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HISTOPATHOLOGICAL AND HISTOCHEMICAL STUDIED ON THE EFFECT OF SODIUM WARFARIN ON THE MOUSE LIVER CELLS

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ABSTRACT

Sodium salt of warfarin ($C_{19}H_{15}O_4Na$) dissolved in distilled water, was administered orally to adult male albino mice through a stomach gauge. The animals were given single (LD_{50}, $1\ ^1/2\ LD_{50}$), or repeated ($1/10\ LD_{50}$ for ten consecutive days) doses.

Histopathological changes, glycogen, and total protein content in the liver tissues were investigated. The liver tissues were examined 72 hours post-treatment. In addition, some specimens of the first group (single LD_{50}) were examined 15 days post-treatment where signs of recovery were observed.

Histopathological lesions of hepatocytes were vacuolar degeneration, necrosis and karyolysis. These were accompanied by severe depletion in both glycogen and total protein content compared with the liver of controls.

The present study indicated that although the drug dose is an effective factor in both histopathological and histochemical changes, yet, warfarin has serious cumulative effect. However, the toxic effect of the drug may show signs of recovery after a while.

INTRODUCTION

As reviewed by Link (1944 and 1959), knowledge of the anti-vitamin K compounds began not with vitamin K but with hemorrhagic disease of cattle. This compound was

known by the trade name Dicoumarol^{R} . While the medical and possible rodenticidal uses of dicoumarol were explored, over 100 analogs of the compound were synthesized.

Warfarin, 3(α-acetonylbenzyl)-4-hydroxycoumarin is one of the most important coumarin compounds. It is a racemic mixture which has the empirical formula $C_{19}H_{16}O_4$ whether it is used as a rodenticide or as a drug. Almost all published toxicity effects are concerned with the mixture. However, West *et al.* (1961) were able to separate the isomers and to determine their absolute configuration.

According to data collected by the French Veterinary Toxicological Information Center, approximately 70% of the calls received in 1981 concerned dogs poisoned by anticoagulant rodenticides, especially warfarin (Lorgue *et al.*, 1985). The use of warfarin as a drug offers greater dosage and, hence, greater opportunity for side effects than pest control operators encounter (Hayes and Laws, 1991).

Bleeding is the most common complication of treatment; most of it is clinically insignificant because many cases, probably, remain unpublished (Pastor *et al.*, 1962). In addition to generalized bleeding, mainly due to deficiency of coagulation, evidence of capillary damage and of parenchymal injury of the liver was found in human cases (Pribilla, 1966).

Ninety-six hours after intraperitoneal injection of warfarin, the concentrations of the drug activity in the liver are 12 times greater than that in the blood (Link *et al.*, 1965).

Kronick *et al.* (1974) studied the effect of sodium warfarin administration during pregnancy in mice. They reported fetal death and congenital malformations following oral anticoagulant therapy during pregnancy. The results of their experiments support

the current clinical practice of avoiding oral anticoagulant during the first trimester of pregnancy.

There is great individual variation in the binding of warfarin by the serum proteins of laboratory rats. The rate of excretion shows a strong positive correlation with the concentration of free drug in the plasma (Yacobi and Levy, 1975).

Histological alteration of the liver tissues due to treatment with coumarin compounds are frequently reported (Hazelton, *et al.*, 1956; Patyza *et al.*, 1965; Feuer, *et al.*, 1966; Hagen *et al.*, 1967; Nievel, 1969; Grasso, *et al.*, 1974, and Ashry *et al.*, 1986).

El-Banhawy *et al.* (1993 a&b) dealt with the histopathological and histochemical effect of the anticoagulant rodenticide "Brodifacoum" on the liver of rat. The authors stated that the magnitude of the lesions on the liver tissue is exponential with both the applied dose and the lapse of time of its application.

The aim of the present study is to evaluate the effect of one of the widely used anticoagulants, warfarin, on the histology and histochemistry of the liver. The role of the liver in metabolic conversion and its susceptibility to chemical injury (Zimmerman and Ishak, 1979) are the main reasons for carrying this work. Extrapolations of the findings to man may help in assessing the potential hazards of this widely used anticoagulant. In the present work, sodium warfarin, which is freely dissolved in water, is used in order to avoid any hazardous effect of using the racemic mixture of warfarin which is practically insoluble in water but readily soluble in acetone and dioxane.

MATERIAL AND METHODS

Fifty adult male albino mice, *Mus musculus*, of an approximate weight 20-22 gm were used in the present investigation. The animals were kept under natural light conditions and ambient temperature (20^{o} - 22^{o}C) during the experimentation period. Food and water were given *ad libitum*.

In the present work The sodium salt of warfarin, a coumarin compound, (Coumadin^{R}, $C_{19}H_{15}O_4Na$) was used because it is fully soluble in water. The drug was administered orally in various doses. The acute oral LD_{50} of warfarin has been determined to be 62-102 mg/kg for male rats (Pyorala, 1968) . However, preliminary tests were made to verify the level of lethality of sodium warfarin in male mice and indicated that the acute oral LD50 is about 40 mg/kg body weight.

