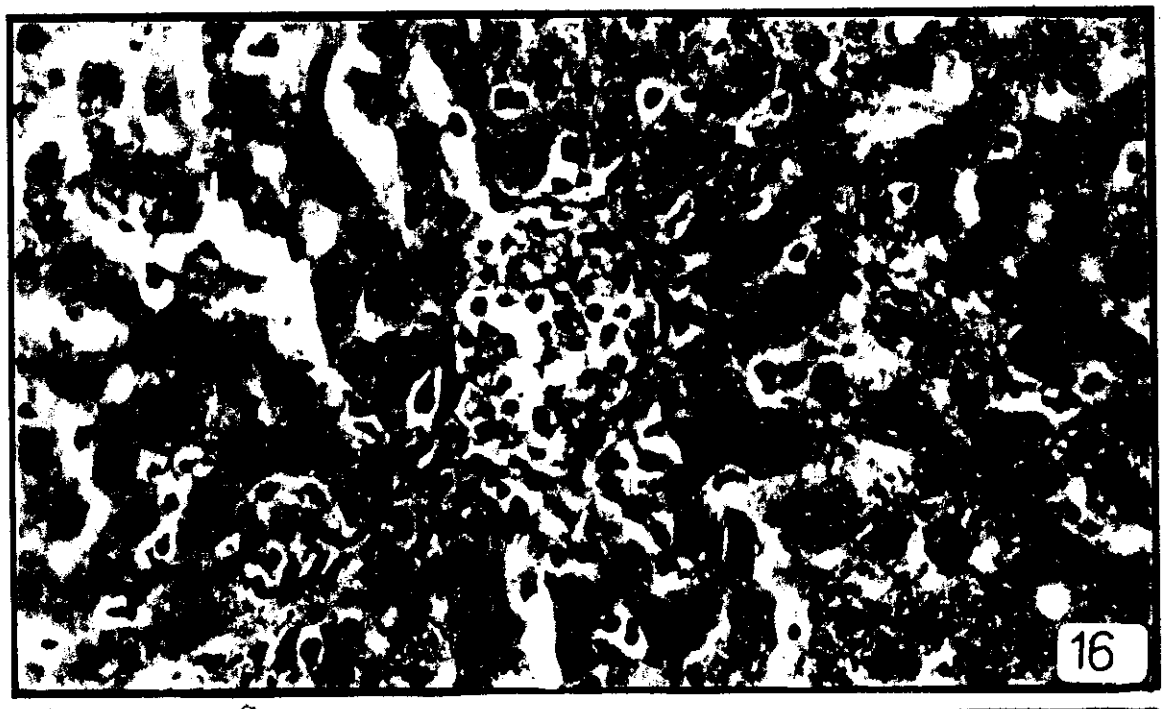
Plate (V) Material fixed in buffered neutral formalin and stained with Haematoxylin and Eosin (Clayden, 1971).

Fig. (13):- Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 10 days shows infiltration of the portal tract with a large number of inflammatory cells (I), congestion (C) of the portal vein radical as well as pyknosis (arrows) of many nuclei of the hepatic cells. x 400

Fig. (14):- Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 20 days shows blocking of the portal tract with an enormous number of inflammatory cells (I) and congestion (C) of the radical of the portal vein. x 400

Fig. (15):- Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 20 days shows an obvious increase in the number of pyknotic nuclei (P) and Von Kupffer cells (K) as well as karyolysis (arrows). The cenral vein (C V) is markedly dilated and congested. x 400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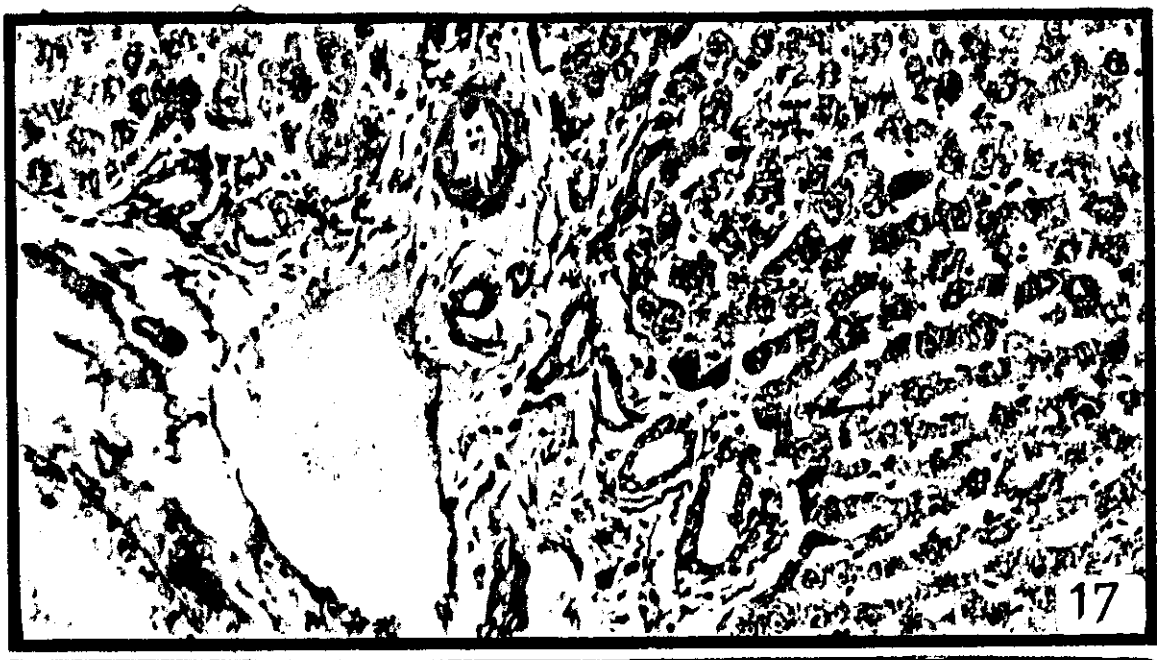
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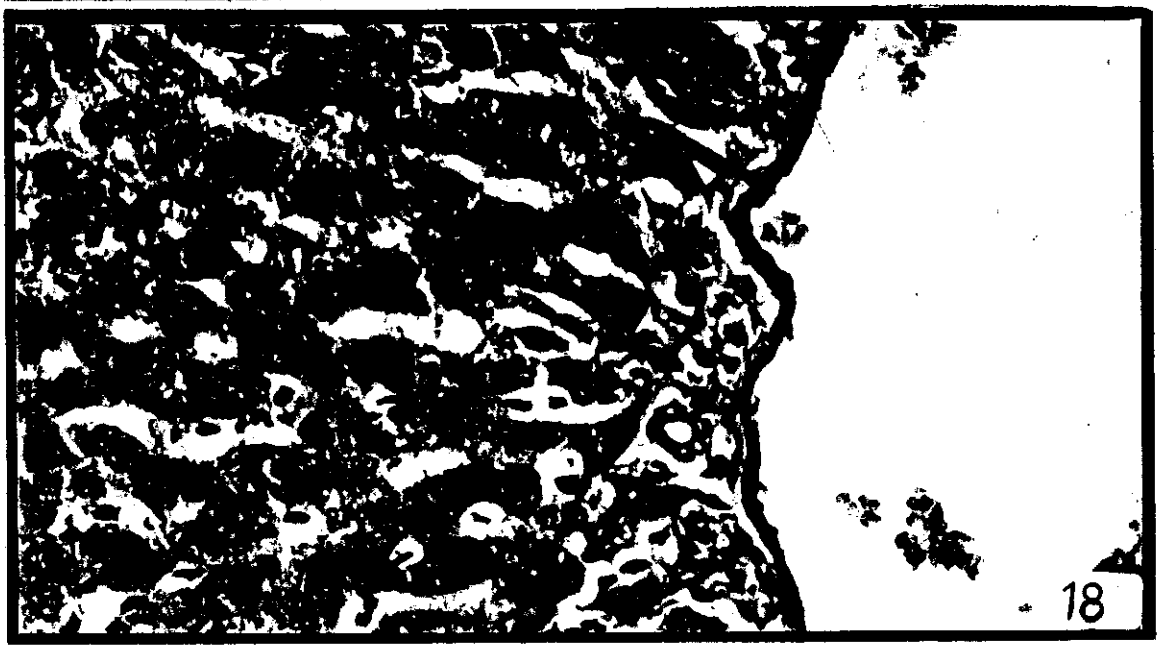
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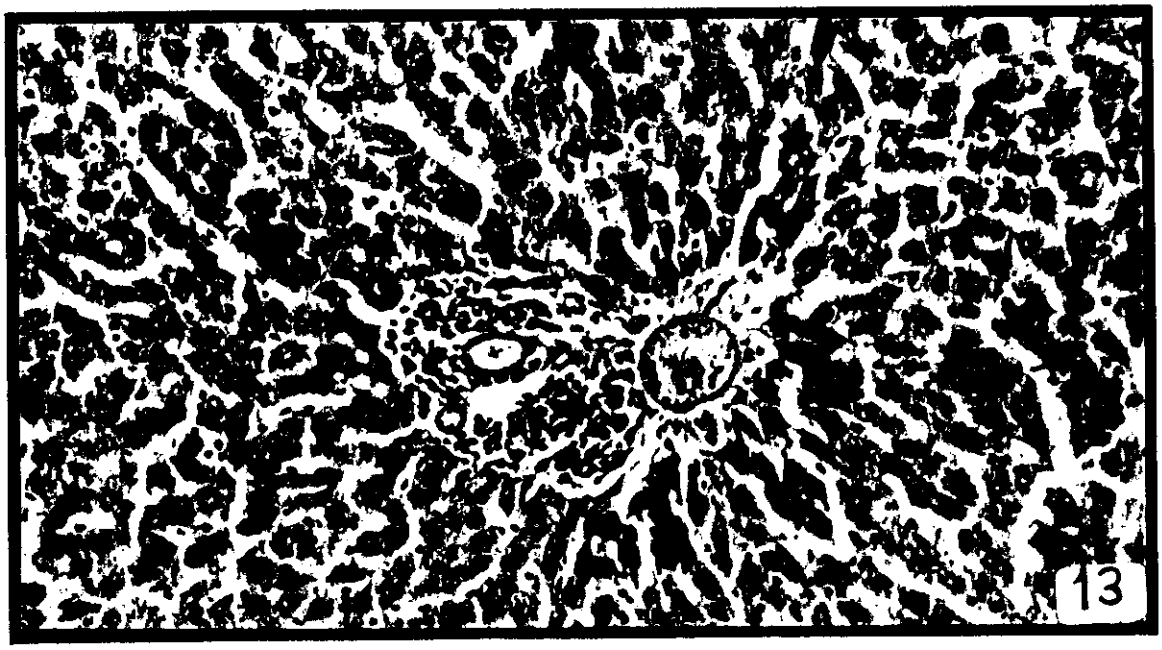
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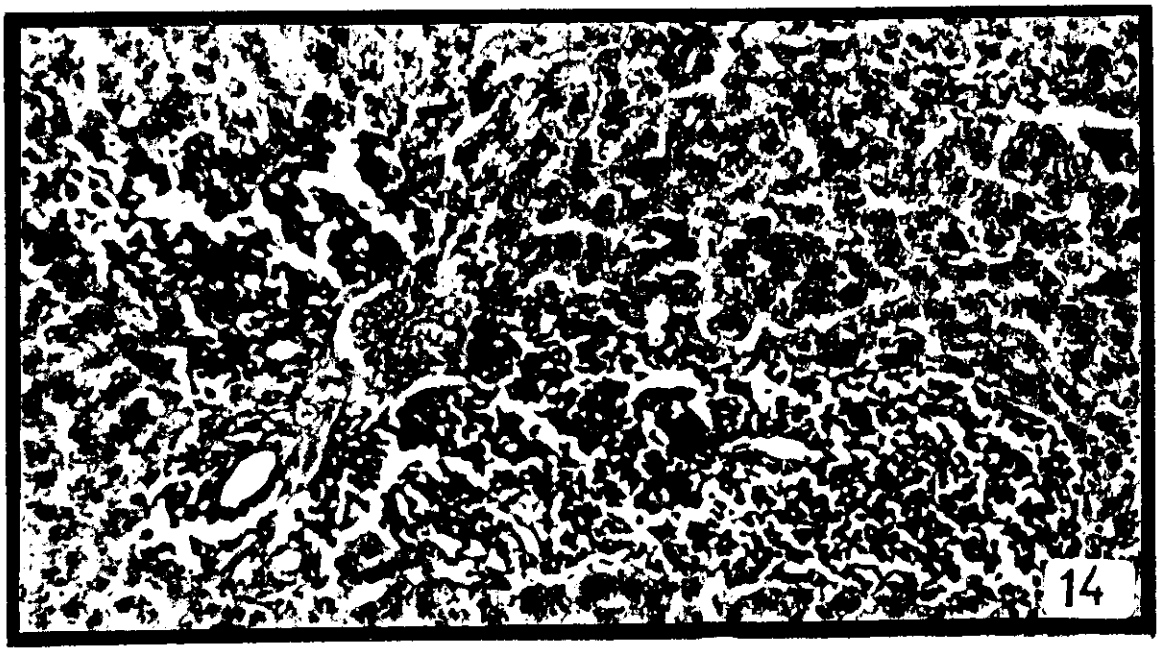
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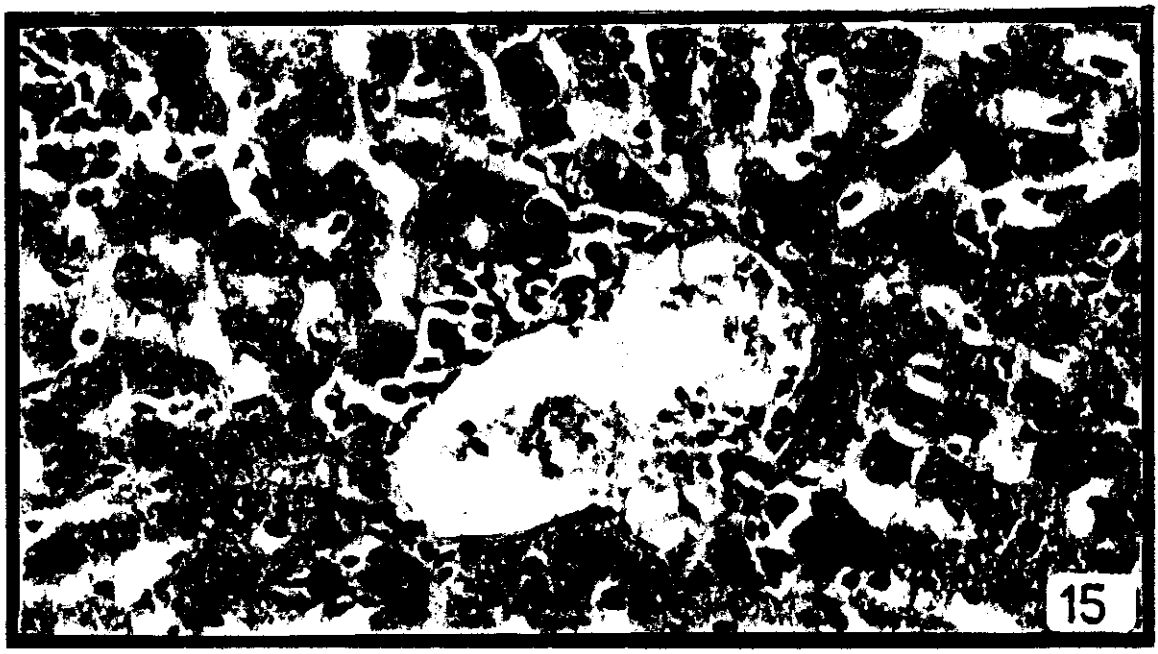
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Plate (VI) - Material fixed in buffered neutral formalin and stained with Haematoxylin and Eosin (Clayden, 1971).

Fig. (16):- Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 20 days shows cytoplasmic swelling (arrows) and pyknotic nuclei (P). Congestion and dilation of the central vein (CV) as well as obliteration of the sinusoids (S O) are also noticed. x 400

Fig. (17):- Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 30 days shows ductal proliferation (arrow) and marked dilation as well as congestion of the portal vein (PV). X 400

Fig. (18):- Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 30 days shows an obvious ductal proliferation (arrows), marked dilation of the portal vein (PV) nuclear pyknosis (P), karyolysis (L) and obliteration of the sinusoids (SO). x 400

Plate (VII) - Material fixed in buffered neutral formalin and stained with Haematoxylin and Eosin (Clayden, 1971).

Fig. (19):- Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 30 days shows marked dilation and congestion of the central vein (CV). Marked increase in the number of Von Kupffer cells (arrows) and margination of the nuclear chromatin are observed. x 400

Fig. (20):- Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 30 days shows marked hyalinization (arrow) of the wall of the central vein which surrounded by large number of inflammatory cells (I) and necrotic cells (N). The central vein (CV) also markedly congested. X 400

Fig. (21):- Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 45 days. Notice the presence of a large number of necrotic cells (N) and binucleated cells (arrows) as well as prominent nucleoli. x 400


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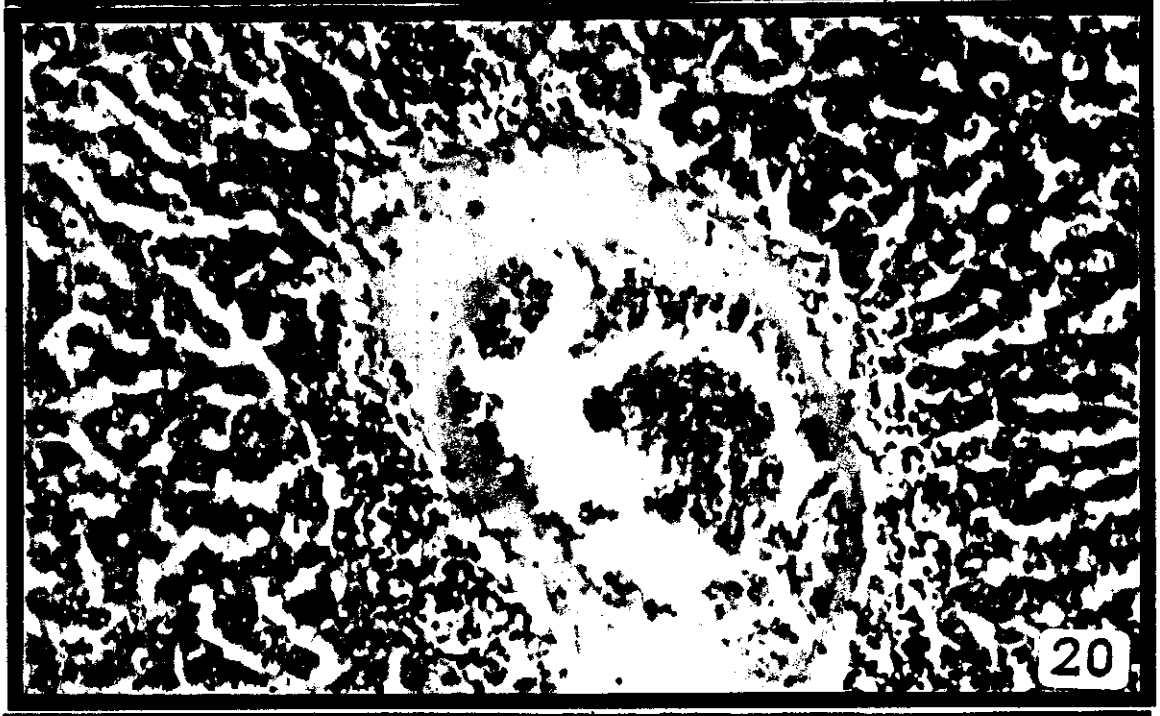
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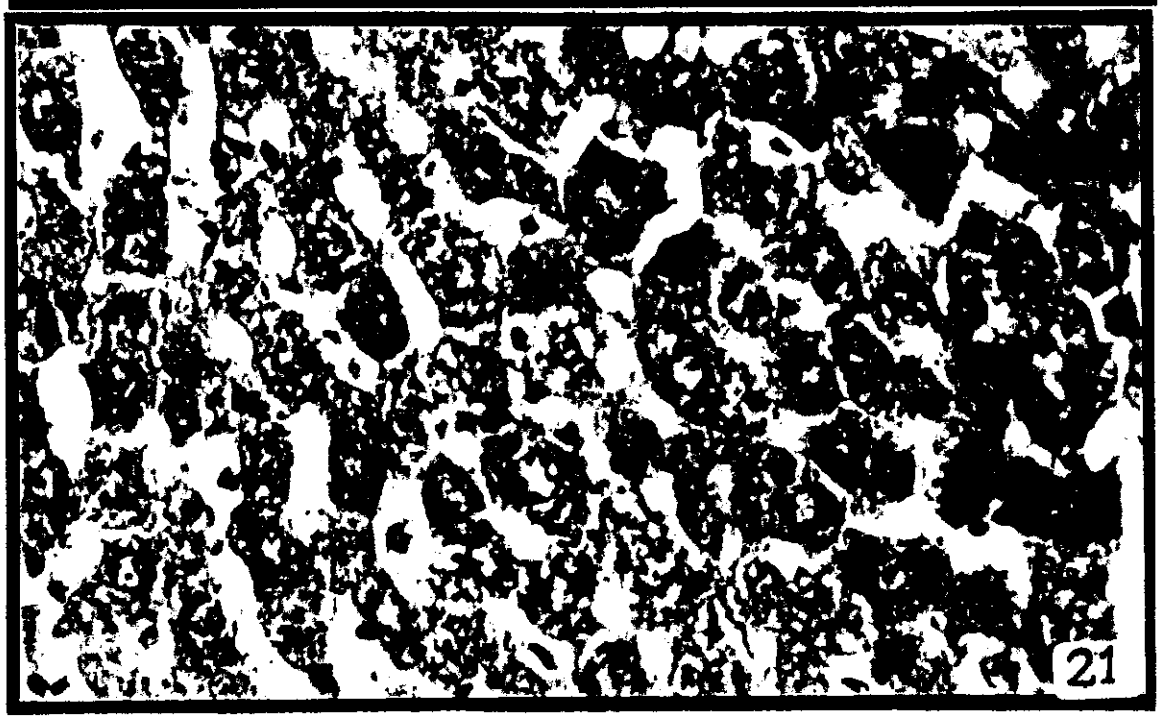
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Plate (VIII) - Material fixed in buffered neutral formalin and stained with Haematoxylin and Eosin (Clayden, 1971).

Fig. (22):- Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 45 days shows large areas of necrotic hepatic cells (arrows). x 250

Fig. (23):- Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 45 days shows marked granulomatous reaction (arrow). x 250

Fig. (24):- Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 45 days shows a marked liver fibrosis (F). x 250


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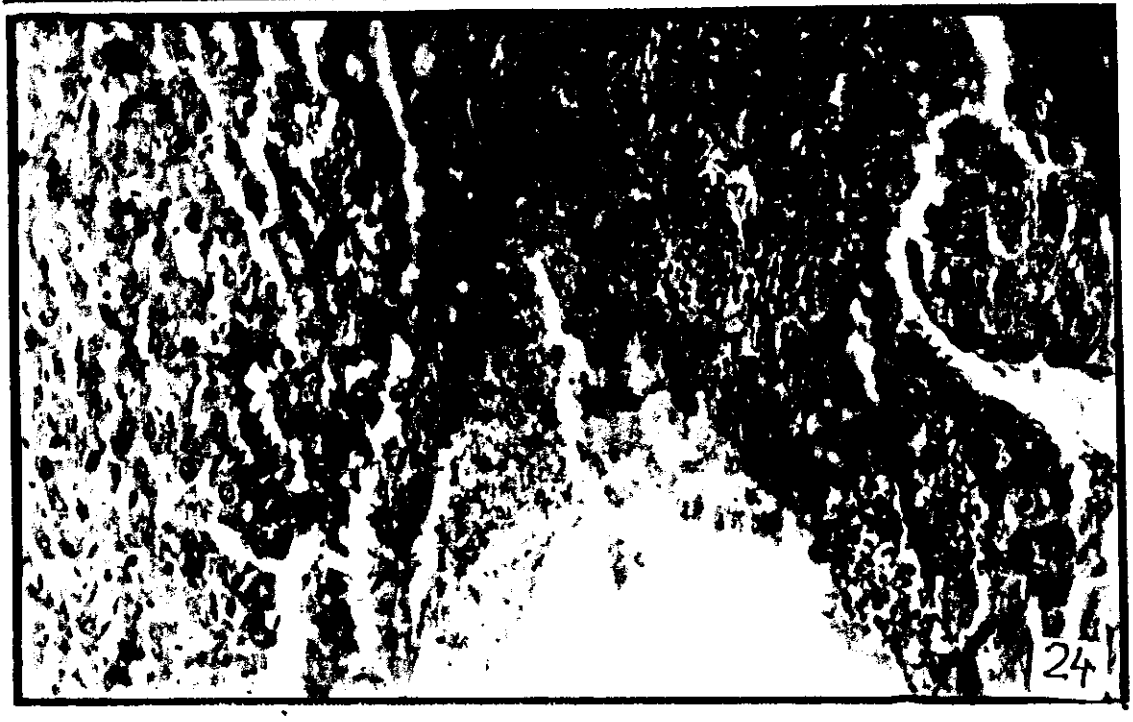
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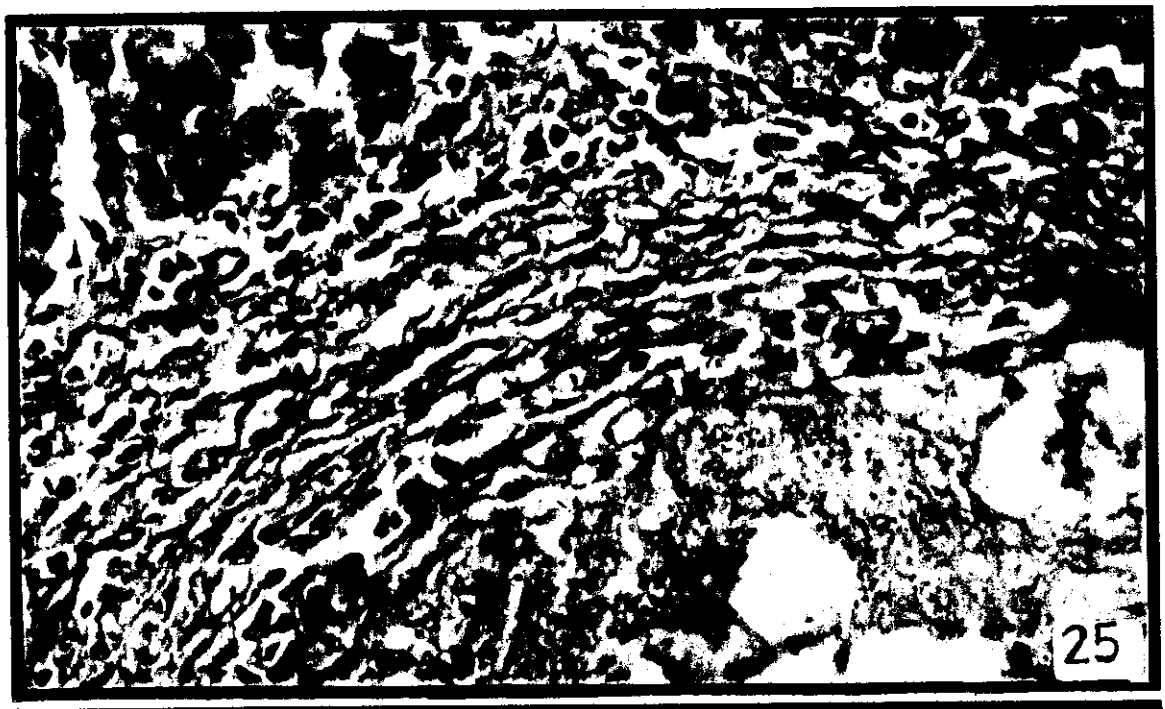
Plate (IX) Material fixed in buffered neutral formalin and stained with Haematoxylin and Eosin (Clayden, 1971).

Fig. (25):- Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 45 days shows marked hepatic fibrosis (F) reacting with inflammatory cells (I) as well as coagulating necrosis (arrows). X 400

Fig. (26):- Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 45 days and left for one month after Cd Cl2 abstainance shows presence of a large number of Von Kupffer cells (hyperplasia) (H), necrotic cells (N) as well as many binucleated cells (arrows). x 400

Fig. (27):- Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 45 days and left for 30 days following $CdCl_2$ abstainance shows atypical dysplastic cells, binucleated (small arrows) cells and abnormal mitotic figures (arrows). x 400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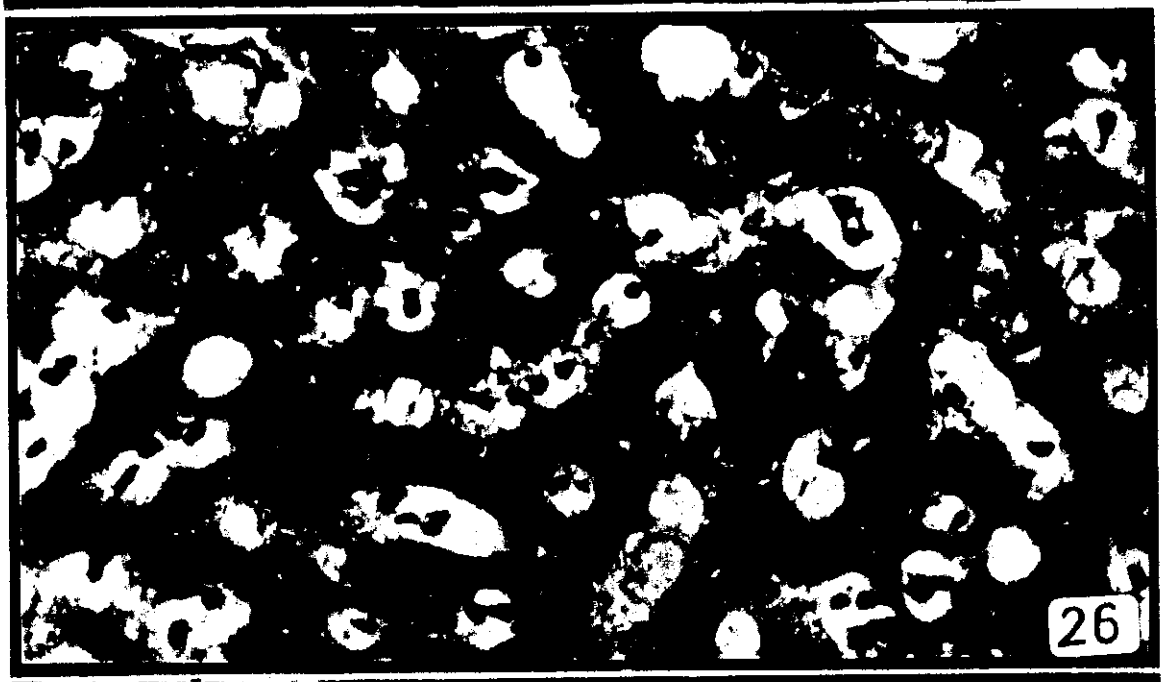
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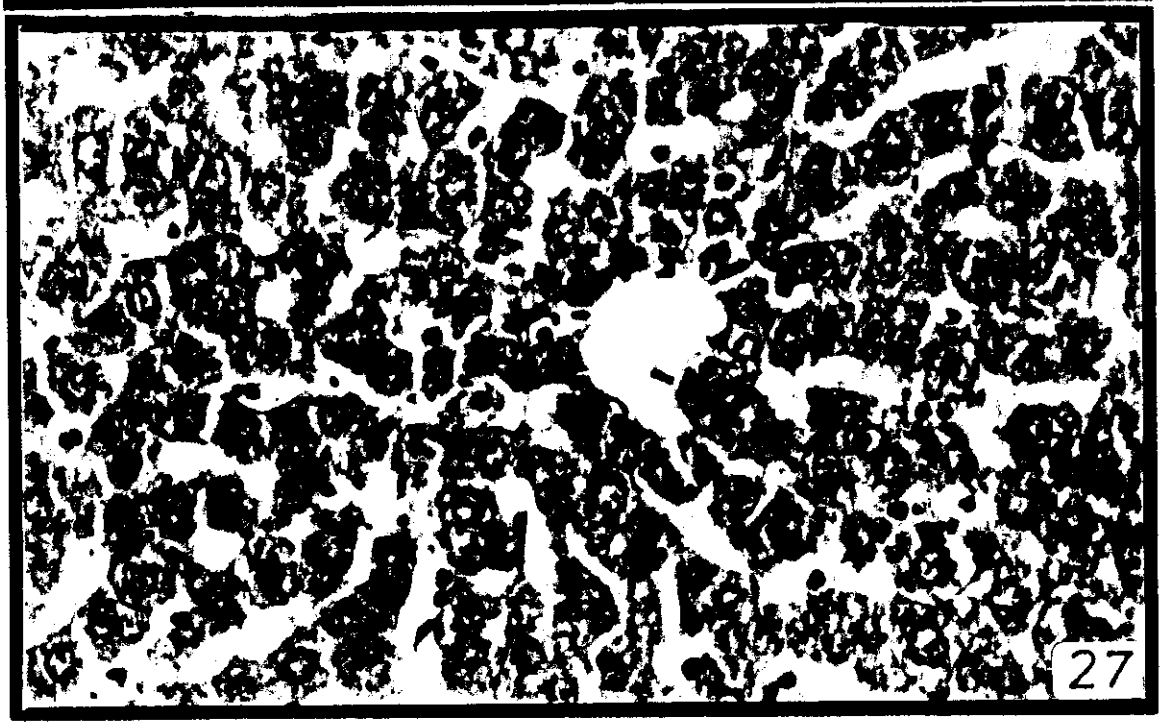
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HISTOCHEMICAL OBSERVATIONS

Glycogen

Control rats

For showing the liver glycogen inclusions of the control group, the Periodic Acid Schiff 's (PAS) method has been used. The cells of the normal liver revealed deep purpple-colored coarse particles of different sizes densely aggregated in the cytoplasm. All such PAS-positively reacting materials have been proved to be glycogen.

