THE UNIVERSITY *of* EDINBURGH

OBSERVATIONS ON EXPERIMENTAL AND HUMAN PARACETAMOL OVERDOSAGE

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SUMMARY

This thesis records my investigations into the consequences of overdosage with the analgesic drug paracetamol. The major complication, that of hepatic necrosis, has been investigated in experimental animals using light-microscopy, electron-microscopy and histochemistry. The pathological findings in 18 fatal human cases are recorded and compared and contrasted with previous reports in the literature. A further 16 fatalities resulting from overdosage with compound preparations containing paracetamol are also reported.

A variety of methods for quantifying the extent of hepatic necrosis have been evaluated, including the use of the image-analysis computer, and these methods applied to experimental and human material.

In addition to morphological studies, the influence of microsomal induction on the toxic metabolism of the drug has been investigated and the effectiveness of a possible antidote, α-tocopherol, has been tested in the rat.

The thesis includes chapters on the history and usage of paracetamol and on the treatment of overdosage based on reviews of the literature.

PREFACE

I was introduced to research into paracetamol overdosage when Dr L. F. Prescott wanted a pathologist to evaluate the structural changes in the livers of experimental animals. Our joint approach to the problem yielded some valuable results which not only stimulated in me a sustained interest in paracetamol hepatotoxicity but initiated an interest in human liver disease in general.

On moving to Leeds I was able to pursue research into the morphological aspects of experimental hepatic necrosis and, through the conduct of post-mortem examinations for H.M. Coroner, obtain first-hand experience of lesions in fatal human overdose cases. At the same time I have been closely involved with research initiated by clinical colleagues in the Department of Medicine at St. James's Hospital, Leeds, and as a member of this team benefitted from their expertise and stimulus. I am therefore grateful to a large number of people who have contributed to the work recorded in this thesis. Most of these colleagues appear as co-authors in the material we have been obliged to publish prior to this presentation. For my part I have been responsible for all the electron-microscopical, histological and histochemical interpretation, and for the development of methods of quantitation used in the experimental studies reported here.

I would like to acknowledge the assistance I have received in relation to the various topics covered:-

Ch. 2. The experiments were performed by Dr L. F. Prescott and Dr J. Nimmo.

Dixon, M.F., Nimmo, J. and Prescott, L.G. (1971) Experimental paracetamol-induced hepatic necrosis: a histopathological study.

J.Path., 103, 225

Ch. 3. The experiments were performed by Dr L. F. Prescott and Dr J. Nimmo.

Nimmo, J., Dixon, M.F. and Prescott, L.F. (1973) Effects of mepyramine, promethazine and hydrocortisone on paracetamol-induced necrosis in the rat.

Clin.Toxicol., 6, 75

Ch. 4. The histological and histochemical preparations were made by Mr D. P. Loney; the electron-microscopy preparation was carried out by Ms Barbara Dixon and Dr S. R. Aparicio.

Dixon, M.F., Dixon, Barbara, Aparicio, S.R. and Loney, D.P. (1975)

Experimental paracetamol-induced hepatic necrosis: a light- and electron-microscope and histochemical study.

J.Path., 116, 17

Ch. 5. The experiments were performed by Dr B. E. Walker; Dr J. Kelleher performed the enzyme estimations, Dr M. J. Fulker gave me assistance with the image-analysis computer.

Dixon, M.F., Fulker, M.J., Walker, B.E., Kelleher, J. and Losowsky, M.S. (1975)
Serum transaminase levels after experimental paracetamol-induced hepatic necrosis.
Gut, 16, 800

Ch. 7. The experiments were performed by Dr B. E. Walker and enzyme measurements were made by Dr J. Kelleher.

Walker, B.E., Kelleher, J., Dixon, M.F. and Losowsky, M.S. (1973)
The effects of phenobarbitone pretreatment on paracetamol toxicity.
Biomedicine, 19, 465

Ch. 9. The experiments were performed by Dr B. E. Walker and enzyme measurements were made by Dr J. Kelleher.

Walker, B.E., Kelleher, J., Dixon, M.F. and Losowsky, M.S. (1974)
Vitamin E protection of the liver from paracetamol in the rat.
Clin.Sci.Molec.Med., 47, 449

Ch. 10. I am grateful to H.M. Coroner for Leeds, the Claro district, Wakefield, York and Halifax, for permission to study their files; to Mr J. Dalley and Mr L. Perkin of the West Yorkshire Metropolital Analyst's Department and to Mr Osborne of the Forensic Science Laboratories, Harrogate, for the toxicological results; and to numerous pathologists in the Region for sending me post-mortem reports and material.

Finally, I would like to express my thanks to Mr S. Toms for photographic assistance, and to Miss B. Sunderland for typing the thesis.

CHAPTER I

INTRODUCTION

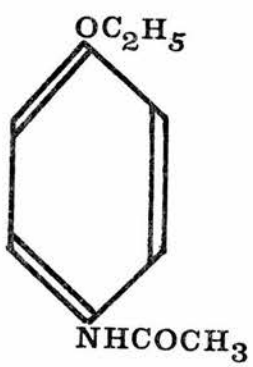
Paracetamol (n-acetyl p-aminophenol, acetaminophen, APAP) is a widely used drug with analgesic and antipyretic actions similar to those of aspirin. It is used for the relief of lesser types of pain such as headache, toothache, and rheumatism.

Although not introduced into the British Pharmacopoeia until 1960 the drug has a long history. In 1889 von Mering studied a series of substituted aminophenols and reported that several of them were active antipyretics in patients with fever. He concluded that, although p-aminophenol was active, it was not suitable for clinical use because of undesirable side effects. He likewise dismissed n-acetyl p-aminophenol for clinical use because, he claimed, it produced similar reactions. It is noteworthy, however, that

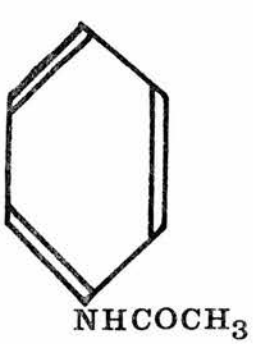
he gave no experimental data or specific observations on patients who had received N-acetyl p-aminophenol, although he gave detailed protocols for the other drugs that he studied.

Hinsberg and Treupel (1894) synthesized N-acetyl p-aminophenol and studied its effects in several species of animals. They found that an oral dose of 3.5 g per kilogram in rabbits, produced only sleepiness and moderate narcosis, but no decrease in body temperature. These were normal rabbits, however, and nothing had been done to produce fever in them. The drug produced convulsions when administered intravenously to rabbits in very large doses of 1.5 to 2 g per kilogram. Dogs were more sensitive to the drug and 0.2 g per kilogram produced hypnosis with 'irritation of kidneys and gastro-intestinal tract'. More recent work has further emphasized these species differences, the mouse and hamster are particularly sensitive to the drug whereas the rat, guinea pig, and rabbit can tolerate a much higher intra-peritoneal dose (Davis et al., 1974).

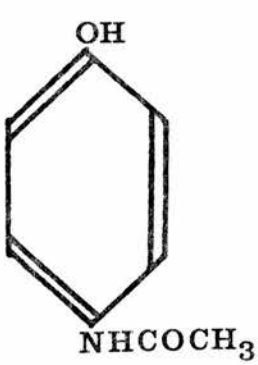
From the outset pharmacological studies on N-acetyl p-aminophenol have been associated with those on acetanilide and acetophenotidin (phenacetin).



Phenacetin



Acetanilide



Paracetamol

Soon after the introduction of acetanilide into therapy Mörner (1889) discovered that some of it was converted in the human to N-acetyl p-aminophenol but it required the development of more sensitive methods to demonstrate the quantitative importance of this metabolite. Such a method was devised by Brodie and Axelrod (1948 a, b) who showed that a minor fraction of acetanilide deacetylates to form aniline, but that the major fraction is oxidized to N-acetyl p-aminophenolwhich is subsequently excreted in a conjugated form. Aniline was shown to be the precursor of the substance which oxidizes haemoglobin to methaemoglobin whereas oral administration of N-acetyl p-aminophenol was not attended by the formation of methaemoglobin. The latter findings suggested an immediate advantage over acetanilide and phenacetin, for although these compounds had a long established reputation as analgesics their clinical use was restricted by their tendency to produce methaemoglobinaemia. Further work established that the analgesic effect of both acetanilide (Flinn and Brodie, 1948) and phenacetin (Brodie and Axelrod, 1949) were largely exerted through their conversion to N-acetyl p-aminophenol, and that a minor product of phenacetin metabolism, p-phenetidine, was the precursor of the substance which resulted in methaemoglobin formation (Brodie and Axelrod, 1949).

Thus by 1950 it had been conclusively demonstrated that N-acetyl p-aminophenol was an active analgesic and antipyretic compound which underwent simple conjugation and was thought not to produce methaemoglobinaemia. In 1962,

however, Keise and Meizel showed that higher doses of paracetamol were associated with methaemoglobin formation, and MacLean et al (1968) reported methaemoglobinaemia in a 20 year old female after taking a therapeutic dose of 'Panadol'. Such cases must be extremely uncommon, and Thomas et al (1966) concluded from their study that "methaemoglobinaemia is not (therefore) a side effect of paracetamol when it is used in normal therapeutic doses, even when it is taken continuously for up to a week. But the compound has the intrinsic ability to induce the formation of methaemoglobin in both cats and man in abnormally high doses."

Since 1950 a number of confirmatory studies validating the safety and efficacy of paracetamol as an analgesic have been reported. Batterman and Grossman (1955) showed it to be more effective than aspirin in the treatment of musculo-skeletal pain and muscle spasm. Chronic toxicity studies with a dose of 3.6 g daily for as long as 116 weeks failed to reveal any blood, kidney or liver disturbances. Newton and Tanner (1956) compared paracetamol with compound codeine tablets in a controlled clinical trial and found that the compound tablet was a superior analgesic in the majority of patients, but a significant minority judged that for them paracetamol was better. Paracetamol was found to have a similar antipyretic effect to aspirin in a comparison using 100 febrile children (Colgan and Mintz, 1957) and its analgesic effectiveness has been demonstrated in cancer patients (Houde, 1956), after orthopaedic operations

(Parkhouse and Hallinon, 1967), in post-partum patients (Lasagna, Davis and Pearson, 1967) and after episiotomies (Hopkinson et al 1973, 1974).

In selecting a mild analgesic for headache or musculo-skeletal pain, the choice lies primarily between aspirin, phenacetin, and paracetamol. Whilst phenacetin and paracetamol have a similar analgesic potency to aspirin, they do not share its anti-inflammatory properties. Therefore aspirin remains the drug of choice for the relief of pain in rheumatoid arthritis and other conditions where an anti-inflammatory effect is desireable. In other situations, however, the choice is governed more by the possible adverse effects of their use.

Aspirin has a number of common and potentially serious toxicity effects. The main ones are dyspepsia and gastro-intestinal haemorrhage, which can in the long term give rise to iron deficiency anaemia, but in some cases can be acute, massive, and fatal. In addition aspirin occasionally causes allergic reactions manifested by asthma and urticaria.

As indicated above, phenacetin may cause the formation of methaemoglobin, Heinz bodies, and acute haemolysis by oxidative damage of red cells (Millar, Peloquin and de Leeuw, 1972). Rarely the drug may cause immune type of haemolytic anaemia (Worrledge 1973). Most importantly, phenacetin has been chiefly responsible for the nephropathy found in chronic analgesic abuse (Koutsaimanis and de Wardener, 1970) although in our present state of knowledge, the habitual consumption of any analgesic should be

considered potentially dangerous in this respect (British Medical Journal 1974).

By comparison paracetamol has proved remarkably non-toxic in therapeutic dosage, although there have been a few cases of hypersensitivity giving rise to dermatitis (Henriques 1970) and single case reports of agranulocytosis (Lloyd 1961), depressed granulopoiesis with fever and mild jaundice (MacKinnon and Menon, 1974) haemolytic anaemia (Mehrotra et al, 1973), and immune thrombocytopenia (Heading; Eisner and Shahidi, 1972), which in the latter report was due to the sulphate conjugation product of paracetamol. These rare instances apart, paracetamol constitutes a safe, effective, mild analgesic which is prescribed with steadily increasing frequency, and in this country is freely available for self-medication.

The popularity of the drug is reflected in the number of proprietary preparations containing paracetamol currently available in this country. There are twelve preparations of paracetamol alone, and no less than twenty nine preparations in which paracetamol is combined with one to seven other constituents (Table 1A). The height of poly-pharmacy seems to be reached by "para-hypon" tablets. These contain paracetamol; caffeine, which is thought to potentiate mild analgesics and stimulate mental activity; codeine phosphate, which is a weak analgesic but tends to produce constipation; and finally phenolphthalin in the hope that its purgative action will nullify the

constipating effect of the codeine.

The proliferation of proprietary preparations containing paracetamol is to some extent a compliment to its effectiveness and safety, but is much more a reflection of the eagerness of manufacturers to enter the very lucrative market in mild analgesics.

TABLE 1. A

PROPRIETARY PREPARATIONS CONTAINING PARACETAMOL

PARACETAMOL ALONE (500 mg)

1. ANPAMOL
2. CALPOL
3. CETADOL (Syrup - 120 mg/5ml)
4. CETAL
5. FEBRILIX (Syrup - 120 mg/5ml)
6. P.C.M.
7. PAMOL
8. PANADOL
9. PANOK
10. SALZONE
11. TABALGIN
12. TETMAL

COMPOUND PREPARATIONS

1.	ADWIN TABLETS	Para. 300 mg, Phenacetin 200 mg, Caffeine 30 mg.
2.	ANTIDOL	Ethosalamide 250 mg, Para. 200 mg, Caffeine 25 mg.
3.	BUDALE	Para. 250 mg, Codeine phosphate 10 mg, butobarbitone 60 mg.
4.	CAFADOL	Para. 500 mg, Caffeine citrate 30 mg.
5.	DISTALGESIC	Para. 325 mg, Dextropropoxyphene hydrochloride 32.5 mg.
6.	DOLALGIN	Para. 375 mg, Codeine phosphate 10 mg, Butobarbitone 60 mg.
7.	ELDO-SED	Para. 500 mg, Dichloralphenazone 325 mg.
8.	FORTAGESIC	Para. 500 mg, Pentazocine base 15 mg.
9.	GEVODIN	Para. 250 mg, Famprofazone (an analgesic) 25 mg, Isopropylphenazone 75 mg, Caffeine 30 mg.
10.	LOBAK	Para. 450 mg, Chlormezanone 100 mg.
11.	MEDOCODENE	Para. 500 mg, Codeine phosphate 8 mg. Phenolphthalein 20 mg.
12.	MYOLGIN	Para. 200 mg, Aspirin 200 mg, Codeine phosphate 5 mg, Caffeine citrate 15 mg, Acetomenaphthone 1 mg, Citric acid 15 mg, Calcium carbonate 60 mg.
13.	NEURODYNE	Para. 500 mg, Codeine phosphate 8 mg.
14.	NORGESIC	Para. 450 gm, Orphenadrine citrate 35 mg.
15.	PALEVA	Aspirin 300 mg, Para. 500 mg.
16.	PANADEINE CO.	Para. 500 mg, Codeine phosphate 8 mg in a base containing sorbitol.
17.	PANASORB	Para. 500 mg in a base containing sorbitol.
18.	PARACODOL	Para. 500 mg, Codeine phosphatase 8mg.

19.	PARAHYPON	Para. 500 mg, Caffeine 10 mg, Codeine phosphate 5 mg, Phenolphthalein 5 mg.
20.	PARAKE	Para. 500 mg, Codeine phosphate 8 mg.
21.	PARALGIN	Para. 450 mg, Caffeine 20 mg, Codeine phosphate 6 mg.
22.	PARAMOL-118	Para. 500 mg, Dihydrocodeine tartrate 10 mg.
23.	PARA-SELTZER	Para. 500 mg, Caffeine 20 mg.
24.	PARDALE	Para. 400 mg, Codeine phosphate 9 mg, Caffeine hydrate 10 mg.
25.	SAFAPRYN	Para. 250 mg, Aspirin 300 mg in an enteric-coated core.
26.	SAFAPRYN-CO	Aspirin 300 mg, Para. 250 mg, Codeine phosphate 8 mg.
27.	SOLPADEINE	Para. 500 mg, Codeine phosphate 8 mg, Caffeine 30 mg in a base containing sorbitol.
28.	TRIPARENE	Para. 500 mg, Caffeine 30 mg, Thiamine HCl 1 mg.
29.	VEGANIN	Aspirin 350 mg, Para. 250 mg, Codeine phosphate 9.58 mg.

CHAPTER II

HEPATOTOXICITY

It has already been stressed that in normal dosage paracetamol is a remarkably safe drug. When a single large overdose is taken, however, the drug produces severe toxic effects on the liver, and death may ensue from hepatic failure.

Hepatic necrosis resulting from massive overdosage with paracetamol was first reported in rats by Boyd and Bereczky in 1966. Later the same year two fatal human cases were reported by Davidson and Eastham and a further non-fatal case was added by Thomson and Prescott.

In 1969 I was approached by Dr. L. F. Prescott with a view to providing a histological assessment of the hepatic changes in rats given a large dose of paracetamol

and subsequently treated with corticosteroids and antihistamines. It was first necessary to conduct pilot experiments to see if we could reproduce paracetamol hepatotoxicity in the rat and to determine the optimum dose of the drug. The material derived from these experiments revealed a number of histopathological changes which had been only briefly described by Boyd and Bereczky, and the presentation of a more detailed account of these changes appeared to be of interest.

Materials and Methods

The experiments were performed in two stages. In the first, two dosages of paracetamol were used, the higher being close to the expected LD_{50} of the drug. Rats surviving 5 days were killed to determine whether or not all the treated animals showed hepatic necrosis. In the second stage a further group were given the higher dosage of paracetamol and examined after longer intervals.

Experiment 1. Twenty male albino Wistar rats weighing 250-400 g were divided into two equal groups; group 1 received paracetamol in a dosage of 2.5 g per kg and group 2 a dosage of 3.5 g per kg. Paracetamol (B.P.C.) was given by gavage, in a suspension of 200 mg per ml stabilised with 0.2 per cent. tragacanth. The rats were fed on a standard Spillers Autoclaved Diet for 2 wk before the experiment. Food was withdrawn 16 hr before paracetamol administration, but water was not restricted. Rats dying within 4 days of drug administration were examined as soon after death as

possible. The surviving rats were killed on the 5th day by cervical dislocation.

Experiment 2. Paracetamol was administered to a further eight male albino Wistar rats under the same conditions as before. It was given as a 300 mg per ml suspension (with 0.2 per cent, tragacanth) by gavage in a dosage of 3.5 g per kg. Two rats died within 48 hr. Of the survivors, two were killed after 7 days, a further two after 14 days, one after 21 days and the last rat on the 28th day.

At necropsy the livers were excised and three representative "blocks" removed and fixed in formol-saline. Frozen sections were prepared from one block and stained for fat by the Sudan IV method. The other two blocks were post-fixed in corrosive-formol solution for 24 hr and then processed and cut, and stained with haematoxylin and eosin (HE) and by silver impregnation, for reticulin. Where indicated further sections were stained by Best's carmine for glycogen.

RESULTS

Animals dying within 24 hr of paracetamol administration

Three rats given paracetamol at the dose-rate of 2.5 g per kg and two given 3.5 g per kg died during this period. There was evidence of hepatic damage in three animals only.

The earliest detectable change is chromatolysis, that is loss of basophilic granules (granules of Berg), normally present in the cytoplasm of hepatocytes.

In parallel with this loss of basophilia, the cells also show evidence of aqueous swelling. The cytoplasm contains numerous small vacuoles which at low power impart a "ground-glass" appearance to the cells. Many centrilobular cells contain larger vacuoles, just visible at low power. The special stains indicate that these vacuoles do not contain fat or glycogen and that the appearances are those of hydropic vacuolation (fig. 2.1, 2.2).

In addition to chromatolysis and hydropic vacuolation there is mild to moderate congestion. The periportal hepatocytes appear healthy. There is no inflammatory infiltration of portal tracts or Kupffer cell hyperplasia.

Animals dying 24-48 hr after paracetamol administration

Three rats given 2.5 g per kg and two given 3.5 g per kg died during this period.

The livers all show marked congestion, the central veins are dilated and the surrounding sinusoids are disrupted and packed with red blood cells. In addition, frank necrosis of hepatocytes is seen. Necrosis is evidenced by nuclear disintegration and increased eosinophilia of the cytoplasm. The nuclei in these dying cells at first show pyknosis and later karyorrhexis, the cells containing discrete fragments of nuclear material (fig. 2.3). The extent of necrosis varies from small foci surrounding the central veins to confluent bands involving centrilobular and midzonal cells (figs. 2.4, 2.5). In these severely damaged livers even the periportal cells

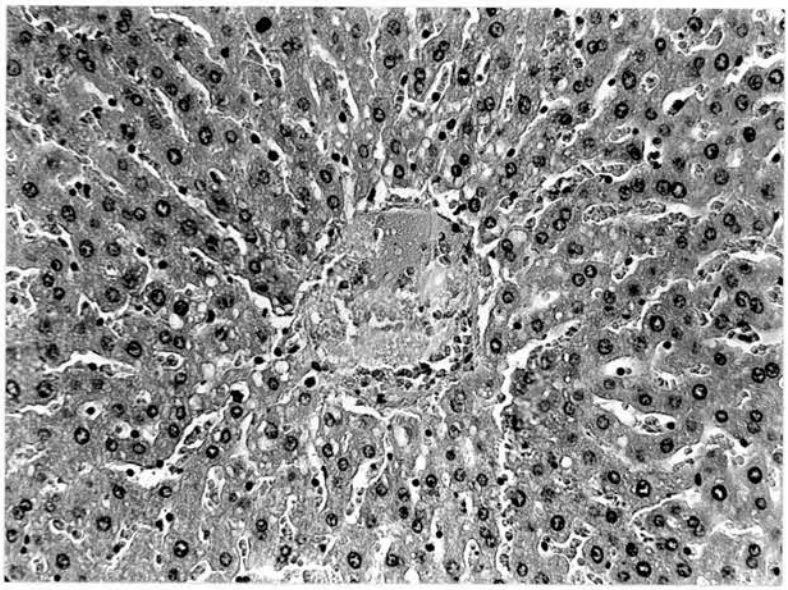


Fig.2.1 Rat liver, less than 24hr after paracetamol. Early damage indicated by variable loss of cytoplasmic basophlia and hydropic vacuolation affecting centrilobular hepatocytes.
Haematoxylin and eosin (HE). X250

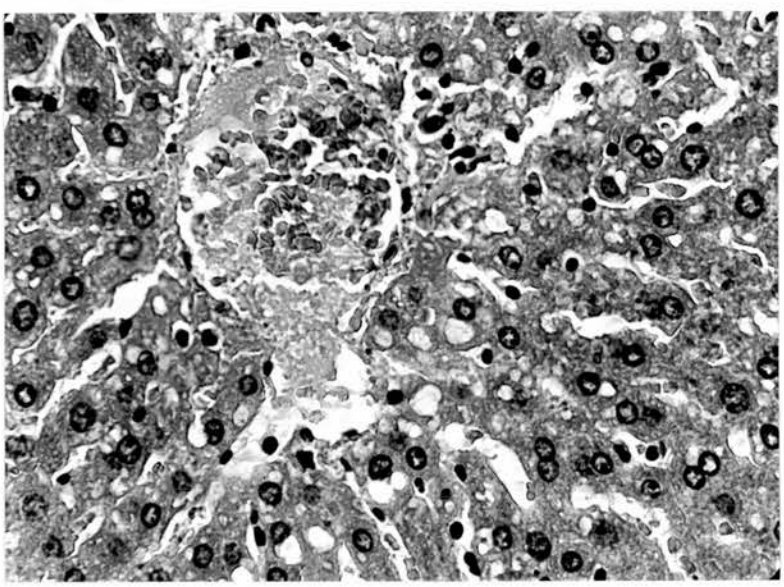


Fig.2.2 Less than 24hr.Hydropic vacuolation in hepatocytes immediately surrounding a small central vein. HE. X450

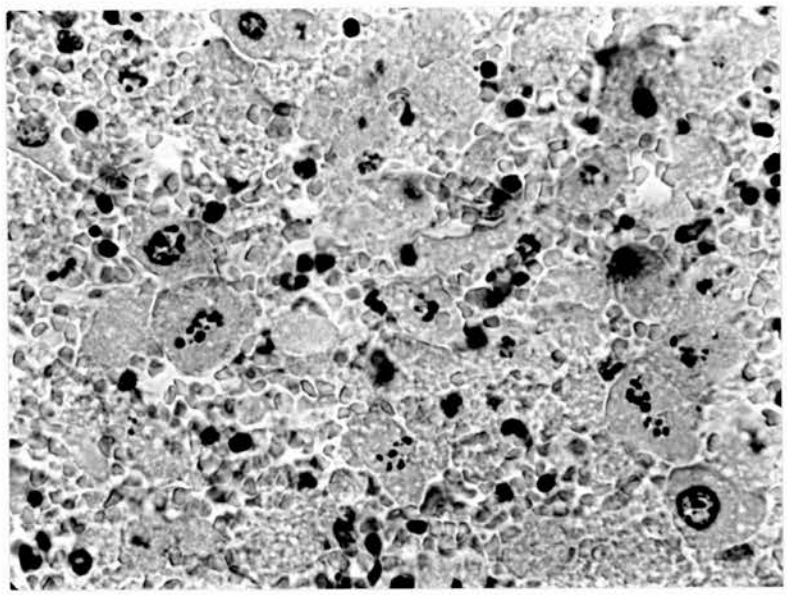


Fig.2.3 24hr. There is marked congestion and necrosis of hepatocytes. Most cells show karyorrhexis and contain discrete fragments of nuclear material. HE. X550

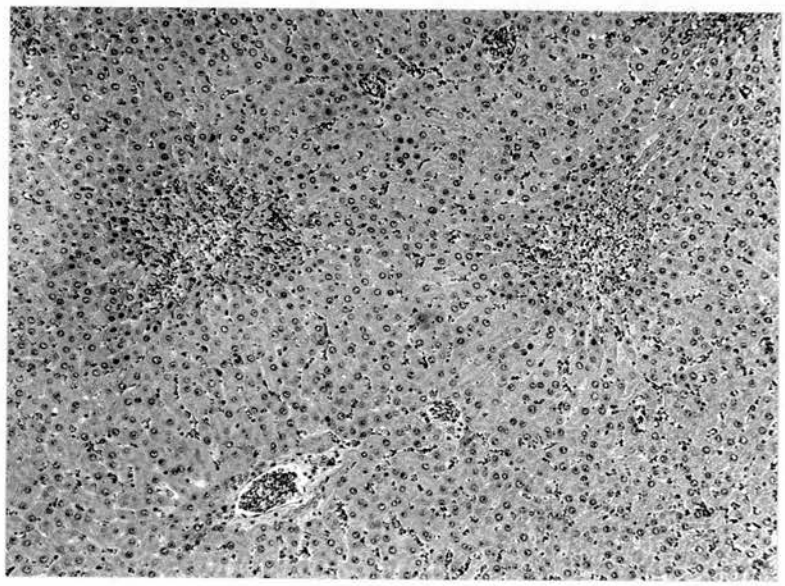


Fig.2.4 24hr. Mild necrosis confined to centrilobular zones. HE. X100

show marked hydropic vacuolation. Two animals show an extreme degree of necrosis, where only the portal tracts are discernible amidst an amorphous mass of necrotic hepatocytes and red blood cells (figs. 2.6, 2.7). The necrotic zones contain variable numbers of neutrophil polymorphs, but there is no inflammatory cell reaction at the margins. The sinusoid-lining cells are largely preserved within the necrotic zones, and reticulin stains show no collapse of the reticulin frame-work. Sudan stains reveal numerous small intracytoplasmic droplets of fat in a narrow band of cells surrounding the necrotic zones.

Animals dying or killed 3-5 days after paracetamol administration

This group comprises four rats given 2.5 g per kg and eight given 3.5 g per kg.

All animals show moderate to marked hepatic involvement. The centrilobular zones are now occupied by large numbers of plump cells possessing bulky granular cytoplasm and indented or "monocytoid"nuclei. The appearances are those of macrophage infiltration (figs 2.8, 2.9). Some animals show evidence of continuing necrosis but on a considerably reduced scale. Scanty residual necrotic hepatocytes together with occasional polymorphs are seen within the infiltrated areas. As expected, the degree of macrophage infiltration parallels the previous extent of necrosis and varies from small foci (fig. 2.10) to confluent bands (fig 2.11).

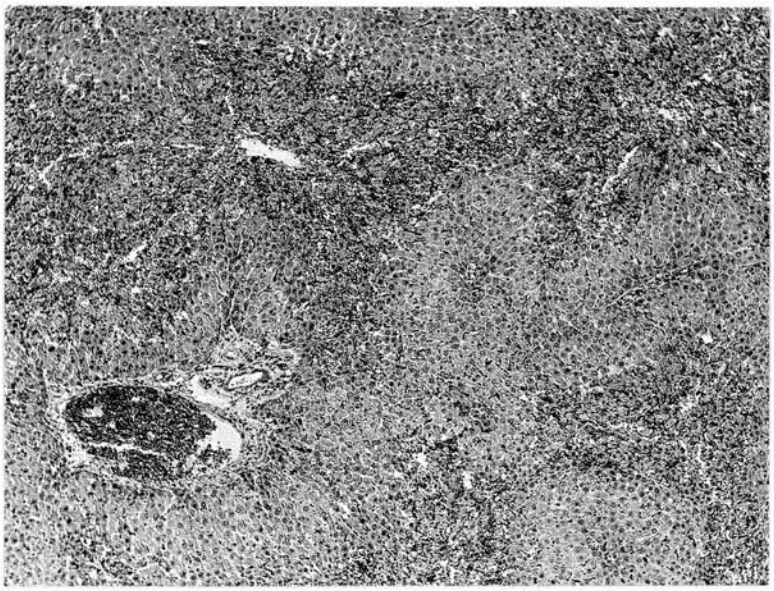


Fig.2.5 36-48hr. The liver shows necrosis involving centrilobular and midzonal hepatocytes producing areas of confluence. HE. X50

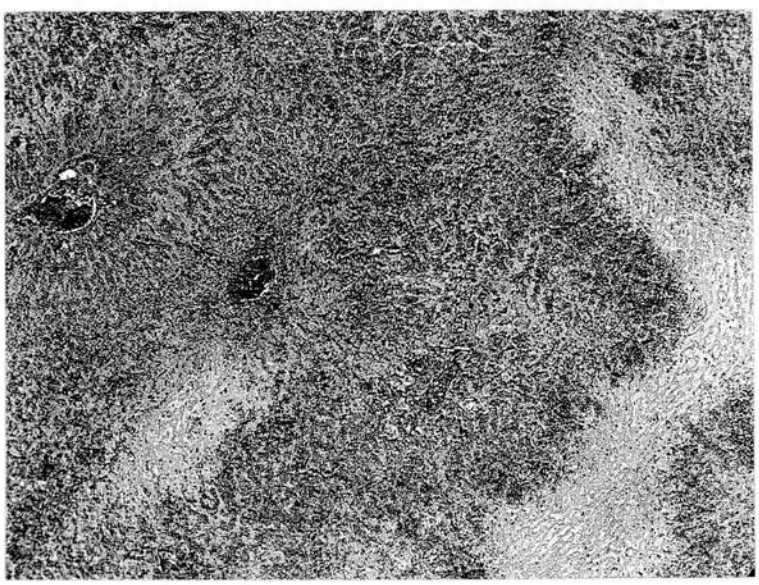


Fig.2.6 36-48hr. Only a narrow band of periportal hepatocytes remain viable in this liver. Elsewhere there is either intense congestion and necrosis or pale-staining coagulative necrosis where the sinusoids are empty. HE. X50

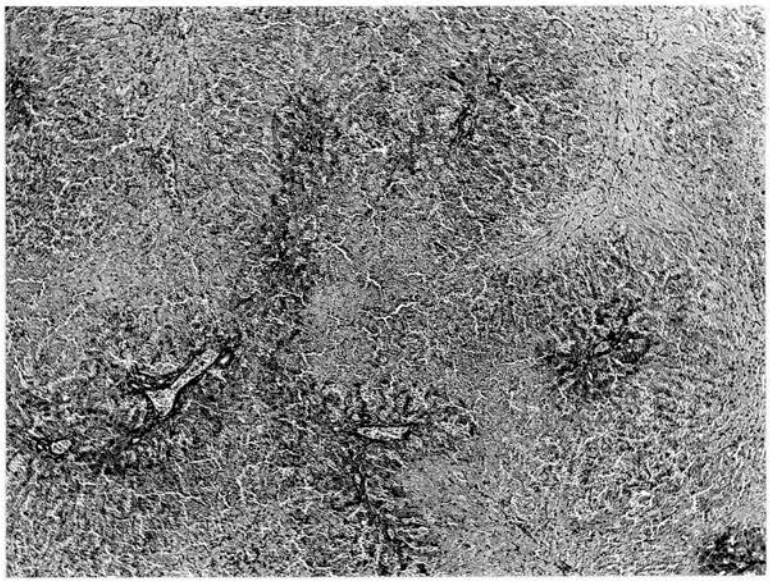


Fig.2.7 36-48hr. Massive necrosis with only a narrow rim of surviving hepatocytes around portal tracts. Reticulin. X50

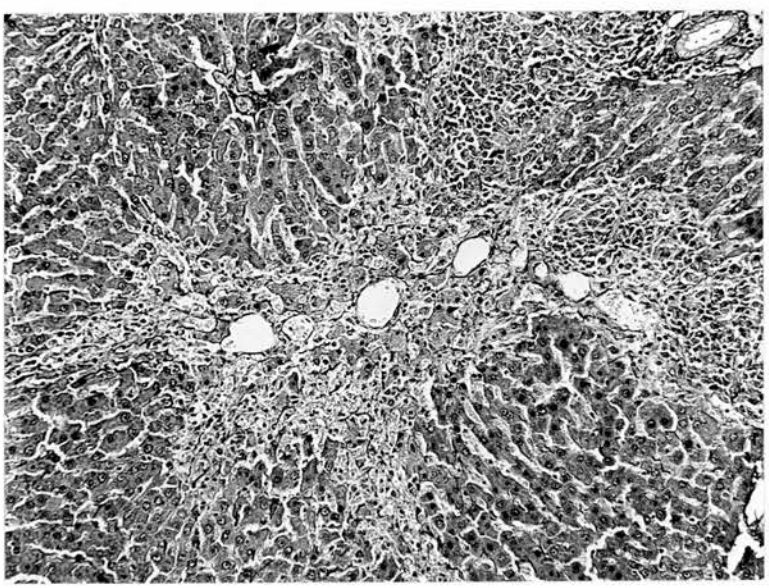


Fig.2.8 5 days. Necrotic area linking central vein and portal tracts now occupied by cellular infiltrate. Retic. X100

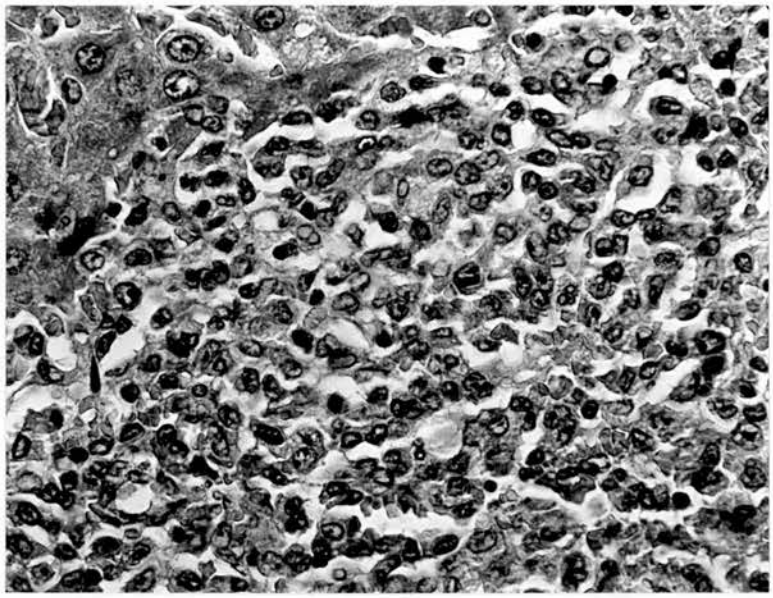


Fig.2.9 5 days. Cellular infiltrate consisting of plump cells with bulky granular cytoplasm and, in many instances, indented "monocytoid" nuclei. HE. X450

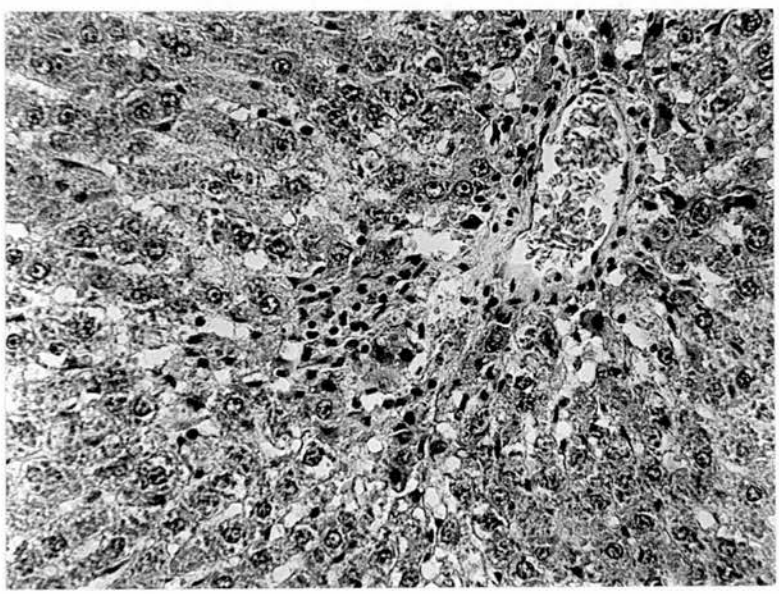


Fig.2.10 5 days. Small focus of macrophage infiltration around a central vein. HE. X250

A further feature is the presence of regenerative activity. Many cells show increased cytoplasmic basophilia, hyperchromatic and double nuclei, and mitotic figures. The mitotic activity is variable, and in some sections as many as two or three figures are seen in each high-power field (fig. 2.12). Whilst the HE-stained sections show widespread loss of hepatocytes, the silver stain reveals complete preservation of the reticulin framework.

Animals killed 7 days after paracetamol administration

There is no evidence of continuing necrosis in two rats given 3.5 g per kg. The hepatocytes show increased basophilia and hyperchromatic nuclei, but there are only occasional double nuclei and very scanty mitotic figures. Macrophage infiltration is considerably less evident than at 5 days and most centrilobular veins are surrounded by only a narrow zone of macrophages (fig. 2.13). The reticulin stains reveal the first evidence of condensation of fine reticulin fibres around a few central veins. The lobular architecture, however, is preserved (fig. 2.14).

Animals killed 14, 21 and 28 days after administration

This group consisted of four rats given 3.5 g paracetamol per kg. Apart from a few widely scattered residual foci of collapsed reticulin and occasional pigment-laden Kupffer cells, the appearances are normal. There is no evidence of fibrosis, inflammatory cell infiltration, or of bile-duct proliferation (figs. 2.15, 2.16).

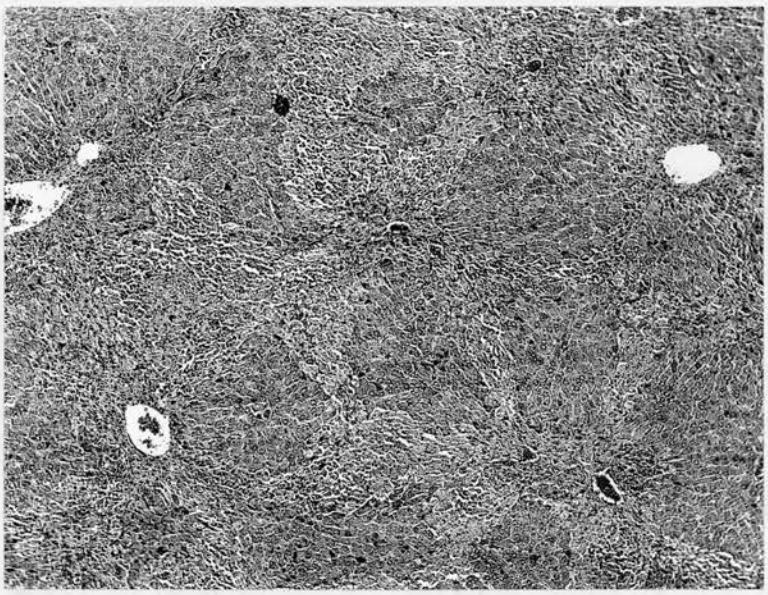


Fig.2.11 5 days. Confluent bands of macrophage infiltration. HE. X50

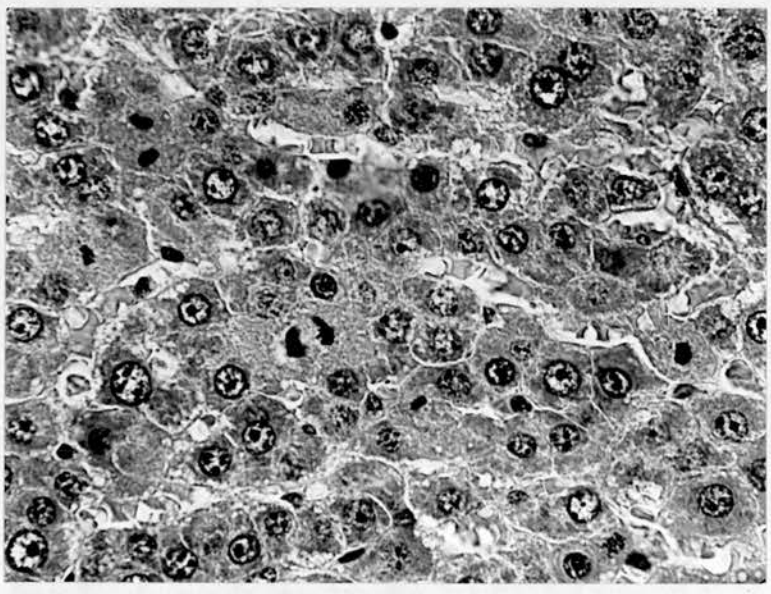


Fig.2.12 5 days. The islands of surviving hepatocytes show marked mitotic activity. HE. X450

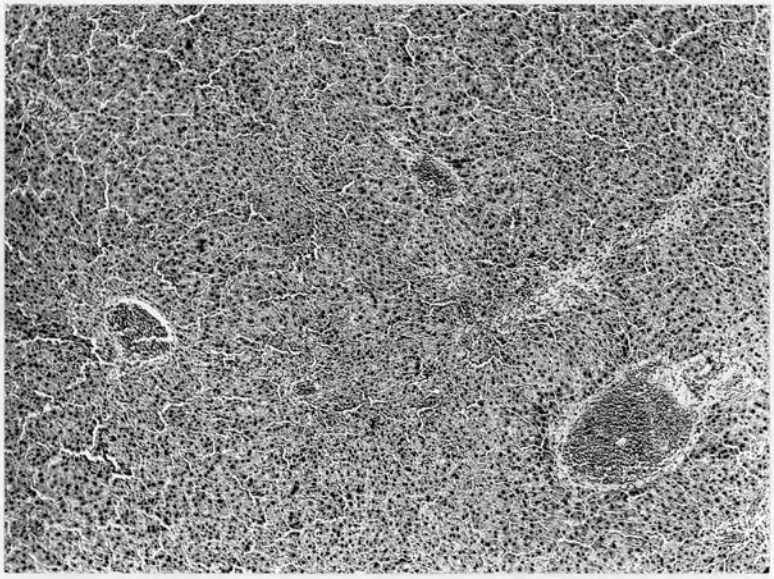


Fig.2.13 7 days. This liver shows a narrow band of macrophage infiltration and nuclear hyperchromatism. HE. X50

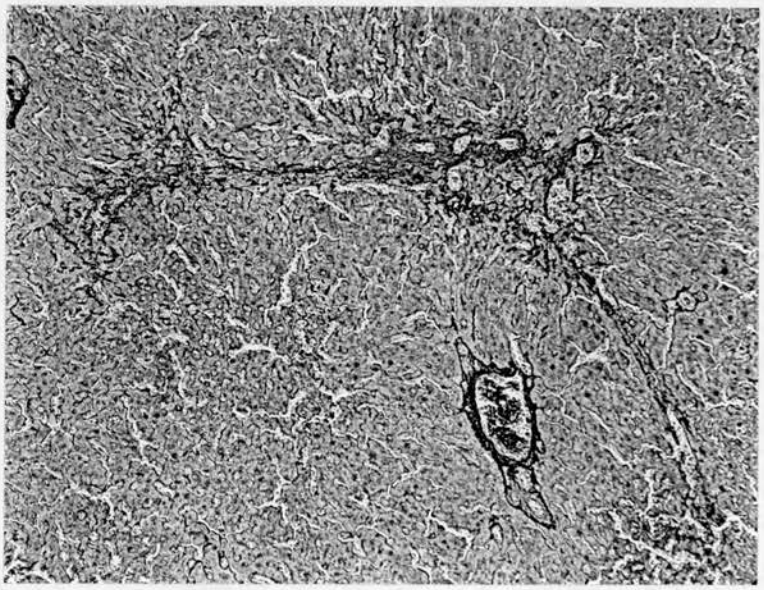


Fig.2.14 7 days. Narrow band of reticulin condensation linking adjacent central veins. Retic. X100

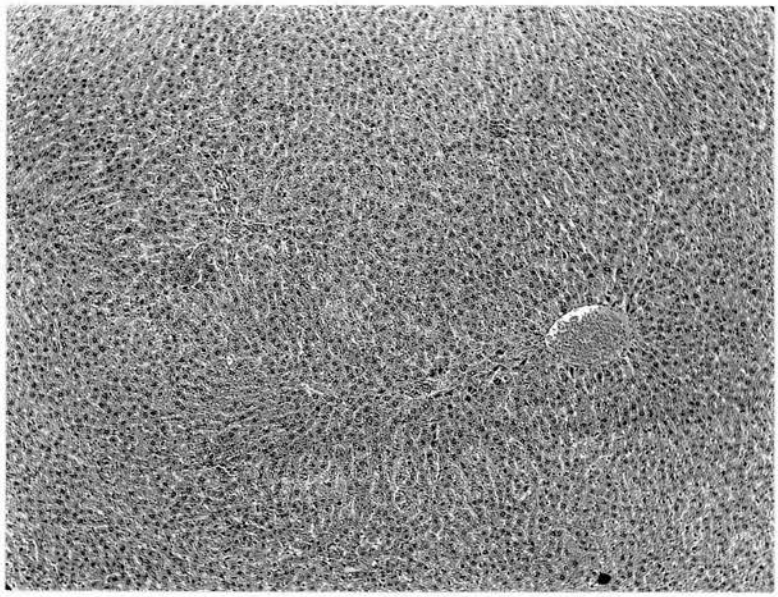


Fig.2.15 Low-power field showing restoration to normal. HE. X50

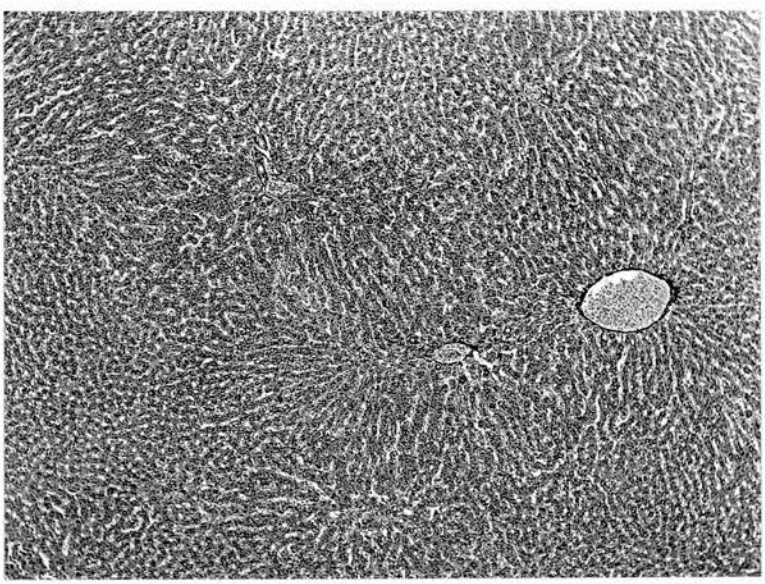


Fig.2.16 The same field stained to show the normal reticulin pattern. Retic. X50

DISCUSSION

Boyd and Bereczky (1966) found centrilobular congestion and "pale staining" in the livers of rats dying up to 24 hr after paracetamol administration; rats dying 1-7 days after administration showed "centrilobular and general hepatic necrosis." Animals were studied only up to 7 days, however, and the further progress of the hepatic lesions in the surviving animals is not described. The effects of prolonged administration of paracetamol at high dosage were studied by Boyd and Hogan (1968). Rats dying after daily administration of doses from 0.5 to 1.1 g per kg up to 100 days showed hepatic congestion, fatty degeneration, and diffuse necrosis, whilst rats given larger daily doses (up to 4 g per kg) showed hepatic necrosis and cirrhosis.

This study has shown that the natural progress of the hepatic lesions after a single large dose in the rat appears to be centrilobular necrosis, followed by macrophage infiltration and regenerative activity, leading quickly to complete recovery. Although occasional foci of collapse were seen in the reticulin stains at 7 days, animals killed after this time showed complete preservation of the lobular architecture and cessation of regenerative activity. Furthermore, macrophage infiltration had disappeared and there was no evidence of fibrosis. These findings, albeit from a small group of animals, indicate that cirrhosis is unlikely to follow a single large dose of paracetamol in the rat.

CHAPTER III

The effect of antihistamines and corticosteroids on paracetamol hepatotoxicity

In a paper entitled "Treatment of Paracetamol poisoning" Maclean and his colleagues (1968) reported hepatotoxicity in 5 cases of paracetamol overdosage and advocated the use of hydrocortisone and mepyramine on the basis that these drugs may limit the degree of hepatic necrosis. This suggestion had some experimental support in that antihistamines had been shown to protect against liver injury caused by a variety of agents, including thioacetamide (Gallagher et al., 1956), carbon tetrachloride (Rees, Sinha, Spector, 1961), murine hepatitis virus (Judha, Bjotvedt, and Vainio; 1960), and against nutritional liver necrosis (McLean, 1960). Furthermore, Dawborn,

Ralston, and Weiden (1961) used diphenhydramine (Bendryl) in the treatment of a 22 year old man suffering from carbon tetrachloride poisoning with apparent success.

Having established that the rat offered a suitable experimental model of paracetamol hepatotoxicity, we set out to determine the efficacy of antihistamines and hydrocortisone in a controlled study.

Materials and Methods

Groups of male albino Wistar rats weighing 200 to 300 gm were maintained on Spiller's Autoclaved Diet. Food was withheld for 16 hr and either paracetamol (2.5 gm/kg as a suspension containing 200 mg/ml with 0.2% tragacanth) or an equivalent volume of suspending agent was given by gavage. At the same time mepyramine, promethazine, hydrocortisone, or 0.9% saline were injected intra-peritoneally as detailed in Table 3A. It was planned to give injections of these drugs twice daily for four days but with the higher doses of mepyramine and promethazine this was not possible because of the poor clinical condition of the animals. The number of doses given is shown in Table 3A.

Autopsies were carried out as soon as possible on rats dying during the experiment. On the fourth day the survivors were killed by cervical dislocation. The livers were removed immediately and prepared for histological examination as described previously.

TABLE 3A Schedule of Drugs and Dosages

Group	Treatment	No. of rats	Dose mg/kg i.p.	Number of doses	Duration of treatment days
1	Paracetamol + Saline (0.4 ml i.p.)	25	--	8	4
2	Paracetamol + Mepyramine	12	20	8	4
		5	37.5	8	4
		10	75	2	1
3	Paracetamol + Promethazine	12	20	8	4
		5	75	1	1
		10	150	1	1
4	Paracetamol + Hydrocortisone	11	100	8	4
5	Suspending agent + Mepyramine	3	20	8	4
		3	75	2	1
	Suspending agent + Promethazine	3	20	8	4
		4	150	1	1
	Suspending agent + Hydrocortisone	4	100	8	4

TABLE 3B Mortality Rates

Group	Treatment	Dose, mg/kl	Number of rats	Mortality, %
1	Paracetamol + Saline		25	0
2	Paracetamol + Mepyramine	20	12	0
		37.5	5	20
		75	10	40
3	Paracetamol + Promethazine	20	12	33
		75	5	100
		150	11	100
4	Paracetamol + Hydrocortisone	100	11	9
5	Suspending agent + Mepyramine	20	3	0
		75	3	0
	Suspending agent + Promethazine	20	3	0
		150	4	25
	Suspending agent + Hydrocortisone	100	4	0

RESULTS

Mortality

None of the 25 control rats receiving paracetamol and saline died during the experiment. In contrast, there was a dose-related increase in mortality in the groups treated with paracetamol and mepyramine or promethazine (Table 3B). All of the rats given paracetamol together with promethazine (75 and 150 mg/kg) died, and the corresponding mortality rate in those given 75 mg/kg of mepyramine was 40%. One rat receiving 150 mg/kg of promethazine alone died, and one receiving paracetamol and hydrocortisone died on the fourth day.

Liver Histology

A detailed histological study was made of the livers from rats receiving paracetamol with saline, mepyramine (20 mg/kg), promethazine (20 mg/kg) and hydrocortisone. Rats receiving higher doses of mepyramine and promethazine were excluded because early deaths prevented meaningful comparisons between groups.

Sections for histological examination were coded and examined "blind". Hepatocellular damage was scored arbitrarily as follows:

(0) No damage to hepatocytes (some livers showed minor degrees of Kupffer cell hyperplasia and lymphocytic infiltration).

(1) Centrilobular hydropic vacuolation without necrosis.

(2) Small foci of centrilobular necrosis.

(3) Necrosis affecting all centrilobular zones with

occasional areas of confluence.

(4) Confluent necrosis with large areas of viable parenchyma.

(5) Massive necrosis with only a narrow periportal zone of surviving hepatocytes.

As judged by the mean histological scores, mepyramine, promethazine, and hydrocortisone did not significantly reduce the degree of hepatic necrosis (Table 3C). Although objective measurements were not made in animals receiving the higher doses of mepyramine and promethazine, their livers all showed gross hepatic lesions, and there was no evidence of protection against paracetamol toxicity.

DISCUSSION

Previous work showing that antihistamines protected rats and mice against hepatic necrosis produced by a variety of injurious agents, and the fact that prednisolone had been recommended in the treatment of fulminant viral hepatitis (Sherlock, 1968) suggested that these drugs might be beneficial in the treatment of paracetamol-induced hepatic necrosis.

MacLean et al.(1968) believed that mepyramine maleate and hydrocortisone restricted hepatic damage in patients with paracetamol poisoning and recommended the use of high doses of hydrocortisone and antihistamines in all such cases. However, they treated only two patients with these drugs, one of whom was apparently not poisoned with paracetamol at all since the plasma paracetamol concentration three hours after ingestion was only a small fraction of that

TABLE 3C: Hepatic Damage - Mean Histological Scores

Group	Treatment	No. of animals	Mean score, ±S.E.
1	Paracetamol + Saline	15	3.3 (±0.2)
2	Paracetamol + Mepyramine (20 mg/kg)	10	3.0 (±0.3)
3	Paracetamol + Promethazine (20 mg/kg)	6*	3.1 (±0.4)
4	Paracetamol + Hydrocortisone (100 mg/kg)	10	3.5 (±0.3)
5	Mepyramine, promethazine, or hydrocortisone	10	0

* Four rats dying within 48 hr were exluded

expected following a therapeutic dose (Prescott et al., 1971). Even without the specific drug therapy proposed by Maclean et al. serum transaminase and bilirubin levels return to normal within a few days in the great majority of patients taking a hepatotoxic dose of paracetamol (Proudfoot and Wright, 1970; Prescott et al., 1971).

In the present study there was no evidence that mepyramine, promethazine, or hydrocortisone administered at the same time as a hepatotoxic dose of paracetamol offered any significant protection against liver damage. Furthermore, there was an alarming dose-related increase in mortality when mepyramine and promethazine were given together with paracetamol.

Clinical studies have failed to demonstrate any protective effect of mepyramine and hydrocortisone against paracetamol-induced hepatic necrosis. In view of the findings in this experimental study the use of anti-histamines and hydrocortisone cannot be recommended for the treatment of paracetamol poisoning.

CHAPTER IV

Electron-microscopy and histochemistry

In our first experiments with paracetamol overdosage a total of 10 rats died within 48 hours of administration of the drug. It is of interest that 2 of these animals died without evidence of liver injury and it must be emphasised that hepatic necrosis is only part of the acute toxicity syndrome. Indeed, in Boyd and Bereczky's study (1966) it was present in only 10% of the animals which died, the remainder were stated to have died as a result of respiratory failure in deep hypothermic stupor. My description of the early histological changes (up to 48 hours) had therefore been based on the findings in only 8 animals. Furthermore these animals had died with hepatic necrosis and could be assumed to illustrate the more severe effects of paracetamol

on the liver. Thus a logical extension of these experiments was a more detailed analysis of the cellular events leading to necrosis and its immediate sequelae. With this objective I next undertook a light- and electron-microscope, and histo-chemical study of the livers of rats killed at various time intervals up to 48 hours after paracetamol overdosage.