Animals were divided into 5 groups, ten mice each. The first group served as a normal control. Group II & III received an oral single dose of 40 mg/kg (LD_{50}). Group IV received an oral daily dose of 4 mg/kg (0.1 LD_{50}). Group V received an oral single dose of 60 mg/kg (1.5 LD_{50}).

The animals of all experimental groups were sacrificed 72 hours post treatment except those of group III which were left for 15 days post treatment for possible recovery after which they were sacrificed.

For histological and histochemical studies the animals were immediately dissected and the liver was carefully removed. Small pieces of the liver were fixed in alcoholic Bouin, Carnoy , 10% neutral formalin or formol saline solutions. Dehydration, clearing and embedding were carried out as usual. Paraffin sections of 6 μm thickness were stained with different staining techniques. Ehrlich's haematoxylin counter stained in eosin was applied for general histology, periodic acid Schiff's (PAS) reagent (Pearse,

1968) for polysaccharide materials, alcian blue-PAS method (Mowry, 1956) for acid and neutral mucins, Best's carmine (Best, 1906) for glycogen, and mercury-bromophenol blue (Mazia *et al.*, 1953) for total proteins.

For more accurate histopathological examination with the light microscope, small pieces of liver were fixed in a 0.1 phosphate buffer at pH 7.4, postfixed in 2% OsO_4 solution in a 0.1 phosphate buffer (pH 7.4). Following dehydration, tissues were embedded in Epon and thin (1 μm) sections were cut with an ultra-microtome and stained with toluidine blue.

RESULTS

Group (1): Normal control mice:

The liver lobules of mice are not well-defined, nevertheless, they appear roughly polygonal. The portal areas, or triads, lying outside the lobules comprise each, a branch of the portal vein, a branch of hepatic artery and an interlobular bile ductule, lying in interlobular connective tissue (Fig. 1). The portal areas are so arranged as to delineate lobules of liver tissue. The interlobular tissue is made up of radiating plates of hepatocytes forming a network around a central vein. The hepatic plates alternate with blood sinusoids that converge towards the central vein (Fig. 1).

The hepatocyte is polyhedral in shape and possesses a homogeneous cytoplasm and a centrally located, spherical nucleus with a distinct nuclear envelope and one or more prominent nucleoli, occasionally together with a number of chromatin granules. According to the distribution of the chromatin particles, two different shapes of nuclei could be detected; a condensed type with relatively numerous chromatin particles distributed in the nucleoplasm, and an open face type with comparatively less peripheral chromatin particles (Fig. 2). Some hepatic cells are binucleated, each of the

two nuclei is of approximately normal size. Small clear areas are scattered throughout the cytoplasm representing areas of glycogen (Figs 1&2).

The hepatic sinusoids make up the intralobular system of blood capillaries which course centripetally through the lobule and convey the blood from the interlobular branches of the portal vein and the hepatic artery to the central vein. They have relatively wide lumina, they anastomose irregularly and separate the hepatic plates one from another (Figs 1&2).

The endothelial lining of the sinusoids is composed of flattened or spindle-shaped cells which project to some extent into the sinusoidal lumen, they contain elongated or rod-shaped dark nuclei surrounded by narrow and greatly attenuated cytoplasmic areas. Between the sinusoidal lining cells and the hepatic cells is a continuous lucent perisinusoidal space "Disse's space" of variable dimensions (Fig.2).

The Kupffer cells are larger in size than the endothelial cells. They are cytoplasm-rich polymorphic cells with indefinite, faintly stained, oval or spindle-shaped nuclei. The bulky bodies of these cells bulge into the sinusoidal lumen. Some of them are found across a sinusoid as if to bridge the opposite walls of the sinusoid by fine cytoplasmic processes (Fig. 2). Besides, numerous lymphocytes could be identified in the sinusoidal lumina (Figs 1&2).

The PAS preparations of the normal liver tissues have revealed the existence of an obvious reactivity for polysaccharides, localized principally in the ground cytoplasm. However, the PAS reactivity is relatively more pronounced in the central cells (Fig.3). All such carbohydrate inclusions have been proved to be glycogen as indicated by Best's carmine staining (Fig.4). However, cells of the same specimen exhibit different intensities with PAS reaction, this may be due to the individual variation in their

polysaccharide contents. The lining epithhelial cells of the bile ductules and the nuclei of the hepatocytes exhibit a negative PAS reaction (Fig. 3).

By the application of alcian blue-PAS technique, only a pink colouration is observed in the liver tissue, but no alcian blue stainability is detected. Such pink-colored inclusions keep the same configuration which is detected previously with PAS reagent (Fig.5).

The normal hepatocytes react positively with bromophenol blue. Cell membranes, cytoplasm, and nuclei are stained darkly blue. Kupffer cells, as well as the endothelial lining of the sinusoids are faintly stained with bromophenol blue (Fig. 6).

Group (II): Treatment with a single LD_{50} (40 mg/kg).