The hepatocytes of this figure differ in their glycogen contents, which appeared by rather different intensities of their PAS reaction. The liver nuclei reacted negatively with PAS indicating non-existence of any polysaccharide particles.

Rats treated with 1/8 LD_{50} $CdCl_2$

The animals treated with 1/8 LD_{50} $CdCl_2$ revealed gradual glycogen depletion proportionally increased with lapse of administration.

As observed in fig. (29) which prepared from liver materials of rats treated with 1/8 LD_{50} of $CdCl_2$ for 10 days revealed a slight decrease in the glycogen inclusions in parenchymal cells particularly in the peripheral hepatocytes.

Twenty days following administration with the same dose showed an obvious decrease of their glycogen content as illustrated in fig. (30). This depletion was markedly observed in the peripheral cells, while pericentral cells are still moderately stained with PAS.

After 30 days of administration with 1/8 LD_{50} $CdCl_2$, glycogen diminution became more pronounced and most of hepatocytes appeared to have lost a considerable proportion of their polysaccharides inclusions as observed in fig. (31). Loss of glycogen was homogenous in almost all hepatocytes except in few hepatocytes, which have a little glycogen particles and still displayed a pronounced positive PAS-reactivity.

The maximize depletion of glycogen inclusions of the parenchymal cells of liver materials of rats inspected 45 days following chronic administration with this low dose as observed in fig. (32). This figure revealed approximately negative PAS-reactivity in large number of the hepatic cells while other cells have a few scattered granules in their cytoplasm.

In a reverse manner a tendency toward restoration of the exhausted carbohydrate inclusions was considerably detected in specimens investigated 30 days after abstainance of Cd although the picture is still less than the normal counterparts as observed in fig. (33).

Rats treated with the 1/4 LD_{50} $CdCl_2$

Rats examined after 10 days following the above $CdCl_2$ dosing have indicated that the glycogen content of the liver parenchymal cells were markedly diminished as illustrated in fig. (34).

Exhaustion of glycogen inclusions became more noticeable in specimens inspected 20 days later as demonstrated in fig. (35). This figure reveals a somewhat moderate to a rather weak PAS-reactivity in some hepatocytes and much weaker reactivity in the others. Apparently, loss of glycogen contents as denoted by the reduced PAS reactivity-has progressed in a pronounced manner being obviously illustrated in the peripheral hepatic cells of rats examined 30 days after treatment with 1/4

LD_{50} $CdCl_2$ as demonstrated in fig. (36). In this figure, most of the hepatocytes appeared faintly stained but nonetheless, there were other ones still manifesting a considerable level of PAS reactivity.

The maximal diminution in the glycogen inclusions was clearly observed in the specimens examined after 45 days of treatment with the high dose. In such specimens, some of parenchymal cells appeared with highly reduced PAS reactivity and most of them were almost devoid of any stainable inclusions as demonstrated in fig. (37)

Later on, 30 days following Cd abstainance, a slight restoring of carbohydrate inclusions was noticed in many hepatocytes. Other hepatocytes still negatively reacted with PAS as observed in fig. (38&39).

Deoxyribonucleic acid (DNA) :

Control rats

Examination of Feulgen-prepared sections of the liver material of control rat have demonstrated that the DNA-containing particles as red purple colored particles (chromatin bodies) located in the nucleoplasm of almost all the liver parenchyma. The nuclear envelopes of these cells are also positively reacted where their cytoplasm does not elucidate any apparent Feulgen-positive reaction denoting the non-existence of any detectable cytoplasmic DNA inclusions.

Rats treated with 1/8 LD_{50} $CdCl_2$

In parallel correlation with proteinic inclusions changes after treatment with the above dose as illustrated in fig. (41) revealed a slight increase in the DNA reactivity in many of the parenchymal cell nuclei after ten days of treatment.

This picture was reversed after 20 days of applied the above dose as demonstrated in fig. (42), where a slight diminution in the DNA inclusions of the nuclei of the hepatocytes was noticed. Such depletion in the amount and reactivity of these inclusions became more noticeable in most of the nuclei of these cells in the materials inspected 30 days following administration with the same dose (fig. 43).

When the rats examined 45 days following the same above treatment, the material under investigation has revealed a further reduction in both amount and reactivity of DNA inclusions in most of the nuclei of the liver parenchymal cells. Because of enlargement and weakness of many nuclei of the hepatic cells as a result of Cd effect, figure (44) revealed weakly colored fine chromatin particles, which observed being aggregated mainly at the peripheries of the nuclear membranes.

A partial restoration of the DNA inclusions was detected in the specimens inspected 30 days following 1/8 LD_{50} $CdCl_2$ abstainance as illustrated in fig. (45). This figure revealed an incomplete picture of recovery, where many hepatic cells had a moderate Feulgen-reactivity. Also, many cells still undergo marked depletion of their chromatin particles.

Rats treated with 1/4 LD_{50} $CdCl_2$

Ten days following the above mode of treatment, a slight increase in the DNA contents of the nuclei of the parenchymal cells of the rat's liver was observed as illustrated in fig. (46). In this figure, the nuclei appeared manifesting a strong Feulgen reaction than before.

On the other hand, The picture was reversed in the specimens examined twenty days after treatment with this dose as observed in fig. (47). This figure demonstrated an obvious diminution of the DNA content of the hepatic cell which manifesting a weaker reactivity with Feulgen stain

Plate (3)

Microscopic findings in the liver of rats treated with 1/8 LD_{50} of $CdCl_2$.

Organ and finding / Days of treatment	10				20				30				45				75 days (recovery)			
liver / Grade	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++
Hydropic degeneration and cytoplasmic granularity	1	5				2	4				3	3				6				6
Karyorrhexis and karyolysis	6				5	1				2	4					6				6
Pyknosis	2	4				1	5				6				2	4			4	2
Focal necrosis	6				6				5	1				2	4				6	
Von kupffer cells increase	6				3	3				2	4					6				6
Infiltration of the portal tract with inflammatory cells		6				1	5					6				6				6
Central vein dilation		6					6				4	2				6				6
Central vein congestion		6					6					6				6				6
Liver fibrosis.	6				6				6				6							
Ductal proliferation	6				6				6				6							
Sinusoidal obliteration regenerating changes		6								4	2					6				6

-	*no lesion*
÷	*mild change*
÷÷	*moderate change*
÷÷+	*severe change*

than the normal picture. These features have become more pronounced in the specimens inspected after thirty days of 1/4 LD_{50} of $CdCl_2$ application as illustrated in fig.(48). In which case, faintly colored nuclei were of common occurrence. The maximal exhaustion in the DNA inclusions was observed in the liver materials of rats inspected 45 days of 1/4 LD_{50} $CdCl_2$ administration as shown in fig. (49). In this figure, many nuclei appeared manifesting approximately negative Feulgen reaction as well as very weak reaction of marginated materials of the others.

Concerning the materials obtained on the thirty day following Cd-abstainance, fig. (50& 51) demonstrated that some of the nuclei have apparently begun to restore some of their DNA inclusions taking a moderate coloration. Other nuclei in the same figure still revealed a marked loss of these inclusions which was reflected by the faint reactivity.

Ribonucleic Acid (RNA)

Control rats

In Pyronein Methyl Green preparations, ribonucleic acid (RNA)-containing particles appeared in the normal rat hepatocytes to be obviously pyroninophilic being demonstrated as a small brightly red-cloured bodies scattered uniformly in the cytoplasm of these cells. The nucleoli also exhibited a deeply red stainability indicating their RNA-constitution while the nuclei were stained greenish blue due to their DNA contents.

Rats treated with 1/8 LD_{50} $CdCl_2$

The rats treated with 1/8 LD_{50} $CdCl_2$ and examined 10 days following administration, revealed a slight increase in the pyroninophylic particles in most of the parenchymal cells. These particles as illustrated in fig. (53) appeared diffusing as red patches in the hepatic cells cytoplasm.

Plate (4)

Microscopic findings in the liver of rats treated with 1/4 LD_{50} of $CdCl_2$.

Organ and finding	Days of treatment	10				20				30				45				75 days (recovery)			
liver	Grade	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++
Hydropic degeneration and cytoplasmic granularity			2	4				6					6				6				6
Karyorrhexis and karyolysis		6					2	4				4	2				6				6
Pyknosis			6					6				6				4	2				6
Focal necrosis		6				6					2	4					6				6
Von kupffer cells increase			6					6					6				6				6
Infiltration of the portal tract with inflammatory cells				6					6				6				6				6
Central vein dilation			6					4	2				6				6				6
Central vein congestion			6					3	3				6				6				
Liver fibrosis.		6				6				6				2	4			1	5		
Ductal proliferation		6				5	1			3	3			2	1	3			2	4	
Sinusoidal dilation		6				6				6				6				4	2		
Sinusoidal obliteration		6					6				6				2	4				4	2
regenerating changes		6				6				2	4				6					6	

-	*no lesion*
+	*slight change*
++	*moderate change*
+++	*severe change*

Contrary to the previous results, the observations of the liver materials of rats inspected 20 days after treatment with the above dose showing a reverse action of long time of administration of Cd on RNA content of the hepatocytes as demonstrated in fig. (54). The RNA inclusions of most hepatic cells revealed a mild decrease comparing to the normal conditions.

Thirty days following administration with the same dose manifested a noticeable diminution of the RNA inclusions in most of the parenchymal cells particularly the peripheral ones. The diminution of these inclusions in the nuclei of these cells was also clearly appeared as observed in fig. (55). The marked loss of these pyronin-reacted inclusions was noticed after 45 day of chronic application of the same above dose in almost of the liver parenchyma as demonstrated in fig. (56).

On the other hand, a partial restoration of the RNA materials for regaining the normal picture was observed in rats inspected 30 days following Cd-abstainance. Figure (57) revealed moderate pyronin reactivity in the cytoplasm of most of the hepatic cells.

Rats treated with 1/4 LD_{50} $CdCl_2$

Following treatment of rats with 1/4 LD_{50} $CdCl_2$ for 10 days, a significant increase could be seen in the RNA-containing granules in the specimens obtained from the central parts of the hepatic lobes as illustrated in fig. (58). Other materials obtained from the peripheral parts revealed similar results such as the normal inclusions.

On the twenty days following application of the above dose, most of hepatic cells apparently underwent a significant decrease in their RNA contents which appeared in the form of diffused patches as illustrated in fig. (59). Such preparations of liver sections appeared to be faintly stained with Pyronin Methyl Green technique.

While those prepared from materials treated with the same dose for thirty days manifested a marked loss in the number and stainability of RNA particles in the hepatocytes as demonstrated in fig. (60). On the 45-th day following 1/4 LD_{50} $CdCl_2$ administration, the RNA contents appeared in the majority of liver parenchymal cells to have lost a considerable proportion, of thir affinity to Pyronin staining and became rather fewer in number and smaller in size than previous examined groups as observed in fig. (61).

Thirty days after the abstainance of $CdCl_2$ treatment of rats with 1/4 LD_{50}, a slight recovery of RNA inclusions was detected in a small number of hepatic cells, while most of them still undergo marked loss of their RNA content as illustrated in fig. (62&63).

Total proteins

Control rats

The proteins were found as fine bluish irregular particles in a weakly to moderately stained cytoplasm. The moderate reaction was observed in the central regions of the hepatic lobules, while the peripheral cells give weak reaction. Both the cell and nuclear membranes were intensively stained bluish. Each nucleus contained moderate positively stained chromatin particles and intensively stained nucleoli. Both Kupffer and endothelial cells were moderately stained.

Rats treated with 1/8 LD_{50} $CdCl_2$

Liver sections obtained on the 10-th days of treatment displayed a slight increase in both cytoplasmic and nuclear total protein content than the normal condition as illustrated in fig. (65).

On the other hand, twenty days following the application of the above dosage of the $CdCl_2$ have rendered the proteinic inclusions relatively less coloured than the normal condition. This is an indication that such materials

have undergone a slight diminution in the different most of the parenchymal cells of the liver as observed in fig. (66). Such protein depletion became rather more marked in the specimens subjected to the same treatment and examined 30 day later as illustrated in fig. (67). These changes were clear in both the cytoplasm and the nuclei of the hepatocytes, although the pericentral cells somewhat persisting dense stainability.

The marked loss of these proteinic inclusions in the rats of this group was seen in specimens obtained 45 days following the 1/8 LD_{50} $CdCl_2$ application. Fig. (68) demonstrated more exhaustion of proteins in the hepatocytes, where the cytoplasm and nuclei of many of these cells appeared weakly stained with bromophenol blue indicating of marked destructive changes of hepatic tissue.

On the other hand, The liver materials inspected 30 days following Cd abstainance revealed some opposite situation of protein diminution which observed in the above treated groups. This was reflected by a partial tendency toward the restoration of the normal picture of protein localization and reactivity in some liver parts as observed in fig. (69).

Rats treated with 1/4 LD_{50} $CdCl_2$

Concerning materials obtained 10 days post-administration of the above dose of this heavy metal, a slight increase in the proteinic contents was observed in the nuclei and of some parenchymal cells as deduced from the intensive bromophenol blue reactivity comparable to the normal counterparts as showed in fig. (70).

Twenty days following 1/4 LD_{50} $CdCl_2$ revealed an obvious depletion which illustrated in fig. (71) as reduction of blue reactivity of the cytoplasm and nuclei of the parenchymal cells particularly the pericentral ones. More diminution of the cytoplasmic and nuclear proteins was detected in the liver

parenchyma of the rats treated with 1/4 LD_{50} $CdCl_2$ after 30 days of treatment. The bromophenol blue reacted particles as observed in fig. (72) appeared weakly stained in the cytoplasm, beside the nuclei revealed a marked loss of these inclusions than the cytoplasm.

The greatest exhaustion of the proteinic materials in the hepatic tissue of all the treated subgroups in this study was demonstrated from specimens inspected 45 days following 1/4 LD_{50} $CdCl_2$ application as observed in fig. (73). This advanced degree of protein depletion appeared in some hepatocytes as a negative reactivity with bromophenol blue. The pericentral cells still showing a moderate bluish stain.

Thirty days following Cd abstainance of the above applied dose revealed a slight tendency toward recovery and restoration of the normal picture of protein localization and reactivity has seemed to have slightly proceeded in a progressive manner to regaining the normal picture as demonstrated in fig. (74& 75).

Plate (X) – Glycogen in the liver of normal and CdCl2 treated rats. Materials were in fixed in alcoholic Bouin 's fluid and treated according to the Periodic Acid Schiff 's (PAS) technique (Hotchkiss, 1948). x 1000

Fig (28) Liver section of a normal rat from the control group shows PAS-positive (glycogen) inclusions as deeply purple colored coarse particles of different sizes densely located in the cytoplasm. The nuclei are negatively stained.

Fig. (29) Liver section of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 10 days shows a strong PAS reaction in some hepatocytes and others stained weakly, while others are faintly stained.

Fig. (30) Liver section of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 20 days shows marked decrease of glycogen granules in most hepatocytes while others are still strongly stained.

Fig. (31) Liver section of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 30 days shows marked glycogen depletion in some cells, while it appears rather less significant in other ones.

Fig. (32) Liver section of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 45 days shows severe depletion of glycogen in the majority of the liver cells.

Fig. (33) Liver section of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 45 days and left for 30 days without injection shows partial recovery of the carbohydrate material.

Plate (XI) – Glycogen in the liver of normal and CdCl2 treated rats. Materials were in fixed in alcoholic Bouin 's fluid and treated according to the Periodic Acid Schiff 's (PAS) technique (Hotchkiss, 1948). x 1000

Fig. (34) Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 10 days shows a slight decrease in glycogen inclusions in many hepatocytes.

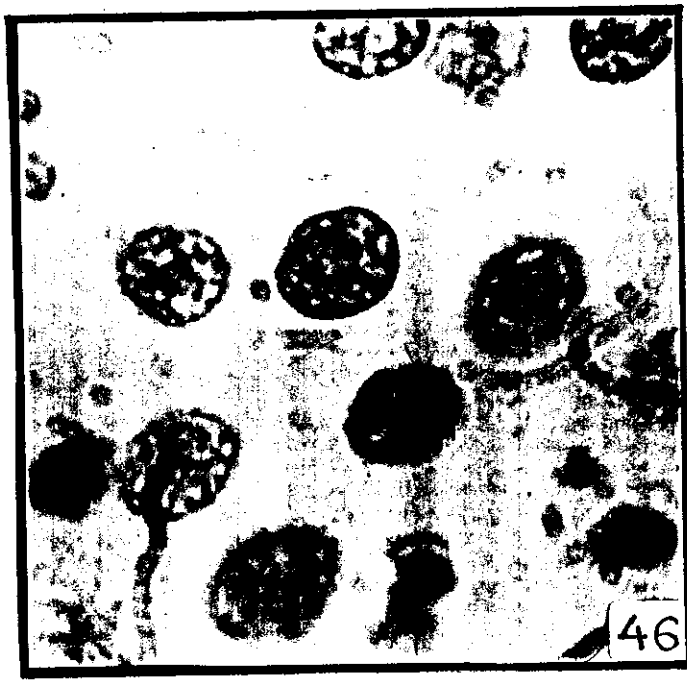
Fig. (35) Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 20 days shows marked depletion of glycogen particles in some cells, while it appears rather less significant in other ones.

Fig. (36) Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 30 days shows a sharp decrease in the carbohydrate content in most liver cells. A weak stainability for glycogen is apparent in most of the hepatocytes.

Fig. (37) Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 45 days shows complete absence of glycogen particles in most liver cells. A mild stainability is revealed by a few numbers of cells.

Fig. (38) Liver section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ and inspected 30 days following Cd-abstainance, reveals a slight restoration of the carbohydrate material in most liver cells.

Fig. (39) Another area of the above specimen elucidates a weak stainability for glycogen in most of the live cells, while few cells are apparently restored their glycogen content.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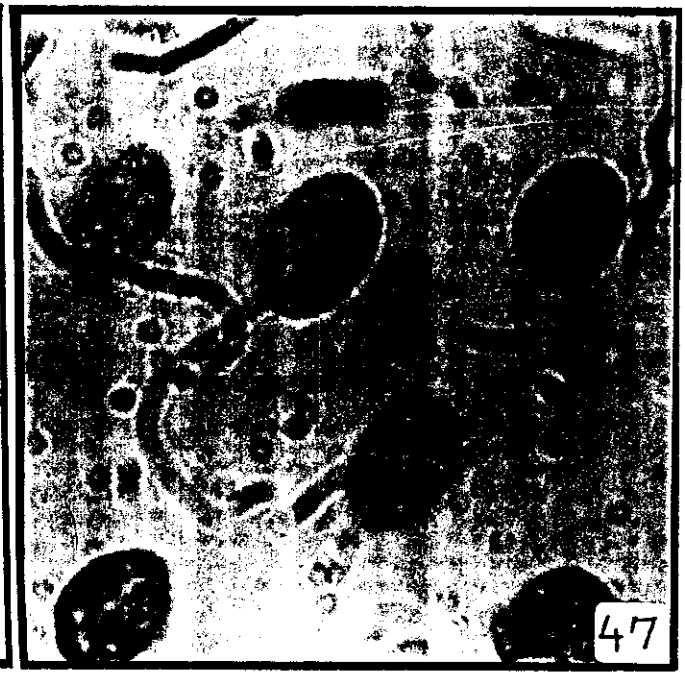
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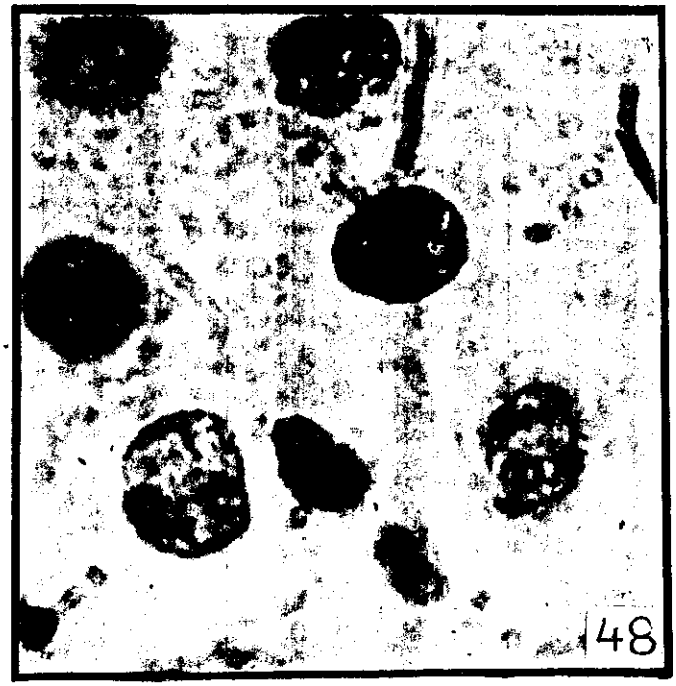
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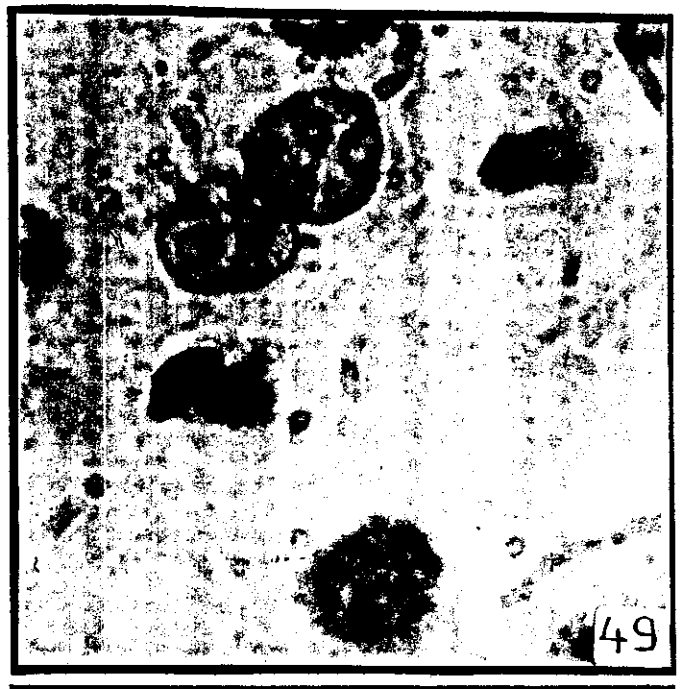
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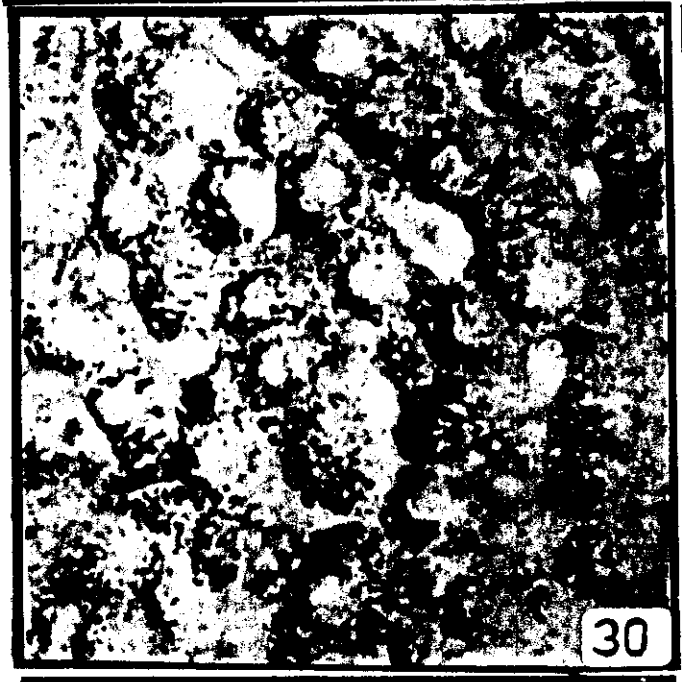
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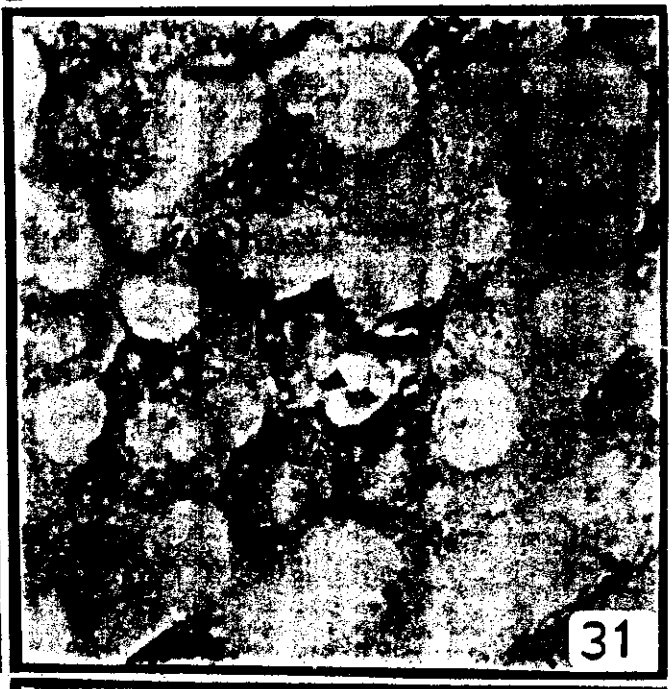
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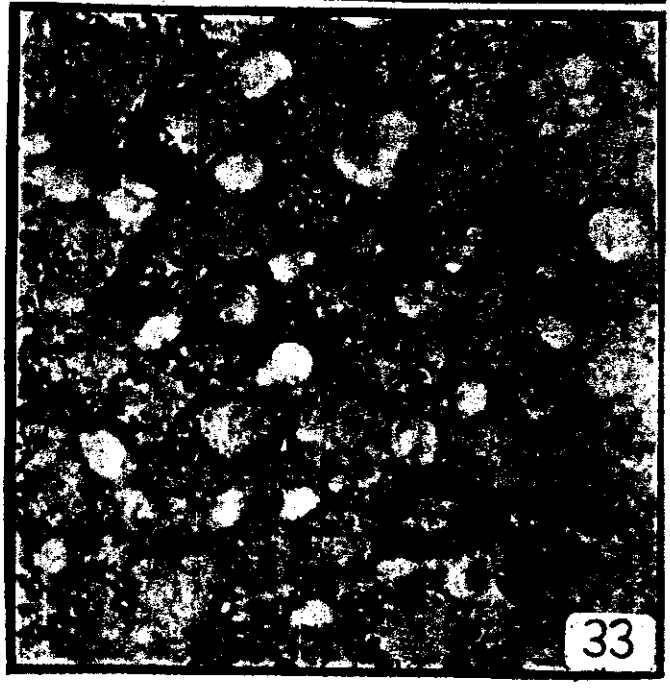
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Plate (XII) - Deoxyribonucleic acid (DNA) containing particles in the liver cells of normal and CdCl2 treated rats. Materials were fixed in Carnoy 's fluid and stained with Feulgen technique (De Tomasi, 1936). x 1000

Fig. (40) Liver section of a control rat shows the chromatin bodies in the form of red stained granules of DNA.

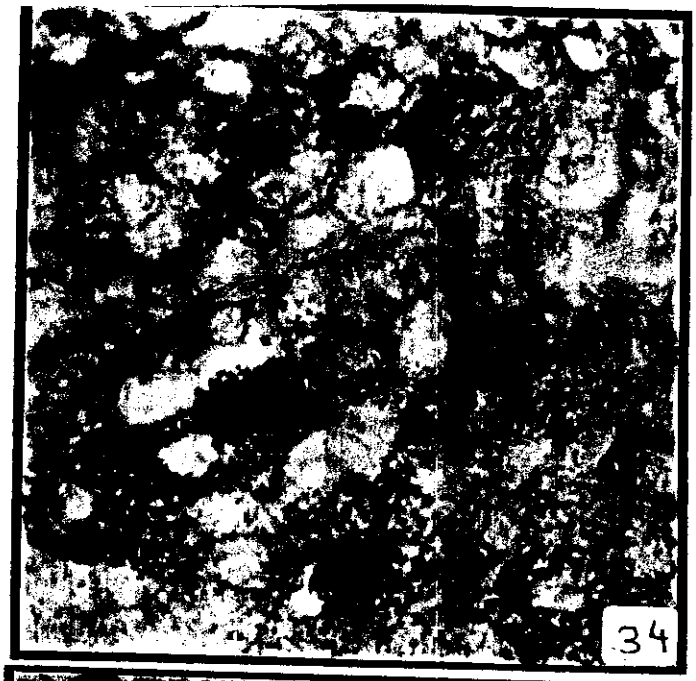
Fig. (41) Liver section of a rat treated day after day with 1/8 LD_{50} $CdCl_2$ for 10 days shows an obvious increase of DNA inclusions.

