RESEARCH ON THE TEST OF HEPATIC FUNCTION WITH CHROMAZUROL S.

REPORT III. RELATIONSHIP BETWEEN HITOLOGICAL CHANGES CAUSED BY CARBON TETRACHLORIDE AND HEPATIC FUNCTION TESTS IN GOATS.

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In the previous report (II), the liver functions following the administration of CCl_4 were studied with various established liver tests as well as with the new CS dye retention test in goats. Considerable dissociation among the results of such tests was also pointed out. The study was extended further to elucidate the relationship between such functional changes and histological changes of the liver under these conditions. An attempt was also made to analyze the histology of various portions of the liver as studied by biopsy. Such results will be reported in the present communication.

METHODS

Under local anesthesia with procaine an incision of about 10 cm. was made along the right posterior costal margin and needle biopsy of the liver was performed on goats. Four specimens were taken at a time, each from the center and the edge of the right lobe and from the center and the edge of the left lobe (**Fig. 1**). The specimen was divided into two pieces, one half being fixed with Levi's solution and the other half fixed with Carnoy's solution. The former was stained (Azan's staining) for the study of fat droplets and mitochondria, the latter was used for methylgreen-pyronin and PAS staining to study RNA and glycogen, respectively. Salivary digestion test was also employed to verify the glycogen. The biopsy specimens were obtained at intervals of 1, 3, 5, 7, and 10 days after the administration of CCl_4 and were immediately fixed.

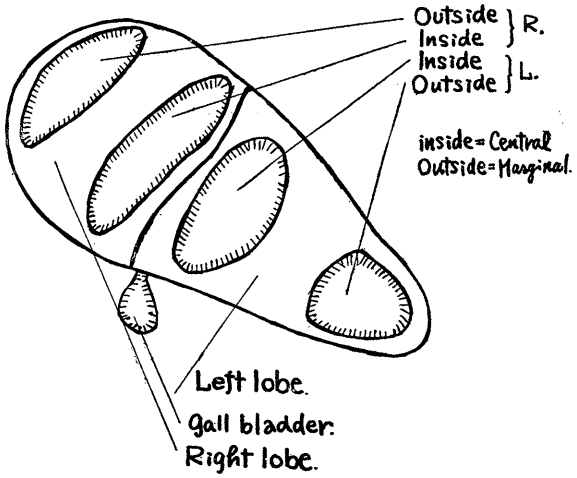


Fig. 1. The sites of biopsy on goat liver.

CYTO-HISTOLOGIC OBSERVATIONS

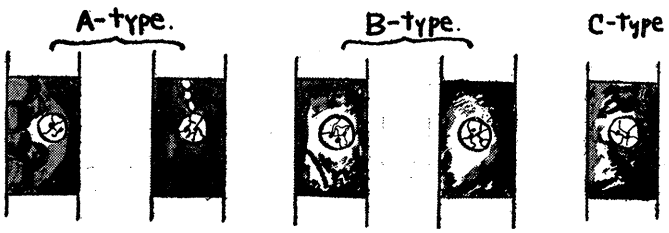
a) Distribution of RNA.

Normal distribution of RNA. RNA distribution under normal conditions may show one of the following patterns: Type A-RNA is densely distributed in one side of the cytoplasm along the cell membrane while the other side of the cytoplasm is coarse and clearer, sometimes containing vacuoles. Type B--RNA is densely distributed in the periphery of the cytoplasm as contrasted by a clear interior. Type C--The general appearance is similar to Type B but RNA is less dense. The predominance of RNA in one side in these types is probably due to a forcced movement of RNA during the fixing process. These three types were considered as the normal distribution (**Fig. 2**).

Abnormal distribution of RNA in damaged liver cells. Damaged cells bore one of the following four types (Fig. 2). Type I–The nucleus is lost and RNA is distributed densely along the membrane. The cell looks as though it embraces a large ball. The RNA content may be similar to that in normal cells, however. Type II–The nucleus is pressed against one side by the center of the cytoplasm which looks like a large vacuole. Cells of this type are readily distinguishable from Type A cells. Type III–Those with small scattered vacuoles. Type IV–Pyroninophile granules have almost disappeared in spite of otherwise normal appearing structure.