72 hours following the treatment with a single dose LD_{50} of sodium warfarin a marked histological alteration in the different constituents of the liver is noticed. In general, there is an impairment of the normal architecture of the hepatic lobules. Some areas of the liver tissue display apparent signs of degenerative changes, while other areas are slightly affected (Fig. 7).

The hepatocytes are characterized by the vacuolated cytoplasm with an obvious inflammatory lymphocytic infiltration with occasional presence of eosinophils. Moreover, some hepatocytes, suffering from cloudy swelling, are detected. Such histological alterations are markedly seen in the peripheral zones of the liver tissue (Figs 7&8).

As regards the nuclei, some of them exhibit clear signs of intense nuclear shrinkage "pyknosis",whereas, some others undergo moderate signs of karyorhexis, in which the nucleus breaks into fragments (Figs 7&8).

Portal veins are distended, these are engorged with stagnant blood indicating signs of hemorrhage.

Histochemical studies showed a marked depletion of glycogen in the liver cells as they exhibit a weaker PAS reactivity compared with the normal control; glycogen content is detected as faintly stained inclusions in the ground cytoplasm. However, the peripheral hepatocytes show more diminution of glycogen than the centrally located ones (Fig. 9).

A slight reduction in the total protein content is indicated by the weak reaction with bromophenol blue. The depletion of the protein content is principally observed in the cytoplasm of the hepatic cells, most of the vacuolar areas react negatively with bromophenol blue. Moreover, the cell membranes reveal a noticeable reduction of the protein content comparable with the normal. However, Kupffer cells exhibit strong reactivity with bromophenol blue stain (Fig. 10).

Group (III): Treatment with a single LD_{50} (40 mg/kg), 15 days post-treatment.

After 15 days post-treatment with single LD_{50} (40 mg/kg) of sodium warfarin, the normal architecture of the liver tissue is, more or less, regained. Cytoplasmic vacuolation is detected on some peripheral cellular areas, whereas most of the cytoplasm exhibits the normal configuration with moderate vacuolation. The nuclei exhibit their normal healthy appearance (Figs 11 & 12).

The central veins are still dilated and posses irregular and incomplete endothelial lining. Hepatic sinusoids are rather wider than those of normal control liver (Figs 11 & 12).

Histological demonstration of glycogen indicates a normal pattern of localization and intensity of reaction with PAS reagent (Fig. 13). This suggests that the glycogen inclusion in the liver cells is regained and that the liver tends to recover and resume its normal activity.

A normal protein inclusion is generally observed in the majority of the hepatocytes with a marked bromophenol blue reactivity. However, few sinusoids are containing more proteinic substances than the normal. Kupffer cells are normally stained for proteins (Fig. 14).

Group (IV): Treatment with 0.1 LD_{50} (4 mg/kg/day) for ten successive days.

Destructive signs are more pronounced and are widely spread over wider areas of the liver tissue after 72 hours following the last applied dose. The distortion of the normal liver architecture is highly remarkable. However, the response of the different liver cells to the drug varies greatly; the peripheral lobular cells are more severely affected (Fig. 15).

Most of the hepatocytes show an advanced degree of hydropic degeneration. Numerous hepatocytes show marked cytoplasmic vacuolization. The nuclei exhibit noticeable signs of chromatolysis, pyknosis, karyorhexis and almost complete karyolysis (Figs 16-18).

Hepatocytes undergoing necrosis lose their lobular arrangement and appear as focal areas or nodules with lymphocytic infiltration. The outer area of such nodules contains a few healthy-looking liver cells and is not separated from the surrounding tissue by any fibrous capsule.

The central veins are obviously dilated and their lining endothelium is severally eroded. These veins are congested with coagulative blood and are extended into the surrounding hepatocytes. Most of the sinusoids are enlarged and endothelial lining is ruptured in some places. Moreover, the Kupffer cells are clearly hypertrophied (Figs 16&18).

Histochemical studies indicate that glycogen is noticeably diminished in the hepatic cells; most of the hepatocytes exhibit scarce glycogen inclusions except those located in the centro-lobular regions which still contain, more or less, moderate amounts of glycogen (Fig. 19).

The protein content of the hepatocytes is noticeably reduced as indicated by the greenish colouration, comparable with the bluish colouration seen in the normal hepatic cells. Nevertheless, such reduction is less conspicuous in the peripheral lobular hepatocytes than the centro-lobular ones. The nuclei are diffusely stained and Kupffer cells exhibit stronger stainability as compared with groups I & II (Fig. 20).

Group (V): Treatment with a single 1.5 LD_{50} (60 mg/kg).

The liver tissue, examined 72 hours post-treatment with a single 1.5 LD_{50} of the drug, shows marked signs of damage with a conspicuous deterioration of the general lobular architecture. Most of the hepatocytes undergo clear sings of cloudy swelling . Such injurious responses are more striking in the peripheral than in the central lobular cells (Fig. 21).

In addition, marked deleterious effects in the nuclei, ranging from pyknosis to karyorhexis are encountered. Moreover, the sinusoids are markedly widened with eroded endothelial lining and intra-luminal hypertrophied kupffer cells (Figs 21&22).