Fig. (42) Liver section of a rat treated day after day with 1/8 LD_{50} $CdCl_2$ for 20 days shows deeply stained chromatin particles in some nuclei, while others are stained with weakly Feulgen technique.

Fig. (43) Liver section of ,a rat treated day after day with 1/8 LD_{50} $CdCl_2$ for 30 days shows marked decrease in the DNA contents.

Fig. (44) Liver section of a rat treated day after day with 1/8 LD_{50} $CdCl_2$ for 45 days shows maximum decrease of chromatin bodies. Many nuclei are shrinked and densely stained, while others are enlarged and showing chromatin margination along the nuclear membrane.

Fig. (45) Liver section of a rat treated day after day with 1/8 LD_{50} $CdCl_2$ for 45 days and left 30 days for recovery shows mild restoration of the DNA contents. Some nuclei are still faintly stained.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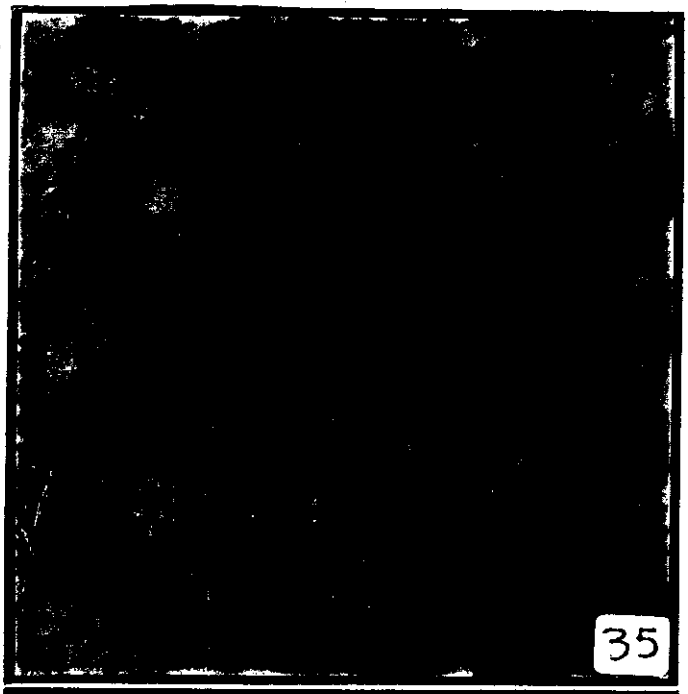
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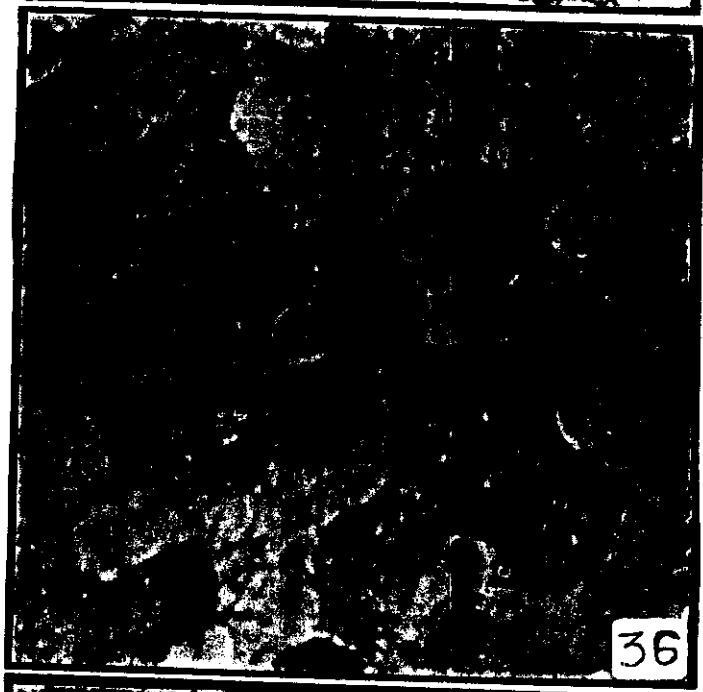
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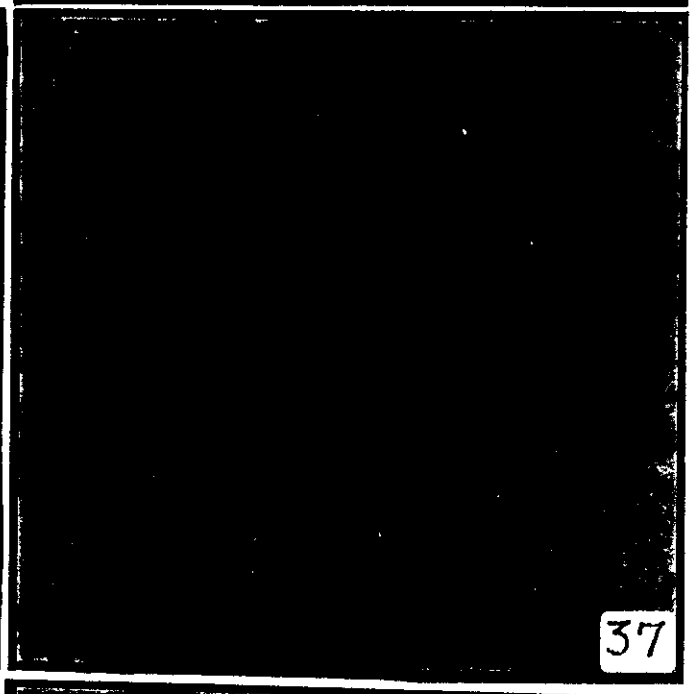
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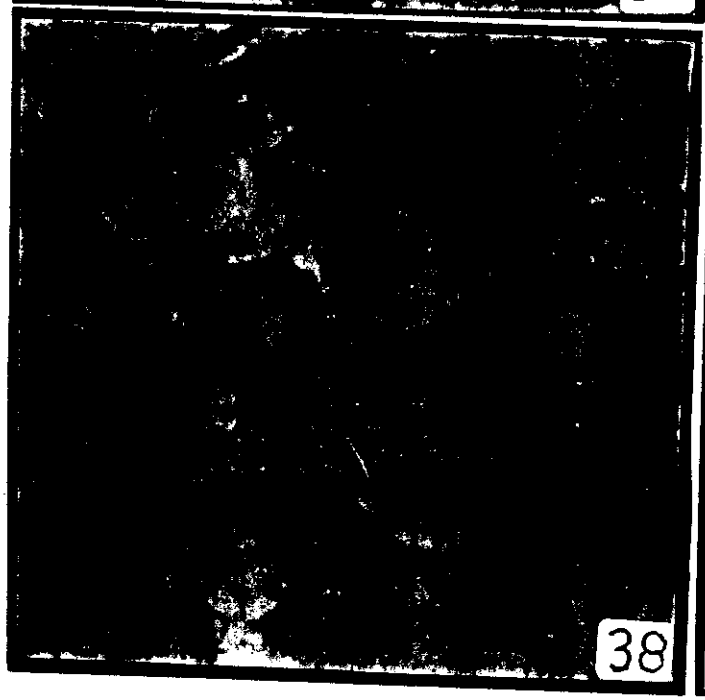
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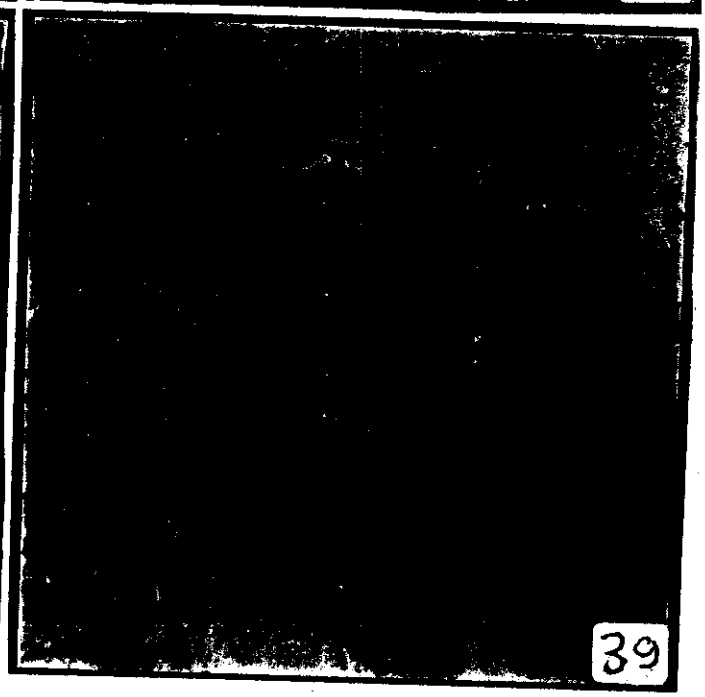
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Plate (XIII) - Deoxyribonucleic acid (DNA) containing particles in the liver cells of normal and CdCl2 treated rats. Materials were fixed in carnoy 's fluid and stained with Feulgen technique (De Tomasi, 1936). x 1000

Fig. (46) Liver section of a rat treated day after day with 1/4 LD_{50} $CdCl_2$ for 10 days shows deeply red- purple stained chromatin bodies. The nuclear membranes are also strongly reacted with Feulgen.

Fig.(47) Liver section of a rat treated day after day with 1/4 LD_{50} $CdCl_2$ for 20 days shows marked depletion in the DNA-containing particles.

Fig. (48) Liver section of a rat treated day after day with 1/4 LD_{50} $CdCl_2$ for 30 days shows marked depletion of the DNA particles. These particles faintly stained with red-purple color and are marginated along the nuclear membrane in some cells, while in others they are scattered in the nucleoplasm.

Fig. (49) Liver section of a rat treated day after day with 1/4 LD_{50} $CdCl_2$ for 45 days shows severe depletion of DNA content, where the chromatin granules disappeared approximately in most degenerated cells and they appear weakly stained with Feulgen.

Fig. (50) Liver section of a rat treated day after day with 1/4 LD_{50} $CdCl_2$ and examined 30 days after Cd withdrawal shows a slight restoring of the normal DNA reactivity in some nuclei. Some cells are still revealing severe depletion of chromatin materials.

Fig. (51)- Another field of the last section shows the same phenomenon of a slight restoration of the DNA contents in the liver cells.

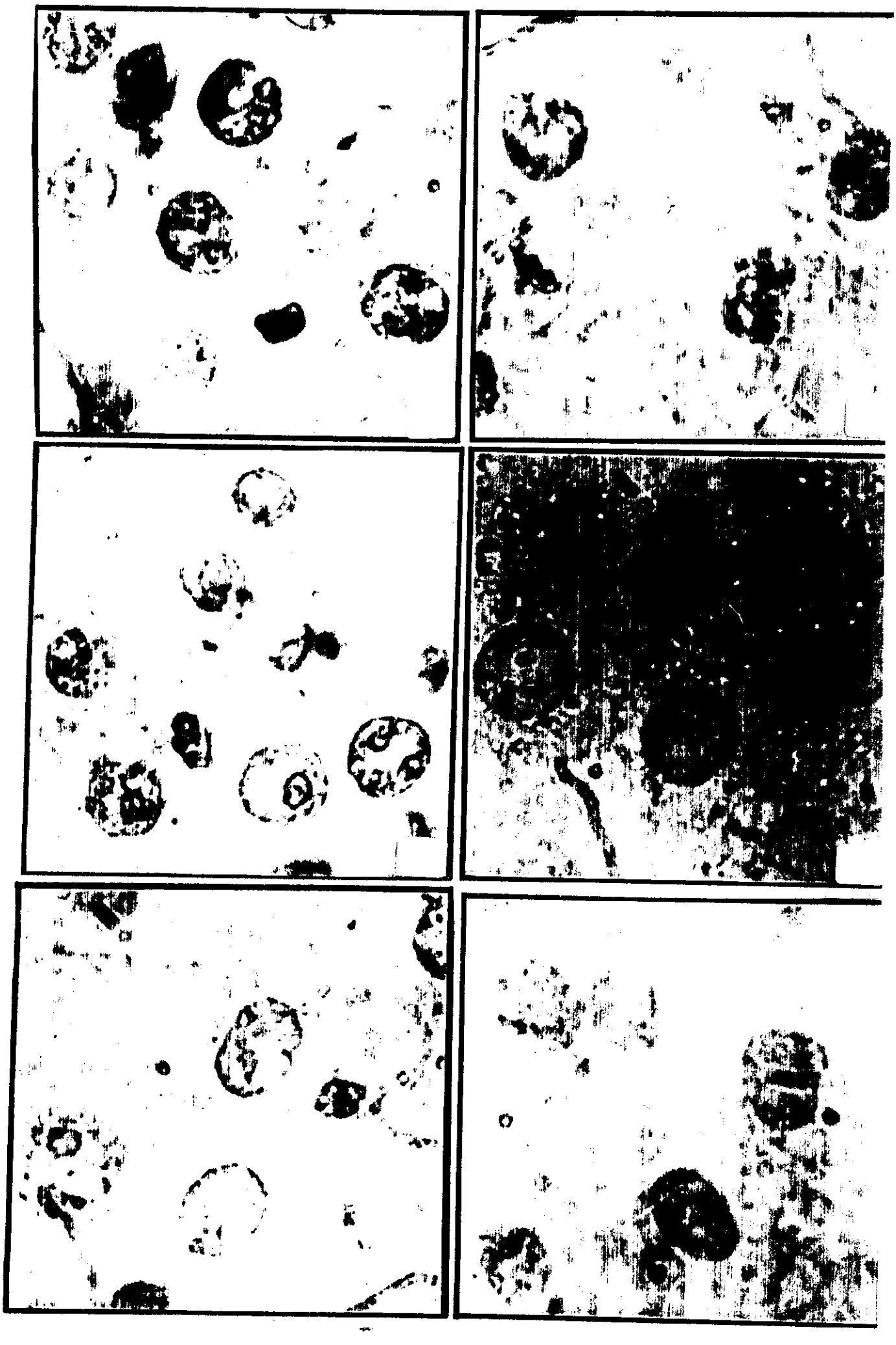


Plate (XIV) - Ribonucleic acid (RNA) containing particles in the hepatocytes of normal and CdCl2 treated rats. Materials were fixed in carnoy 's fluid and treated according to the pyronin-methyl green technique (Kurnick,1955). x1000

Fig. (52) Liver section of a normal rat shows RNA content in the form of small patches scattered at random in the cytoplasm. The nuclei are also positively stained. The nuclei are stained greenish blue indicating their DNA constitution.

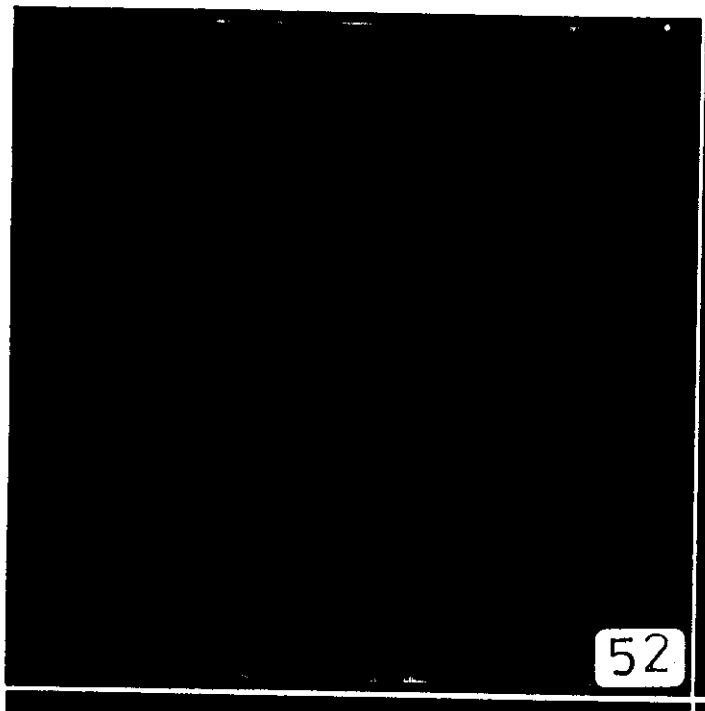
Fig. (53) Liver section of a rat treated day after day with 1/8 LD_{50} $CdCl_2$ for 10 days shows a mild increase in RNA inclusions in most of the hepatic parenchymal cells.

Fig. (54) Liver section of a rat treated day after day with 1/8 LD_{50} $CdCl_2$ for 20 days shows a mild diminution in the pyronin-stained particles (RNA) particularly in the peripheral hepatocytes.

Fig. (55) Liver section of a rat treated day after day with 1/8 LD_{50} $CdCl_2$ for 30 days shows a noticeable decrease in the RNA inclusions in many hepatocytes especially the degenerating ones.

Fig. (56) Liver section of a rat treated day after day with 1/8 LD_{50} $CdCl_2$ for 45 days reveals a pronounced loss of RNA inclusions in almost all the hepatocytes.

Fig. (57) Liver section of a rat treated with 1/8 LD_{50} for 45 days and examined after 30 days of the heavy metal withdrawal shows partial recovery of RNA contents.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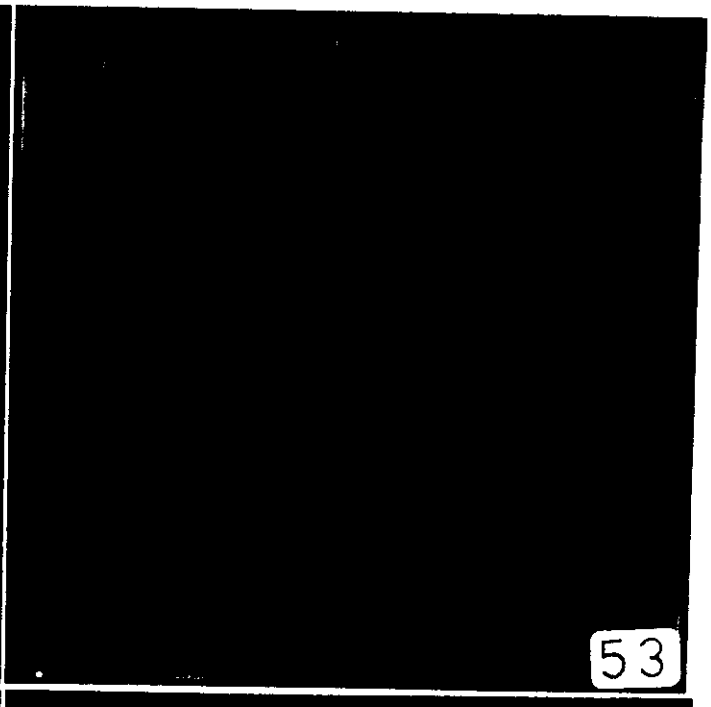
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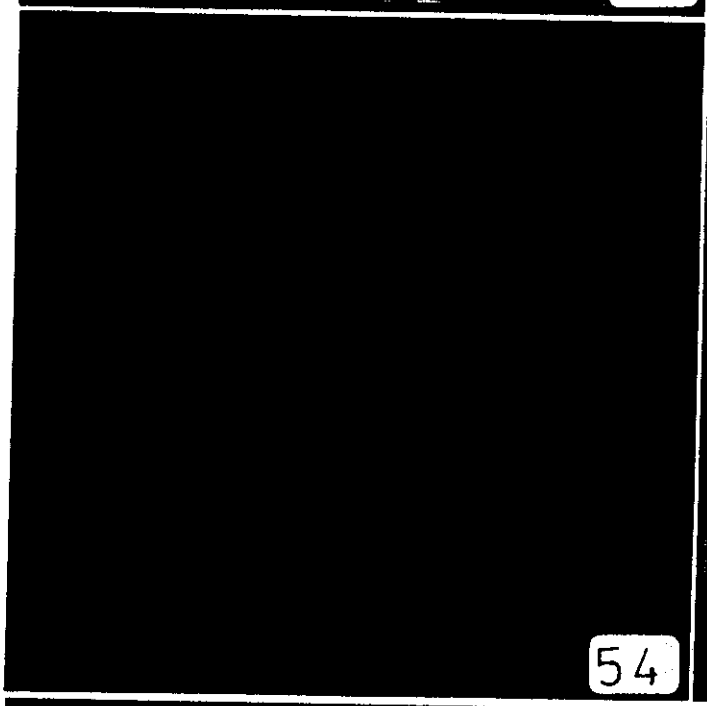
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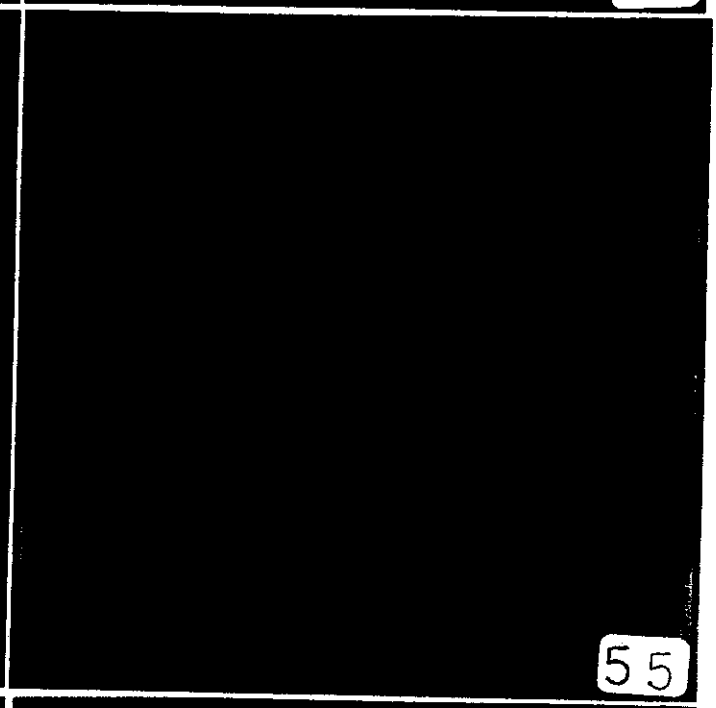
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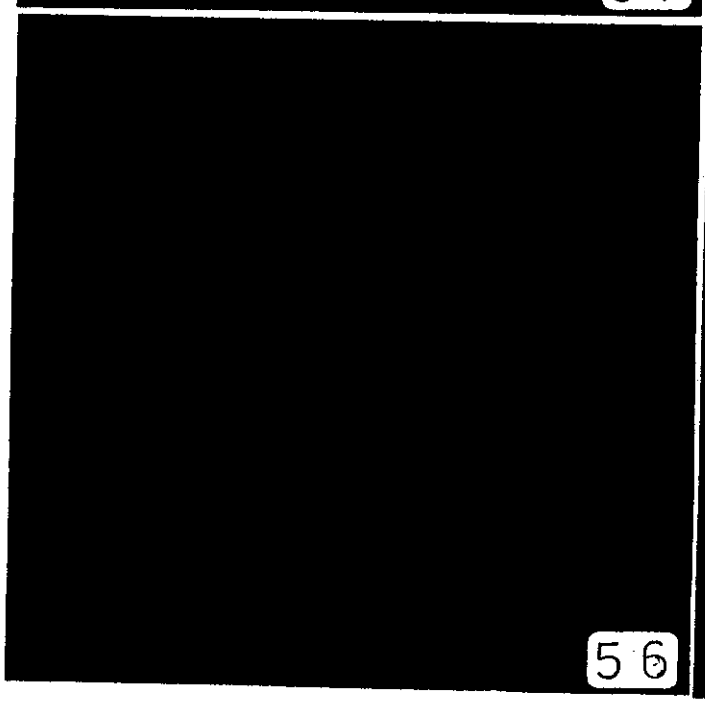
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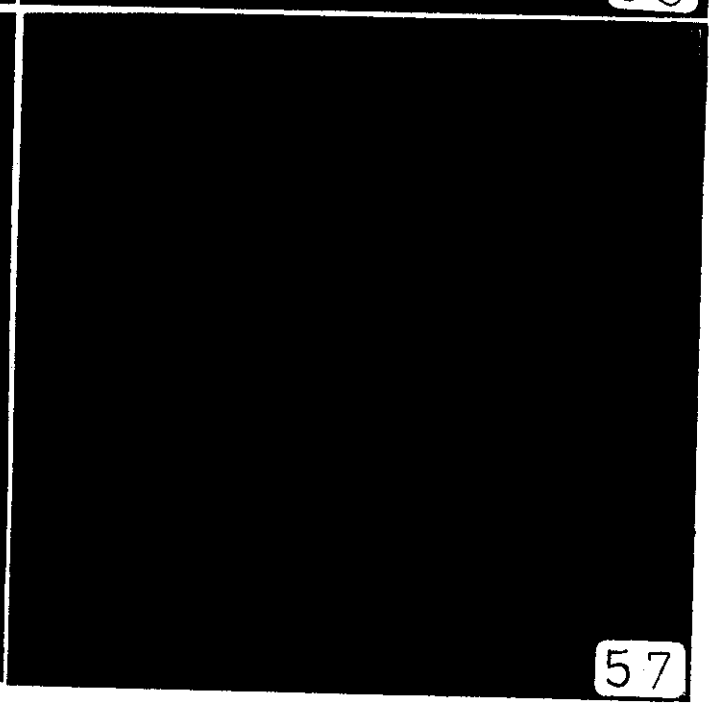
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Plate (XV) - Ribonucleic acid (RNA) containing particles in the hepatocytes of normal and CdCl2 treated rats. Materials were fixed in carnoy 's fluid and treated according to the pyronin-methyl green technique (Kurnick,1955). x1000

Fig. (58) Liver section of a rat treated day after day with 1/4LD_{50} $CdCl_2$ for 10 days reveals a slight increase in both cytoplasmic RNA and nuclear DNA contents in most hepatocytes.

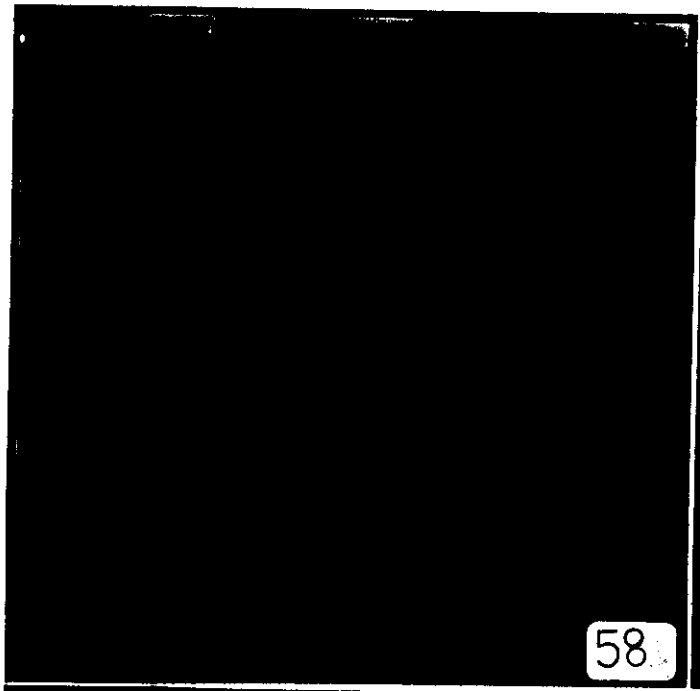
Fig. (59) Liver section of a rat treated day after day with 1/4 LD_{50} $CdCl_2$ for 20 days shows that most hepatocytes are slightly decreased in their RNA contents which appear in the form of week pyronin stained diffused patches.

Fig. (60) Liver section of a rat treated day after day with 1/4 LD_{50} $CdCl_2$ for 30 days shows marked loss in the RNA contents, which are weakly stained with pyronin.

Fig. (61) Liver section of a rat treated day after day with 1/4 LD_{50} $CdCl_2$ for 45 days shows that the majority of hepatic cells are markedly decreased in their RNA particles which lost a considerable proportion of their affinity to pyronin stain.

Fig. (62) Liver section of a rat treated day after day with 1/4 LD_{50} $CdCl_2$, for 45 days and examined 30 days after withdrawal of the heavy metal, shows mild restoration of the RNA inclusions. Other cells are still showing marked loss of their RNA contents.