RNA distribution in the lobules following CCl_4 administration. RNA distribution in various parts of the lobule following the administration of CCl_4 is shown in Table 1. One day after the administration changes in RNA were minimal in the central zone regardless of the site of biopsy, whereas the changes were most marked

Fig. 2.
Normal distribution of R.N.A. in livere cell.



Abnormal distribution

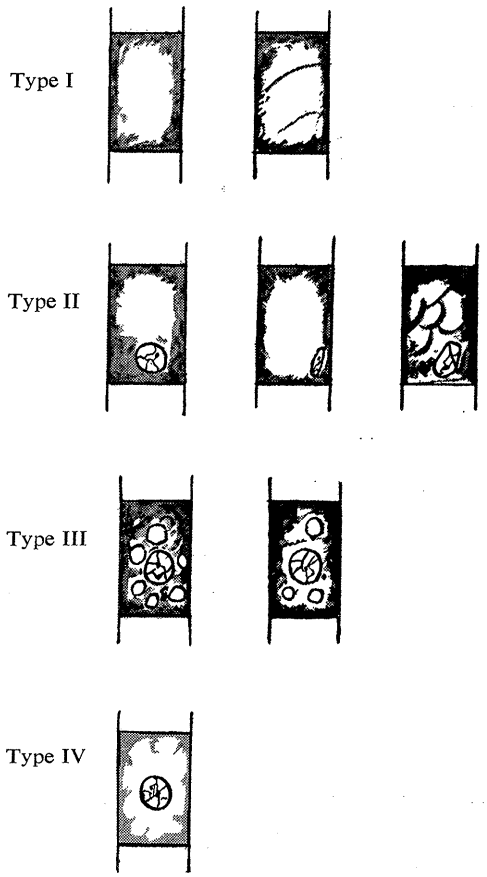


Table. 1.

Injection of CCl_4	Position of punch biopsy	Fatty Degeneration				Abnormal Distribution of R.N.A.				Abnomal Distribution of glycogen			
		Central zone of Lobule.	Middle zone of L.	Peripheral zone of L.	Numeral coefficient of degree.	Cent. Z.	Midd. Z.	Periph. Z.	Numeral coef. of degree.	Cent. Z.	Midd. Z.	Periph. Z.	Numeral Coef. of degree.
Before	R. Outside	−	−	−	0	−	−	−	0	−	−	±	1
	R. Inside	−	−	−	0	−	−	±	1	−	−	±	1
	L. Inside	−	−	−	0	−	−	±	1	−	−	±	1
	L. Outside	−	−	−	0	−	−	±	1	−	−	±	1
	Sammary of Co.	0	0	0	0	0	0	2	2	0	0	3	3
After 1 day.	R. O.	+	†††	††	9	±	†††	††	8	†††	±	††	8
	R. I.	±	†††	††	8	±	††	+	6	†††	+	††	9
	L. I.	±	†††	††	8	±	†††	†††	9	†††	±	+	7
	L. O.	±	†††	††	8	±	††	†††	8	††	−	+	5
	Sum. coeff.	5	16	12	33	4	14	13	31	15	4	10	29
After 3 days.	R. O.	±	†††	††	8	±	††	††	7	†††	−	+	6
	R. I.	+	†††	††	9	±	††	+	6	†††	±	+	7
	L. I.	+	†††	††	9	−	††	+	5	†††	†††	+	10
	L. O.	±	†††	††	8	−	+	+	4	††	±	±	5
	Sum. coeff.	6	16	12	34	2	11	9	22	15	6	7	28
After 5 days.	R. O.	+	††	±	6	+	††	+	7	+	−	−	2
	R. I.	+	+	−	4	††	†††	±	8	+	−	−	2
	L. I.	+	††	−	5	+	††	−	5	+	−	±	3
	L. O.	±	††	±	5	+	††	±	6	+	−	−	2
	Sum. coeff.	7	11	2	20	9	13	4	26	8	0	1	9
After 7 days.	R. O.	±	−	±	2	−	+	−	2	−	+	†††	6
	R. I.	−	††	−	3	−	+	±	3	−	±	††	4
	L. I.	±	††	−	4	−	±	−	1	−	−	±	1
	L. O.	−	−	±	1	±	+	±	4	±	±	+	4
	Sum. coeff.	2	6	2	10	1	7	2	10	1	4	10	15
After 10 days.	R. O.	−	−	−	0	−	±	±	2	−	−	−	0
	R. I.	−	−	±	1	±	−	−	1	−	±	††	4
	L. I.	−	−	±	1	±	±	−	2	−	±	†††	5
	L. O.	−	−	−	0	±	−	−	1	±	−	±	2
	Sum. coeff.	0	0	2	2	3	2	1	6	1	2	8	11