Histochemical studies of the liver tissue support the histopathological observations. Glycogen depletion is prominently remarkable in the hepatic cells being more apparent in the peripheral lobular cells. The diminution is spread over wider areas involving most of the hepatic cells (Fig. 23).

A pronounced depletion of proteinic inclusions is observed, it is more striking in comparison with that detected in group (II). Nonetheless, some centro-lobular hepatocytes are obviously less influenced and still have considerable amount of protein content as indicated by their moderate bromophenol blue reaction. Kupffer cells exhibit stronger reaction for protein in comparison with those of groups I & II (Fig. 24).

DISCUSSION

As regards the histopathological effects of anticoagulant drugs on different organs, it has been found that the liver is the most susceptible organ (Carlisle and Blaschke, 1981).

The present study indicates that warfarin has an obvious adverse effect on the liver tissue. The histological investigations elucidated that there is a direct relationship between the dose of warfarin and the degree of the pathological alterations. However, the consecutive small doses are more effective, comparatively, than one single larger dose. However, Hayes and Laws (1991) mentioned that coumarin compounds have relatively no untoward effects when used therapeutically and have been given for long periods without signs of toxicity. Occasional adverse reactions include gastrointestinal disturbances (especially diarrhea), necrosis of the small intestine, elevated transaminase, urticaria, dermatitis, leukopenia, and alopecia, but not all of these with every compound (Hazelton *et al.*, 1956 and Coon & Willis, 1972).

In the present investigation, the most prominent histopathological change displayed by the liver under the effect of sodium warfarin is cytoplasmic vacuolization. This is supported by the results presented by Ashry *et al.* (1986) and El-Banhawy *et al.* (1993a) in the liver of albino rats treated with the rodenticides warfarin and brodifacoum, respectively. Cytoplasmic vacuolization has also been described by Evans *et al.* (1979) in their ultrastructural study on mammalian hepatocytes after coumarin treatment.

Similar histopathological alterations in liver cells were reported by El-Banhawy (1974) in rats treated with different insecticides including DDT, BHC, pyrethrum and sodium arsenate.

The interpretation of vacuolar cytoplasm, observed in such cases, has been subjected to wide speculations by many investigators. Martin *et al.*(1983)correlated this vacuolar degeneration with the disturbed enzyme activities especially GPT and GOT transaminases. In addition, Zhang and Wang (1984) considered these vacuoles to be resulting from a reduction in ATP supply which occurs in rodenticide-intoxicated animals, which in turn leads to impairment of lipid metabolism.

Some investigators correlated the vacuolar degeneration of the intoxicated hepatocytes to the nucleolar abnormalities at the level of synthesis and assembly of ribosomal precursors and to the change in the ribosome-ergastoplasm complex in the cytoplasm (Svoboda and Higginson, 1968). Moreover, other reports have elucidated that hepatocellular damage may be correlated with the inhibition of protein synthesis due to decrease in the ribosome content of the endoplasmic reticulum (Williams and Hultin, 1973).

El-Banhawy (1974) and El-Banhawy *et al.* (1993a) suggested that cytoplasmic vacuolization is mainly a consequence of a considerable disturbance in fat metabolism and such vacuoles correspond to the areas previously occupied by lipid droplets before the pathological impacts.

In the present investigation, the cytoplasmic vacuolization observed in the damaged liver cells can be explained as a disturbance of fat metabolism. It is well known that fatty change is a reflection of the key position which the liver occupies in fat metabolism. In addition to to manufacturing fatty acids, the hepatocytes remove free fatty acids from the blood stream and utilize them in the production of phospholipids. These are bound to protein, and passed back into the blood as lipoproteins by an active transport mechanism. If liver cells are damaged, protein synthesis is impaired; lipoprotein manufacture is impeded and the fat taken up by the liver is not exported. It accumulates and appears as droplets. Such cytoplasmic vacuoles correspond to the areas occupied by extensive lipid droplets due to routine paraffin preparation (Walter and Israel, 1978).

West *et al.* (1967) stated that mammalian liver normally contains about 5% lipid, found in hepatocytes and kupffer cells. Under the influence of various pathological lesions the lipid content may rise to 25-30%. Fat droplets increase in size from 2 to 10 μm. Proper and Schaffner (1957) added that many agents cause fatty livers and the increased fat found in liver may be resulting from impaired removal of fat from the liver due to a number of factors including toxicity.

The present study revealed that the injurious effects are more striking in the peripheral lobular cells. This may be explained by the fact that the peripheral cells in a lobule have a good blood supply loaded with the drug, but those near the central vein are farther away from their blood supply. These observations receive support from the

results presented by many authors for the hepatocytes post-treatment with various rodenticides or insecticides (El-Banhawy & El-Ganzuri, 1980, El-Banhawy *et al.*, 1984 & 1993a).

Coon & Willis (1972) and Leck & Park (1981) reported that Warfarin and coumarin anticoagulants inhibit synthesis of vitamin K-dependent factors and decreases the production of prothrombin in the liver. In addition, warfarin induces capillary damage and leads to bleeding upon every slightest trauma. This statement explains, more or less, explains the hemorrhagic features detected after warfarin treatment.

