STUDIES ON THE REGENERATION OF BLOOD AND TISSUES

II. Histopathology of Liver, Kidney and Spleen in Acute Hemolytic Anæmia of Rats, Induced by Phenylhydrazine

By Mrs. V. V. Chiplunkar (née Miss L. S. Kale), A. S. Ramaswamy and M. Sirsi

(Pharmacology Laboratory, Indian Institute of Science, Bangalore-3)

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Abstract

Acute hemolytic anæmia of a severe degree induces biochemical alterations and pathological lesions in various organs. A detailed characterization of the hemolytic anæmia and the bone marrow changes induced by the maximum tolerating dose of phenylhydrazine in albino rats has in earlier studies shown close similarity to those occurring in other pathological conditions associated with liver injury.

The sequential course of the pathological lesions occurring in the liver, spleen and kidney during the acute phase and the recovery period after phenyl hydrazine anæmia has been reported in this communication.

An understanding of the degree and type of damage induced in the various organs is essential to understand the biochemical alterations and the pathogenesis of the acute hæmolytic syndrome. Besides the direct toxic effect of the chemical on the tissue cells, the other likely causes to be operative in producing the lesions are, the acute diminution of red blood cells and hæmoglobin resulting in anoxæmia and also the effect of the effete products of the red blood cells. These play a prominent role in the extent of damage caused and in the time of recovery.

The sequential course of the pathological lesions occurring in the liver, kidney, and spleen during the acute phase of phenylhydrazine anæmia and the recovery period is described in this note.

Experimental

The selection of animals and procedure evolved to produce acute hæmolytic anæmia associated with liver and other tissue injury has been described earlier.[1] Before and at varying intervals after phenylhydrazine (12 mg./kg. body weight) administration, rats were sacrificed and the required organs (Liver, Kidney and Spleen) taken out, fixed in Bouin's fluid, sections prepared by the standard paraffin embedding technique and stained with hæmotoxylin eosin. The following changes were observed at the stated intervals.

Macroscopically liver, spleen and kidney showed dark chocolate red colour as compared to that of normal. The enlargement of the tissues was observed. The tissue surface appeared slightly granular and friable.

A. *Liver*

(i) *Six hours.*—Cloudy swelling and signs of congestion in the parenchymal cells, and dilatation of sinusoids were observed.

(ii) *Eighteen hours.*—By this time, the cloudy swelling and congestion of liver parenchymal cells had become more marked and the stagnation of blood in the sinusoids was also noticed.

(iii) *Twenty-four to seventy-two hours.*—The changes were similar to those observed previously, but for the intensity. Sinusoids were depleted of blood after 36 hours and the deposition of pigment throughout the tissue was noted. There was no evidence of phagocytic activity. Degeneration and necrosis of the liver parenchymal cells were observed in scattered manner, but was more marked around the central vein (Fig. 1).

(iv) *Seven days.*—Vascular congestion in the parenchymal cells was almost absent at this time; the pigment deposition in the tissue had been increased; Extensive fatty degeneration of the tissue especially around the central vein was observed. The portal tract was normal and no signs of its degeneration were observed (Figs. 2, 3 and 4).

(v) *Ten to fourteen days.*—Scanty deposition of the pigment was noted. The fatty infiltration of the tissue was retrogressive after ten days, and the evidence of cellular regeneration was noted. The parenchymal tissue was showing almost 40% recovery (Fig. 5).

(vi) *Twenty-three to forty-two days.*—But for the scattered deposition of the pigment, the liver tissue was observed more or less normal by the end of forty-two days.

B. *Kidney*

(i) *Six hours.*—Kidney showed signs of vascular congestion throughout the cortex, and the cells were markedly hyaline.

(ii) *Eighteen hours.*—The vascular congestion had lessened by this time, but the early signs of glomerulonephritis were observed.