Materials and methods

Thirty male albino Tuck-Wistar rats weighing 280-300 gm were divided into six equal groups. They had been housed under identical conditions and maintained for two weeks prior to the experiment on Oxoid pasteurised diet and water ad libitum. Food was withdrawn 16 hours before paracetamol administration but water was not restricted. The test animals received paracetamol (B.P.C.) in a suspension of 300 mg per ml stabilised with 0.2 per cent. tragacanth. The drug was given by stomach tube without anaesthetic in a dosage of 3.0 gm per kg. One or two animals in each group acted as controls and they were given a similar volume of a 0.2 per cent. solution of tragacanth in distilled water alone. After administration, the animals were again given access to food.

Groups of rats were killed by cervical dislocation 1½, 3, 6, 12, 24 and 48 hours after paracetamol administration. One of the rats in the 24 hour group became moribund and had to be killed at 22 hours. The abdominal cavities were

opened quickly and the anterior part of the right lateral lobe of the liver was removed for histology and histochemistry.

The portal vein was then cannulated with a thin-walled 21-G needle and secured with a ligature. After cutting the hepatic veins, the liver was perfused with a solution of 2.5 per cent. glutaraldehyde in 0.1M phosphate buffer pH = 7.4, from an elevated reservoir at a pressure approximately equivalent to 10 cm of water. Perfusion was maintained for about 10 min. by which time the liver had become uniformly hard. It was then removed and random small (1 mm) cubes were taken into ice-cold 2.5 per cent. buffered glutaraldehyde for electron microscopy. After fixation for 1 hour, the cubes were washed in phosphate buffer, post-fixed in a 2 per cent. osmium tetroxide solution for a further 1 hour and embedded in Epon after dehydration in a graded series of alcohols. Semi-thin sections were cut with a Cambridge ultramicrotome and stained with methylene blue Azure II (Richardson, Jarett and Finke, 1960) for light microscopy. Selected areas of the blocks were trimmed and 50 nm-thick sections cut on a LKB ultratome I these were mounted on uncoated 400 mesh copper grids, stained with 8 per cent. uranyl acetate followed by lead citrate, and examined with Philips' EM 100 and EM 300 electron microscopes.

The unperfused tissue for light microscopy and histochemistry was dealt with in two ways:-

1. Slices 2-3 mm thick were mounted on a cryostat chuck, quenched immediately in liquid nitrogen, and stored in the liquid nitrogen until required. 8µm cryostat sections were then cut, picked up on gelatinised slides, air-dried, and fixed in Lillie's calcium acetate formalin (Lillie, 1965) at 4°C for 5 min. Sections were well washed and Gomori's method for acid phosphatase (Gomori, 1950) was carried out.

6µm cryostat sections were picked up on coverslips, briefly dried, and rinsed in acetone at 4°C for 10s to remove lipid artefacts. Succinate dehydrogenase (SHD) was demonstrated by the 3 - (4,5-dimethyl-thiazolyl-2,5-diphenyl tetrazolium bromide (MTT) method of Pearse (1960).

Other cryostat sections were stained with methyl-green pyronin (MGP); Sudan black B; oil-red O; and periodic acid-Schiff (PAS).

2. Slices 2-3 mm thick were fixed in Lillie's calcium acetate formalin and processed to paraffin. 5µm paraffin sections were cut and stained by the following methods:- Harris's haematoxylin and eosin (HE); periodic acid-Schiff; Gordon and Sweets' reticulin method; and the Martius, Scarlet, Blue method (MSB) (Lendrum et al., 1962).

RESULTS

Control Animals

Whilst the normal appearances of the rat liver are well known, three features have to be stressed in regard to controls; these are vacuolation, glycogen content, and lipid globules.

All control livers show some degree of extremely fine peripheral vacuolation in hepatocytes (4.1, 4.2). In some animals they are scanty and are only found in a few randomly distributed hepatocytes, in others they are seen in the majority of cells. On electron-microscopy, the vacuoles range from 0.5 to 0.8 μm in diameter and have a single-membrane lining. They are considered to be pinocytotic in nature.

The glycogen content varies considerably in control animals, a feature probably resulting from the period of starvation prior to the experiment. The 1½- and 3-hr control animals, for example, show some loss of glycogen in all periportal areas so that even when depletion is exaggerated in test animals the finding must be interpreted with caution (4.3). After 6 hr, however, the controls have a relatively even distribution of glycogen with only occasional hepatocytes showing depletion.

Small lipid globules are seen in scattered hepatocytes in control livers (4.4). They are found mainly in periportal and midzonal cells, and although never conspicuous, are most prominent in the 48-hr control.

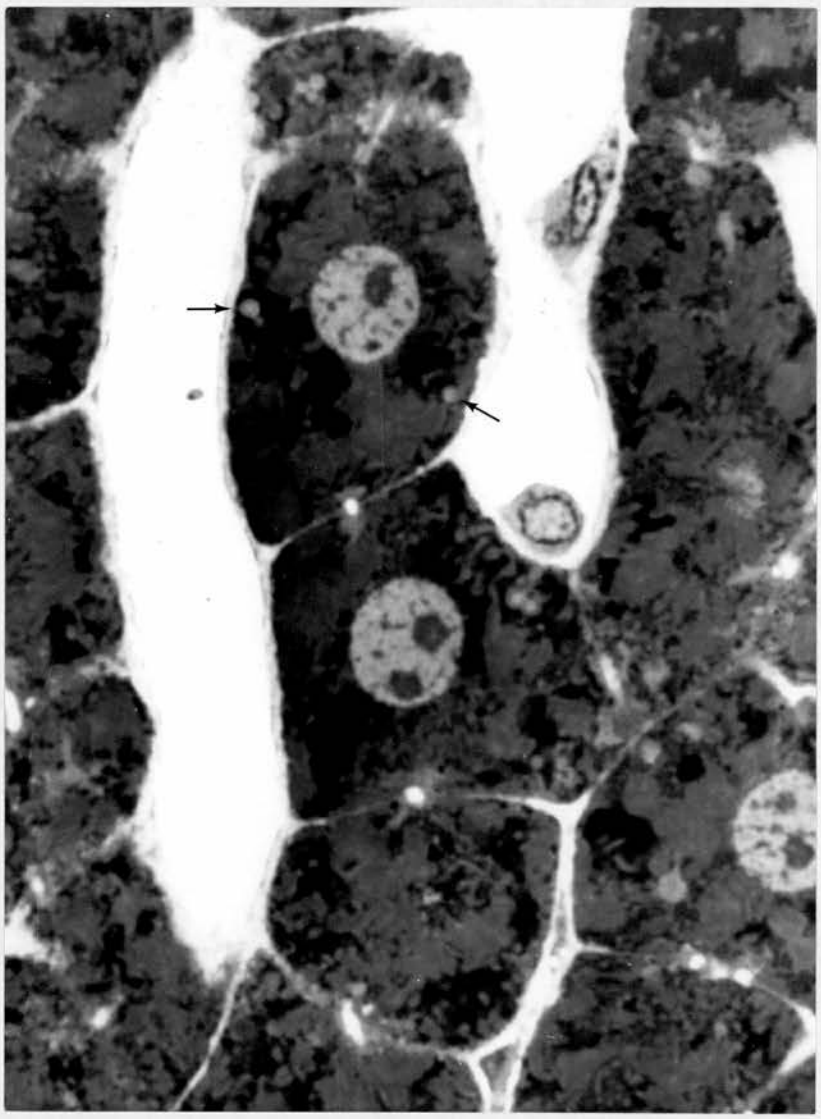


Fig.4.1 Control. The hepatocytes contain a few, mainly peripheral, pinocytotic vacuoles (arrowed). The empty bile canaliculi between adjacent hepatocytes are clearly seen at this magnification, and the cells contain conspicuous dark masses of glycogen.
Semi-thin plastic section (STS). Azure Blue II (AB). X1600

Fig.4.2 Control. Further pinocytotic vacuoles are seen in this field which also contains two budding acidophil bodies. These were extremely scanty in control livers. STS. AB. X1600

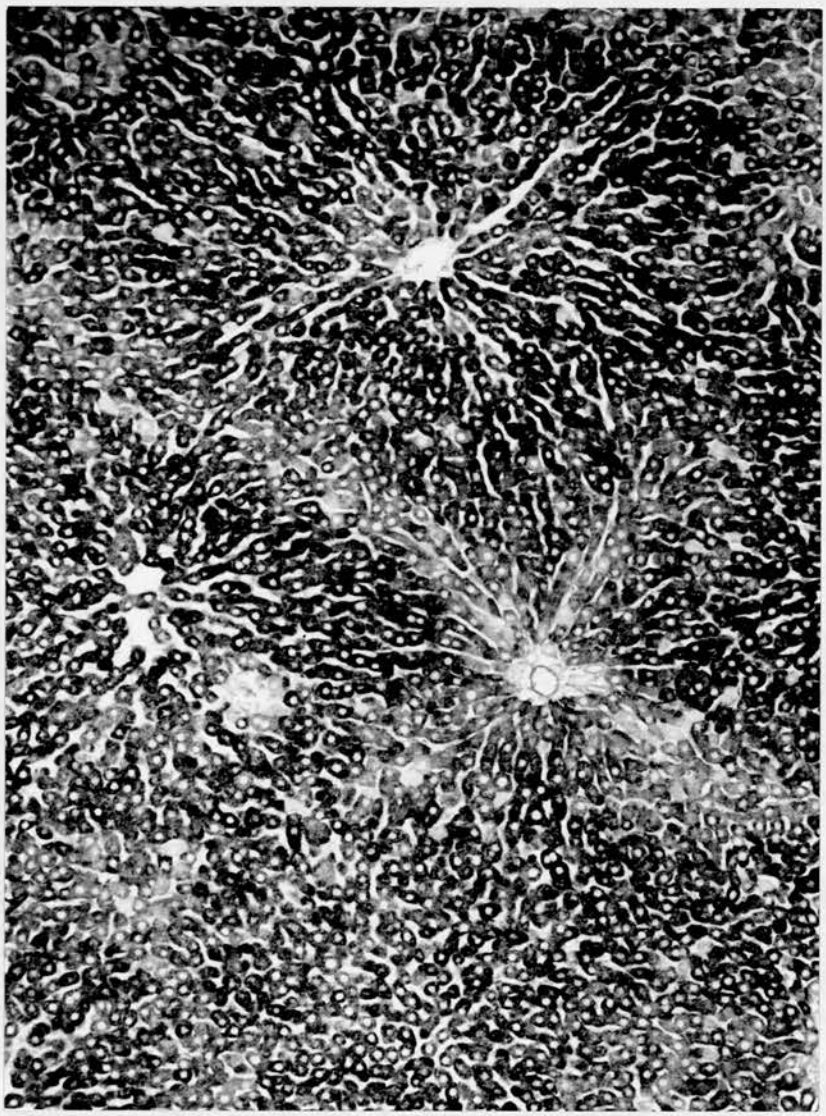


Fig.4.3 Control. PAS stain reveals some loss of glycogen in periportal areas. Paraffin section (PS). X100

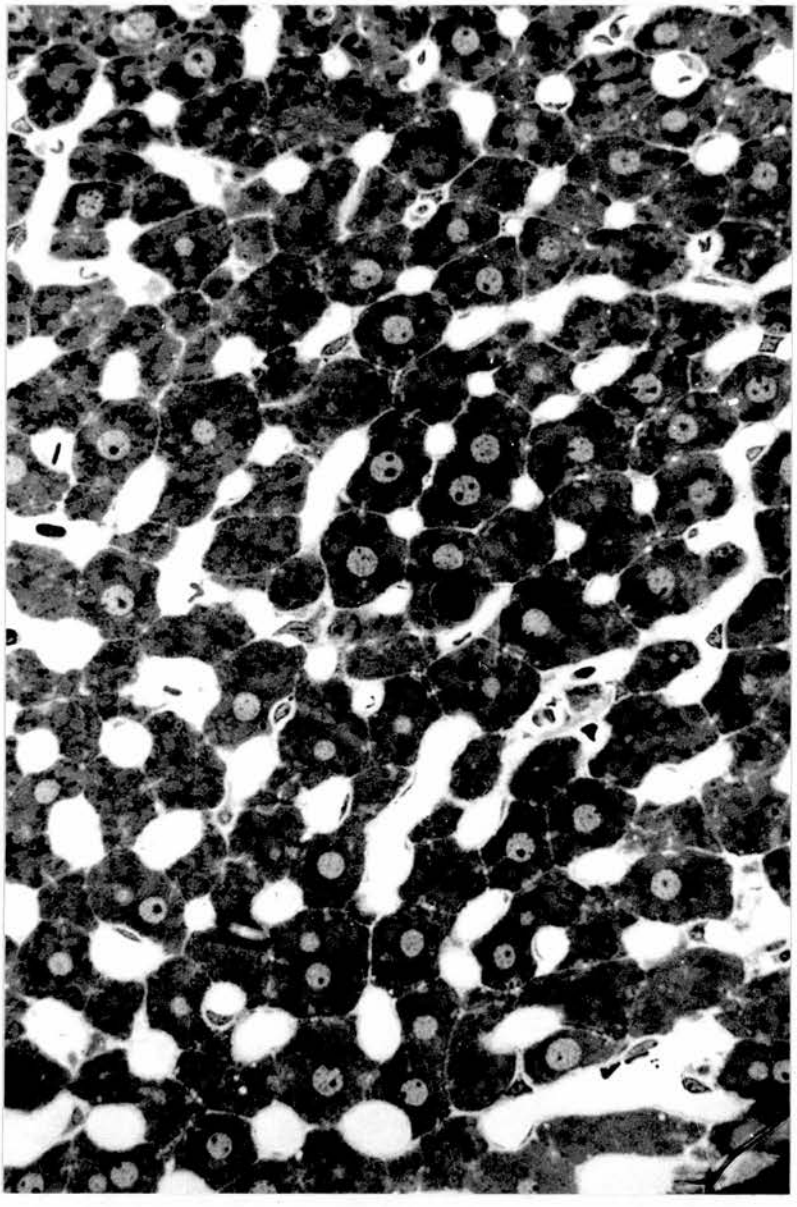


Fig.4.4 Control. A single dark-staining lipid vacuole is seen in this field. STS. AB. X640

The methyl-green-pyronin stain demonstrates cytoplasmic ribonucleoprotein and all control animals show an even distribution of pyroninophilia throughout the parenchyma (4.5).

In the Gomori preparations, acid phosphatase activity is identified by lead deposition which appears as dense "granules" of variable size. The granules are predominantly aggregated towards the contiguous surfaces of the hepatocytes in relation to bile canaliculi, but other small deposits are scattered throughout the cytoplasm.

The MTT method demonstrates mitochondrial succinate dehydrogenase activity by the production of small blue-black formazan dots. Control animals show an even distribution of such dots within the cytoplasm of individual hepatocytes and in the hepatic parenchyma in general.

The normal ultrastructural appearances are illustrated in figs. 4.6, and 4.7.

Test Animals

1½ hr group. The semi-thin and HE appearances do not differ from those in controls. The PAS preparations, however, show a variable degree of glycogen depletion in excess of the controls but always in periportal or midzonal areas. There are no significant differences from the controls either on electron-microscopy (4.8) or in the histochemical preparations.

3 hr group. Again, the semi-thin and HE sections do

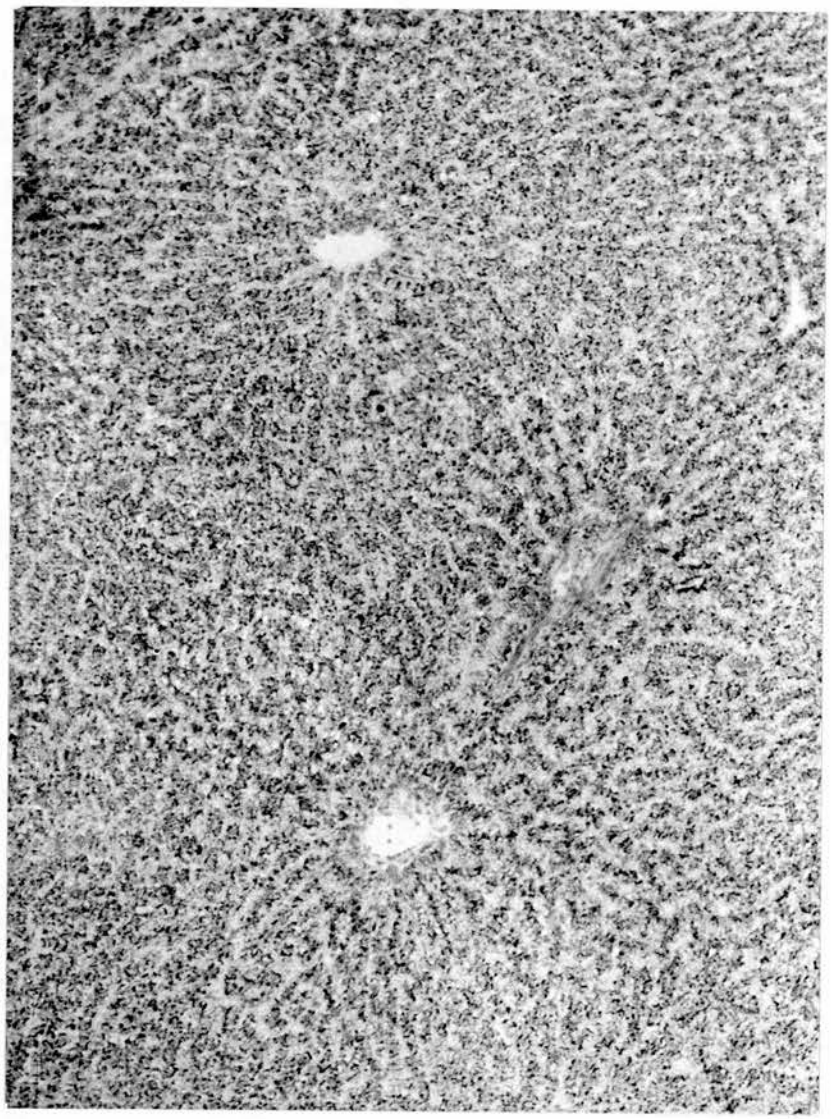


Fig.4.5 Control. The MGP stain reveals an even content of cytoplasmic RNA throughout the parenchyma. Frozen section (FS). X100

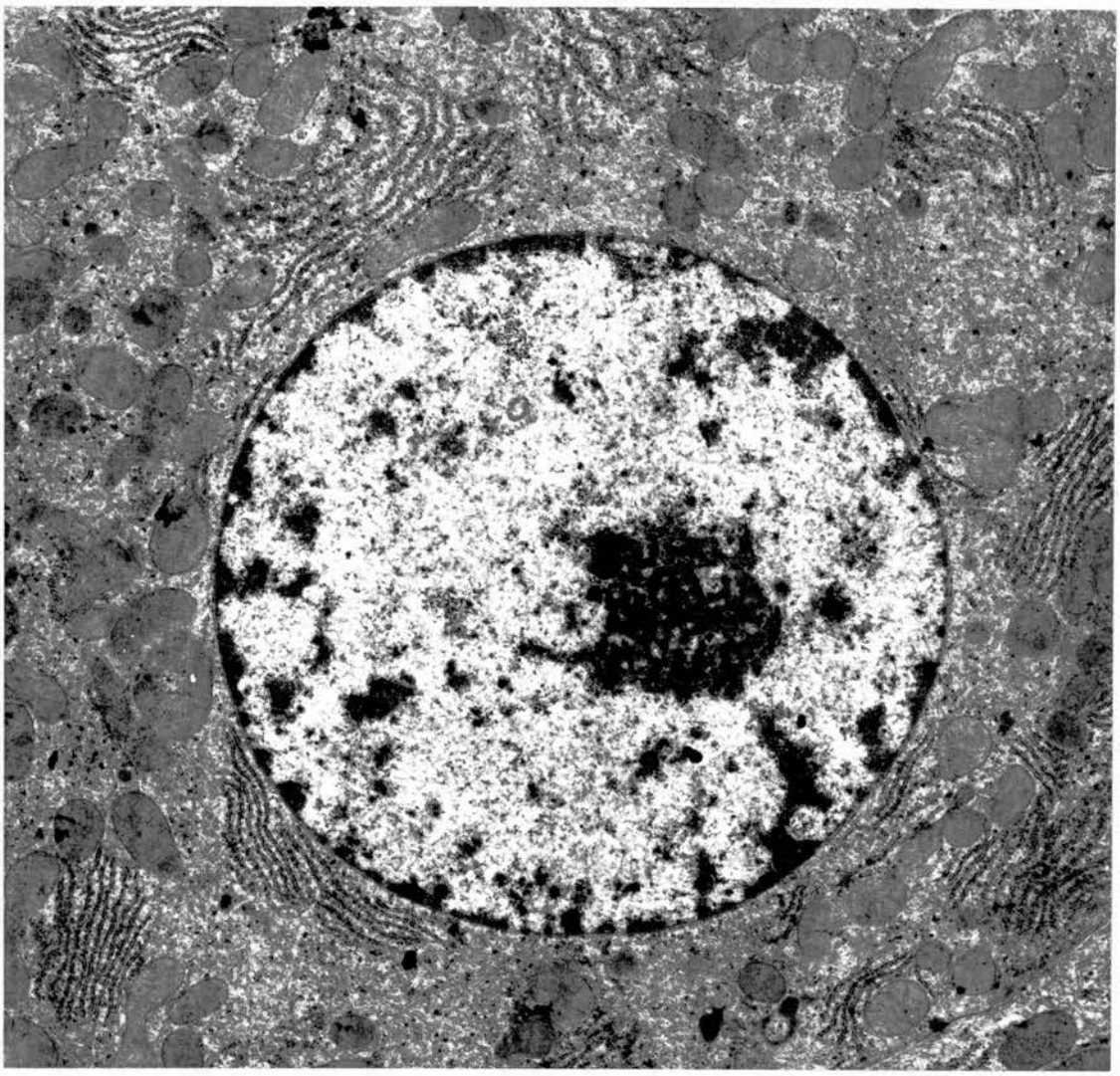


Fig.4.6 Control.This relatively low-powered electron micrograph illustrates a normal hepatocyte nucleus with its peripheral chromatin and prominent nucleolus, and in the surrounding cytoplasm, parallel arrays of RER and mitochondria of varied shape. EM. X9250

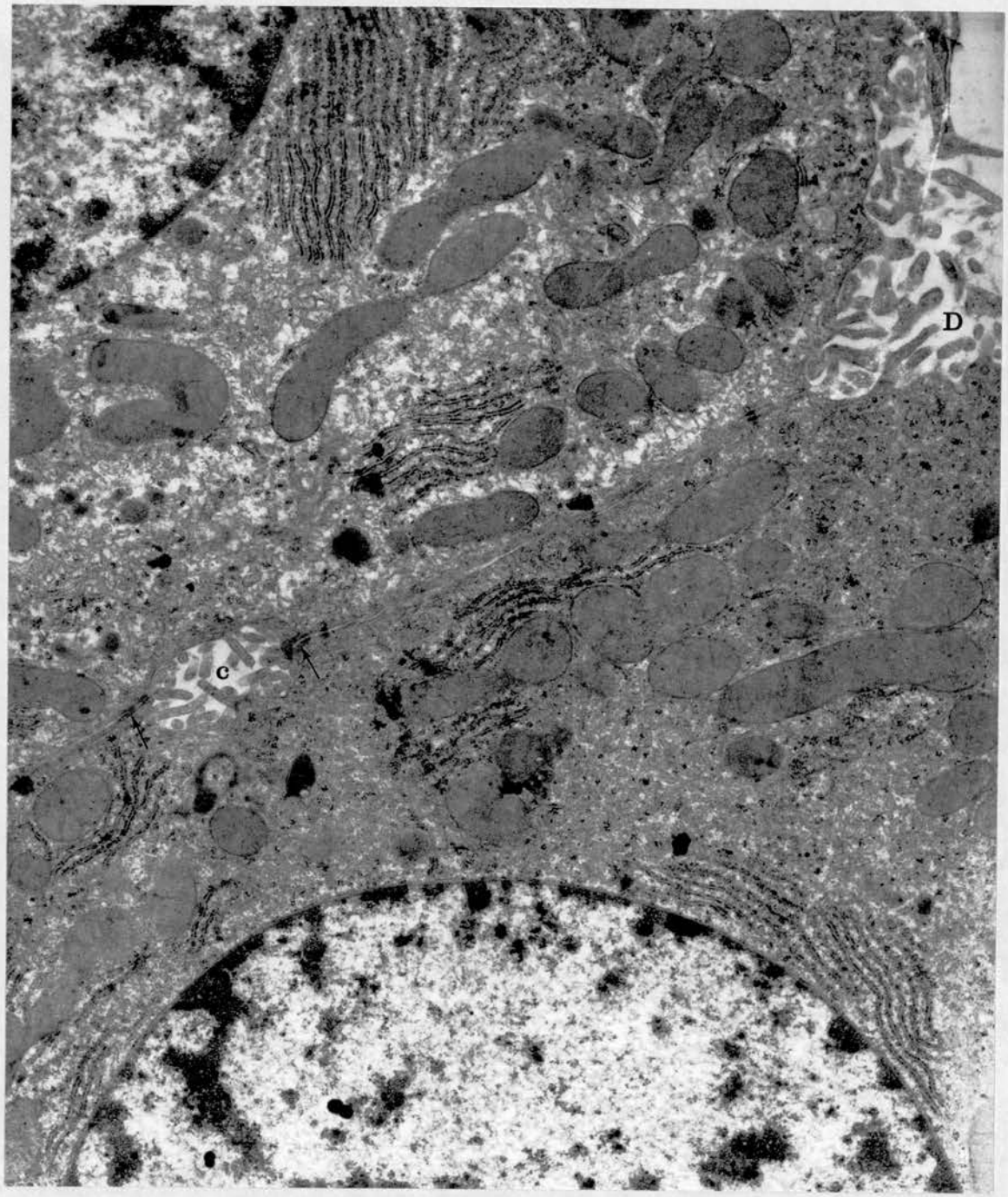


Fig.4.7. Control. Parts of two contiguous hepatocytes are shown. A bile canaliculus with fine microvilli projecting into it (c) is enclosed by two tight junctions (arrowed). Larger microvilli project into the space of Disse (D). The fine vesicles and cisternae of the SER can be discerned , and the small dark intra-cytoplasmic granules are glycogen rosettes. EM. X12,900

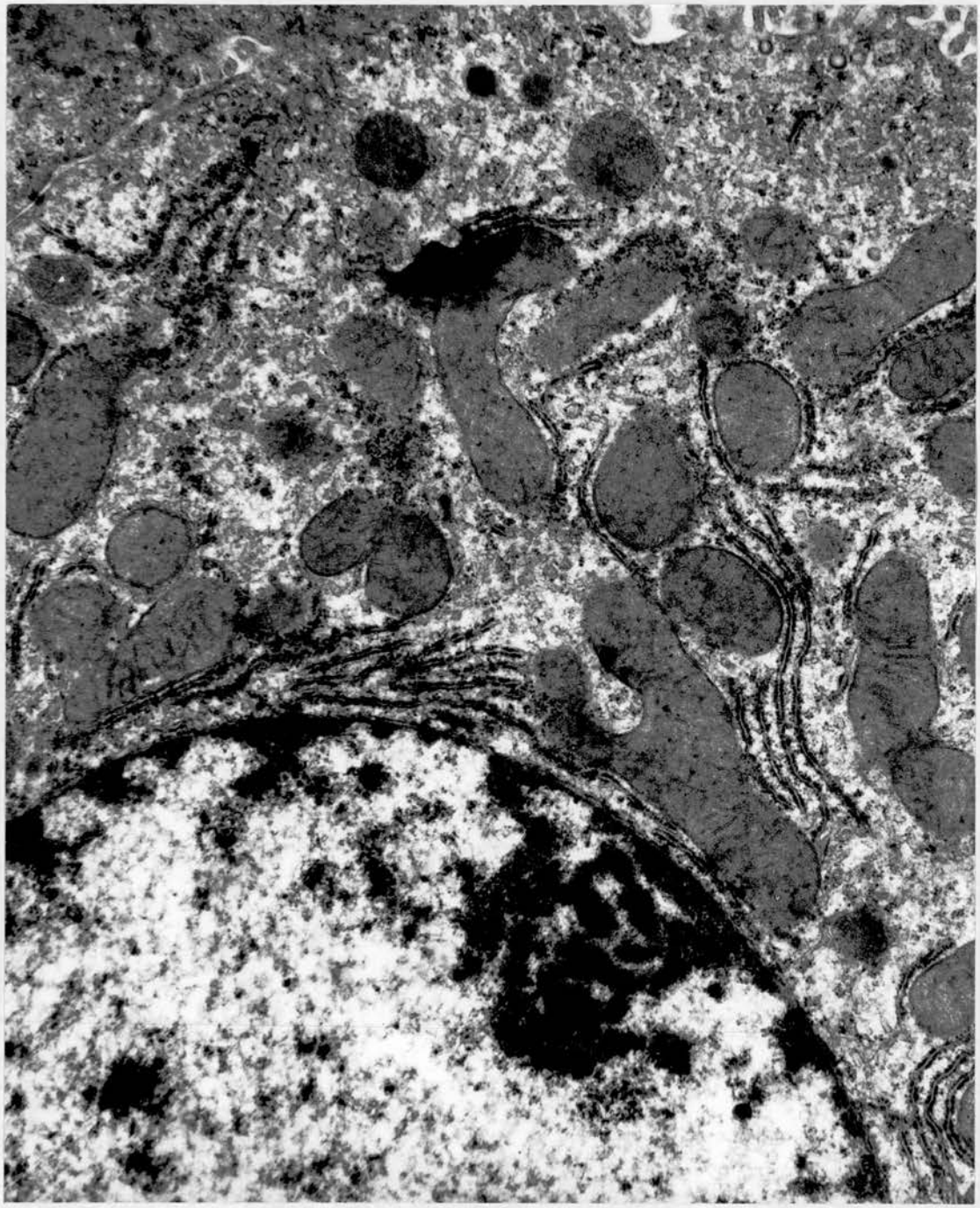


Fig.4.8 1½ hours. There is no apparent difference from the controls. In particular, there is a normal glycogen content and no swelling of the SER or mitochondria. EM. X16,500

not differ from controls. One animal, however, shows marked diffuse glycogen depletion (PAS) and the MTT method reveals a slight increase in the size of formazan dots in centrilobular zones. Despite this finding, the ultrastructural appearances are within normal limits. The other MTT and the acid phosphatase preparations are normal.

6 hr group. On electron microscopy the earliest change noted is slight exaggeration of the peripheral vacuolation seen in controls. The vacuoles are about 1.5 μm in diameter and a few show apparent detachment of the plasma-membrane lining.

In the semi-thin sections the centrilobular and midzonal areas are pale with loss of the normal cytoplasmic "granularity" mainly as a result of loss of glycogen. In the HE sections, hepatocytes in these areas show loss of cytoplasmic basophilia, and parallel loss of pyroninophilia in the MGP stain (4.9).

The pallid areas show a variable degree of aqueous (cloudy) swelling and vacuolation. Aqueous swelling is seen predominantly in midzonal cells, in the HE sections as a uniform "ground-glass" appearance in the cytoplasm, and in the semi-thin sections as a fine, diffuse cytoplasmic "vesiculation" (4.10). Under the electron-microscope, hepatocytes in the immediate vicinity of the central vein exhibit glycogen depletion and uniform cellular matrix swelling whilst midzonal cells show in addition, dilatation of the rough and smooth endoplasmic

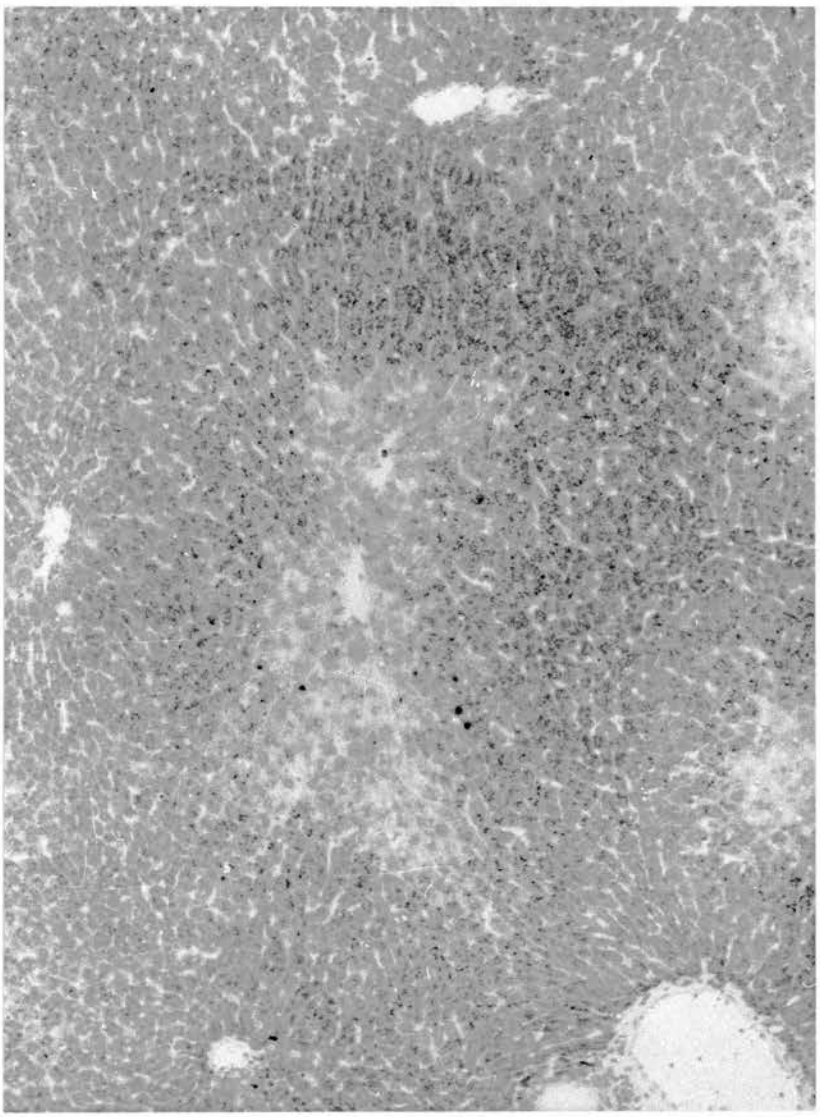


Fig.4.9 6 hours. Loss of pyroninophilia in centrilobular zones. FS. MGP. X100

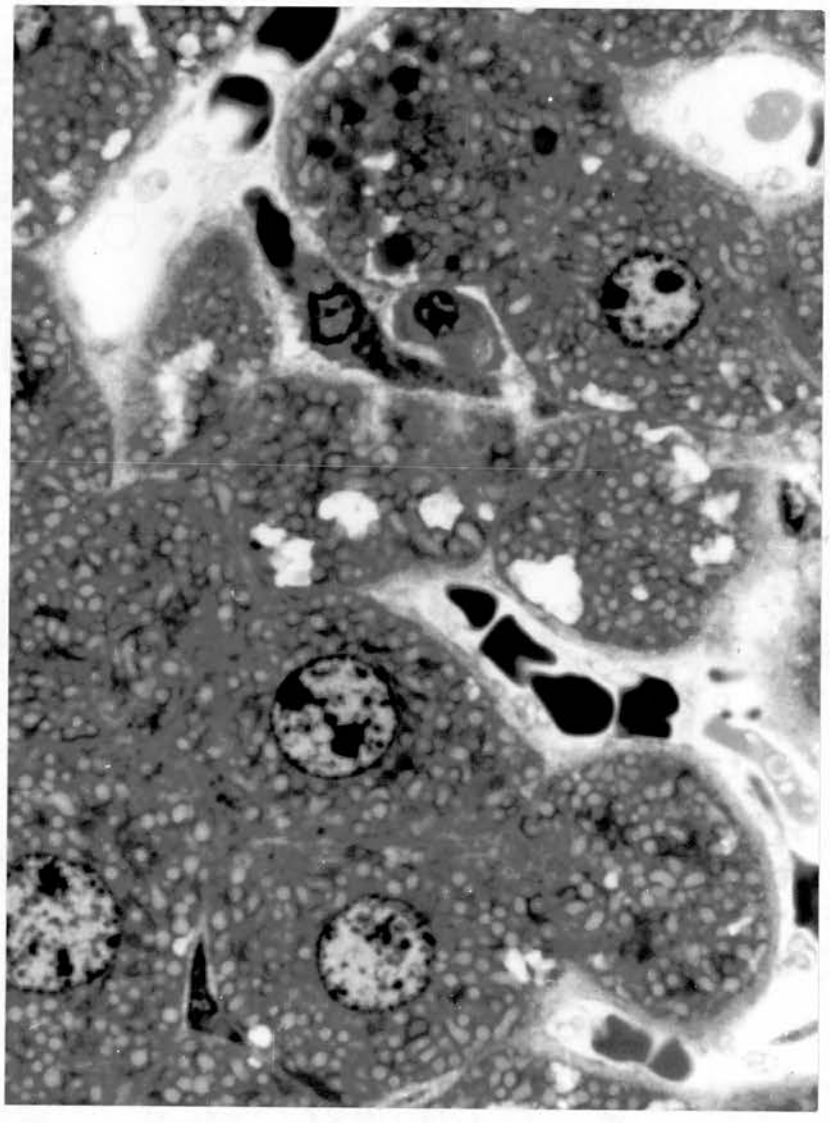


Fig.4.10 6 hours. Midzonal cells showing diffuse cytoplasmic vesiculation indicating aqueous swelling. STS. AB. X1600

reticulum and the Golgi apparatus (4.11, 4.12, 4.13). The swelling of rough surfaced endoplasmic reticulum is accompanied by detachment of ribosomes, which underlies the loss of cytoplasmic basophilia and pyroninophilia seen on light microscopy. Some cells appear to have a predominance of smooth membranes, a finding partly due to the loss of ribosomes from the rough membranes and partly to a uniform swelling of the entire canalicular system of the cell. These changes are seen in all animals in the 6-hr group but at different stages of development in adjacent cells (4.14, 4.15).

One animal in this group shows a more advanced type of degeneration more commonly seen in the 12-hr group, revealing numerous large vacuoles in centrilobular and midzonal areas (4.16). These clear vacuoles, which do not contain lipid, appear to be formed by coalescence of vesicles in the smooth endoplasmic reticulum (4.17). In the MTT preparations of this liver, the centrilobular zones are pale as a result of hydropic vacuolation and loss of mitochondrial staining but on electron microscopy there is good preservation of the overall architecture of these organelles, although some show swelling of the matrix.

12-hr group. These animals show considerable variation in the character and extent of damage.

One animal shows only a minor loss of glycogen. Another shows changes similar to those seen in the 6-hr group, namely pallor and glycogen depletion with aqueous

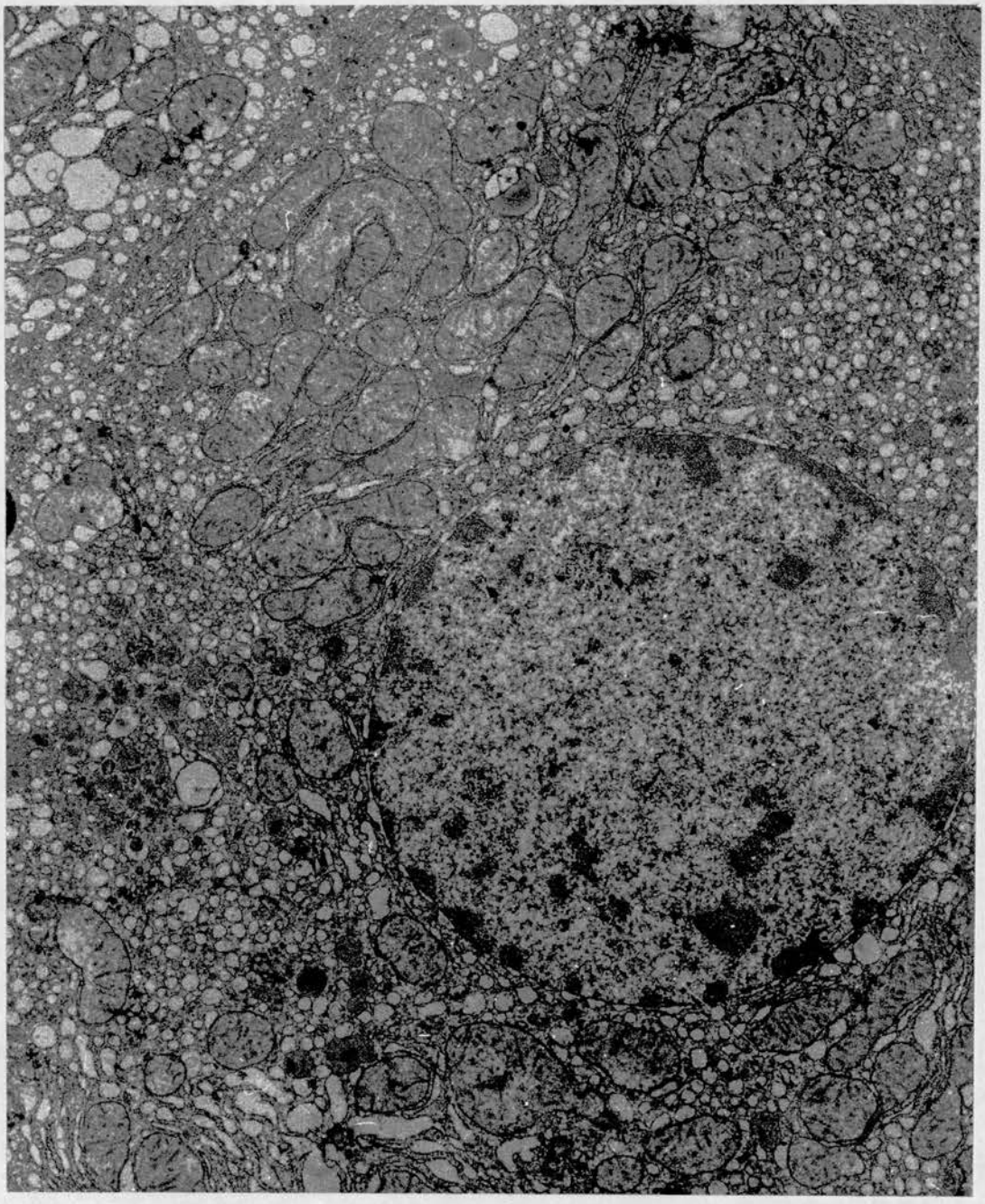


Fig.4.11 6 hours. Centrilobular hepatocyte showing diffuse swelling and vesiculation of the endoplasmic reticulum and loss of glycogen. EM. X8,000

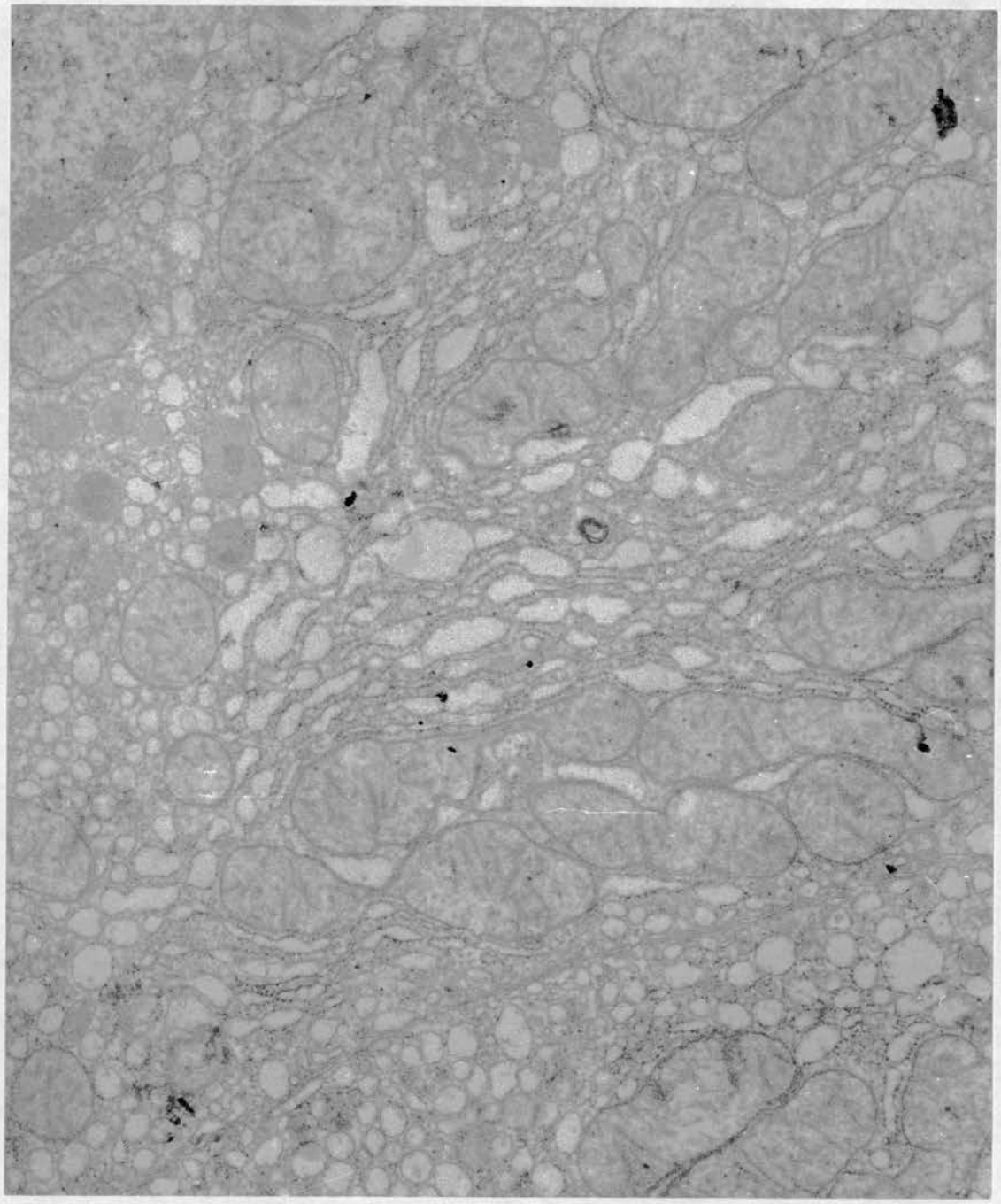


Fig. 4.12 6 hours. Midzonal cell. At high magnification the swelling is seen in both smooth and rough ER. In the latter there is associated loss of ribosomes. The mitochondria are apparently normal. EM. X20,000

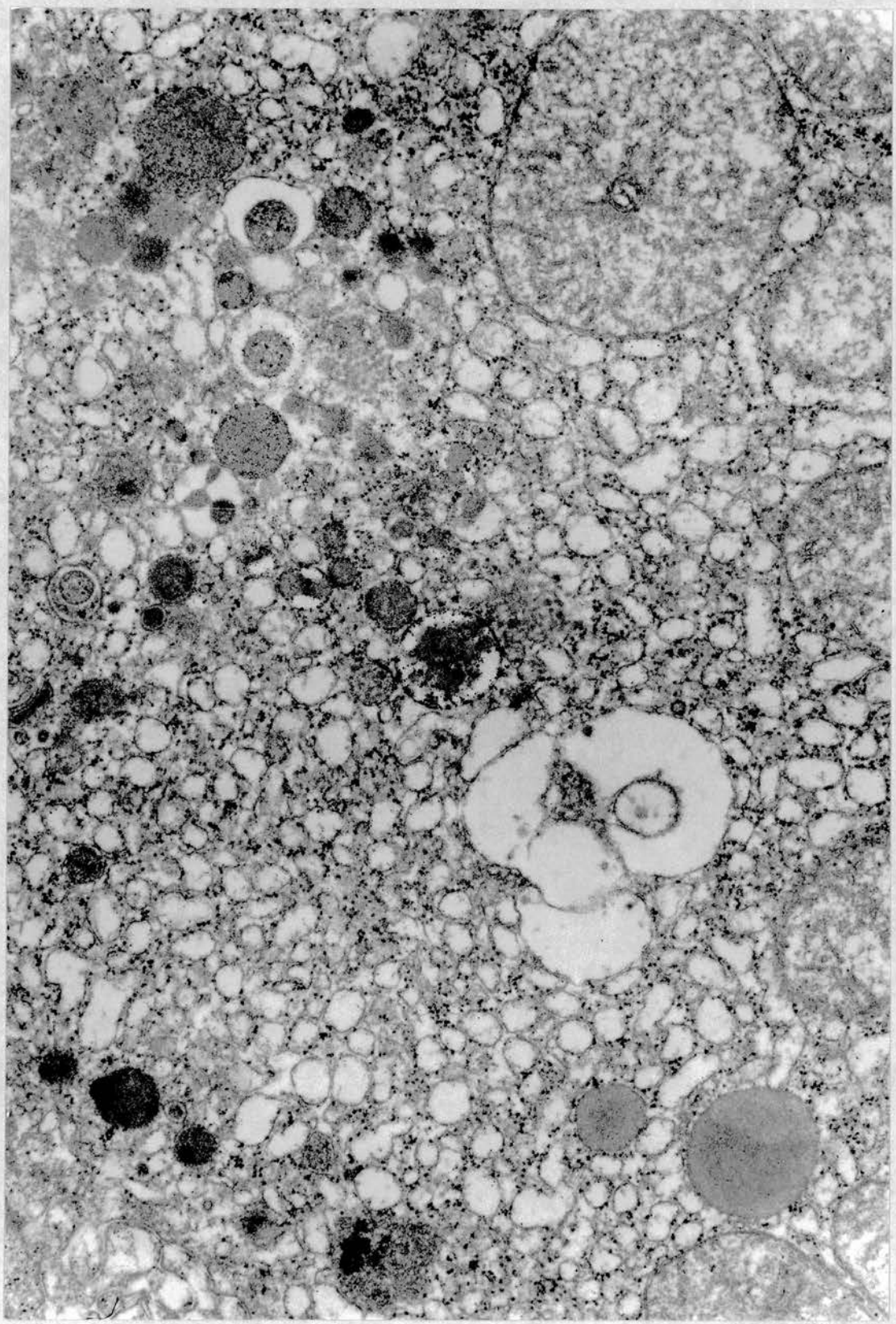


Fig.4.13 6 hours. Midzonal cell. Swelling involving the Golgi apparatus in addition to the ER. There is also some flocculation of the mitochondrial matrix. EM. X21,000

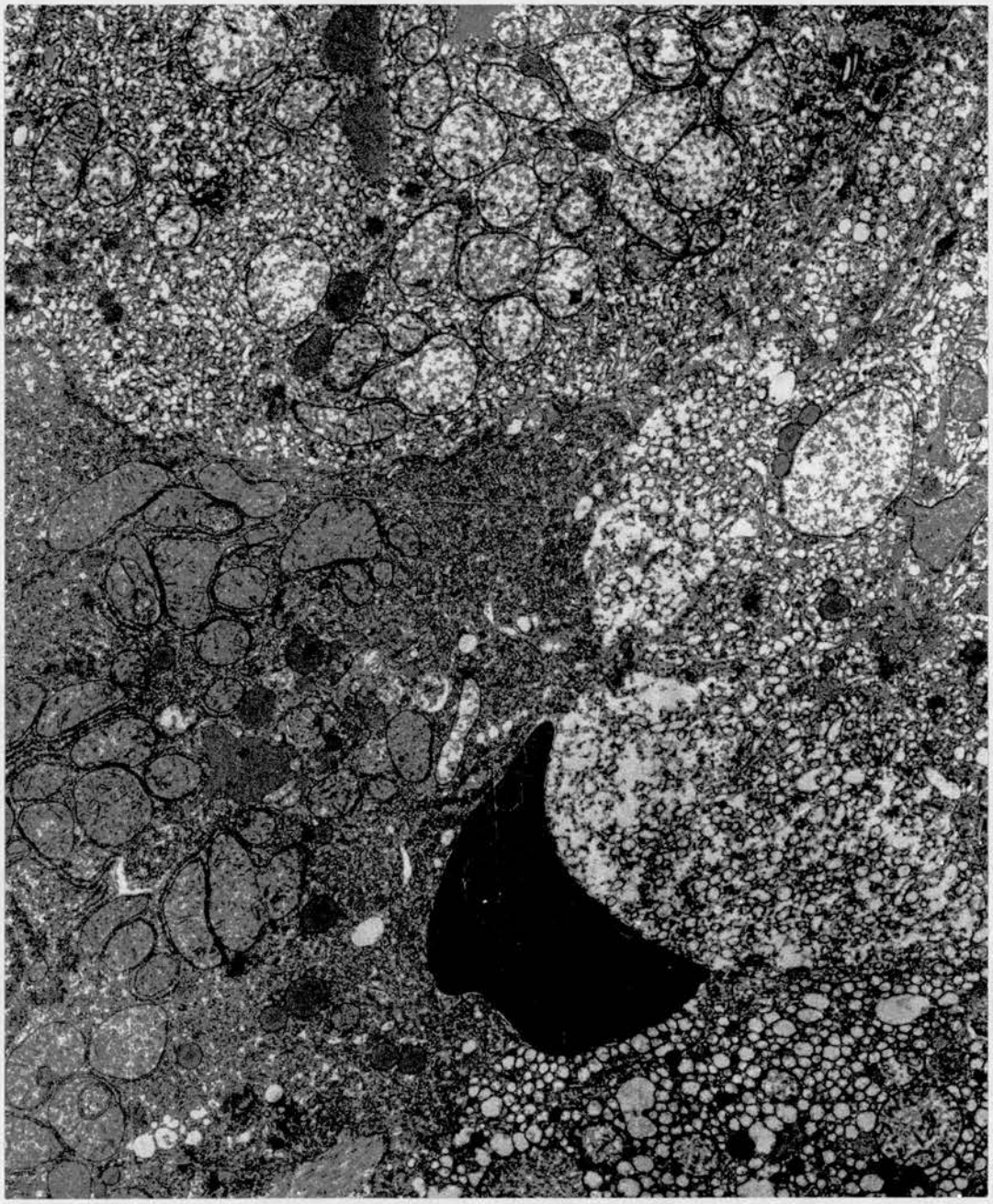


Fig.4.14 6 hours. Three midzonal hepatocytes showing glycogen depletion and varying degrees of dilatation of the canalicular system. This dilatation, together with an increase in cytoplasmic matrix, has led to narrowing of an intervening sinusoid which contains a distorted erythrocyte. EM. X7,950

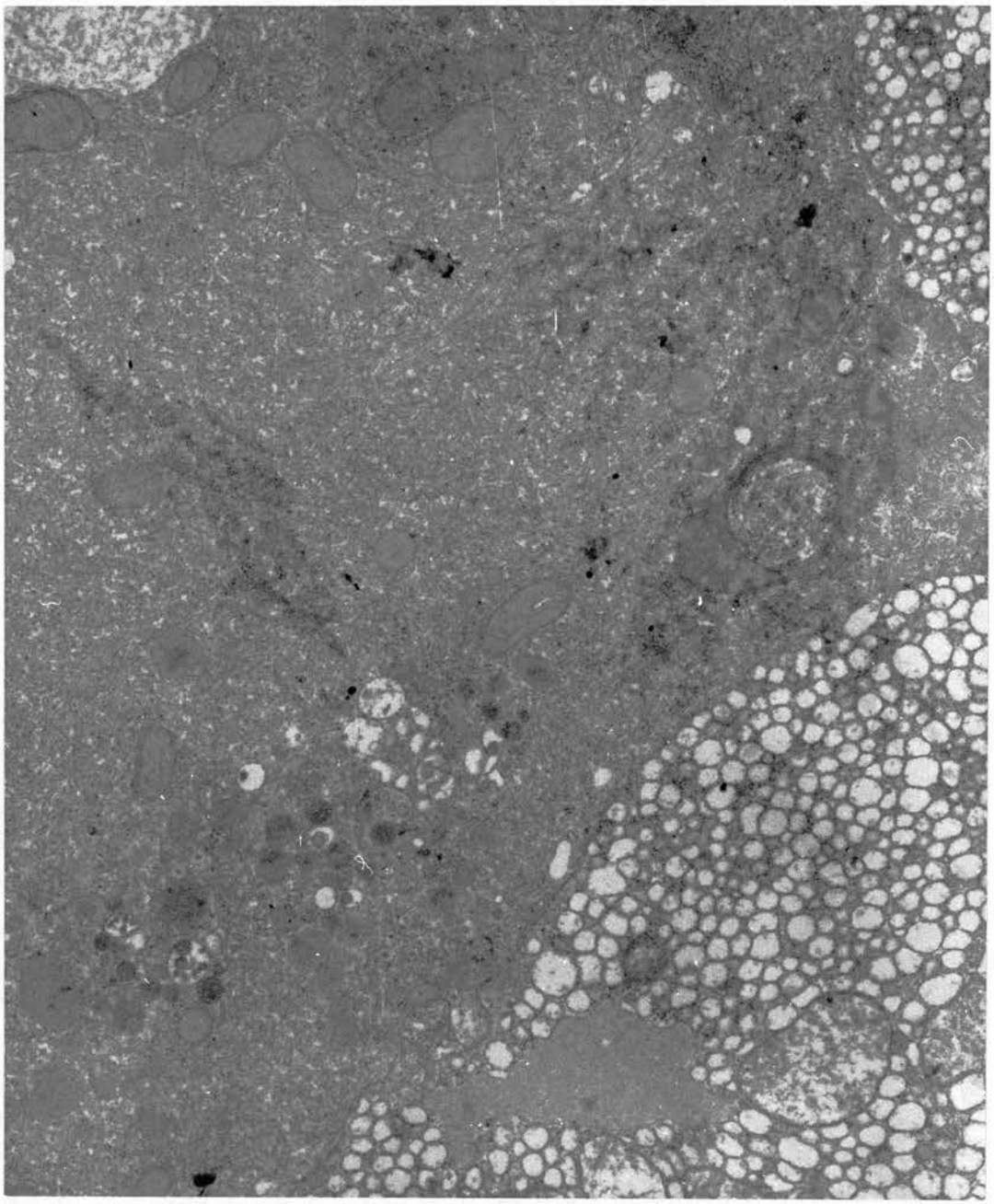


Fig. 4.15 6 hours. Two adjoining cells show a conspicuous difference in dilatation of the E.R., and in one an apparent proliferation of smooth membranes. EM. X12,900