The present work revealed lymphocytic infiltration in the liver tissues post-treatment with warfarin. The present results confirm and support the findings of Abdel-Raheem *et al.*(1986a), Mohamed *et al.* (1986) and El-Banhawy *et al.* (1993a) on using brodifacoum or warfarin insecticides. In contrast, Abdel-Raheem *et al.*(1986a) detected a reduction in the white blood cell count after treatment of rats with the anticoagulant diphacinone.

Gresham (1984) mentioned that the first event, in tissue injury is a transient arteriolar constriction followed rapidly by a conspicuous and prolonged vasodilation mainly of capillaries and venules. These dilated vessels may have such a sluggish flow that the blood clots within them. If there is no thrombosis, the lucocytes, espicially lymphocytes, move to the endothelial surface and migrate between the endothelial cells, leaving the vessels and entering the inflamed tissue.

The present study revealed that the histochemical alterations in the liver tissues proceed in parallel manner to the histo-pathological ones. The liver glycogen, as well as, the total proteins, are markedly decreased in different degrees directly proportional

with the applied dose. However, similar to histopathological alterations, they are more pronounced when several small repeated doses are applied than when a single large dose is used. Many other anticoagulants, namely warfarin and racumin (Abdel-Raheem *et al.*, 1986b), warfarin (Ashry *et al.*, 1986) and brodifacoum (El-Banhaway *et al.*, 1993b) have been reported to induce an obvious decline in glycogen content in hepatocytes of treated rats.

The glycogen depletion after warfarin treatment may be explained, as reported by O'Reilly *et al.*(1963), by the fact that absorption and metabolism of warfarin from the gastrointestinal tract is apparently complete. In addition, Fouts (1965) postulated that the capacity of liver cell to ensure glycogen storage lies behind their ability to metabolize drugs to a certain extent. Thus, when such ability is disturbed, this will be reflected in the non-ability of these cells to perform this job in a perfect manner. In this concern, Feuer *et al.* (1965 & 1966) reported that, in coumarin treatment, minor-abnormalities of the hepatic tissue and disturbance in carbohydrate metabolism have been observed.

Again, Abdel-Raheem *et al.* (1986a), Ashry *et al.* (1986) and Mohamed *et al.* (1986) reported severe depletion in glycogen content, in the rat hepatocytes post-treatment with anticoagulant rodenticides. This was parallel to a significant and gradual increase in the liverglucose-6-phosphatase activity, and was accompanied by an increase in the blood glucose level, and a significant decrease in the plasma insulin content, leading to pronounced state of glycogenolysis in the liver cells.

Moreover, in the present work, the presence of nearly normal liver glycogen content 15 days post-treatment, may denote that the remarkable toxicity of warfarin on the hepatic tissue, may show some recovery after a while. Ashry *et al.*(1986) stated

that the newly formed glycogen, during the recovery period, may be a result of enhanced hepatic gluconeogenesis by the endogenous glucagon.

The present investigation revealed that the total protein content exhibited an obvious diminution in the liver cells following treatment with warfarin. Warfarin has been reported to inhibit the synthesis of liver microsomal protein and other liver protein (Biezunski, 1970). In contrast, Bernacki and Bosmann (1970) reported that warfarin promots the synthesis of such liver proteins. The contradictory results may be explained by differences in procedure, but exactly how is unclear.

Generally, the present observations receive support from the results presented by El-Banhawy *et al.* (1993b), describing simultaeous alterations of proteins, as well as, nucleic acids (RNA and DNA) in the hepatocytes of rats under the effect of the anticoagulant brodifacoum. They explained that the primary target in such unusual circumstances is DNA; its decline will result a reduced amount of RNA leading eventually to a corresponding reduction of the synthesized proteins.

The presently reported depletion of glycogen and proteins, post-treatment with warfarin, were apprent in the peripheral lobular hepatocytes than those of the centrally located ones. In this concern, Kelly *et al.* (1985) described that the liver lobule may be divided into three zones; an inner, hepatic zone around the central vein, an outer, portal zone at the periphery of the lobule and an intermediate zone between the central and peripheral regions. The zones differ metabolically as exemplified by their storage and release of glycogen. Glycogen is usually given up from the peripheral region of the lobule first, leaving a central region with stored glycogen.

Finally, it is concluded that warfarin has serious cumulative effect and the disturbance in carbohydrate and protein metabolism might be attributed to tissue

injury. Such effects may be responsible for impairment of liver functions due to the intoxication with warfarin. Moreover, the toxic effect of warfarin on the hepatic tissue, may recover after a while.

ABBREVIATIONS USED IN FIGURES

I: Nucleus of a condensed type; **II**: Nucleus of an open face type; **B**: Bile duct; **C**: Central vein; **Ch**: Chromatolysis; **CyV**: Cytoplasmic vacuolization; **D**: Disse's space; **E**: Endothelial cell; **Fn**: Focal nodule; **HA** : Branch of hepatic artery; **HD**: Hydropic degeneration; **K**: Kupffer cell; **L**: Lymphocyte; **PV**: Branch of portal vein; **S**: Sinusoid; **X**: Karyorhexis; **Y**: Pyknotic nucleus.