Fig. (63) Another field of the previous section.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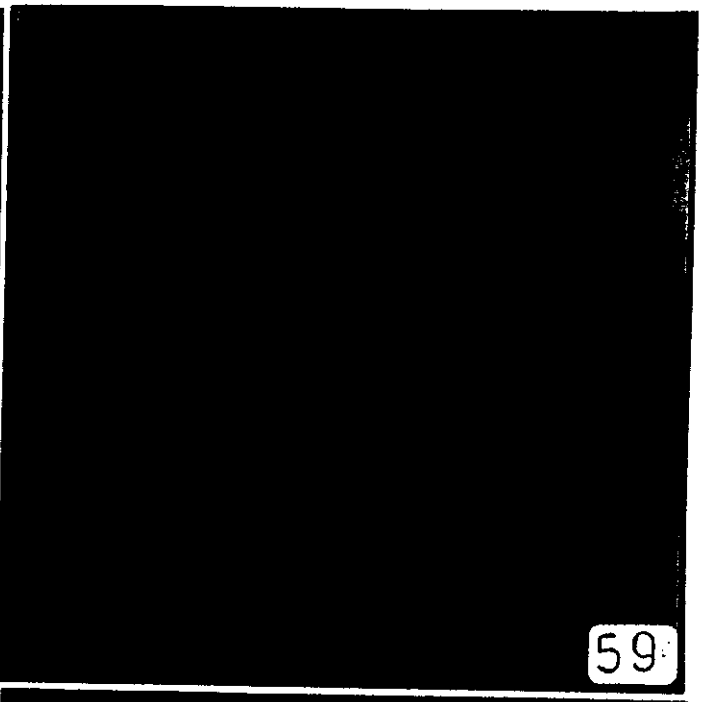
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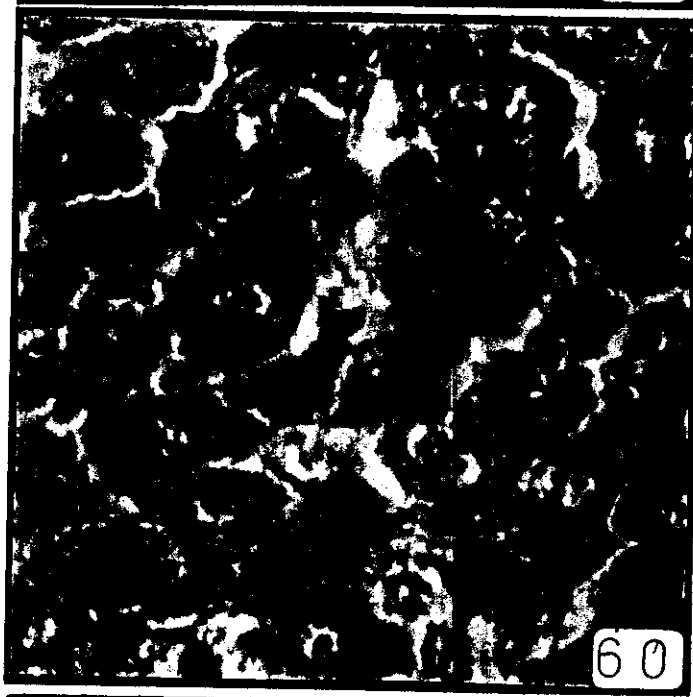
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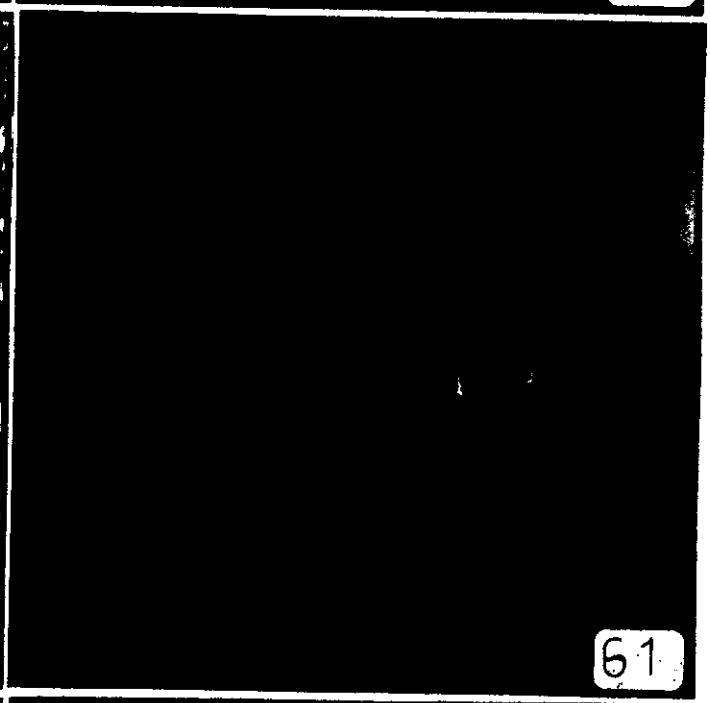
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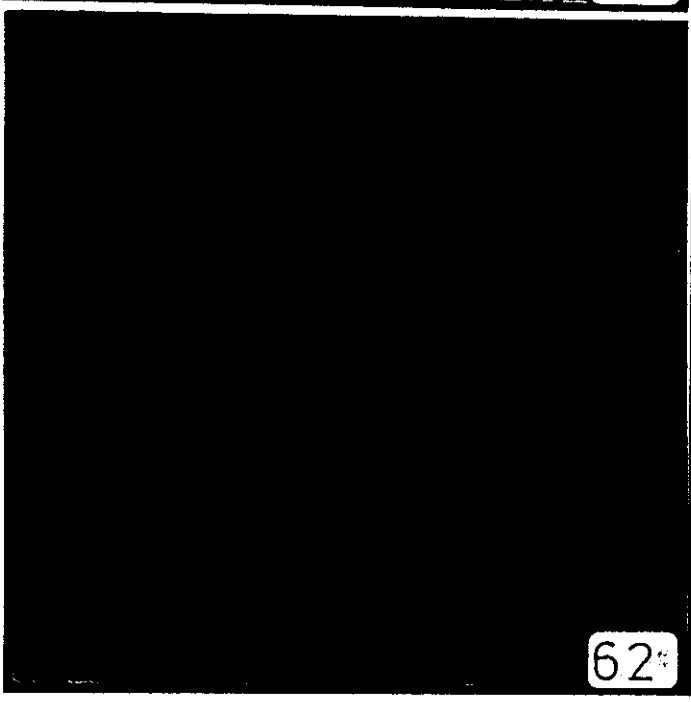
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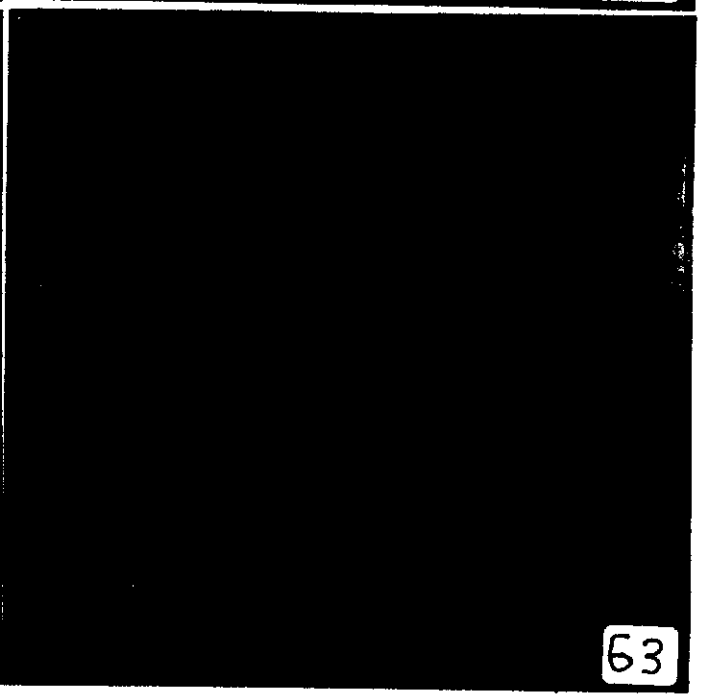
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Plate (XVI) - Total proteins in the liver cells of normal and $CdCl_2$ treated rats. Materials were fixed in Carnoy 's fluid and stained with bromophenol blue technique (Mazia et al., 1953). x 1000

Fig. (64) Liver section of a normal rat (control) shows protein granules scattered randomly in the cytoplasm. The nuclei, nucleoli and nuclear and cellular membranes are intensively stained. The Von Kupffer cells and endothelial cells are moderately stained.

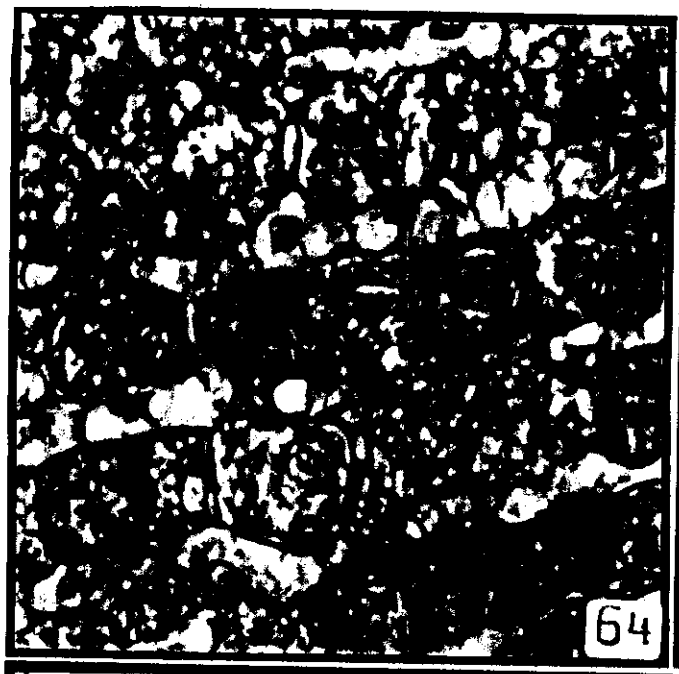
Fig. (65) Liver section of a rat treated day after day with 1/8 LD_{50} $CdCl_2$ for 10 days shows a slight increase in protein granules in the cytoplasm and nuclei. The nuclear and cellular membranes are also more stained than the normal.

Fig. (66) Liver section of a rat treated day after day with 1/8 LD_{50} $CdCl_2$ for 20 days shows a slight decrease in the fine bluish granules in the cytoplasm and nuclei. Nuclear and cellular membranes still deeply stained than normal.

Fig. (67) Liver section of a rat treated day after day with 1/8 LD_{50} $CdCl_2$ for 30 days shows marked decrease in the nuclear and cytoplasmic protein granules. Nuclear and cellular membranes and Von Kupffer cells are also less stained than normal.

Fig. (68) Liver section of a rat treated day after day with 1/8 LD_{50} $CdCl_2$ for 45 days shows marked loss in the total protein contents in the nuclei and cytoplasm of most hepatocytes. Some hepatocytes are still moderately stained.

Fig. (69) Liver section of a rat treated day after day with 1/8 LD_{50} for 45 days and inspected 30 days following abstainance of the heavy metal shows an obvious recovery and nearly restoration of the normal content of the total protein.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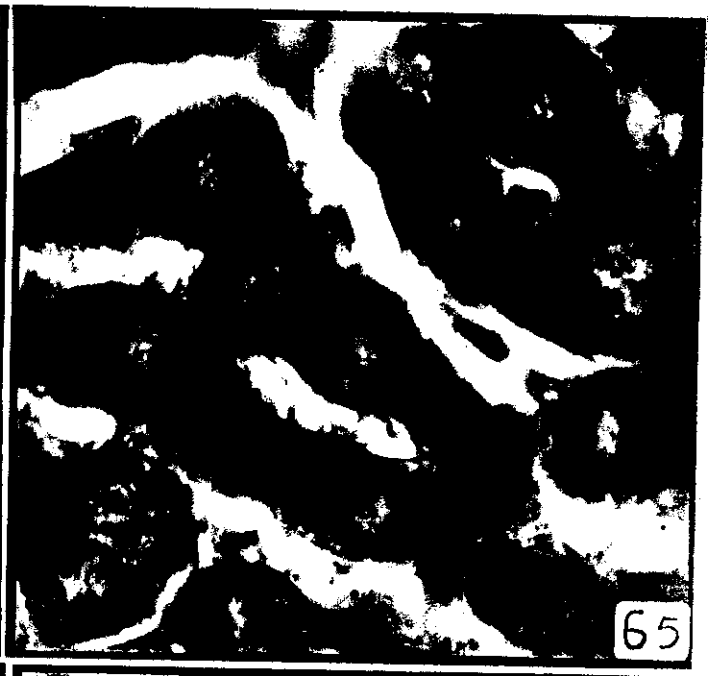
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Plate (XVII) - Total proteins in the liver cells of normal and $CdCl_2$ treated rats. Materials were fixed in Carnoy 's fluid and stained with bromophenol blue technique (Mazia et al., 1953). x 1000

Fig. (70) Liver section of a rat treated day after day with 1/4 LD_{50} $CdCl_2$ for 10 days reveals an obvious increase in the total protein content of the nuclei and cytoplasm.

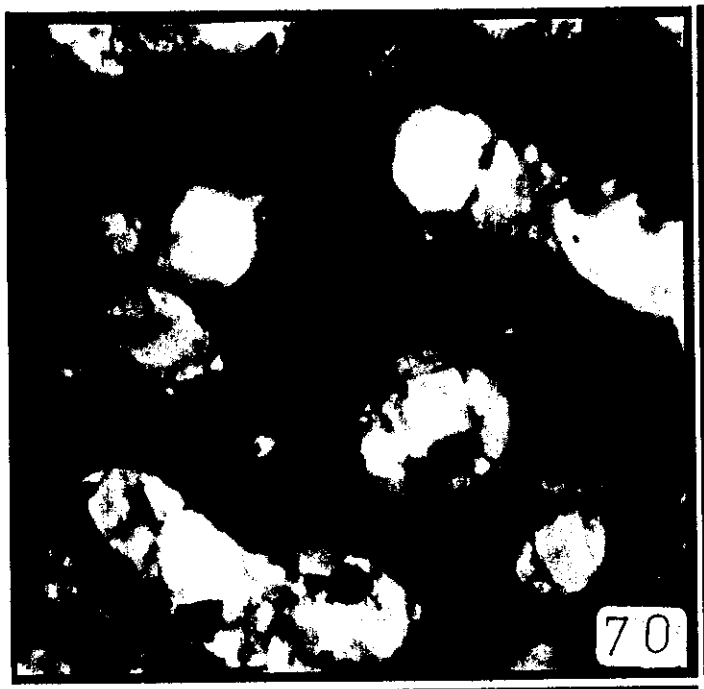
Fig. (71) Liver section of a rat treated day after day with 1/4 LD_{50} $CdCl_2$ for 20 days shows a mild decrease in the protein particles in the cytoplasm and nuclei, comparing to the control. The cell and nuclear membranes as well as the nucleoli are still deeply stained with blue colour.

Fig. (72) Liver section of a rat treated day after day with 1/4 LD_{50} $CdCl_2$ for 30 days shows marked decrease in the cytoplasmicand nuclear protein content.

Fig. (73) Liver section of a rat treated day after day with 1/4 LD_{50} $CdCl_2$ for 45 days shows maximum signs of proteinic depletion in the cytoplasm and nuclei. The protein contents in most of hepatic cells to be limited to the narrow cytoplasmic areas lying in-between unstained vacuoles.

Fig. (74) Liver section of a rat treated day after day for 45 days and examined 30 days after abstainance of $CdCl_2$ reveals a slight restoration of the total protein inclusions in the cytoplasm and nuclei.

Fig. (75) Another field of the last section shows the same phenomenon of a slight restoration of the total proteinin the liver cells.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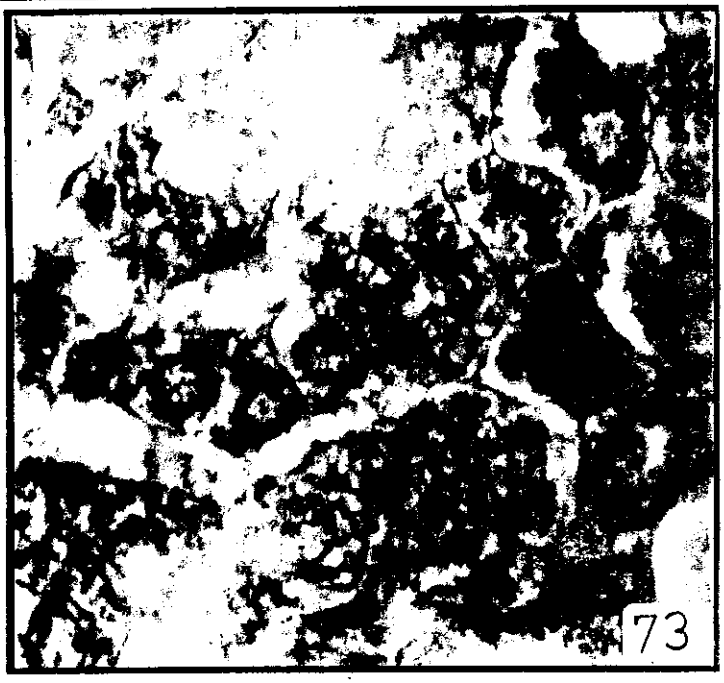
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RENAL CHANGES

HISTOPATHOLOGICAL OBSERVATIONS

Control rats

The kidney consists of a large number of urineferous tubules, each being formed of two main parts, the nephron and the collecting tubule. The nephron is the structural and functional unit of the kidney. It is made up of two principal portions, the renal (Malpighian) corpuscle and the nephric tubule. The nephric tubule is in turn mainly comprised of the proximal convoluted tubule, loop of Henle (descending and ascending limbs) and distal convoluted tubule.

The renal corpuscles, the proximal and distal convoluted tubules are located in the cortical region in the kidney, whereas the medullary region encloses the descending and ascending limbs of the loops of Henle, in addition to scattered parts of the collecting tubules.

The renal (Malpighian) corpuscle is roughly spherical in shape consisting of a double membraned corpuscle-Bowman's capsule, enclosing a tuft of blood capillaries (the glomerulus). The space in-between the glomerulus and the capsule is called the urinary space.

Each renal corpuscle has two a characteristic poles; a vascular pole, where the afferent and efferent arterioles enter and leaf the glomerulus respectively and a urinary pole which is located at the opposite site of the corpuscle where the capsular space is continuous with the lumen of proximal convoluted tubule.

The outer wall of Bowman's capsule, the parietal layer is composed of a single layer of squamous epithelial cells. Their nuclei are slightly,

protruding into the capsular space. The inner wall i.e. the visceral layer is also formed flattened epithelial cells closely investing the glomerular capillaries. The nuclei of these cells are located on the capsular side of the basal lamina covering the glomerular capillaries.

The proximal convoluted tubule begins at the urinary pole of the renal corpuscle and extends along the cortex in highly tortuous manner. Generally the proximal convoluted tubules are numerous and their cross sections a round or oval outline with a narrow lumen. Their lining coat is comprised of large pyramidal cells with markedly basophilic cytoplasm. A distinct brush border marks their free narrow apices. The border bases of these cells rest on the prominent basement membrane. However, cells are not clearly demarcated, though they embody conspicuous centrally located nuclei.

The descending limb of Henle's loop appears in cross sections being formed of flattened epithelial (squamous) cells bounding a rather wide lumen. They have a homogeneously eosinophilic cytoplasm. The cells themselves are generally mononucleate and their nuclei are bulging into the lumen.

The ascending limb of Henle's loop is lined by cuboidal epithelial cells enclosing a narrow lumen. The ground cytoplasm of these cells is also generally eosinophilic. Their cell boundaries are ill defined, but their nuclei are prominent, centrally situated and exhibiting deep basophilia enclosing distinct nucleoli.

The distal convoluted tubule has a wide lumen bordered by cubical or low columnar epithelial cells and their basophilic nuclei are basally located. However it is worthy of pointing out that such portions (distal convoluted

tubules) are not so long, hence cross section of them are not so often met with in the cortical portions of the kidney.

The collecting tubules having rounded or oval outlines formed of low cuboidal epithelial cells enclosing a wide cavity. These cells have a lightly stained cytoplasm with large oval nuclei located in the mid-position of the cells.

Treated rats:

Macroscopical examination, revealed that the changes was the same during the whole study where the kidney size was approximately similar to the normal case. Also the Kidney became more dark brownish in their color with increase the time of administration. They have soft consistency and smooth on cut section.

Rats treated with 1/8 LD_{50} $CdCl_2$

The sections prepared from the kidneys of rats treated for 10 days and stained with (H&E) revealed that the capillary tuft of the glomeruli and their capsules have a normal structure similar to the control, also the distal convoluted tubules were slightly affected, fig. (77). The same figure exhibited mild cloudy swelling of the epithelial cells of proximal convoluted tubules, infiltration of the tissue with a small number of mononuclear inflammatory cells as well as no hyaline casts has been observed.

Twenty days of administration with the same dose as showed in figure (78), revealed that the glomeruli had normal structure although presence of some inflammatory cells around them. There was moderate cloudy swelling of the cytoplasm of the proximal tubular cells, as well as single cell necrosis in many tubular epithelia. Also, there were hypertrophy of the nuclei of some of these cells , beside presence of prominent nucleoli. In the same figure, mild hyaline casts of some proximal tubules were

markedly observed. The distal tubules and medullary cells still have normal structure.

The proximal tubular cells degeneration increased markedly as observed from the materials inspected from kidneys of the rats treated for 30 days with 1/8 LD_{50} $CdCl_2$, (fig. 79& 80). This figure showed mild hypercellularity of the glomeruli. The inflammatory lymphocytes appeared with large number in the intestinal tissue. Proximal convoluted tubules exhibited marked cloudy swelling with cytoplasmic granulation, also there were some nuclear pyknosis, karyolysis and focal necrotic areas. The epithelial cells of the distal convoluted tubules have minimal cytoplasmic granulation, but the tissue of medulla has normal structure .

Microscopical examination of the 45 days treated groups revealed also some hypercellularity of glomeruli, and severe cloudy swelling of cells of the proximal convoluted tubules, marked hydropic degeneration, beside marked increase in the number of pyknotic nuclei, karyorrhexis and karyolysis as observed in fig.(81). Fig.(82) showed focal necrotic areas which appeared as marked feature of most of rats' kidneys of this group. Also the epithelial cells of the distal convoluted tubules observed have marked cloudy swelling, beside the lumens of some proximal convoluted tubules (PT) were approximately blocked with hyaline casts. In comparison with the previous treated groups, there was moderate increase in the number of the inflammatory cells inside the interstitial tissue.

On the other hand, the materials examined 30 days after abstainance of 1/8 LD_{50} $CdCl_2$ administration revealed a partial incomplete restoration of the normal nephric tissue architecture. The observations as illustrated in fig. (83) were increase of the mitotic figures as well as increase in the new regenerative tubules and approximately restoring of the normal renal corpuscle structure. The generative changes are also still observed such

Dose	Reaction / periods	10d.	20 d.	30 d.	45 d.	75 d.
Contral	Carbohydrates	+++++	++ +++	+++++	+++++	+++++
	DNA	++++	++++	++++	+++	+++
	RNA	++++	+++	+++	+++	+++
	T.Protien	++++	+++	+++	+++	+++
1/8 LD_{50} $CDCL_2$	Cardohydrates	++++	+++	++	+	++
	DNA	+++++	++++	+++	++	++
	RNA	+++++	++++	+++	++	++
	T.Protien	+++++	++++	+++	++	++
¼ LD50 CDCL2	Cardohydrates	+++	++	+	-	+
	DNA	+++++	+++	+++	++	++
	RNA	+++++	+++	++	++	++
	T.Protien	+++++	+++	++	++	++

-	No reaction	+++	Moderate reaction.
+	Minimal reaction	++++	Severe reaction.
++	Mild reaction	+++++	Most severe reaction.

Plate (5)

Histochemical changes in the liver of control rats and those treated with 1/8 and 1/4 LD_{50} of $CdCl_2$.

as, severe cloudy swelling, pyknosis, karyorrhexis, karyolysis as well as single cell necrosis and hydropic degeneration of the epithelial cells of the proximal and distal convoluted tubules. Adding to, complete tubular necrosis of many proximal tubules and severe hyaline casts. The same examined section showed a marked interstitial fibrosis and marked increase in the number of inflammatory cells.

1/4 LD_{50} $CdCL_2$ Treated Groups :

The incidence and severity of the tubular damage and other tissue changes in the kidneys of rats treated with (1/4 LD_{50}) was greater than those observed in the groups treated with (1/8 LD_{50} $CdCl_2$). The epithelial cells of the proximal convoluted tubules were early affected component of the kidney tissues obtained from the first group which treated with this dose for 10 days, where these cells underwent mild cloudy swelling as well as the inflammatory cells were seen in the interstitium between the tubules as illustrated in fig.(84). The same figure revealed the presence of minimal hyaline casts in some (PT) lumens and normal structure of distal convoluted tubules as well as renal corpuscles.

The marked cytoplasmic swelling of the epithelial cells of the proximal tubules were exhibited in those rats treated with the same dose for 20 days. Also, there were single cell necrosis and nuclear pyknosis of the lining epithelium of these tubules, beside mild focal necrotic areas. Also, the distal convoluted tubules revealed mild cloudy swelling. It was also clear that the glomeruli have undergone an obvious hypertrophy and hypercellularity with narrowing of the urinary space, as illustrated in fig. (85). It is also apparent in this figure increasing the number of the inflammatory cells around the vein. Figure (86) showed many necrotic cells of the proximal and distal convoluted tubules epithelia. Adding to congestion and dilation of the vein.

The section prepared from 1/4 LD_{50} $CdCl_2$ treated rats for 30 days revealed marked tissue deformation where there were strong cloudy swelling of the epithelial cells of proximal and distal convoluted tubules beside moderate cytoplasmic granularity, enlargement of most of their nuclei displaying clear signs of pyknosis, some of them had protruded into the the internal lumens. Also, there were marked nuclear karyolysis and focal area of necrosis (fig. 87). The Bowman's capsules became markedly affected than those of the previous treated groups, where there were widening of the intratubular spaces, Bowman's capsule were expanded enclosing swollen glomeruli with marked reduction of the urinary spaces in the same specimen, the interstitium was moderately invaded with inflammatory cells and there were obvious interstitial fibrosis. It was also observed some regenerative tubules, which have hypertrophy of the epithelial cells with conspicuous nuclei.

The severe tubular degeneration was illustrated from the sections prepared from 1/4 LD_{50} treated rats for 45 days as in fig. (88) .The architecture of the lining epithelial cells of the proximal and distal convoluted tubules were not homogenous, where, their cytoplasm underwent strong swelling beside almost indistinct boundaries. There was an obvious variable in their nuclear size large number of them actually underwent pyknosis and karyolysis. Also there were many single cell necrosis, complete necrotic tubules and large area of focal necrosis. The same figure showed that Bowman's capsule were remarkably bulging and their glomeruli became obviously hypertrophied being comparatively larger in size than the normal cases. Large number of inflammatory cells filled the interstitial tissue along all the section. An marked of hydropic degeneration has been observed in some of the proximal convoluted tubules.

All of these deteriorations of the nephric tissue still observed in those rats treated and inspected 30 days following abstainance of 1/4 LD_{50} $CdCl_2$.

Fig. (89) revealed that most of the nephric tissue components had lost a marked proportion of their normal characteristic stainability. The development and restoration of the normal structure of this tissue was slightly appeared, where we observed a few regenerating tubules as illustrated in fig. (90).

Plate (XVIII) - Kidney material fixed in buffered neutral formalin and stained with Haematoxylin and Eosin (Clayden, 1971).

Fig. (76) T.S. in the kidney of a normal control rat showing normal Bowman 's capsule which containing a tuft of blood capillaries (glomerulus) (g), surrounded by renal space (s) constricted by a capsule (c). There are many proximal (p) and distal convoluted tubules (d). x400

Fig. (77) T.S. in the kidney of a rat treated day after day for 10 days with 1/8 LD_{50} $CdCl_2$ showing cloudy swelling of the epithelial cells of the proximal convoluted tubules (arrows) and mild infiltration of the kidney tissue with inflammatory cells (I). x400

Fig. (78) T.S. in the kidney of a rat treated day after day with 1/8 LD_{50} $CdCl_2$ for 20 days shows cloudy swelling of proximal tubular epithelia (arrows), many pyknotic nuclei (P) and invasion of the interstitial tissue with inflammatory cells (I). x 400


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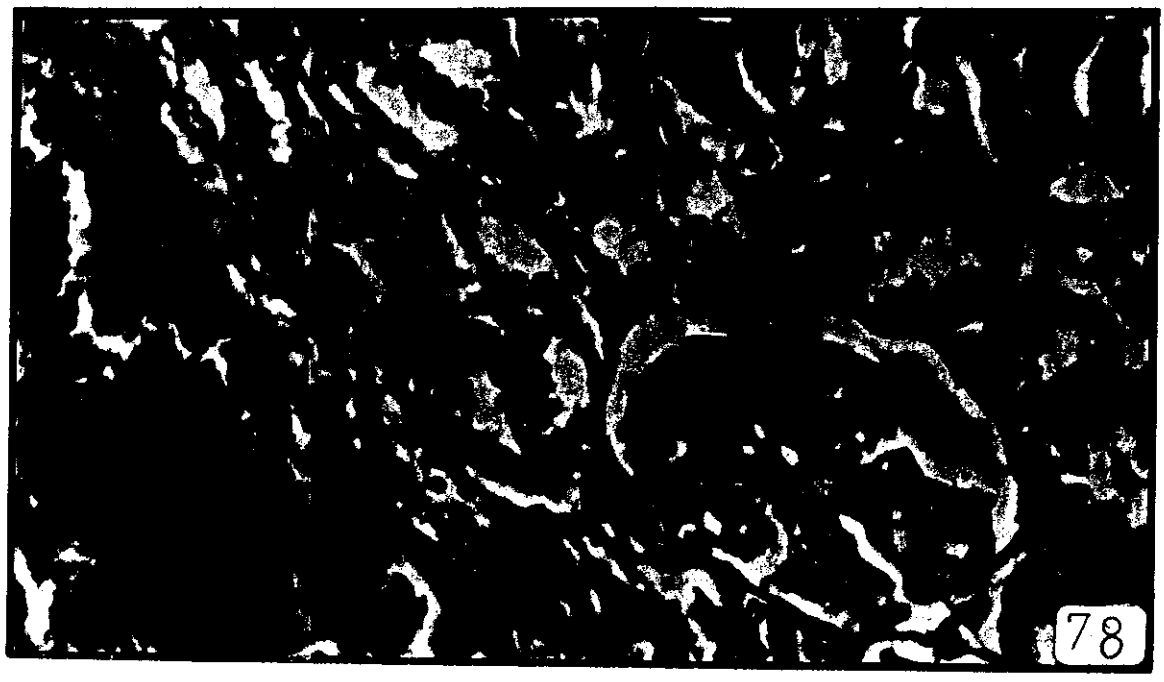
78

Plate (XIX) - Kidney material fixed in buffered neutral formalin and stained with Haematoxylin and Eosin (Clayden, 1971).

Fig. (79) T.S. in the kidney of a rat treated day after day with 1/8 LD_{50} $CdCl_2$ for 30 days shows , marked hypercellularity of the glomerului (h) , narrowing of the renal space (RS), cloudy swelling (arrows) of the epithelia of the PT and DT cells, pyknosis (P) of most of their nuclei as well as hyalinization of some tubules (Z).. X 400

Fig. (80) T.S. in the kidney of a rat treated day after day with 1/8 LD_{50} CdCl2 for 30 days shows marked cloudy swelling (c) of PT and DT., karyolysis (L) and karyorrhexis (x) as well as pyknosis of the nuclei. Also single cell necrosis (arrows) markedly appearent in the epithelia of almost all the tubules and hydropic degeneration. x 400

Fig. (81) T.S. in the kidney of a rat treated day after day with 1/8 LD_{50} $CdCl_2$ for 45 days shows pyknosis (arrows) of some nuclei of the tubules and necrosis of many cells (N) lead to marked deterioration in the nephric tubules, and marked hydropic degeneration (H). x 400

Plate (XX) - Kidney material fixed in buffered neutral formalin and stained with Haematoxylin and Eosin (Clayden, 1971).

Fig. (82) T.S. in the kidney of a rat treated day after day with 1/8 LD_{50} $CdCl_2$ for 45 days shows marked hypercellularity (h) of the glomeruluar cells, a large number of inflammatory cells (I), severe congestion of the renal veins (c) as well as marked hyaline casts (y). x 400

Fig. (83) T.S. in the kidney of a rat obtained 30 days after abstainance of $CdCl_2$ shows hypercellularity of the glomerulus (h) and of regenerative tubule (arrows). Hydropic degeneration and necrosis of many tubular cells (arrows) as well as pyknosis (p) of the nuclei are still observed. x 400

Fig. (84) T.S. in the kidney a rat treated day after day for 10 days with 1/4 LD_{50} $CdCl_2$ shows mild cloudy swelling (CS) of the epithelia of the proximal tubules. x 400


82


83


S
84

Plate (XXI) - Kidney material fixed in buffered neutral formalin and stained with Haematoxylin and Eosin (Clayden, 1971).

Fig. (85) T.S. in the kidney of a rat treated day after day for 20 days with 1/4 LD_{50} $CdCl_2$ shows congestion and dilation of the blood vessel (BV) and mild hypercellularity of the capillary tuft (h) as well as infiltration of the nephric tissue with inflammatory cells (I). x 400

Fig. (86) T.S. in the kidney of a rat treated day after day for 20 days with 1/4 LD_{50} $CdCl_2$ shows marked cloudy swelling (CS) and single cell necrosis (N) as well as pyknosis (P) of many cells of the (PT). Dilation and congestion of the vessels are also apparent. X 400

Fig. (87) T.S. in the kidney of a rat treated day after day for 30 days with 1/4 LD_{50} $CdCl_2$ shows nuclear pyknosis (arrows) of a large number of the tubular epithelial cells, also their cytoplasm was markedly swelled. single cell necrosis (N) and marked hypercellularity (h) of the capillary tuft. x 400

Plate (XXII) - Kidney material fixed in buffered neutral formalin and stained with Haematoxylin and Eosin (Clayden, 1971).