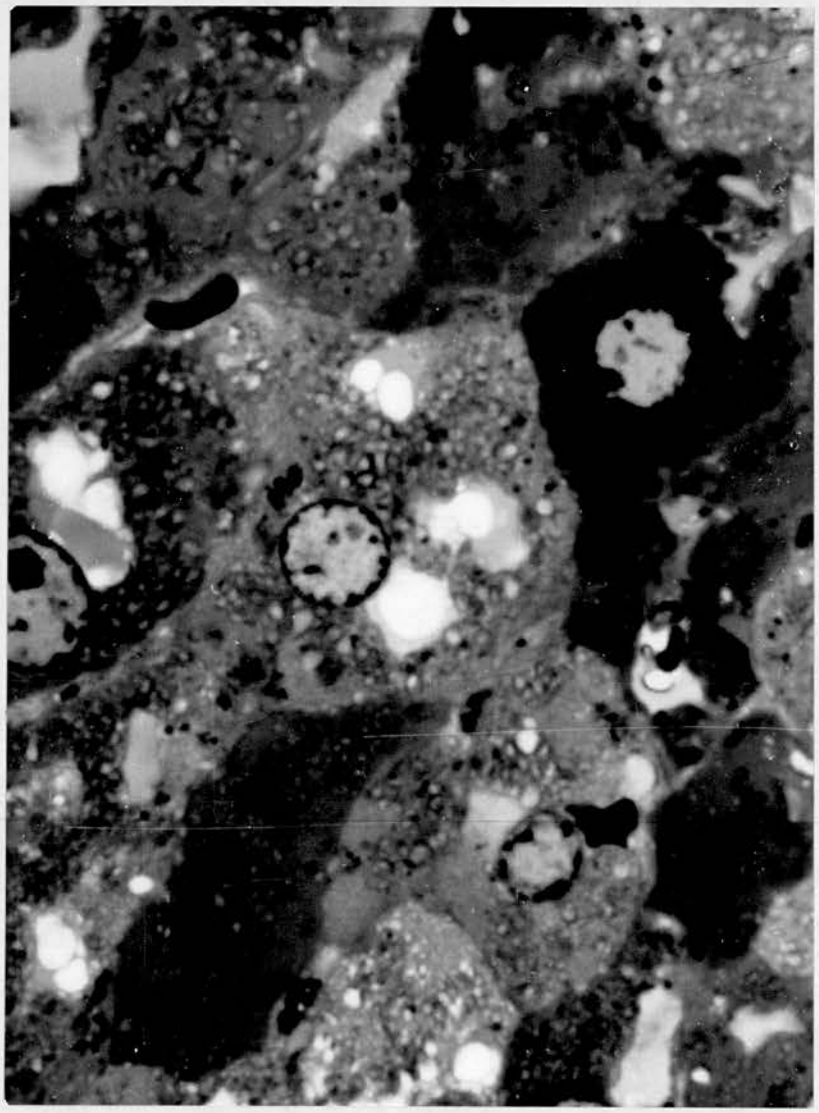


Fig. 4.16 6 hours. More advanced degeneration in pale mid-zonal cells has led to the formation of hydropic vacuoles. STS. AB. X1,600

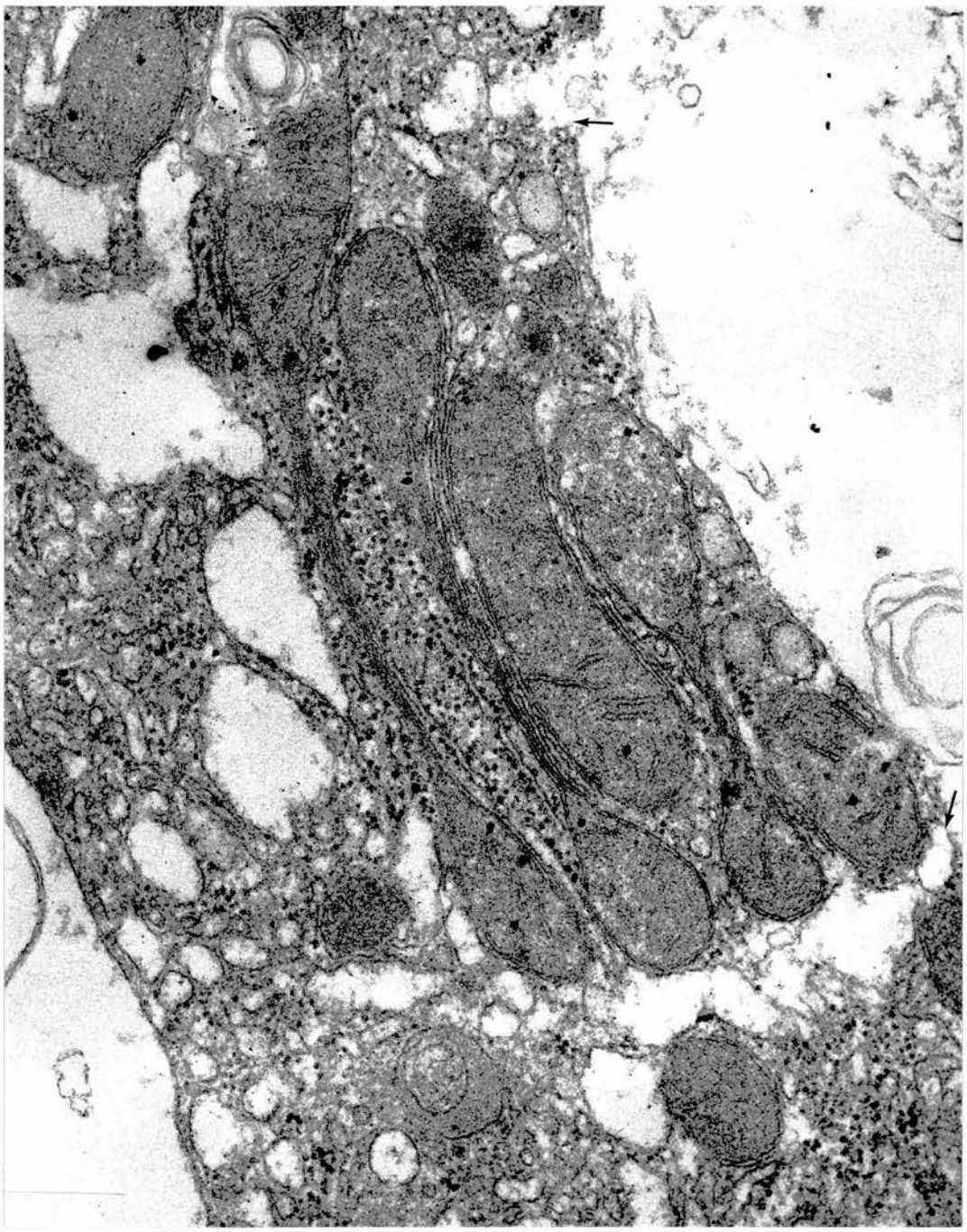


Fig.4.17 6 hours. Detail of a grossly vacuolated hepatocyte showing communications between the swollen E.R. and a large intracellular vacuole, (arrows). EM. X43,605

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swelling and mild vacuolation in midzonal cells (4.19). Two animals, however, reveal much more advanced changes amounting to confluent centrilobular necrosis in the blocks examined (4.20, 4.21,4.22). Such necrosis is expressed ultrastructurally as disintegration of the canalicular system with formation of small electron-clear vesicles, gross mitochondrial swelling accompanied by disruption of cristae, and breakdown of the plasma membrane which appears interrupted (4.23, 4.24). Only a minority of necrotic cells show evidence of previous gross vacuolation. The nuclear changes vary from clumping of chromatin to pyknosis and karyorrhexis.

A few of the "surviving" hepatocytes contain ingested red blood cells (erythrophagocytosis), and hepatocytes immediately adjacent to the necrotic areas contain large clear vacuoles (4.25).

Congestion in sinusoids is prominent especially towards the periphery of the necrotic areas. In addition to large numbers of red blood cells, the sinusoids contain membrane fragments, vesicular structures enclosing remnants of rough endoplasmic reticulum, and occasional small fibrin-like deposits. In one animal there is a marked leucocytic reaction around the necrotic areas.

There is diffuse loss of glycogen in the two severely affected livers involving even the apparently viable periportal cells, and a complete absence of pyroninophilia in the necrotic areas (4.26).

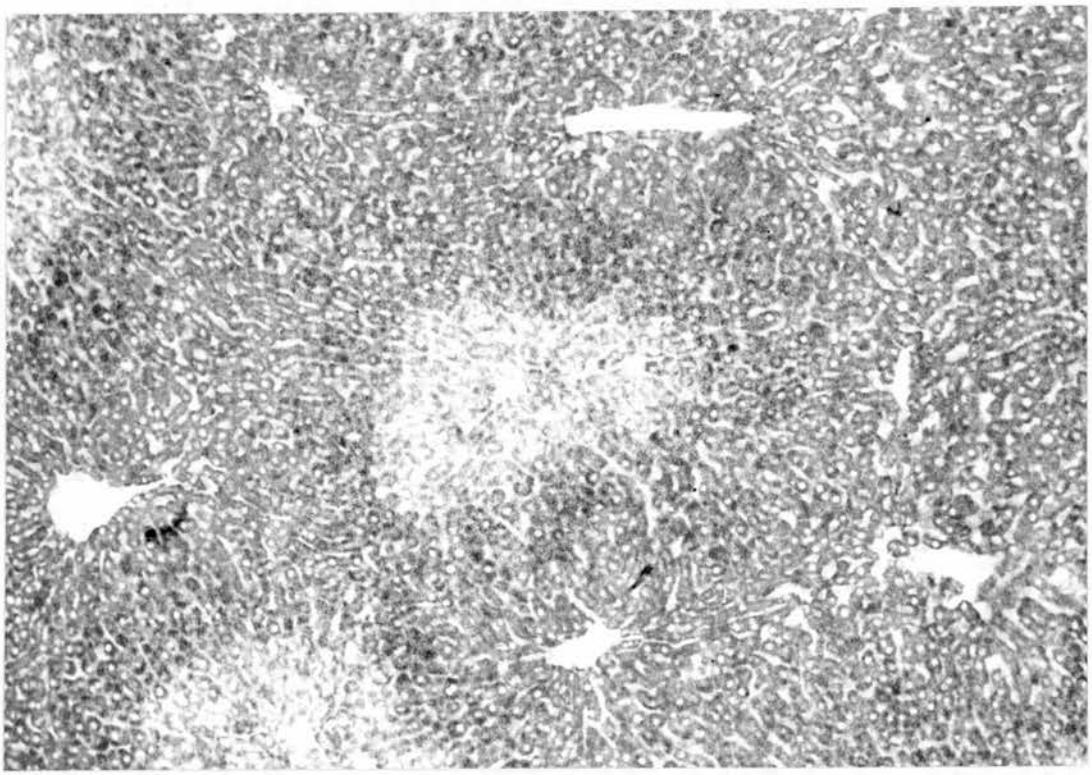

Fig. 4.18 6 hours. Loss of succinate dehydrogenase activity in centrilobular zones. FS. MTT. X100

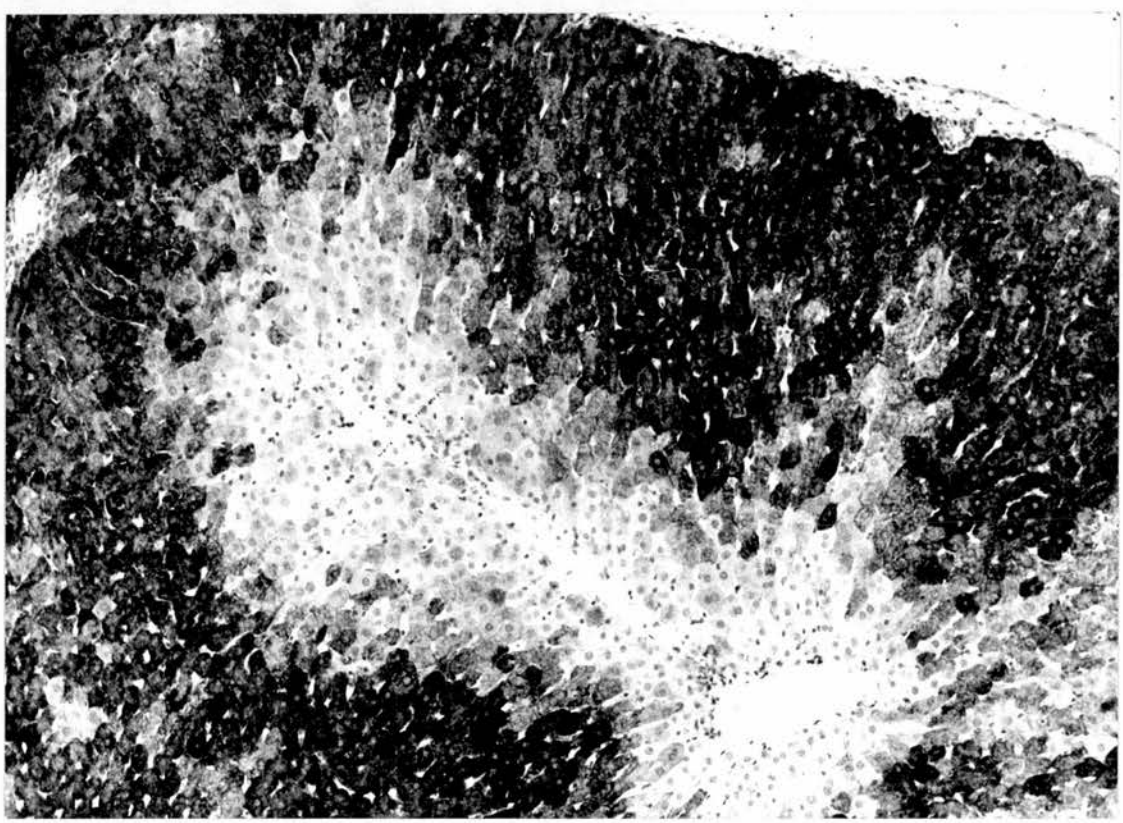

Fig. 4.19 12 hours. Loss of glycogen from centrilobular cells. The mid-zonal (bordering) cells reveal partial loss of glycogen, and in the HE sections show mild vacuolation. FS. PAS. X100

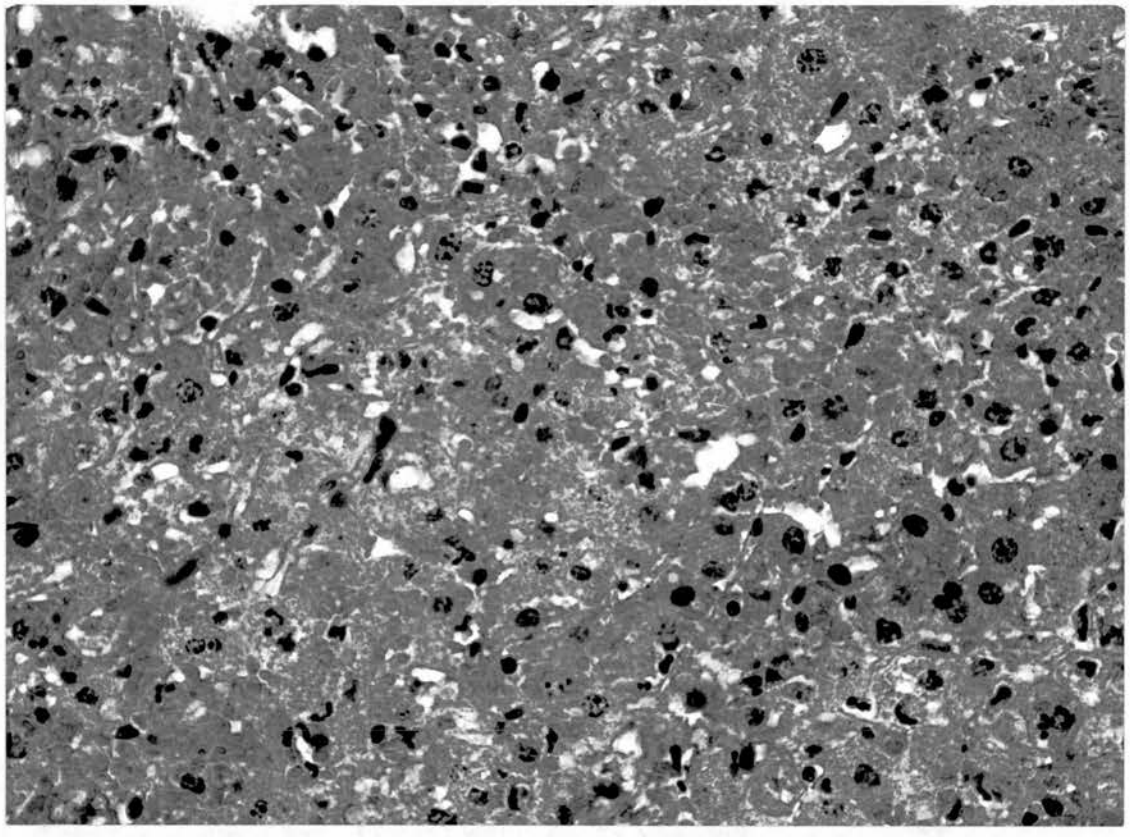

Fig.4.20 12 hours. Early coagulative necrosis of hepatocytes, with clumping of chromatin and nuclear pyknosis and karyorrhexis. PS. HE; X400

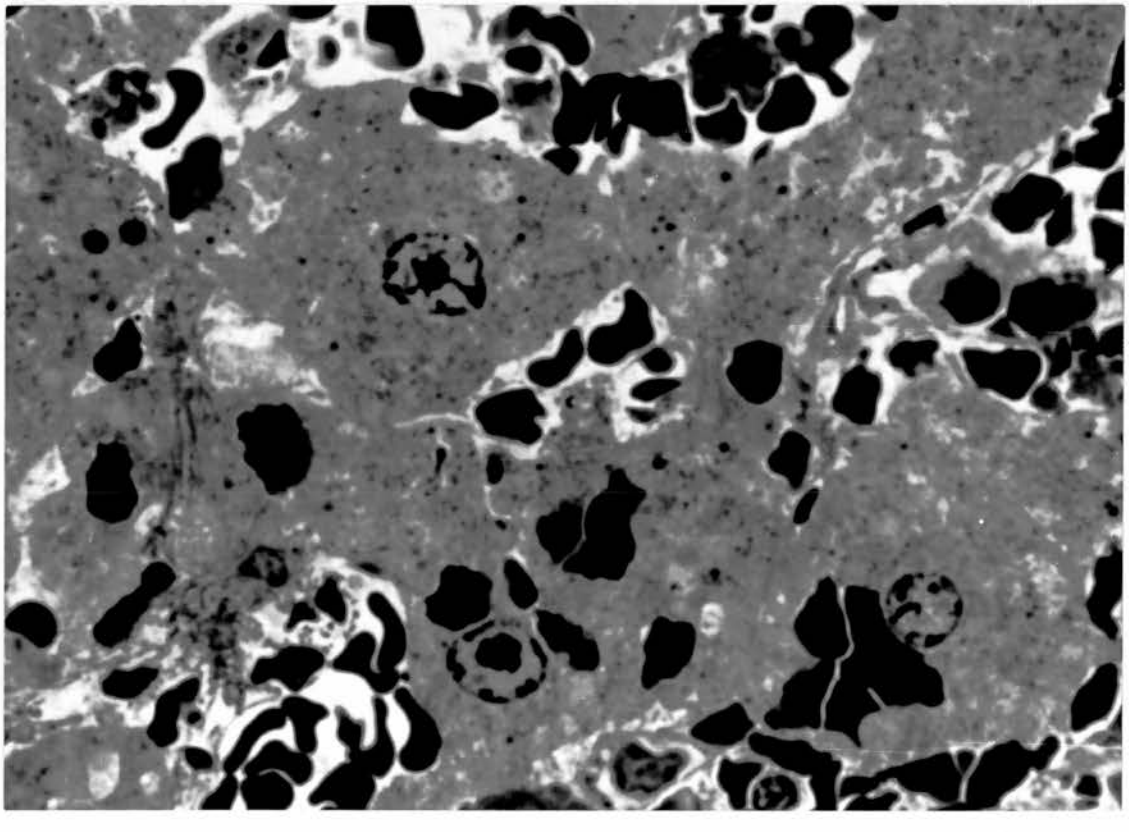

Fig.4.21 12 hours. Centrilobular cells with granular cytoplasm (which showed increased eosinophilia) and indistinct borders. There is marked sinusoidal congestion. STS. AB. X1,600

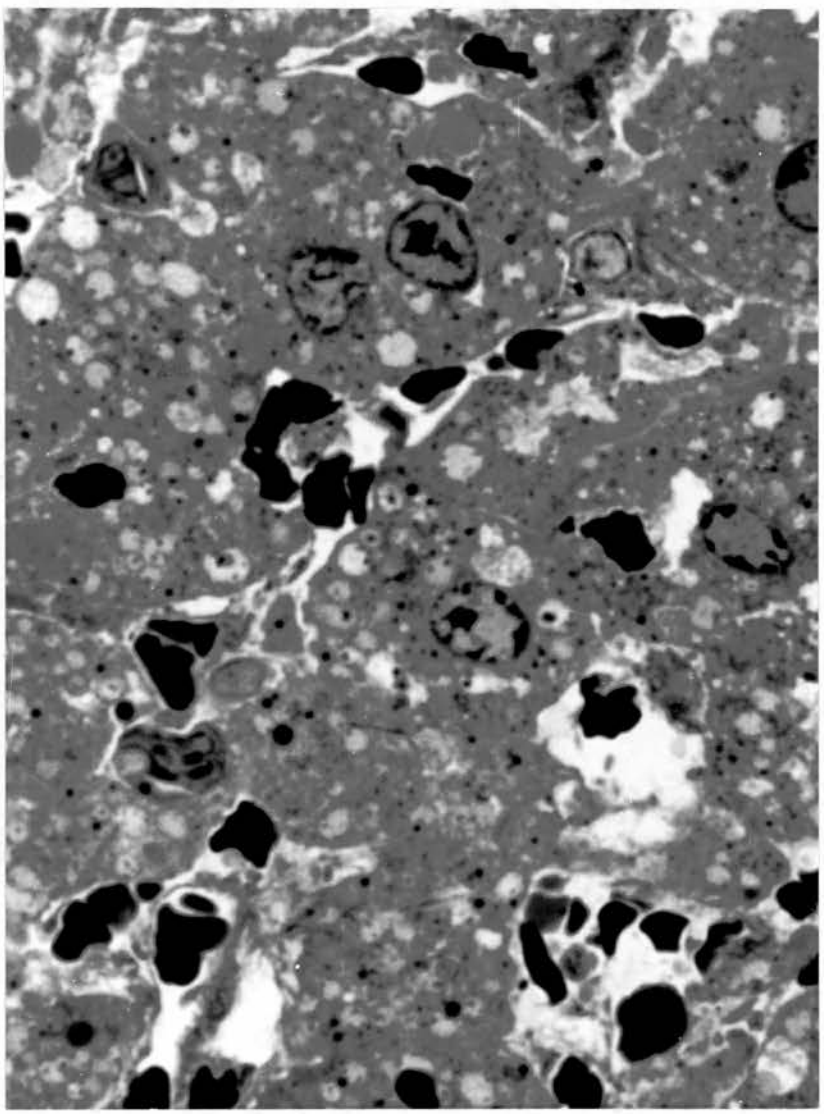

Fig.4.22 12 hours. Dying centrilobular cells which have undergone hydropic vacuolation. STS; AB. X1,600

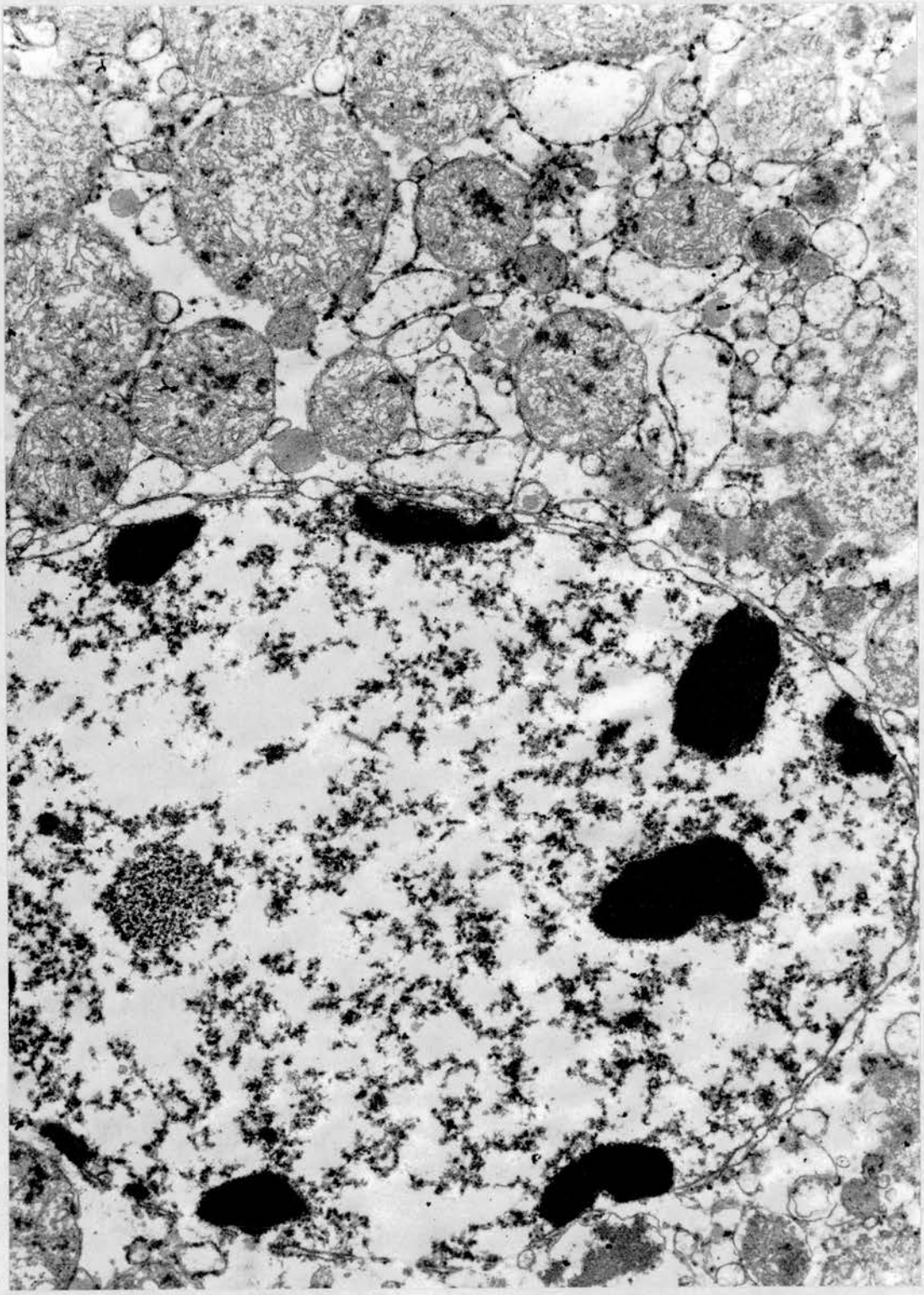

Fig.4.23 12 hours Centrilobular hepatocyte revealing clumping of chromatin, focal expansions of the nuclear membrane, dilatation of the E.R. with vesiculation, and distortion and swelling of mitochondrial cristae. EM. X12,900

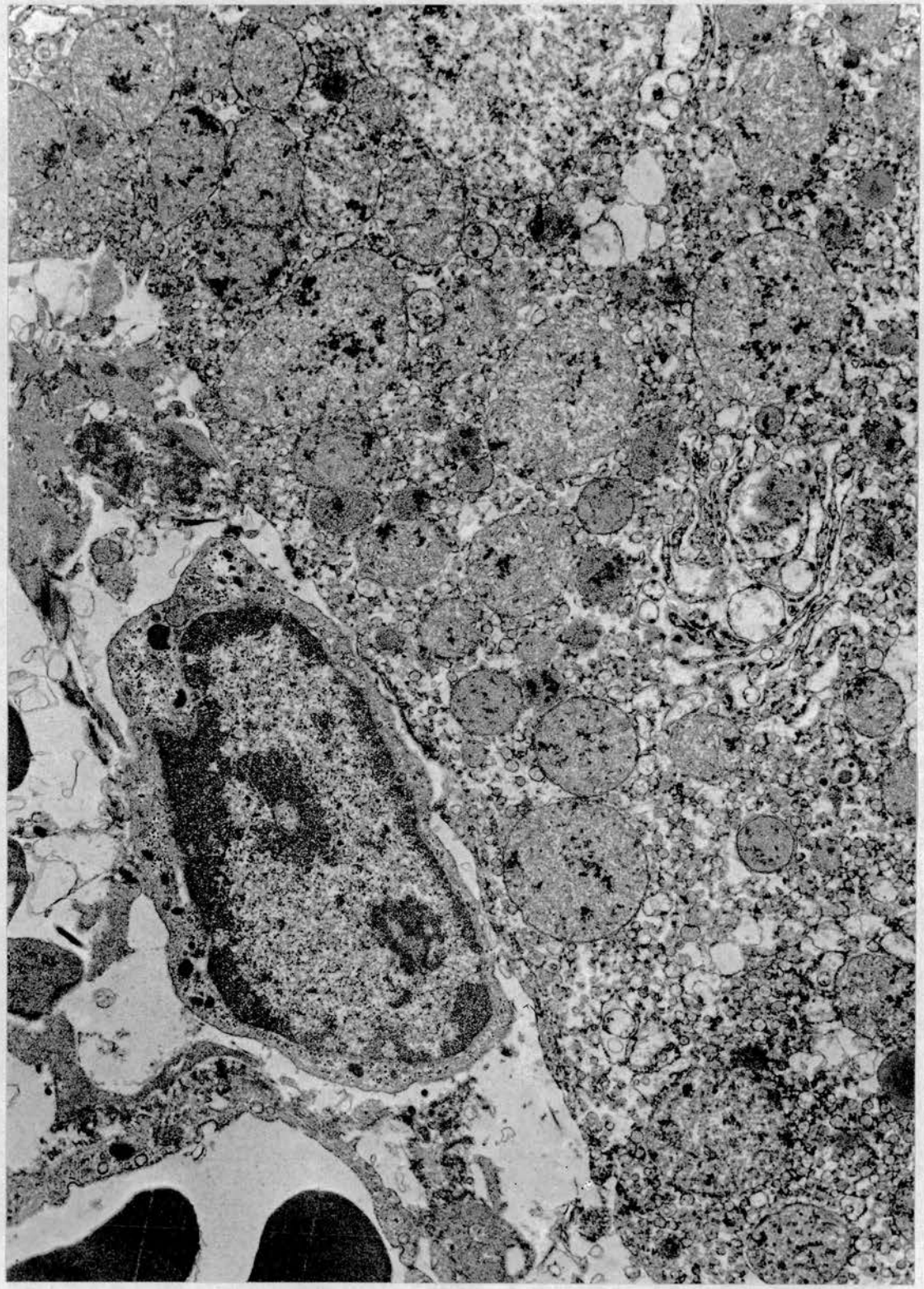

Fig.4.24 12 hours. Part of a centrilobular hepatocyte showing swelling of the matrix and mitochondria and fragmentation of the canalicular system with formation of discrete vesicles. An apparently viable macrophage is seen in the enlarged space of Disse. EM. X12,540

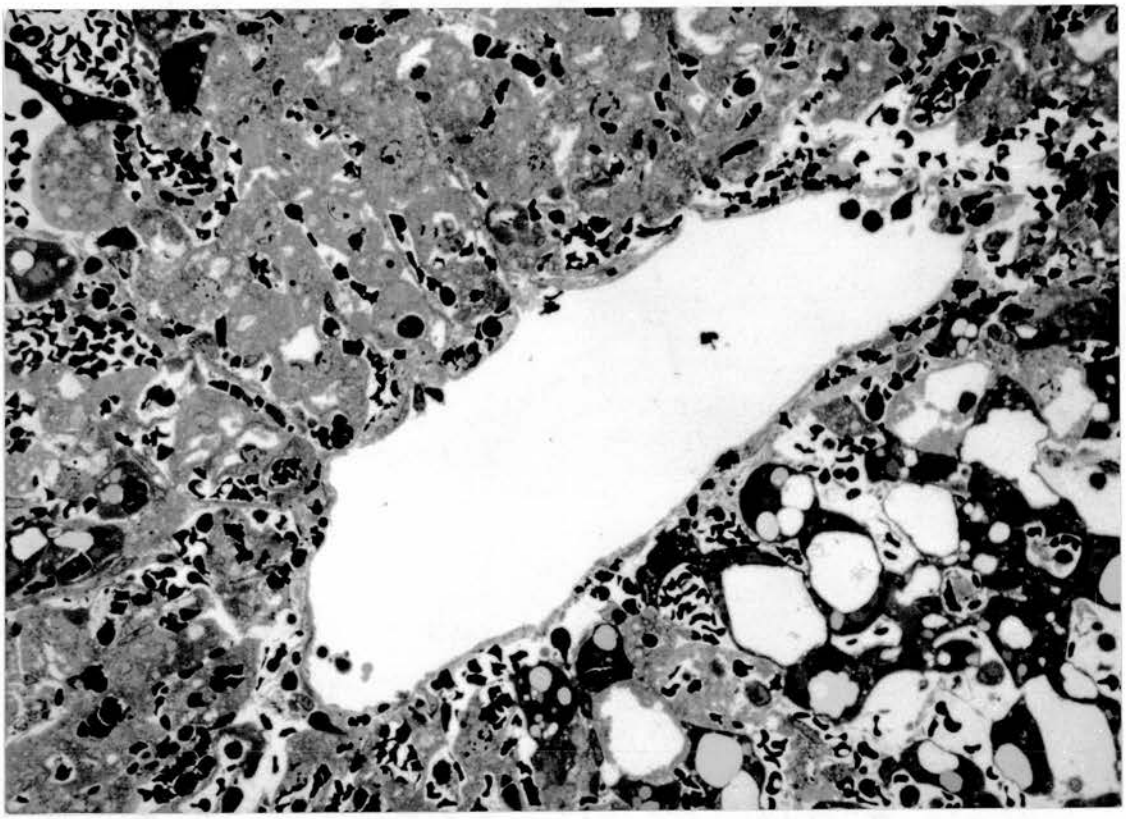

Fig. 4.25 12 hours. Centrilobular area showing coagulative necrosis, intense sinusoidal congestion, and clear vacuolation of some bordering hepatocytes. STS. AB. X400

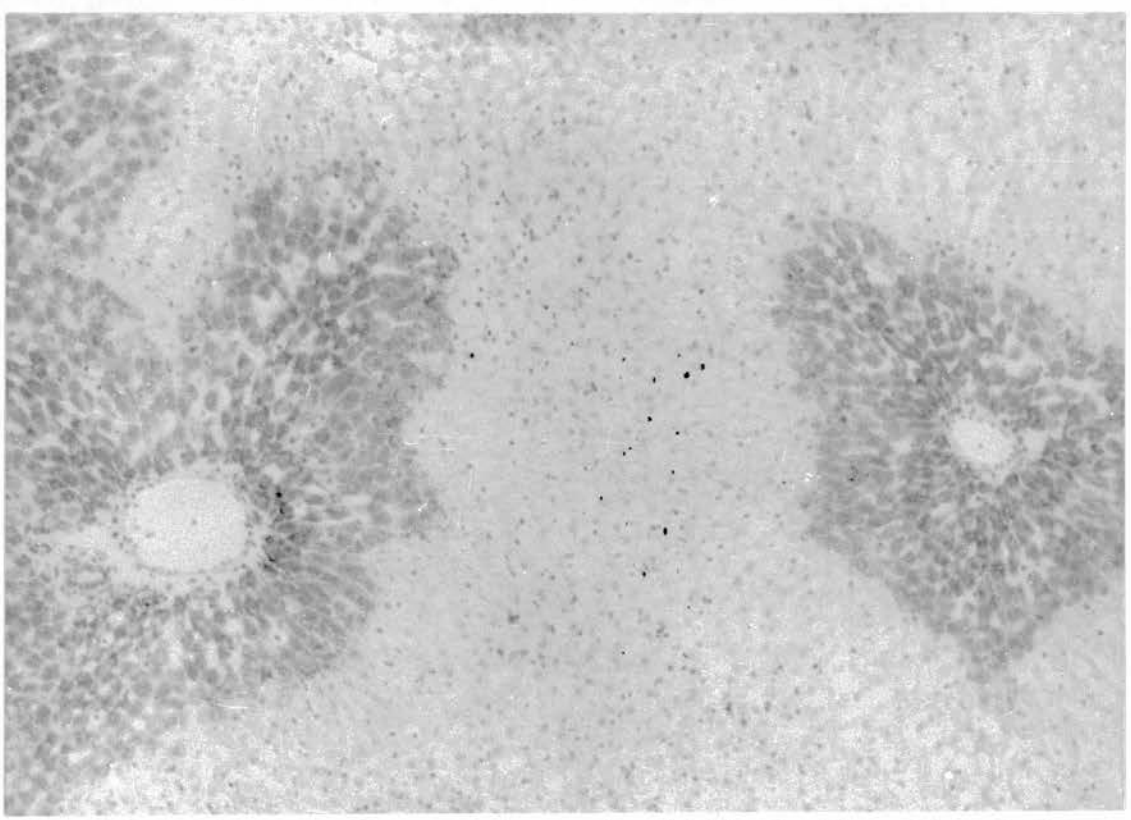

Fig. 4.26 12 hours. Absence of pyroninophilia in the cells of the necrotic zones. FS. MGP. X100

Histochemistry reveals a variety of appearances paralleling the morphological findings. In the MTT preparations, one of the animals appears normal, whilst in another there is patchy increase in size and density of formazan dots in centrilobular areas. The two necrotic livers show widespread loss of enzyme activity with accentuated activity in the few surviving periportal cells.

The acid phosphatase preparations show no change from the control in the two animals with minor histological abnormalities, but in the other two, coarse clumping of granules in periportal cells and a progressive loss of activity towards the central veins in necrotic hepatocytes is seen.

Fat stains on the necrotic livers show an increase in small lipid globules in surviving hepatocytes. The other livers show no abnormality.

24-hr group. These animals have a more uniform appearance than the 12-hr group in regard to both the extent and progress of the lesion.

All show more advanced coagulative necrosis. The plasma membranes of affected hepatocytes are frequently absent and it is often difficult to distinguish individual cell borders. The sinusoids contain numerous multivesicular bodies. Nuclear degeneration is most apparent in this group and karyorrhexis is frequently seen. The necrotic areas are infiltrated by small numbers of neutrophil polymorphs and macrophages, many of which contain

phagocytosed cytoplasmic debris.

A few hepatocytes bordering the necrotic areas have an acidophilic shrunken appearance with condensed pyknotic nuclei. On electron microscopy in addition to condensation of the nucleus and swelling of the nuclear membrane, the cytoplasm contains numerous small vacuoles separated by a dense matrix (4.27). These distinctive degenerative changes which do not seem to involve breakdown of the endoplasmic reticulum, represent the ultrastructural features of "shrinkage necrosis" a form of cell death resulting in the formation of spherical acidophilic (Councilman) bodies composed of compacted organelles and sometimes nuclear material (Kerr, 1971). Acidophil bodies are scanty but are much more readily found than in controls (4.28).

The necrotic zones are also bordered by dense cells either deeply indented by large extracellular vacuoles or apparently containing intracellular vacuoles which often impinge on the nucleus (4.29). The latter type contain loosely-packed electron-dense amorphous material but, as determined by serial sections, are in continuity with the clear peripheral and extracellular vacuoles. The plasma membrane lining many of the vacuoles is clearly seen. The cytoplasm of these vacuolated cells is glycogen depleted but contains well formed, regular, rough-surfaced endoplasmic reticulum frequently surrounding mitochondria. The mitochondria are of normal size but have a very dense matrix and slightly irregular cristae (4.30, 4.31, 4.32). The

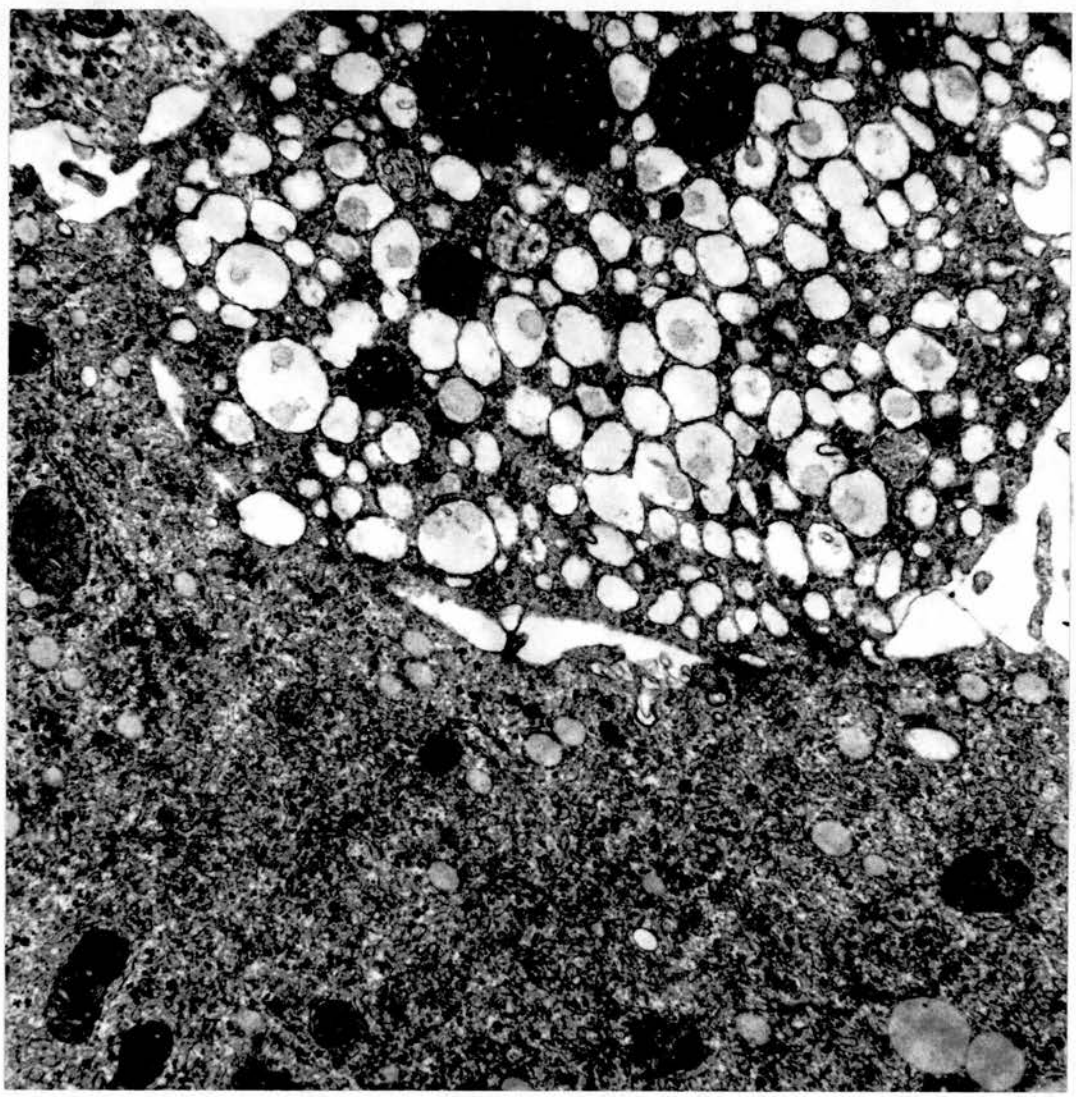

Fig. 4.27 24 hours. Part of a shrunken hepatocyte in which there is increased mitochondrial density and regular vesiculation of the E.R. EM. X16,500

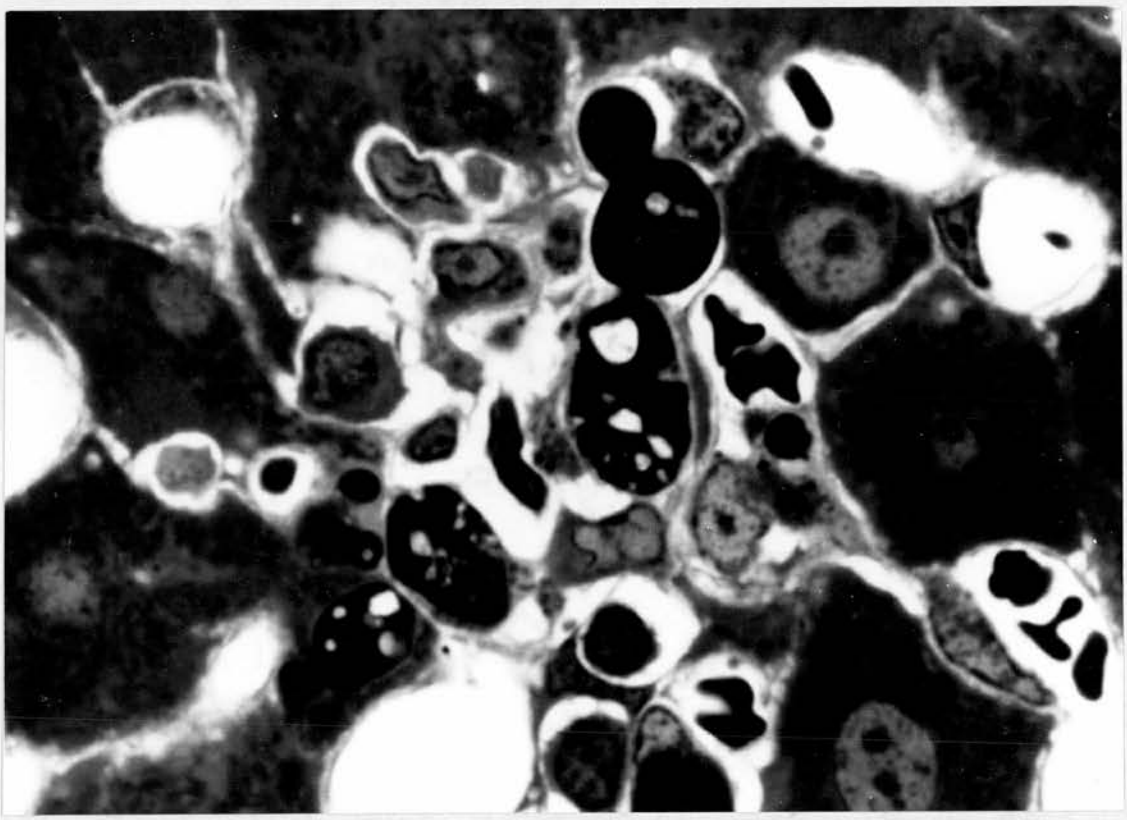

Fig.4.28 24 hours. A group of four acidophil bodies in an area of early macrophage infiltration. The upper body shows apparent "budding". STS. AB. X1,600

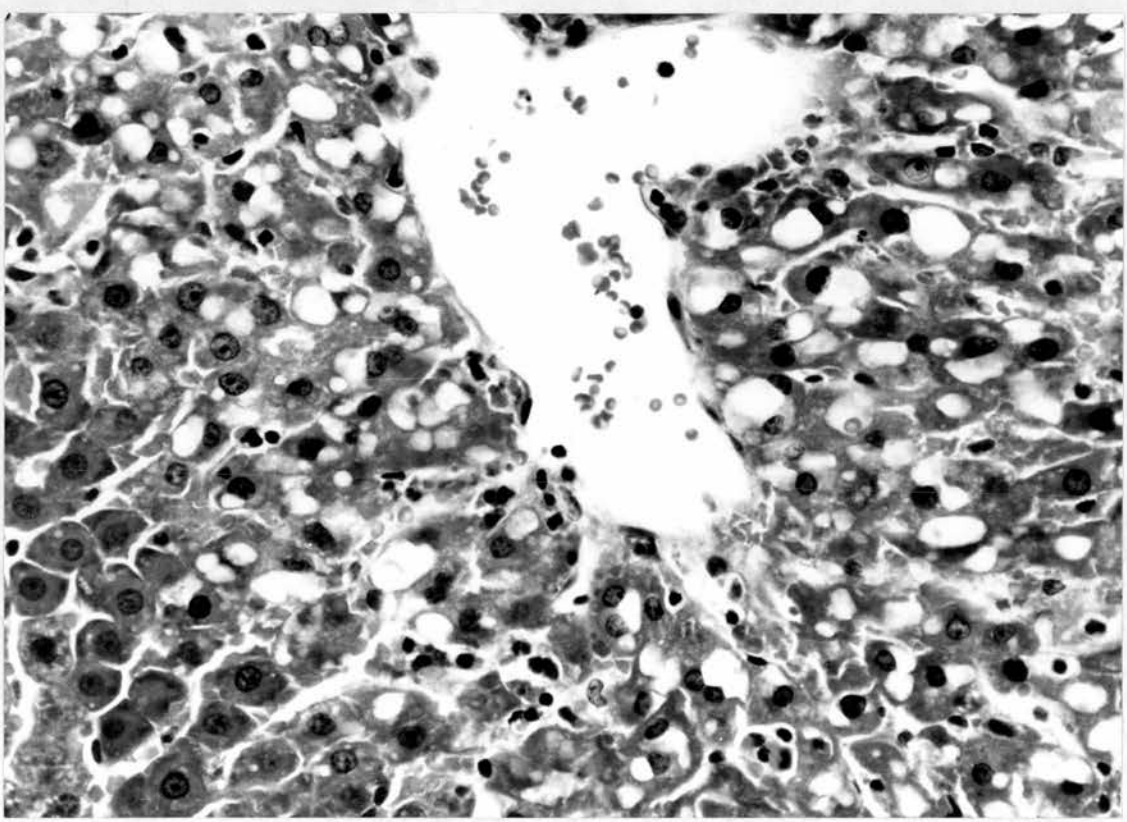

Fig.4.29 24 hours. Centrilobular area containing large clear vacuolated cells. Similar cells were found around the margins of the necrotic zones. PS; MSB. X400

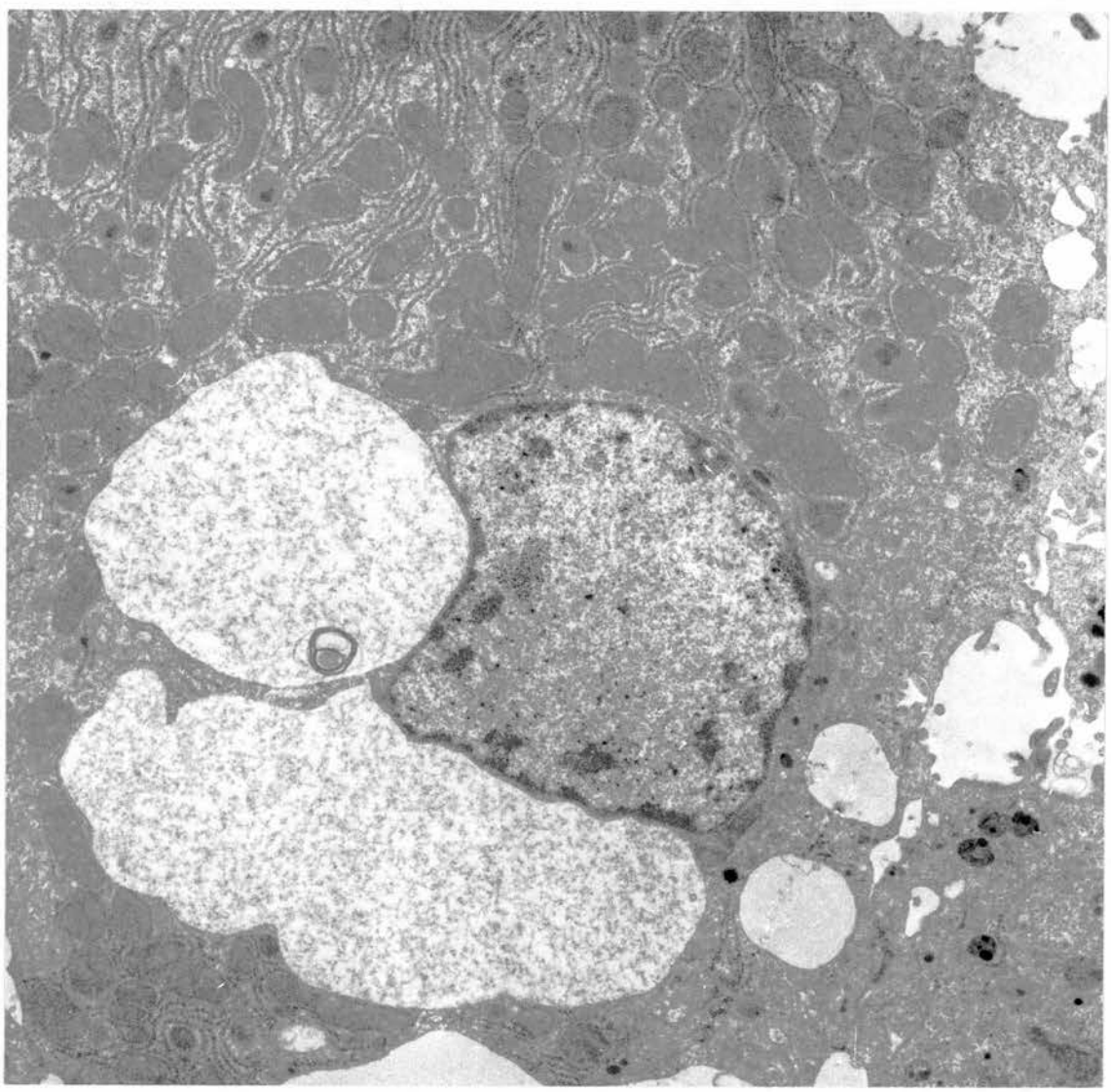

Fig.4.30 24 hours. A vacuolated cell containing regular arrays of R.E.R and dense mitochondria, with no evidence of aqueous swelling elsewhere in the cell. EM. X9,250

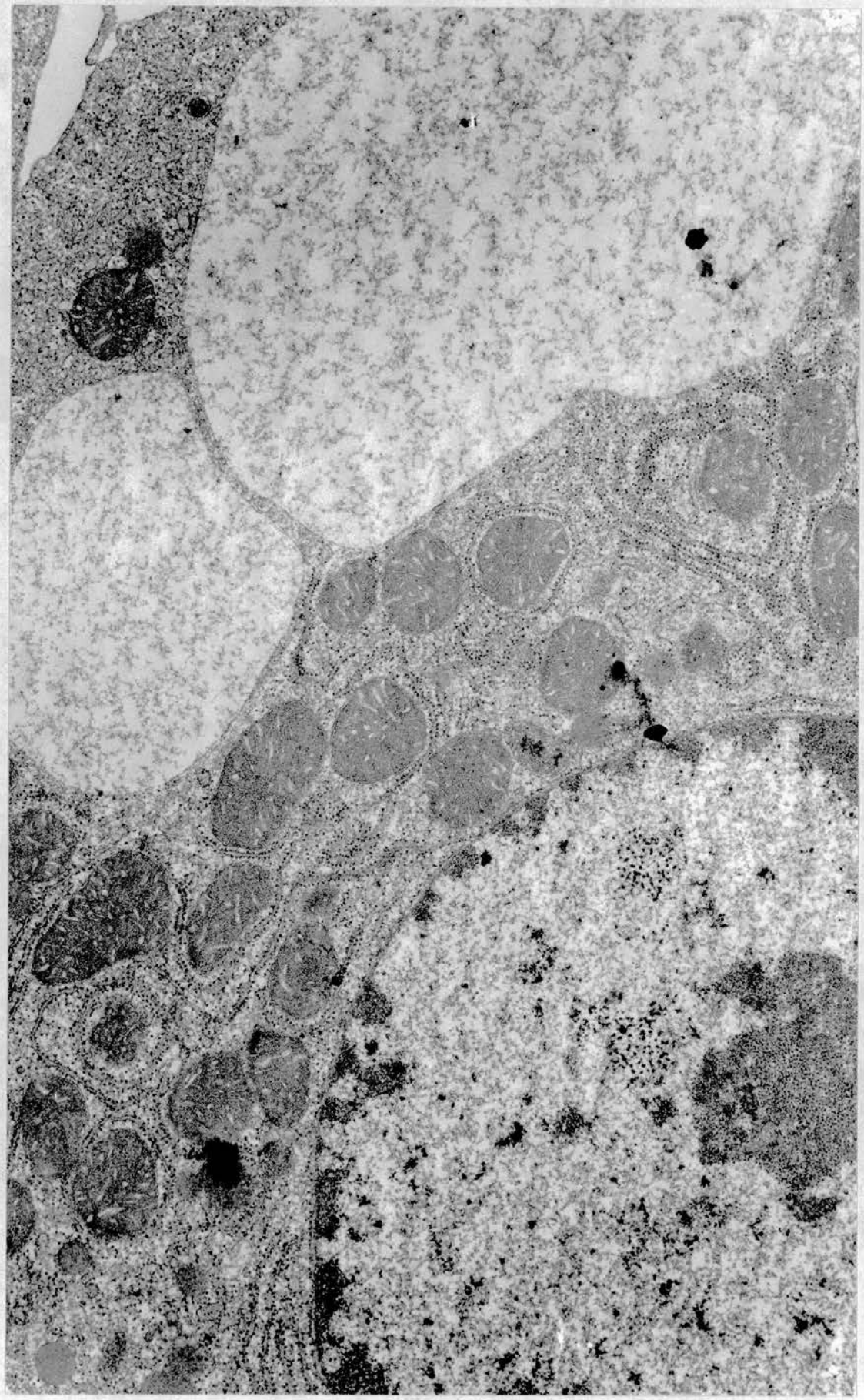

Fig.4.31 24 hours. Vacuolated hepatocyte. The single plasma membrane lining the vacuoles can be identified at this magnification. EM. X12,540

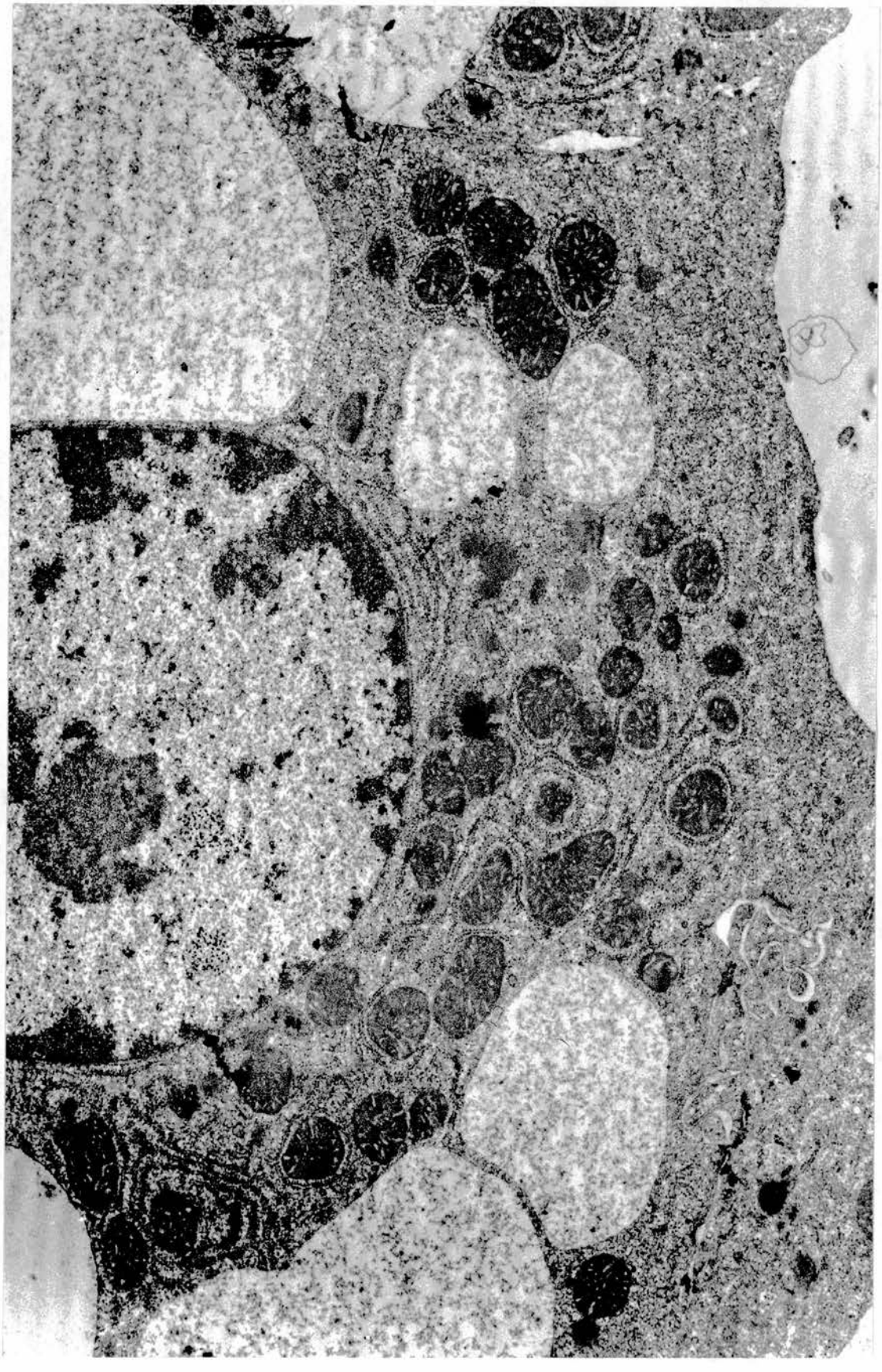

Fig.4.32 24 hours. Part of a vacuole lying between two adjacent cells is included in this field (arrowed). EM. X12,540

microvilli are diminished in size and number.