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EXPLANATIONOF FIGURES

Fig. 1 : Photomicrograph of a section of the liver of a normal control mouse. F., n.formalin; S., HE. X 215

Fig. 2 : Photomicrograph of a thin section of the liver of a normal control mouse. F0., OsO_4; S., Toloudine blue. X 540

Fig. 3 : Photomicrograph of a section of the liver of a normal control mouse. F., Carnoy; S., PAS. X 215

Fig. 4 : Photomicrograph of a section of the liver of a normal control mouse. F., formol saline; S., Best's carmine. X 215

Fig. 5 : Photomicrograph of a section of the liver of a normal control mouse. F., Alcoh.Bouin; S., alcian blue-PAS technique.X 215

Fig. 6 : Photomicrograph of a section of the liver of a normal control mouse. F., n.formalin; S., bromophenol blue. X 215

Fig. 7 : Photomicrograph of a section of the liver of warfarin treated mouse (group II). F., n.formalin; S., HE. X 215

Fig. 8 : Photomicrograph of a thin section of the liver of warfarin treated mouse (group II).F.,OsO4 ; S., Toloudine blue. X 540

Fig. 9 : Photomicrograph of a section of the liver of warfarin treated mouse (group II). F., Carnoy; S., PAS. X 215

Fig. 10: Photomicrograph of a section of the liver of warfarin treated mouse (group II). F., n.formalin; S., Bromophenol blue. X 215

Fig. 11: Photomicrograph of a section of the liver of warfarin treated mouse (group III). F., n.formalin; S., HE. X 215

Fig. 12: Photomicrograph of a thin section of the liver of warfarin treated mouse (group III). F., OsO4 ; S., Toloudine blue. X 540

Fig. 13: Photomicrograph of a section of the liver of warfarin treated mouse (group III). F., Carnoy; S., PAS. X 215

Fig. 14: Photomicrograph of a section of the liver of warfarin treated mouse (group III). F., n.formalin; S., Bromophenol blue. X 215

Fig. 15: Photomicrograph of a section of the liver of a warfarin treated mouse (group IV). F., n.formalin; S., HE. X 215

Fig. 16: Photomicrograph of a thin section of the liver of warfarin treated mouse (group IV).F.,OsO4; S., Toloudine blue. X 540

Fig. 17: Photomicrograph of a section of the liver of warfarin treated mouse (group IV). F., n.formalin; S., HE. X 215

Fig. 18: Photomicrograph of a thin section of the liver of warfarin treated mouse (group IV). F.,OsO4; S.,Toloudine blue. X 540

Fig. 19: Photomicrograph of a section of the liver of warfarin treated mouse (group IV). F., Carnoy; S., PAS. X 215

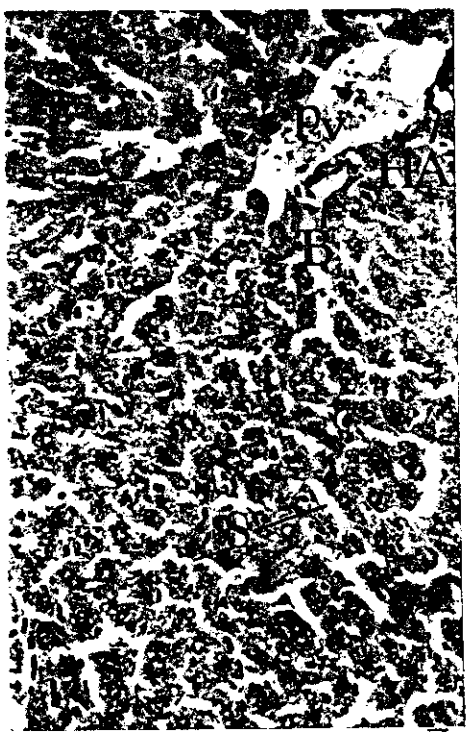
Fig. 20: Photomicrograph of a section of the liver of warfarin treated mouse (group IV). F., n.formalin; S., Bromophenol blue. X 215

Fig. 21: Photomicrograph of a section of the liver of warfarin treated mouse (group V). F., n.formalin; S., HE. X 215

Fig. 22: Photomicrograph of a thin section of the liver of warfarin treated mouse (group V). F., OsO4; S..Toloudine blue. X 540

Fig. 23: Photomicrograph of a section of the liver of warfarin treated mouse (group V). F., Carnoy; S., PAS. X 215