in the middle zone and relatively so in the peripheral zone. These changes were probably a result of the development of lipid droplets in these areas and the decrease in RNA could not be very rapid. The degree of degeneration in the four parts of the liver from which the specimens were taken was in the following order; the central portion of the lobe>left marginal portion=right marginal portion>right

central portion. The changes after 3 days were; right marginal portion>right central portion>left central portion>left marginal portion. The changes at 3 days differed from those at one day in that reappearance of RNA was marked in the left lobe but was slow in the right lobe. The changes after 5 days were; right central portion>right marginal portion=left marginal portion>left central portion. It is of interest to note that the relationship between the central and the marginal portion in terms of severity of changes was reversed if the changes at 5 days were compared with those at 3 days. Changes 7 days after the administration were; left marginal portion>right central portion>right marginal portion>left central portion. Normal cells were increased in each portion of the liver. Changes at 10 days; left central portion>right marginal portion>right central portion>left marginal portion. If one grades the degree of changes into 5 grades of 0, 1, 2, 3 and 4, the changes in the central, middle and peripheral zones of the lodule would be expressed as in **Fig. 3.** Namely, the RNA changes when added were 49 grades in the right lode and 46 grades in the left, no significant difference being observed. However, the changes in the marginal portion in both lobes were in an inverse relation with those in the central portion; when RNA in the former was decreased between the 3rd and the 7th day, RNA in the latter was incresed. These changes gradually disappeared and the cells returned to a near normal state in 10 days.

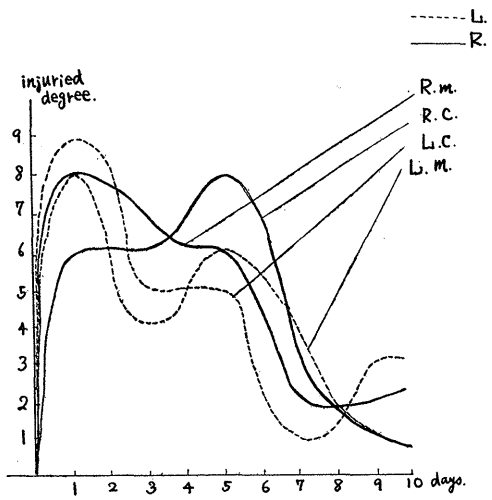


Fig. 3. Abnormal distribution of R.N.A. in 4 parts of goat's liver treated with CCl_4.

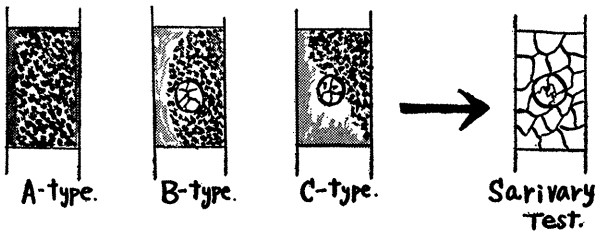
b). Distribution of glycogen

Patterns of glycogen distribution.

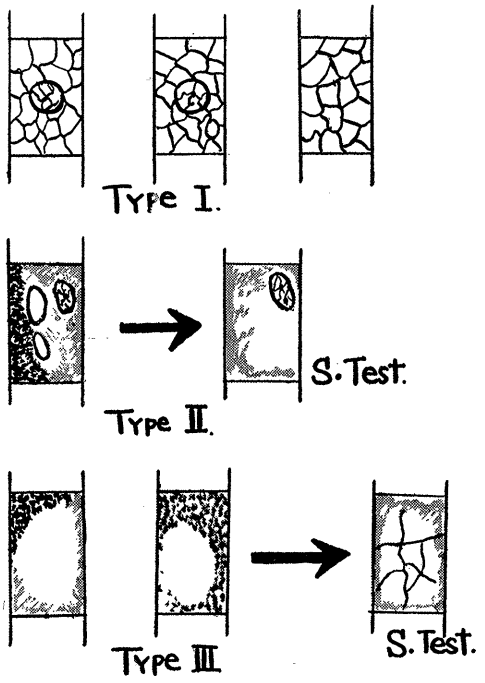
The PAS stained granules which disappeared completely by the salivary digestion