In addition to vacuolated cells, other hepatocytes adjacent to the necrotic areas contain increased numbers of fine, dispersed lipid globules, and a few reveal large, pale globules which are faintly Schiff-positive in the PAS stain (4.33).

The MTT preparations contain bands of extreme pallor indicative of almost complete loss of activity in the necrotic zones. These areas are surrounded by a narrow band of exaggerated activity, the bordering hepatocytes containing increased numbers of large formazan dots. The periportal cells remain normal (4.34).

Loss of acid phosphatase activity has progressed so that the majority of necrotic cells contain only a few coarse, dense granules in the paracanalicular areas and some cells show a complete loss of activity. In addition, macrophages contain discrete granules of variable size and some Kupffer cells show increased activity. Occasional heavily stained spherical "bodies" are seen within macrophages and these presumably represent heterolysosomes or ingested acidophil bodies.

48-hr group. Although necrosis is fully established in each liver there is moderate variability in its extent. The smaller necrotic areas contain numerous discrete deposits of eosinophilic debris apparently within the cytoplasm of macrophages (4.35). The larger areas show persistence of amorphous cellular debris, and infil tration by macrophage

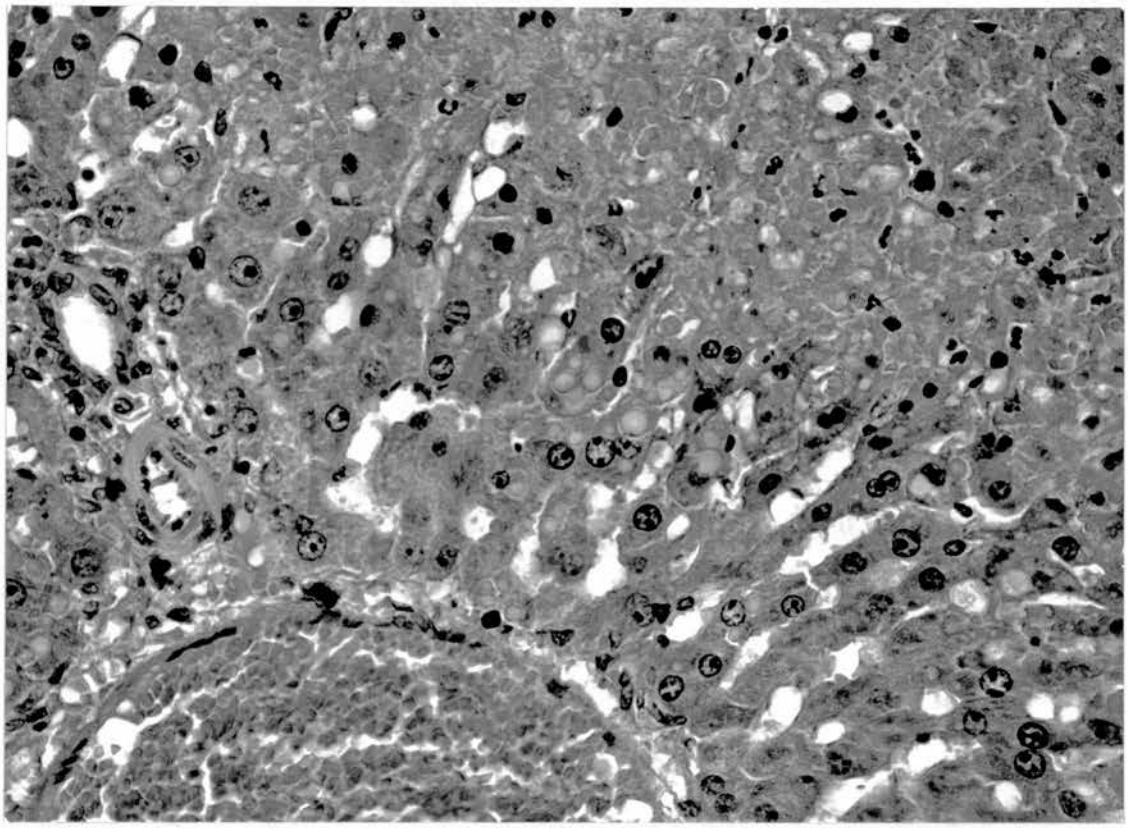

Fig.4.33 24 hours. Pale staining "globules" in bordering hepatocytes. PS. HE. X400

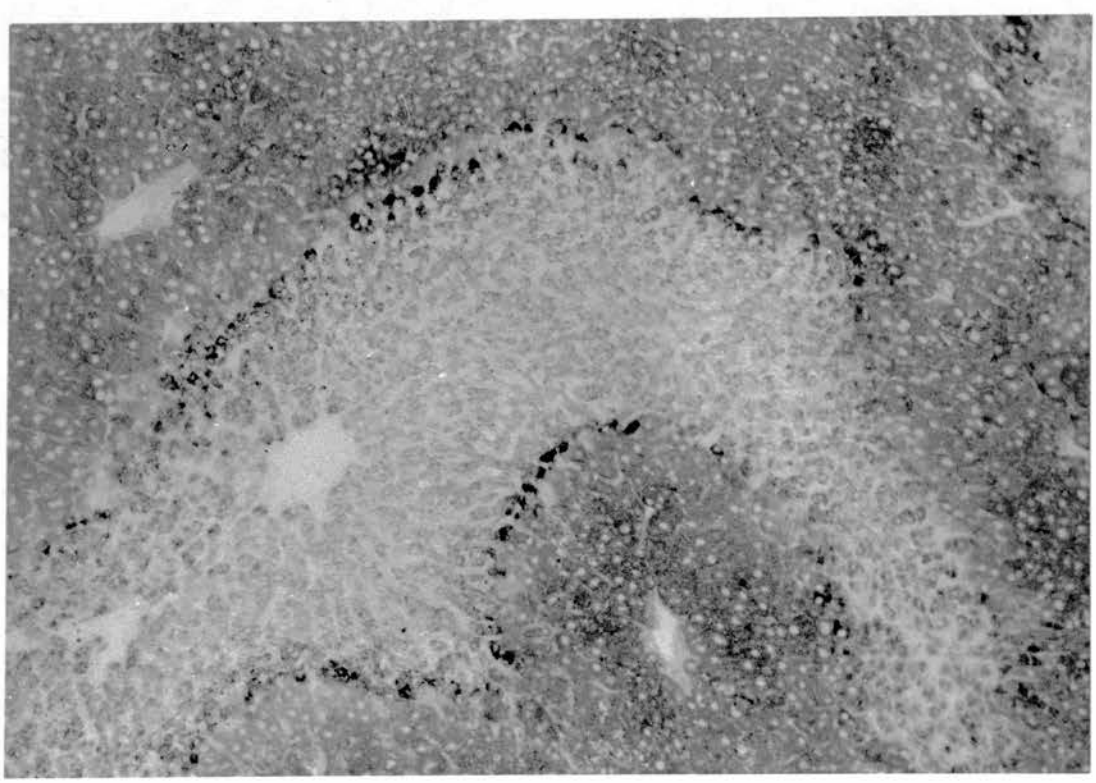

Fig.4.34 24 hours. Loss of succinate dehydrogenase activity in the necrotic zones with a narrow band of heightened activity in bordering cells. FS. MTT. X100

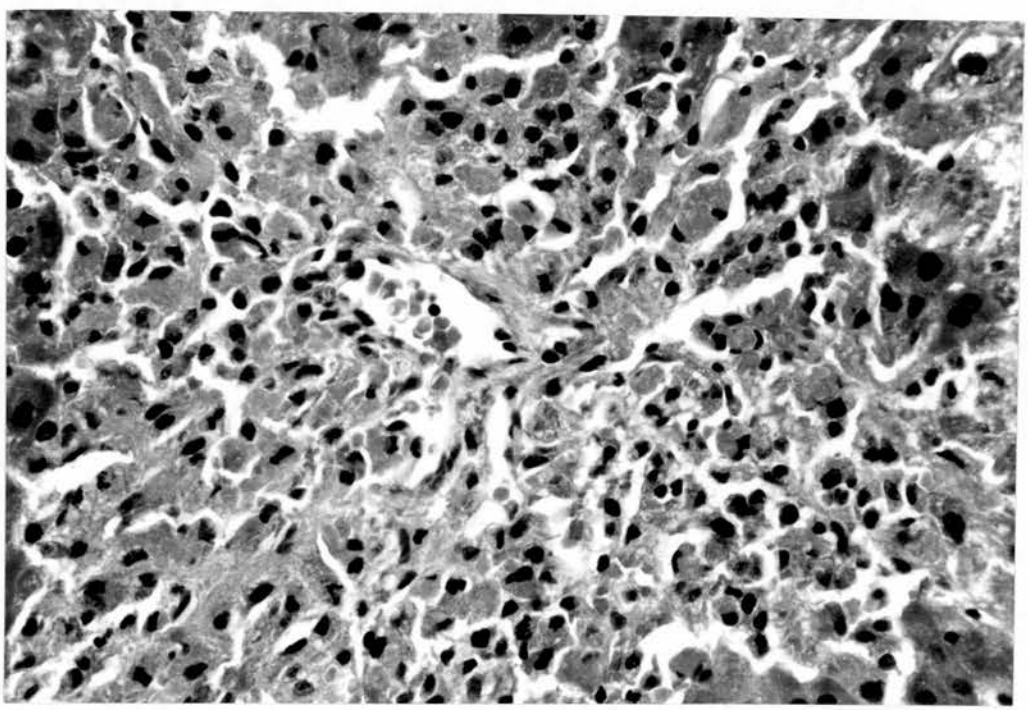

Fig. 4.35 48 hours. Centrilobular area infiltrated by macrophages which have phagocytosed much of the cellular debris. PS. HE. X100

is less pronounced.

Electron microscopy reveals spherical collections of cyto plasmic debris in which degenerate mitochondria are the predominant recognisable particle. Such collections are found either within macrophages or in the process of engulfment by macrophages (4.36). Other particulate cellular debris is seen in macrophages (4.37) or lying free in sinusoids. After phagocytosis, the collections of debris become aggregated and large, generally spherical, electron-dense inclusions are formed.

The necrotic areas are devoid of glycogen and show marked loss of pyroninophilia, surrounding hepatocytes show loss of glycogen but the midzonal and periportal cells have, in the main, a normal glycogen content.

Transitional forms between irregularly shaped, shrunken hepatocytes and spherical acidophil bodies are seen. The bodies are found either within macrophages (4.38), in hepatocytes, or more frequently, lying free in sinusoids around the margins of the necrotic zones. Electron microscopy shows that they are composed of compacted mitochondria, endoplasmic reticulum with ribosomes, and a variety of small clear vesicles (4.39, 4.40). Nuclear remnants are not identifiable. In some instances, the intracellular bodies show evidence of continuing breakdown when the internal components are much more electrondense and less distinct (4.41).

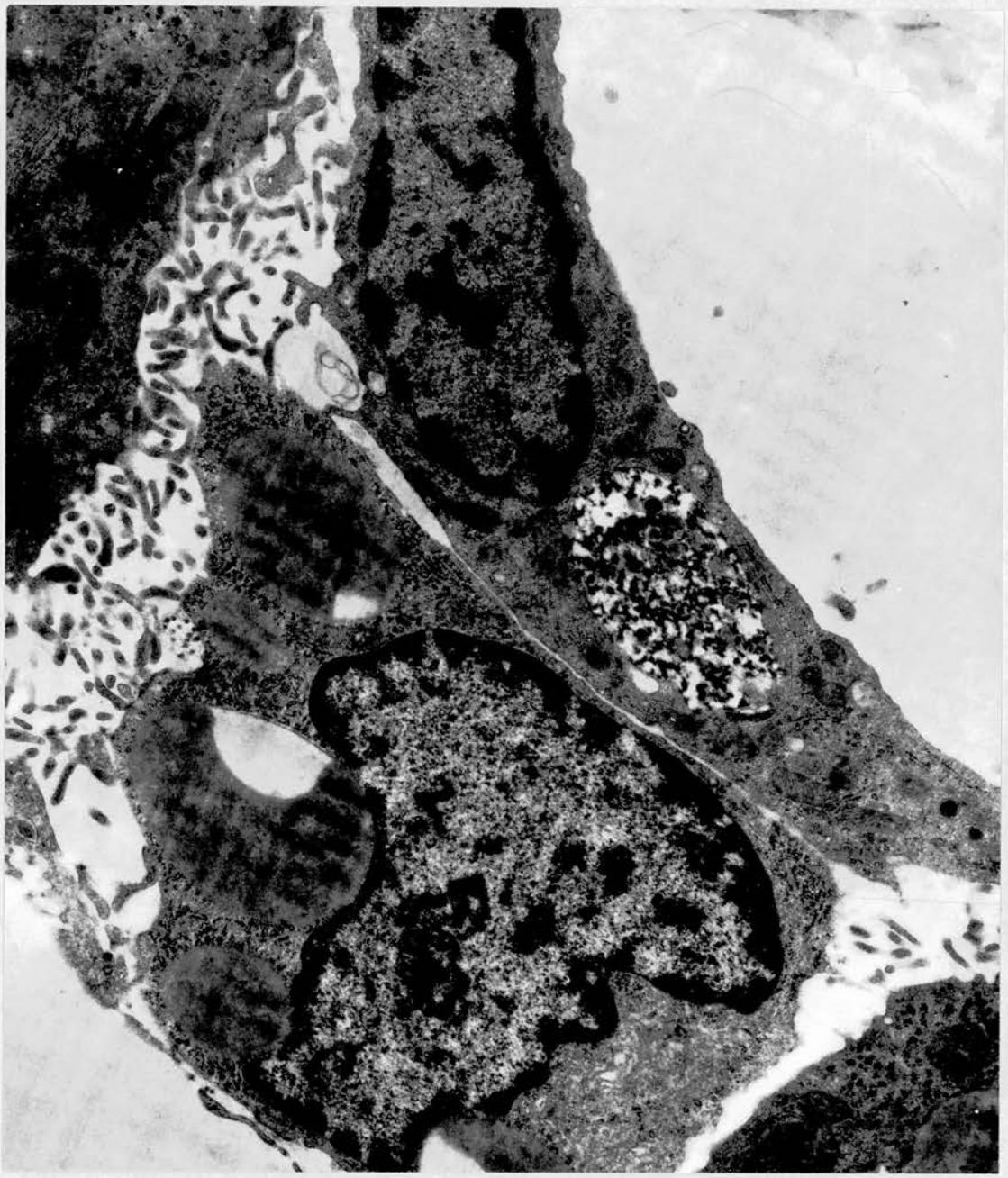

Fig. 4.36 48 hours. Large spherical phagosome in the cytoplasm of a sinusoid-lining macrophage. EM. X10,750

Fig.4.37 48 hours. Particulate debris (arrowed) within a macrophage. Most appears to be aggregated in poorly-defined phagosomes. EM. X10,750

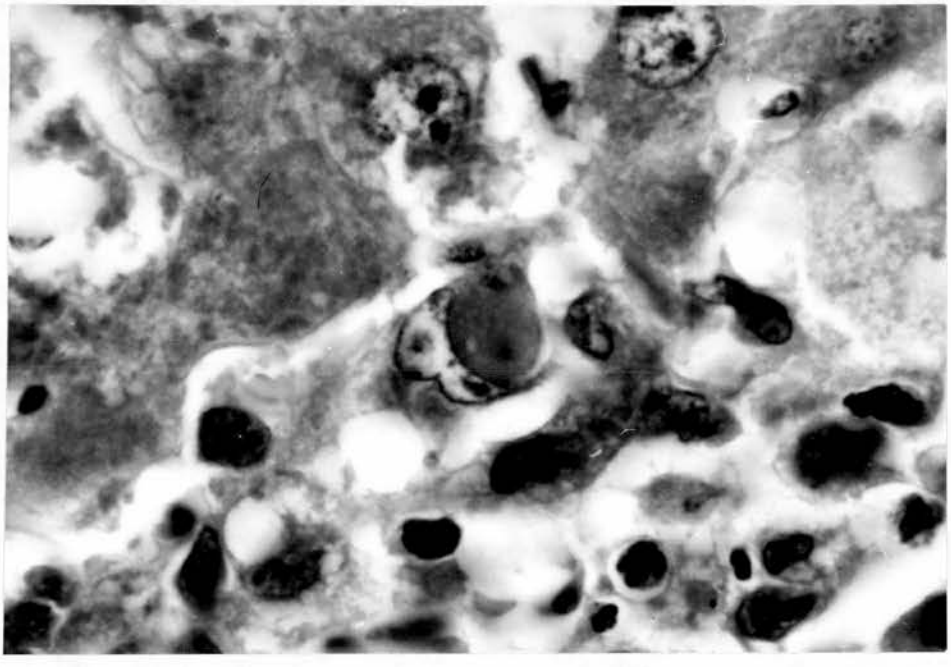

Fig.4.38 48 hours. Macrophage containing a spherical acidophil (apoptotic) body which is indenting the bilobed nucleus.
PS. MSB. X640

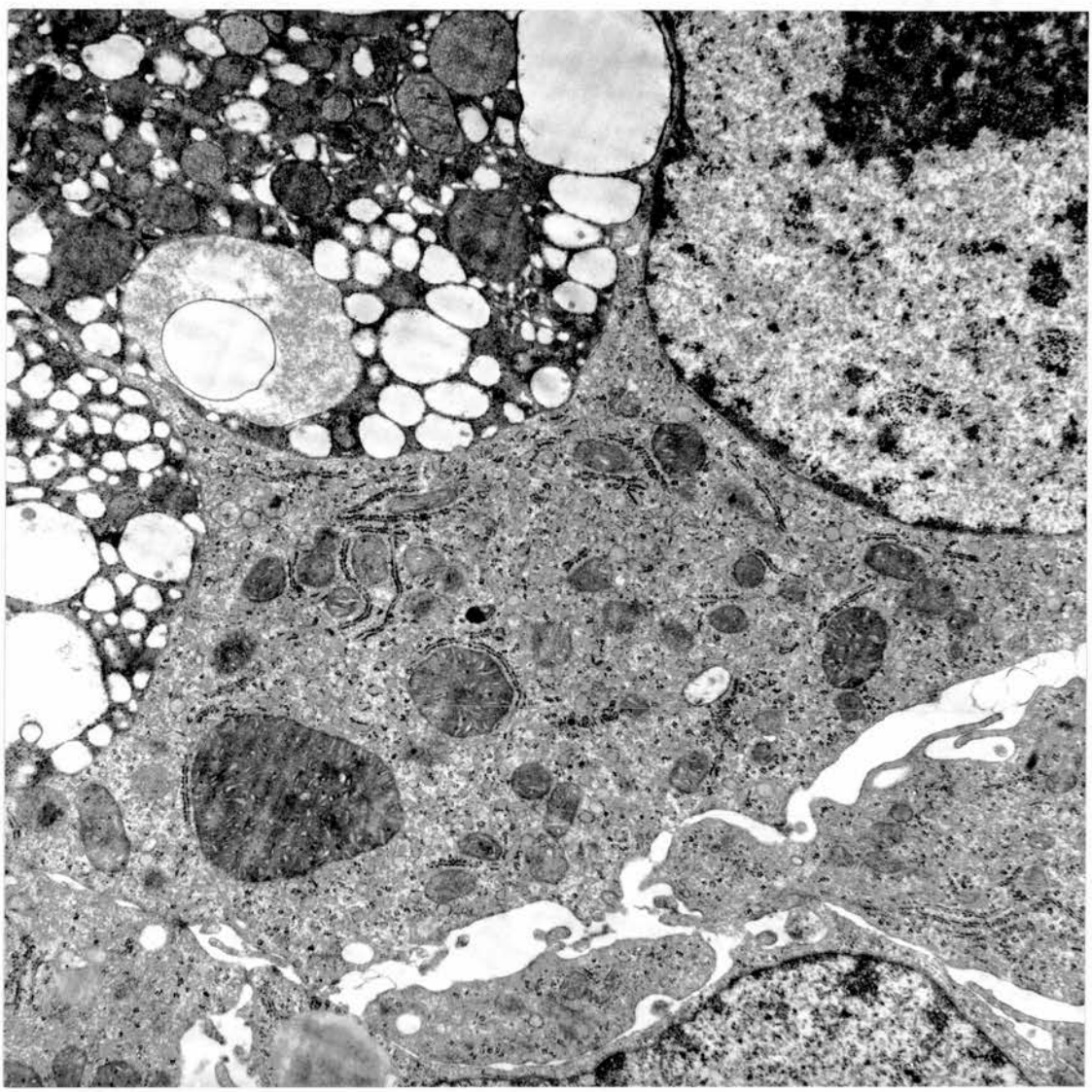

Fig.4.39 48 hours. Part of a hepatocyte containing two round membrane-bound inclusions composed of compacted mitochondria, endoplasmic reticulum, and a variety of small vesicles. EM. X10,750

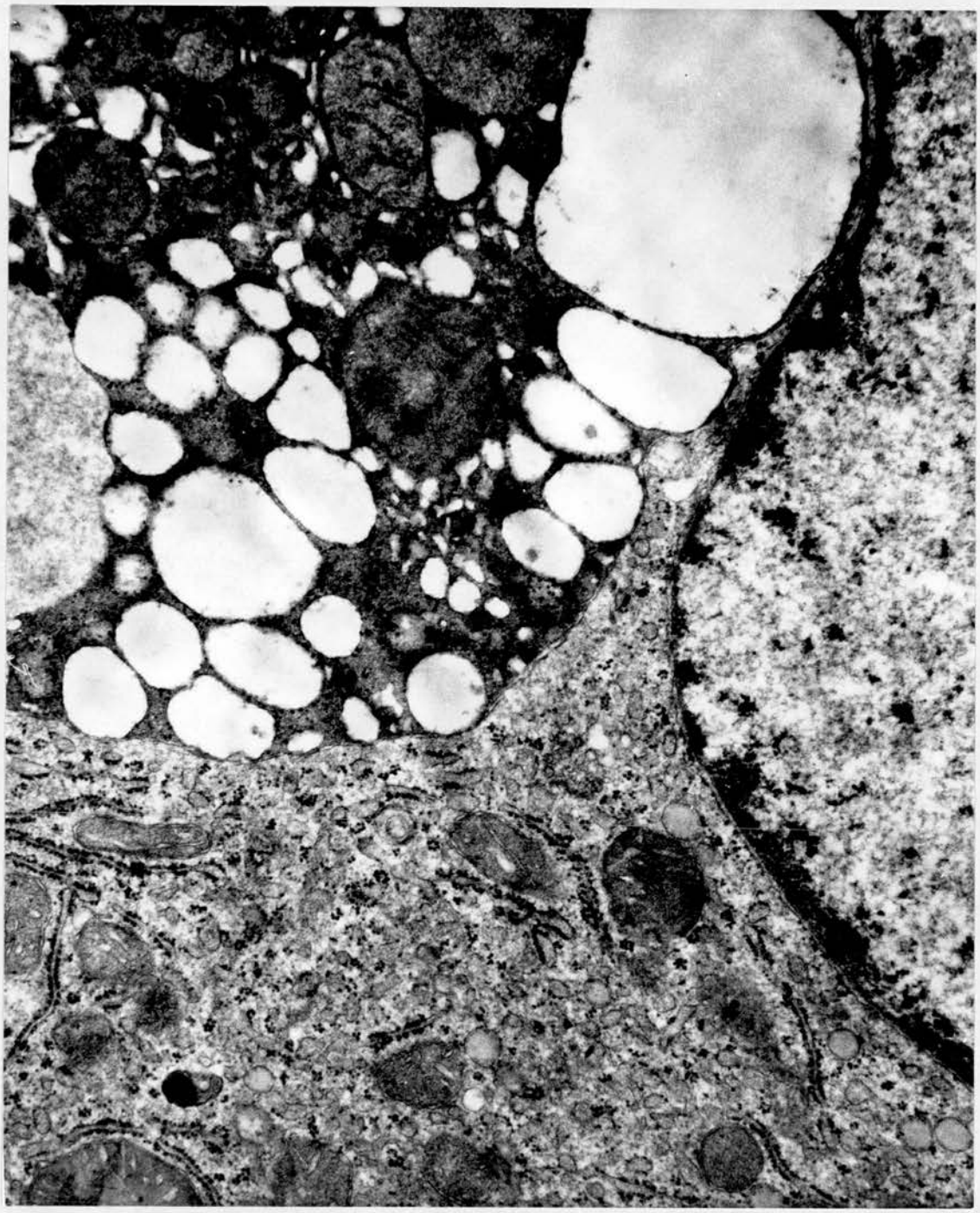

Fig.4.40 48 hours. Higher magnification showing relatively normal mitochondria within the apoptotic body and its bilaminar bounding membrane. EM. X16,500

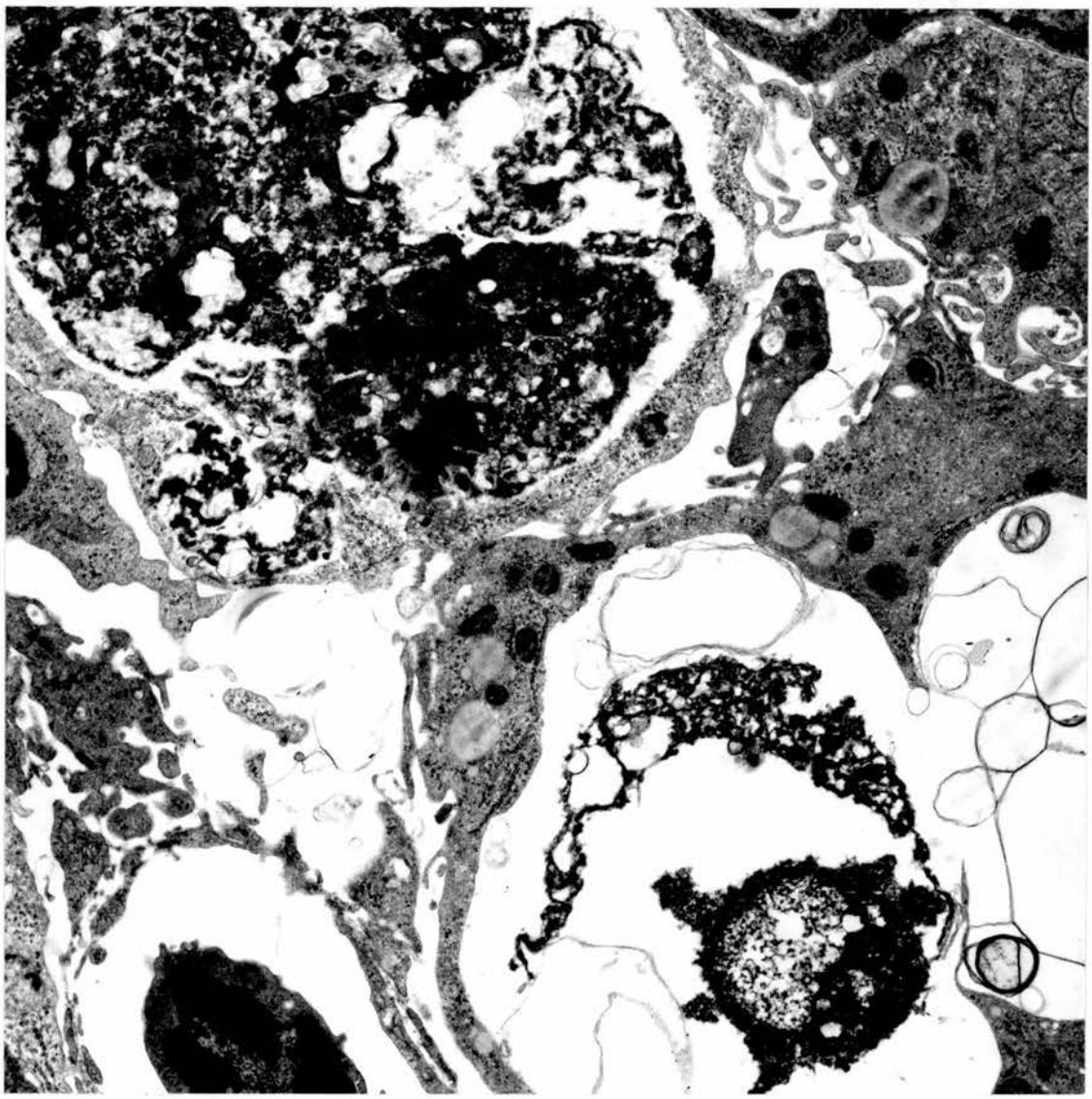

Fig.4.41 48 hours. Irregular inclusions in hepatocytes reveal a considerable increase in electron density and breakdown of internal structure. EM. X10,750

In the fat stains some increase in lipid is seen in midzonal cells and a few globules are found in the necrotic areas and in macrophages. One liver contains abundant black-staining material within necrotic areas in the Sudan Black preparation, but stains much less impressively with Oil Red O, suggesting the presence of oxidised lipids in the hepatocyte debris.

The centrilobular loss of succinate dehydrogenase activity persists but the peripheral exaggeration of activity seen at 24 hr. is now inconsistent and not seen in one test animal.

The macrophage infiltrate reveals conspicuous acid phosphatase activity containing numerous discrete granules of variable size (4.42). Kupffer cells lining sinusoids close to the necrotic areas also show increased activity (4.43). Densely-staining intracellular spherical "bodies" are more readily found than at 24 hr but are still scanty. The majority probably represent phosphatase activity associated with the ingestion of acidophil bodies.

The above features of resolution are accompanied by regeneration, evidenced by high mitotic activity and an increase in size of hepatocytes which show an increased nuclear diameter and new endoplasmic reticulum formation. Ribosomes are of normal size and are attached to regular parallel arrays of endoplasmic reticulum which, although distributed throughout the cytoplasm, are closely related to normal-looking mitochondria (4.44). Glycogen particles are reduced in number in many of these hepatocytes. The cell surfaces possess normal microvilli and new bile canaliculi are formed.

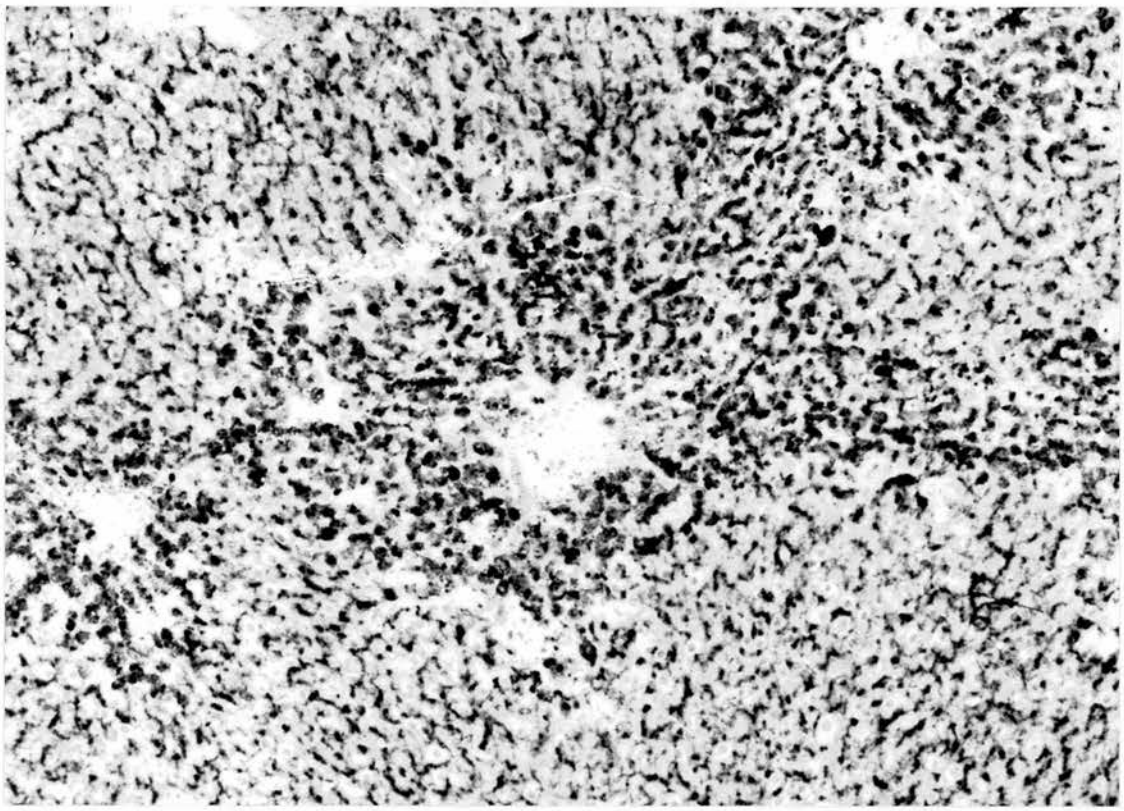

Fig. 4.42 48 hours. Increased acid phosphatase activity in the zones of macrophage infiltration. FS. Gomori. X100

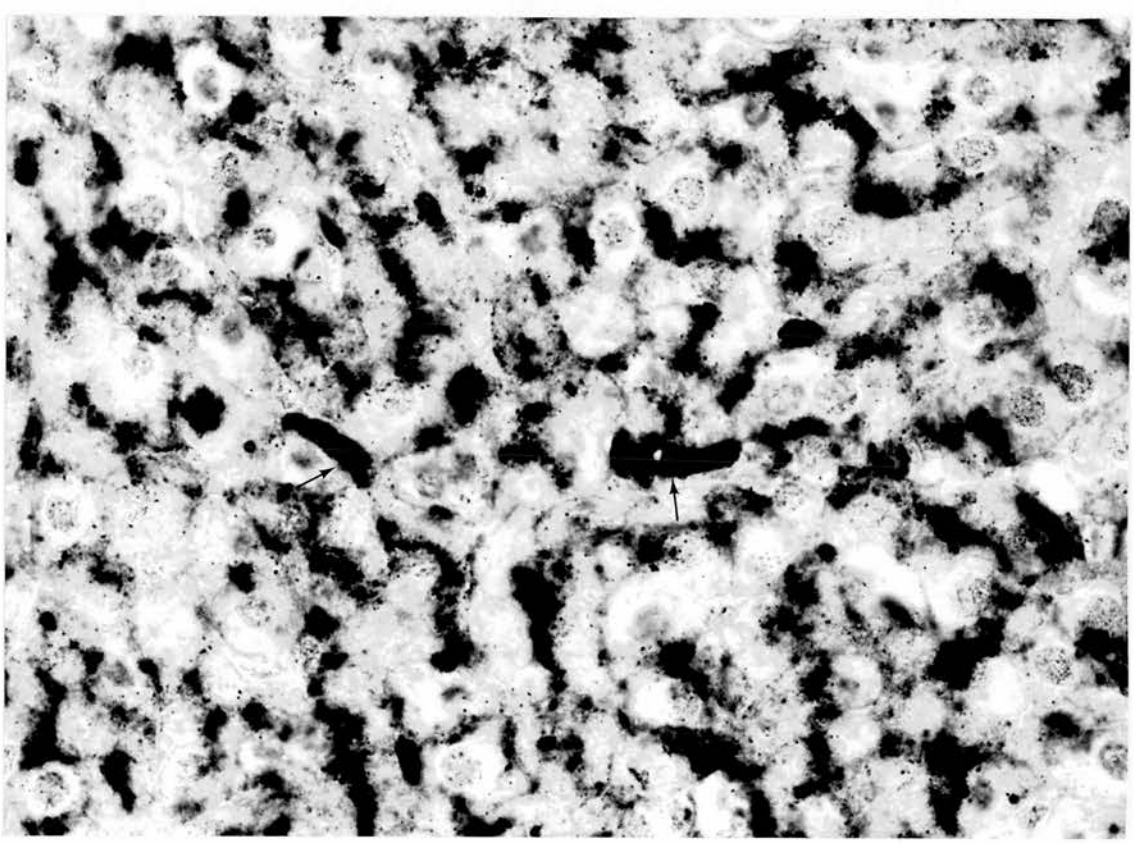

Fig. 4.43 48 hours. Incresed acid phosphatase activity in Kupffer cells (arrowed) close to the necrotic zones. FS. Gomori. X400

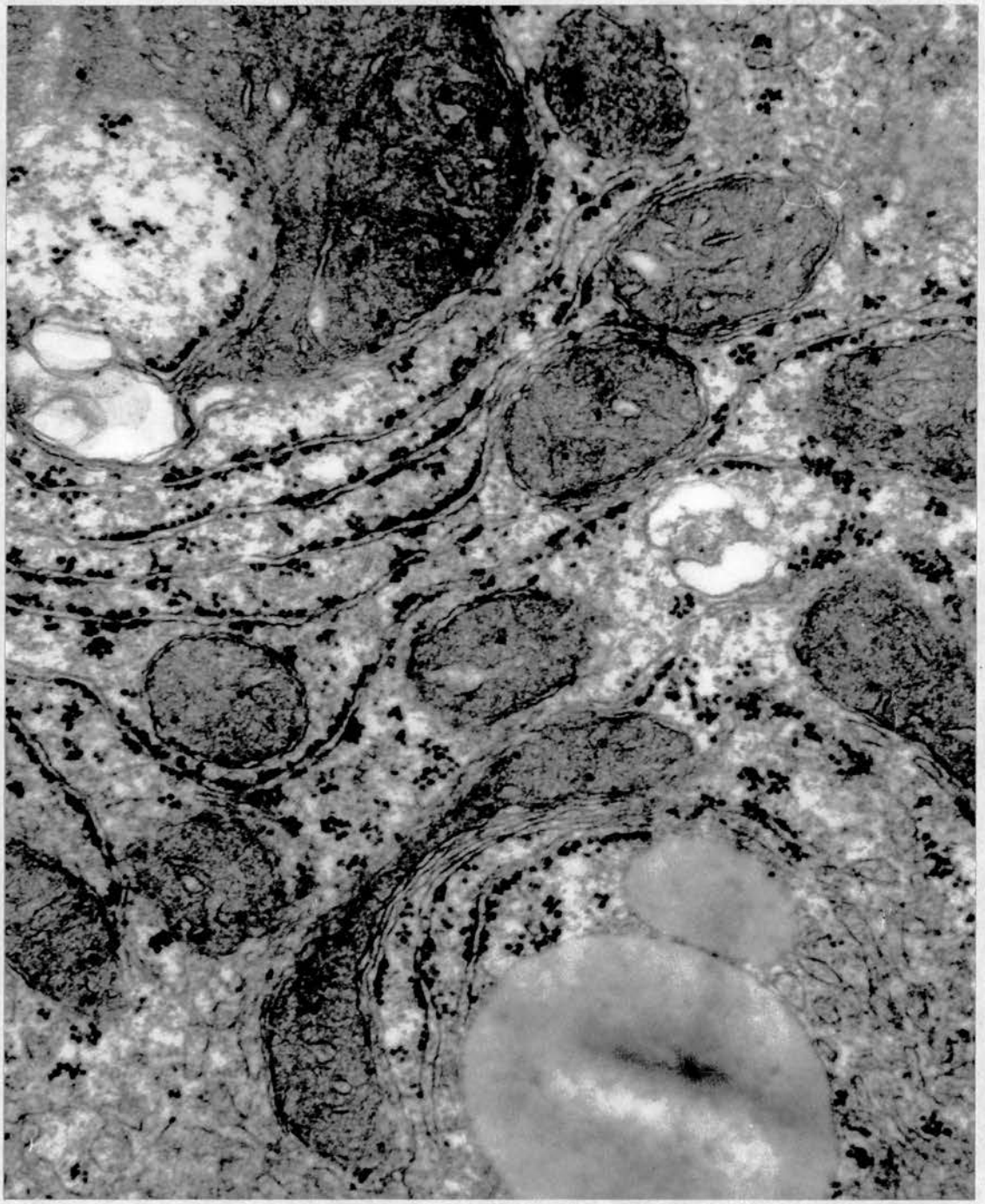

Fig.4.44 48 hours. Irregular attachment of ribosomes to otherwise normal parallel arrays of endoplasmic reticulum. This may represent regeneration of the RER. EM. X48,000

DISCUSSION

The original experiments in which the evolution of paracetamol-induced hepatic necrosis had been studied up to 28 days after administration revealed hydropic vacuolation, centrilobular necrosis, macrophage infiltration and regenerative activity with a rapid restoration to normal. The present study has considerably amplified these changes and provided possible explanations for them.

The earliest morphological changes seen in the development of paracetamol-induced liver injury are loss of glycogen and cytoplasmic matrix swelling followed by loss of ribosomes and mitochondrial abnormalities. These changes are entirely non-specific and are common to liver injury provoked by a wide variety of agents. Similar appearances have been described in ischaemia (Bassi and Bernelli-Zazzera, 1964), hypovolaemic shock (Blair et al., 1968), and as a result of such diverse poisons as carbon tetrachloride (Smuckler and Arcasoy, 1969), tannic acid (Arhelger, Broom and Boler, 1965), galactosamine (Scharnbeck et al., 1972), and the azo-dye 2-methyl-4-dimethylamino azobenzene (La Fontaine and Allard, 1964).

In the centrilobular cells there appeared to be a rapid progression from these early changes to frank coagulative necrosis. This contrasted with cells in mid-zonal and occasional periportal areas where vesiculation of the endoplasmic reticulum was a conspicuous feature. In some cells this appeared to progress through gross hydropic

vacuolation to cell-death, but the fact that at 48 hr necrosis was in general confined to centrilobular areas, suggests that in the majority of midzonal and periportal cells vesiculation represents a reversible form of injury. Indeed, although vesiculation of the endoplasmic reticulum is frequentlyseen in many types of liver injury, its previous interpretation as one of the sequence of events leading to necrosis has been disputed (Judah Ahmed and McLean, 1965; Magee, 1966). Conclusive evidence of reversible injury is the finding of autophagosomes within recovering hepatocytes. Whilst these form a prominent feature of some types of liver injury, for example in that due to heliotrine (Kerr, 1969) and glucagon (Artila and Trump, 1968), we found only a few autophagosomes in midzonal cells in the later groups, certainly fewer than would be expected if sublethal cell injury is an important feature of paracetamol hepatotoxicity.

Vacuolation was a conspicuous finding in this study and four distinct types could be recognised. Firstly, very small vacuoles indenting the sinusoidal margins of some hepatocytes were found in both control and test animals. These are interpreted as pinocytotic in nature, and have been previously described in control material in an electron-microscope study of anoxic changes in liver cells (Oudea, 1963). The exaggeration of these vacuoles in the 6-hr test animals may reflect early damage to the plasma membrane, but where the injury was more advanced and swelling of the cell had occurred, such pinocytotic vacuoles were not seen.

The second type of vacuolation was that found predominantly in midzonal cells in association with vesiculation of the endoplasmic reticulum. These vacuoles appear to arise by coalescence of smaller dilatations of the cisternae reflecting continued accumulation of fluid within the cells, and represent the ultrastructural counterpart of the ' hydropic' vacuoles seen on light microscopy. In carbon tetrachloride hepatotoxicity, correlations between light microscope changes and ultrastructural alterations have also established that hydropic vacuolation corresponds to an extensive dilatation of cisternae of the endoplasmic reticulum. (Stenger, 1969; 1970). Swollen mitochondria have been claimed to contribute, albeit to a lesser degree, to the overall swelling of the injured cells (Ashworth et al., 1963) but we found little or no evidence of mitochondrial swelling in hydropic cells. Mitochondrial changes were only conspicuous in central necrotic cells which remained remarkably free from vacuolation. This failure of centrilobular cells to show hydropic swelling in contrast to the less severely injured cells in the midzone has been previously emphasized by Dixon and McCullagh (1957).

Large clear vacuoles were also found within hepatocytes bordering the necrotic areas in the 12- and 24-hr groups but they differed from the hydropic vacuoles just described in that apart from minor mitochondrial changes and loss of glycogen, the remainder of the cell appeared normal. Serial sections revealed that many of these vacuoles communicated

with the extracellular space. The appearances therefore indicated that a mechanism other than the progressive intracellular accumulation of fluid was responsible. A minority of these large clear 'bordering' vacuoles were apparently entirely extracellular, being situated between adjacent hepatocytes. The possibility that the latter two forms of vacuolation were artefacts produced, or exaggerated, by perfusion-fixation was considered, but their presence in parallel paraffin sections of unperfused liver, their absence in controls, and the consistency of their location around necrotic areas, argued against an artefactual origin. Similar vacuolation of liver cells had been described in a classical series of experiments by Trowell (1946-47), who showed that such "watery vacuoles" resulted from a combination of anoxia and congestion and that the entry of fluid into liver cells was due to the hydrostatic pressure in the sinusoids and not to osmotic absorption. More recent ultrastructural studies of the changes produced in liver cells by respiratory hypoxia have confirmed the presence of large clear vacuoles with a single-membrane lining which communicate with the space of Disse (Oudea) and other vacuoles which are entirely extracellular (Brewer and Heath, 1965). The centrilobular necrosis seen in this study was invariably accompanied by marked sinusoidal congestion. Thus the consistent presence of clear vacuoles around the necrotic and therefore congested zones, suggests that stasis of blood leading to hypoxia and raised hydrostatic pressure

might be responsible for their formation. Glynn and Himsworth (1948) were sufficiently impressed by changes in blood flow through the liver in carbon tetrachloride injury to suggest that the centrilobular necrosis was a result of the restriction of blood supply consequent upon swelling of the cells. Whilst such a mechanism is now considered improbable (Magee), the presence of morphological changes related to secondary vascular effects rather than to the primary hepatotoxin has been largely overlooked in many recent studies of liver injury. Furthermore, possible exaggeration of the injury by these secondary vascular changes might explain why a reduction in mortality after paracetamol over-dosage in mice has been achieved by treatment with vasoactive compounds (Dixon, Dixon and Aparicio, 1973: Rosner, Romero-Ferret and Mottot, 1973).

Lipid accumulation is not a prominent feature of paracetamol liver injury, but a narrow band of midzonal cells bordering the necrotic areas showed fine lipid globulation at 24 and 48 hrs. This may be indicative of a sub-lethal injury or a much slower form of cell-death, as the accumulation and aggregation of fat within cells requires continuing metabolic activity. It is also conceivable, however, that this fatty change is a further manifestation of secondary hypoxic damage.

The principal changes found in the MTT stains for succinate dehydrogenase were an inconsistent rise in activity in centrilobular areas first seen at 6 hr followed by a

uniform loss of activity in degenerating cells. At 24 hr the necrotic areas were surrounded by a narrow zone of increased activity. These changes are identical to those found in experimental carbon tetrachloride and thioacetamide intoxication by Smith and Coote (1963). The initial transient rise in activity has been interpreted by Kerr (1965) in a study of ischaemic liver injury, as reflecting increased accessibility of substrate to enzyme in damaged mitochondria. Whilst we found some increase in mitochondrial matrix density at this stage, there was good preservation of the architecture of the organelle with no evidence of enlargement such as that described as early as 1-2 hr after a single large dose of carbon tetrachloride (Reynolds, 1963). Loss of succinate dehydrogenase activity, however, was associated with mitochondrial swelling and disruption of cristae but was inevitably accompanied by advanced changes in the remainder of the cell amounting to frank necrosis. We conclude, therefore, that significant mitochondrial injury is a relatively late feature of paracetamol hepatotoxicity which follows an initial and more conspicuous injury to the endoplasmic reticulum. This interpretation is compatible with the finding that covalent binding of tritiated paracetamol *in vitro* is predominantly directed towards the microsomal fraction of the cell, (Potter *et al*, 1973).

The acid phosphatase preparations did not suggest a role for lysosomes in the early paracetamol injury; loss of activity was first seen at 12 hr and was only found in

already necrotic cells. This parallels the findings of Slater (1969) in carbon tetrachloride hepatoxicity and may account for the failure of drugs known to stabilise lysosomes, namely corticosteroids and antihistamines, to modify experimental paracetamol-induced necrosis (Nimmo, Dixon and Prescott, 1973).

Whilst the general sequence of events leading to necrosis appeared to be consistent, the time course and ultimate extent varied considerably. Most animals, however showed the earliest evidence of damage at 6 hr and by 24 hr all showed frank centrilobular necrosis. The variation in the extent of necrosis between animals was not unexpected, as other studies on paracetamol induced liver injury in the rat in which multiple blocks have been examined by light microscopy, have revealed considerable variation in the extent of necrosis within the same liver (Walker et al., 1974).

Thus far we have been mainly concerned with the events leading to necrosis but detailed study of the later groups revealed some important aspects of healing. Between 24 and 48 hr there was a rapid accumulation of macrophages in the necrotic areas which showed marked acid phosphatase activity probably associated with the formation of heterolysosomes. Acidophil bodies, although numerous in certain types of liver injury, for example following ligation of the portal vein (Kerr, 1971), were scanty even at 48 hr. They are also removed by phagocytosis and degradation in macrophages,

"fixed" Kupffer cells and hepatocytes. These findings indicate a very active phase of phagocytosis and absorption immediately following injury, but such processes do not operate in isolation and healing is largely achieved through a concurrent phase of rapid regeneration. This was evidenced by the finding of numerous mitotic figures at 48 hr when often two or three mitoses could be found in a single high-power field of midzonal cells. The capacity of the liver to regenerate rapidly and the prompt macrophage response must be borne in mind when undertaking comparative or quantitative studies on liver necrosis. The present findings suggest that in the case of paracetamol hepatoxicity in the rat such studies should preferably be carried out 24-36 hr after administration. During this period necrosis is well established, although possibly not complete, and macrophage activity and regeneration are still at a low level.

CHAPTER V

The quantitation of hepatic necrosis and its relation to serum enzyme levels

In studying the effects of antihistamines and hydrocortisone on paracetamol-induced necrosis I used an arbitrary grading method to quantify the extent of necrosis in each liver. Whilst this demonstrated that there were no significant differences between the various treatment groups and I believe this to be a valid conclusion, I had some misgivings about the method of grading. Firstly, the animals were killed four days after administration by which time the necrotic areas were diffusely infiltrated by macrophages. Furthermore some regeneration would have occurred and it is likely that the degree of regeneration would have varied from animal to animal. For these reasons it was suggested

in the preceeding chapter that the optimum time for quantifying necrosis in the rat is between 24 and 36 hours. Secondly, ths degree of necrosis varied markedly within each section of liver and a compromise grade had to be given. It therefore seemed likely that sampling errors were an important consideration and multiple sections should be examined.

With these reservations in mind, I was determined to compare three methods of histological quantitation applicable to hepatic necrosis, namely arbitrary grading, point-counting, and the image-analysis computer. At the same time, the opportunity was taken to study the relation between the degree of necrosis and the serum transaminase levels in these animals.

Elevation of serum transaminase levels has invariably been used as an index of liver damage in detecting hepatic necrosis complicating cases of self-poisoning with paracetamol. Striking increases in these enzymes have been found in a few such patients, who, however, judging by other liver function tests and their clinical state do not appear to have correspondingly severe liver damage (Prescott et al., 1971; personal observations). The relation between peak serum enzyme values and the subsequent development of hepatic failure has not been studied in these patient; other parameters such as the plasma paracetamol half-life (Prescott et al., 1971) and the increase in serum bilirubin and prothrombin ratio (Clark et al., 1973) have been proposed as

better prognostic indices.

Previous experience indicated that significant increases in serum transaminases do not occur before the appearance of frank necrosis which is first seen at 12 hr and that maximum elevation usually occurs 24 hr after administration. Thus serum transaminase levels were correlated with histological necrosis from 24 hr after a large dose of paracetamol until well into the recovery phase.