Fig. 24: Photomicrograph of a section of the liver of warfarin treated mouse (group V). F., n.formalin; S., Bromophenol blue. X 215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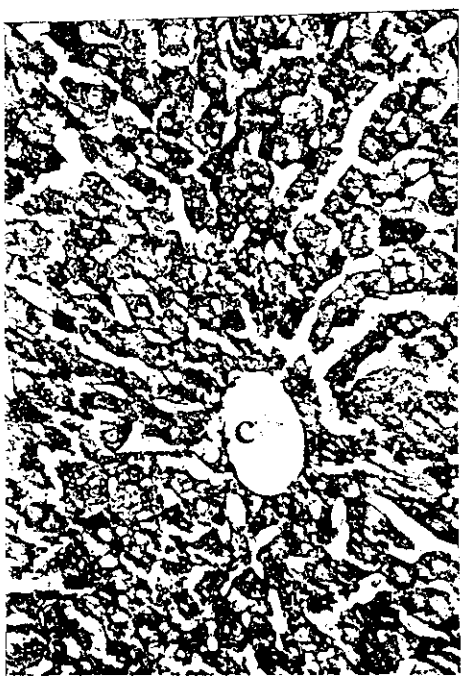
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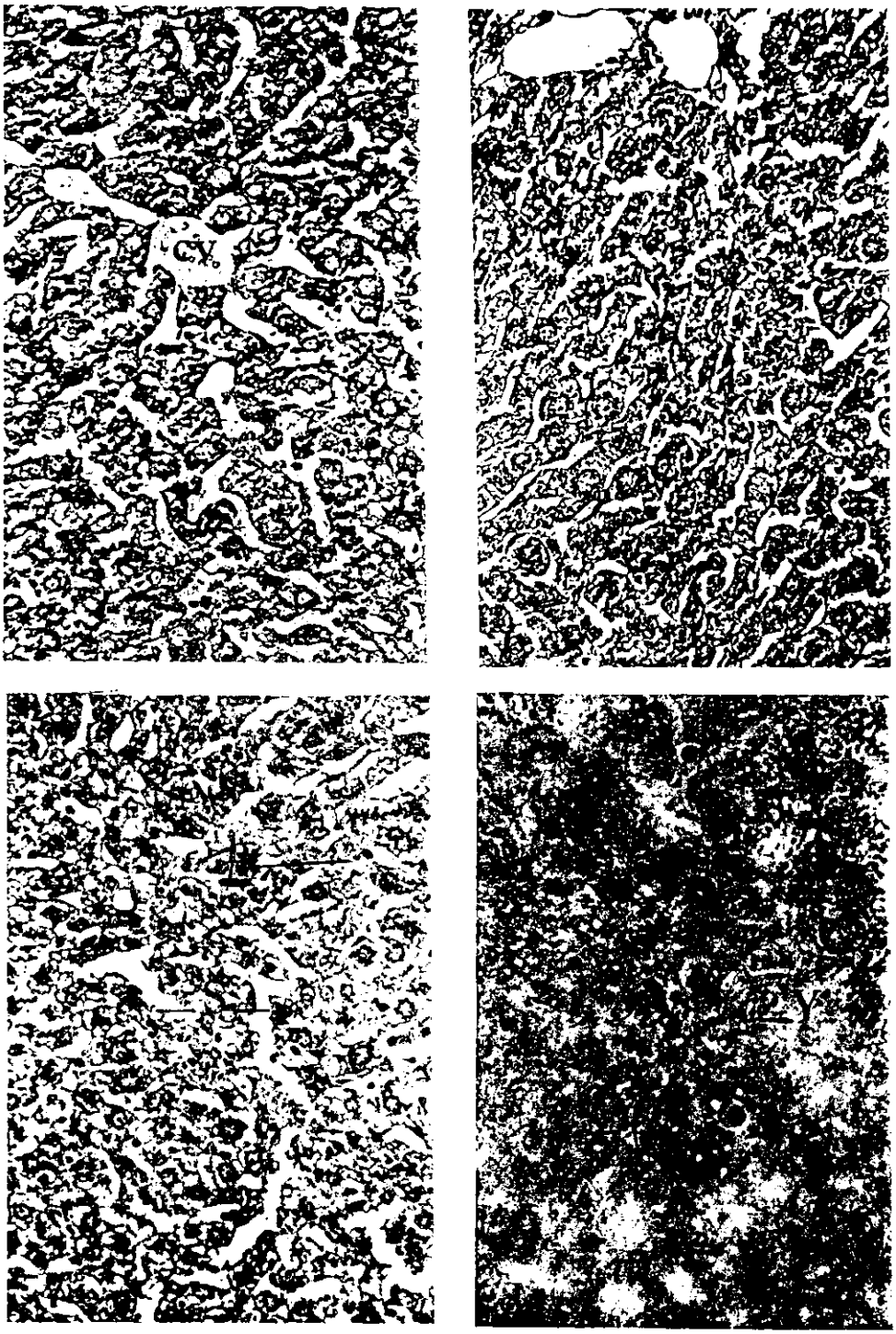
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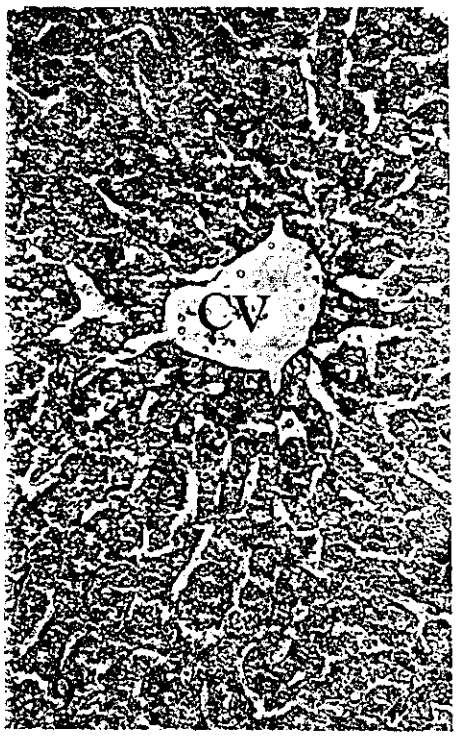