test were considered as glycogen, and its distribution was studied. Normal cells showed one of the patterns of the three types of A, B, and C as in the distribution of RNA. The glycogen contents in these cells were rather high. The distribution of glycogen along one side of the cell in Types of B and C may be due to sedimentation during the fixation and staining processes. The cells with abnormal contents of glycogen were classified into the following 3 types: Type I–Only reticular matrix is seen without glycogen and looks like normal cells after salivary digestion. Type II–The nucleus is in one side, contains vacuoles, and the glycogen granules are located in one side. Type III–The nucleus is lost and there is a large vacuole instead. Glycogen granules are pressed against the cell membrane.

Fig. 4.
Normal distribution of glycogen.



Abnormal distribution



Distribution of glycogen after the administration of CCl_4.

The distribution after 24 hours showed reduction of glycogen in the order of right center>right margin>left center>left margin, reduction being more pronounced in the right lobe. The reduction 3 days after the administration was; left center> right center>right margin>left margin, a considerable difference being seen between the center and the margin with less difference between the lodes. Distribution at 5 days; left center>right center=left margin=right margin. In general, the reduction of glycogen was small. At 7 days; right margin>right center=left margin>left center, the changes being generally greater than those after 5 days. At 10 days; right center=left center>right margin.

If the abnormality of glycogen distribution was graded 0, 1, 2, 3 and 4 as was in the case of RNA, the changes would be expressed as **Fig. 5:** the right lobe had 51 grade changes and the left lobe 44 grades, a noticeable difference being found between the two lobes. Abnormality was marked in the early stage of the damage in both the marginal and the central portion of the right lobe and the change thereafter was rather equal, whereas in the left lobe, there was a difference in the degree of abnormality between the two portions.

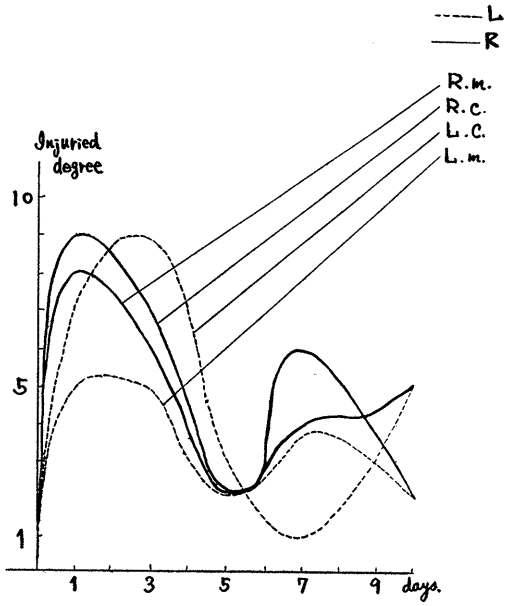


Fig. 5. Abnormal distribution of glycogen in 4 parts of goat's liver treated with CCl_4.

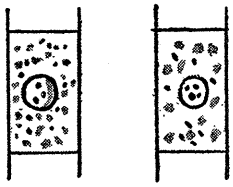
c). Alterations of fat content and mitochondria in liver cells.

The liver cells may be grouped into the same patterns as before with respect to fat and morphology.