Materials and methods

In the first experiment rats were killed 24 hr after a large hepatotoxic dose of paracetamol, and serum transaminase levels at the time of death were correlated with the degree of hepatic necrosis. Two further experiments were performed in which groups of rats were killed at 36 hr and 72 hr after paracetamol to study the relation in the recovery phase.

Female Tuck-Wistar rats weighing approximately 200 g were used throughout. Paracetamol BPC was administered by stomach tube without anaesthesia in a dose of 4g/kg body weight as a suspension (300 mg/ml) in 0.2% tragacanth and water. The animals were fasted for 16 hr prior to administration. The 20 rats in the main group were killed 24 hr after paracetamol, and groups of 6 and 10 rats were killed at 36 and 72 hr respectively.

Samples of blood for transaminase estimations were taken by cardiac puncture under light ether anaesthesia immediately before the animals were killed. Serum aspartate aminotransferase (ASAT, formerly SGOT) and alanine aminotransferase (ALAT, formerly SGPT) were determined by a modification (Henry et al, 1960) of the methods of Karmen (1955) and Wroblewski and LaDue (1956) respectively.

The animals were killed by cervical dislocation. The livers were immediately removed and a series of cuts was made into the lobes to ensure adequate penetration of the fixative (10% buffered formol-calcium) in which they were immersed. After fixation for 48 hr the lobes were separated and cut into thin slices (2-3 mm) along their long axes and alternate slices were taken for histology. This provided 8-16 (mean 10.1) pieces of liver ('blocks') for each animal. The blocks were identified separately. A further 24 hr in fixative was followed by paraffin embedding and 5-μm sections were cut and stained with Harris's haematoxylin and eosin (HE) and with Pyronin Y (see below).

The extent of necrosis in the HE section of each block was graded on a scale 1-5 which attempted to represent four equal increments between scattered small centrilobular foci of necrosis (Grade 1) and complete destruction of the parenchyma (Grade 5). Often there was considerable variability in the extent of necrosis within the same block and a compromise grade had to be allocated. The mean of the grades for individual blocks gave the final grade of necrosis for each

animal. In order to assess reproducibility of this method, a secondgrading was performed 'blind' several weeks after the first.

The second method of quantitation employed was a 'point-counting' technique using a random 25-point array eyepiece graticule. The sections from the 24 hr group were examined by this method in addition to grading. Twenty non-overlapping consecutive fields were counted with a x 10 objective and x 10 eyepiece giving a total of 500 points for each block. The number of points falling on viable hepatocytes, necrotic hepatocytes, and on portal tracts, central veins or sinusoids was recorded. The point-counts for these various components are directly proportional to the volume that each component occupies in the liver, so that the proportion of the total parenchyma occupied by necrotic cells can be calculated for each block. The mean of the results for individual blocks gave the percentage necrosis for each animal.

The theoretical accuracy of point-counting can be estimated using the Gauss equation for the standard error $S_{\bar{x}}$ (expressed as a percentage of the component being measured) which is given by the formula

$$S_{\bar{x}} = 67.45 \sqrt{\frac{100-c\%}{nc}}$$

where n is the number of observations made for the presence or absence of a component type that occupies c% of the whole area (Curtis, 1960). On the basis of 500 observations per block, for levels of necrosis greater than 25% the standard

error will be less than 5%, and taken over the average of 10.5 blocks for each animal in the 24 hr group even at low levels of necrosis the theoretical accuracy of the method will be acceptable, e.g., for 5% necrosis $S_{\bar{x}}$ = 4%.

The reproducibility of the method was tested by counting 'blind' the HE-stained sections from one of the animals a second time.

Finally, 10 of the 24 hr livers, having over 25% necrosis by point-counting, were examined on the Quantimet Image Analyser (720D, Image Analysing Computers, Ltd). The computer utilises a digitally controlled plumbicon scanner to provide a video-screen image of the histological section viewed through an optical microscope. By setting three grey-level thresholds, various components of the image can be identified and quantified separately. The instrument used was fitted with a shading corrector to compensate for the inevitable slight unevenness of the microscope illumination and sensitivity of the scanner. This technique has not been previously employed to quantitate liver necrosis and it was first necessary to choose a staining technique which was susceptible to analysis in monochrome. I have utilised the loss of pyroninophilia in degenerating hepatocytes to distinguish viable cells (bright crimson) from necrotic cells (pale pink) after staining with 1% Pyronin Y in 0.2 M Walpole's acetate buffer (pH 4.2) for 10 min. The relation between the degree of necrosis as demonstrated by the Pyronin Y stain and by HE was checked by point-counting

the Pyronin sections. The sections were viewed through a green filter (Wratten no. 58) to improve the contrast and facilitate detection of the necrotic areas and 'clear' areas separately. Using the x 5 objective with the full frame equivalent to a specimen area of 2.1 mm^2, three areas were measured for each field corresponding theoretically to:-

a = 'Clear' areas, i.e., portal tracts, central veins, sinusoids, and areas not occupied by tissue such as cracks in the section, or around the margins of the block (Fig 5.1)

b = Necrotic parenchyma + a (Fig 5.2)

c = Viable parenchyma + b (i.e., everything but occasional small fragments of dense stain debris or other foreign material).

The three thresholds were set for each field and the areas a, b and c measured in picture points by the programmer and recorded on 8-track tape. The settings were carried out without knowledge of the point-counting results. The microscope was fitted with a scanning stage, set in this instance to scan the specimen on a square raster in steps of 2.5 mm, corresponding approximately to almost complete coverage of the section with non-overlapping fields as viewed through an x 5 objective. Empty fields or fields where the tissue occupied less than half of the displayed area were not measured. In this way the number of fields measured for each block was approximately proportional to the section area.

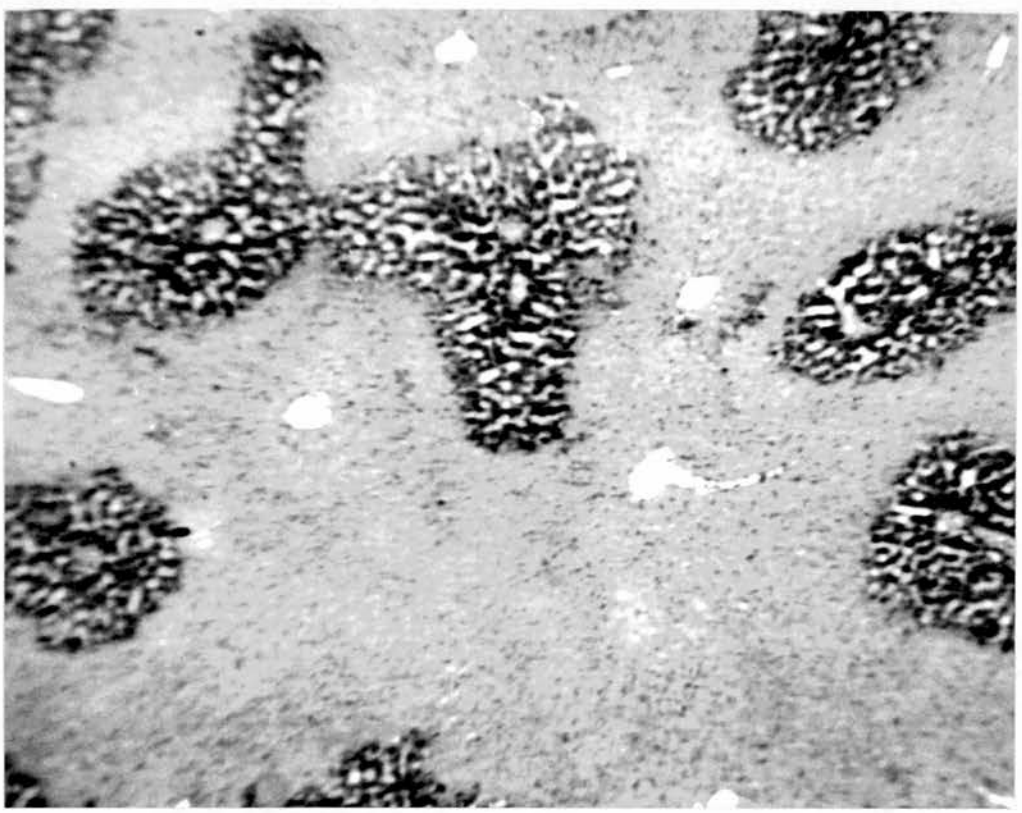

Fig.5.1 Video-screen image of Pyronin Y-stained section showing the first threshold setting which detects central veins, vessels in portal tracts, and sinusoids. The area detected (a) is measured in picture points and the figure displayed above the image and given as a 'print-out'.

Fig.5.2 The second threshold setting detects area a together with the necrotic tissue (= area b). It is evident that this setting in addition to detecting necrotic tissue is detecting more sinusoidal spaces than the first setting and failing to detect dense nuclear material in the necrotic zones. The latter contributes to the area c measured by the third threshold setting.

Fig.5.3 The Quantimet Image Analyser. The dials for setting the three grey-level thresholds can be seen in the centre of the main console.

For each frame

The area of necrotic tissue = $\underline{b} - \underline{a}$

The total area of parenchyma = $\underline{c} - \underline{a}$

Therefore, the percentage necrosis (N) = $\frac{\underline{b} - \underline{a}}{\underline{c} - \underline{a}} \times 100$

The picture-point areas of each component were fed into an ICL 1906A computer which calculated the percentage necrosis for each field, and the mean percentage necrosis of all the fields scanned for each animal.

Results

Assessment of necrosis

The degrees of hepatic necrosis as assessed by grading and point-counting at 24 hr are given in Table 5A. It can be seen that the grades of necrosis given on two separate occasions by the same observer show only minor differences and are clearly reproducible (Pearson correlation coefficient $r = 0.98$ significance $P<0.001$ for the mean grades of runs 1 and 2).

The reproducibility of point-counting the HE sections from one animal (18) is illustrated in Table 5B. Whilst the mean percentage necrosis as determined on two occasions is almost identical, there is in two out of the 10 blocks considerable variation between the two determinations. However, the overall correlation between run 1 and run 2 was very close ($r = 0.86$, $P<0.001$). The table also illustrates the marked variation in the degree of necrosis

TABLE 5A: Mean grade of hepatic necrosis, mean percentage necrosis (point-counting) and serum transaminase at 24 hours.

RAT	MEAN GRADE OF NECROSIS ± S.E. RUN 1	RUN 2	MEAN % NECROSIS (Point Counting) ± S.E.	SERUM TRANSAMINASE LEVELS i.u./l ASAT	ALAT
1	3.0 ± 0.26	2.5 ± 0.38	40.4 ± 8.2	3520	1056
2	2.8 ± 0.29	2.2 ± 0.42	29.0 ± 5.0	3410	1717
3	3.2 ± 0.3	3.1 ± 0.29	53.6 ± 6.1	11730	3040
4	0	0	0	746	85
5	2.2 ± 0.3	2.1 ± 0.29	28.9 ± 6.3	12800	2293
6	3.3 ± 0.28	3.0 ± 0.24	51.6 ± 4.0	11090	3157
7	2.3 ± 0.15	2.2 ± 0.2	28.1 ± 3.2	4693	1578
8	4.5 ± 0.25	4.4 ± 0.24	67.0 ± 5.6	11300	2810
9	1.7 ± 0.37	1.7 ± 0.33	16.6 ± 6.5	2069	661
10	2.2 ± 0.2	2.5 ± 0.17	25.8 ± 2.8	1920	853
11	3.5 ± 0.15	3.0 ± 0	46.0 ± 2.5	7680	1920
12	2.6 ± 0.15	2.5 ± 0.16	28.4 ± 2.4	3093	1269
13	1.1 ± 0.23	1.0 ± 0.25	12.2 ± 5.2	2026	1120
14	1.0 ± 0.13	1.0 ± 0.13	4.8 ± 1.3	261	58
15	2.2 ± 0.25	2.0 ± 0.26	32.0 ± 4.0	9490	1344
16	0.9 ± 0.14	0.7 ± 0.18	0.2 ± 0.1	300	69
17	2.9 ± 0.09	2.6 ± 0.15	31.4 ± 3.0	3330	1700
18	2.5 ± 0.31	1.9 ± 0.28	25.0 ± 5.7	1620	500
19	0	0	0	464	148
20	2.9 ± 0.1	2.8 ± 0.13	36.4 ± 2.1	7250	3500

TABLE 5 A

CORRELATION COEFFICIENTS AND SIGNIFICANCE :

RUN 1 v. RUN 2: $r = 0.98$, $P<0.001$

RUN 1 v. ASAT: $r = 0.67$, $P<0.001$

RUN 1 v. ALAT: $r = 0.77$, $P<0.001$

RUN 1 v. Point Count %: $r = 0.96$, $P<0.001$

RUN 2 v. ASAT: $r = 0.70$, $P<0.001$

RUN 2 v. ALAT: $r = 0.79$, $P<0.001$

RUN 2 v. Point Count %: $r = 0.96$, $P<0.001$

% necrosis v. ASAT: $r = 0.80$, $P<0.001$

% necrosis v. ALAT: $r = 0.84$, $P<0.001$

TABLE 5 B ; REPRODUCIBILITY OF POINT-COUNTING AND IMAGE-ANALYSIS IN RAT 18

BLOCK	1	2	3	4	5	6	7	8	9	10	MEAN % NECROSIS ± S.E.
POINT-COUNTING HE RUN 1	17.1	48.5	46.1	17.9	12.6	4.9	7.3	54.1	15.4	25.9	25.0 ± 5.1
POINT-COUNTING HE RUN 2	7.0	47.0	50.2	13.3	14.9	26.2	5.3	41.8	17.9	22.9	24.6 ± 5.2
IMAGE ANALYSIS PYRONIN Y RUN1	15.7	33.4	41.7	12.0	12.1	23.2	8.6	38.6	22.8	31.0	23.7 ± 1.8
IMAGE ANALYSIS PYRONIN Y RUN2	19.1	39.6	45.9	17.9	17.8	34.6	17.5	41.8	25.2	29.3	27.9 ± 1.7

from block to block, a feature seen throughout the material, and underlines the need for multiple samples. Had only two blocks been taken from this liver, for example, they could have given results ranging from 6.1% to 51.3%, compared with the overall mean of 25.0%. Table 5B also shows the image-analysis results for this animal determined on two separate occasions. There is a very close correlation between the two runs ($r=0.952$, $P<0.001$), which demonstrates the good reproducibility of this method.

In order to test the validity of arbitrary grading as a measure of necrosis, the mean grades (run 1) were correlated with the corresponding percentage necrosis obtained by point-counting the HE sections. The correlation coefficient of 0.96 is highly significant ($P<0.001$).

The Quantimet image analyser was used to measure the percentage necrosis in sections of 10 livers stained by the Pyronin Y method. It was first necessary to establish that this stain gave an accurate representation of hepatic necrosis as compared with the orthodox HE method. The results of point-counting the two stains and of the image analysis are given in Table 5C. There was a highly significant correlation between the point-counting and image-analysis results which, as would be expected, was marginally better for Pyronin Y than for HE ($r = 0.92$ and 0.88 respectively; $P<0.001$ for each).

Despite the good correlation and near equivalence of the final results the image-analysis method had some

RAT	% NECROSIS (± S.E.) AS ASSESSED BY:-		
	POINT-COUNTING - HE	POINT-COUNTING - PYRONIN Y	IMAGE ANALYSIS - PYRONIN Y
1	40.4 ± 8.2	38.4 ± 7.8	37.3 ± 2.9
2	29.0 ± 5.0	27.6 ± 7.4	26.0 ± 1.8
3	53.6 ± 6.1	43.3 ± 5.2	34.8 ± 2.1
5	28.9 ± 6.3	32.0 ± 5.9	31.2 ± 2.6
6	51.6 ± 4.0	44.7 ± 5.6	40.3 ± 2.2
7	28.1 ± 3.2	24.9 ± 3.0	32.5 ± 1.4
8	67.0 ± 5.6	60.5 ± 6.7	57.2 ± 2.4
10	25.8 ± 2.8	26.6 ± 3.9	17.0 ± 1.6
15	32.0 ± 4.0	29.1 ± 3.8	32.5 ± 1.9
18	25.0 ± 5.7	22.3 ± 4.7	23.7 ± 1.8

TABLE 5 C : COMPARISON OF POINT-COUNTING AND IMAGE-ANALYSIS METHODS.

Point-counting (HE) v. point-counting (Pyronin): $r = 0.976$, $P<0.001$

Point-counting (HE) v. image-analysis (Pyronin): $r = 0.88$, $P<0.001$

Point-counting(Pyronin) v. image-analysis(Pyronin): $r = 0.92$ $P<0.001$

Percentage necrosis (image analysis) v. ASAT: $r = 0.622$, $P\leq0.05$

Percentage necrosis (image analysis) v. ALAT: $r = 0.657$, $P<0.05$

shortcomings. The threshold for area a (portal tracts, central veins and sinusoids) could not always be set to detect all these components without including some of the necrotic tissue. Thus some areas which theoretically ought to contribute to a would be excluded and only contribute to b and c. There was also grey-level overlap across the second threshold so that pyknotic nuclei in necrotic areas contributed to c only, instead of b and c. The effect of these detection errors is that firstly incomplete detection of component a increases both $\frac{b - a}{c - a}$; but since $\frac{c - a}{c - a} > \frac{b - a}{c - a}$ the percentage necrosis (N) derived from $\frac{b - a}{c - a} \times 100$ will be too high. Secondly, incomplete detection of nuclear material in b will result in values of b and $\frac{b - a}{c - a}$ which are too low, giving a low value of N. Thus, the two most important shortcomings of the detection procedure tend to balance each other out.

Relation between necrosis and serum enzymes

The serum transaminases at 24 hr in the first experiment are shown in Table 5A and correlated with the grade of necrosis and percentage necrosis as determined by point-counting. Although for both enzymes there are extreme variations in the response of individual animals, the enzyme levels nevertheless closely reflected the subsequently determined severity of necrosis. The correlation between ALAT or ASAT was closer when compared with percentage necrosis as determined by point-counting than with grade of necrosis but all correlations (Table 5A) were highly

TABLE 5 D : Mean grades of necrosis and serum transaminase levels at 36- and 72 hours after paracetamol.

RAT	36 Hr.			RAT	72 Hr.		
	Mean Grade ± S.E.	ASAT i.u./l	ALAT i.u./l		Mean Grade ± S.E.	ASAT i.u./l	ALAT i.u./l
21	2.6 ± 0.18	4690	2730	27	1.0 ± 0	94	38
22	1.3 ± 0.17	1380	117	28	1.0 ± 0	128	42
23	1.6 ± 0.18	1810	1237	29	2.4 ± 0.18	450	188
24	2.7 ± 0.24	3701	1194	30	1.6 ± 0.27	118	77
25	3.0 ± 0	2997	192	31	1.6 ± 0.25	99	30
26	3.5 ± 0.28	9160	4370	32	1.2 ± 0.15	152	68
				33	2.2 ± 0.19	480	215
				34	0.8 ± 0.16	115	229
				35	2.2 ± 0.3	850	289
				36	2.1 ± 0.23	1546	290

Correlation coefficients and significance:
Mean Grade 36hr v. ASAT: $r = 0.823$ $P<0.05$
Mean Grade 36hr v. ALAT: $r = 0.611$ $P<0.1, >0.05$
Mean Grade 72hr v. ASAT: $r = 0.640$ $P<0.05$
Mean Grade 72hr v. ALAT: $r = 0.575$ $P<0.1, >0.05$

significant ($P<0.001$). ALAT gave a better correlation than ASAT with both methods of assessing necrosis but the advantage of using one or other of the two enzymes were only minimal. As expected the serum enzyme values at 24 hr also correlated well with image analysis method of assessing necrosis (Tables 5A and 5C).

In the recovery phase the correlation between serum enzymes and severity of necrosis is less close. The results at 36 and 72 hours are shown in Table 5D. The correlation between the mean grade of necrosis and serum ASAT at both times reached statistical significance at the 5% level, but with ALAT just failed to reach significance at this level. Nevertheless these results indicate that even in the recovery phase the serum enzyme levels can be used with reasonable confidence to assess the degree of necrosis that has taken place.

DISCUSSION

Serum transaminases were first used as an index of hepatocellular injury by Molander, Wroblewski and LaDue (1955) in a study of carbon tetrachloride-induced necrosis in rats. Since then there have been several studies of transaminase levels after various types of liver injury. Friend, Wroblewski and LaDue (1955) examined SGOT activity in mice with viral hepatitis, and further studies have been carried out using carbon tetrachloride alone (Block and Cornish, 1958; Richarz and Schoetensack, 1959; Wirtschafter

and Tsujimura, 1961; Fox, Dinman and Frajola, 1962; Gabrieli and Orfanos, 1968; and Magos et al., 1974) or together with other hepatotoxins such as thioacetamide (Grice et al., 1971), sodium selenate (Cutler, 1974), various chlorinated hydrocarbons (Klaassen and Plaa, 1967) and a wide range of toxic compounds (Blazs et al., 1961). In addition Strubelt, Breining and Praël (1973) have studied serum transaminase changes after mechanical injury to the liver.

These experiments have usually included histological examination of the liver but in the majority a purely morphological analysis has been compared with the enzyme changes. In 5 studies the histological changes have been graded (Balazs et al., 1961; Grice et al., 1971; Strubelt et al., 1973; Cutler, 1974; Magos et al., 1974) and, in general, the serum transaminase levels reflected the presence and severity of hepatic necrosis. Zelman, Wang and Appelhanz (1959) compared the grade of hepatic necrosis in 143 human aspiration biopsies with simultaneous determinations of SGOT and SGPT. They found an excellent correspondence between the extent of necrosis of liver cells and the rise in serum activity of either enzyme.

Previous experience of grading paracetamol-induced hepatic necrosis had revealed considerable variation in the extent of necrosis in different parts of the liver, a finding possibly related to the irregular lobar distribution of microsomal enzymes (Lawson and Pound, 1974). In order to minimise sampling errors multiple blocks have been examined in this study. In view of this wide variation, the

estimation of necrosis based on 1 or 2 blocks or on a needle biopsy sample must be viewed with circumspection, unless it has been conclusively established that a lesion is diffusely and regularly distributed throughout the liver.

The wedge-shaped areas of confluent necrosis found in rats given paracetamol in high dosage have been termed "infarcts" by Gazzard et al. (1974) and were noted separately in their assessment of liver necrosis as they considered that such areas represented ischaemic damage. I feel that localised areas of confluent necrosis reflect the marked variation in the degree of toxic injury within the liver rather than resulting from secondary vascular effects, although it is possible as I have indicated previously that the extent of injury is exaggerated by congestion and hypoxia. Whatever the precise pathogenesis of these confluent lesions, they are nevertheless part of the totality of liver injury and should therefore be included in any quantitation of liver necrosis. It is of interest that McLean and Day (1975) assessed the degree of paracetamol-induced necrosis by "weighing the visibly affected portions on the basis of the very striking speckled white appearance" and expressing this as a percentage of the total liver weight. These portions probably correspond to the "infarcts" of Gazzard et al. (1974). Despite the arbitrary nature of the grading used in this study, repetition after an interval of several weeks indicated that the method is reproducible when used by an experienced observer.

A more accurate and objective method of histological quantitation is 'point-counting' based on the method originally proposed by Chalkley (1943). Variations on this method have been applied to the quantitation of experimental carbon tetrachloride-induced liver necrosis (Beck and Hughes, 1970; Adam and Thorpe, 1972) and to experimental paracetamol-induced necrosis (Mitchell et al., 1973). Point-counting has also been used to estimate hepatocyte volume in liver biopsies from patients in acute hepatic failure (Scotto et al., 1973), with the assumption that the biopsy forms a representative sample.

Although we found a very good correlation between the mean grade and the mean percentage necrosis as determined by point-counting, the theoretical percentage necrosis equivalent to the mean grade (1 = 20%, 2 = 40%, etc) consistently exceeded the corresponding point-count result. This over-estimation is attributable to the allocation of Grade 1, equivalent to 20%, to blocks in which there was any necrosis whatsoever, and to the natural tendency of an observer to exaggerate the extent of the 'sought' component.

The image analysis computer is a new approach to the measurement of liver necrosis. Whereas in theory the analysis of 500,000 picture points per field would appear to offer a very accurate method of quantitation, in practice certain shortcomings detracted from its accuracy. Although the Pyronin Y stain in most cases enabled clear discrimination between viable and necrotic hepatocytes, when the viable

cells had a high glycogen content the intensity of staining was reduced to a level that made distinction in the video image very difficult. Several staining techniques were tried, usually based on staining the necrotic areas with a variety of anionic dyes. None of these complex methods was found to be consistently superior to staining with Pyronin Y alone. Problems in discrimination led to overlap of the grey-level ranges of the different tissue components and the setting of the detection thresholds remained to some extent a subjective process. Improvements in the specificity and consistency of stains are therefore of great importance if the technique of image analysis is to gain in accuracy and usefulness.

Of the two quantitative methods more generally available, grading and point-counting, the latter is more accurate but is very time-consuming and tedious. I feel that for experiments involving large numbers of animals where a relatively simple comparative assessment of necrosis is required, arbitrary grading of multiple samples is the most acceptable method.

Following paracetamol over-dosage patients have very striking rises in serum enzymes, levels which are rarely seen in any other form of liver disease. Despite these extreme elevations the patients may have little or no clinical evidence of liver disease. Though enzyme levels may be higher in those patients who develop fulminant hepatic failure, they may be of less help than other liver

function tests in assessing eventual outcome (Clark et al., 1973), and the variability in response of serum enzymes in individual patients may make their prognostic value unreliable (Stewart and Simpson, 1973). Despite this it is agreed by most authorities that serum ASAT is useful for detecting liver cell injury (Perez, Shaffner and Popper, 1972; Sherlock, 1972). A detailed investigation of the usefulness of serum transaminase in predicting liver cell necrosis after paracetamol over-dosage is not available.

The results of the present investigation suggest that the serum enzyme level gives a reliable indication of the severity of necrosis at that time, and this is true even in the early recovery phase. The peak serum enzyme rise in rats after paracetamol usually occurs at about 24 hr, and is probably more predictable than the peak rise in humans which occurs later (Clark et al., 1973) and may be difficult to detect because of its very transient nature (Stewart and Simpson, 1973). Whilst measurement of the peak enzyme level gives a more accurate index of liver damage, levels obtained during the recovery phase are still closely related to the severity of the damage. It must be borne in mind, however, that for any given degree of histological necrosis the enzyme value at 72 hr is considerably lower than at 24 hr. The results of this study are in agreement with those of Balazs et al. (1961), who, using a variety of hepatotoxins in the rat, found that serum enzyme levels at specified times following dosing

generally reflected the severity of necrosis. Perhaps in the human situation if the time of paracetamol ingestion is known, the serum transaminase levels also reflect accurately the severity of liver damage but not necessarily the prognosis in these patients.

After the completion of this work, James et al., (1975) reported a comparison of liver function tests, fasting serum bile acids, and liver histology in 54 cases of paracetamol overdose. The biopsies were allocated to one of three grades of abnormality.

They found that 6 patients showed moderate histological changes (Grade II in their classification) despite normal liver function tests, and concluded that transaminase levels are not a very sensitive indicator of mild or moderate degrees of liver damage. Serum bile-acid measurement was claimed to be more sensitive,being abnormal in all patients with grade II biopsies and in all but 2 of the patients with grade I changes. They found, however, a large overlap in bile-acid levels in all three histological groups, and the peak level in an individual patient was a poor predictor of the abnormality found at histology. Where more severe liver damage had been sustained A.S.T. was more discriminatory; there was no overlap between levels seen in patients with grade I and II changes (all under 400 units/l) and levels in patients with grade III changes (all over 400 units/l). Thus A.S.T. levels over 400 units/l gave a good prediction of severe liver damage.

It would appear that these authors were attaching too much functional significance to relatively minor changes (Grades I and II) in liver histology, changes which although consistently associated with raised serum bile acids were only in a minority associated with elevated transaminase levels or prolongation of the prothrombin time. A more accurate quantitation of the liver injury, using point-counting for example, would have probably resulted in a closer correlation between necrosis and enzyme level. Certainly their general conclusions regarding the value of transaminase levels in predicting severe injury are in keeping with our experimental results.

On the other hand, Portmann et al., (1975) carried out an accurate quantitation of the viable hepatocyte volume fraction (HVF) in 76 biopsies within 10 days of overdosage and found no correlation with the serum aspartate amino-transferase values. The enzyme values varied widely, however, and it is possible that peak values were missed. Furthermore only 4 of the biopsies were performed before the 4th day after which time the relationship between enzyme levels and the extent of necrosis would be expected to be poor. They did show a significant inverse correlation between the HVF and the maximum prolongation of the prothrombin time (which probably occurs later than peak enzyme rise and is less labile), and between the HVF and the peak level of serum bilirubin.

CHAPTER VI

Extra-Hepatic findings in experimental overdose

Although Boyd and Bereczky's study on acute paracetamol toxicity in the rat included histological examination of a wide selection of organs, their descriptions are brief and do not satisfactorally answer the many questions which have arisen concerning extra-hepatic lesions of paracetamol overdosage. Experience with human overdosage has raised the possibility of renal, cardiac, and cerebral injury by paracetamol and it was felt that detailed study of tissues removed from rats given a hepatotoxic dose of the drug might provide further useful information.

The major problems which I wished to examine were;

1. Is there any evidence of direct cardiac toxicity?
2. Are there any CNS changes which might explain death in the absence of hepatic necrosis?
3. What is the nature of the renal injury, if any, in early paracetamol overdosage?
4. Do lesions in the pancreas explain why elevated serum amylase levels are found?
5. In view of the fact that gastro-intestinal haemorrhage complicates some cases of overdose, does the drug have any primary effect on the gastric mucosa?

Material and Methods

Rats given paracetamol alone at a dose of 3.0 g/kg were utilised from other experiments. Control rats were given a similar volume of the tragacanth solution as before.

After killing the rats by cervical dislocation a full dissection was carried out. The organs were removed and fixed prior to taking blocks for histology. The skull was opened using small bone forceps and the brain, supported by the base of the skull, removed and fixed en bloc. After fixation blocks were taken from heart, lungs, kidneys, spleen, adrenals, stomach, pancreas and brain. The following numbers of animals were examined.

TIME	PARACETAMOL	CONTROL
90 mins	3	1
3 hrs	3	1
6 hrs	4	1
12 hrs	5	1
24 hrs	3	1
48 hrs	4	1
72 hrs	6	1
5 days	6	1
3 weeks	5	-
	39	8

Several paraffin sections stained by H & E supplemented by special stains when required, were examined from each organ.

Results

1. Heart

In two of the controls, the myocardium contained very small interstitial infiltrates of leucocytes which appeared to be unrelated to fibre damage. One of the test animals contained a similar infiltrate, but none showed any evidence of toxic injury.

2. Brain

Both control and test animals showed mild to moderate meningeal congestion with no consistent increase in the

the latter group. Examination of neurones and glial cells in representative coronal sections through the cerebral hemispheres and cerebellum revealed no significant differences between controls and test animals.

3. Kidneys

The control animals showed no abnormality. One animal (90 min) showed a single small focus of interstitial nephritis and another (3 hr) had more widespread chronic pyelonephritis.

Degenerative changes in tubule lining cells were first seen at 12 hrs. These comprised increased granularity and cloudy swelling of proximal tubular cells associated with the presence of granular cell-debris in the lumina. The changes were focal and found in only 2 of the 5 animals in this group.

One of the 24 hr group showed established, but focal, acute tubular necrosis affecting proximal tubules. The other two animals showed earlier (or milder) degenerative changes, with only scanty single cell involvement and luminal debris, some of which appeared to contain calcium salts.

In the 72 hr group, 3 animals showed granularity and vacuolation of tubule cells maximal towards the surface of the kidney but no other abnormality, and this was interpreted as a fixation artifact.

One animal in the 5 day group showed focal, and minor regenerative activity but nothing else of note. All other

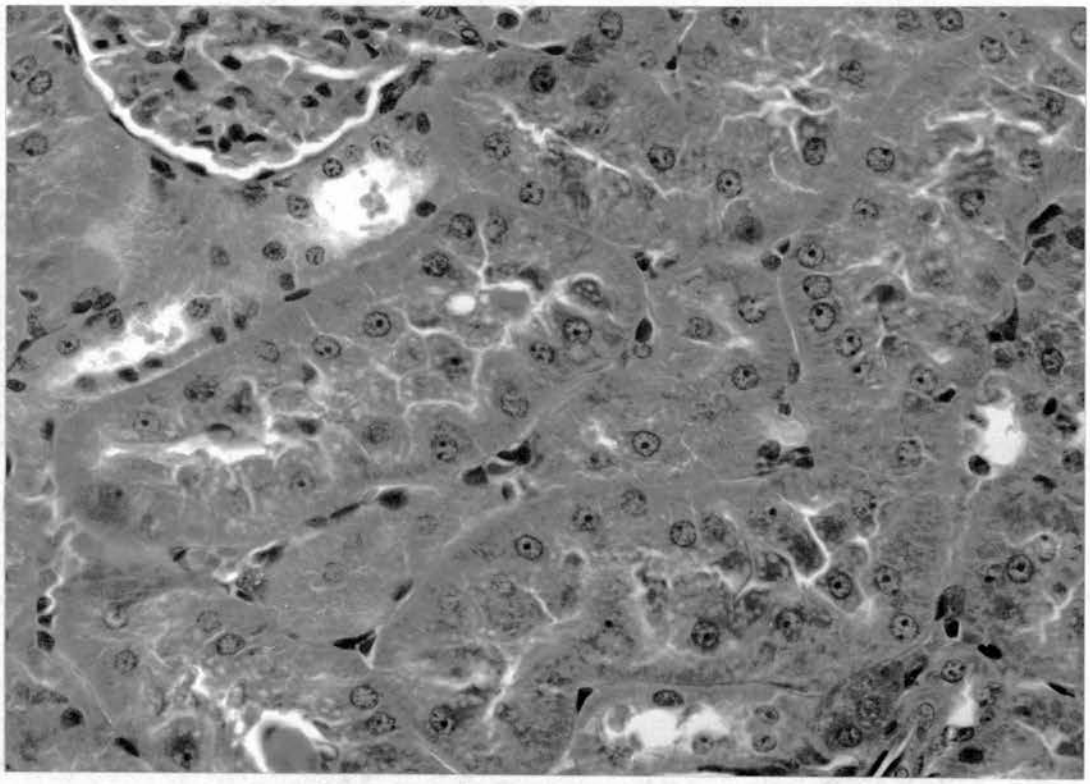

Fig.6.1 Kidney. 12 hours. Tubule lining cells are swollen and show mild hydropic vacuolation. The tubular lumina contain desquamated cells. HE. X450

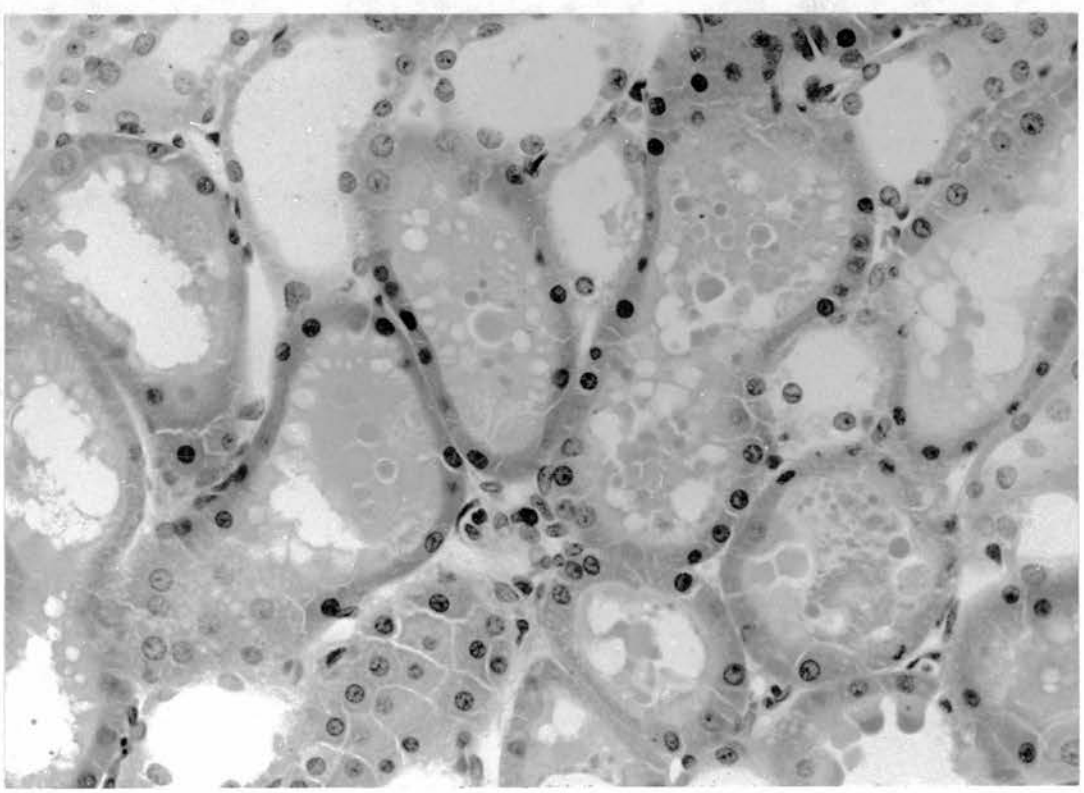

Fig.6.2 Kidney. 12 hours. Dilated tubules lined by degenerate cells with pyknotic nuclei and containing granular debris. HE. X450

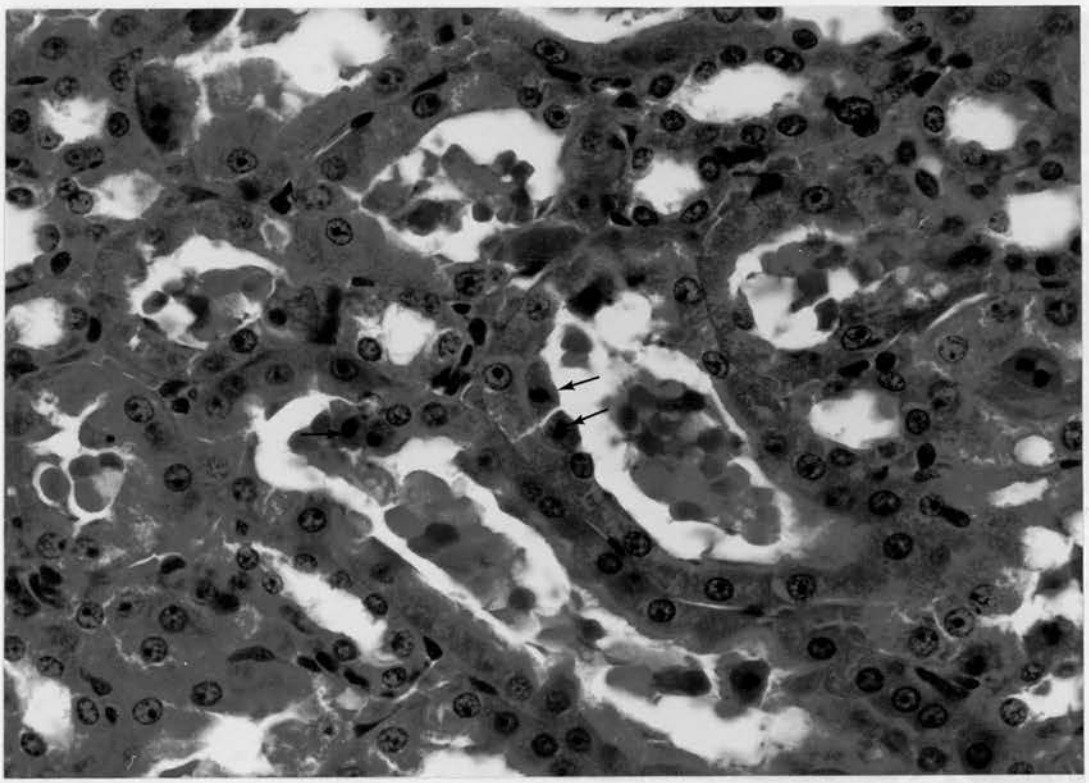

Fig.6.3 Kidney. 24 hours. Early degenerate changes indicated by nuclear pyknosis (arrowed) and desquamation into the lumina. HE. X450

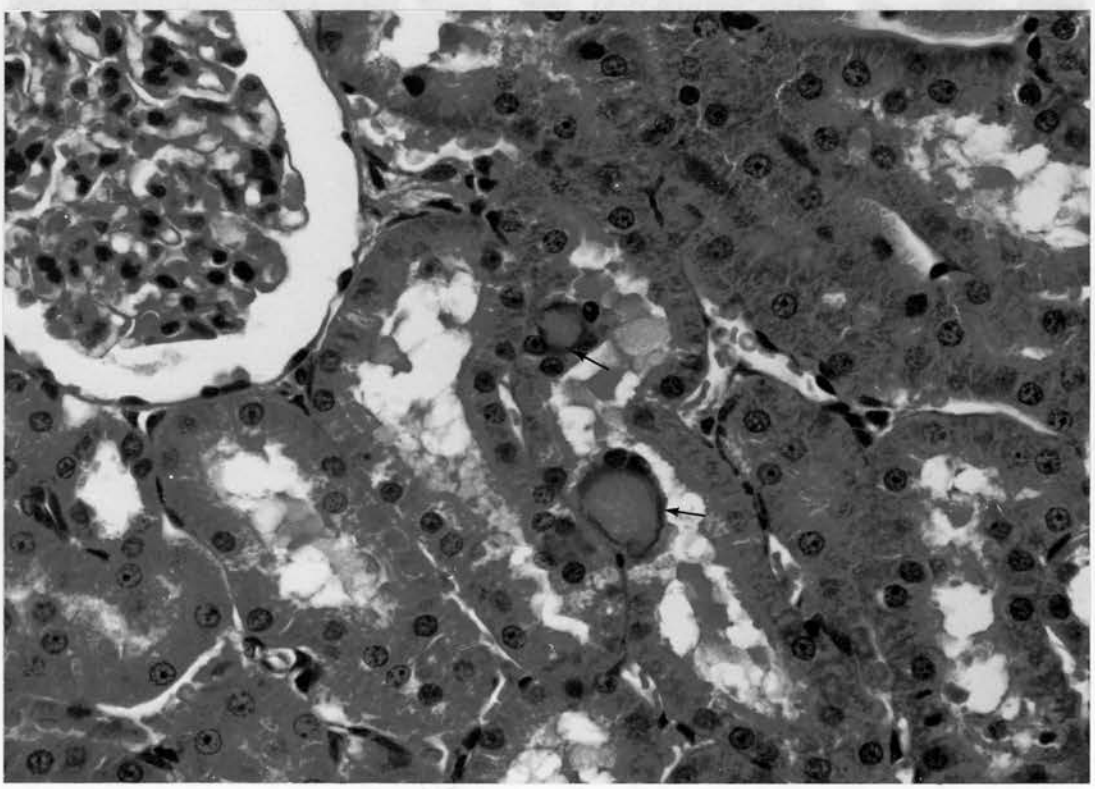

Fig.6.4 Kidney. 24 hours. Luminal debris containing calcium salts (arrowed). HE. X450

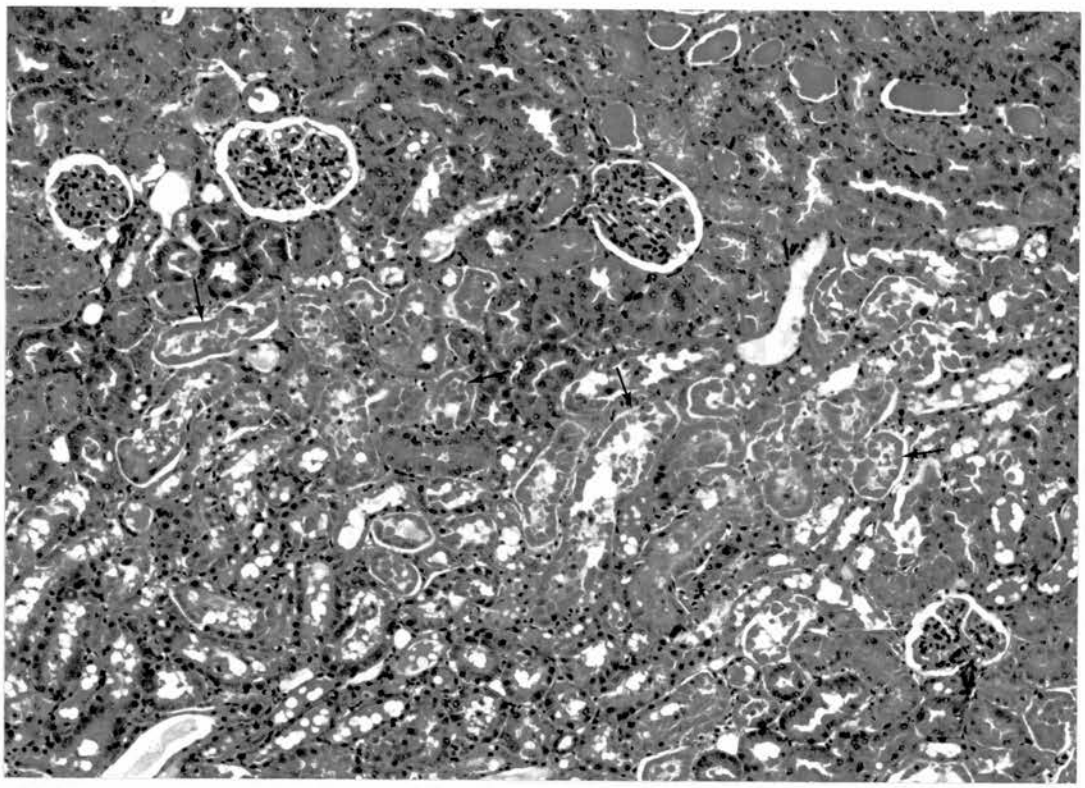

Fig.6.5 Kidney. 24 hours. Low power view showing foci of necrotic tubules, (arrowed). HE; X112

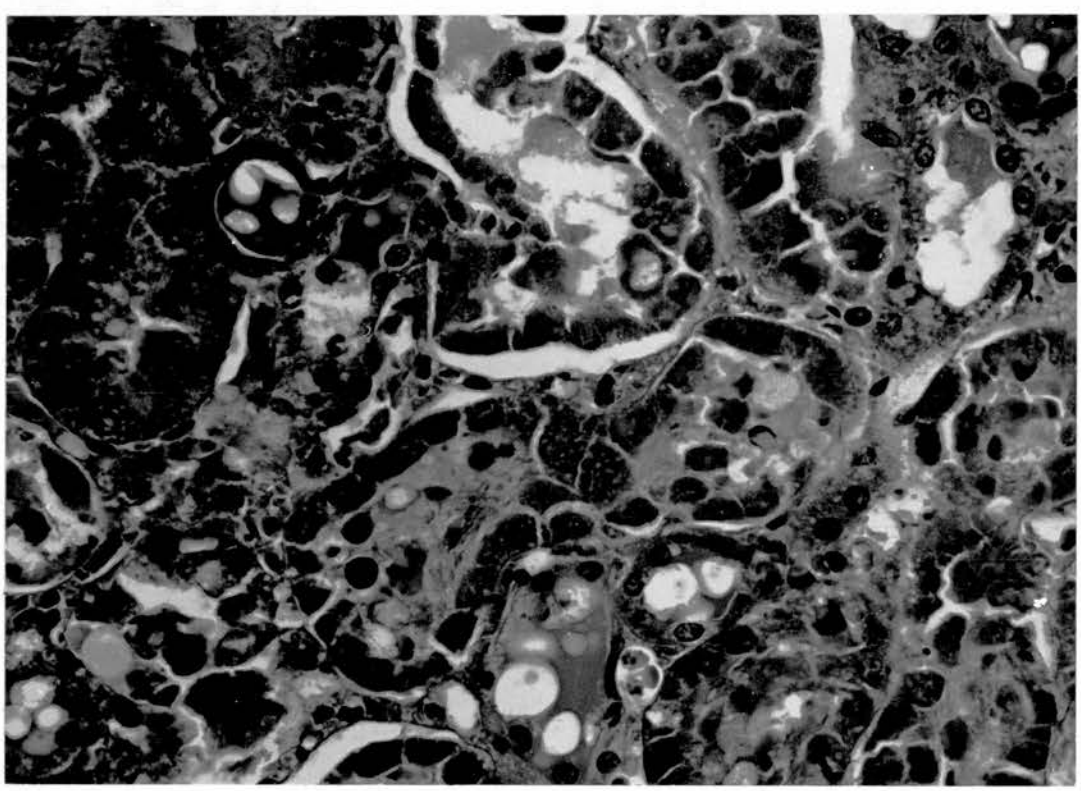

Fig.6.6 Kidney. 24 hours. Tubules lined by cells showing coagulative necrosis and containing cell debris. Adjacent tubular epithelium shows swelling and mild hydropic vacuolation. HE. X450

animals were normal.

To summarise, one animal (24 hrs) showed definite acute tubular necrosis. Lesser degenerative changes were found in 4 animals, and one (5 days) showed mild regenerative activity.

4. Pancreas

One of the control animals showed mild capillary dilatation in the islets of Langerhans, and another revealed early hydropic vacuolation in islet cells. The exocrine elements were all normal.

Similar capillary dilatation was found in 4 test animals, two at 12 hrs and two at 24 hrs. One of the 5 day group contained a single focus of vacuolated exocrine cells.

Apart from these minor abnormalities assumed to be insignificant there was no evidence of a toxic lesion.

5. Stomach

One animal (90 min) revealed a mild chronic gastritis, but none showed evidence of any acute degenerative or inflammatory changes.

In the 5 day group, one animal had a small superficial ulcer in the squamous portion and another revealed a foreign body granuloma in the submucosa of the glandular portion of the stomach. These lesions may be a response to trauma resulting from gastric intubation.