(iii) *Two to three days.*—Urinary tubules and Malpighian corpuscles showed signs of degeneration. However no evidence of hæmorrhage was noticed. Scanty deposition of pigment was also observed (Figs. 6 and 7).

(iv) *Seven days.*—By this time marked degeneration of the tubules was observed. Malpighian corpuscles showed early stage of glomerulonephritis. Deposition of pigment was increased extensively and had blocked the tubules completely (Fig. 8).

(v) *Fourteen days.*—Though some signs of past degeneration were present at this period, the general tendency of recovery regarding tubular damage, glomerular nephritis and the pigment deposits were noticed.

(vi) *Twenty-three to forty-two days.*—But for the scattered deposition of pigment after twenty-three days, the tissue seemed to be normal.

C. *Spleen*

(i) *Six to eighteen hours.*—Vascular congestion was markedly present in sinusoids.

(ii) *Two to three days.*—Congestion had cleared up to a great extent by this time, but deposition of pigment was observed. Though the lymph cells were observed to have been damaged in some places, hypertrophy of the splenic vessels was not seen.

(iii) *Seven days.*—The congestion was confined to the medulla only and the cortex was free. Deposition of pigment had become more widespread and extensive.

(iv) *Fourteen days.*—The congestion in the medulla had almost been cleared. The deposition of pigment was distributed along the perimalpighian areas only (Fig. 9).

(v) *Twenty-three to Forty-two days.*—The splenic tissue was observed to be almost normal (Fig. 10).

DISCUSSION

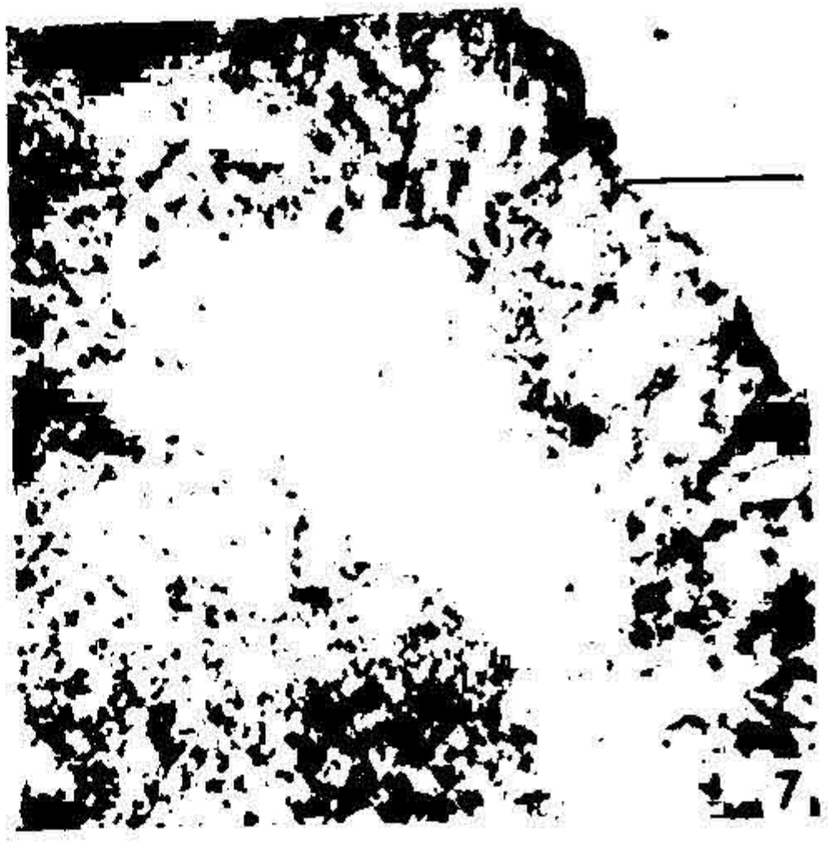
The histopathological studies reveal that the tissues, *viz.*, liver, spleen and kidney are affected to a great extent after the administration of phenylhydrazine, especially on the seventh day.