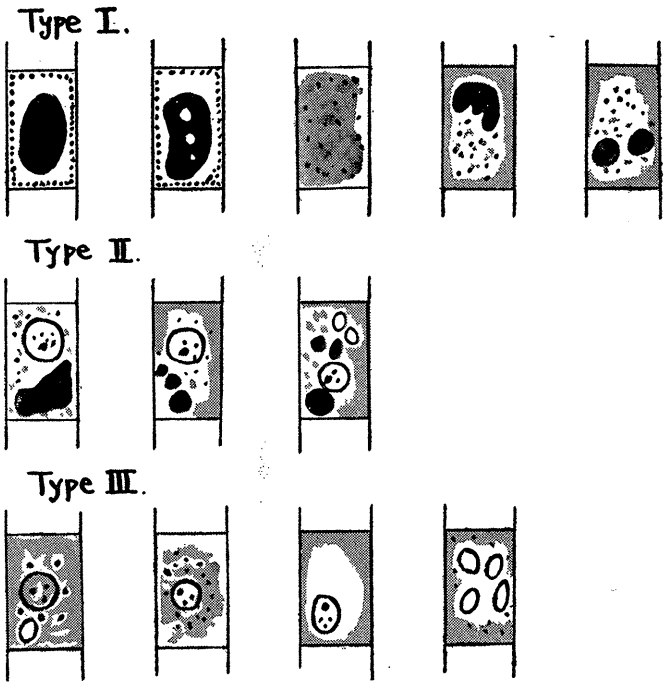
Histology before the CCl_4 administration.

No fatty degeneration or alteration of the mitochondria was found in any portion of the liver.

Fig. 6.
Fatty degeneration. Normal Liver cells.



Degenerative cells.



Histology 24 hours after CCl_4 administration.

Fatty metamorphosis was found in a patchy distribution in the periphery and in the area extending toward the middle zone of the lobule without reaching the center. In the periphery, the cells surrounding the interlobular space, particularly those surrounding blood vessels and bile ducts were swollen without advanced degeneration, neither was there any marked changes of the mitochondria. The cells situated toward the middle zone were more seriously involved, coarse fat droplets appearing in the ones near the middle zone. No uniform shape of fat droplets was seen. In some cells, the nucleus had disappeared and mitochondria distributed along the cell membrane. There was no difference severity of the changes among the four portions of the liver from which specimens were taken.

Histology 3 days after CCl_4 administration.

Fatty metamorphosis of severer degree was seen in a wider area, such an area radiating in a band-like distribution from the interlobular vessels. The swollen cells around the vessels were filled with fatty substance, mitochondria were shaped like drops and distributed along the cell membrane. No demonstrable difference in severity was seen among various liver portions.

Histology 5 days after CCl_4 administration.

Fatty changes in the periphery became less marked but some abnormal cells appeared in the central zone of the lobule. There was little difference among the various portions except that the left lobe margin had more definite alterations.

Histology 7 days after CCl_4 administration.

Histology was almost normal in general except for the presence of a few cells with fatty degeneration. There was a slight difference in the degree of fatty changes among the studied portions.

Histology 10 days after CCl_4 administration.

The histology was completely normal. **Figure 8.** represents the fatty changes in various parts of the liver in relation to time after CCl_4 administration. It will be clear that there was little difference at the beginning but significant variations in severity were seen among these portions thereafter. The histology was completely normalized after 10 days.

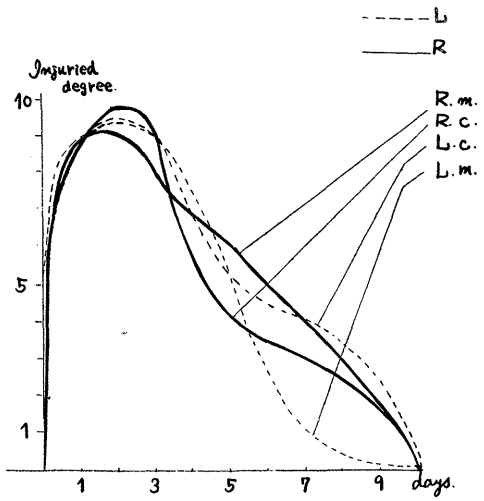


Fig. 7. Fatty degeneration in 4 parts of goat's liver treated with CCl_4.

DISCUSSION

While a large quantity of CCl_4 causes marked central necrosis in the lobule, a

small amount of CCl_4 such as used on goats in this study (0.5 ml. intramuscularly) causes only slight liver damage. CCl_4 carried via blood vessles into the liver tissue first affects perivascular liver cells in the interlobular space. The affected liver cells swell because of fat accumulation and press the capillaries. Thus, the initial alteration advances along the capillaries leading to the center of the lobule. Since the dose of CCl_4 used was small, such alteration barely reached the center as reflected by the demonstrated zone-wise distribution of fat. Should large quantities of CCl_4 have been given, central necrosis must have developed. Dynamic mechanism of these phenomenon was observed by Hosokawa and his coworkers with a vital transillumination method.