Fig. (88) T.S. in the kidney of a rat treated day after day for 45 days with 1/4 LD_{50} $CdCl_2$ shows completely necrotic tubules (N) marked hypercellularity (h) of the capillary tuft, disappearance of the renal space (arrows) and hyaline casts (y). x 400

Fig. (89) T.S. in the kidney of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 45 days and examined 30 days following Cd withdrawal shows hypercellularity of the capillary tuft (arrows). Tubular necrosis (N), marked congestion of blood vessels (C) and infiltration of the interstitial tissue with inflammatory cells (I) are still observed. x250

Fig. (90) T.S. in the kidney of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 45 days and examined 30 days following Cd withdrawal shows many regenerated tubules(R), marked hypercellularity (h) of the glomeruli and disappearance of the renal space. Many PT and DT lost their normal characteristic stainability (arrow) x 400


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89


90

Plate (6)

Microscopic findings in the kidney of rats treated with 1/8 LD_{50} of $CdCl_2$.

Organ and findings	Days of treatment	10				20				30				45				3Dd. For reeovery			
Kidney	Grade	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++
Glomerular hypercellularity		6		-		4	2				6					3	3			2	4
Infiltration with inflammatory cells		2	4				3	3				6					6				6
Cloudy swelling and cytoplasmic granularity.		6					2	4				6					6				6
Pyknosis, karyorrhexis and karyolysis		6				5	1				3	3					6			4	2
Focal necrosis		6				5	1			3	2	1			3	3		2	2	2	
Hyaline casts		6				5	1			3	1	2			2	3	1	2	3	1	
Degeneration of the distal tubular cells		6				6				4	2			2	4			3	3		
Tubular regeneration		6				6				6				4	2				4	2	
Interestitial fibrosis		6				6				6				6				6			

-	*No lesion*
+	*Mild change*
++	*Moderate change*
+++	*Se vere change*

HISTOCHEMICAL OBSERVATIONS

Polysaccharides

Control rats

In the renal corpuscles, the PAS reactivity was rather marked in the basement membranes lining the capillary walls of the glomerular structures and the outer membranes of Bowman 's capsule, in contrast to the negative reactivity observed in the inner Bowman 's capsule membranes. Also, the proximal convoluted tubules, the basement membranes, as well as the brush borders of their component cells were obviously coloured with PAS technique in comparison with feeble stainability of their ground cytoplasm and the lack of any trace of PAS reactivity in their nuclei. The distal convoluted tubules displayed a somewhat mild PAS reactivity in their basement membranes and a weaker coloration in their ground cytoplasm of their constituent cells and non-stainability of their nuclei.

Concerning the descending and ascending limbs of loop of Henele, their lining cells exhibited a weak reaction for polysaccharides being mainly restricted to their limiting membranes. The nuclei of these cells did not display any apparent PAS reactivity in these preparations reflecting their complete lack of any carbohydrate inclusions. A mild PAS reactivity was indicated in the basement membranes of the collecting tubules with a weaker coloration in the ground cytoplasm of their component cells and non-stainability of their nuclei.

Treated rats

Rats treated with1/8 LD_{50} $CdCl_2$

All of the rats treated with $CdCl_2$ revealed a progressively gradual depletion in their polysaccharides content. These observations were clearly demonstrated with increase the exposure time to this heavy metal.

Plate (7)

Microscopic findings in the kidney of rats treated with 1/4 LD_{50} of $CdCl_2$.

Organ and findings	Days of treatment	10				20				30				45				Recovery			
kidney	Grade	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++
Glomerular hypercellularity		6				2	4				5	1				1	5				6
Infiltration with inflam. cells			6				2	4				4	2				6				6
Cloudy swelling and cytoplasmic granularity			6				2	3	1			3	3				6				6
Pyknosis, karyorrhexis and karyolysis		6					5	1				2	4			1	5				6
Focal necrosis		6				6				2	1	3			2	1	3	1	2	2	1
Hyaline Casts		6				1	3	2			1	4	1		1	3	2		3	2	1
Degeneration of distal tubular cells		6				6				2	4				6			1	5		
Tubular regeneration		6				6				6				3	3			1	5		
Interstitial fibrosis		5	1			6				4	2			1	2	3		3	3		

-	*no lesion*
+	*mild change*
++	*moderate change*
+++	*severe change*

Kidney materials inspected 10 days after chronic administration with the above dose revealed a mild diminution of the positive PAS-reacted materials. This diminution as illustrated in fig. (92) was clearly observed in the basement membranes of many nephric tubules. Other components such as the renal corpuscles, collecting tubules as well as each limb of Henle appeared similar to the normal preparations.

Twenty days following repeated application of the same dose showed approximately a pronounced decrease in the basement membranes of the proximal and distal tubules as well as their brush borders as manifested in fig. (93). The renal corpuscle wall and the cells of the glomerulus are still stained moderately with PAS but weak in comparable to the normal conditions. Other components, which markedly affected by Cd are reflecting also a weak PAS reactivity. Nevertheless, the decrease in PAS reactivity appeared more obvious in the basement membranes of the proximal convoluted tubules of the materials inspected 30 days following 1/8 LD_{50} $CdCl_2$ administration. But their basal portions of their component cells were weakly stained, while their luminal portions still displaying a striking PAS-reactivity as demonstrated in fig. (94). Also, the distal convoluted tubules appeared retaining a weak coloration. PAS reactivity became markedly diminished in the descending and ascending limbs of Henle's loop as well as the collecting tubules.

As the time of administration with1/8 LD_{50} $CdCl_2$ was prolonged 45 days, the reduction of the carbohydrate inclusions became more pronounced as illustrated in fig. (95). In that case, all of the nephric tissue components revealed considerably very weak PAS coloration.

Kidney materials inspected thirty days following Cd-abstainance demonstrated a partial restoring of PAS-stained particles of their cells to

regaining the normal picture as illustrated in fig. (96). In this figure, the renal corpuscle as well as the proximal convoluted tubules revealed a remarkable development of their PAS reactivity, which appeared as a moderate magenta color of their brush borders and their basement membranes. Other renal constituents still appeared weakly reacted with PAS method.

Rats treated with1/4 LD_{50} $CdCl_2$

Materials inspected 10 days following administration of the rats with the above dose showed a slight diminution of the general carbohydrate inclusions in most of the renal tissue components as illustrated in fig. (97).

Twenty days of repeated treatment with the same dose revealed a significant decrease in the PAS-stained materials in the walls of Bowman 's capsule while the capillary tuft still moderately with magenta colour, as illustrated in fig. (98).

The basement membranes of the proximal convoluted tubules revealed a pronounced diminution in their polysaccharides content but the basal portions of their component cells were weak to moderately with PAS. The tubular lumens still stained moderately with PAS, while other nephric components reacted weakly with faint magenta colour.

Also, 30 days of treatment with the high dose revealed a marked loss in the renal corpuscles membranes and the cells of the capillary tuft as well as the proximal convoluted tubules contents of carbohydrate inclusions as demonstrated in fig. (99). Other components were approximately negative reacted with PAS.

The maximal depletion of normal carbohydrate level in the glomeruli and their outer capsules as well as the proximal and distal convoluted tubules was observed in the materials examined 45 days of treatment.

Other kidney constituents were negatively stained with Periodic Acid Schiff reaction as illustrated in fig. (100).

On the other hand, the materials prepared from the rats inspected 30 days following Cd-abstainance showed a slight restoration of the general carbohydrate inclusions. The capillary tuft of Bowman 's capsule and many of the basement membranes of the proximal convoluted tubules as well as their lumens were stained moderately with magenta. Other components (distal convoluted tubules, ascending and descending limbs of Henle 's loop as well as the collecting tubules) still showed marked poverty in their carbohydrates level comparing with the normal conditions as illustrated in fig. (101&102)

Deoxyribonucleic acid (DNA)

Control rats

Each of glomerular and proximal tubules nuclei as well as nuclei of other kidney components appeared contain red-purple colored particles after staining with Feulgen stain.

These particles which stained with Feulgen reaction indicated the presence of DNA. They appeared either evenly distributed in the nucleoplasm in the condensed type nuclei or in a peripheral localization in the open face nuclei. Some of these particles appeared attached to the nucleoli. the cytoplasm of all the kidney cells revealed negative Feulgen reaction indicating complete lack of detectable DNA inclusions.

Treated rats

Rats treated with 1/8 LD_{50} $CdCl_2$

The materials stained with Feulgen method and inspected 10 days following 1/8 LD_{50} $CdCl_2$ application revealed a slight increase in the

chromatin inclusions in almost all the nuclei of the components of the kidney sections. These changes as illustrated in fig. (104) demonstrated deeply stained particles with magenta colour in all of the nuclei of the lining epithelial cells of the renal tubules as well as other nuclei of the renal corpuscles.

This picture was reversed 20 days following administration with the same above dose, where the DNA materials particularly in the nuclei of the epithelia of the proxirnal as well as the distal convoluted tubules. The nuclei of the renal corpuscles still revealed a strong Feulgen-reaction as observed in the normal conditions. Other components cells of the kidney revealed a somewhat a mild diminution in their DNA content as manifested in fig. (105).

Thirty days after chronic application of the above dose revealed an obvious diminution in the amount and intensity of the DNA-containing particles in many cells of these materials particularly the proximal as well as the distal convoluted tubules. Fig (106) demonstrated that the nuclei of the renal corpuscles revealed a slight depletion in chromatin materials.

The major loss of this nucleic acid containing particles was appeared in most of the nuclei of the renal tubules of the materials prepared from the 45 days treated rats with the low dose as illustrated in fig. (107). In this figure, the chromatin materials of these nuclei present adhesived to the nuclear membrane, while the nuclei of the renal corpuscles revealed considerably weak coloration in comparable to the normal preparations. Other components nuclei revealed very weak reaction with Feulgen stain.

A partial development and recovery was observed in most of the renal constituents' nuclei of the materials inspected thirty days following Cd-abstainance after 45 days of treatment as manifested in fig. (108). This

restoration was markedly obviated in the DNA inclusions of the cells of the capillary tuft than the other components.

Rats treated with 1/4 LD_{50}

Treatment of rats with the high dose 1/4LD_{50} $CdCl_2$ for 10 days produced a significant increase in the DNA content in each of the proximal and distal convoluted tubules' nuclei as well as the nuclei of glomerular cells which indicated by deep red-purple colour comparing to the normal preparations as illustrated in fig. (109).

On the other hand, when materials were examined after 20 days of treatment, a slight decrease in the DNA contents was detected in the nuclei of tubular epithelial cells and glomerular cells when compared to the normal levels as observed in fig (110)

Thirty days of treatment revealed a significant decrease in the DNA content which obviously observed in the pathologically affected nuclei particularly degenerating ones which appeared poor in their chromatic material level comparing to normal tissue. Regenerating cells have Fulgen-positive material similar to the normal cells as shown in fig (111). As shown in figure (112) a sharp depletion of the DNA content in materials examined on the 45-days, following treatment with the same dose. The remnants of DNA bodies were mostly found adjacent to the nuclear membranes.

Thirty days following the last injection after 45 days of treatment revealed a slight restoration of the nuclear chromatin of almost all the epithelial cells of each of proximal and distal convoluted tubules as illustrated in fig. (113&114). Also the same figures still manifested a marked diminution in these inclusions of many nuclei.

Ribonucleic Acid (RNA)

Control rats

In Pyronin-Methyl Green preparations, ribonucleic acid (RNA)-containing particles appeared in the normal rat lining epithelial cells of distal and proximal convoluted tubules and the glomerular cells as well as the cells of other components to be obviously demonstrated as a small brightly red colored bodies scattered randomly in their cytoplasm. Also, the nucleoli a deeply red stainability indicating their RNA constitution, while the nuclei were stained greenish blue due to their DNA contents.

Treated rats

Rats treated with 1/8 LD_{50} $CdCl_2$

The materials prepared from rats treated with the above dose for 10 days manifested a slight increase of cytoplasmic RNA inclusions in most of the cells of the renal corpuscles as well as the lining epithelial cells of the proximal as well as the distal convoluted tubules. Other components cells of the kidney appeared similar to the normal conditions as demonstrated in fig. (116).

Twenty days following the chronic application of the same dose revealed that kidney materials prepared from rats showing approximately normal RNA-pyroninic particles content in the cytoplasm of most of the cells of the renal corpuscles and many renal tubules. Other tubular cells revealed a mild reduction in their RNA inclusions as illustrated in fig. (117).

A conspicuous diminution of RNA inclusions was observed in those materials examined 30 days following 1/8 LD_{50} $CdCl_2$, fig. (118). This figure demonstrated that the RNA-containing particles, which appeared aggregated in a considerably weak Pyronin-staining patches in most of the component cells of the kidney.

Forty five days of treatment of rats showing a marked loss in the cytoplasmic and nucleic RNA inclusions in almost all the nephric components cells which appeared as a weak in the amount and intensity of coloration as manifested by fig. (119).

A partial restoration of the RNA inclusions was observed in many epithelial cells of the proximal and distal convoluted tubules as well as the renal corpuscles was demonstrated in the materials inspected 30 days following Cd-abstainance after treatment with the same above dose for 45 days. This incomplete recovery clearly observed in fig. (120).

Rats treated with 1/4 LD_{50} $CdCl_2$

It was clearly observed after treatment of rats with the above dose for 10 days, marked increase in the RNA content of the renal corpuscles, proximal and distal convoluted tubular cells as shown in fig. (121). This increase in the amount of RNA-positive materials was reversed gradually with increase the time of application of this dose. The kidney materials inspected after 20 days of administration revealed a slight diminution in these materials in comparable to the normal preparations as illustrated in fig. (122).

Materials examined thirty days following application of this dose manifested a significant diminution in the cytoplasmic RNA inclusions of most of the epithelial cells of the renal tubules as well as the cells of the renal corpuscles as illustrated in fig. (123). Other components revealed also a conspicuous decrease in the amount and intensity of coloration of Pyronin-staining particles.

On the 45-th day post-treatment with the high dose, almost all the cells of the renal tissue components underwent a sharp loss of their cytoplasmic and nucleic RNA contents which appeared as diffused patches as

demonstrated in fig. (124). Other components cells of the kidney appeared to be stained faintly with Pyronin.

The materials examined 30 days following the last Cd injection showed a slight restoration of the RNA inclusions in comparable with the normal picture. The regenerated tubules and some cells of the renal corpuscles are strongly reacted with Pyronin-Methyl Green stain. These observations were clearly illustrated in fig. (125& 126).

Total Proteins

Control rats

Total proteins were detected in the parts of the nephrous in the kidney of control rats exhibiting a positive stainability with Mercuric Bromophenol Blue as illustrated in fig. (127).

The renal corpuscles including the glomeruli as well as the two epithelial layers of Bowman's capsule were moderately coloured with bromophenol blue indicating the existence of an average amount of proteins. The glomerular tuft stained strongly than the epithelial layers of Bowman's capsule.

The cytoplasm of the proximal and distal convoluted tubular cells stained with intense blue color detecting the majority of total protein content in these cells, while their nuclei stained weakly comparing with the cytoplasm.

A mild reaction was observed in the lining cells of the descending limb of Henle's loop but a comparatively more apparent reactivity in the cells of the ascending limb of that loop. Also the lining cells of the collecting tubules stained with faint blue color.

Treated rats:

Rats treated with 1/8 LD_{50} $CdCl_2$

Treatment of rats with the above dose for 10 days induced a slight increase in both the mode of occurrence and pattern of stainability of the total proteins in the different constituent of the renal tissue in comparison with the control material as illustrated in fig. (128). This figure manifested a rather strong bluish coloration of the lining epithelial cells of the proximal convoluted tubules as well as the cells of the renal corpuscles indicating a noticeable increase of their proteinic inclusions in their nuclei and cytoplasm than the normal conditions. This stainability also increased in all of other kidney components than the control tissue.

This picture gradually reversed with increase the time of the above dose application as illustrated in fig. (129). In this figure a slight diminution of the proteinic material is observed in most of the renal components cells (the renal corpuscles and the lining epithelial cells of the proximal and distal convoluted tubules).In comparison with normal preparations. the descending and ascending limbs of the loop of Henle as well as the collecting tubules were less reactive with bromophenol blue.

Thirty days following treatment with the same above dose showed a remarkable depletion in approximately most of the component cells of the kidney which appeared as a low level of reactivity for bromophenol blue in their cytoplasm with a lighter colouration of their nuclei as demonstrated in fig. (130). After treatment of rats for 45 days, a more notable reduction in both amount and intensity of colouration in most of the nephric tissue cells of all components as illustrated in fig. (131).

On the other hand, materials inspected 30 days following Cd-abstainance after 45 days of treatment revealed a partial restoration to regaining the normal picture of protein inclusions as demonstrated in fig.

(132). In this figure, the cells of the renal corpuscle as well as some epithelial cells of the nephric tubules revealed a moderate Bromophenol blue reactivity.

Rats treated with the 1/4 LD_{50} $CdCl_2$

The sections prepared from the materials of rats treated for 10 days showed a noticeable increase in the total protein content of most of kidney components as illustrated in fig (133). There was a conspicuous increase in the glomeruli reaction with bromophenol blue as compared to the normal tissues.

The basement membranes as well as the cytoplasm of the epithelial cells of the proximal convoluted tubules were intensively stained with bluish colour than the normal cells while their nuclei also stained strongly but lighter than cytoplasm. The bromophenol blue stainability of the component cells of the distal convoluted tubules, the descending and ascending limbs of Henle's loop and the collecting tubules was much increased than normal denoting an obvious protein increase under such condition.

The reverse observations were observed 20 days following 1/8 LD_{50} $CdCl_2$ administration as illustrated in fig (134). This figure revealed a significant decrease the total protein content comparing with the normal tissue. the lining epithelial cells (cytoplasm and nuclei) stained moderately with bromophenol blue while, the basement membranes still stained deeply with bluish colour. The capillary tuft showed a moderate reaction. All of the other components revealed very weak bromophenol blue reaction.

Thirty days following treatment with the same above dose demonstrated a pronounced loss of their total protein content in most of kidney tissue components as shown in fig (135). This figure illustrated remarkable depletion of proteinic material in each of the layers of Bowman's

capsule and the capillary tuft. On the other hand, the basement membranes of proximal convoluted tubules still stained deeply with bromophenol blue, but thel cytoplasm of their lining epithelial cells revealed a slight reactivity and intensity. Their nuclei stained weakly with blue colour. Other cells of different components (distal tubules, ascending and discending limbs of Henle's loop) as well as the collecting tubules) appeared weakly reacted with bromophenol blue as compared to the previous treated and normal tissues indicating marked loss of the proteins in the cytoplasm and nuclei.

The maximal exhaustion of proteinic material was clearly appeared from the material of 45 days treated rats. These reduction appeared in both amount and intensity of colouration in most of the renal tissue cells as demonstrated in fig (136).

Specimens invistigated after 30 days of Cd abstainances revealed a slight development and restoration of almost all the nephric tissue constituents of proteinic materials as illustrated in fig. (137& 138).

Plate (XXIII) - Polysaccharides in the Kidney of normal and CdCl2 treated rats. Materials were fixed in alcoholic Bouin 's fluid and treated according to the Periodic Acid Schiff 's (PAS) technique (Hotchkiss, 1948). x400

Fig. (91) T.S. in the Kidney of a normal rat shows a marked PAS reactivity in the basement membrane and in the outer membrane of Bowman,s capsule of the renal corpuscles. In the proximal convoluted tubules, the basement membranes as well as the brush borders of their cells are deeply coloured with PAS reagent. Distal convoluted tubules display a mild PAS reactivity in their basement membranes.

Fig. (92) T.S. in the kidney of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 10 days shows a mild decrease in PAS-positive inclusions in the basement membranes of the tubules as well as the glomerulus.

Fig. (93) T.S. in the kidney of a rat treated day after day with 1/8 LD50 of CdCl2 for 20 days shows an obvious reduction of the polysaccharides reactivity in the renal tubules. Also, the renal corpuscles are still moderately stained.

Fig. (94) T.S. in the kidney of a rat treated day after day with 1/8 LD50 of CdCl2 for 30 days shows a noticeable decrease of the polysaccharides inclusions in the Bowman's capsule and in the basement membranes and the brush borders of the proximal convoluted tubules.

Fig. (95) T.S. in the kidney of a rat treated day after day with 1/8 LD50 of CdCl2 for 45 days shows an obvious diminution of PAS positive inclusions in the renal corpuscles, the distal convoluted tubules and the collecting tubules.

Fig. (96) T.S. in the kidney of a rat treated day after day with 1/8 LD_{50} $CdCl_2$ for 45 days and examined 30 days after withdrawal of the metal shows an obvious restoration of PAS positive materials.


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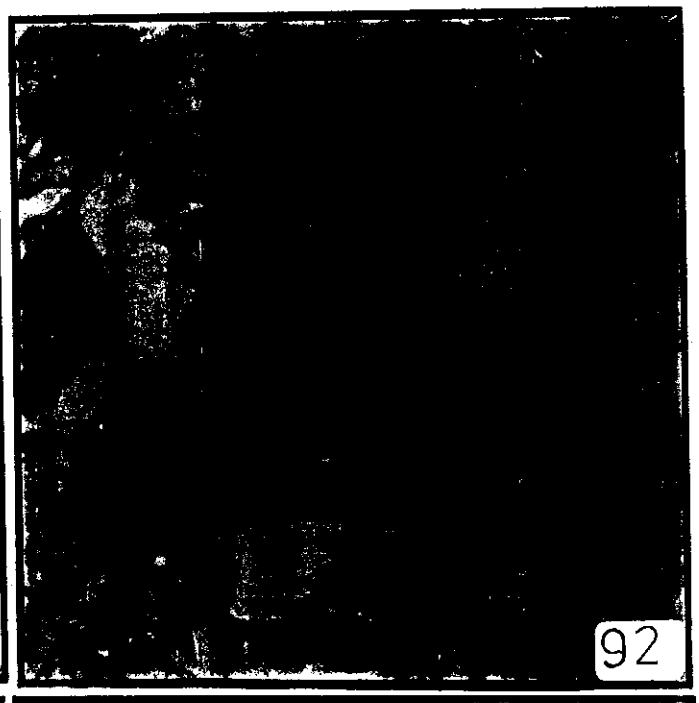
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Plate (XXIV) – Polysaccharides in the Kidney of $CdCl_2$ treated rats. Materials were fixed in alcoholic Bouin 's fluid and treated according to the Periodic Acid Schiff 's (PAS) technique (Hotchkiss, 1948). x400

Fig. (97) T.S. in the kidney of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 10 days shows a noticeable diminution of the polysaccharides reactivity in almost all the tubular basement membranes, brush borders of the PT and glomerulus comparing to the normal tissue.

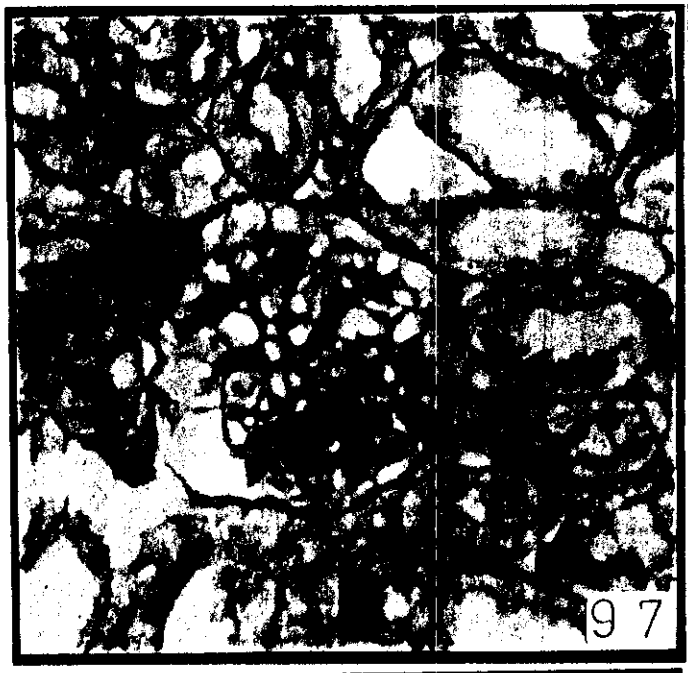
Fig. (98) T.S. in the kidney of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 20 days shows a marked decrease of polysaccharides inclusions in all the tissue components.

Fig. (99) T.S. in the kidney of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 30 days shows an obvious reduction of the polysaccharides inclusions in both the tubular basement membranes and brush borders while the renal corpuscles are moderately stained with PAS.

Fig. (100) T.S. in the kidney of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 45 days shows maximum decrease of the polysaccharides materials in the different parts of the kidney including the renal corpuscles, the proximal and distal convoluted tubules and the collecting tubules.

Fig. (101) T.S. in the kidney of a rat Kidney section of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 45 days and examined 30 days post-treatment shows a slight improvement and restoration of PAS- positive particles in the basement membranes and brush borders of the (PT) as well as the cells of the renal corpuscles.

Fig. (102) Another field of the last specimen shows the same phenomenon of partial restoration of the polysaccharides content in the kidney cells.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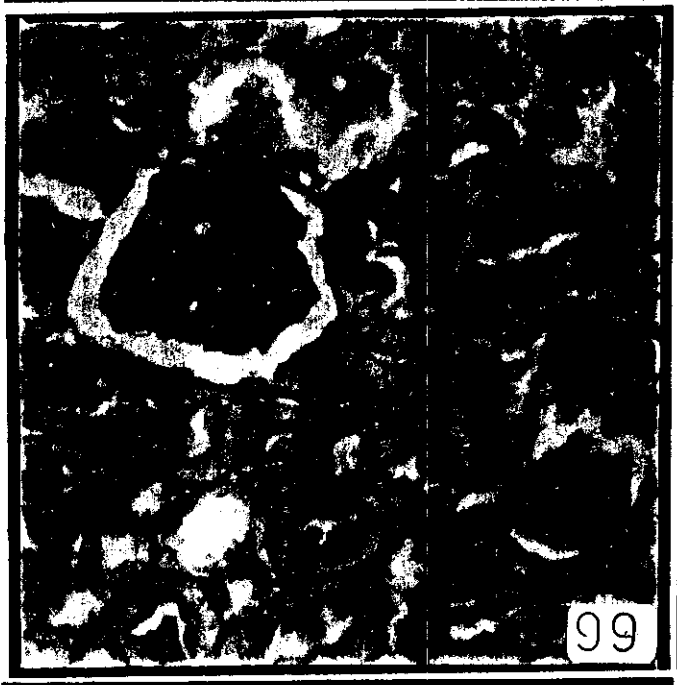
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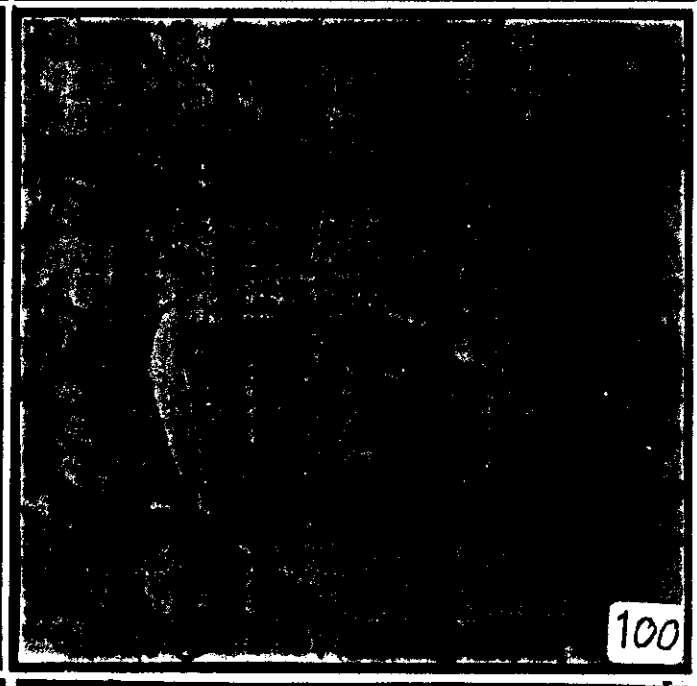
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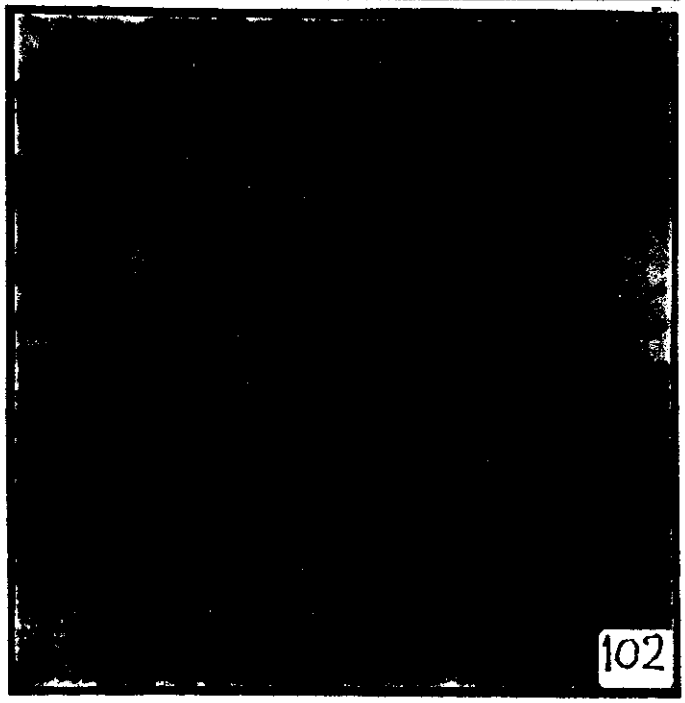
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Plate (XXV) - Deoxyribonucleic acid (DNA) containing particles in the kidney cells of normal and $CdCl_2$ treated rats. Materials were fixed in carnoy's fluid and stained with Feulgen technique (De Tomasi,1936). x1000

Fig. (103) Kidney section of a control rat revealed that all the tubular nuclei contain homogeneously distributed line red- purple coloured DNA particles. Some of them appeared attached with the nucleoli.

Fig. (104) Kidney section of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 10 days showing a mild increase in DNA (chromatin) reactivity in the nuclei of the epithelial cells of the renal tubules. This increase was obviously observed in the nuclei of the cells of the capillary tuft.

Fig. (105) Kidney section of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 20 days showing approximately normal reaction in the nuclei of the glomeruli while the nuclei of the epithelial cells of DCTs and PCTs revealed a mild diminution in the chromatin materials.

Fig. (106) Kidney section of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 30 days showing an obvious decrease of DNA inclusions.

Fig. (107) Kidney section of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 45 days showing a striking weakness of Feulgen reactivity in most of the nuclei of the epithelial cells. The nuclei of the cells of the capillary tuft are still markedly stained.

Fig. (108) Kidney section of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 45 days, and examined, 30 days following abstainance of Cd treatment showing a partial recovery in the DNA (amount and reactivity) in most of the nuclei.