6. Lungs

One of the controls and 5 of the test animals revealed intra-pulmonary haemorrhage probably related to trauma associated with cervical dislocation. Another 5 test

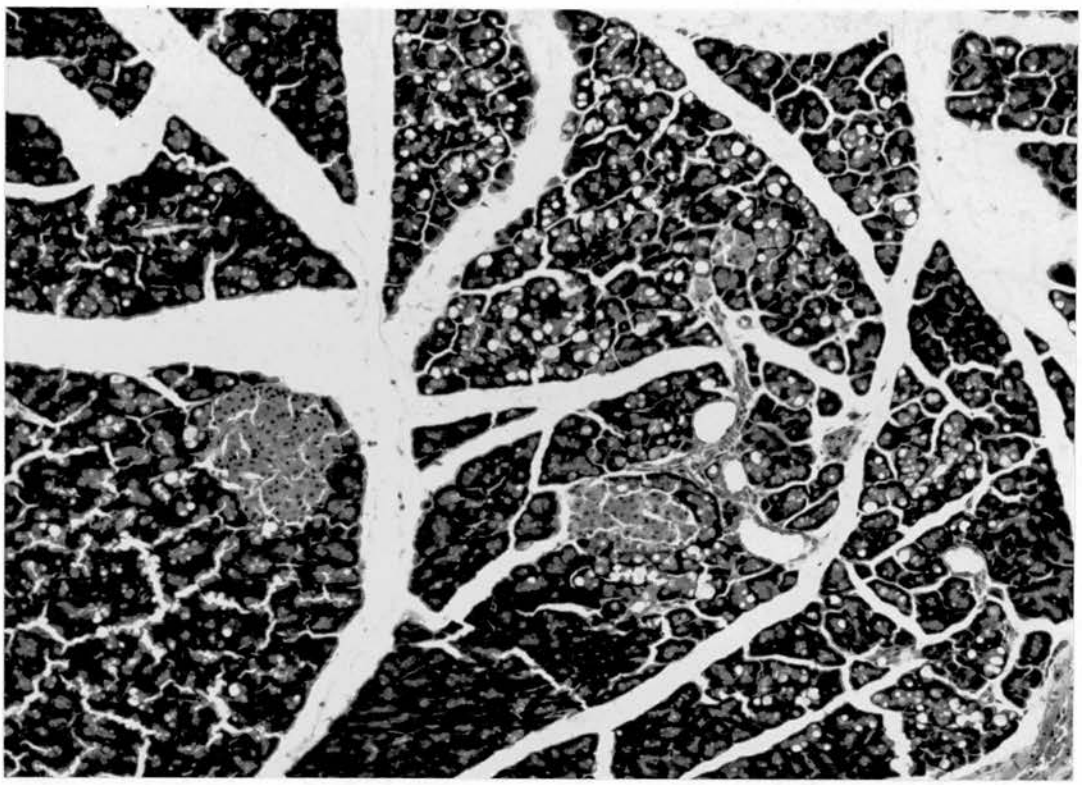

Fig.6.7 Pancreas. 5 days. Small focus of vacuolated exocrine cells. HE. X112

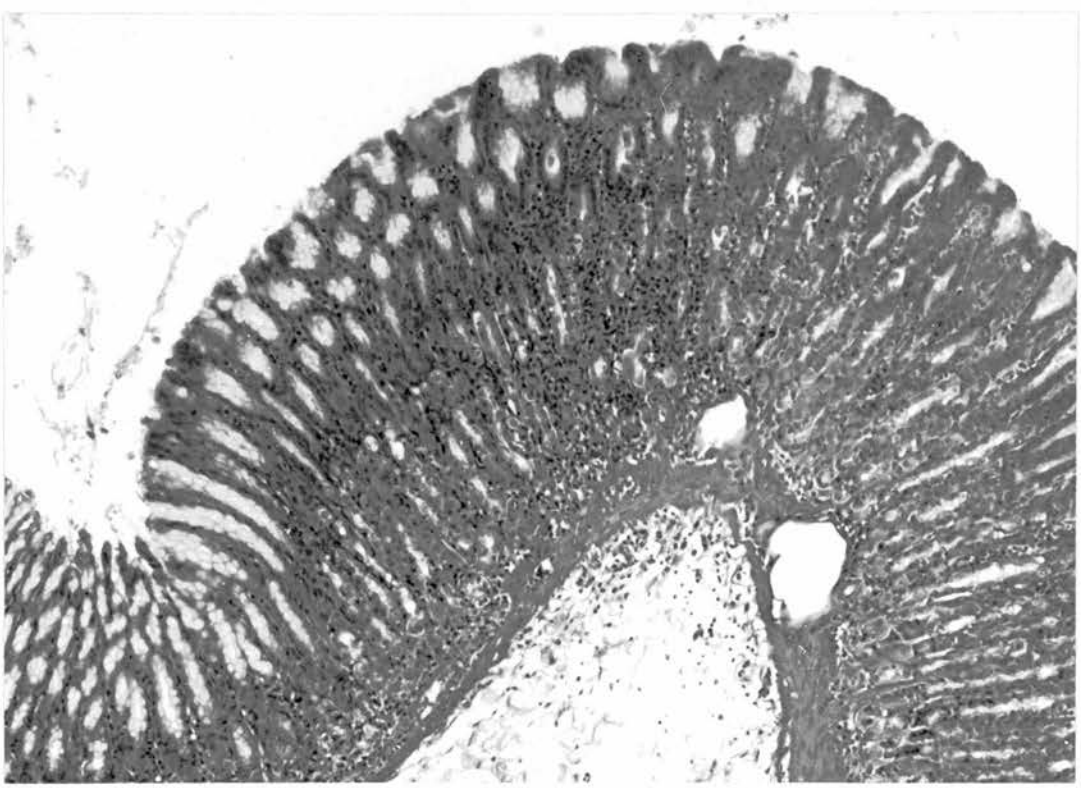

Fig.6.8 Gastric mucosa. 90 mins. Area of chronic inflammation involving the full thickness of the mucosa. HE. X112

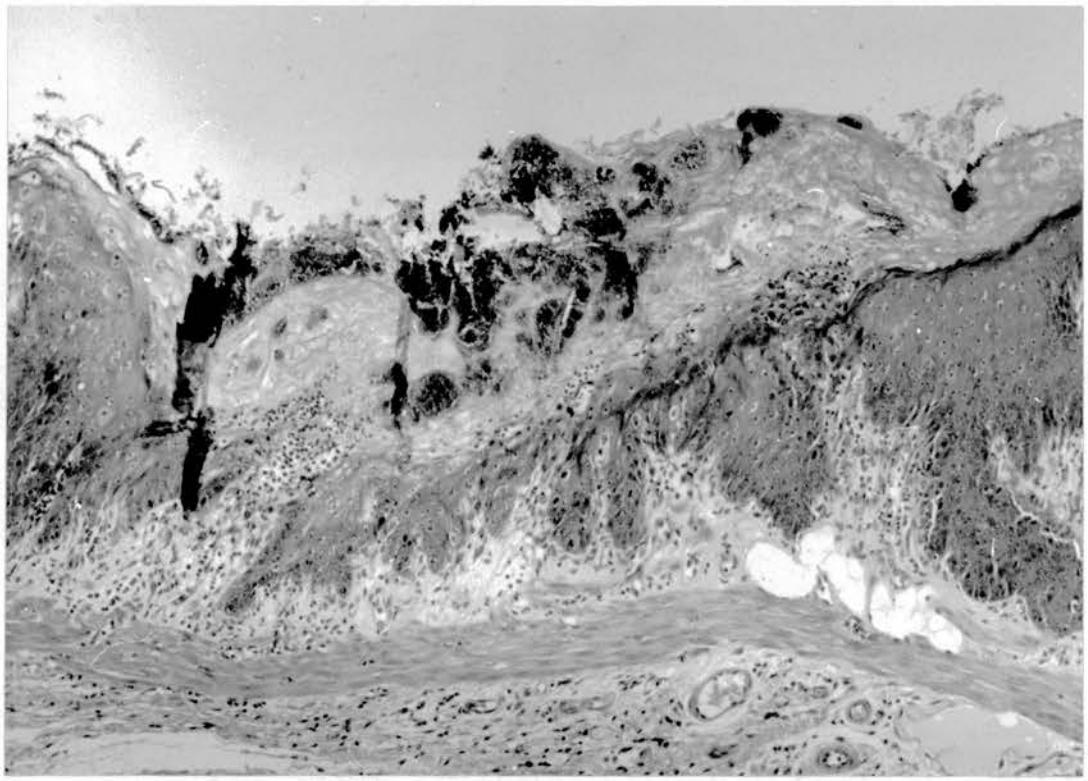

Fig.6.9 Gastric mucosa, 5 days. A small superficial ulcer in the squamous portion of the stomach. The overlying necrotic slough contains abundant bacteria. HE. X112

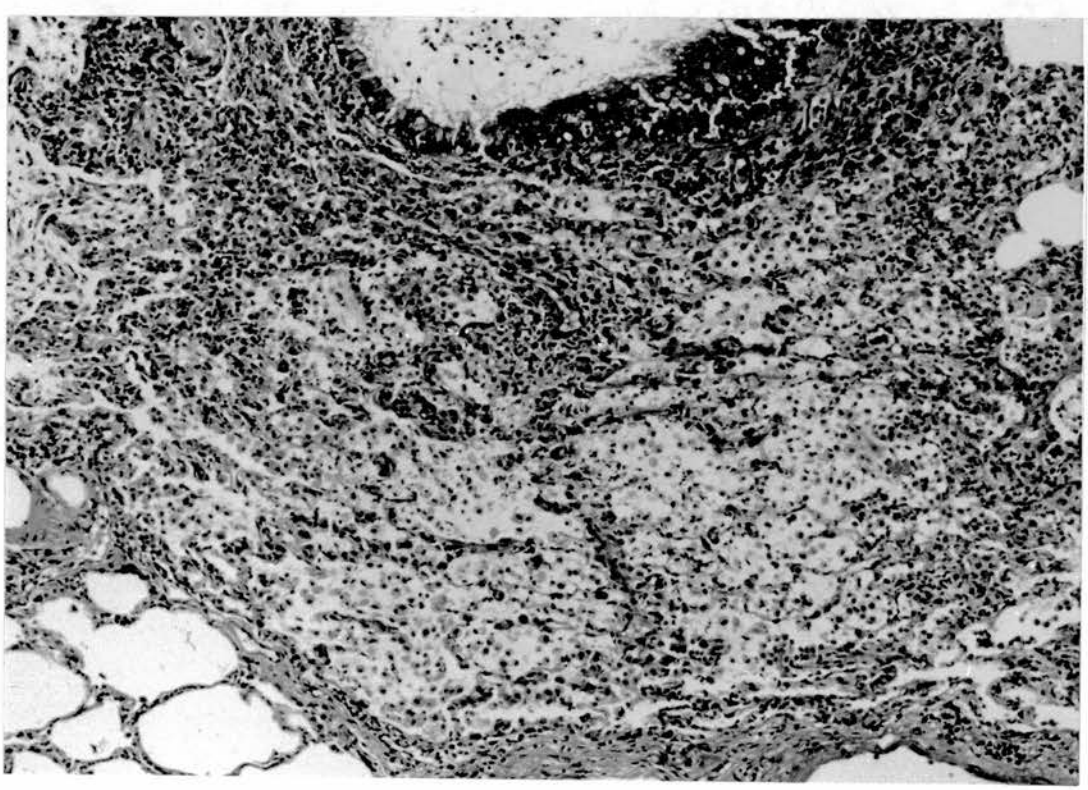

Fig.6.10 Lung. 72 hours. Numerous intra-alveolar macrophages in an area of lung around a mildly inflamed bronchiole. HE. X112

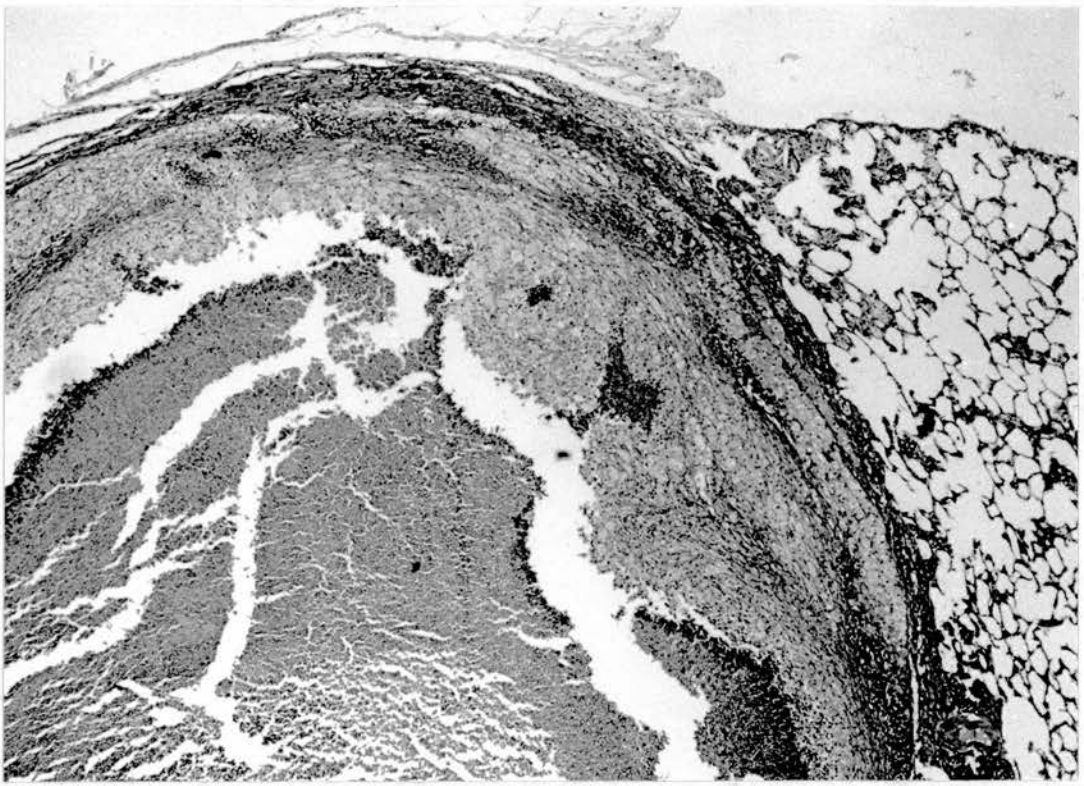

Fig.6.11 Lung. 72 hours. Pus-filled cavity lined by a thick layer of plump macrophages. HE. X45

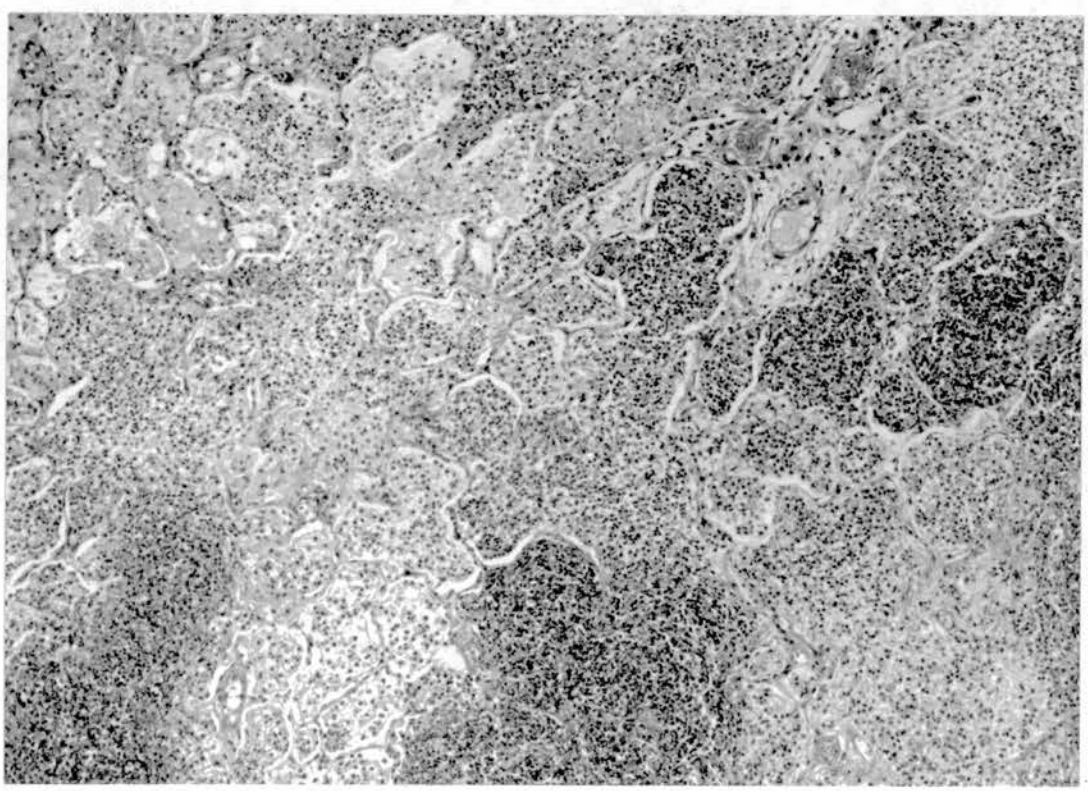

Fig.6.12 Lung. 3 weeks. Confluent bronchopneumonia. HE. X112

animals showed evidence of aspiration of the drug suspension despite administration by gavage. Four contained focal collections of macrophages which in one of the 5 day group were associated with early fibrosis. The other animal (72 hr) had two small cavities in the lungs containing polymorphs and macrophages some of which were lightly pigmented. The cavities showed early re-epithelialisation.

One animal in the 3 weeks group had evidence of suppurative bronchopneumonia.

7. Spleen

In order to appreciate the changes found in the spleen the normal structure will be briefly described.

Control spleens consist of lymphoid follicles, composed of a compact collection of mainly small lymphocytes aggregated around a central arteriole, separated by red pulp. The follicles are surrounded by a mantle of large mononuclear cells which have been termed histiocytes but may represent more primitive "stem-cells". Variable numbers of follicles contain central or eccentrically placed germinal centres showing plentiful mitotic activity. The red pulp consists of a mixed cell population, predominantly endothelial cells but including small numbers of macrophages, lymphocytes, and plasma cells together with haemopoietic cells amongst which megakaryocytes are conspicuous.

Up to 12 hrs the test animals showed no significant differences from controls, but the 12 hr group showed some

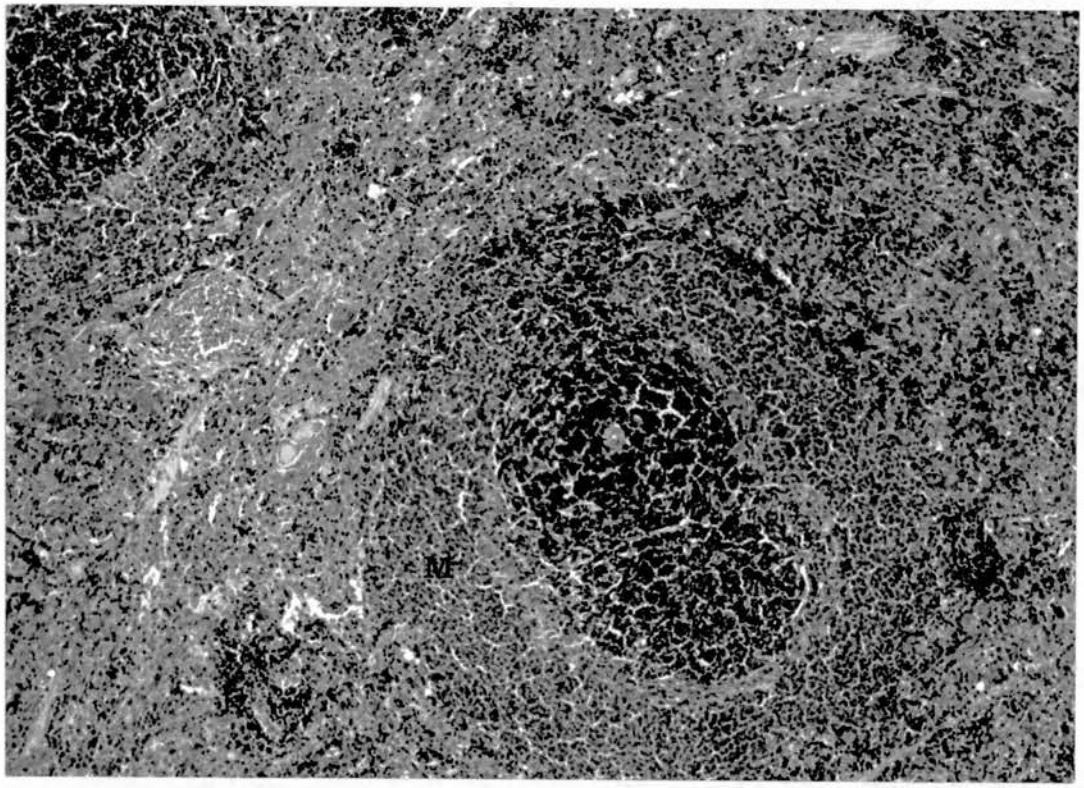

Fig.6.13 Spleen. Control. Follicle composed of small lymphocytes around a small arteriole (arrowed) and surrounded by a mantle (M) of large mononuclear cells.The follicles are separated by a heterogeneous mixture of cells. HE. X112

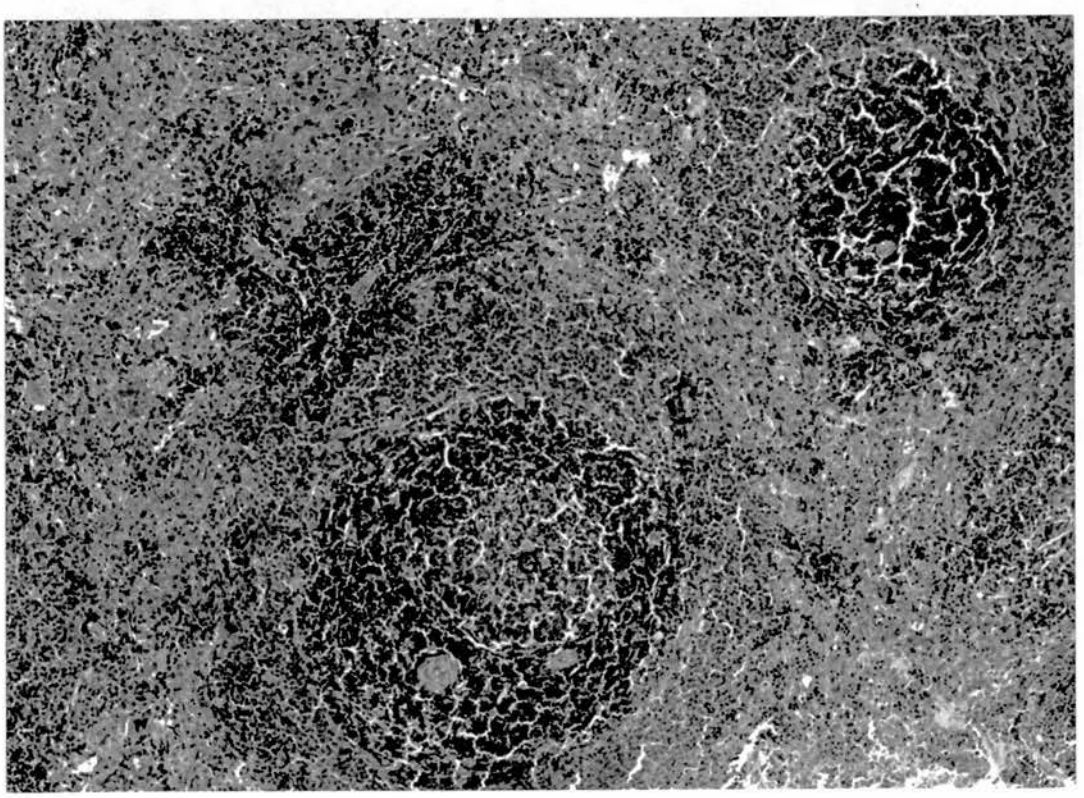

Fig.6.14 Spleen. Control. This follicle contains a small germinal centre (G). HE. X112

reduction in the number and size of germinal centres.

At 24 hrs the lymphoid follicles contained increased numbers of macrophages most of which had phagocytosed nuclear (karyorrhectic) debris. The mantle of mononuclear cells was generally reduced in thickness but showed marked mitotic activity.

By 48 hrs there were very few germinal centres, the mononuclear mantle was more prominent but had ill-defined borders, and there was an increase in interfollicular lymphocytes and plasmacytoid cells.

At 72 hrs increased numbers of small germinal centres showing plentiful mitoses were present and broad mononuclear mantles had developed. The red pulp contained numerous pigment-laden macrophages. This pigment was in the form of discrete small granules which were pale golden-brown in colour and resembled the ceroid pigment present in the liver at this stage.

At 5 days mitotic activity was conspicuous in the few small germinal centres, in the mononuclear mantles, and in scattered inter-follicular cells. Plentiful pigment-laden macrophages were present, but the most noteworthy feature was the marked increase in lymphocytes and plasmacytoid cells in the red pulp. This last feature was also noted in the 3 week group although it was less marked in 4 of the 5 animals. Only small numbers of pigmented macrophages were present at this time.

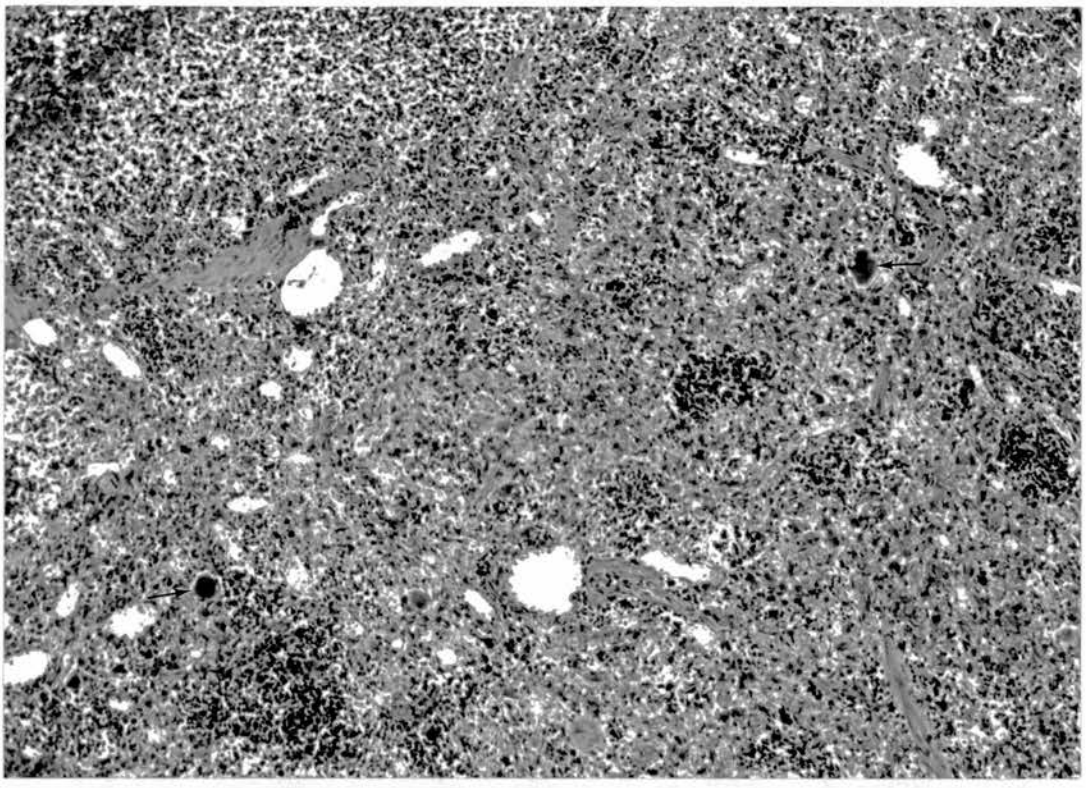

Fig.6.15 Spleen. Control. Interfollicular area consisting of predominantly endothelial cells, together with lymphocytes, plasma cells, and haemopoietic cells, including megakaryocytes (arrowed). HE. X112

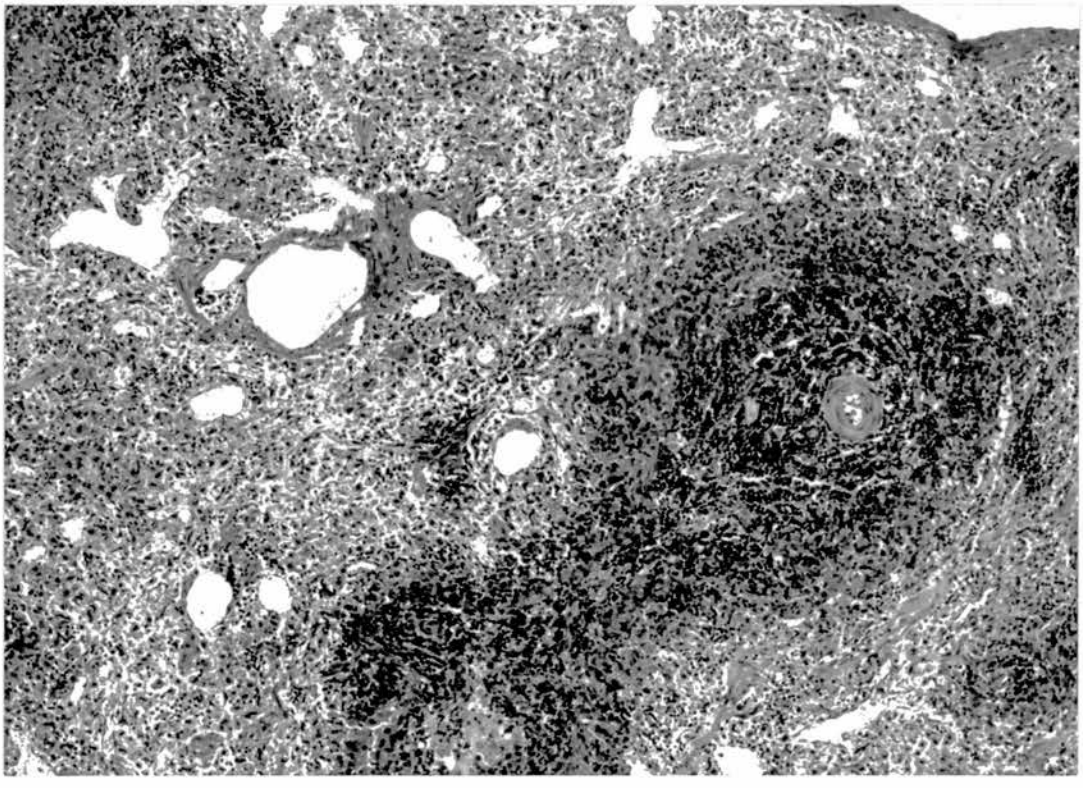

Fig.6.16 Spleen. 24 hours. Follicles show reduced thickness of their mantles but no apparent change in the interfollicular areas. HE. X112

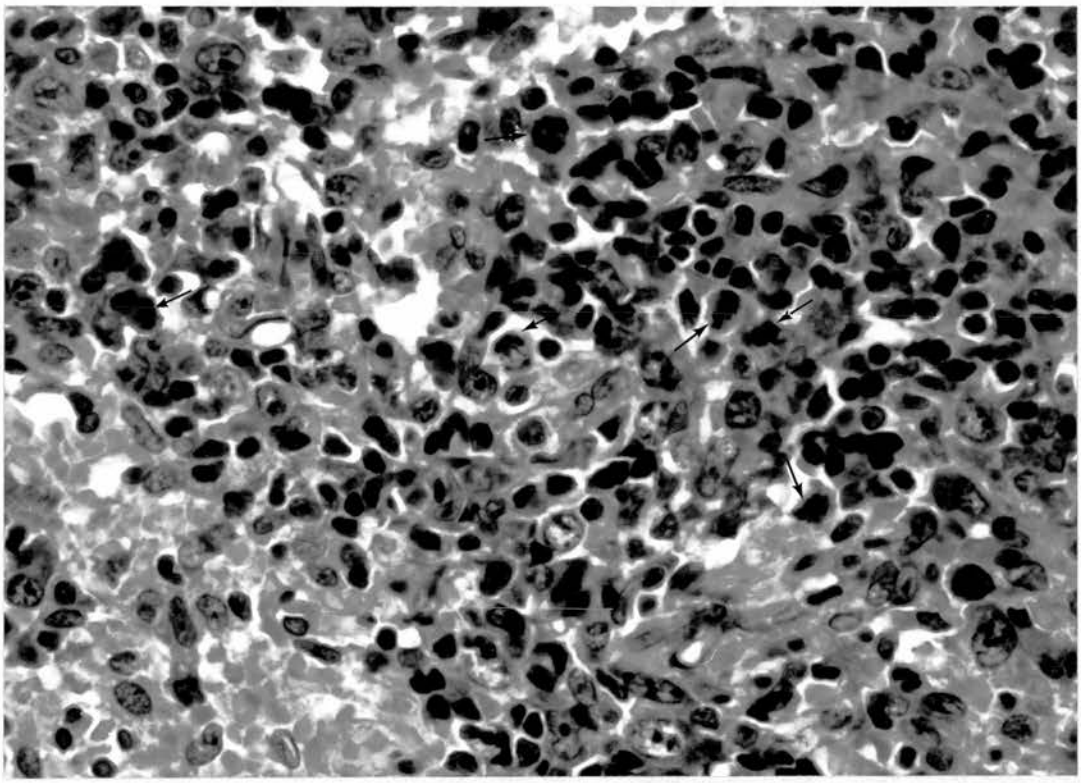

Fig.6.17 Spleen. 24 hours. At higher power the thinned mantle reveals marked increase in mitotic activity,(arrows). HE. X560

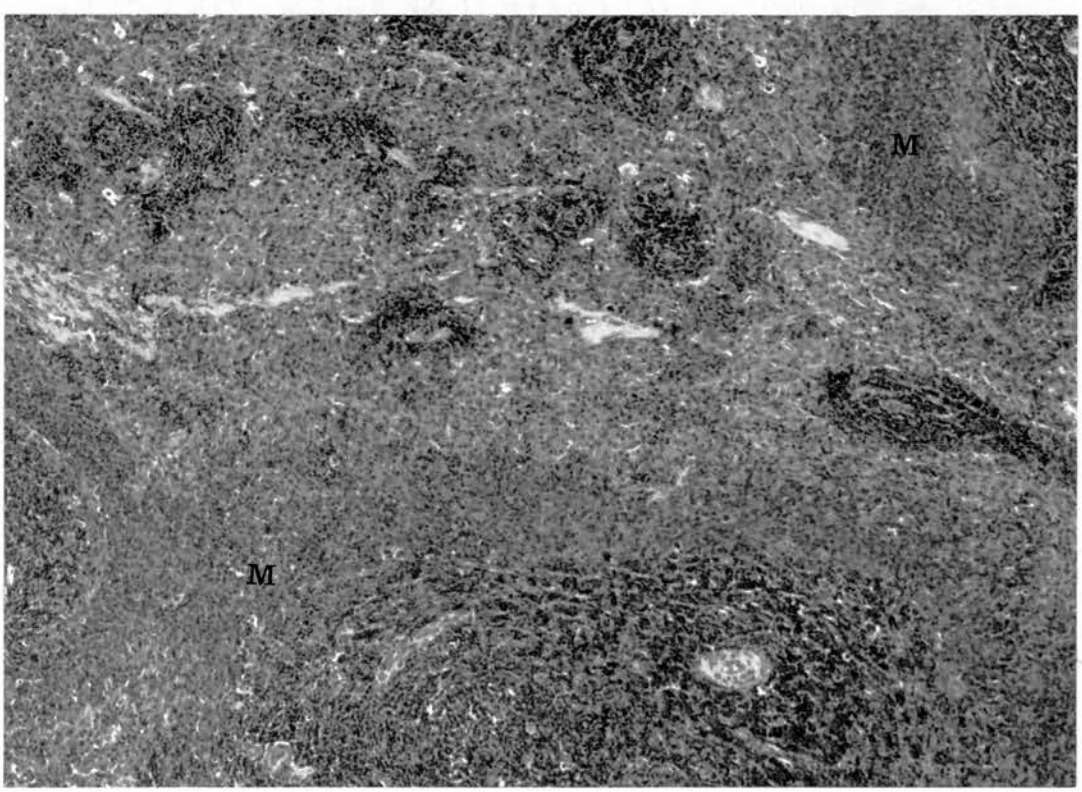

Fig.6.18 Spleen. 48 hours. Increase in thickness of the mantles and in the numbers of lymphocytes and plasmacytoid cells in the interfollicular zones. HE. X112

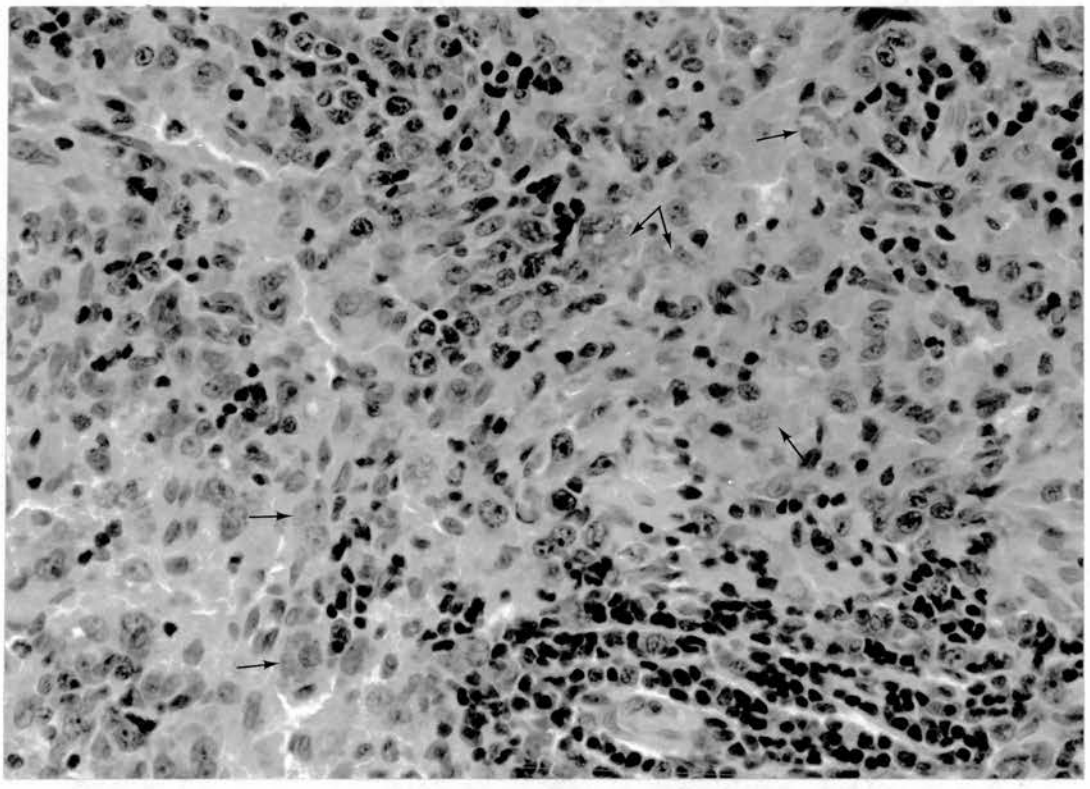

Fig.6.19 Spleen. 72 hours. Marked increase in interfollicular macrophages many of which contain pale-staining pigment granules (arrows) HE. X450

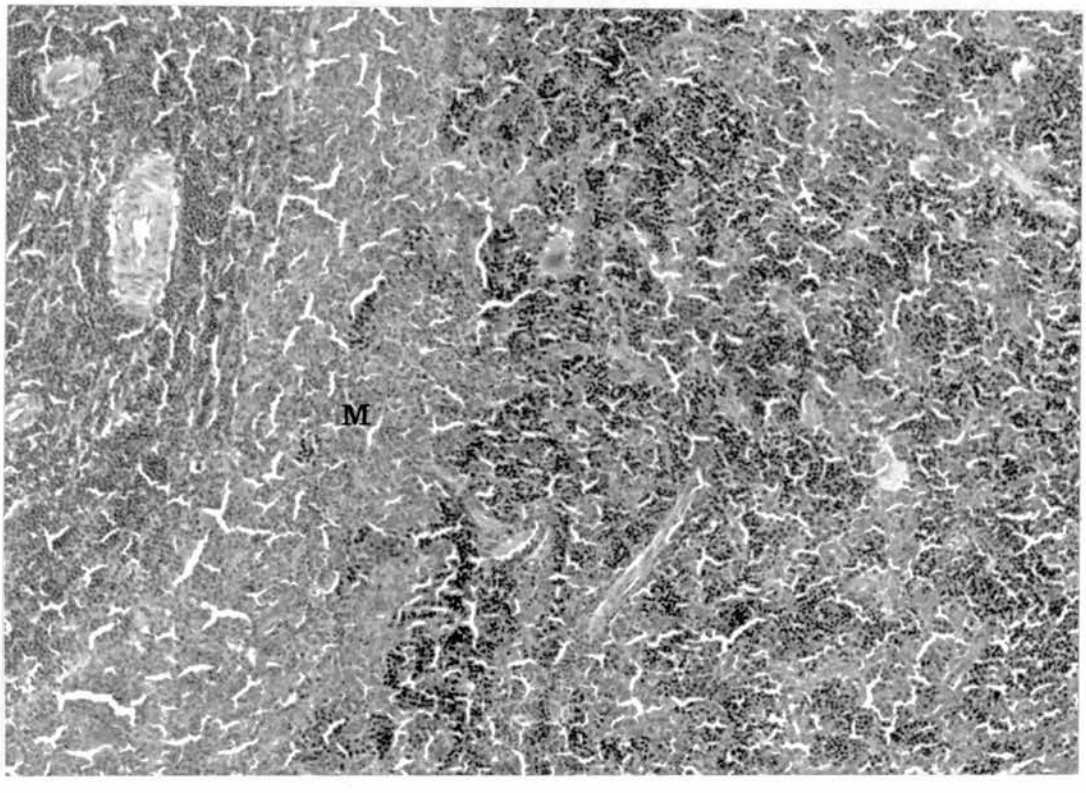

Fig.6.20 Spleen. 5 days. Marked increase in lymphocytes and plasmacytoid cells in the interfollicular areas together with apparent thickening of the mantle (M). HE. X 112

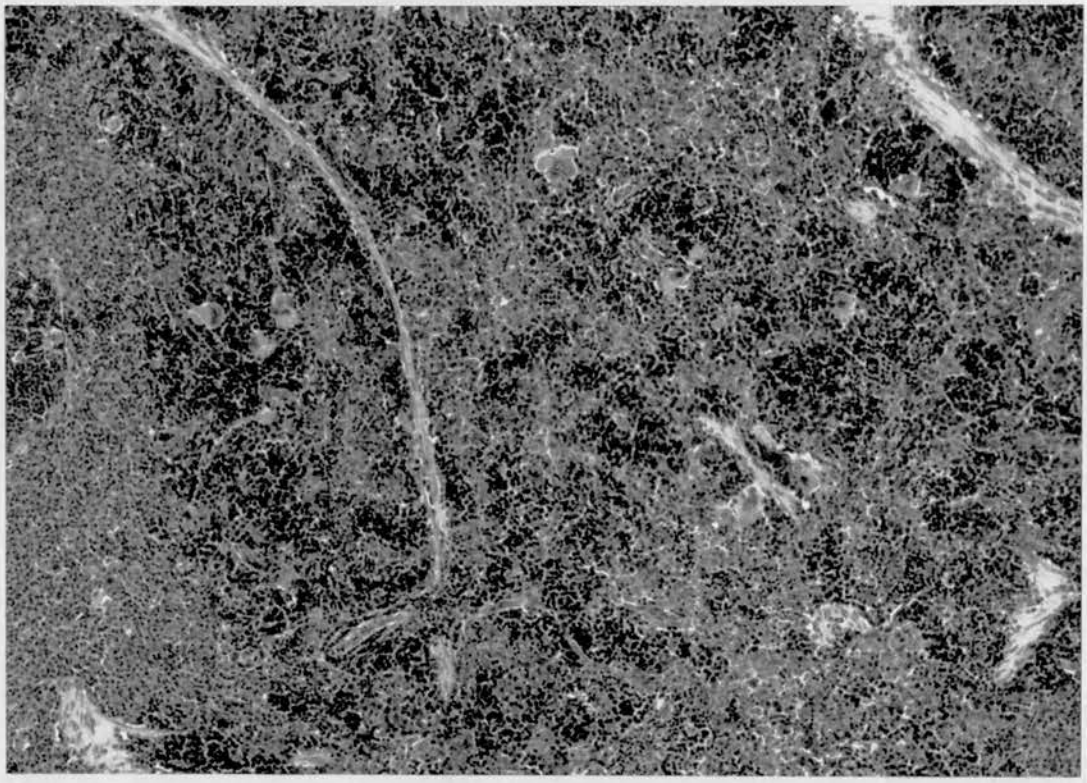

Fig.6.21 Spleen. 5 days. A similar marked increase in interfollicular lymphocytes and plasmacytoid cells is seen in this animal. HE. X112

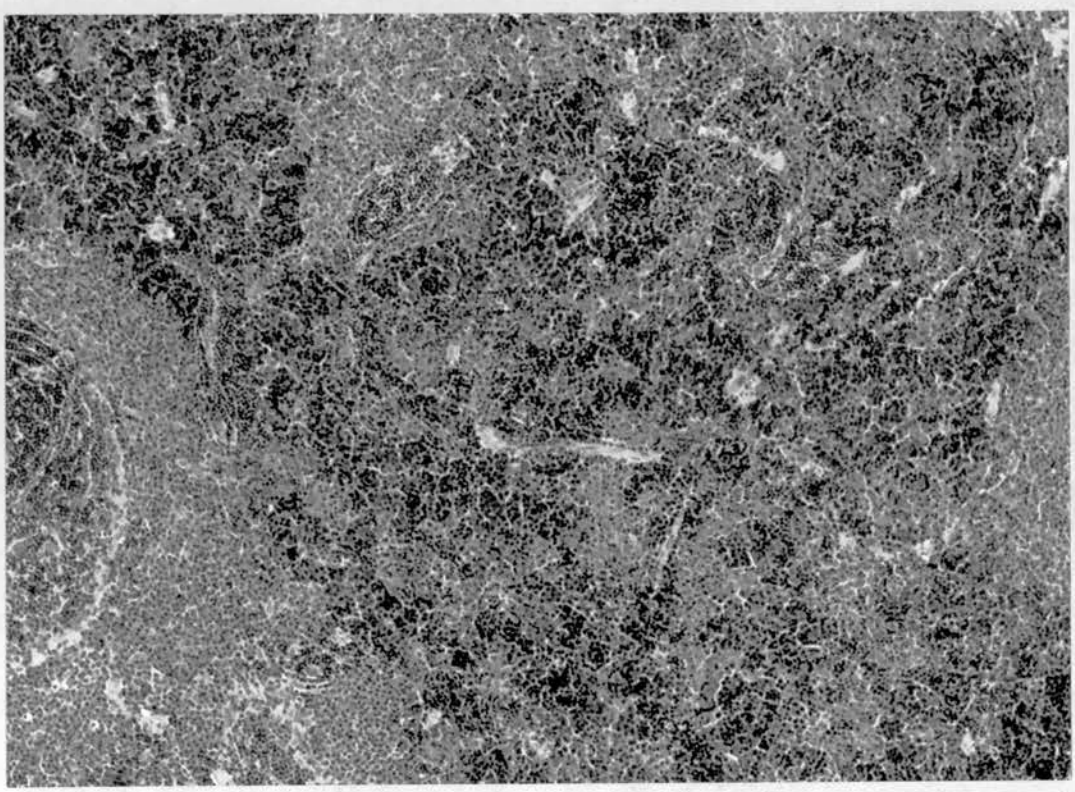

Fig.6.22 Spleen. 3 weeks. The increased cellularity is still evident in this animal. HE. X112

8. Adrenals

The glands from test animals showed no significant differences fron controls.

DISCUSSION

1. Heart

Boyd and Bereczky reported that rats dying less than 24 hrs after a large oral dose of paracetamol showed no lesions in the heart, whilst those dying between 1 and 7 days showed "vacuolar degeneration of coronary arterioles". The authors do not enlarge upon this statement, but changes in arterioles were not seen in the present study. Indeed, there were no noteworthy abnormalities in any constituent of the heart.

It would appear that there are no myocardial lesions detectable by light-microscopy following acute paracetamol overdosage in the rat and there is therefore no support for direct cardio-toxocity.

2. Brain

Apart from variable meningeal congestion, no significant abnormalities were found in the brain. Boyd and Bereczky mention "minute areas of granular degeneration" in rats dying between 1 and 7 days but this finding was not confirmed in the present series. Although the absence of morphological changes on light microscopy by no means excludes functional abnormalities and changes in fine structure, this study does not offer any support for a

direct toxic action by paracetamol on the brain.

3. Kidneys

Most experimental work and indeed, most controversy has been attached to the possible nephrotoxicity of paracetamol.

Patients suffering from either papillary necrosis or "interstitial nephritis" are often found to have taken considerable amounts of analgesic mixtures usually over many years prior to the onset of the renal disease. Such mixtures usually contain several analgesic or anti-inflammatory drugs combined with caffeine or other substances. Practically all the mixtures incriminated have contained phenacetin and it is generally concluded that phenacetin given in large doses over a prolonged period has a specific toxic effect on the kidneys. The epidemiological foundations for this conclusion are weak (Prescott, 1966; Abel, 1971) and if phenacetin, or its main metabolite paracetamol, had a specific nephrotoxic effect then it should be possible to reproduce it in animal experiments.

Animal studies purporting to demonstrate nephrotoxicity by phenacetin have generally been poorly controlled or poorly evaluated (Eisalo and Talanti, 1961; Dawborn et al., 1964; Fordham et al., 1965). Calder et al., (1971) compared the acute nephrotoxicity of aspirin and phenacetin derivatives in rats and concluded that the latter were much more toxic. They suggested that the nephrotoxicity of some phenacetin derivatives might be related to the para-arrangement of

amino and hydroxyl groups on the benzene ring, and showed that acetylation of the amino group and alkylation of the hydroxyl group of the phenacetin molecule each independently masks the nephrotoxicity. They found for example, that N-acetyl-p-aminophenol and p-phenetidine were not nephrotoxic. Since phenacetin cannot be made water-soluble, however, it was not studied in their experiments.

Peters et al., (1972) studied the possible nephrotoxicity of large doses of phenacetin, paracetamol, and other constituents of analgesic mixtures, in a large number of rats. Neither of the analgesics depressed glomerular filtration rate, maximal urinary concentration after dehydration plus vasopressin, urinary dilution after hypotonic expansion, or urinary acidification. Very large doses of paracetamol slightly increased proteinuria and the excretion of tubular cells. Phenacetin and paracetamol did induce degenerative changes in cortical proximal and, to a lesser extent, distal tubules. These changes comprised vacuolar degeneration, hyaline inclusions, and mild haemosiderosis, but as there was no interference with the reabsorption of sodium, water or potassium, or with urinary acidification, they were considered to be functionally irrelevant. There were no inflammatory changes, nor any medullary or papillary lesions. The authors concluded that neither phenacetin nor paracetamol exert major nephrotoxic effects in rats.

Whilst it is probably true that long-term administration of paracetamol is not associated with nephrotoxicity, the

absence of acute toxicity has more recently been challenged by Chenery, Fisher and McLean (1976). They argued that by manipulating the diet of laboratory animals so that it more closely resembled a human diet, principally by increasing the fat content, acute tubular necrosis could be produced by large doses of paracetamol.

In the present study 6 animals showed evidence of tubular degenerative lesions but in 5 these were minor and by analogy with Peters' study probably of no functional consequence. One animal did show acute tubular necrosis but this animal had evidence of widespread hepatic necrosis at death, and the renal damage may, therefore, have an alternative explanation.

It would appear that paracetamol can produce morphological changes in the kidneys when given in a single large dose but the functional significance of these changes is dubious. By manipulation of dietary factors, it seems possible to exaggerate the tubular damage and produce frank necrosis, but the co-existent hepatic damage may be modifying any renal effects and has to be carefully considered.

4. Pancreas

Raised serum amylase levels were found in 22% of 201 patients admitted for paracetamol overdosage in Newcastle (Hamlyn, personal communication). There were no episodes of clinical pancreatitis, however, and the elevated enzyme levels were attributed to hepatic dysfunction. In keeping with this conclusion, no significant histological abnormalities were found in the sections of pancreas in this study.

5. Stomach

Although paracetamol is frequently recommended as a substitute for aspirin to avoid gastric mucosal injury, there have been very few studies showing that paracetamol causes less damage to the gastric mucosa than aspirin.

The studies of Boyd and Bereczky, and Boyd and Hogan, revealed submucosal capillary-venous congestion in the stomach, with occasional ulcers found in the long term experiments. The present series showed no evidence of acute erosions or inflammatory changes. One animal had a mild chronic gastritis but this was obviously present before the administration of paracetamol.

Recently Ivey, Silvoso, and Krause (1978) studied the effect of paracetamol on the gastric mucosa of seven healthy volunteers using light and scanning electron-microscopy on biopsy samples. After a single dose of 1950 mg (six tablets) they found degenerative changes in 3.5% of surface cells. This did not differ significantly from the number of degenerating cells in control samples. In contrast, two aspirin tablets (600 mg) damaged 20-25% of the surface cells within 10 mins. Paracetamol produced none of the microscopic erosions seen after aspirin administration.

It would appear that paracetamol has no significant deleterious effects on the gastric mucosa. When gastrointestinal haemorrhage is seen after human paracetamol overdosage it cannot be ascribed to direct drug injury and must be a consequence of hepatic failure and/or uraemia.

6. Lungs

Boyd and Hogan found oedema, occasional abscesses and pneumonitis, in their long term experiments and attributed these changes to an increased susceptibility to infection. Similar changes were found in 5 test animals in the present study but here the explanation appeared to be that some aspiration of the drug suspension had occurred around the time of intubation.

7. Spleen

Changes in the weight of the spleen were demonstrated by Boyd and Bereczky after acute overdosage with paracetamol. Between 4 and 6 hours after administration the weight had gone down by 26.7% compared to controls, and by 24 hrs, 21.5%. On histology they found the red pulp contracted but do not mention any cellular changes. One month later the spleens had returned to the same mean weight as the controls.

The present series revealed interesting changes in the proportions of the various cell types found in the spleen.

Up to 24 hrs there was an overall reduction in the number and size of germinal centres and depletion of cells in the perifollicular mononuclear mantle. At 48 hrs the mantle had increased together with interfollicular lymphocytes and plasmacytoid cells and these were present in increased numbers at 3 weeks.

The best explanation of these changes is that from 24 hrs there is a marked proliferative response in the spleen. An initial depletion of lymphocytes and macrophages is

rapidly compensated for by mitotic division and migration of mononuclear cells from the perifollicular areas into the red pulp. Further cell division occurs in the red pulp so that by 5 days there is a considerable increase in cellularity involving all the cell lines native to the spleen.

The changes described here are very similar to the appearances encountered following the provocation of an immune response to foreign particulate antigens in experimental animals. Jandl et al., (1965) and Craddock et al., (1967) studied the responses in rat spleens following injection of sheep red blood cells, and found hyperplasia of all the interfollicular cell-types several days later. They suggested that the initial proliferative response may be an entirely non-specific reaction to the administration of particulate material and pointed to the enhancement of antibody synthesis brought about by simultaneous injection of particulate or colloidal materials and antigen. This explanation seems plausible when restricted to macrophages but the proliferation of lymphocytes and plasmacytoid cells which dominates the histological picture at 5 days demands an alternative hypothesis. The proliferation of these immunocompetent cell lines suggests that a specific reaction has been mounted, presumably as a consequence of tissue destruction in the liver. There is ample evidence that such immune reactions occur after injury to a wide variety of tissues and in particular following toxic liver injury. Weir (1964, 1967) has suggested that after cell death,

particulate subcellular components are taken up by phagocytic cells and induce the formation of IgM anti-tissue antibodies. These antibodies, detected by complement fixation, were found in maximum titre on day 4, and had returned to normal 7-10 days after injection of the liver damaging agent. Weir proposed that the IgM antibodies may combine with subcellular particulate antigens and in conjunction with complement release leucotaxins attracting polymorphs into the area of tissue breakdown. As far as the paracetamol injury is concerned, polymorphs are generally present in only small numbers which argues against this theory. It may be that the auto-antibody is acting as an opsonic factor which promotes the phagocytosis of cell debris by macrophages. This alternative role is consistent with the cytological events described above, namely

1. Particulate sub-cellular constituents of dead liver cells are taken up by local (Kupffer cell) and splenic macrophages.
2. There is a non-specific proliferation of macrophages in these sites.
3. Phagocytosis and processing of antigenic (? altered) self-constituents by macrophages is followed by proliferation of lymphocytes and plasmacytoid cells and synthesis of IgM antibodies.
4. Maximal opsonic activity occurs around 4 days when phagocytosis is at its height.
5. Thereafter, the amount of particulate antigen

delivered to the R.E.S. diminishes and there is a concomitant fall in antibody synthesis.

The investigation of the possible opsonic role of these auto-antibodies could be undertaken by in-vitro techniques but is outside the scope of the present study.

The changes found in the test spleens have given some insight into the general responses of animals to major tissue injury but there are no grounds for suggesting any direct effect by paracetamol.

Conclusion

This study of a range of tissues from paracetamol overdosed rats has unfortunately provided little new information. I have confirmed that minor morphological changes are produced in the kidneys but that the only instance of frank tubular necrosis was associated with severe hepatic necrosis, The interplay between hepatic and renal necrosis must not be overlooked in the experimental situation.

There are no grounds for suggesting a direct toxic action by paracetamol on any of the other tissues examined.

The spleens revealed an interesting sequence of cellular changes which illustrates the general response to tissue injury.

CHAPTER VII

Microsomal induction and hepatotoxicity

Despite the rapid absorption of paracetamol and its widespread distribution throughout the body (Gwilt, Robertson, and McChesney, 1963) the drug has a selective toxic effect on the liver. Primary damage to other organs such as the kidneys, heart, or C.N.S. has not been conclusively demonstrated. One explanation for this selectivity is that the liver is more "sensitive" than other tissues to paracetamol, but a much more likely possibility is that the drug is converted in the liver to a toxic metabolite - so-called "lethal synthesis". Examples of compounds which may undergo such enzymatic conversions are dimethylnitrosamine (Magee, 1956), the carcinogenic azo dyes (Mueller and Mueller, 1952), and carbon

tetrachloride (Recknagel and Ghoshal, 1966). It has been shown that pre-treatment with phenobarbitone, a known inducer of microsomal enzymes, potentiates the toxic effects of carbon tetrachloride and chloroform by enhancing production of their toxic metabolites (Judah et al., 1970). A logical first step in the examination of the mechanisms of paracetamol hepatotoxicity was to study the effects of phenobarbitone pre-treatment on the liver injury.

Materials and Methods

Two groups of six female Tuck-Wistar rats, each weighing about 200 g, were housed under identical conditions All animals were fed standard pelleted diets (Diet 41B, Oxoid Ltd) and were allowed water ad lib. One group of six rats was given drinking water containing phenobarbitone (1 mg/ml) for three weeks prior to the experiment.

After a 12 hour fast, each rat was given 4 g of paracetamol per kg body weight, made up in a suspension with 0.2% tragacanth, containing 300 mg paracetamol per ml. The suspension was given directly into the stomach, without anaesthesia.

At 1, 12 and 18 hours after the dose, approximately 0.5 mlblood was withdrawn from the tail without anaesthesia, into heparinised tubes.

Free paracetamol was determined in whole blood by gas-liquid chromatography as described by Prescott (1971). Plasma aspartate amino transferase (ASAT) and alanine

amino transferase (ALAT) were determined as previously described.

There were three deaths in the phenobarbitone treated group at 12, 18 and 24 hours following paracetamol. Livers of two of these were removed immediately and fixed in formol-saline. In one animal there was a maximum delay of three hours following death before removal of the liver. The remaining animals from both groups were killed at 76 hours by cervical dislocation, their livers removed and fixed immediately in formol-saline.

After fixation, the lobes of the liver were separated, cut into thin slices (2-3 mm), and alternate slices taken for microscopy, providing up to 15 "blocks" from each liver. Paraffin sections of these blocks were stained with haematoxylin and eosin, and by the periodic acid-Schiff method.

Quantitation of liver damage was carried out "blind" using a point-counting technique with a 25 point random-array eyepiece graticule and a X10 objective. Five-hundred points were counted for each block and the degree of necrosis expressed as a percentage of the total hepatic parenchyma. The mean of these results gave the extent of necrosis for each liver.

RESULTS

The mean blood paracetamol levels for each group of rats at 0, 1, 12 and 18 hours after the dose are shown in Fig. 7.1. The mean value at 1 hour for the control group

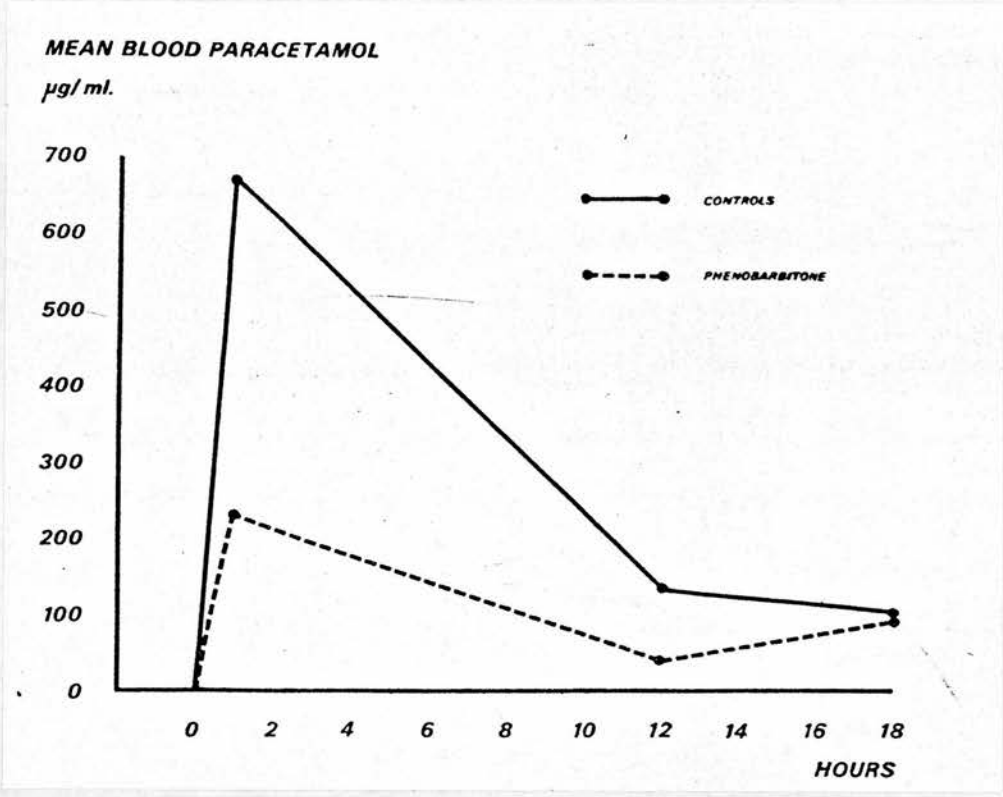

Fig.7.1 Mean blood paracetamol levels for phenobarbitone treated and control animals at 0,1,12 and 18 hours following oral dosage.

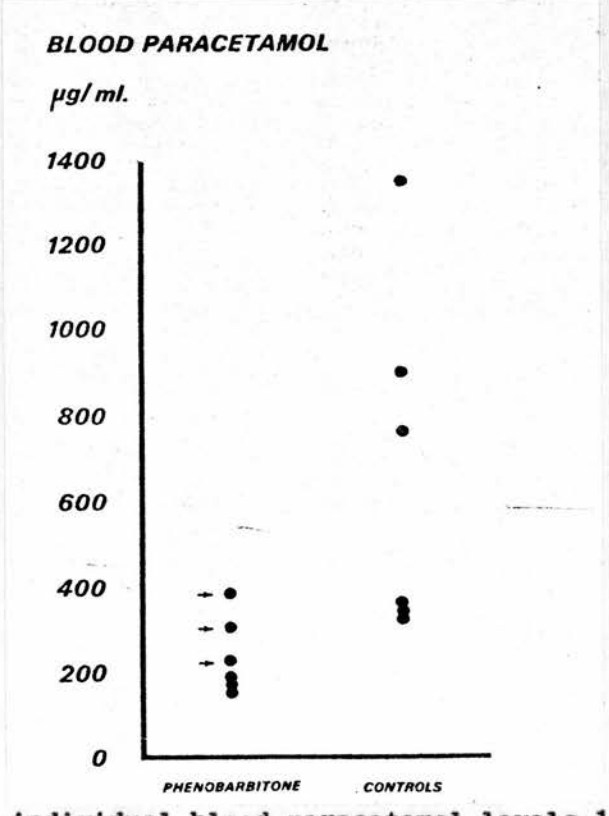

Fig.7.2 The individual blood paracetamol levels 1 hour after dosage. Arrows indicate the animals which died.

was 670 µg/ml and for the phenobarbitone group 234 µg/ml. The difference between these two results is significant ($t = 2.3$, $p < 0.05$).