Alteration of RNA distribution was most marked 1 day after CCl_4 administration, gradually improved with some fluctuation thereafter and quickly normalized in 7 to 10 days. Abnormal glycogen distribution was marked during the lst and the 3rd day, recovering in 5 days, and aggravated again about the 7th day. The three histological criteria in their relation to time is shown in **Fig. 8.** A marked dissociation between RNA and glycogen may be noted in the figure between the 4th and the 6th day of CCl_4 administration.

The right and left lobes were compared with respect to severity of damage and the alterations were expressed by arbitrary grades. No significant difference was noted between the two lobes with respect to fatty degeneration and RNA distribution, but a significant difference was found in glycogen content, the right lobe showing 51 grade change and the left, 44. The fact that the glycogen alteration was less marked in the left lobe may be explained that the CCl_4 was administered intramuscularly, and more of it reached the right lobe. The changes in various portions of the liver seem to suggest an inverse correlation between RNA and glycogen,

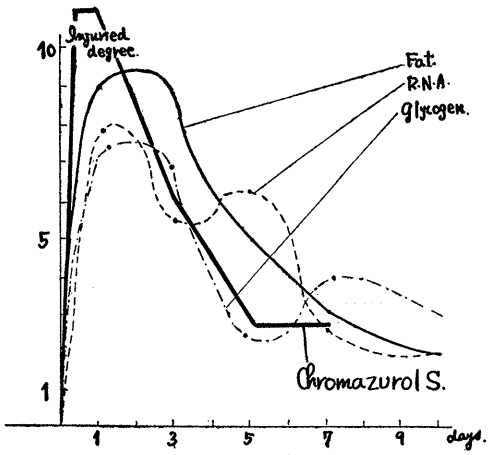


Fig. 8. Relation among fatty degeneration, and abnormal distribution of R.N.A. and glycogen in goat's liver treated with CCl_4

i. e., where glycogen alteration was marked, RNA alteration was not severe. It may be that if one component was affected seriously, the other component was affected to a lesser extent.

These findings were slighter than the results obtained in mice and rats by Hosokawa and his co-labolaters. However, the changes were very similar in both studies.

It is to be re-emphasized that the CS retention test showed a good correlation with fatty degeneration, abnormal distributions of RNA and glycogen during the first three days of CCl_4 administration. This correlation was not significant after 5 days.

Saito reported that the CS is excreted rapidly and in large quantities into bile of normal mice and rats, but its excretion is seriously disturbed after 1 day following CCl_4 administration and recovered in 6 to 8 days. This result was in close agreement with the above data obtained in goats.

SUMMARY

The relationship between liver functions and morphology of the liver based on needlelbiopsy was studied in goats following CCl_4 administration.

CONCLUSIONS

1) The chromazurol S retention test in blood was found to be closely correlated with liver morphology as studied by fat, RNA and glycogen staining during an early stage of CCl_4 intoxication.
2) Chromazurol S retention test is superior to other established tests except that the recovery of retention occurs rather early as judged from histological findings of the liver.
3) There is a good correlation between fructose tolerance and disappearance of glycogen from the central zone of the lobule.
4) The various tests for protein metabolism did not have any clear correlation with other function tests or histological distribution of RNA.
5) Bromsulphalein retention test reflected degenerative changes in peripheral zone of the lobule, but its return to normal occurred earlier than that of histology.

ACKNOWLEDGEMENT

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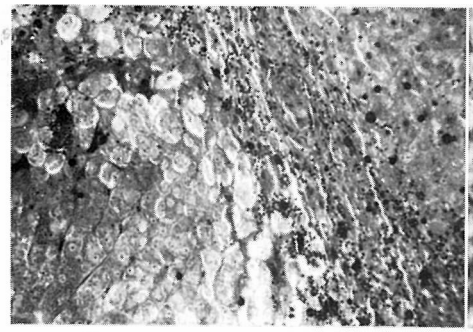
EXPLANATION OF PHOTO MICROGRAPHS