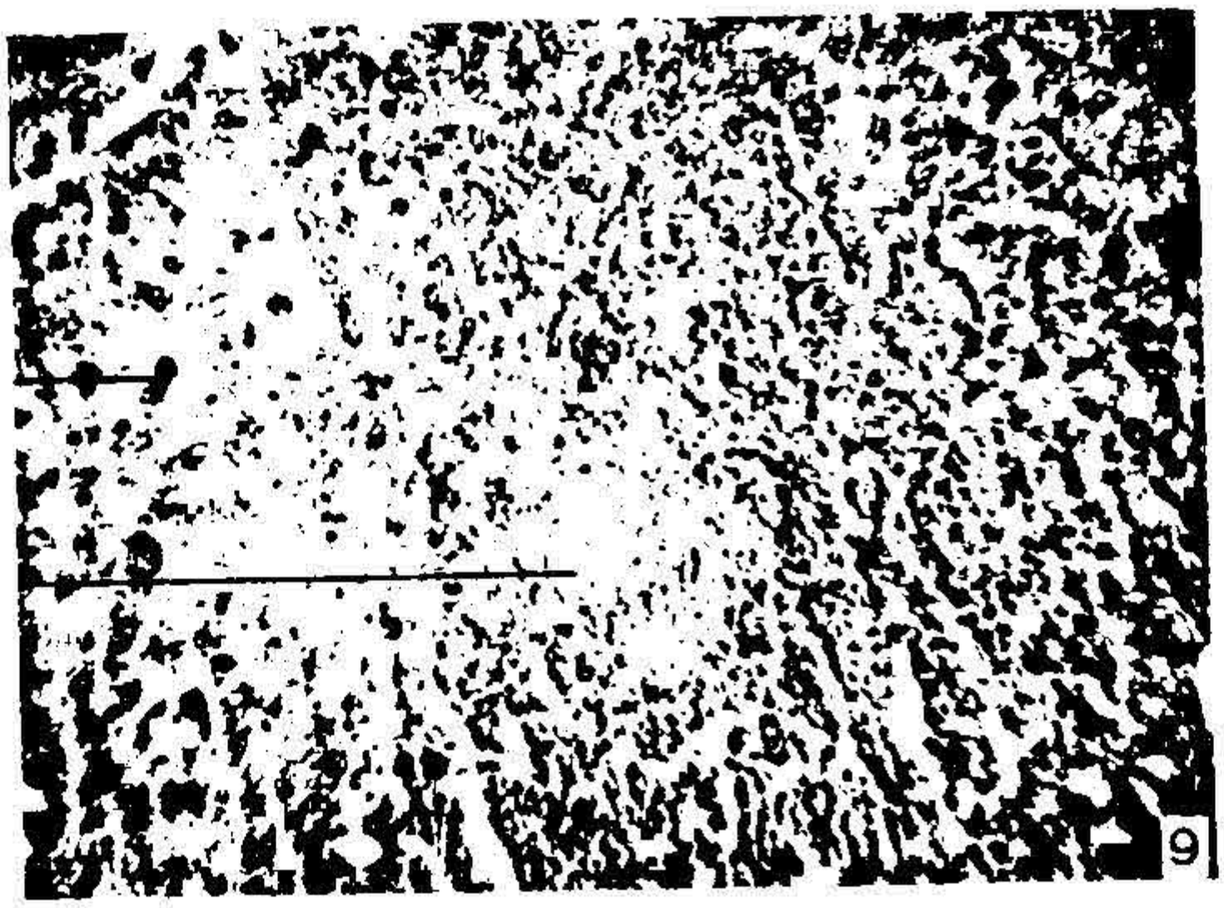
In the liver though on the seventh day the vascular congestion in the parenchymal cells was absent, overall extensive deposition of pigment and fatty infiltration extending to an area of about $\frac{1}{3}$ the lobule around the central vein were the main features of the lesion. No damage was noticed to the area surrounding the portal tract. Therefore, centrilobular zonal necrosis was a characteristic type of damage in the liver tissue.