Three of the phenobarbitone treated animals died 12, 18 and 24 hours after the dose of paracetamol (fig. 7.2). Three of the control animals had paracetamol levels very much higher and yet did not die up to 76 hours, when all surviving animals were killed.

The maximum enzyme levels obtained in each animal are shown in table 7A. All rats showed some elevation in the ASAT although the rise was very variable. In the phenobarbitone treated group the 3 rats which died showed the highest ASAT levels. In the control group similar and even higher ASAT values were found but none of these animals died within the experimental period. One rat in the control group showed a very high ASAT level (21,000 IU/L) yet did not appear in any way different from the other animals within the group. The animal with the highest paracetamol level (1,350 µg/ml) had a maximal ASAT level of 7,890 IU/L, a value not as high as two of the phenobarbitone treated rats which died with very much lower paracetamol levels.

In both the phenobarbitone treated group and the control group, the maximum ALAT was lower than the corresponding ASAT value, in all except one animal. In the phenobarbitone treated animals the three which died showed the highest values for ALAT in that group. Two of the three phenobarbitone treated rats which died showed

TABLE 7A: The degree of hepatic necrosis, and the maximum value attained for ASAT and ALAT in phenobarbitone and control rats

Phenobarbitone pretreated				Control			
% ± 1 SE Necrosis		IU/L ASAT	IU/L ALAT	% ± 1 SE Necrosis		IU/L ASAT	IU/L ALAT
55.2	3.8	2197	160	30.0	17.1	9490	5010
35.7	4.2	778	106	30.6	8.4	7890	1120
81.8	3.7	9810	5010*	27.4	3.3	21760	5120
87.1	1.9	9810	6080*	36.1	9.4	9810	4900
91.3	1.4	7360	9810*	22.4	4.0	881	252
54.5	5.2	7250	1600	33.9	13.9	10800	3754

* Animals which died during the experiment

values for ALAT (9,810, 6,080 IU/L) which were in excess of the highest value (5,120 IU/L) attained in the control animals and the third animal which died had a value (5,010 IU/L) which was only slightly below the highest value (5,120 IU/L) attained in the control animals.

All animals showed histological evidence of hepatic necrosis. The greatest degree of necrosis was seen in those animals which died (table 7A). In the phenobarbitone group the animals with the least rise in ASAT and ALAT showed, in general, the least necrosis. Of the control animals, in those which had values for ASAT greater than 9,000 IU/L showed considerably less necrosis than the phenobarbitone animals which died, even though some of these had lower values for ASAT.

DISCUSSION

The metabolism of foreign compounds has been extensively studied by Brodie, Gillette and La Du (1958). They demonstrated that there is a system of enzymes located in the microsome fraction of the liver (equivalent to the smooth endoplasmic reticulum) which acts upon a wide range of foreign molecules tending to convert them into more water-soluble forms. The enzyme system hydroxylates or dealkylates a large number of substrates, such as steroids, barbiturates, polycyclic hydrocarbons and insecticides like DDT. The system requires reduced nicotinamide-adenine dinucleotide phosphate (NADPH) and oxygen, and is inhibited

by carbon monoxide which binds onto its cytochrome component (P-450). Cytochrome P-450 is central to the system, as it is there that the substrate (probably held by one of a number of substrate-binding proteins), oxygen, and the reducing action are brought together (Conney, 1967). This drug-metabolising enzyme system can be enhanced by phenobarbitone pretreatment and is paralleled by a proliferation of smooth endoplasmic reticulum in hepatocytes.

The hypothesis that toxicity is due to 'lethal synthesis' and not to unchanged paracetamol is supported by the results of this experiment in that there was increased mortality in those animals pretreated with phenobarbitone, although having lower plasma levels of the drug.

Plasma levels of ASAT and ALAT were raised in all animals, both phenobarbitone treated and controls. There was, however, considerable variation within each group in the maximum value obtained. The 3 phenobarbitone treated rats which died exhibited the highest values for each of the amino-transferases in that group, which suggests that their death was related to a more severe hepatic lesion. Within the control group, however, higher levels were seen in animals which did not die and which had less hepatic necrosis. Thus in this experiment the maximum enzyme levels did not accurately reflect the degree of hepatic necrosis. Apart from the animals which died, however, necrosis was estimated from the appearances at 72 hours by which time significant regeneration would have occurred.

To overcome this problem the periodic acid-Schiff stain was used in order to identify recently regenerated hepatocytes surrounding residual areas of centrilobular necrosis and macrophage infiltration in the surviving animals. Regenerated cells have a low content, or are devoid of glycogen and contrast with the glycogen-rich cells of the uninvolved peri-portal areas. By quantitating the regenerated tissue along with the residual necrotic areas, a measure of the maximal extent of damage was obtained. In this way, it was hoped that valid comparisons could be made with those animals dying early in the experiment. The fact that there was a poor correlation between estimates of necrosis and the peak enzyme values probably reflects the disparity between the time of maximum enzyme elevation (usually 24 hours) and sacrifice of the animals (72 hours) with a consequently less accurate assessment of liver necrosis.

CHAPTER VIII

The toxic metabolism of paracetamol

Our understanding of the toxic metabolism of paracetamol has been greatly advanced by the findings of Mitchell and his colleagues at the National Institute of Health, Bethesda. In a series of four important papers published in 1973 they demonstrated not only that paracetamol -induced hepatic necrosis was enhanced by phenobarbitone pre-treatment, but that known inhibitors of cytochrome P-450 activity, namely cobaltous chloride and piperonyl butoxide, protected against the necrosis, (Mitchell et al; 1973 a). Using tritium-labelled paracetamol they showed that covalent binding of large amounts of radio-labelled material to mouse liver protein in vivo and that the degree of binding in individual mice was always directly proportional

to the severity of necrosis regardless of the biological variation among animals. They therefore proposed that paracetamol-induced hepatic necrosis may be caused by the covalent binding of a chemically reactive metabolite to vital hepatic macromolecules (Jollow et al; 1973 a). In a further paper they showed that the binding occurred by covalent linkage to amino-acids or protein, and that reduced NADP and oxygen were necessary for the binding. On the other hand, carbon monoxide or cobaltous chloride pretreatment inhibited the binding, indicating that it was mediated by a cytochrome P-450 dependent, mixed function oxidase. The authors had previously shown that the binding of 2-acetylaminofluorene was also dependent on cytochrome P-450 and that its hepatotoxicity resulted from its conversion to a N-hydroxy derivative, and they therefore suggested that the toxic metabolite of paracetamol may be an N-hydroxy derivative (Potter et al; 1973).

Probably influenced by previous reports of glutathione conjugation with foreign aromatic or unsaturated compounds and subsequent excretion of their detoxified metabolites as mercapturic acids in animals (Boyland and Chasseaud, 1967, 1968; Clapp and Young, 1970; Gilham, 1971), Mitchell et al., examined the possiblity that glutathione may prevent paracetamol-induced necrosis. They showed that the alkylation of hepatic macromolecules by the toxic metabolite can be inhibited in vitro by the addition of glutathione and cysteine, and they next examined the effects of these

compounds in vivo. Diethyl maleate, which depletes hepatic glutathione, potentiates paracetamol-induced necrosis, whereas pretreatment with cysteine, a glutathione precursor, prevented hepatic damage. They further demonstrated that covalent binding of the toxic metabolite to hepatic macromolecules did not occur until the availability of glutathione was depleted by at least 70% through conjugation with the metabolite (Mitchell et al., 1973 b).

In man there has been some difficulty in establishing the role of glutathione in preventing hepatic damage induced by paracetamol and other aryl and unsaturated drugs. Conjugation of drugs with glutathione does occur, but enzyme activities are reported to be lower in man than in rats and mice (Grover and Sims, 1964). Warner and Lorincz (1963) were unable to isolate a glutathione conjugate after administering bromobenzene to human subjects. Jagenburg and Toczko (1964) reported the identification of S-(1-acetamido-4-hydroxyphenyl) cysteine as a metabolite of both paracetamol and phenacetin in man; however, there was no evidence cited that indicated glutathione participation in the formation of this metabolite. Jagenburg, Nagy and Rodger (1968) reported the isolation of another cysteine-containing metabolite excreted in amounts ranging from 4.5 to 6.1% of the ingested dose of paracetamol. Based on the absence of reaction with ninhydrin and also on "the well known fact that mono-halogenated benzenes form N-acetyl-cysteine derivatives (mercapturic acids) in the animal

body," Jagenburg et al., assumed the metabolite to be S-(1-acetamido-4-hydroxyphenyl)-mercapturic acid. Mrochek et al. (1974), however, using mass spectrometry failed to idenfity any mercapturic acid among the seven urinary metabolites obtained from human subjects (Table 8.A). The absence of a mercapturic acid metabolite argues against a reaction between a toxic metabolite and glutathione, and the authors suggest that the protective role may be played by cysteine or methionine.

A role for glutathione now appears to be confirmed in recent work by Andrews et al (1976). Using two dimensional thin-layer chromatography they have identified seven urinary metabolites (Table 8.A) together with very small amounts of 3-methylthioparacetamol. In general terms, the metabolites identified in their study compared closely in number and type with those reported by Mrochek et al., but the assignment of structures differed markedly. The most important difference was the failure of Andrews et al., to identify a cysteine-glucuronide conjugate. The identification of this compound by Mrochek et al., was based on the fact that it gave a positive reaction with ninhydrin, even though their compound was completely resistent to hydrolysis with β-glucuronidase and the mass spectral data was confused. Andrews et al., made considerable efforts to find a compound with the expected properties of a cysteine-glucuronide conjugate totally without success. They concluded therefore, that the compound noted by

TABLE 8A: Assigned structures - Andrews et al. (1976) vs. Mrochek et al.(1974)

Compound	Andrews et al.	Mrochek et al.
P	Paracetamol	Paracetamol
G	Paracetamol glucuronide	Paracetamol glucuronide
S	Paracetamol sulphate	Paracetamol sulphate
G1	3-methoxyparacetamol glucuronide	2-methoxyparacetamol glucuronide
S1	3-hydroxyparacetamol-3-sulphate	2-hydroxyparacetamol sulphate
S2	3-methoxyparacetamol sulphate	2-methoxyparacetamol sulphate
C	Paracetamol -3-cysteine	Paracetamol 3-cysteine
M	Paracetamol -3-mercapturate	Paracetamol -3-cysteine glucuronide

Mrochek et al., was the mercapturate contaminated with an unknown ninhydrin-positive compound.

The positive identification of a mercapturate is of considerable importance as it lends much support to the hypothesis that glutathione is the principal nucleophilic sulphydril compound protecting the liver against the toxic metabolite of paracetamol. Mercapturic acid formation derives from an initial conjugation of aromatic hydrocarbons with glutathione, a reaction catalysed by glutathione-S-transferase. Subsequent degradation of the S-substituted glutathione to an S-substituted cysteine is followed by acetylation and excretion of the mercapturic acid.

Quantitation of urinary metabolites

Although Mrochek et al., appear to have been in error in their identification of the urinary metabolites, their high-resolution anion-exchange chromatographic technique offers the most accurate method so far developed of measuring the urinary concentrations of paracetamol and its seven metabolites. Their results for the urinary metabolites of two clinically normal men who ingested 950 mg paracetamol are shown in Table 8.B. Also included are the results of Jagenburg and Toczko (1964) obtained using a strong cation-exchange column; Cummings, King and Martin (1967) using silica-gel plates and spectrophotometric measurement; and Jagenburg et al. (1968) using gel filtration on Sephadex G.10.

Table 8B Paracetamol Metabolites in Therapeutic Urines

Subject (all male)	Dose (mg)	Collection Period	Urinary metabolites (as % of dose)								% recovery	Source
			free paracetamol	S2	S1	S	G	G1	C	M		
1	1,950	24	1.3	3.6	2.5	29.4	60.1	3.4	*	5.0	105	Mrocheck et al (1974)
2	1,950	24	1.9	1.3	1.8	29.3	57.8	2.3	*	1.6	96	
3	2,000	24	3.0	-		17.5	58.5		0.4	4.5	84	
4	1,500	24	4.5	-		22.9	49.4		3.0	6.1	91	Jagenberg, Nagy and Rodjer (1968)
5	1,500	24	3.4	-		33.9	30.7		5.9	4.6	82	
6	2,000	24	*						2.8	*	-	Jagenberg and Toczko (1964)
7	762	15	4.1	25.5			59.4			*	89	
8	684	15	3.8	26.2			47.0			*	77	Cummings, King and Martin (1967)
9	840	15	3.1	23.8			42.1			*	69	
10	993	15	3.2	22.2			46.6			*	72	

The results quoted in Table 8B reveal that after a therapeutic dose of paracetamol the concentration of the urinary metabolites expressed as a percentage of the dose is as follows:-

Free paracetamol	1-4%	
Paracetamol glucuronide	40-60%	simple conjugates
Paracetamol sulphate	20-30%	
Sulphur conjugates	5-10%	oxidised metabolites
Catechol derivatives	5-10%	
(Total recovery	70-100%)	

The relative proportions of metabolites appearing after overdosage have been studied by Davis et al (1976). In a preliminary experiment they measured the urinary metabolites from three male volunteers given 1, 2, 3 and 4 g of paracetamol. These revealed that 85-90% of the drug was excreted, as conjugates, in the first 24 hours after ingestion. Paracetamol glucuronide accounted for 45-55% of the total metabolites, paracetamol sulphate 20-30%, and the cysteine and mercapturic acid conjugates 15-25%. Whereas the excretion of the glucuronide and of the cysteine/mercapturic acid conjugates increased progressively as the dose was raised, the excretion of the sulphate reached a plateau and did not rise with the increase in dose from 3 to 4g.

In the 30 patients studied after overdosage, the total quantities of paracetamol metabolites increased with the

severity of ensuing liver damage. With one exception, in both groups all patients who excreted 10.5 g or more developed moderate or severe liver damage, and those in which less than this amount was recovered developed mild hepatotoxicity or showed no biochemical evidence of liver damage. When the quantity of metabolites was compared in patients with similar degrees of liver damage, however, there was considerable variation in the amounts excreted.

In keeping with the levelling-off of sulphate excretion in the volunteers, the proportion of sulphate in the over-dose urines fell with increasing total quantity of metabolites. It appears that sulphate conjugation becomes rapidly saturated and can occur even with therapeutic doses. This is initially compensated for by an increased conjugation with glucuronide, but at high hepatotoxic doses this path-way also becomes saturated. When this occurs there is an increased proportion of the drug conjugated with mercapturic acid and cysteine. These changes in the relative proportions of the metabolites could be correlated with the severity of liver necrosis. Thus, those patients who excreted more than 30% of the total as the sulphur conjugates, with less than 60% appearing as glucuronide, developed moderate or severe liver damage.

On the basis of these findings, Davis et al., suggested that when the rate of presentation of paracetamol to the liver exceeds the capacity of the microsomal enzymes to form glucuronide and sulphate conjugates, then the cysteine

and mercapturic acid conjugates are formed via the chemically reactive intermediate at an increased rate. If the oxidative metabolism of paracetamol occurs faster than glutathione can be synthesized, the levels of the latter substance will fall, and the reactive metabolite combine with liver cell proteins instead.

Nature of the toxic metabolite

Two main candidates have been proposed as the toxic metabolite, the N-hydroxy derivative, or an epoxide metabolite of paracetamol. If the latter compound is responsible, this may be acting through free-radical formation. Free-radicals may be formed in some other way, however, and constitute a third possible toxic intermediate.

Unequivocal evidence that monocyclic N-acylarylamines (of which paracetamol is an example) undergo N-hydroxylation has been obtained by Hinson, Mitchell and Jollow (1974) using p-chloroacetanilide incubated with hamster liver microsomes. The reaction required NADPH and oxygen, and was inhibited by a CO: O_2 (9:1) atmosphere, again suggesting that the enzyme responsible is a cytochrome P-450 mixed function oxidase. Their findings are consistent with the hypothesis that N-oxidation is important for the toxicities caused by many monocyclic N-acylarylamines including paracetamol.

Andrews has been able to synthesise the N-hydroxy derivative and considers on purely chemical grounds that

this is unlikely to be the toxic intermediate (Personal communication, 1976). It would be most interesting to examine the hepatotoxicity (or otherwise) of this compound.

Our group have considered the possibility of free-radical formation. These injure cells by a number of actions, but a major cause of damage is the peroxidation of lipid in cell membranes. Breakdown of lipid peroxides leads to an increased concentration of malonaldehyde (Jose and Slater, 1972) but we have not been able to demonstrate convincing increases in this compound after paracetamol overdosage in the rat (unpublished observations). This argues against a major role for lipid peroxidation in the mediation of paracetamol hepatotoxicity.

Thus in the present state of knowledge we do not know the identity of the toxic metabolite of paracetamol. Conclusive proof of its nature must presumably await the isolation or capture of the reactive intermediate.

Cellular effects of the toxic metabolite

The rate of accumulation and final concentration of this toxic metabolite might explain the various forms of cell injury seen in paracetamol liver damage and described in Chapter 4.

In the earliest stages a relatively low concentration of the reactive metabolite could impair synthetic activity by damage to ribosomes and the endoplasmic reticulum and result in a net catabolism of macromolecules. This could

account for the loss of glycogen, and a rise in osmotic pressure within the cell. The consequent influx of water leading to cytoplasmic matrix swelling. Later, mitochondrial damage would result in failure of the energy-dependent sodium-pump,and the entry of sodium ions, with their larger hydration shells than potassium ions, bring more water into the cell.

In centrilobular cells, which are richest in drug metabolising enzymes (Wattenburg and Leong, 1962; Koudstaal and Hardonk, 1969, 1970), there is likely to be a more rapid accumulation of the metabolite with accelerated destruction of membranes, and, in particular loss of semi-permeability of the plasma membrane. The influx of water would then cease and continuing disruption of membranes and organelles give rise to coagulative necrosis. Where the build-up of free metabolite was slower (in midzonal cells) and insufficent to destroy the semi-permeability of the plasmalemma, water accumulation would predominate producing vesiculation of the endoplasmic reticulum and, in some cells, hydropic vacuolation. Such aqueous swelling may, however, be an expression of sub-lethal injury which elsewhere is manifested by fatty change. Other scattered cells may react to the toxic metabolite by continued metabolic activity, with progressive dehydration, budding, karyorrhexis, and eventually, acidophil-body formation.

CHAPTER IX

Vitamin E protection against paracetamol hepatotoxicity

Having failed to modify the extent of hepatic necrosis using either antihistamines or corticosteroids and rejecting their use in the treatment of paracetamol overdosage, other methods of preventing hepatoxicity were sought. It is known that antioxidants provide some protection against the hepatotoxic effects of carbon tetrachloride which gives a similar hepatic necrosis, (Gallaher 1962; DiLuzio and Costales, 1965). Therefore, the possibility that the antioxidant α-tocopherol might provide some protection against the hepatotoxic effect of paracetamol was investigated.

Materials and Methods

Female Tuck-Wistar rats, weighing about 200 g, were used for all experiments.

In the first experiment twelve rats were housed under identical conditions. Six were fed a synthetic diet deficient in vitamin E (Kelleher, Davies, Smith, Walker and Losowsky 1972) for sixteen weeks. The remaining six rats were given the same diet but supplemented with 100 mg α-tocopherol acetate /kg diet, which was intended to restore the intake to normal, for sixteen weeks. Both diets were readily accepted by the animals, the consumption of each approximating to 20 g per rat per day. Each animal then received paracetamol 4 g/kg body weight in a suspension (300 mg/ml) with 0.2% tragacanth by stomach tube without anaesthesia. The animals were fasted for 16 hrs from 6 pm on the previous day prior to paracetamol administration at 9 am.

In the second experiment thirteen rats were housed under identical conditions and fed a standard pelleted diet (diet 41B, Oxoid Ltd) containing 37.4 mg vitamin E/kg. Six rats were given 50 mg of α-tocopherol acetate by stomach tube at 9 am each day for eight days prior to the experiment. The six vitamin E treated animals, along with the remaining seven control rats, were then each given paracetamol at a dose of 4 g/kg body weight, by stomach tube without anaesthesia, at 9 am following an overnight fast from 6 pm the previous day. The last dose of α-tocopherol acetate

was given 24 hrs previously.

In the third experiment groups of rats (12 in most instances) fed the standard pelleted diet were fasted overnight from 6 pm and dosed by stomach tube without anaesthesia at 9 am. Paracetamol was fed at three dose levels, 4, 3, and 2 g/kg body weight, with and without added α-tocopherol acetate to six separate groups of rats. The paracetamol was prepared as a suspension 300 mg/ml in 8% Cremophor E.L. with or without α-tocopherol acetate at a concentration of 100 mg/ml.

In each experiment, at 1 hr following the paracetamol, 1 ml of blood was withdrawn from the tail for paracetamol, aspartate amino-transferase, (ASAT), alanine aminotransferase (ALAT), vitamin E and red cell haemolysis estimations. The enzyme levels at 1 hr were not influenced by paracetamol and were used as control levels for each rat. For all rats used in these experiments the mean 1 hr enzyme values were 129 i.u./l, SD 64 for ASAT and 29.4 i.u./l, SD 10.5 for ALAT.

Samples of blood for paracetamol and enzyme estimations were also taken immediately before killing the animals by cardiac puncture under light ether anaesthesia at 24 hr. The experiments were terminated at 24 hr because earlier studies had shown this to be the usual time of maximum enzyme elevation in the rat.

Free paracetamol was determined in 0.1 ml whole blood by gas liquid chromatography as described by Prescott (1971).

Serum ASAT and ALAT were determined as previously described. Plasma α-tocopherol was determined by the spectrofluorimetric micromethod of Hansen and Warwick (1966). The susceptibility of red cells to haemolysis by hydrogen peroxide in vitro was determined by the Horwitt (1962) modification of the method of Rose and György (1952).

Histology was taken as previously described. In these experiments the mean number of blocks was 10.7 per animal. Hepatic necrosis was graded on a scale 0-5 as before, and a mean grade calculated for each animal.

RESULTS

First Experiment

In the first experiment the vitamin E deficient animal had a mean plasma vitamin E of 188 µg/100 ml (SD 120 n=6,) which was significantly lower ($p<0.001$) than that for the vitamin E supplemented animals 740 µg/100 ml, (SD 118 n=6). The animals in the vitamin E deficient group all showed 100% red cell haemolysis with hydrogen peroxide, whereas the red cells from the supplemented group did not haemolyse.

The mean 1 hr and 24 hr blood paracetamol levels for the six supplemented animals (189 µg/ml, SD 96.7 and 212 µg/ml, SD 153 respectively) did not differ significantly ($p> 0.2$ and $p<0.8$) from those of the deficient group (266 µg/ml, SD 93.7 and 227 and 227 µg/ml, SD 101.3 respectively).

The values of ASAT and ALAT at 1 hr and 24 hr in the vitamin E deficient and vitamin E supplemented animals are shown in Fig. 9.1. and the individual enzyme values at 24 hr in Table 9A. All rats in these groups had higher values for ASAT and ALAT at 24 hr than at 1 hr. In the vitamin E deficient group, five of the six animals had values for ASAT at 24 hr which were very much higher than the highest value for the supplemented group. For ALAT at 24 hr five of the six vitamin E deficient rats had values exceeding 1000 i.u./litre whereas only two of the vitamin E supplemented group exceeded this value.

Second Experiment

In the second experiment the mean plasma vitamin E for the control animals 396 µg/100 ml (SD 48, n=7) was significantly lower ($p<0.001$) than that for the vitamin E treated group (1240 µg/100 ml SD 540 n=6). The mean 1 hr and 24 hr paracetamol levels in the control animals (199.5 µg/ml SD 93.7, n=7 and 68.0 µg/ml, SD 36.3 n=7 respectively) did not differ significantly ($p>0.3$, and $p>0.8$) from those in the treated animals (152.7 µg/ml, SD 57, n=6 and 72.6 µg/ml SD 39.3, n=6 respectively). Although the 1 hr paracetamol levels for rats in this second experiment did not differ significantly from the 1 hr levels in the first experiment, the 24 hr levels (mean 69.5 µg/ml SD 40.8, n=13) were significantly lower in the rats of this second experiment compared to the 24 hr levels (mean 219 µg/ml SD 124 n=12) in the first experiment ($p<0.005$).

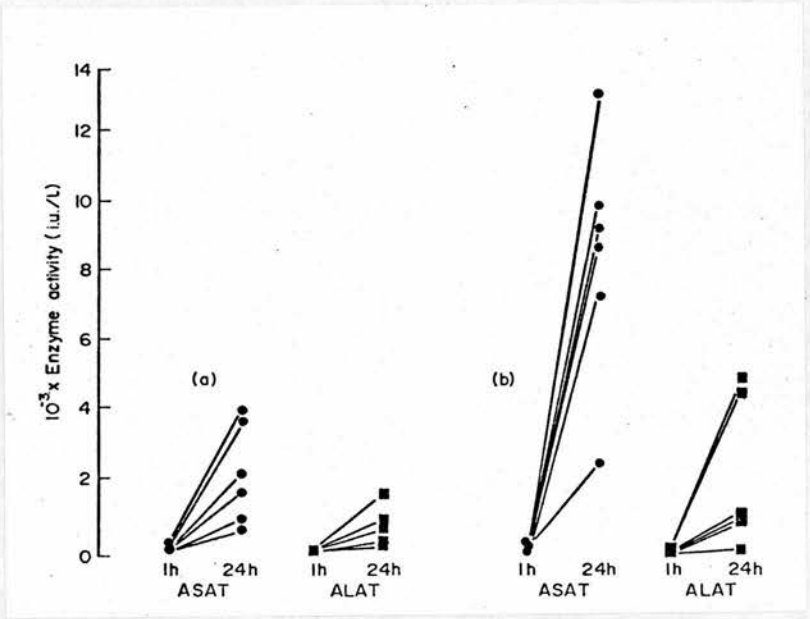

Fig. 9.1 Plasma concentration of ASAT and ALAT 1 and 24 hr after dosage with paracetamol (4g/kg) in (a) six vitamin E supplemented and (b) six vitamin E deficient animals.

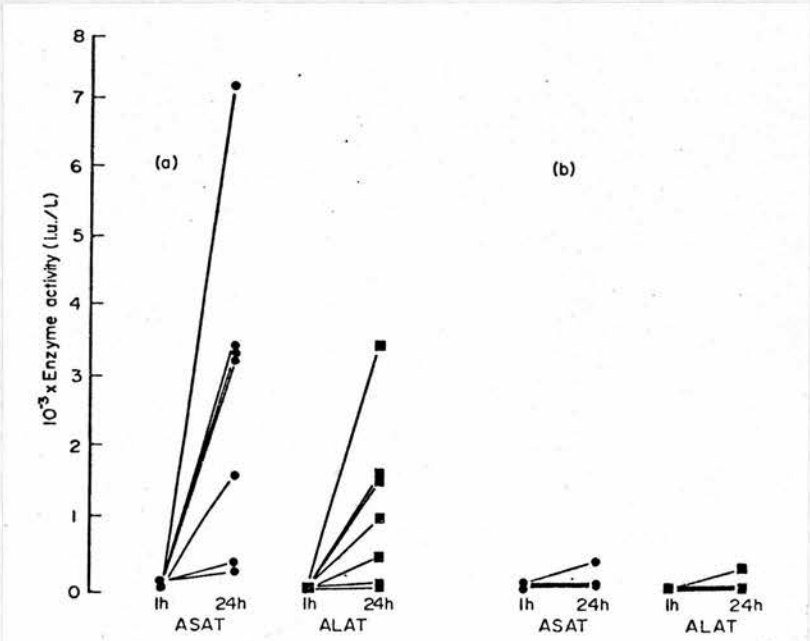

Fig. 9.2 Plasma concentration of ASAT and ALAT 1 and 24 hr after paracetamol (4g/kg) in (a) seven contrl animals (fed on standard pelleted diet) and (b) six animals fed on the same diet, but each in addition receiving a daily dose of 50mg of α-tocopherol for 8 days before the paracetamol.

The results of the plasma ASAT and ALAT in these rats are shown in Fig. 9.2. and the individual enzyme values at 24 hr in Table 9A. In the control group (those fed a standard diet) all rats showed a rise in both ASAT and ALAT at 24 hr compared with the 1 hr value. For ASAT five out of seven rats had 24 hr values of greater than 1,000 i.u./litre. Two rats showed much smaller rises in ASAT (134 to 464 i.u. /litre and 153 to 300 i.u.Elitre). For the group of rats which received daily doses of vitamin E prior to paracetamol the rises in enzyme values were much smaller than for the control group. Only one rat showed substantial rises (87 to 475 i.u./litre for ASAT and 58 to 365 i.u./litre for ALAT) and even these were very small when compared to the rises found in the majority of rats in the control group.

Histology

Table 9A shows the severity of necrosis and the serum enzyme levels at 24 hr in each animal and also the means and SD for each group in both experiments. The vitamin E deficient group, which showed the greatest mean increase in each of the serum enzymes, also showed the most severe degree of necrosis. All animals within this group had severe necrosis affecting most centrilobular areas. The vitamin E supplemented animals which had a lesser increase in serum enzyme levels also generally showed less severe necrosis, while the vitamin E treated animals which showed only very slight increases in serum enzymes had no evidence

TABLE 9A. Serum aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) activities and grade of liver necrosis in rats 24 hr after an oral dose of paracetamol (4g/kg)

ANIMAL GROUP	VITAMIN E DEFICIENT			VITAMIN E SUPPLEMENTED			VITAMIN E TREATED			CONTROL		
RAT NO.	GRADE OF NECROSIS	ASAT	ALAT	GRADE OF NECROSIS	ASAT	ALAT	GRADE OF NECROSIS	ASAT	ALAT	GRADE OF NECROSIS	ASAT	ALAT
1	3.0	9490	1344	1.3	2400	896	0.8	169	41	0.9	300	64
2	2.5	2730	222	1.9	1984	469	0.4	110	35	3.0	3330	1700
3	3.9	8960	1280	2.5	3946	1088	0.8	475	365	2.5	1620	500
4	3.0	7680	1109	2.9	1056	341	0	104	46	0	464	148
5	3.2	13300	5220	1.5	864	266	0	74	59	2.9	7250	3500
6	3.2	10130	4800	2.9	4256	1833	0	133	41	3.0	3520	1056
7	-	-	-	-	-	-	-	-	-	2.6	3410	1717
	*‡	*‡								**	**	**
MEAN	3.13	8715	2330	2.16	2417	815	0.3	177	98	2.13	2842	1241
S.D.	0.456	3480	2119	0.701	1426	583	0.39	149	131	1.19	2382	1204

x‡ =Significantly greater (P>0.05) than the corresponding means for supplemented and vitamin E-treated groups

xx =Significantly greater (P>0.05) than corresponding means for vitamin E-treated group.

of necrosis in three animals and only scattered small foci of centrilobular necrosis in the remaining three. In the control group two of the seven animals showed only a slight increase in serum enzymes and little if any histological evidence of necrosis and the other five had large increases in serum enzymes, and severe necrosis affecting most centrilobular areas.

There was a close correlation between the log serum enzyme levels at 24 hr and the severity of necrosis at 24 hr ($r = 0.922$, $p<0.001$ and $r = 0.859$, $p<0.001$, for ASAT and ALAT respectively).

Third Experiment

The mean plasma paracetamol level at 1 hr in the group given 2 g/kg (137.6 µg/ml, SD 49.9, n=24) is significantly less ($p<0.001$) than either of the other two groups (mean 225 µg/ml, SD 66.5, n=25 in the 3 g/kg group, and mean 257 µg/ml, SD 115, n=24 in the 4 g/kg group). There was no significant difference ($p>0.15$) between the mean paracetamol levels at 1 hr in the latter two groups. The mean paracetamol level at 24 hr in the 2 g/kg group (24.2 µg/ml, SD 12.0, n=23) is significantly less ($p<0.01$) than either of the other two groups (mean 63.5 µg/ml, SD 43.8, n=22 in the 3 g/kg group, and mean 128.5 µg/ml, SD 81.6, n=20 in the 4 g/kg group). The mean 24 hr paracetamol level in the 3 g/kg group was significantly less ($p<0.01$) than in the 4 g/kg group .

The plasma enzyme values at 24 hr for this experiment are shown in Table 9B. As in the first two experiments the enzyme responses at all dose levels of paracetamol were variable. At 2 g/kg in the absence of α-tocopherol 10 out of 12 animals had an ASAT greater than 1000 i.u./l while in the group given α-tocopherol only 2 out of 10 rats had levels above this value. For ALAT 3 out of 6 rats without α-tocopherol had levels above 1000 i.u./l while none of 10 rats given α-tocopherol had levels above this value. The mean ASAT and ALAT for the group not given α-tocopherol were significantly greater ($p<0.01$) than the group given α-tocopherol simultaneously.

At 3 g kg 6 out of 12 rats not given α-tocopherol had ASAT values above 2000 i.u./l while only 1 out of 13 rats given α-tocopherol had levels above this value. For ALAT 6 out of 12 rats had values above 1000 i.u./l while only 1 out of 13 in the group given α-tocopherol had levels above this. The mean ASAT and ALAT values for the group not given α-tocopherol were significantly greater ($p<0.025$ and $p<0.05$ respectively) than the means for the group given α-tocopherol simultaneously.

At 4 g/kg 7 out of 12 rats not given α-tocopherol had ASAT values above 2000 i.u./l while only 2 out of 12 in the group given α-tocopherol had levels above this. For ALAT 7 out of 12 rats not given α-tocopherol had values above 1000 i.u./l while only 1 out of 12 in the group given

TABLE 9B. Serum aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) activities in rats 24 hr after oral doses of paracetamol with or without added α-tocopherol acetate.

RAT NO.	2g/kg				3g/kg				4g/kg			
	E -		E +		E -		E +		E -		E +	
	ASAT	ALAT	ASAT	ALAT	ASAT	ALAT	ASAT	ALAT	ASAT	ALAT	ASAT	ALAT
1	5000	-	-	-	1010	800	660	130	800	160	1080	140
2	960	-	-	-	416	101	1730	787	4000	1460	7500	3720
3	1600	-	500	128	10100	5460	132	65	8100	6208	500	83
4	2700	447	550	101	5760	2080	245	91	1500	1700	580	283
5	1750	-	202	99	16050	3840	182	71	600	130	661	661
6	690	64	341	153	437	69	1546	299	6600	2380	9060	5280
7	1340	-	220	175	550	230	323	53	680	158	1980	587
8	1550	-	690	171	13807	2730	192	60	-	-	137	51
9	1600	92	790	138	2890	1920	314	69	10200	2000	1845	960
10	13300	1710	350	127	6400	2500	4800	4600	3100	980	630	54
11	5500	2700	1120	126	640	320	326	290	13600	3200	1002	426
12	6400	2430	1310	530	352	157	384	230	5800	1790	630	33
13	-	-	-	-	-	-	1707	150	1800	590	-	-
MEAN	3685	1240	607	174	4872	1663	964	526	4740	1707	2126	1676
S.D.	3666	1191	374	127	5654	1753	1299	1239	4261	1727	2925	1033
P*	<0.01	<0.01			<0.025	<0.05			>0.05	>0.15		

α-tocopherol had values above this. At this dose level however, the mean ASAT and ALAT for the group not given α-tocopherol were not significantly greater ($p>0.05$ and $p>0.15$ respectively) than the means for the group given α-tocopherol.

DISCUSSION

These experiments have again emphasised that after paracetamol overdose in the rat histological findings correlate well with plasma enzyme evidence of liver necrosis both from group to group and from animal to animal, although there was the expected variability in the response of individual animals to paracetamol. Even among our control animals two out of seven showed little if any increase in serum enzymes after paracetamol and these two animals also had minimal necrosis. In all animals the serum enzymes at 24 hrs reflected very faithfully the severity of necrosis at 24 hrs.

Although the mean 1 hr blood paracetamol levels were similar in all groups, the mean 24 hr levels differed in the first two experiments. In the first experiment, in which the animals were maintained on a synthetic diet, the mean 24 hr levels were significantly greater than in the second experiment in which the animals were fed a standard pelleted diet. This suggests, perhaps, that some constituent of the diets may have induced alterations in the microsomal drug metabolising enzyme system but this

suggestion can only be speculative. The plasma paracetamol levels at 1- or 24 hr bore no relationship to the ultimate extent of necrosis.

There are many similarities between the hepatotoxic effects of paracetamol and carbon tetrachloride. For example, modification of microsomal enzyme function produces analogous results in paracetamol and carbon tetrachloride poisoning (Garner and McLean, 1969; Stenger, Miller and Williamson, 1970). Likewise, large doses of carbon tetrachloride produce extensive centrilobular necrosis both in man and the rat (Hashinoto, Glende, and Recknagel, 1968; Cameron and Karunaratne, 1936), and ultrastructural similarities have already been mention. Although the degree of fatty change induced by paracetamol is not as great as that produced by carbon tetrachloride, in the latter case the mechanism responsible for fatty change is thought to be different from that causing necrosis (Rees, 1964). One point of distinction appears to be in their dose relationship. The response to paracetamol is much more of an 'all-or-none' reaction than that found with CCL_4 where the extent of injury is closely related to dose (McLean and Day, 1973).

While the mechanism of the hepatotoxic effect of CCl_4 is not clear, much work indicates that it may involve lipid peroxidation (Recknagel and Ghoshal, 1966). Part of the evidence for this is that anti-oxidants, including vitamin E, seem to provide some protection against the toxic effect.

The lipid peroxidation mechanism however, has been disputed by Green, Bunyan, Cawthorne and Diplock (1969) who concluded from a detailed study of vitamin E and hepatotoxic agents that no tangible evidence exists to support this hypothesis and suggested that the mode of action may be indirect and operate by several mechanisms including an effect on drug processing enzymes (Cawthorne et al 1970).

Judged by plasma ASAT and ALAT levels and by histological evidence, vitamin E deficient rats are, on average, more susceptible to the hepatotoxic effect of paracetamol than rats given a standard diet or an adequate nutritional supplement. Rats given large doses of α-tocopherol for several days prior to the paracetamol, although showing similar plasma paracetamol levels to the other groups showed very much smaller rises in plasma enzyme values and little hepatic necrosis. α-tocopherol when administered simultaneously with the paracetamol also protects against its hepatotoxic effect as judged by plasma ASAT and ALAT levels. This protection was not as effective at very high paracetamol intake but this may be explained by insufficient α-tocopherol being administered or absorbed.

The protective action of α-tocopherol suggests that the mechanism of paracetamol toxicity may be mediated by lipid peroxidation. It is of interest that glutathione, which reduces paracetamol hepatotoxicity, is another potential antioxidant present in the liver and may act by protecting nucleophilic protein groups from a toxic

metabolite (Mitchell et al. 1973 b). On the other hand, we have already seen that treatment with promethazine, another powerful antioxidant, afforded no protection against paracetamol suggesting that an action other than the prevention of autoxidation may underlie the protective effect of ∝-tocopherol. It may be that its action as a stabiliser of selenide-containing enzymes (Giassuddin et al, 1975) is enhancing the activity of glutathione peroxidase which in turn is increasing the rate at which the detoxication pathway can trap the toxic metabolite (McLean and Day, 1973). The possibility remains, however, that ∝-tocopherol acts by inhibiting the toxic metabolism of paracetamol or promoting its conjugation to glucuronide and sulphate, rather than operating after formation of the toxic metabolite. In these experiments protection was obtained either by prior treatment with ∝-tocopherol, or by simultaneous administration of ∝-tocopherol with the paracetamol, McLean and Day have more recently (1975) shown that ∝-tocopherol pretreatment reduced the lethality of paracetamol in rats fed a low protein diet. On the other hand, Gazzard et al (1974) claimed that simultaneous intra-peritoneal ∝-tocopherol did not reduce paracetamol-induced liver necrosis although it did maintain the levels of cytodrome P450 which normally fall with the development of necrosis. The method of quantitation used by these authors has already been criticised however, and they did note that the number of wedge-shaped areas of necrosis (infarcts) in the liver was reduced.

The protection afforded by ∝-tocopherol may form a basis for preventing or reducing the hepatoxic effect of paracetamol in man. If ∝-tocopherol were incorporated into paracetamol tablets than its effect would be simultaneous with that of the paracetamol, the dose taken would be related to the amount of paracetamol taken, and it might be possible to incorporate sufficient to reduce or even prevent toxic effects on the liver.

CHAPTER X

HUMAN PARACETAMOL OVERDOSAGE

The number of patients admitted to hospital because of overdosage has risen dramatically over the past 10 to 15 years and is currently estimated to be in excess of 100,000 admissions per annum (Volans, 1976). It is difficult to ascertain the proportion of these admissions resulting from paracetamol overdosage, however, as they are grouped with aspirin and reported under "mild analgesics". This category accounts for approximately 20,000 hospital admissions and between 200 and 300 deaths annually. If present trends are continued about one half of these admissions will involve paracetamol overdosage.

The increase in admissions and the number of deaths after paracetamol overdosage is not merely a result of

increased availability. During the period 1960-69 a considerable increase in consumption was not paralleled by any increase in overdose mortality. In fact, the number of deaths reported before 1968 was negligible. It was only after considerable publicity was given to its possible dangers that the number of overdose admissions and deaths became significant. Indeed, one patient who died attached her suicide note to a newspaper cutting in which the hazards of the drug to the liver were described, (Clark et al., 1973).

The increase in deaths from paracetamol overdose is detailed in the Registrar General's Statistical Review. In 1968 there were only 7 deaths after paracetamol alone and this had risen to 61 deaths in 1974. Paracetamol is increasingly prescribed as a compound preparation, however, and this is reflected in a similar rise in deaths from paracetamol mixtures which accounted for 71 deaths in 1974. Some impression of the overall mortality rate is given by the figures of the National Poisons Information Service which in 1975 received notice of 755 cases of paracetamol poisoning of which 24 were fatal (Crome et al 1976).

As regards the published literature on human paracetamol overdosage scattered case reports first appeared in the late 1960's and recorded the major clinical, biochemical and pathological findings. Since then, most of the published cases are contained in three large series, from Edinburgh(Proudfoot and Wright, 1970; Prescott et al.,1971),

from Newcastle (James et al., 1975) and from King's Collage Hospital (Portmann et al., 1975). These total 345 cases of which 34 were fatal.

The paper by Portmann et al., gives an excellent account of the histological changes in the liver in 104 cases (27 fatal), and a few of the early reports give reasonably full autopsy findings, but in general there has been little attempt to describe the morbid anatomy in a series of fatal cases and to examine the histological changes in a systematic manner. The major post-mortem findings noted in the earlier reported fatalities are listed in Table 10A.

With a view to a more detailed consideration of the histological findings at autopsy I have collected a series of previously unreported fatal cases of paracetamol over-dosage occurring in West Yorkshire over the past six years.

TABLE 10A

AUTHORS	AGE/SEX	Time between ingestion and death	Main P.M. Findings
Davidson and Eastham, 1966	30F	72 hrs	Hepatic necrosis Casts in collecting tubules of kidneys
	28M	60 hrs	Hepatic necrosis Tubular necrosis, mainly proximal
Maclean et al., 1968	40F	5 days	Hepatic necrosis Tubular degeneration with focal necrosis
Pimstone and Uys, 1968	26F	8 days	Hepatic necrosis, Myocardiopathy Kidneys - normal
Rose, 1969	46M	80 hrs	Hepatic necrosis Tubular necrosis, mainly proximal
Toghill et al, 1969	33F	18 days	Hepatic necrosis Tubular necrosis
Proudfoot and Wright, 1970	18F	-	Hepatic necrosis Gastric haemorrhage
Prescott et al., 1971	32F	16 days	Hepatic necrosis Renal failure (?tubular necrosis)
Sanerkin, 1971	15F	40 hrs	Hepatic necrosis. Myocardial necrosis. Tubular focal necrosis
Barnes and Prichard, 1972	30F	48 hrs	'Gross hepatic and renal damage'
Benson and Boleyn, 1974	59M	4½ days	Hepatic necrosis Proximal tubular vacuolation

Case Material

Fatal cases of paracetamol overdosage were traced through the records of the Leeds Public Analyst's Laboratory and the West Riding Forensic Sciences Laboratory at Harrogate. These laboratories analyse specimens submitted from cases of suspected poisoning arising in Coroner's or Forensic practice in West Yorkshire, and all cases in which higher than therapeutic levels of paracetamol were detected were followed-up.

In many cases although the pathologist (and subsequently the Coroner) had attributed death to paracetamol overdosage, toxicological and police evidence indicated that a compound preparation had been taken. There were 16 deaths in this category and where the combination was with dextropoxyphene (Distalgesic), death should have been attributed to the toxic effects of this drug rather than paracetamol. These deaths are considered in more detail in a later section.

In 18 cases death was attributed to paracetamol taken in overdosage either alone (8 cases) or in association with alcohol or other drugs, and these cases form the basis of my detailed study. The cases are listed in Table 10.B.

TABLE 10B

CASE	Age	Sex	Overdose	Time between ingestion and death
1	48	M	Paracetamol Alcohol	<12 hrs
2	24	M	Paracetamol	<12 hrs
3	33	F	Paracetamol Therapeutic dose of Librium	12 hrs
4	54	F	Paracetamol	13 hrs
5	24	M	Paracetamol Small quantity of oxazepam	16 hrs
6	45	M	Paracetamol Alcohol	<24 hrs
7	57	F	Paracetamol	24 hrs
8	61	F	Paracetamol Methaqualone	28 hrs
9	32	F	Paracetamol	36 hrs
10	23	F	Paracetamol	42 hrs
11	40	M	Paracetamol	48 hrs
12	21	F	Paracetamol Tuinal	60 hrs
13	24	F	Paracetamol Glutethimide	70 hrs
14	40	F	Paracetamol	72 hrs
15	19	F	Paracetamol	75 hrs
16	43	F	Paracetamol Alcohol	84 hrs
17	24	F	Paracetamol Methaqualone	96 hrs
18	32	F	Paracetamol Salicylate	5 days

CASE HISTORIES

CASE 1 Male: aged 48 died Dec. 1974

The deceased was a miner in good general health until 1968 when he sustained a fracture of the skull. Following this accident he complained of headaches and dizzy spells and was in receipt of Disablement Benefit. After an evening out drinking (during which time he is said to have consumed 2 pints of beer) he returned home and became involved in a family argument. His wife and other members of the family left him alone in the house and he was last seen alive at 12.40 am. At 1.58 pm on the same day the house was found locked and the curtains drawn. An entry was forced and the deceased was found upstairs dead in bed. Glasses containing sherry and crushed tablets and an empty bottle of PANADOL were found in the room. The body was cold.

Time between ingestion and death: < 12 hours

Post-mortem findings:

Aspiration of vomit
Liver - acute congestion
Brain - congested but otherwise normal
Kidneys - normal

Immediate cause of death: Inhalation of vomit

CASE 2 Male: aged 24 died July 1975

This man had been receiving treatment for an anxiety state for 18 months. His father had suffered from schizophrenia for many years and one of the patient's anxieties was that he would end up in the same condition as his father.

During the past 18 months he was admitted to hospital on two occasions after taking overdoses of tranquillizers.

A few days prior to his death he developed a headache and on the evening of his death went to see his Doctor and returned home about 6 pm. At 7.30 pm. he went to bed. He didn't complain and was apparently in normal health. At 5.30 am. the next morning he was found lying in bed. He had obviously been sick and was dead. Toxicology revealed paracetamol overdosage.

Time between ingestion and death: < 12 hours

Post-mortem findings

Aspiration of vomit
Lungs - normal
Liver - pale and "exsanguinated"
Kidneys - pale
Heart - normal

Immediate cause of death: Inhalation of vomit

CASE 3 Female: aged 33 died Feb 1973

The deceased was a married woman with 2 children who suffered from depression since 1970 when she was admitted to Doncaster Royal Infirmary after an overdose (? barbiturates). In 1971 she was again admitted to hospital after cutting her throat with a razor blade. Later that year she moved to York and was admitted to Clifton Hospital for treatment of depression. On the evening before her death she went to the cinema with a friend who saw her onto a bus at 10.40 pm. At 9.35 am the next morning having failed to return home she was found collapsed in some public toilets. An ambulance was summoned but she was moribund and died soon after arrival at the County Hospital at 11.45 am. Toxicology revealed an overdose of paracetamol.

Time between ingestion and death: about 12 hours

Post-mortem findings

Lungs - Right (700 g) showed marked oedema
Left (370 g), oedema in lower lobe

Liver
Brain
Kidneys
Heart
looked normal

Immediate cause of death Not known

CASE 4 Female: aged 54 died Feb 1975

The deceased was a heavy drinker who had been in good health until November 1974 when she was admitted to hospital as a suspected overdose but was later found to have had intracerebral and subdural haemorrhages. Burr-holes were made and the blood clot evacuated, and she made a good recovery.

On the evening prior to her death she had a disagreement with her husband and went upstairs at about midnight. Soon afterwards she threw down some pill bottles, some of which contained tablets. Her husband noted that they were paracetamol which she had bought "over the counter". She told him that she had not taken any tablets but she had some in her hand and the husband took these and threw them down the toilet. He called the GP who arranged her admission to hospital.

She arrived at hospital at 4.00 am in a fully conscious state and was detained for observation. Stomach wash-out was performed in Casualty. This produced no tablets but the washings were streaked with blood. The pulse rate increased to 130/min and an ECG revealed ST segment depression in leads V2-V5. At 12.40 pm the patient had a cardiac arrest and resuscitation was unsuccessful. Toxicology revealed overdosage with paracetamol. No other drugs were detected.

Time between ingestion and death: 13 hours

Post-mortem findings:

Brain - showed degeneration and pigmentation beneath burr-holes in the right temporal region

Stomach - contained fresh blood arising from numerous haemorrhages over the surface of the gastric mucosa

Liver - "was pale (yellow) and suggested fatty infiltration with possible toxic changes"

Kidneys - normal

Immediate cause of death cardiac arrest

CASE 5 Male: aged 24 died Oct 1972

The deceased was in generally good health although he had suffered from asthma for a considerable time. He had been depressed of late because he had been unable to obtain a council house, and his wife (aged 16) had left him, taking their small child with her, and was living with her mother.

On the day before his death he visited his wife's sister, returning to his lodgings at about 6 pm. At 9.15 pm he was found seated in a chair in a very dazed and confused condition. He was given salt and water and black coffee to induce vomiting and an ambulance was called. He arrived in hospital at about 10 pm. A stomach wash-out was performed in Casualty and during this procedure he became cyanosed and his respirations slow and shallow. Artificial respiration was started.

Over the next few hours he became deeply comatose and his blood pressure fell from 130/80 mmsHg to 90/50. At 10.45 am he had a cardiac arrest and resuscitation was abandoned at 11.15 am.

Time between ingestion and death: about 16 hours

Post-mortem findings

Lungs - show areas of collapse and widespread bronchopneumonia
Liver - congested
Brain - congested and swollen
Kidneys - normal
Heart - normal

Immediate cause of death bronchopneumonia

CASE 6 Male: aged 45 died July 1973

The deceased was a married man who had been legally separated from his wife. He had suffered from paranoid schizophrenia for 3 or 4 years for which he was taking Librium. Over the last year he had been treated with Warfarin for an episode of deep vein thrombosis.

He was last seen alive at 2.0 pm on the 10th July. At 8.15 pm on 11th July he was found dead in bed by a friend. The body was very cold. Toxicology revealed paracetamol overdose.

Time between ingestion and death: about 24 hours

Post-mortem findings

Lungs - right (840 gms) and left (600 gms) were congested and oedematous
Liver - congested
Kidneys - congested
Brain - normal
Heart - normal

Immediate cause of death not known

CASE 7 Female: aged 57 died Sept 1971

The deceased had suffered from depression since 1948 and had received regular psychiatric treatment. She had threatened to commit suicide on several occasions but had never made an attempt to do so. Two days before her death she bought a bottle of 50 Paracetamol tablets from a chemist At 12 noon the following day she informed her sister that she had taken all the Paracetamol tablets earlier that day. She said that she had vomited and brought all the tablets back. As she did not seem to suffer any ill effects and she acted normally throughout the day a Doctor was not called. She went to bed at 10.15 pm that night.

At about 8.00 next morning her sister took her a cup of tea whilst she was still in bed. The deceased told her that she did not feel any better.

At 9.20 am that morning her sister left the house to call a Doctor, leaving the deceased upstairs in bed. She returned to the house about 9.50 am to find her sister lying dead on the floor at the side of her bed.

An empty Paracetamol bottle was found in the house.

Time between ingestion and death: about 24 hours

Post-mortem findings

Lungs - moderate oedema
Stomach - distended with numerous acute mucosal erosions
Heart
Brain looked normal
Kidneys

Liver - (1600 g) showed accentuation of the normal lobular pattern

Immediate cause of death not known

CASE 8 Female: aged 61 died April 1973

The deceased was a divorced woman, but resided with her common-law husband. She had had a hysterectomy in 1962, and for the past 5 years had been treated with oral hypoglycaemic drugs for diabetes. She had suffered from depression over the last 5 years and in 1968 had been admitted to hospital for an overdose.

Two days before her death she received a telegram stating that her mother was seriously ill. Her husband returned home from baby-sitting at 10.15 pm and found that his wife was already in bed. He also went to bed. At 3.00 am he awoke and heard his wife gurgling and saw blood on her pillow. An ambulance was called and she was admitted to hospital at 4.45 am.

She remained unconscious and died at 3.25 am on the following day. Toxicology revealed overdosage with paracetamol and mandrax.

Interval between ingestion and death: about 28 hours

Post-mortem findings;

Aspiration of vomit
Lungs - congestion and oedema
Liver - gross 'fatty change'
Cholelithiasis
Kidneys - pale on section
Brain - generalised oedema
Osteoarthritis

Immediate cause of death aspiration of vomit

CASE 9 Female: aged 32 died Feb 1974

The deceased was a married woman who lived with her husband and three children. During the past 5 years she had taken overdoses of barbiturates on two occasions and had received psychiatric treatment.

On the morning of her death the deceased stayed in bed and did not appear well. Around lunch time her husband called a Doctor who arranged her admission to hospital.

On admission at 2.43 pm she was unconscious with biochemical evidence of a metabolic acidosis. Her blood pressure was unrecordable. A stomach wash-out was performed. and she was put on a respirator. She remained deeply unconscious with dilated pupils and flaccid muscles. At 7.55 pm she had a cardiac arrest from which she could not be resuscitated.

Toxicology revealed overdosage with Paracetamol, and a suicide-note was found on the day of her death. Paracetamol had been prescribed for back-ache 12-18 months before in a quantity of 500. It was not possible to determine the time of ingestion from the evidence, but the liver histology suggested that the ...

Time between ingestion and death: about 36 hours

Post-mortem findings

Lungs - moderate congestion and oedema

Liver - 'showed a striking reticular pattern similar to nutmeg liver but the pale areas were of

cream colour and the organ was soft in consistency'.

Kidneys - very pale with a sharp distinction between cortex and medulla

Heart
Brain - looked normal

Immediate cause of death Cardiac arrest

CASE 10 Female: aged 23 died Oct 1972

The deceased was a single woman who had attempted suicide on numerous occasions by slashing her wrists and taking overdoses of various drugs.

In June 1972 she was committed to prison for theft and there was discovered to be about 6 months pregnant. The identity of the father was not known but may have been a patient at a mental hospital to which she had been admitted. Her own father had served 2 years in prison following an incestuous assault on her when she was 14 years old.

She was admitted to hospital at 4.15 pm on the 8th October stating that she had taken about 100 Paracetamol tablets at 2 pm. She had a stomach wash-out and was taken into a general ward. At 2 pm on the following day she suddenly became semiconscious and pale, and her blood pressure fell to 80/50. She was transferred to the Intensive Care Unit. Her blood pressure continued to fall slowly and she became oliguric. On the next morning she had two cardiac arrests, the second proving irreversible, and she was declared dead at 8.00 am.

Time between ingestion and death: 42 hours

Post-mortem findings

Lungs - marked oedema
multiple petechiae
blood stained effusion in the right pleural cavity

Heart - pericardial haemorrhage

multiple petechiae

Liver - marked chronic passive congestion

Kidneys - congested

Uterus - contained a normal male foetus, approximately 6 months gestation

Immediate cause of death Cardiac arrest

CASE 11 Male: aged 40 died Dec 1972

The deceased had enjoyed reasonably good health until 1959 when he underwent a partial gastrectomy for peptic ulcer. In 1967 he was operated on for a perforated stomal ulcer.

He had lived on his own since his divorce in 1966.

In November 1972 he was beaten-up in a fight and detained overnight in hospital. Some days later he still seemed unable to walk properly and complained of headaches.

At midday on 11th December he was re-admitted via the Casualty Department with an apparent history of haematemesis but apart from epigastric tenderness physical examination was unremarkable. At 8.30 am on 13th December he suddenly became unresponsive, all four limbs were flaccid and the pupils dilated. He died at 11.40 am and toxicology revealed paracetamol overdose.