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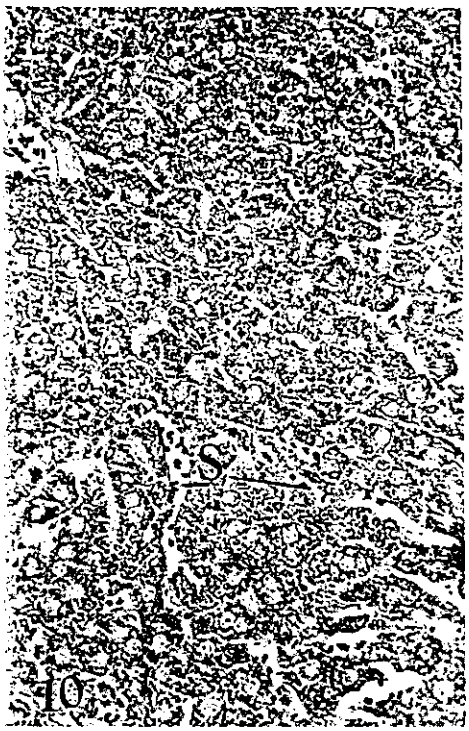
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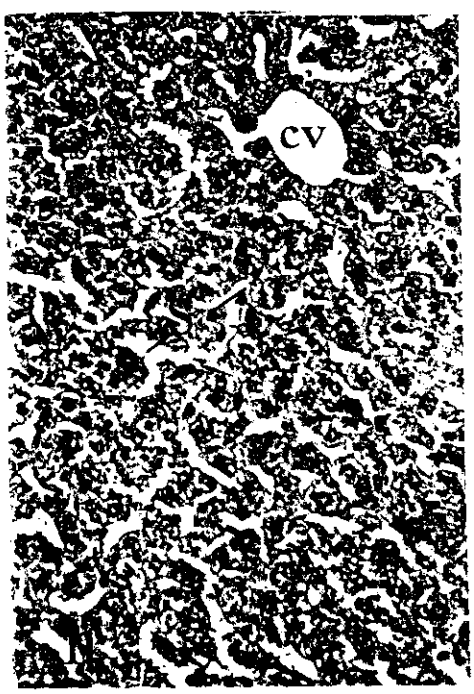
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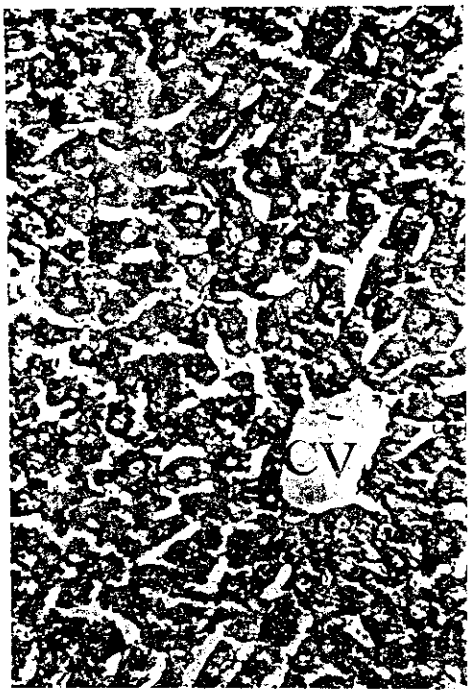
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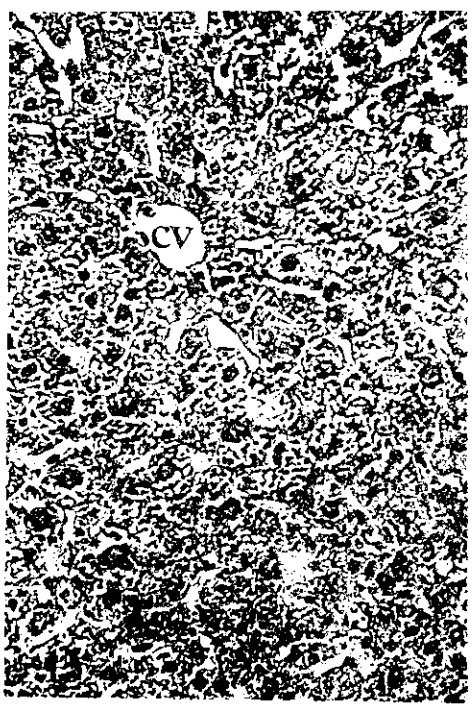
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