1. Azan's staining: Before CCl_4 administration, the liver cells at the central portion of right lobe.
2. After 1 day treated with CCl_4, at the right margin.
3. After 3 days, at the middle zone of right margin.
4. After 3 days, at the right margin.
5. After 5 days, at the left margin.
6. After. 5 days, at the middle zone of left margin.
7. After 7. days, at the right margin.
8. After 10 days, at the right central.
9. PAS staining: Before CCl_4 administration, at the left margin.
10. After 1 day, at the peripheral zone of the right margin.
11. After 3 days, at the left margin.
12. After 7 days, at the right margin.
13. After 7 days, at the right margin.
14. After 10 days, at the left central.
15. Methylgreen-pyronin reaction: After 1 day, at the left central.
16. After 5 days, at the right central.



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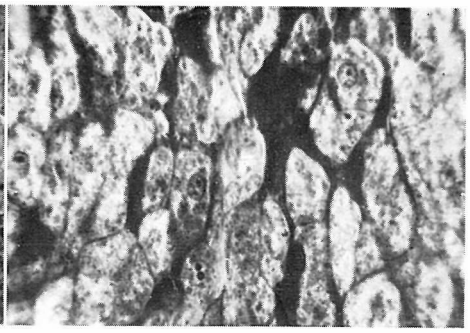

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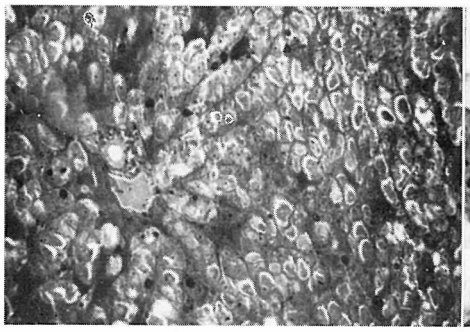

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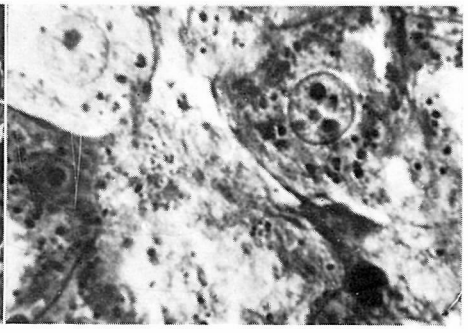

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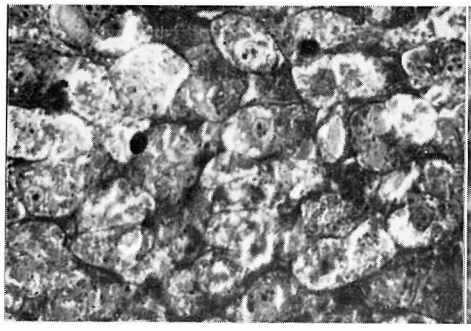

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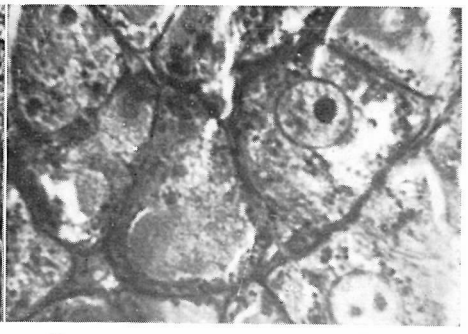

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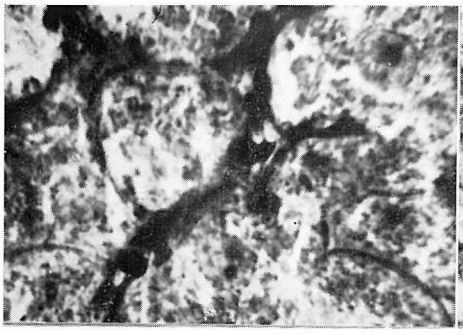

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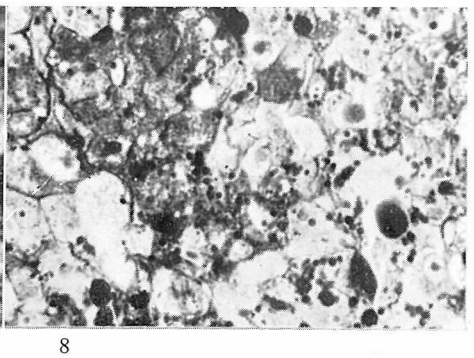