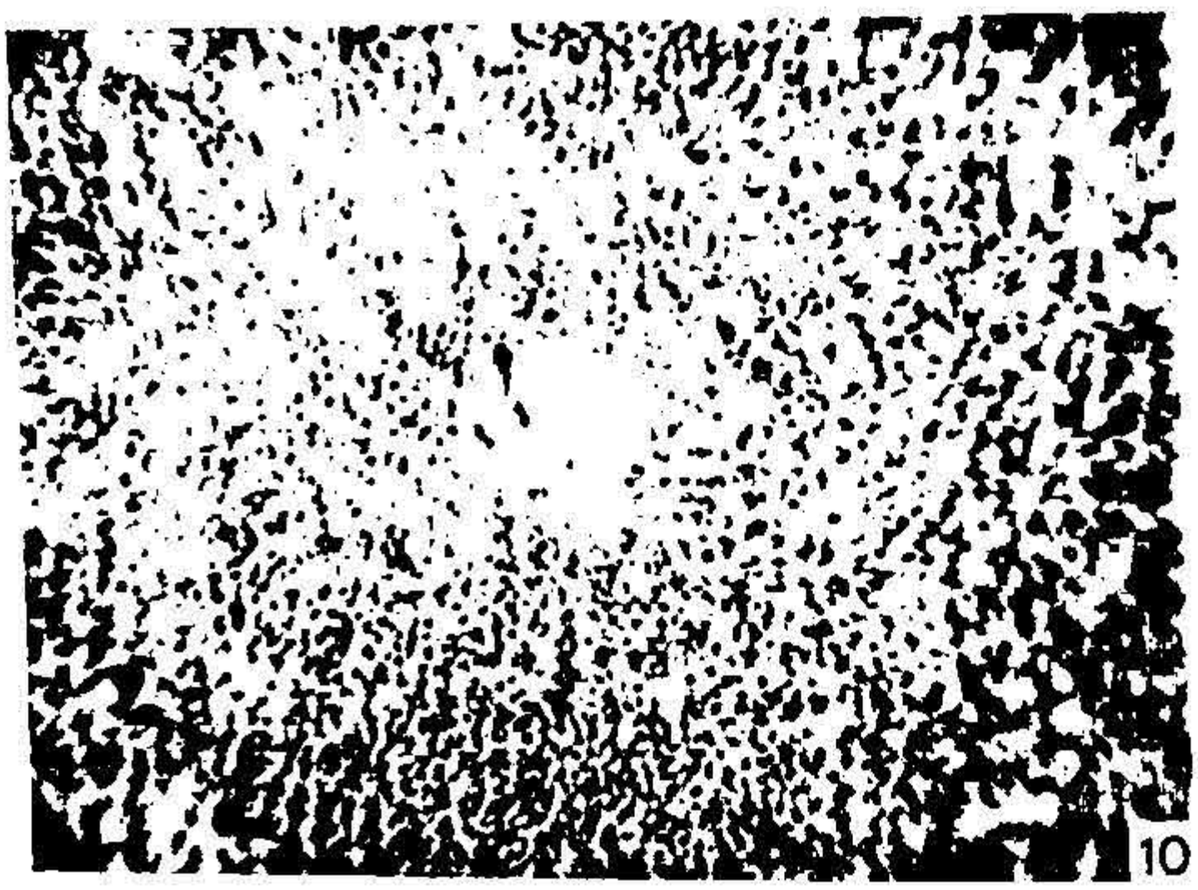
Damage to the liver tissue has been observed in man[2] as well as in animals,[3] after exposure to the atmospheres deficient in oxygen. A similar lesion has also been observed when the oxygen supply to the liver is diminished by circulatory failure.[4] Himsworth has concluded that the lack of oxygen, whether due to a deficient supply, as in exposure to low pressures of oxygen, or circulatory failure, or to the increased demands by the liver parenchyma leads to centrilobular necrosis of the liver. During the biochemical investigations low uptake of oxygen has been observed,[5] perhaps due to the intensive hemolysis which had occurred after the phenylhydrazine administration. Consequently besides hemolysis, anoxia might be also a contributory factor to the existing liver damage.