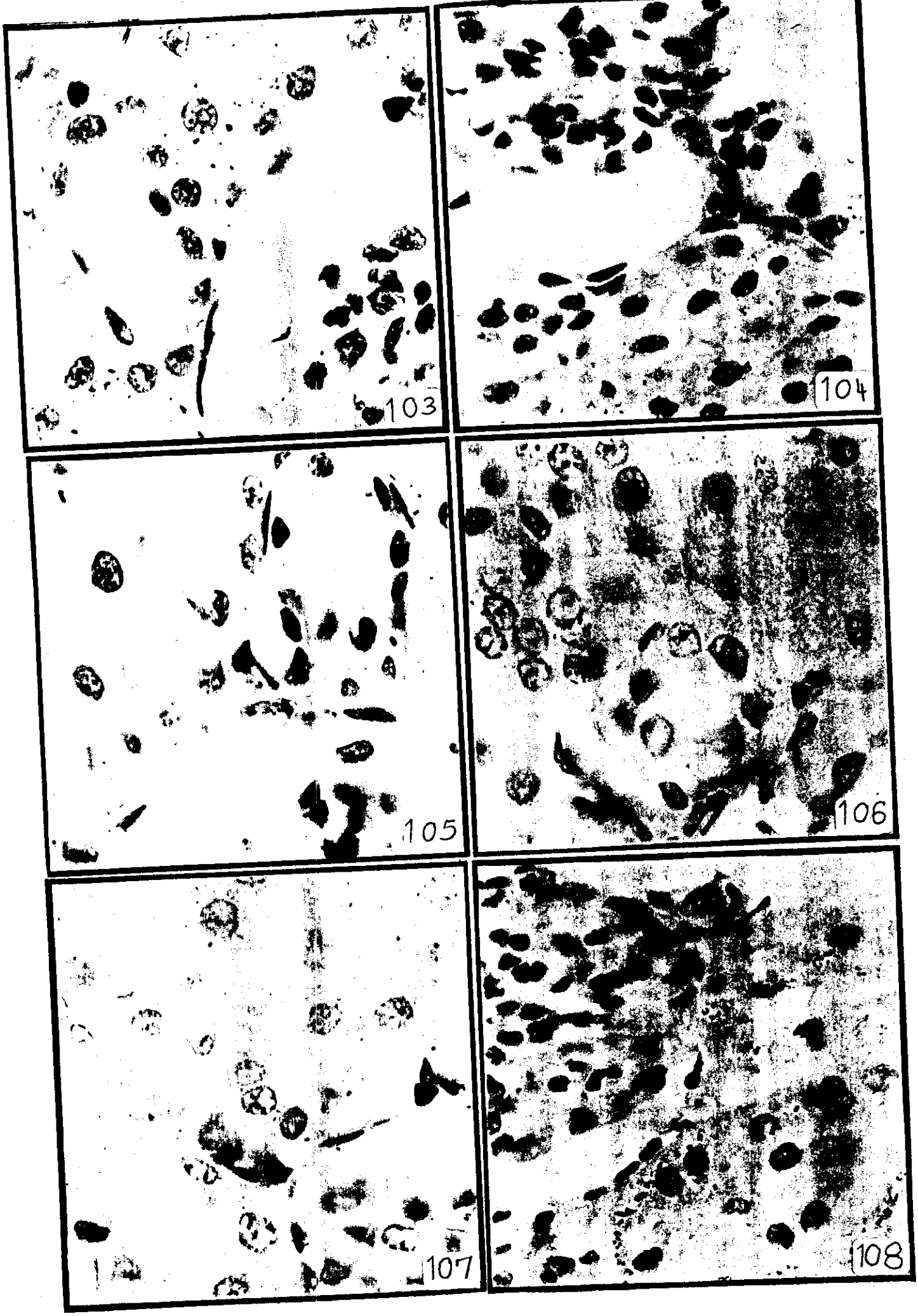
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Plate (XXVI) - Deoxyribonucleic acid (DNA) containing particles in the kidney cells of $CdCl_2$ treated rats. Materials were fixed in carnoy 's fluid and stained with Feulgen technique (De Tomasi, 1936). x1000

Fig. (109) Kidney section of a rat treated day after day with 1/4 LD50 of CdCl2 for 10 days, shows approximately mild increase in the chromatin materials which appeared as a strong positive reaction of the DNA particles with Feulgen.

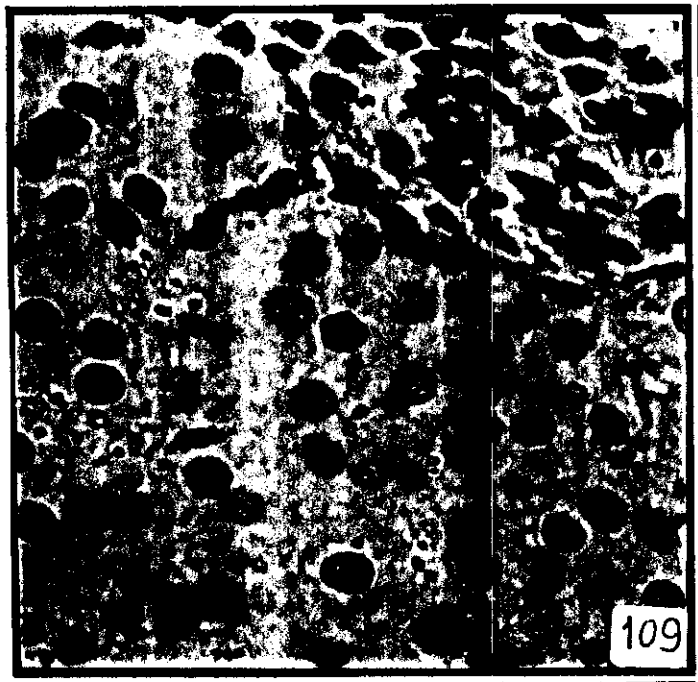
Fig. (110) Kidney section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 20 days showing a slight decrease in the DNA content of the nuclei of the epithelial cells of the renal tubules and glomerular cells comparing to the normal tissue.

Fig. (111) Kidney section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 30 days showing a significant reduction of the DNA-containing particles of most of the renal tissue nuclei.

Fig. (112) Kidney section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 45 days revealed an intensive diminution of DNA. The chromatin granules situated in the periphery of most of the nuclei attached to the nuclear membrane and moderately stained with Feulgen. Other nuclei were weakly stained .

Fig. (113) Kidney section of a rat treated day after day a slight recovery of chromatin materials observed in many nuclei of the epithelial cells of the renal tissue, 30 days following treatment with 1/4 LD_{50} $CdCl_2$ for 45 days.

Fig. (114) Kidney section of a rat treated day after day showing a slight recovery of chromatin materials observed in many nuclei of the epithelial cells of the renal tissue, 30 days following treatment with 1/4 LD_{50} of $CdCl_2$ for 45 days.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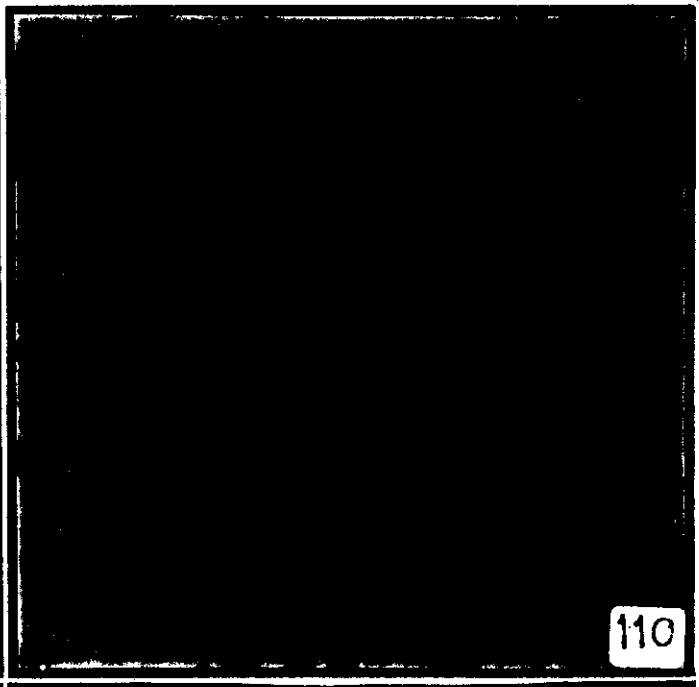
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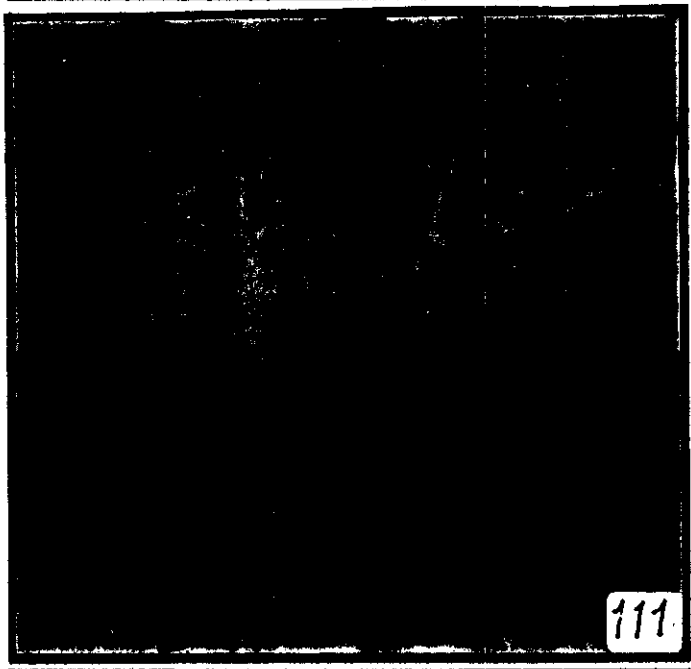
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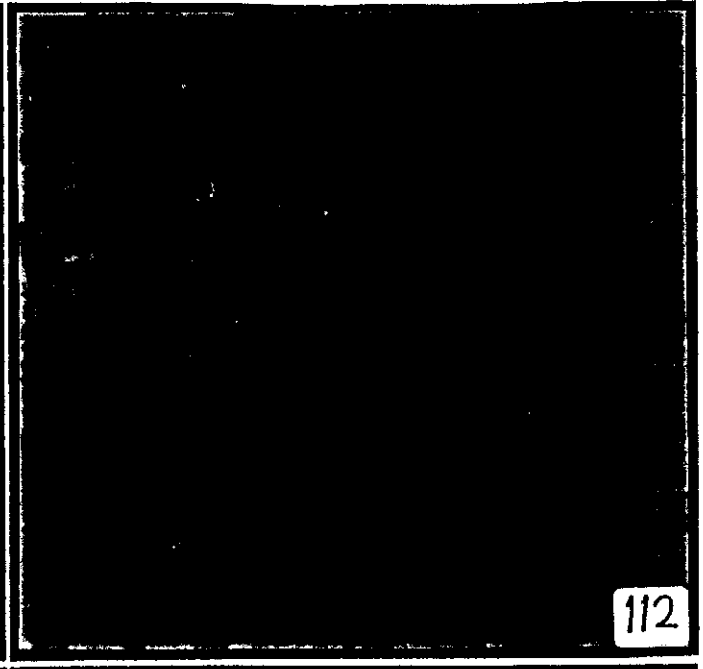
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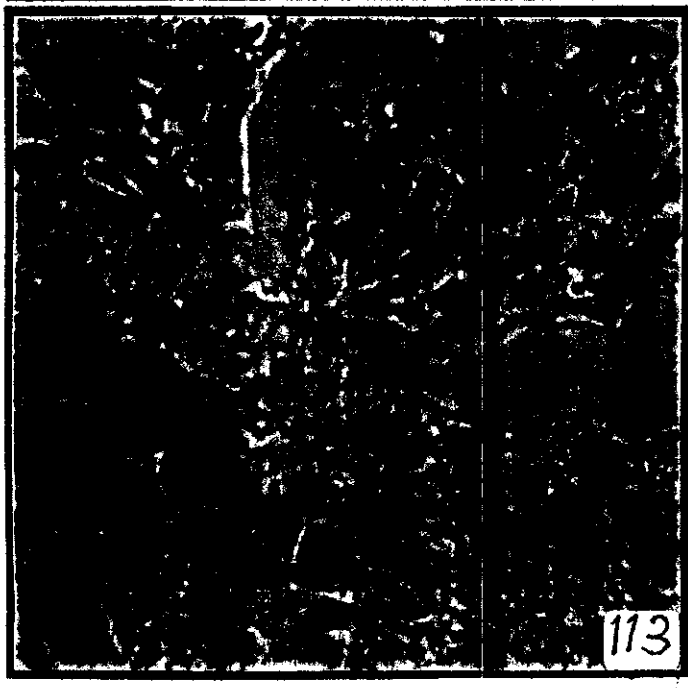
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Plate (XXVII) - Ribonucleic acid (RNA) containing particles in the kidney cells of normal and $CdCl_2$ treated rats. Materials were fixed in Carnoy 's fluid and stained with Pyronin Methyl Green. x1000

Fig. (115) Kidney section of a normal rat showing the mod of localization and pattern of reactivity of RNA inclusions in epithelial cells of the renal tubules appearing as bright red-coloured particles located in the cytoplasm of these cells. The nuclei are exhibiting a greenish blue colouration indicating their DNA content.

Fig. (116) Kidney section of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 10 days showing a noticeable increase of RNA inclusions in each of nuclei and cytoplasm of epithelial cells of the renal tubules.

Fig. (117) Kidney section of a rat treated day after day with 1/8LD_{50} of $CdCl_2$ for 20 days showing a mild decrease in the pyronin stained inclusions as well as DNA inclusions.

Fig. (118) Kidney section of a rat treated day after day with 1/8LD_{50} of $CdCl_2$ for 30 days showing a noticeable decrease in RNA inclusions particularly in the epithelial cells of the renal tubules.

Fig. (119) Kidney section of a rat treated day after day with 1/8LD_{50} of $CdCl_2$ for 45 days showing a remarkable loss of RNA inclusions in most of the cells of the kidney tissue.

Fig. (120) Kidney section of a rat treated day after day and examined 30 days following 1/8LD_{50} dose of the $CdCl_2$ withdrawal indicating a partial recovery of RNA inclusions in the epithelial cells of PCTs and DCTs and more pronounced in the cells of glomerulus.

Plate (XXVIII) - Ribonucleic acid (RNA) containing particles in the kidney cells of normal and CdCl2 treated rats. Materials were fixed in carnoy 's fluid and stained with Pyronin Methyl Green. X1000

Fig. (121) Kidney section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 10 days showing a noticeable increase in the RNA inclusions, which intensively stained with pyronin.

Fig. (122) Kidney section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 20 days showing an obvious decrease in the pyronin-stained particles in the renal tissue cells comparing to the normal tissue.

Fig. (123) Kidney section of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 30 days showing a pronounced diminution in the RNA - containing particles in the epithelial cells PCTs and DCTs as well as the cells of the glomerulus.

Fig. (124) Kidney section of a rat treated day after day with1/4 LD_{50} of $CdCl_2$ for 45 days showing a sharp loss of RNA inclusions in almost all the renal tissue cells.

Fig. (125) Kidney section of a rat treated day after day showing a slight improvement of RNA inclusions of the epithelial cells of the renal tissues of a rat studied 30 days following treatment with successive day after day doses of 1/4 LD_{50} of CdCl2 for 45 days.

Fig. (126) Kidney section of a rat treated day after day showing a slight improvement of RNA inclusions of the epithelial cells of the renal tissues of a rat studied 30 days following treatment with successive day after day doses of 1/4 LD_{50} of $CdCl_2$ for 45 days

Plate (XXIX)- Total proteins in the kidney cells of normal and $CdCl_2$ treated rats. Materials were fixed in Carnoy 's fluid and stained with bromophenol blue (Mazia et al., 1953). (M. B. B. x1000)

Fig (127) Normal renal tissue having the total proteins in the epithelial cells of the PCTs, DCTs and the cells of the glomerulus as deeply blue coloured closely packed fine granulations lying in positive reactive cytoplasm. Their nuclei are also strongly stained.

Fig. (128) T.S. in the kidney of a rat treated day after day with 1/8 LD_{50} of $CdCl_2$ for 10 days showing a significant increase in the protein reactivity in both nuclei and cytoplasm of the epithelial cells of both PT and DT as well as the cells of the capillary tuft. The basement membranes of the tubules are somewhat strongly stained.

Fig. (129) T.S. in the kidney of a rat treated day after day with 1/8 $_{LD5050}$ of $CdCl_2$ for 20 days showing a mild diminution of the total proteins in the cytoplasm and nuclei of the PT and DT. The basement membranes of these tubules are still retaining a deep stainability.

Fig. (130) T.S. in the kidney of a rat treated day after day with 1/8 $LD_{50}50$ of $CdCl_2$ for 30 days showing an obvious diminution of the protein in the cytoplasmic and nuclei of most of the renal tissue components.

Fig. (131) T.S. in the kidney of a rat treated day after day with 1/8 LD_{50} $CdCl_2$ of for 45 days showing the highest depletion of the total proteins in the cytoplasm and nuclei of most cells of the renal tissue components.

Fig. (132) T.S. in the kidney of a rat treated day after day for 45 days with 1/8 LD_{50} of $CdCl_2$ and obtained 30 days after abstainance of Cd treatment showing a partial restoration of the total protein content in the nuclei and the cytoplasm of many cells of the renal tubules and glomeruli.

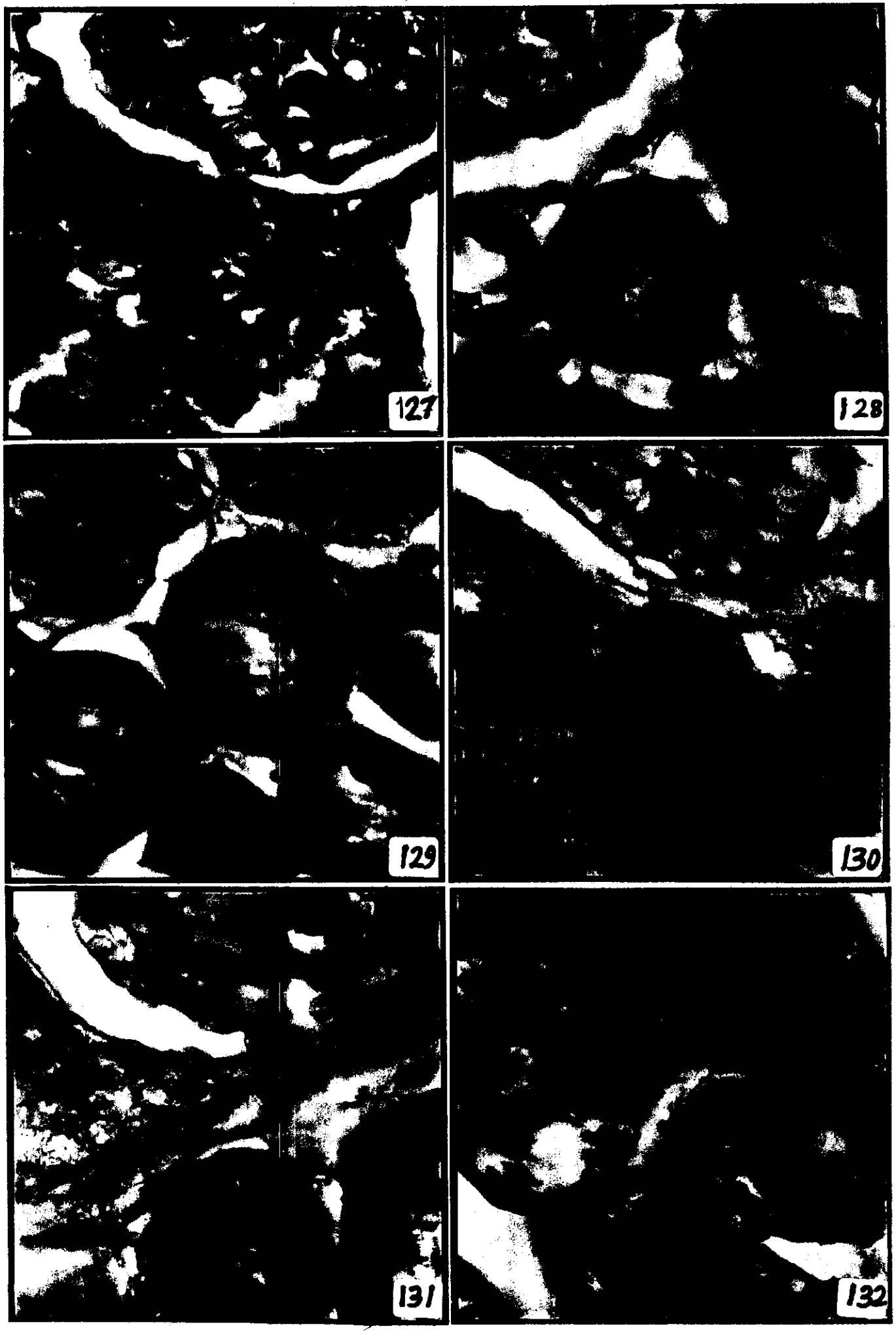
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Plate (XXX) - Total proteins in the kidney cells of $CdCl_2$ treated rats. Materials were fixed in Carnoy 's fluid and stained with bromophenol blue (Mazia et al., 1953). x1000

Fig. (133) T.S. in the kidney of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 10 days showing a significant increase in the total protein content of the cells of the renal tissue comparing to the control rat. The basement membranes of the tubules are stained more deeply than other components.

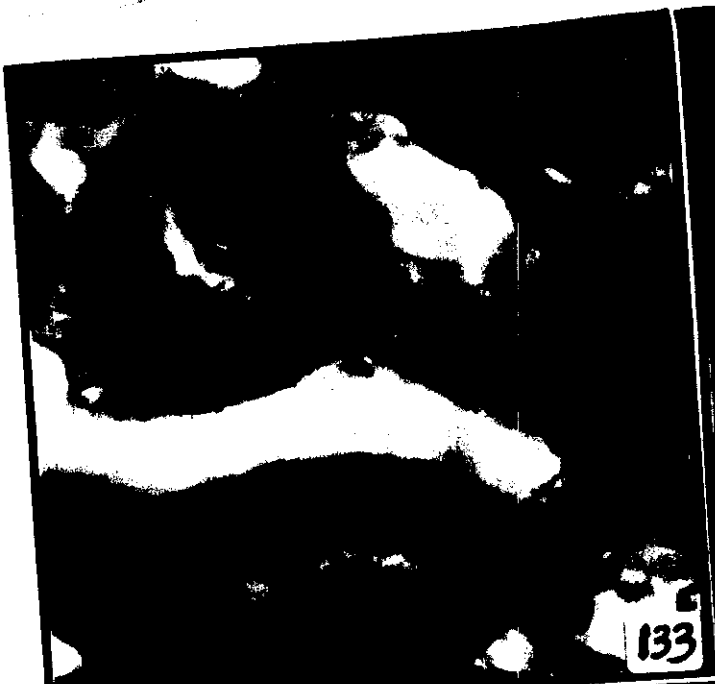
Fig (134) T.S. in the kidney of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 20 days revealing a slight decrease in the total protein contents of the epithelial cells of the PT & DT. The cells of the glomerulus are moderately stained while the basement membranes are deeply stained with M.B.B..

Fig. (135) T.S. in the kidney of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 30 days showing a marked loss of the total proteins in the nuclei and the cytoplasm of the epithelial cells of the PT and DT.

Fig. (136) T.S. in the kidney of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 45 days showing a sharp depletion in the total protein contents of most of the renal component cells especially in the degenerated cells.

Fig. (137) T.S. in the kidney of a rat treated day after day with 1/4 LD_{50} of $CdCl_2$ for 45 days and left for 30 days following abstainance of Cd treatment showing a mild restoration of the total protein contents.

Fig. (138) Another field of the last specimen showing the same phenomenon of partial restoration of the total protein contents in the kidney cells.



133

134

135

136

Dose	Reaction	Polysaccharides					DNA				
	Days Renal comp.	10	20	30	45	75	10	20	30	45	75
Control	P.T.	++++	++++	++++	++++	++++	+++++	+++++	+++++	+++++	+++++
	D.T.	+++	++	++	++	++	+++++	+++++	+++++	+++++	+++++
	B.C.	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	C.T.	+++	++	++	++	++	++++	++++	++++	+++++	++++
	A. &D. LH	++	++	++	++	++	+++++	+++++	+++++	+++++	+++++
1/8 LD_{50} $CdCl_2$	P.T.	+++	+++	++	++	+++	+++++	++++	+++	+++	++++
	D.T.	++	+	+	+	++	+++++	++++	+++	+++	++++
	B.C	++++	+++	+++	++	+++	++++	+++	++	++	+++
	C.T.	++	+	+	+	++	++++	+++	++	++	+++
	A. &D. LH	++	++	+	+	++	++++	+++	++	++	+++
¼ LD_{50} $CdCl_2$	P.T	+++	++	++	+	++	+++++	+++	++	++	++
	D.T	++	+	+	+	++	+++++	+++	++	+	++
	B.C	+++	++	++	+	++	+++++	+++	++	+	++
	C.T	++	+	+	+	++	++++	++	++	+	++
	D&A.LH	++	+	+	+	++	++++	+++	++	+	+++

Polysaccharides and DNA changes in the kidney of control rats and those treated with 1/8 LD_{50} and ¼ LD_{50} $CdCl_2$

Dose	Reaction	RNA					Protein				
	Days Renal comp.	10	20	30	45	75	10	20	30	45	75
Control	P.T.	++++	++++	++++	++++	++++	+++++	+++++	+++++	+++++	+++++
	D.T.	++++	++++	++++	++++	++++	+++++	+++++	+++++	+++++	+++++
	B.C.	++++	++++	++++	++++	++++	+++++	+++++	+++++	+++++	+++++
	C.T.	++++	++++	++++	++++	++++	+++	+++	+++	+++	+++
	D.&A. LH	++++	++++	++++	++++	++++	+++	+++	+++	+++	+++
1/8 LD50 CdCl2	P.T.	+++++	++++	+++	++	+++	+++++	++++	+++	++	+++
	D.T.	+++++	++++	+++	++	+++	+++++	++++	+++	++	+++
	B.C	+++++	++++	+++	++	+++	+++++	++++	+++	++	+++
	C.T.	++++	+++	+++	++	+++	++++	+++	+++	++	+++
	A.&D. L. H.	++++	+++	+++	++	+++	++++	+++	+++	++	+++
¼ LD50 CdCl2	P.T	+++++	+++	++	+	+++	+++++	+++	++	+	+++
	D.T	+++++	+++	++	+	+++	+++++	+++	++	+	+++
	B.C	+++++	+++	++	++	+++	+++++	+++	++	++	+++
	C.T	++++	+++	++	++	+++	++++	+++	++	++	+++
	A.&D. L. H.	++++	+++	++	+	+++	++++	+++	++	++	+++

RNA and Protein changes in the kidney of control rats and those treated with 1/8 LD_{50} and ¼ LD_{50} $CdCl_2$

DNA IMAGE CYTOMETRY

Liver cells

In the present study, DNA image cytometric analysis was performed on a variety of liver samples from the rats of all the studied groups.

In the liver tissue, most of the hepatocytes appeared flattened On the glass surface without adhesions or overlapping with others, but if there were any aggregates, we avoided recording them. Therefore, evaluation of single cell DNA-content by DNA image cytometry was often very easy. Fifty-micrometer sections showed good results versus the number of cut nuclei.

According to the criteria of ***Hedely et al., (1984)***, a single G1 peak was regarded as diploid DNA and two or more G1 peaks as aneuploid.

On the basis of the fact that the liver cells in their normal state revealed an euploid polypolidization phenomenon, (2C, 4C, 8C...etc.). We observed that liver materials prepared from control rats have a large number of tetraploid nuclei (4C), beside the diploid (2C) cells as illustrated in figure (A). Most of the diploid and tetraploid nuclei has been considered in (G_0/ G_1) phase meaning that most of these cells in a quiescence state. The CV values were relatively broad ranged from (2.51- 7.78), but were still within an acceptable range in all of the analyzed cases. The DNA index for the normal diploid peaks as reported in the previous studies ranged from (0.9 to 1.1), while, in the present study, we recorded that it ranges from (0.91 to 1.01). The DNA index of the tetraploid peaks ranged from (1.90 to 2.10).

We noted in this study that DNA index decreased gradually with dose and duration of all the groups treated with $CdCl_2$, which become below (0.9) for the diploid peaks and below (1.9) for the tetraploid ones.

The control group, which represented in fig. (A), revealed that the S-phase (DNA synthesis phase) was low (15.77). Also the second histogram in the same plate is showing that reference cells (lymphocytes) which normally have the diploid number of chromosomes were situated on the diploid peak.

The materials prepared from liver of rats treated with 1/8 as well as 1/4 from the LD_{50} $CdCl_2$ for 10 days revealed an obvious increase in the DNA synthesis (S- phase) which as illustrated in fig. (B) equal (34.91 %).

The following treated groups revealed a gradual decrease in the (S-phase) accompanied with increase (Go/G1) phase. Also, the cytometry of these cases showing that there was a marked loss in the DNA content of their hepatocytes (hypodiploidy). The hypodiploidy is an aneuploid case and considered as abnormal change may lead to cancer incidence.

The hypodiploidy of the hepatic cells has been clearly observed after 45 days of treatment particularly in the rats dosed with 1/4 LD_{50} as shown in fig. (C). The (G0/G1) phase was (52 %) which became larger than the normal (20.3 %), while the tetraploid peak has been disappeared and the (G2/M) phase was markedly low (20 %) comparing to the normal state (62.8 %). The DNA index of this case was shifted and lower than the normal, equal (84 %).

In the materials sampled from the recovery groups, we noted continuing of the hypodiploid case, beside a small S- phase (17 %) as illustrated in fig. (D). The (Go/G1) was still higher (49%) than the normal

one. Also the diploid peak was shifted and appeared before (2C) and the DNA index was (0.84).

This figure showed also a partial recovery to the normal DNA content of the hepatic cells, which appeared by presence of the tetraploid peaks, which disappeared previously in the 1/8 and 1/4, LD_{50} treated rats for 45 days. This means that stopping of Cd injection for along time leads to DNA repairing.

Kidney Cells

The results obtained from DNA image cytometry of the kidney cells revealed mild changes comparing to those observations of liver materials.

The smears prepared from the kidneys of the control rats revealed a typical normal diploid histogram, where all of these nuclei have diploid number of chromosomes (2 C) content and the reference cells (2C) typically situated on the diploid peak. The data letter of this histogram clarified that most of the kidney cells were in (Go/ G_1) phase which equal (96.5 %), also, (G_2/M) phase equal (0%), while S-phase was (3.5). This means that these cells were in a quiescence state and there were no mitotic division, fig. (E).

The kidney materials of rats treated for 10 days with 1/8 and others treated with 1/4 LD_{50} $CdCl_2$ showed that most of these cells were in (Go/ G1) phase although there were a mild increase in the S-phase than the normal state.

Figure (F) shows the cytometric data acquired from examined materials of rats treated with 1/4 LD_{50} revealed a high (G0/ G1) which was (89.76 %) but lower than the normal case. While the DNA synthesis slightly increased than the normal because the S-phase equals (10.49 %), and the (G2/ M) phase approximately absent.

Other cases examined from the kidneys of the rats treated with 1/8 or 1/4 LD_{50} $CdCl_2$ for 20, 30, and 45 days revealed a gradual mild decrease in the DNA content (hypodiploidy) which reached the maximum after 45 days of injection especially with 1/4 LD_{50} as illustrated in fig. (G). This table shows a decrease in the DNA index (0.8) and the peak was shifted behind the (2C) (hypodiploidy) and representing an aneuploid peak. The aneuploid state of hypodiploidy represented an early sign of tumor incidence.

On the other hand, our present data manifested that the materials examined from rats treated with the 1/8 LD_{50}, 30 days after abstainance of Cd treatment showed complete DNA restoration.

A pronounced recovery was observed in those materials examined after 30 days and treated with the 1/4 LD_{50} by the same manner as illustrated in fig. (H). This plate showed that most of the kidney cells were in Go/G1, which equals (91.13 %), and the DNA index is (1.07) representing a standard diploid peak.