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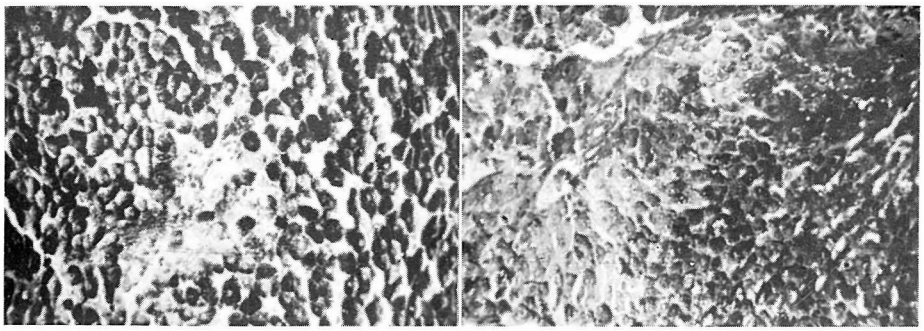

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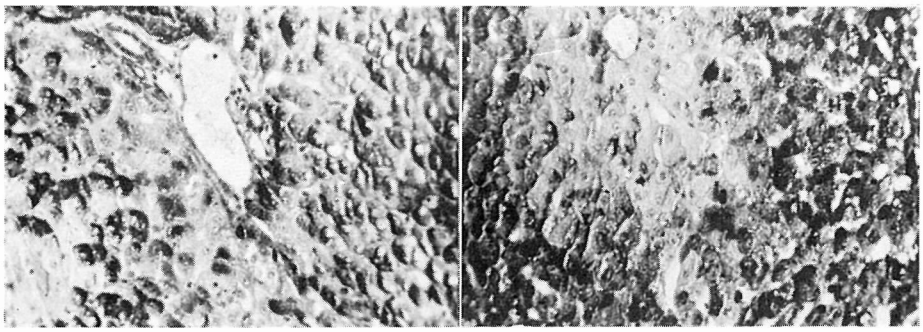

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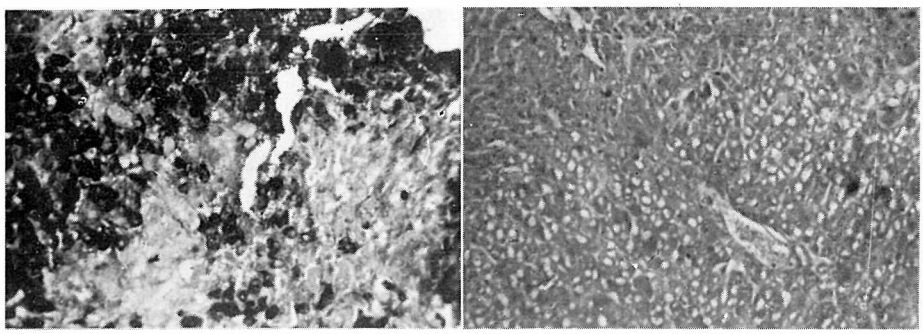

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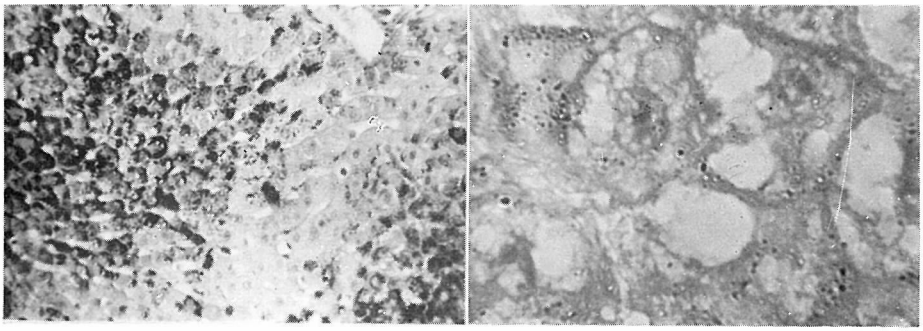

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