The necrosis might also be due to action of phenylhydrazine on the tissue cells, as reported by Bodansky.[6]

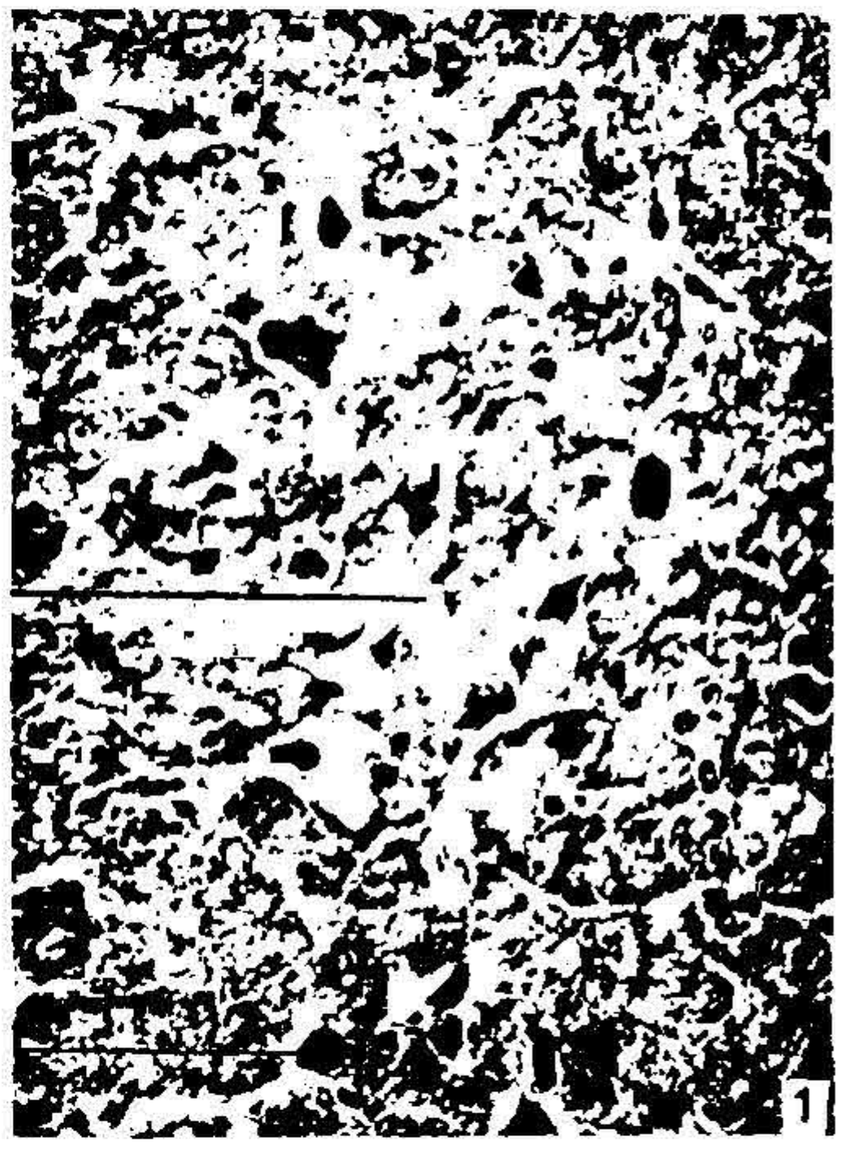
Biochemical investigations are confirming the histologically observed damage to the liver. There is maximum deposition of fat on the seventh day as determined