Fig. (A) A histogram of DNA cytometry of hepatic cells of rat of control group of normal diploid (2C) and tetraploid (4C) peaks (euploid polyploidization).

Fig. (B) A histogram of DNA cytometry of hepatocytes of rat treated with 1/4 LD_{50} $CdCl_2$ for 10 days revealed an increase in the DNA synthesis (S phase).

Fig (C) A histogram of DNA cytometry of hepatocytes of rat treated with 1/4 LD_{50} $CdCl_2$ for 45 days showing a marked hypodiploidy.

Fig (D) A histogram of DNA cytometry of hepatic cells of rat of 1/4 LD_{50} recovery group revealed DNA hypodiploidy and normal tetraploid peak.

Fig (E) A histogram of normal kidney cells of control rat revealed a normal diploid (2C) peak.

Fig. (F) A histogram of DNA cytometry of rat treated with 1/4 LD_{50} for 10 days revealed presence of a slight increase in the DNA synthesis (S phase).

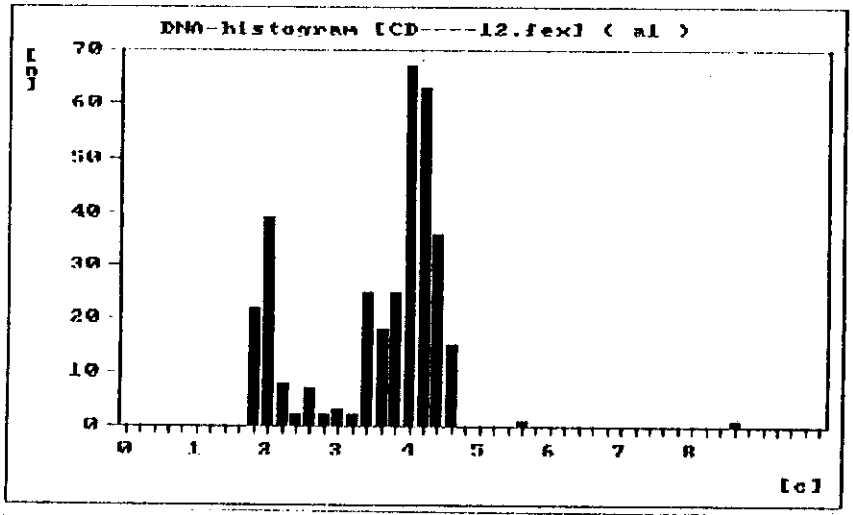
Fig (G) A histogram of DNA cytometry of rat treated with 1/4 LD_{50} $CdCl_2$ for 45 days showing a slight hypodiploidy.

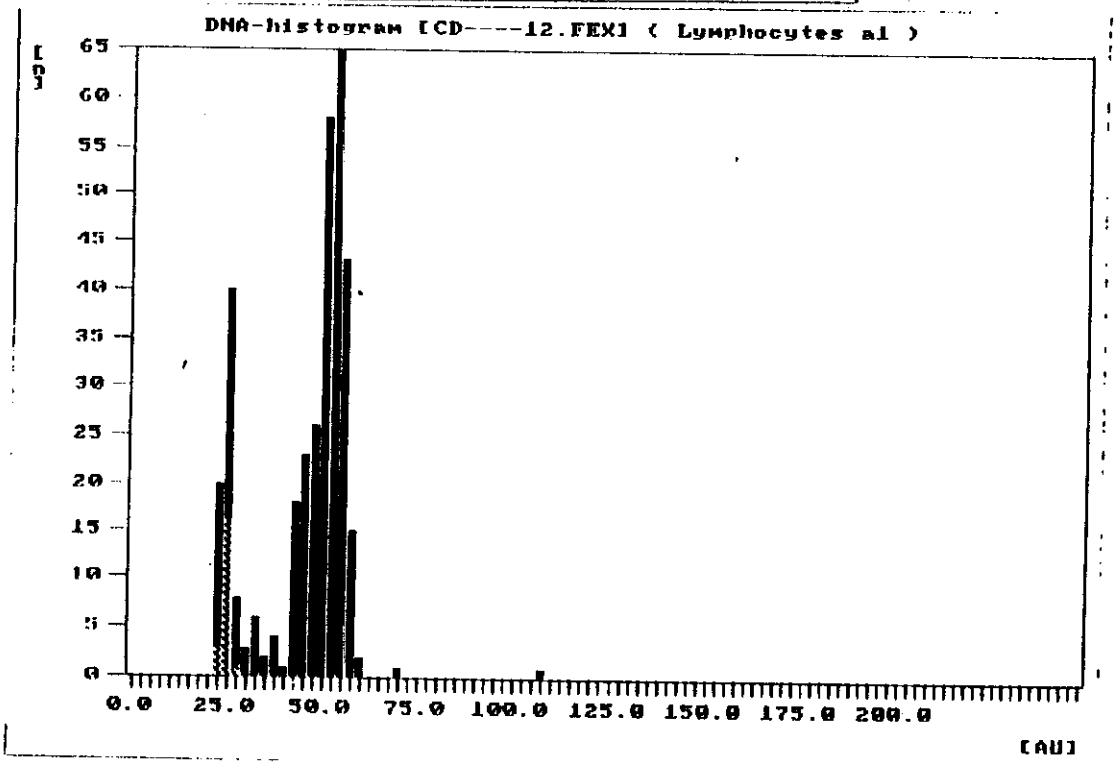
Fig (H) A histogram of DNA cytometry of rat treated with 1/4 LD_{50} $CdCl_2$ for 45 days and examined 30 days following Cd-abstainance showing a approximately complete recovery of the normal picture (normal diploid peak).

D N A - C Y T O M E T R Y

.cycle indices		
. fraction	[%]	20.83
fraction	[%]	15.77
l fraction	[%]	62.80
.iferation fraction	[%]	80.06

DNA-INTERPRETATION: non aneuploid.





Cytometric parameters:

iber of cells [n]:	336	2c-Deviation Index [c^2]:	3.29
Stemline ploidy [c]:	2.02	DNA index:	1.01
Stemline ploidy [c]:	4.12	DNA index:	2.06
loid Deviation Quotient:	3.59	Entropy of DNA:	4.28
Exceeding Events [n]:	2	Mean ploidy [c]:	3.55
euploid Events d [n]:	31	Mean area [micron^2]:	46.21

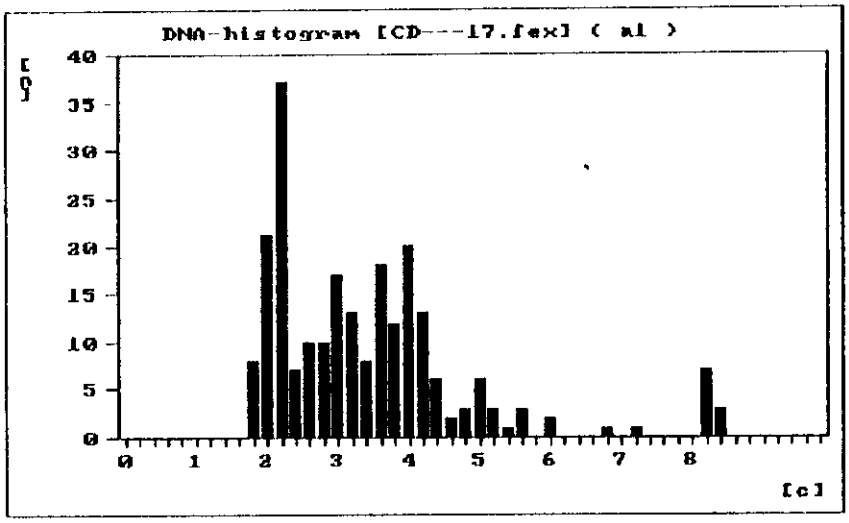
e statement on DNA-aneuploidy is based on the DNA-single-cell interpre-
ion according to Kropff et al. 1991 using the Aneuploid Events diploid
d) as a threshold, and is only valid if euploid polyploidisation >4c,
ologically detectable virus infection, cytostatic and radiation therapy
be excluded.
cells were measured and 0 with a DNA-content > 8.62[c] were found.

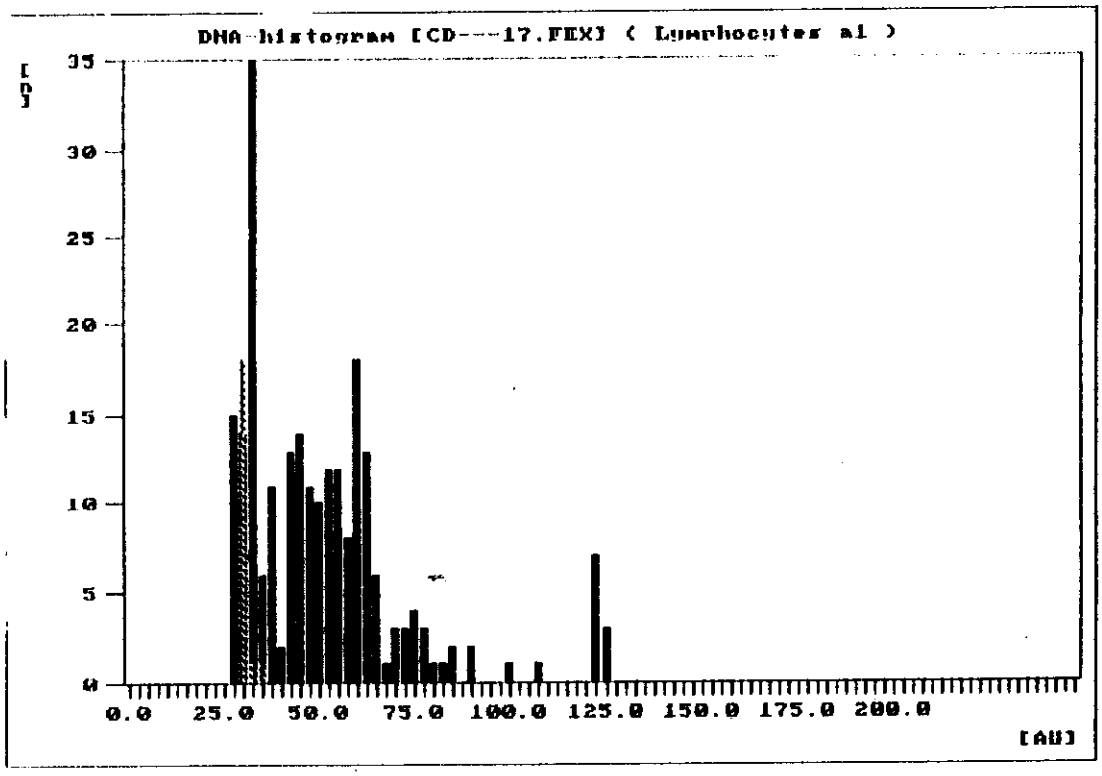
(A)

D N A - C Y T O M E T R Y

Cellcycle indices		
G0/1 fraction	[%]	29.31
S fraction	[%]	34.91
G2/M fraction	[%]	22.84
Proliferation fraction	[%]	69.83

DNA-INTERPRETATION: aneuploid.





Cytometric parameters:

Number of cells [n]:	232	2c-Deviation Index [c^2]:	4.21
1. Stemline ploidy [c]:	2.14	DNA index:	1.07
2. Stemline ploidy [c]:	4.00	DNA index:	2.00
Diploid Deviation Quotient:	3.43	Entropy of DNA:	4.89
5c Exceeding Events [n]:	24	Mean ploidy [c]:	3.45
Aneuploid Events d [n]:	29	Mean area [micron^2]:	60.71

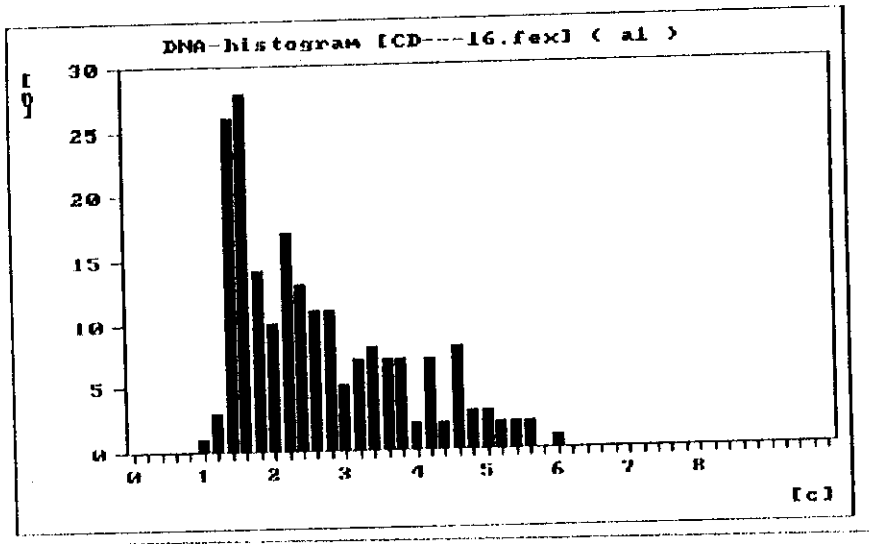
The statement on DNA-aneuploidy is based on the DNA-single-cell interpretation according to Kropff et al. 1991 using the Aneuploid Events diploid (AEd) as a threshold, and is only valid if euploid polyploidisation >4c, cytologically detectable virus infection, cytostatic and radiation therapy can be excluded.
232 cells were measured and 28 with a DNA-content > 4.84[c] were found.

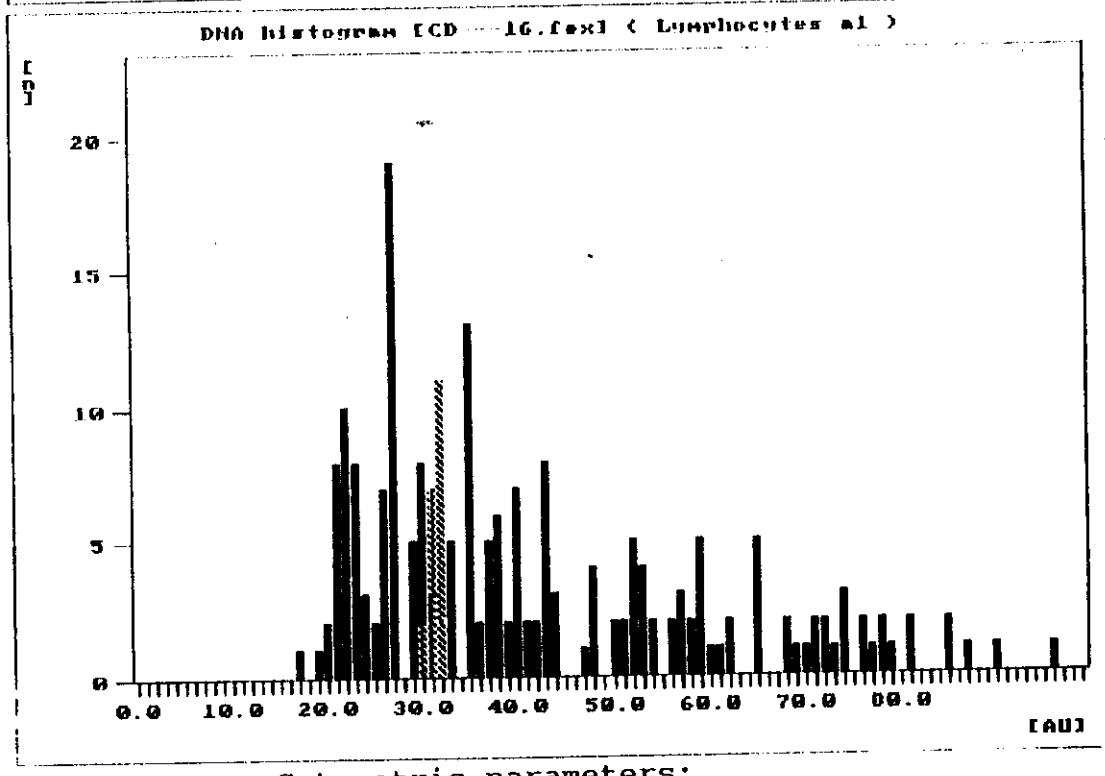
(B)

DNA-CYTOMETRY

ellcycle indices		
0/1 fraction	[%]	52.00
fraction	[%]	25.00
2/M fraction	[%]	20.00
roliferation fraction	[%]	64.00

DNA-INTERPRETATION: aneuploid.





Cytometric parameters:

Number of cells [n]:	200	2c-Deviation Index [c^2]:	1.70
Stemline ploidy [c]:	1.69	DNA index:	0.84
Diploid Deviation Quotient:	2.65	Entropy of DNA:	4.96
5c Exceeding Events [n]:	7	Mean ploidy [c]:	2.63
Aneuploid Events d [n]:	45	Mean area [micron^2]:	41.80

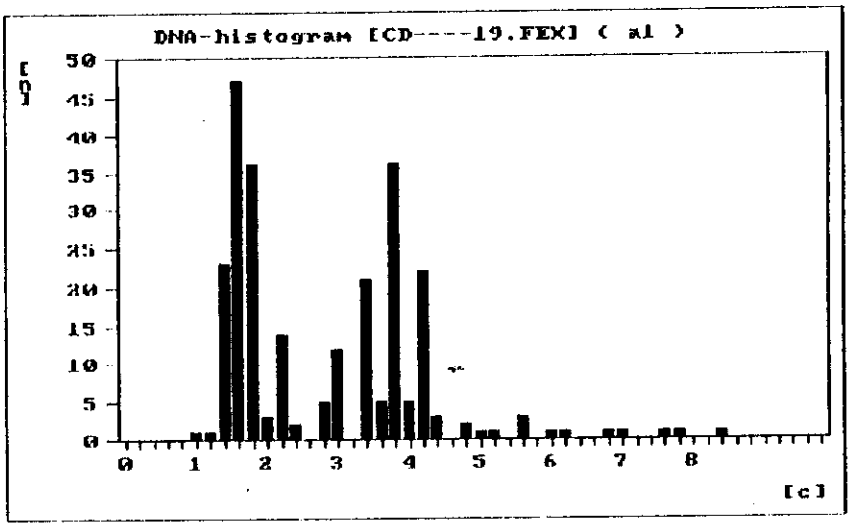
The statement on DNA-aneuploidy is based on the DNA-single-cell interpretation according to Kropff et al. 1991 using the Aneuploid Events diploid (AEd) as a threshold, and is only valid if euploid polyploidisation >4c, cytologically detectable virus infection, cytostatic and radiation therapy can be excluded.
200 cells were measured and 46 with a DNA-content > 3.44[c] were found.

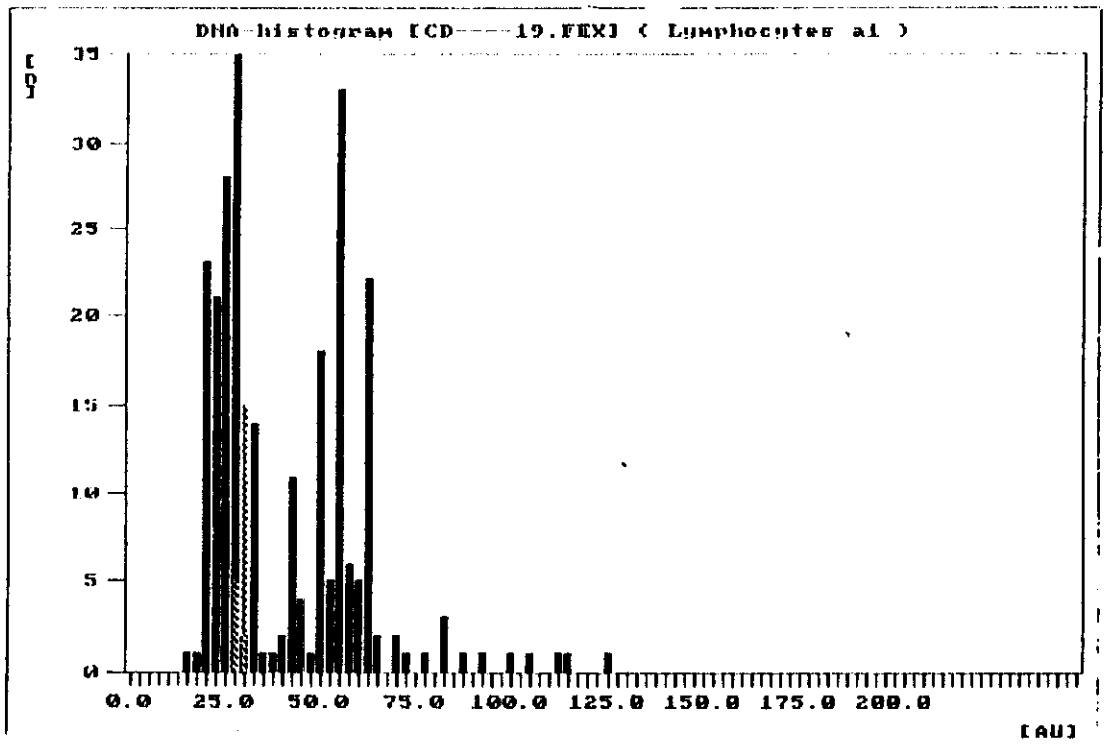
(C)

D N A - C Y T O M E T R Y

Cellcycle indices		
G0/1 fraction	[%]	49.60
S fraction	[%]	17.60
G2/M fraction	[%]	26.80
Proliferation fraction	[%]	56.40

DNA-INTERPRETATION: aneuploid.





Cytometric parameters:

Number of cells [n]:	250	2c-Deviation Index [c^2]:	2.45
1. Stemline ploidy [c]:	1.67	DNA index:	0.84
2. Stemline ploidy [c]:	3.80	DNA index:	1.90
Diploid Deviation Quotient:	2.83	Entropy of DNA:	4.20
5c Exceeding Events [n]:	11	Mean ploidy [c]:	2.82
Aneuploid Events d [n]:	104	Mean area [micron^2]:	37.94

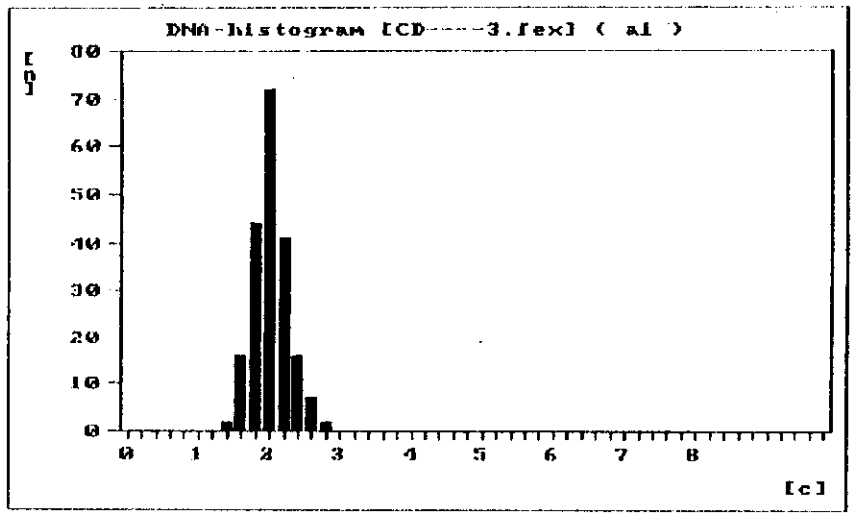
The statement on DNA-aneuploidy is based on the DNA-single-cell interpretation according to Kropff et al. 1991 using the Aneuploid Events diploid (AEd) as a threshold, and is only valid if euploid polyploidisation >4c, cytologically detectable virus infection, cytostatic and radiation therapy can be excluded.
250 cells were measured and 82 with a DNA-content > 3.67[c] were found.

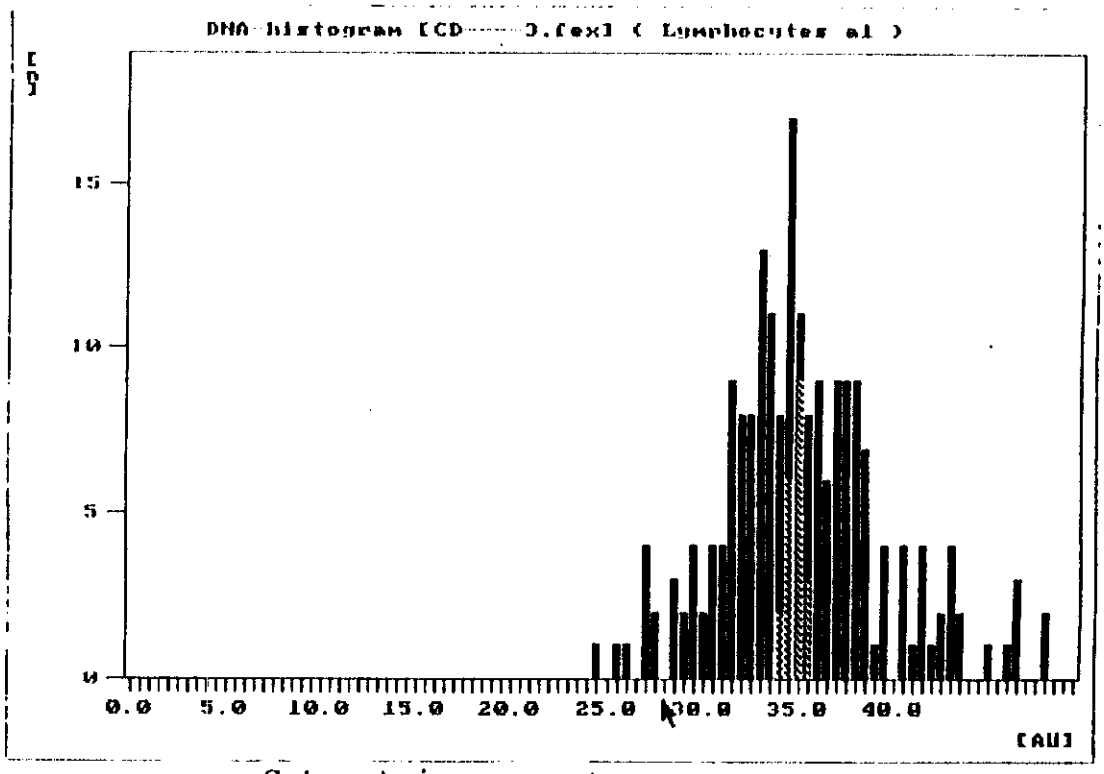
(D)

D N A - C Y T O M E T R Y

Cellcycle indices		
G0/1 fraction	[%]	96.50
S fraction	[%]	3.50
G2/M fraction	[%]	0.00
Proliferation fraction	[%]	19.50

DNA-INTERPRETATION: non aneuploid.





Cytometric parameters:

Number of cells [n]:	200	2c-Deviation Index [c^2]:	0.0(
Stemline ploidy [c]:	1.94	DNA index:	0.9
Diploid Deviation Quotient:	2.02	Entropy of DNA:	3.2
5c Exceeding Events [n]:	0	Meah ploidy [c]:	2.0
Aneuploid Events d [n]:	0	Mean area [micron^2]:	39.1

200 cells under analysis were measured at random.

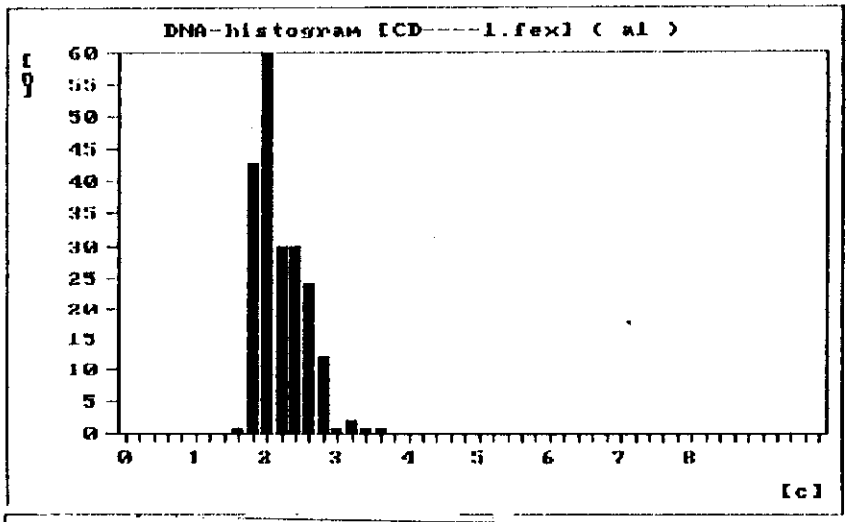
The statement on DNA-aneuploidy is based on the DNA-stemline interpretation according to Boecking et al., 1992 (p<0.001, Kolmogoroff-Smirnow test

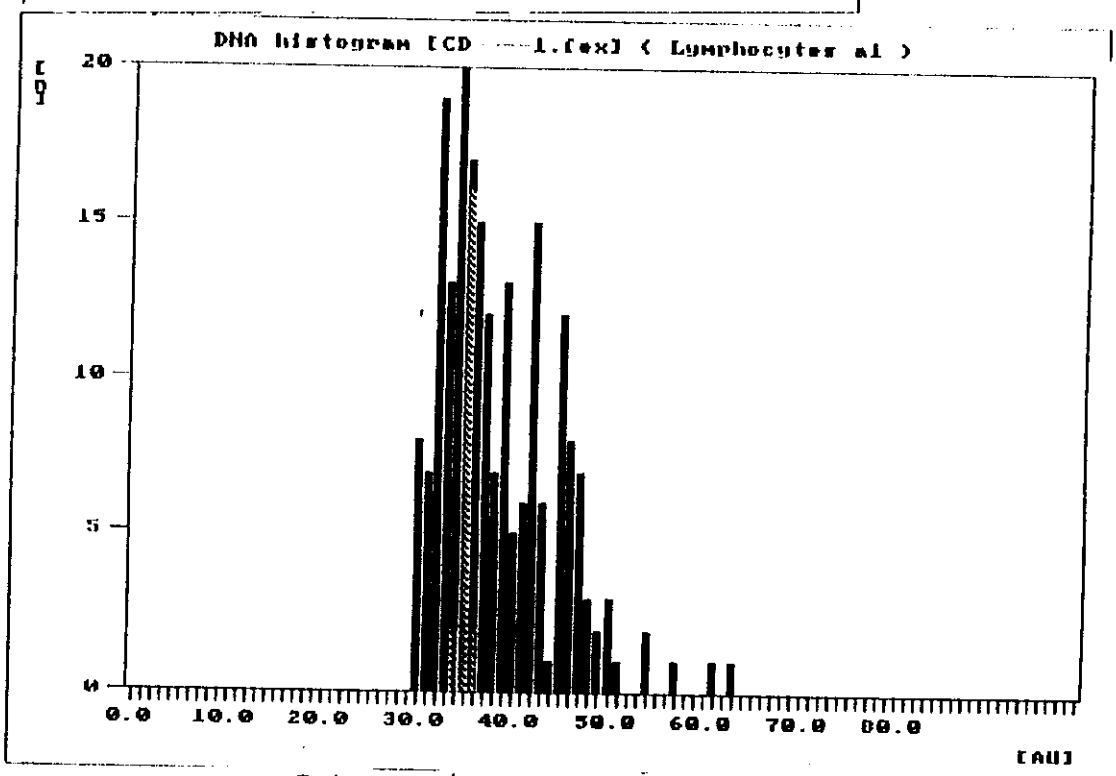
(E)

D N A - C Y T O M E T R Y

lcycle indices		
1 fraction	[%]	89.76
fraction	[%]	10.24
M fraction	[%]	0.49
liferation fraction	[%]	42.44

DNA-INTERPRETATION: non aneuploid.