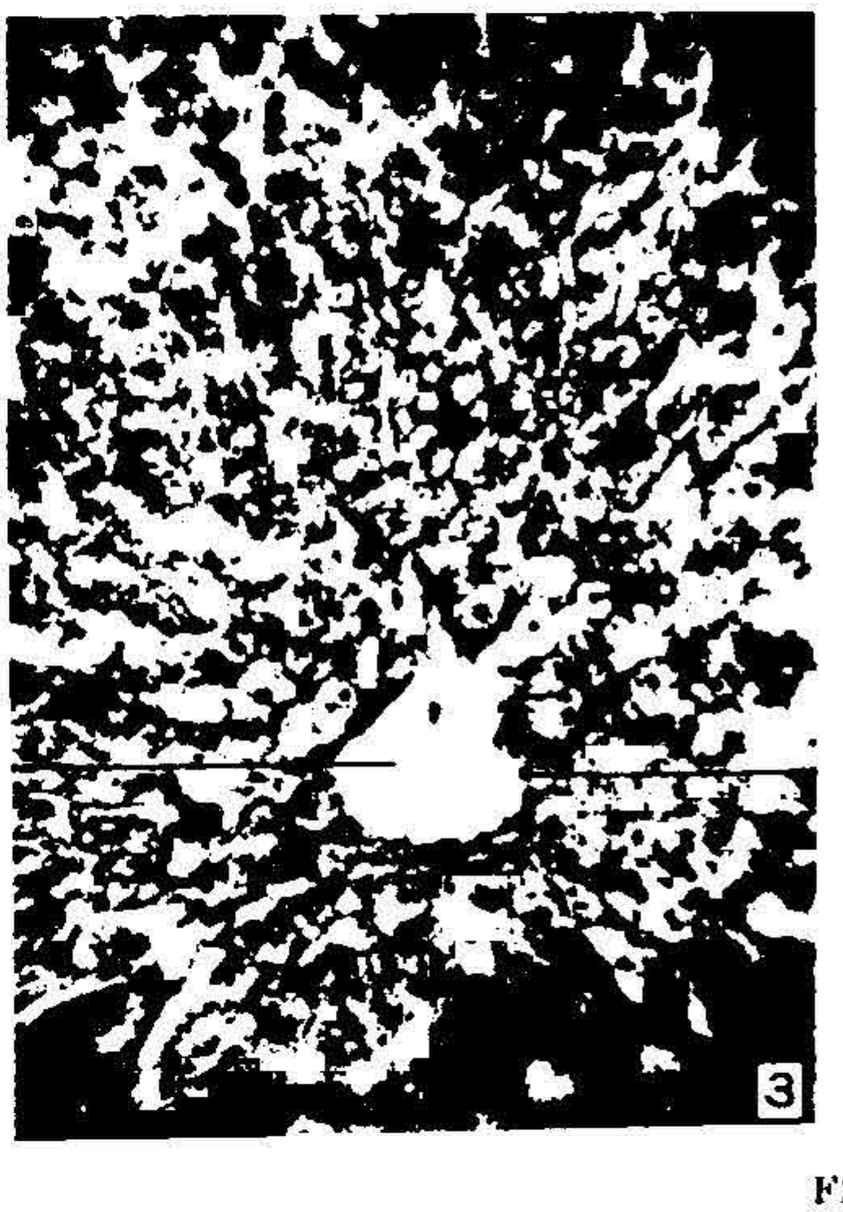


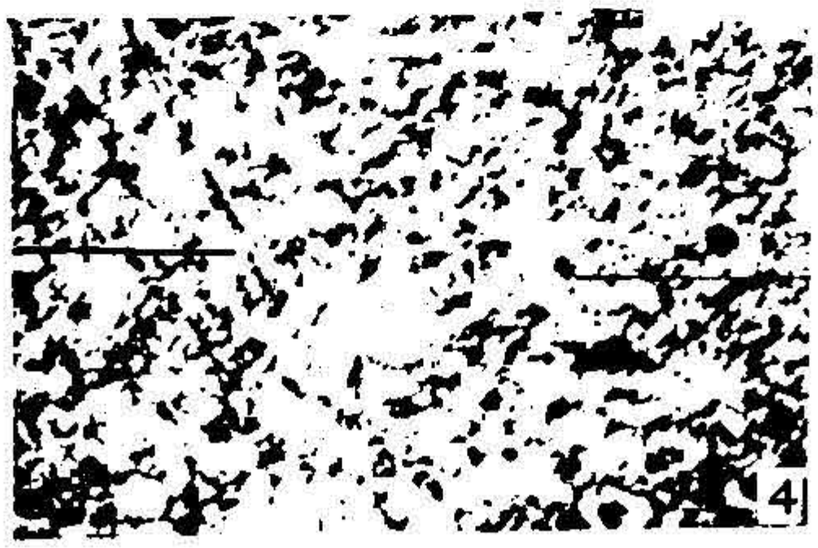




FIGS. 6–10







FIGS. 1–5

biochemically, which is in close agreement with histological findings. The evidence of the damage to the tissue on the seventh day is also proved by the other biochemical findings such as glycogen, blood sugar, nucleic acids, etc., since the maximum changes occurred in these constituents on the same day.[5] Renal and splenic lesions also parallel the changes that were observed in the case of the liver cited above.

In dogs after phenylhydrazine administration, Bodansky has observed extensive degeneration and necrotic and fatty changes in the liver and also fatty degeneration in the cortical portion of the kidney,[6] and atrophy of liver parenchymal cells has been reported by Allen.[7] Studies carried out after the phenylhydrazine administration in rats and presented in this paper are in close agreement with the observations in dogs reported previously.

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Explanation of Plates

Plate XV

Photomicrographs showing histopathological changes occurring after the administration of phenylhydrazine in rat (12 *mg.*/100 *g. body weight*)

[*Reduced to ¾ the original magnification*]

Fig. 1. Rat liver: 3 days. The dilatation of venous sinusoids and pigment deposits may be seen. The liver cells show early signs of fatty degeneration and the sinusoids contain hyaline material. H & E, × 500; P = Pigment; S = Sinusoids.

Fig. 2. Rat liver: 7 days. Fatty degeneration around the central vein is seen; ⅓ of the area around the portal tract is not affected. Sinusoids still show dilatation. H & E × 100; C.V. = Central Vein; P.T. = Portal Tract.

Fig. 3. Rat liver: 7 days. The fatty degeneration around the central vein and the fine granular nature of the lipoid material may be noted. Deposition of pigment is also marked. H & E, × 250; C.V. = Central Vein; P = Pigment.

Fig. 4. Rat liver: 7 days. Extensive fatty degeneration, pigment deposition and scanty cytoplasm in the liver cells are noticed. H & E, × 340; P = Pigment; F = Fatty degeneration.

Fig. 5. Rat liver: 14 days. Absence of fatty degeneration and pigment deposition. H & E, ×340.

PLATE XVI

FIG. 6. Rat kidney: 2 days. The tubular damage and scanty deposition of pigment can be noted. H & E, × 250.

FIG. 7. Rat kidney: 2 days. Showing the cortical congestion. H & E, × 250; C = Cortex of the kidney.

FIG. 8. Rat kidney: 7 days. Early signs of glomerulo-nephritis showing splitting of vascular tufts in renal tubules. The tubules are filled with hyaline material. H & E, × 250.

FIG. 9. Rat spleen: 14 days. The perimalphigian distribution of pigment may be noted. Splenic corpuscles are atrophied in some places. Follicular artery is slightly thickened. H & E, × 250; P = Pigment; F = Follicular artery.

FIG. 10. Rat spleen: 23 days. Tissue has returned to normalcy. H & E, × 250.