Cytometric parameters:

mber of cells [n]:	205	2c-Deviation Index [c^2]:	0.16
emline ploidy [c]:	1.95	DNA index:	0.98
ploid Deviation Quotient:	2.19	Entropy of DNA:	3.56
Exceeding Events [n]:	0	Mean ploidy [c]:	2.19
euploid Events d [n]:	0	Mean area [micron^2]:	37.23

5 cells under analysis were measured at random.

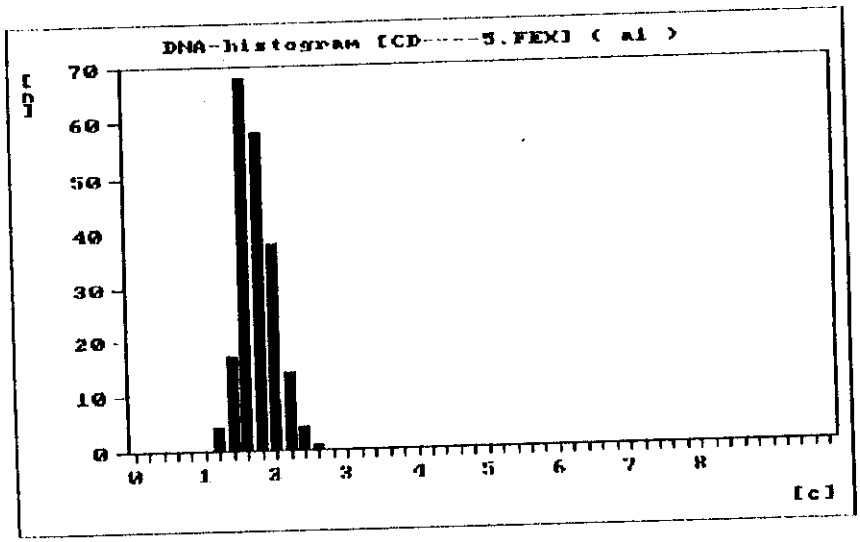
e statement on DNA-aneuploidy is based on the DNA-stemline interpreta-
on according to Boecking et al., 1992 (p<0.001, Kolmogoroff-Smirnow test)

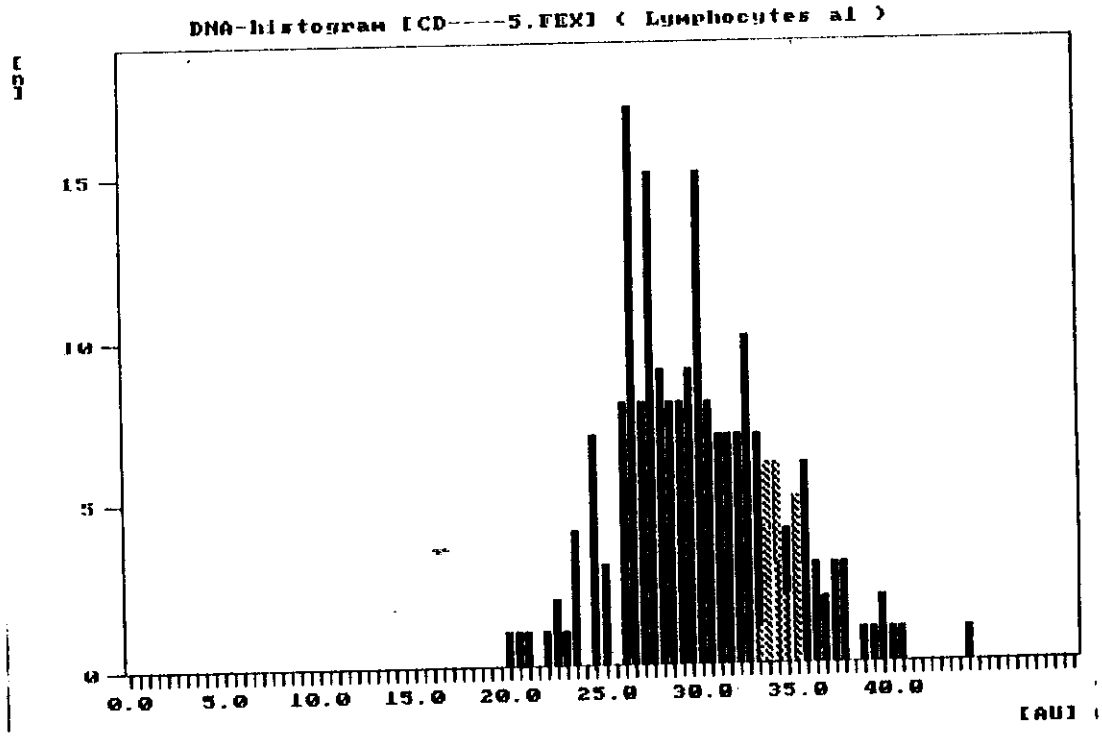
(F)

D N A - C Y T O M E T R Y

Cellcycle indices		
G0/1 fraction	[%]	98.04
S fraction	[%]	1.96
G2/M fraction	[%]	0.00
Proliferation fraction	[%]	39.22

DNA-INTERPRETATION: aneuploid.





Cytometric parameters:

Number of cells [n]:	204	2c-Deviation Index [c^2]:	0.11
Stemline ploidy [c]:	1.60	DNA index:	0.80
Diploid Deviation Quotient:	1.76	Entropy of DNA:	3.24
5c Exceeding Events [n]:	0	Mean ploidy [c]:	1.76
Aneuploid Events d [n]:	0	Mean area [micron^2]:	37.95

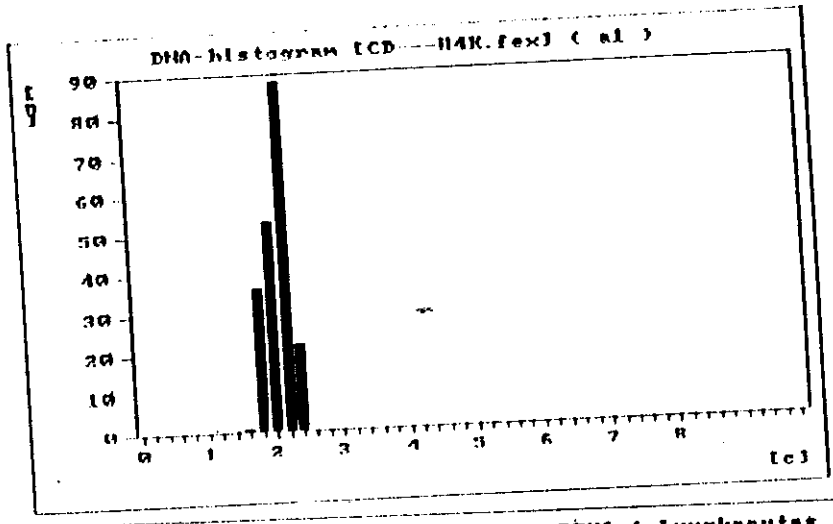
204 cells under analysis were measured at random.

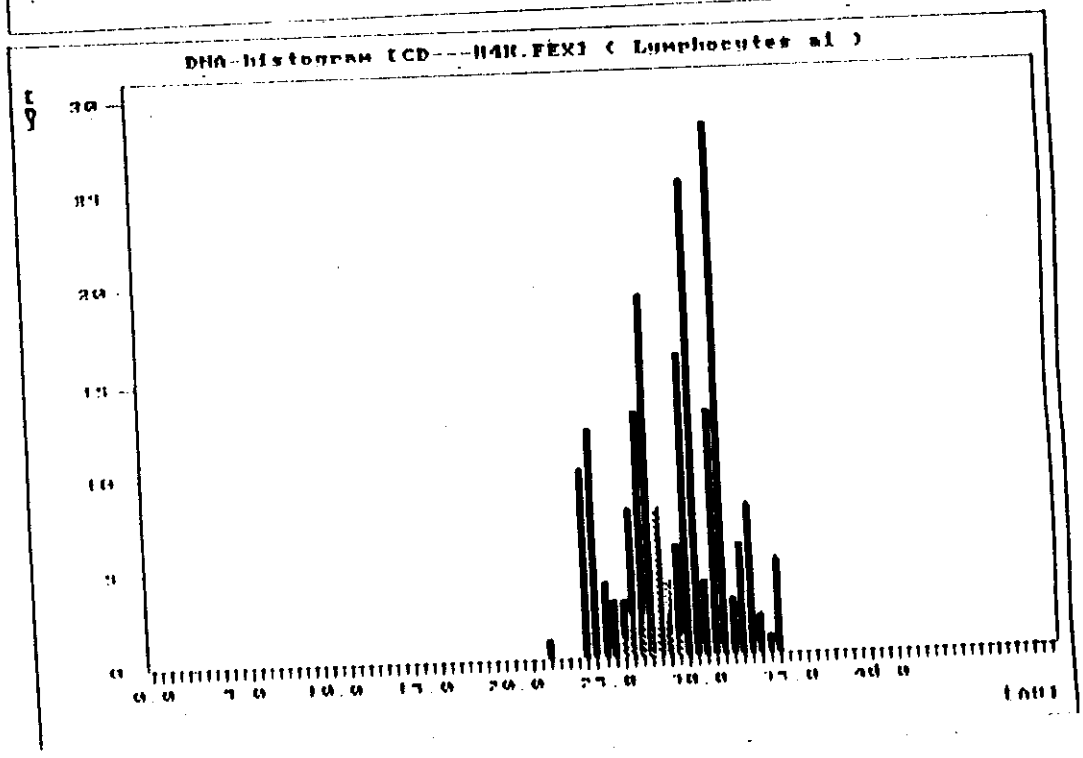
The statement on DNA-aneuploidy is based on the DNA-stemline interpretation according to Boecking et al., 1992 ($p<0.001$, Kolmogoroff-Smirnow test)

(G)

Cellcycle indices		
G0/1 fraction	[%]	97.00
S fraction	[%]	3.00
G2/M fraction	[%]	0.00
Proliferation fraction	[%]	4.00

DNA-INTERPRETATION: non aneuploid.





Cytometric parameters:

Number of cells [n]:	200	2c Deviation Index [c^2]:	0.04
Stemline ploidy [c]:	2.13	DNA Index:	1.06
Diploid Deviation Quotient:	2.10	Entropy of DNA:	3.00
5c Exceeding Events [n]:	0	Mean ploidy [c]:	2.08
Aneuploid Events d [n]:	0	Mean area [micron 2]:	32.29

200 cells under analysis were measured at random.

The statement on DNA-aneuploidy is based on the convetional DNA-stemline interpretation which annument aneuploidy if the stemline ploidy differs more than +- 10% from the median of the reference cells.

(H)

CHROMOSOMAL CHANGES

The percentages of chromosomal aberrations of our study are illustrated in tables (9&10). In the majority of cases, only one abnormality per nucleus was found, but nuclei with up to 8 abnormalities were observed. The number of abnormalities increased not only with period of treatment but also with increasing the dose level.

Chromosomal examination of the bone marrow cells of the control rats revealed approximately no chromosomal abnormalities. As illustrated in table 9 and 10, we noticed that the total aberrations were not significant after 10 days of treatment with the 1/8 LD_{50} and 1/4 LD_{50} of $CdCl_2$, where structural aberrations included only isocentric gaps, chromatid gaps centromeric attenuation and fragments. The percentage of the total chromosomal aberrations after treatment with 1/8 LD_{50} $CdCl_2$ for 10 days was (8.6 %), while it was increased to (12.6 %) in case of 1/4 LD_{50}.

Cadmium chloride induced a significant level of total damaged bone marrow cells, which included structural types of aberrations after 20 days of treatment. As shown in tables 9 and 10, the cells with structural chromosomal aberrations examined 20 days after treatment with 1/4 LD_{50} $CdCl_2$ were significantly increased over those treated with 1/8 LD_{50} for the same period of time. The total chromosomal aberration percentages of 1/8 LD_{50} treated group for 20 days was 25.9 % while that of the cells treated with 1/4 LD_{50} was 38%. The main structural aberrations recorded after 20 days were centromeric attenuation, acentric rings, beaded chromosomes, isocentric gaps, centric fusion, deletion, fragments and chromatid gaps.

From table 9 and 10 recorded data, we noticed increase in the number of aberrant cells in the groups treated for 30 days with 1/8 and 1/4 LD_{50}

than the previous mentioned groups. Also, there were significant increase of aberrant cells of the group treated with high dose was (66%) than those treated with the low dose which was also (50.7 %). All the structural aberrations present here similar to the others treated for 20 days.

In the present study the highest increase in chromosomal aberrations as shown in table 10 was recorded in the groups of rats treated with 1/4 LD_{50} for 45 days which presented equal (91.3 %). While the highest percentage of abnormalities of all the groups treated with 1/8 LD_{50} was (70.8 %) after 45 days of treatment (table 9). Both structural and numerical aberrations were observed in each of the two groups after 45 days of $CdCl_2$ administration. The chromosomal aberrations which observed in these groups were centromeric attenuation, rings, isocentric gaps, beaded forms and centric fusion, while chromatid type aberrations was deletion, fragments and chromatid gaps.

Our observations revealed presence of a slight partial recovery, which appeared as a decrease in the percentages of abnormalities due to chromosomal repairing after stopping Cd injection for 30 days. These observations were illustrated in table 9 and table 10 and showing that the percentage of chromosomal aberrations became (52 %) for the low dose while it was (68.6 %) for the high dose treated rats. In these groups all of the chromosomal aberrations mentioned in this study were observed.

In the following part we will speak about each type of chromosomal aberrations, which we noted in the present study:

Structural aberrations

Chromosomal aberrations

1- Centromeric Attenuation: In which the centromere starts to stretch and the two chomatids join together by very thin chromatid threads (Fig, 141) . The percentage of centromeric attenuation was higher as compared to other aberrations.

It was clear that, there was a highly significant difference in the percentage of cells with centromeric attenuation in treated animals according to the dose and time of treatment. The percentage of bone marrow cells with centromeric attenuation after treatment with $1/8LD_{50}$ for 10 days was (4%), while it was 7,3 % in case of $1/4LD_{50}$ (tables, 9, 10). After 20 days , the percentage of cells with centromeric attenuation was 9,3 % after treatment with $1/8LD_{50}$, also , it was 14.7 % after treatment with $1/4LD_{50}$ for the same period (tables, 9,10). This abnormality type was markedly increased in the rats injected with $1/8LD_{50}$ for 30 days. It represented 14 %, while it was 20% in the rats treated with $1/4LD_{50}$ for the same period of time. The maximum increase of the cells with centromeric attenuation had been observed after 45 days of treatment with the high dose. It was 24.7% while it was 18 % in others treated with the low dose ($1/8LD_{50}$) as illustrated in fig. (150).

2- Ring form : In which the two chromatids join together at the distal end and the chromosome appears as a ring during mitosis. A ring chromosome usually gives rise to daughter rings of equal size which are regularly distributed to each daughter nuclei .

As shown in tables (9& 10), the ring form chromosome was not apparent after 10 days of treatment. Twenty days of treatment with 1/8 and $1/4LD_{50}$ $CdCl_2$ revealed many cells have ring form chromosome, which was

1.3% and 2% respectively . After treatment with the low and high doses for 30 days, the percentage of metaphases with ring chromosomes was proportionally increased with the dose size, where it was 2.7 % and 3.3%. Following treatment with 1/8 LD_{50} for 45 days, the percentage of ring chromosome was 4.7 % while its maximal increase in the present study was observed in case of treatment with 1/4 LD_{50} for the same period of time and it was 5.3%, (tables 9& 10 and Fig, 145, 146 &153).

3- Chromosome gap : It is an achromic region in both chromatids the size of the gap is equal or smaller than the width of chromatid. The percentage of metaphases with chromosome gaps in bone marrow cells of animals treated with both the 1/8LD_{50} and the 1/4LD_{50} was the same 1.3% after treatment for 10 days. Treatment with these low and high doses for 20 days resulted in an obvious increase in the percentage of this aberration, which was (3.2 % and 4.7%) respectively.

The percentage of cells with chromosome gaps was 6.7 % in case of treatment with 1/8 LD_{50} for 30 days, while it was increased to 8% in case of treatment with 1/4LD_{50} for the same period of time. This type of aberrations was markedly increased after 45 days of treatment with 1/4LD_{50}. It was 11.7% while the percentage was 8.7 % in the case of treatment with 1/8LD_{50} the same period of time,(tables 9& 10 and Fig,142 & 150)

4- Discentric fusion : In which two chromosomes are attached together in the centromoes are attached together in the centromeric region .In telocentric chromosomes appears as metacentric chromosomes . In this case the total number of chromosomes appears lacking one chromosome, Fig (143). As shown in tables (9& 10), the centric fusion aberrations did not present in the first period (10 days) of treatment in each of 1/8 LD_{50} treated groups, but they found increased proportionally with the dose and duration

of treatment. The percentages of centric fusion chromosomes were (1.3%) after 20 days for 1/8 LD_{50} treated groups but (2.0%) for 1/4 LD_{50} dose. Also, after 30 days the percentage was (3.3%) for 1/8 LD_{50} dose but (4.7%) for 1/ LD_{50} dose. The maximum increase of this type of aberrations was after 45 days of treatment, where the highest percentage was (6.7 %) for 1/4 LD_{50} treated groups, while it was (4.7 %) for others treated with 1/8 LD_{50}. This type of aberrations was still markedly observed after 30 days of Cd-abstainance. It was (4%) for 1/8 LD_{50} treated rats, while it was (4.7%) for the high dose treated rats.

5- Beaded chromosomes : These chromosomes appeared shattered or segmented, having a peculiar appearance.The chromatic and achromatic segments are weakly attached together at the point of gaps. fig (141,).

As shown in table 9 and 10, the groups treated for to days with 1/8 and 1/4 LD_{50} had no beaded chromosomes. The groups treated with 1/4 LD_{50} showed a high percentages for beaded chromosomal aberrations at different periods of treatment and they were (1.3 %, 4 .0%, 4.7%, 3.3 %) for 20 ,30, 45 days and groups left for studying recovery, respectively . The low dose 1/8LD_{50} treatment was lower that high dose (1/4LD_{50}) and they were (2% , 4.7% , 6% , 4.7%) for 20 ,30,45 days and groups left for recovery , respectively (fig. 144).

Chromatid type Aberrations

1- Chromatid gap , It is an achromatic region in a single chromatid . The percentage of metaphases with chromatid gaps in cells of animals treated with both 1/8LD_{50} for 10 days was the same (2%).

Twenty days following administration of the rats with 1/8LD_{50} showed 4.7 % percentage of cells with chromatid gaps. This percentage increased to 5.3 % when applying the high dose. The percentage of this aberration

increased gradually to become (8% and 10 %) for the 1/8LD_{50} and 1/4LD_{50} respectively. The chromatid gaps was 14 % after 45 days of administration with 1/4LD_{50}. It was (12%) for the animals treated with the low dose for the same interval (Tables, 9 & 10 and Fig, 142, 147 & 150)

2- Fragments : These are single chromatids without evident centromeres arising as a result of structural chromosomal changes of breaks . As shown in tables (9&10), the percentage of cells with fragments was (1.3% and 2%) for the rats administered with 1/8LD_{50} and 1/4LD_{50} for 10 days, respectively. Twenty days of treatment with 1/8LD_{50} revealed an increase in the cells have this abnormal change 2.7 %, while it was 4% for the others treated with 1/8 LD_{50}. The percentage of cells with fragments (Fig ,) for animals treated with the low dose for 30 days was 6%, while it was 7.3 % for animals treated with both of the low and high doses, for 45 days. It was (8.7% and 10.7%) for the low and high doses respectively, as illustrated in tables (9 &10) and figures (149& 150).

3- Deletion : It represents the loss of the chromosomal material. The separated segment may remain as a fragment in the cell or may be attached to other chromosome (fig. 146, 148& 151). Deletion was firstly represented after 20 days of treatment where its percentage was (2% & 2.7) for the rats treated with the 1/8LD_{50} and 1/4LD_{50} respectively. This percentage, after treatment with 1/8 LD_{50} for 30 days was 4.7%, while it was 6% in case of animals treated with 1/4LD_{50}. The rats which had been treated for 45 days with 1/4LD_{50} showed the maximum increase of the percentage of the metaphases with deletion in the present study (8.7%), while, it was 6.7% for the animals treated with 1/8LD_{50} for the same treatment as illustrated in tables (9& 10).

Numerical aberrations:

The present results also revealed that the administration of both the used low and high doses of $CdCl_2$ caused only hypoploidy. This type of the numerical aberrations means decrease in the normal chromosomal number. Sometimes, this decrease was more than one chromosome. As illustrated in table (9 & 10), hypoploidy is represented only after 30 and 45 days of treatment. The percentage of cells with hypoploidy after treatment with $1/8LD_{50}$ for 30 days was 1.3%, while it was 2% in case of $1/4LD_{50}$. The more obvious hypoploidy alteration was observed, 45 days both of $1/8\ LD_{50}$ and $1/4LD_{50}$ of administration and the percentage of this aberration was (3.3% and 4%), respectively (fig. 152)

Later on, when the 45 days treated rats were left for 30 days post-$CdCl_2$ withdrawal, they have indicated an obvious degree of recovery and improvement regarding the structure of chromosomes of the bone marrow cells. This improvement appeared much better in rats treated with $1/8\ LD_{50}$ than those treated with $1/4LD_{50}$. This phenomenon was indicated from the data of the total aberrations for cells of rats treated with $1/8LD_{50}$ was 52 % after 30 days of the heavy metal withdrawal as illustrated in table (9). For the same internal, the percentage of the total aberrations was 68.6 % for animals treated with $1/4LD_{50}$ (table, 10).

All of the structural and numerical aberrations observed in the present study were represented in these recovery groups.

Plate (XXXI)- Materials prepared from the bone marrow for showing the chromosomal aberrations in the normal rats and others treated with LD_{50} and ¼LD_{50} and stained with Giemsa. (ISCN, 1985 X2000

Fig. 139- A photomicrograph of a normal metaphase chromosomal spread prepared from the bone marrow of a control normal rat.

Fig . 140- A photomicrograph of a metaphase chromosome spread with chromatid gap prepared from a rat treated with ¼ LD_{50} day after day for 10 days.

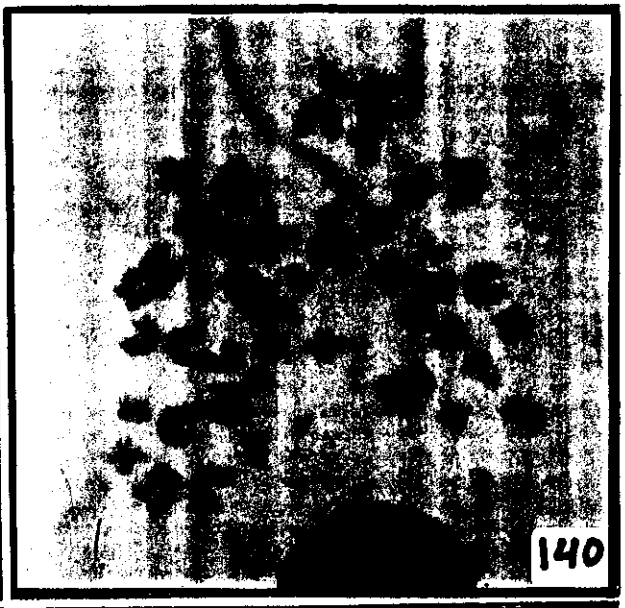
Fig 141- A photomicrograph of a metaphase chromosome spread with centromeric attenuation (arrows) prepared from a rat treated with $_{50}$ day after day for 10 days.

Fig 142- A photomicrograph of a metaphase chromosome spread with chromosomal and chromatid gaps (G), centromeric attenuation (arrows) and fragments (F) prepared from a rat treated day after day with ¼LD_{50} $CdCl_2$ for 20 days.

Fig 143- A photomicrograph of a metaphase chromosome spread with disenteric fusion (arrow) prepared from a rat treated day after day with $_{50}$ $CdCl_2$ for 30 days.

Fig 144- A photomicrograph of a metaphase chromosome spread with beaded chromosomes prepared from a rat treated day after day with ¼LD_{50} $CdCl_2$ for 30 days.


139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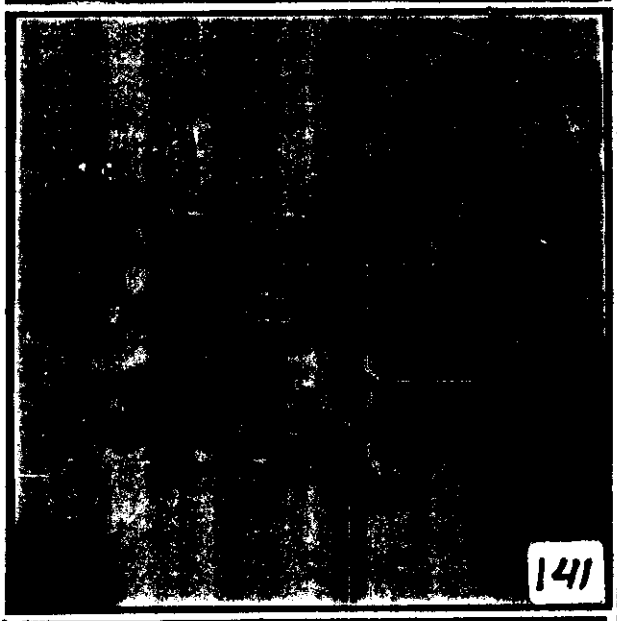
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Plate (XXXII)- Materials prepared from the bone marrow for showing the chromosomal aberrations in the male rats treated with ⅛ LD_{50} and ¼LD_{50} and stained with Giemsa. (ISCN, 1985) x2000

Fig 145- A photomicrograph of a metaphase chromosome spread with acentric ring (arrows) and chromatid gap (G) prepared from a rat treated day after day with ¼LD_{50} $CdCl_2$ for 30 days.

Fig 146- A photomicrograph of a metaphase chromosome spread with ring form chromosome (R), deletion (arrow) and fragments (F) prepared from a rat treated day after day with ¼LD_{50} $CdCl_2$ for 45 days.

Fig 147- A photomicrograph of a metaphase chromosome spread with chromatid gap (arrow) prepared from a rat treated day after day with $_{50}$ $CdCl_2$ for 20 days.

Fig 148- A photomicrograph of a metaphase chromosome spread with fragments (F) and deletion (arrows) prepared from a rat treated day after day with $_{50}$ $CdCl_2$ for 45 days.

Fig 149- A photomicrograph of a metaphase chromosome spread with fragments prepared from a rat treated day after day with ¼LD_{50}. $CdCl_2$ for 45 days.

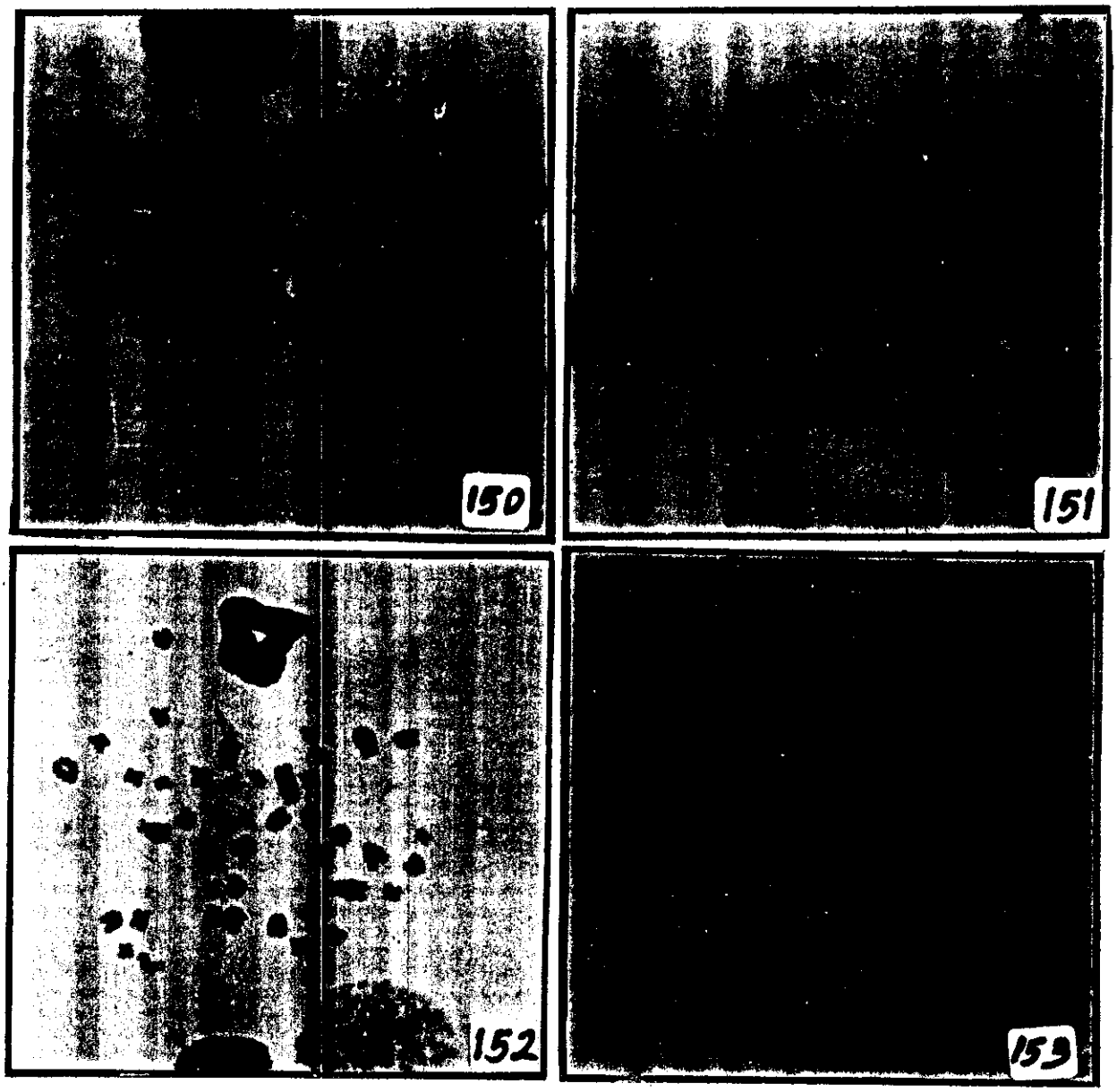
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Plate (XXXIII)- Materials prepared from the bone marrow for showing the chromosomal aberrations in the male rats treated with LD_{50} and ¼LD_{50} and stained with Giemsa. (ISCN, 1985) x2000

Fig 150- A photomicrograph of a metaphase chromosome spread with chromosomal and chromatidal gaps (arrows) and deletion (D) prepared from a rat treated day after day with $_{50}$ $CdCl_2$ for 45 days.

Fig 151- A photomicrograph of a metaphase chromosome spread with deletion (arrows) prepared from a rat treated day after day with ¼LD_{50}. $CdCl_2$ for 45 days

Fig 152- A photomicrograph of a metaphase chromosome spread with hypoploidy prepared from a rat treated day after day with $_{50}$ $CdCl_2$.

Fig 153- A photomicrograph of a metaphase chromosome spread acentric ring (arrow) prepared from a rat treated day after day with ¼LD_{50} $CdCl_2$ and left for 30 days for Cd withdrawal.