Time between ingestion and death: about 48 hours

Post-mortem findings

Mild jaundice (skin and conjunctivae slight yellow colour)
Lungs - acute haemorrhagic bronchopneumonia
Liver - (1340 g) was firm, but very pale yellow in colour and mottled in a lobular pattern by reddish-brown areas
Kidneys - slightly enlarged and pallid with a few irregular scars. The cortex had a pale yellow colour
Brain - old haemosiderin staining on undersurfaces of of frontal and temporal lobes. On section rather soft and slightly congested

Immediate cause of death acute haemorrhagic bronchopneumonia

CASE 12 Female: aged 21 died June 1970

The deceased was an unmarried student who had suffered from depression since 1967.

After spending an evening drinking with a party of students she returned to her flat with a male friend where she consumed half a bottle of whisky and eight double gins. The friend left the flat at 3.00 am. She did not appear to be depressed when he left.

Another friend became anxious when she failed to appear for an examination at 9.00 am that morning. At 11 am two student friends called at the flat and found a note pinned to the door. They broke into her room and found the deceased unconscious in bed. An empty 100 tablet canister of paracetamol was found by her bed.

A stomach wash-out was performed in Casualty at 1.45 pm. She remained unconscious and her blood pressure was 110/80. By the following morning her blood pressure had fallen to 100/60, and her temperature was 100^{o}F. At 8.0 pm she had a cardiac arrest. Sinus rhythm was restored and she was put onto a ventilator. On the following day she became anuric and was given peritoneal dialysis. At 5.20 pm she had a further cardiac arrest and resuscitation was abandoned at 5.45 pm.

Time between ingestion and death: about 60 hours

Post-mortem findings

Bronchopneumonia

Liver - very pale, 'necrosis and fatty degeneration'
Brain - small subdural haematoma without compression of the brain which was slightly oedematous
Kidneys - pale and swollen

Cause of death cardiac arrest associated with bronchopneumonia and renal failure

CASE 13 Female: aged 25 died Aug 1971

The deceased was a married woman who had suffered from severe depression since the birth of her first child in September 1969. In December of that year she was admitted to hospital following an overdose and was discharged 6 weeks later. She had at least four subsequent admissions for overdose during 1970.

On the day of her final admission she had been to see a psychiatrist with a view to obtaining an abortion. She was 9 weeks pregnant. The psychiatrist told her that he could not help, so her husband took her to their General Practitioner but he advised against termination at that time. They returned home at 6.10 pm and she went upstairs for a few minutes. A little later she collapsed and told her husband she had taken some pills. He called an ambulance and she was admitted at 7.30 pm in an unconscious state.

Blood taken on admission contained 13mg% of paracetamol and gave a positive reaction for glutethimide.

She remained deeply unconscious with low blood pressure (80/40 mmHg) until her death 3 days later. Terminally she developed epistaxis, vaginal bleeding, and bronchopneumonia. The prothrombin time was prolonged to 105 sec (N = 12 sec).

Time between ingestion and death: 70 hours

Post-mortem findings

Lungs - congested and oedematous with lower lobe bronchopneumonia

Stomach - contained a large quantity of altered blood
Liver - was pale and fatty (1400 g)
Kidneys - congested
Uterus - contained a foetus of crown-rump length 55 mm
consistent with a gestation of 9-10 weeks
Brain - showed considerable oedema (1380 g)
Heart - congested and rather soft

Cause of death Bronchopneumonia and cerebral oedema

CASE 14 Female: aged 40 died Nov 1975

This woman was a known alcoholic who had had several admissions for "drying-out", and in 1973 had been admitted twice for drug overdoses. On 3rd November she was seen by her GP when she complained of vague abdominal pain maximal in the right hypochondrium. She was also somewhat confused. She was prescribed ampicillin in the belief that she was suffering from acute cholecystitis. When she was seen by the GP the next day he found her much more confused and unable to give a history. On admission to hospital at 2.15 pm that day she was unconscious. She showed multiple bruises particularly over the anterior aspects of the legs but also on the arms, together with superficial skin loss over the postero-lateral aspect of the left leg. She was mildly jaundiced. She was found to be hypoglycaemic and her prothrombin time was markedly increased. She was given intravenous dextrose and vitamin K but her general condition deteriorated and she died at 8.50 pm

Toxicology revealed paracetamol overdosage.

Time between ingestion and death: 72 hours

Post-mortem findings

Liver - (1750 g) Pale, soft and exaggerated pattern
Kidneys - Right(155 g) Pale with linear scars
Left (170 g) Contained foci of suppuration
Brain (1295 g) Normal in appearance
Pancreas - scattered small foci of haemorrhage
Heart - (295 g) mild left ventricular hypertrophy

Cause of death Hepatic failure

CASE 15 Female: aged 19 died Feb 1975

The deceased was a single girl living in a Local Authority Home. She had been very depressed and withdrawn since her brother's death in a car accident in August 1974.

She presented herself at the Casualty Department stating that she had taken 50 paracetamol tablets about 6 hours before. She was given an emetic (?) but no tablets were recovered, and she was admitted.

Apart from vomiting she was well until 36 hours after admission when she appeared to be having hallucinations. Biochemical tests revealed

Blood urea	=	4.1 mmol/l (25 mg%)
Serum sodium	=	138 mmol/l
HCO_3^-	=	13 mmol/l
ALAT	=	285 iu/l
Glucose	=	4.7 mmol/l (85 mg%)

On the following day she lapsed into semi-coma. She responded to her name but could not speak coherently. She was drowsy and irritable. Her blood pressure was 110/65. She was dehydrated, jaundiced, menstruating and bleeding from a venepuncture site. The prothrombin time was 100 secs, ALAT was over 1000 iu/l and the serum bilirubin was 82 µmol/l (4.9 mg%).

On the day after, the bilirubin had risen to 109 µmol/l (6.3 mg%) but the ALAT was down to 245 iu/l. Despite treatment with vitamin K her coagulation tests were still abnormal

Prothrombin time = 94 sec (N = 12 sec)
Thrombin time = 26 sec (N = 14 sec)
Fibrinogen titre = 1/128
Fibrin degradation products = 8 mg/l (normal)

Later that day she became more deeply unconscious with hyper-reflexia in the legs and upgoing plantar responses. She developed subconjunctival haemorrhages and her neck became extended and rigid. She died at 1.45 on the third day after admission.

Time between ingestion and death = 75 hours

Post-mortem findings

Conjunctival petechiae
Jaundice
Lungs - moderately congested and oedematous
Liver - 'is pale and shows a faint nutmeg pattern'
Kidneys - pale, especially the cortices which are more clearly demarcated from the medulla than usual
Brain - very oedematous with coning of the medulla and cerebellum
Stomach - petechial haemorrhages

Cause of death Cerebral oedema due to hepatic failure

CASE 16 Female: aged 43 died Feb 1969

The deceased was an inpatient at a York psychiatric hospital undergoing treatment for chronic alcoholism. She had been divorced 2 years before.

Her past medical history was of repeated miscarriages, an appendicectomy in 1946 and rheumatoid arthritis since the early 1950's. In 1961 she underwent a laparotomy for intestinal obstruction and later that year developed depression. In 1962 there was a recurrence of severe depression, and in 1965 she was admitted for alcoholism. Between 1966 and 1969 she had a number of admissions to psychiatric hospitals as an informal patient.

At 9.30 pm on 27.2.69 she approached a Staff Nurse and said that she had taken 100 PANADOL. She also smelled of alcohol. It was discovered that her locker contained an empty bottle of whisky and a 3/4 empty bottle of sherry. She was given salt and water and she vomited 'a reasonable amount'. She was transferred to a General Hospital where a stomach-wash-out was performed. On examination at midnight she was unconscious and her pupils were dilated and fixed.

On 1.3.69 she had two cardiac arrests, from which she was successfully resuscitated but she remained hypotensive and unconscious.

On the following day her blood pressure fell further (to 60 mmHg systolic) and she went into renal failure.

Blood urea = 82 mg%
Sodium = 146 mEq/l

Potassium	= 3.8 mEq/l	pH	= 7.5
Chloride	= 100 mEq/l	pCO_2	= 112
CO_2	= 30	pO_2	= 94

By the next day her blood urea had risen to 133 mg/%, her blood pressure was 50/20 mmHg, and she had developed a pyrexia of 104.6°F. At 10.30 am that morning she died.

Time between ingestion and death: 84 hours

Post-mortem findings

Lungs - Right (600 g), left (400 g)
Bronchopneumonia in both lower lobes

Heart - (400 g) 'pale and flabby'

Stomach - small amount of altered blood

Liver - (2500 g) enlarged, cut surface shows alternating red haemorrhagic areas and yellow necrotic areas

Kidneys - pale, slightly enlarged

Brain - (1400 g) soft in consistency. On section there haemorrhagic changes in the pons and medulla

Cause of death Cerebral anoxia due to
Cardiac arrests associated with
Bronchopneumonia and renal failure

CASE 17. Female: aged 24. died December 1970

The deceased was a schoolteacher who had been depressed and anxious for many years. She had taken a number of overdoses in the past and in 1968 she required medical attention after slashing her wrists.

Her mother had committed suicide in 1966 and she had always had a difficult relationship with her father, a headmaster, who was autocratic and dominating.

She got married during her first year at University but her husband turned out to be 'a pervert'. On one occasion he poured acid between her breasts which resulted in permanent scarring. The marriage lasted less than a year.

On 27/11/70 she was admitted to the Dermatology Department for treatment of psoriasis. She made satisfactory progress and was discharged on 12/12/70.

Four days later she was re-admitted to a general ward having taken an overdose of PANADOL, SODIUM AMYTAL and MANDRAX. She had last been seen at lunch time on the day before, but when a neighbour found her door locked that night and on the morning of 18/12/70, he broke into the flat and found her unconscious.

On admission her blood pressure was 120/70 mmHg and her temperature was 94.7°F. On the following day her level of consciousness appeared to have improved but her temperature had risen to 103°F. 500ml of altered blood was aspirated from her stomach.

On 20/12/70 her condition was unchanged. The serum bilirubin was 5mg% and the blood urea 58mg%. By the following day the bilirubin had risen to 9.3mg% and the urea to 101mg%, and her blood pressure had fallen to 60 mmHg systolic. Other laboratory findings were:-

Sodium	151 mEq/l	Total Protein	= 4.8 g/l
Potassium	2.8 mEq/l	Albumin	= 2.9 g/l
CO_2	= 22 mEq/l	Alk. Phos.	= 20 KA units
Chloride	= 100 mEq/l	SGPT	= 7,600 iu/l

She died at 4.30 pm on 21/12/70

Time between ingestion and death = about 96 hours

Post-Mortem findings

Jaundice

Lungs - marked oedema, especially in left lung (1,350g)

Heart - petechial epicardial haemorrhages

Liver - patchy congestion and diffuse yellow mottling (1960g)

Brain - marked oedema

Kidneys - looked normal

Cause of death

Cerebral and pulmonary oedema
due to hepatic failure
associated with renal failure.

CASE 18. Female: aged 33. died November 1975

This women worked as a secretary. She was separated from her husband, and had formed a relationship with a younger man.

Some years ago she had been involved in a serious car crash and underwent plastic surgery to her face and neck. These facial injuries tended to depress her and more recently she had financial problems arising out of her separation.

At about 10.30 pm on 7/11/75 she telephoned her boyfriend and told him that she had just taken an overdose - about 20 Valium and 100 paracetamol tablets. He summoned her parents who went round to her flat and called an ambulance.

She was seen at the Casualty Department of a Leeds hospital at 2 am on 8/11/75 where a stomach wash-out was performed. She was then transferred to a second hospital.

Three hours after admission the plasma paracetamol level was 11.6 mg/100 mls and five hours later had risen to 30 mg/100 mls.

Two days after admission her general condition deteriorated with the onset of hepatic coma. Liver function tests revealed bilirubin = 92 µmol/l, SGOT = approx. 11,900 i.u. SGPT = approx. 5,400 iu/l and alkaline phosphates of 8 KA units. On 10/11/75 she had a period of prolonged hypo - tension, and the following day she developed Cheyne-Stokes respiration and anaemia. An EEG revealed only minimal cortical activity. She died at 2.30 pm on 12/11/75.

Time between ingestion and death = 5 days

Post-mortem findings

Mild jaundice

Bleeding in the nasal cavity

Heart - moderately enlarged (320g)

Lungs - R = 570g; L = 480g

Oedema and intra-pulmonary haemorrhage

Liver - (1370g) had a pale surface and on section an exaggerated pattern

Small intestine - multiple petechial haemorrhages but no significant blood loss into the bowel

Kidneys - (each 155g) - pale

Brain - (1250g) mild cerebral oedema

Cause of death

Hepatic failure

HISTOLOGY

1. LIVER

Samples of liver were available from 14 of the 18 cases. Sections were stained by HE, Gordon and Sweets' method for reticulin, PAS before and after diastase digestion, methyl-green pyronin Y, and the MSB method for fibrin.

Where coagulative necrosis was present, the extent of necrosis was assessed using point-counting. The method was the same as that employed in the rat experiments described previously. 500 points were counted for each block available. The results are listed in Table 10C.

Cases dying up to 24 hrs after ingestion

Of the 7 cases in this category liver histology was available in only 3.

Less than 12 hr after overdose (case 1) the liver shows no abnormality. Case 6 (12-24 hr) reveals centrilobular pallor, mild fatty change and mild to moderate hydropic vacuolation (fig. 10.1). There is diffuse loss of glycogen but this probably reflects autolysis rather than the in-vivo content.

Case 7 (about 24 hr) shows early focal centrilobular necrosis, with pallor and mild hydropic vacuolation in mid-zonal hepatocytes (fig. 10.2). The viable areas contain occasional cells undergoing shrinkage necrosis. The centri-lobular cells contain moderate amounts of lipofuscin.

Cases dying after 24 hrs

Liver histology was available in all 11 cases in this

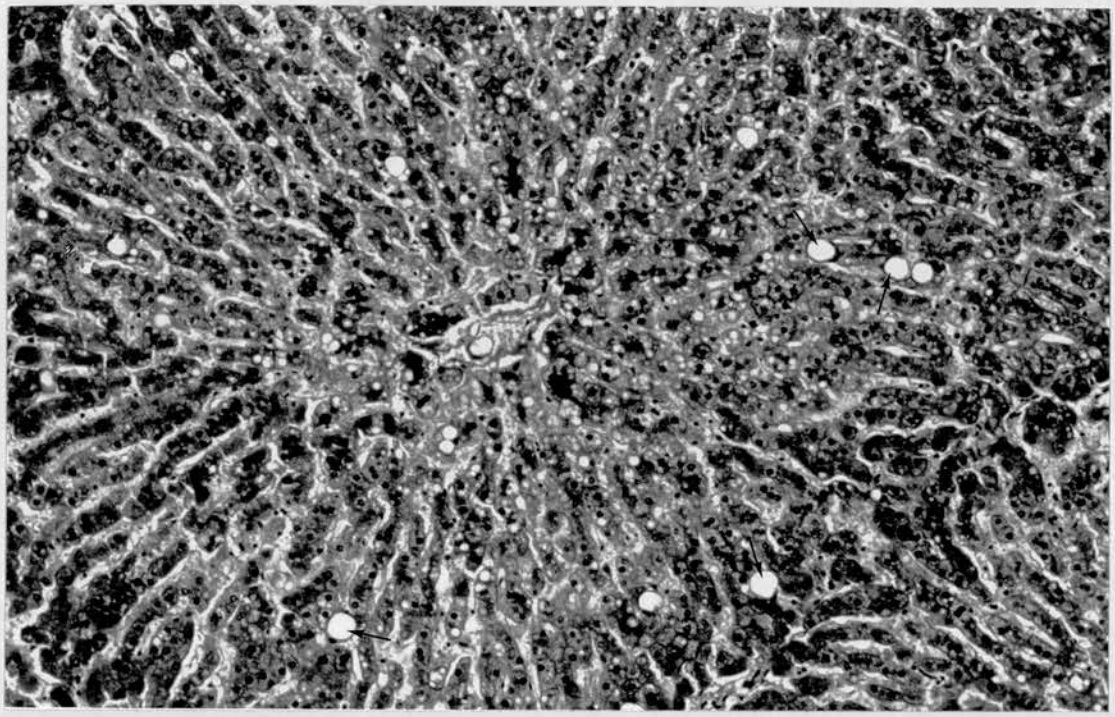

Fig.10.1 Case 6. Liver. Centrilobular areas showing moderate hydropic vacuolation. Scattered cells contain larger fat-filled vacuoles (arrowed) HE. X100

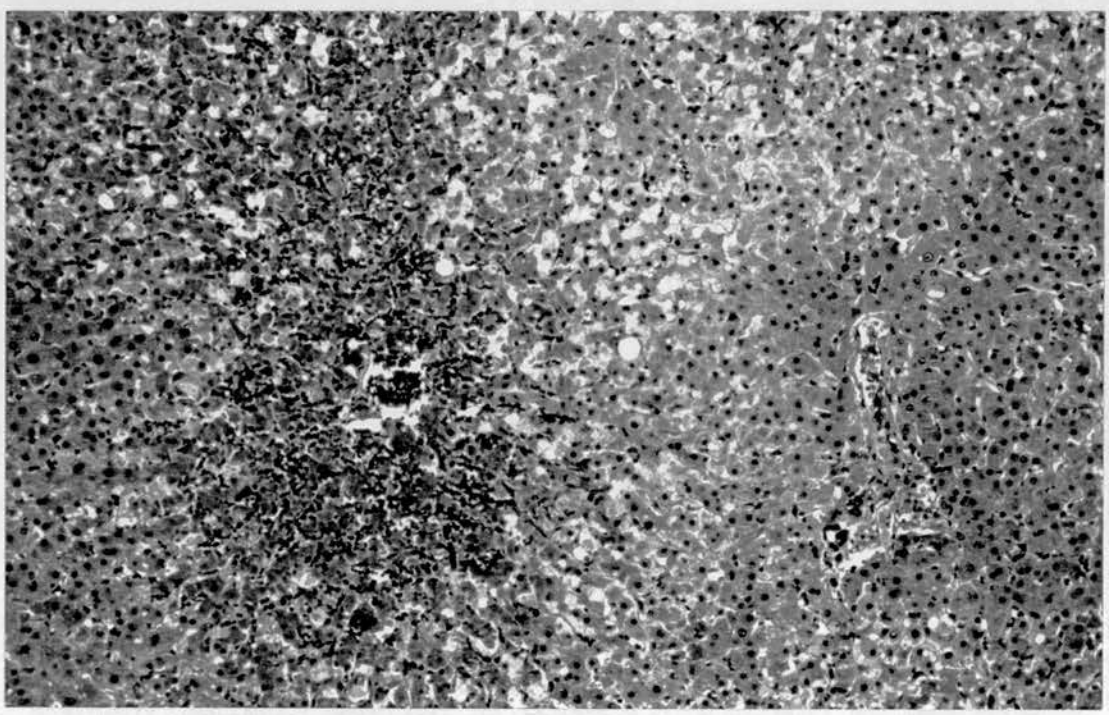

Fig.10.2 Case 7. Liver. Small centrilobular focus of necrosis and sinusoidal congestion surrounded by midzonal pallor and mild hydropic vacuolation. HE. X100

TABLE 10C: LIVER HISTOLOGY AND QUANTITATION

Case	Time Ingestion - death	Histology	Percentage Necrosis
1	<12 hr	Normal	-
6	<24 hr	Hydropic vaculation Fatty change	-
7	24 hr	Early necrosis	33.0
8	28 hr	Coagulative necrosis	72.2
9	36 hr	Coagulative necrosis	55.9
10	42 hr	Coagulative necrosis	92.4
11	48 hr	Coagulative necrosis	94.1
12	60 hr	Coagulative necrosis	72.4
13	70 hr	Coagulative necrosis	58.4
14	72 hr	Coagulative necrosis	79.0
15	75 hr	Coagulative necrosis	68.1
17	96 hr	Coagulative necrosis	72.4
18	5 days	Coagulative necrosis	89.8

group.

Case 8 (28 hr) shows extensive necrosis and intense sinusoidal congestion. The centrilobular hepatocytes remain discrete with pyknotic nuclei and there is no inflammatory reaction (fig. 10.3). PAS stains reveals diffuse loss of glycogen. The reticulin framework is preserved but there is a slight increase in inter-connecting fibrils. Lipofuscin is not seen.

In case 9 (36 hr) the picture is one of more extensive necrosis where even the surviving periportal hepatocytes show gross hydropic vacuolation. This liver contains increased amounts of lipofuscin which is seen in disintegrating hepatocytes but appears to be more concentrated in Kupffer cells within the necrotic zones (fig. 10.4). The reticulin framework is preserved throughout.

Case 10 (42 hrs) shows almost complete hepatic necrosis with only a narrow rim of hydropic liver cells around the portal tracts. The necrotic cells are discrete and retain their pyknotic nuclei (fig. 10.5). A few neutrophil polymorphs are seen in periportal zones and there is intense congestion. There is some focal condensation of reticulin around central veins. Lipofuscin is absent.

Case 11 (48 hrs) show similar, almost complete, necrosis. The borders of necrotic hepatocytes are less easily distinguished and there is more nuclear karyorrhexis. A normal amount of lipofuscin is present. There is a slight increase in polymorphs within the compressed sinusoids.

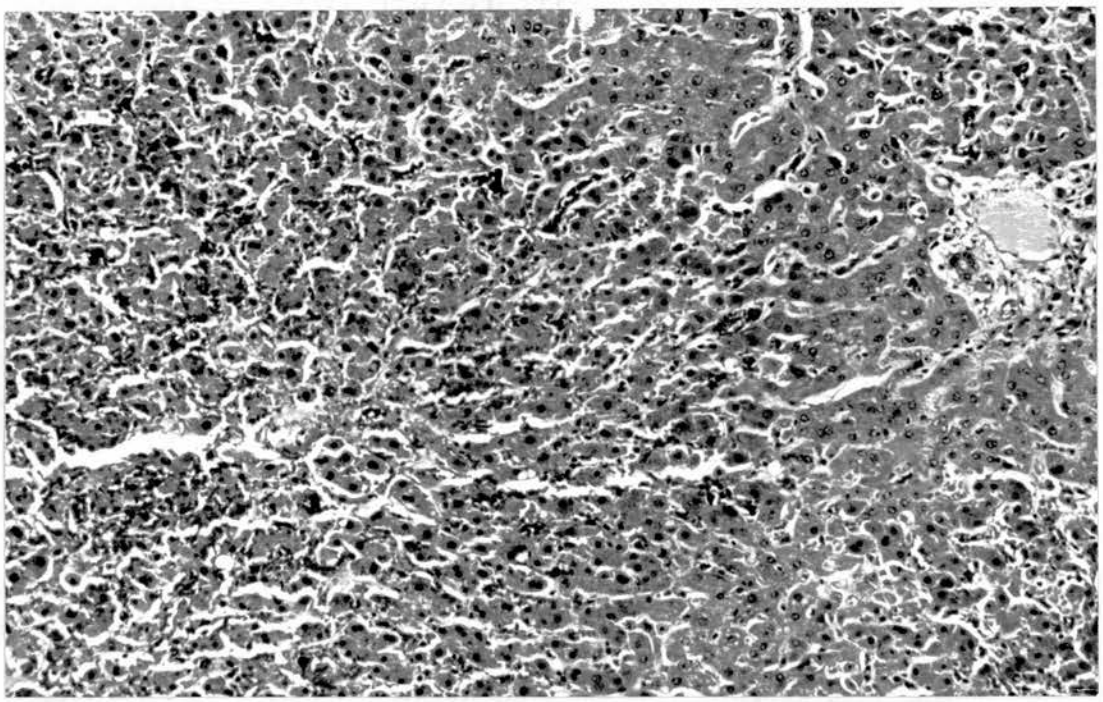

Fig.10.3 Case 8. Liver. More extensive centrilobular and midzonal necrosis showing retention of pyknotic nuclei in the necrotic cells. Periportal cells (right) remain viable. HE. X100

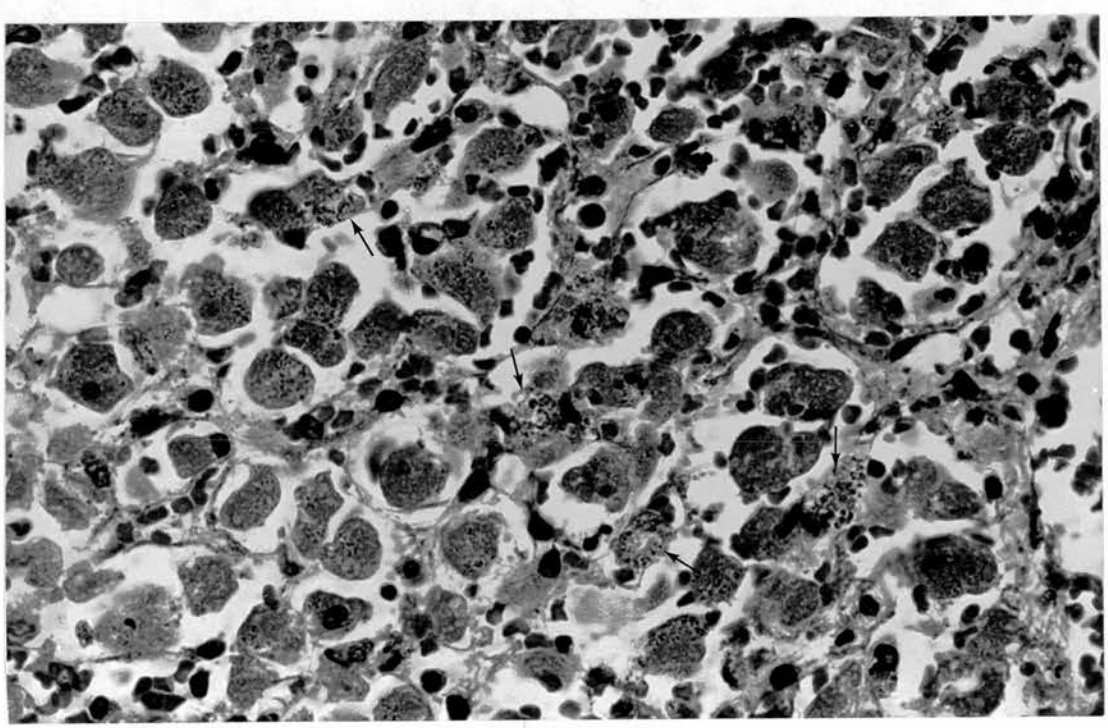

Fig.10.4 Case 9. Liver. Necrotic cells show loss of nuclei and contain conspicuous granules of lipofuscin. The pigment is particularly abundant in Kupffer cells in the necrotic zones (arrowed). HE. X400

Case 12 (60 hrs) shows less extensive necrosis than 10 and 11. Although the strongly eosinophilic necrotic cells remain discrete many are anuclear. The surviving cells are hydropic. Lipofuscin is absent. The reticulin pattern is normal.

Case 13 (70 hr) shows a moderate degree of necrosis which is less well developed than in the preceeding two cases and resembles most closely that seen in case 10. The hepatocytes remain discrete with retention of pyknotic nuclei. The necrotic areas are bounded by a narrow band of mid-zonal cells which under low magnification are swollen and clear (fig.10.6). Under high power this is seen to be a result of gross hydropic swelling and vacuolation which in some cells is accompanied by nuclear degeneration. The periportal cells show lesser degrees of hydropic vacuolation. There is, as usual, diffuse absence of glycogen. The reticulin pattern is normal.

Case 14 (72 hrs). There is extensive confluent necrosis together with a mild polymorphonuclear inflammatory reaction. In addition, there is diffuse and moderately severe fatty change (fig. 10.7).

Case 15 (75 hrs). There is extensive necrosis. The hepatocytes are granular, eosinophilic and discrete, and some possess pyknotic nuclei. The majority, however, are anuclear. There is moderate infiltration of the necrotic areas by polymorphs and conspicuous macrophage activity. The small groups of viable periportal cells show moderate

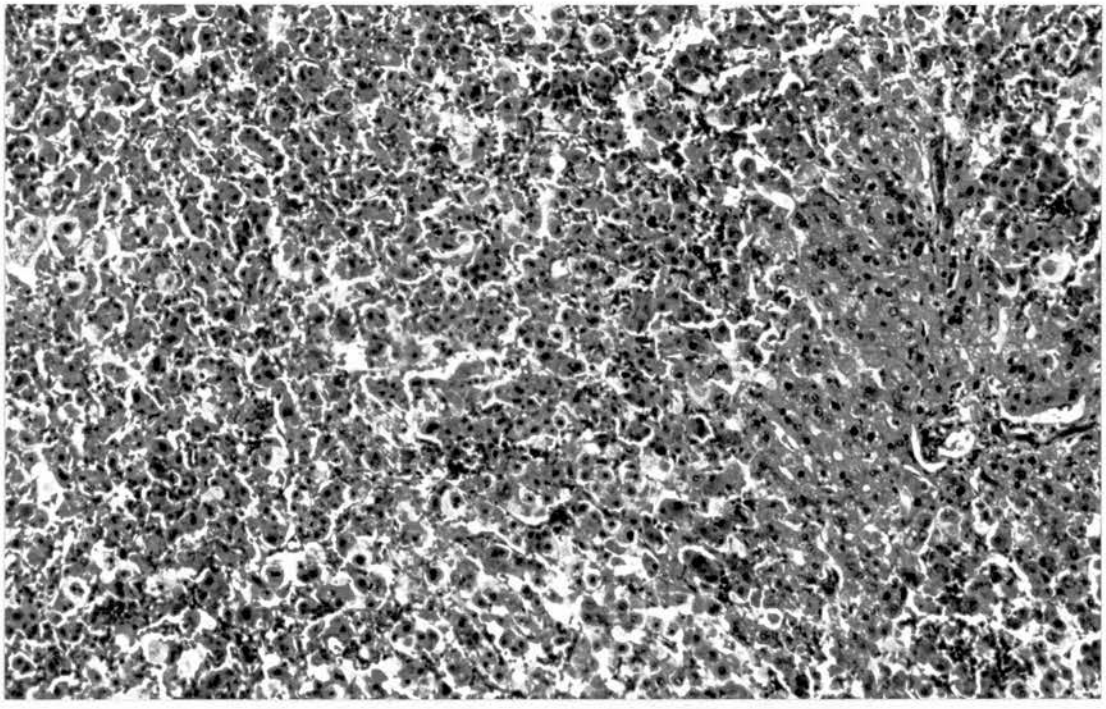

Fig.10.5 Case 10. Liver. Almost complete necrosis with only a narrow zone of surviving periportal cells (right). HE. X100

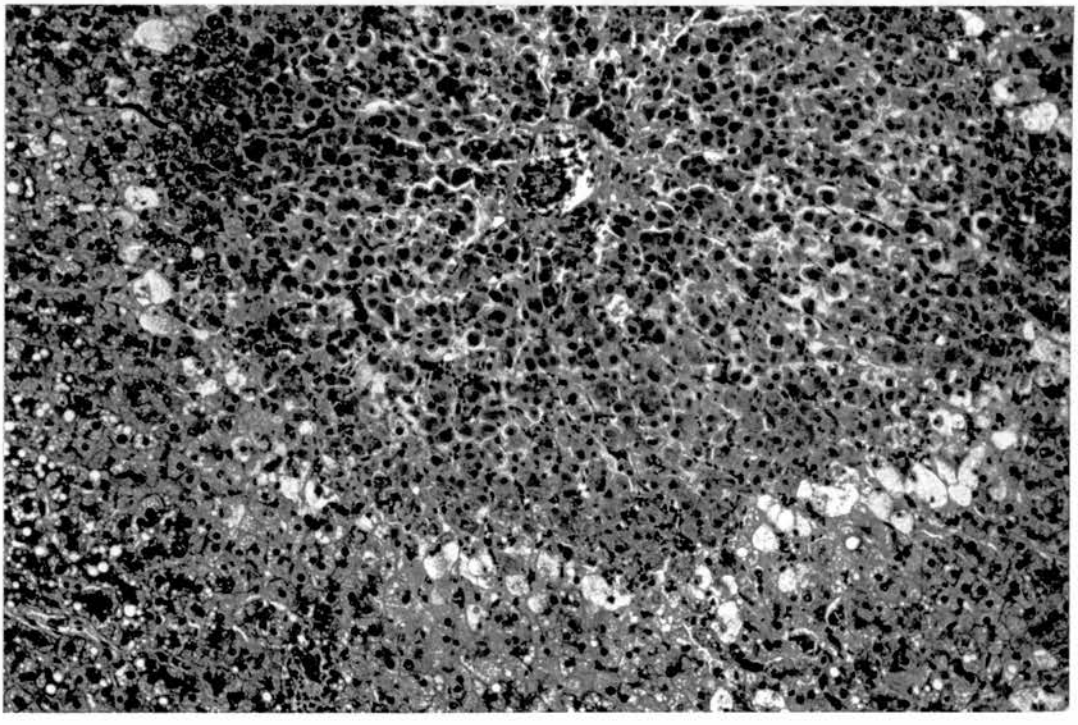

Fig.10.6 Case 13. Liver. Boundary of vacuolated cells around the necrotic zone. HE. X100

hydropic vacuolation and regenerative activity is evidenced by the finding of pseudo-ductules composed of proliferating hepatocytes. This liver shows a minor increase in lipofuscin, again concentrated in macrophages. The reticulin pattern is normal.

Case 16 (84 hrs). In this liver the necrosis is of unusually irregular distribution with relatively large areas of intact lobules (fig.10.8,9). In the necrotic areas the cells are globular and discrete with retention of pyknotic nuclei in the majority (fig. 10.10). Although necrosis is therefore not fully developed there are increased numbers of macrophages, and the areas of normal hepatocytes contain scattered mitotic figures (fig.10.11).

Case 17 (96 hrs). Here the well-developed necrosis is accompanied by a prominent macrophage response, dead hepatocytes being interspersed with numerous elongated macrophages (fig.10.12). The small islands of surviving periportal hepatocytes show marked hydropic vacuolation but nevertheless contain frequent mitotic figures (fig.10.13). The reticulin framework is intact.

Case 18 (5 days). There is widespread confluent necrosis with only a narrow rim of surviving hepatocytes around the portal tracts and even these show vacuolation (fig.10.14). There are a few small foci of inflammatory cells and only an early macrophage response. The centrilobular areas contain a mild increase in lipofuscin pigment.

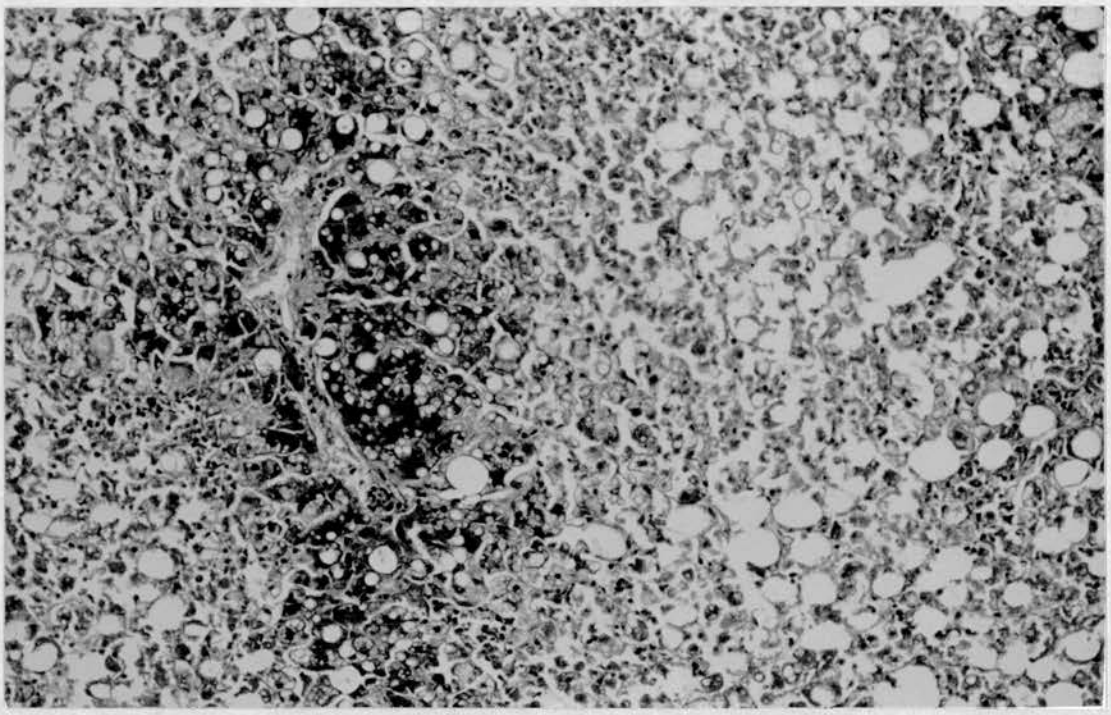

Fig.10.7 Case 14. Liver. Extensive necrosis accompanied by moderately severe fatty change. HE. X100

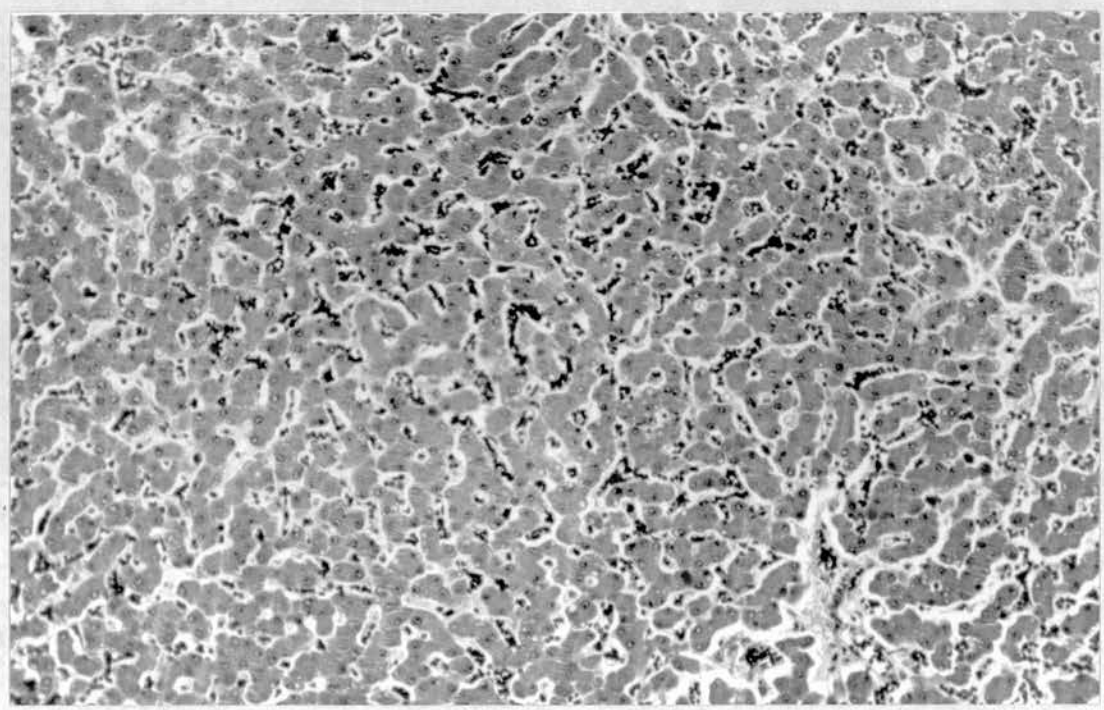

Fig.10.8 Case 16. Liver. In this field there is sinusoidal congestion but good preservation of architecture and normal cytological features. HE. X100

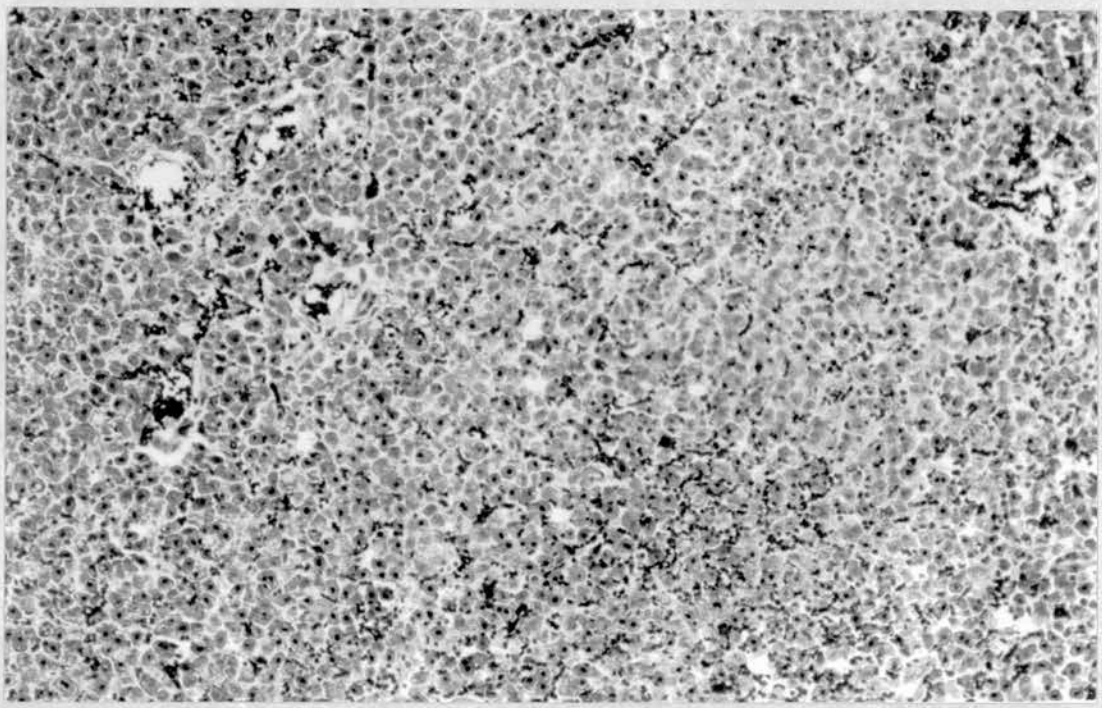

Fig. 10.9 Case 16. Liver. This field from another part of the same section reveals total necrosis with loss of cellular pattern and nuclear pyknosis. HE. X100

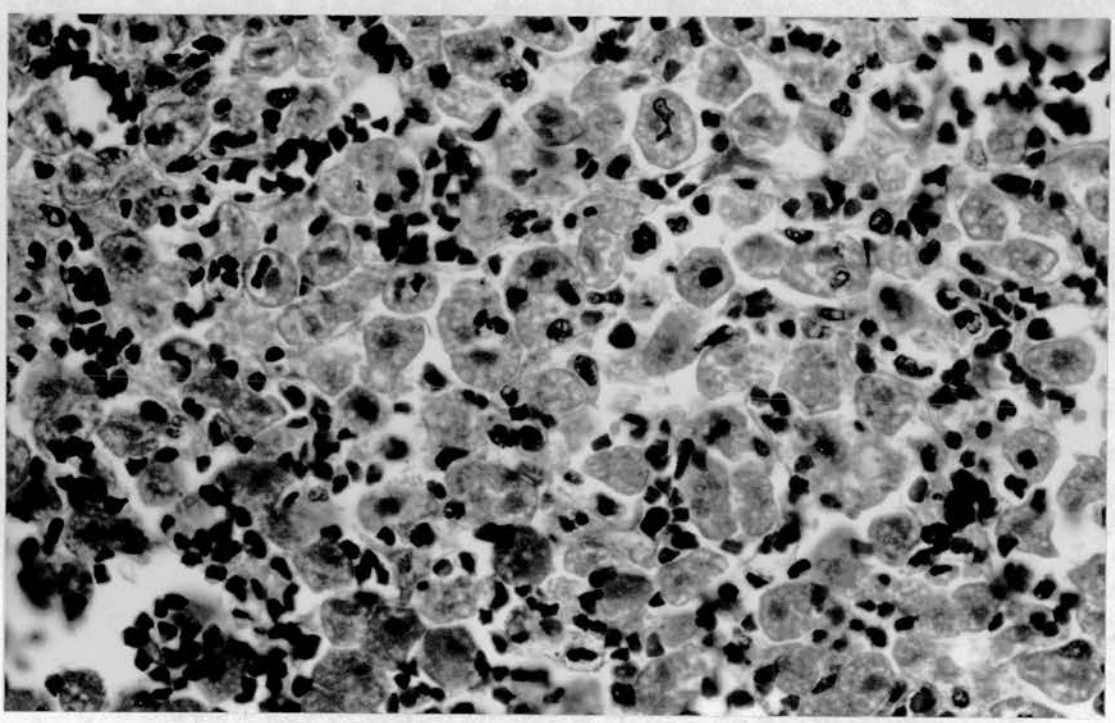

Fig.10.10 Case 16. Liver. Some necrotic cells show retention of pyknotic nuclei and persisting hydropic vacuolation. There is marked congestion. HE. X400

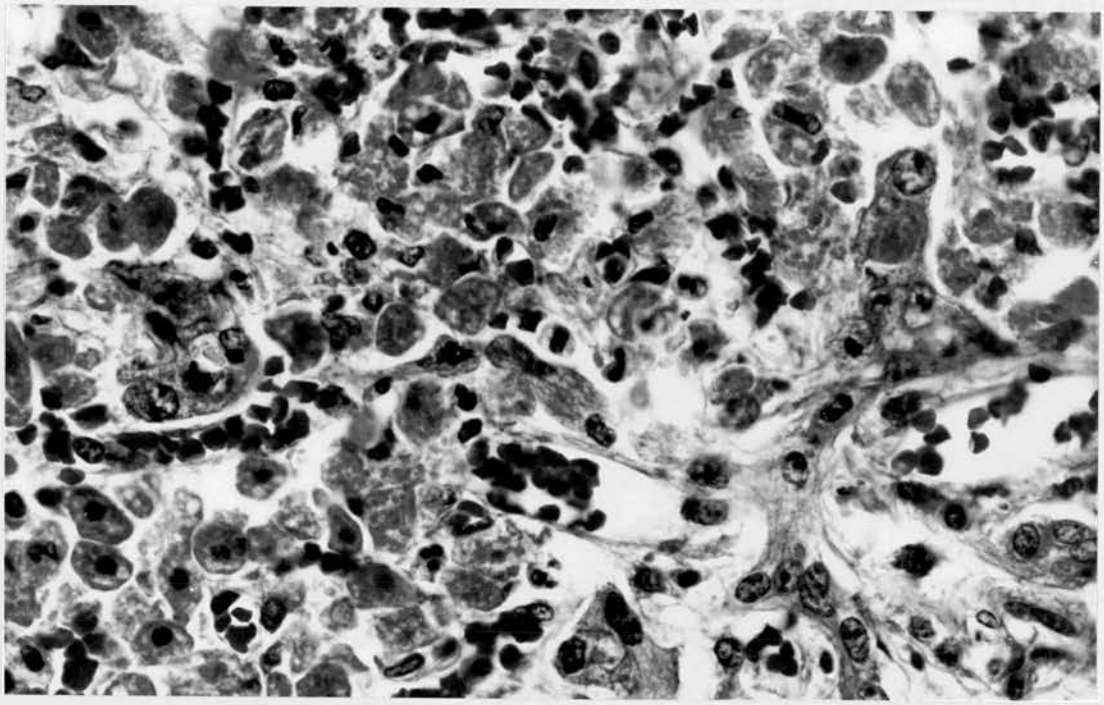

Fig.10.11 Case 16. Liver. Infiltration by macrophages in the necrotic areas. HE. X400

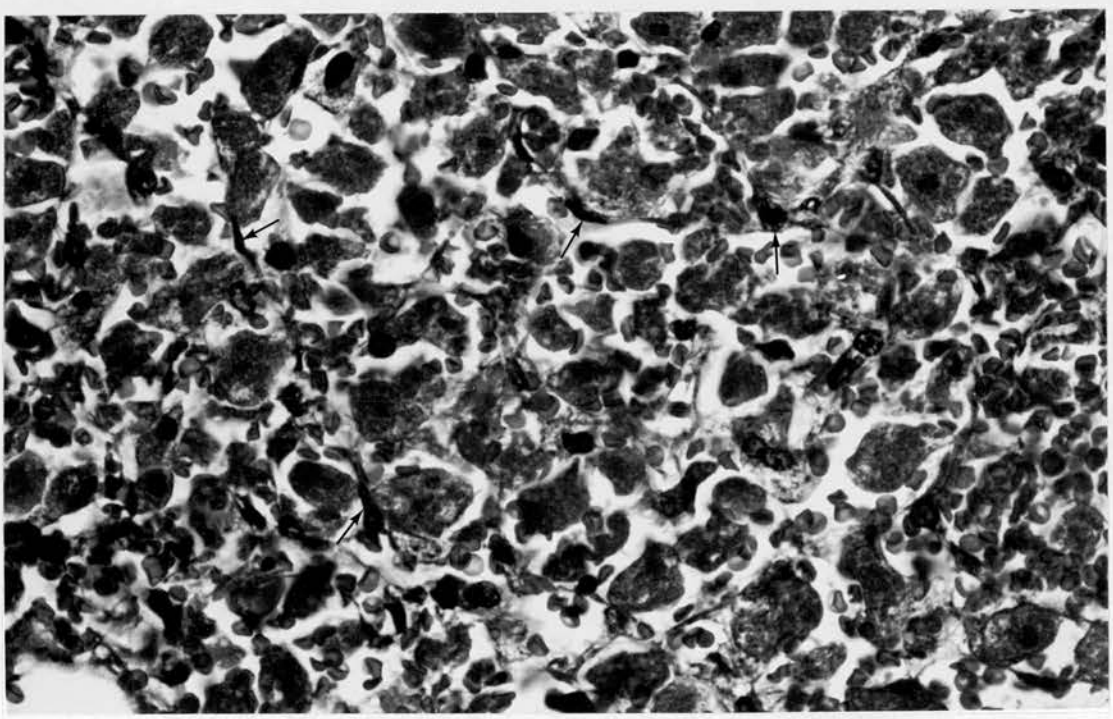

Fig.10.12 Case 17. Liver. The dead hepatocytes are interspersed with RBCs and elongated macrophages (arrowed). HE. X400

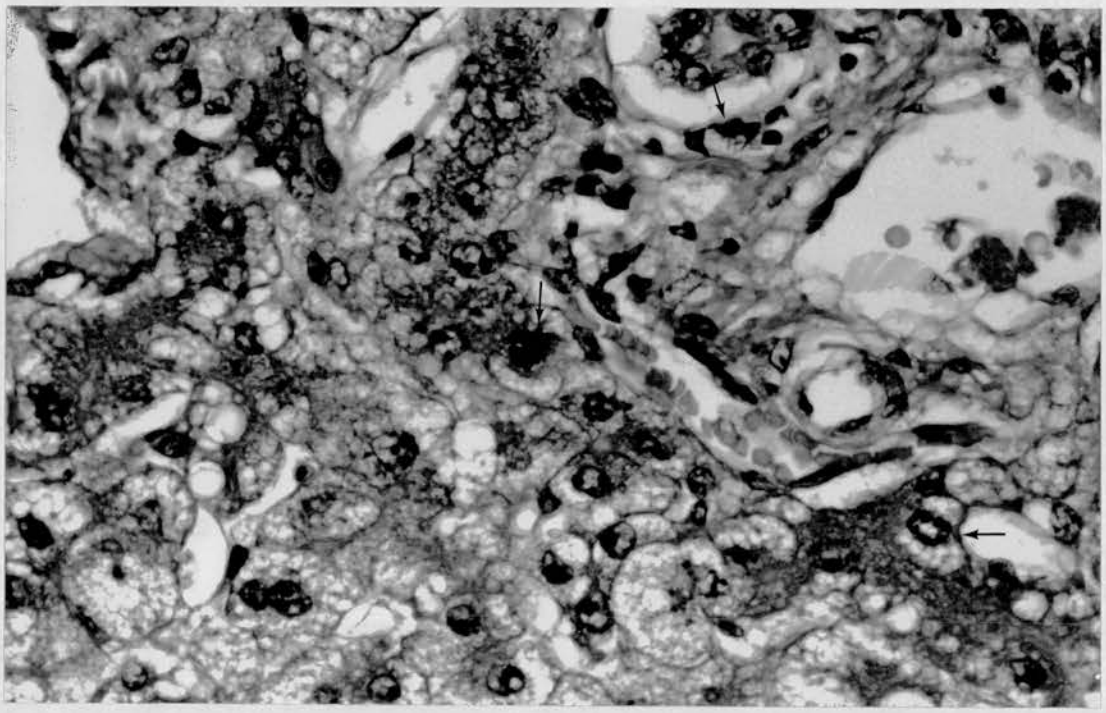

Fig.10.13 Case 17. Liver.The grossly hydropic periportal cells contain occasional mitotic figures (arrowed) HE. X640

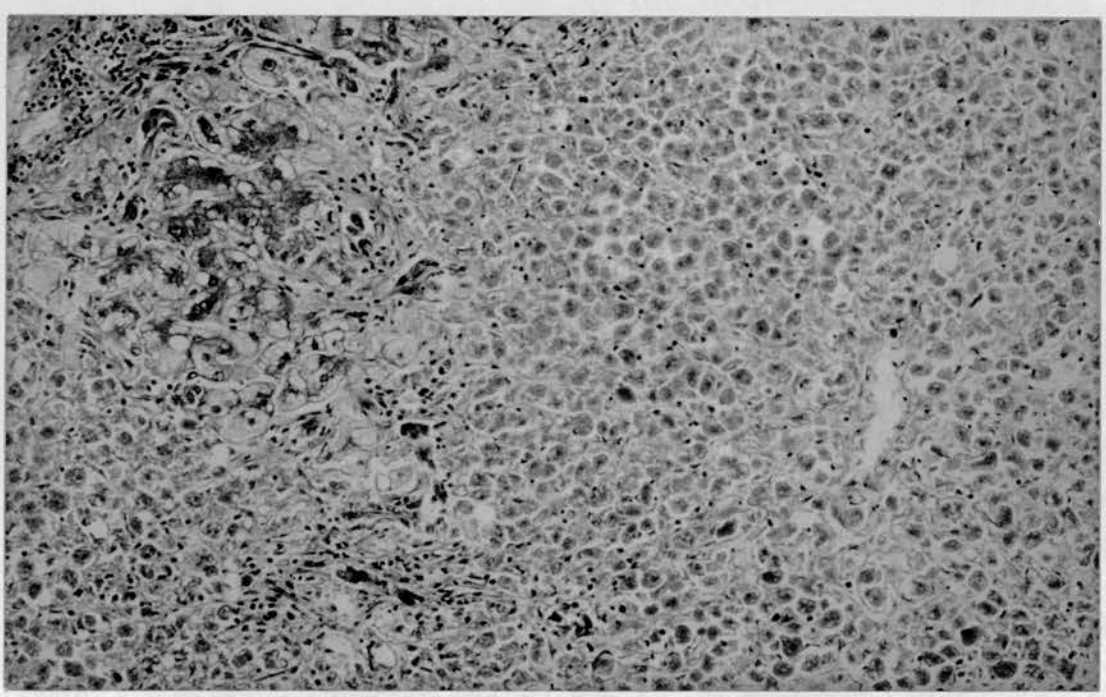

Fig.10.14 Case 18. Liver. Confluent coagulative necrosis with a narrow periportal zone of surviving, but markedly hydropic, hepatocytes. HE. x100

Hepatotoxicity

With the basic assumption that individual cases form part of a continuum one can build up a picture of the evolution of the paracetamol lesion in the human. This assumption may not be strictly valid but to do otherwise would be unduly nihilistic.

In the one case dying before 12 hrs post-ingestion there was no abnormality. Less than 24 hrs after overdose the liver showed hydropic vacuolation, fatty change and pallor maximal in, but not confined to, centrilobular areas. The patient dying at 24 hrs showed early centrilobular necrosis. Thereafter, the coagulative necrosis became progressively more established and the breakdown of necrotic hepatocytes continued over the next 3 days with, in general, a gradual increase in the number of macrophages in the liver. There was little or no collapse of the reticulin framework. After 72 hrs regenerative activity in surviving hepatocytes became apparent, and much of the acidophilic cell debris appeared to be within macrophages. The residual parenchyma frequently showed hydropic vacuolation.

When fatty change was present this was invariably associated with a preceding history of alcohol consumption.

A review of liver histology in the reported cases reveals that the shortest interval between ingestion and death or biopsy is 40 hours that being the fatal case described by Sanerkin (1971). The liver showed centrilobular

necrosis and fatty change in both the viable and necrotic liver cells, polymorphonuclear infiltration in portal tracts, and 'definite but early bile ductular proliferation'. This latter change is difficult to reconcile with a 40 hr interval between ingestion and death. Even if they were the hepatocyte pseudo-tubules or ductules seen as a regenerative feature in massive necrosis one would not expect to see them at 40 hrs. Between 72-96 hrs (Davidson and Eastham; Rose Case 1) centrilobular necrosis and vacuolation and early degenerative features in periportal cells were found, and at 5 days (McLean, Case 1; Benson and Boleyn, Case 4) there was widespread necrosis but the reticulin framework was preserved. At 8 days (Pimstone & Wys) the liver showed centrilobular necrosis, macrophage infiltration, ceroid in Kupffer cells, regeneration in periportal cells, and small numbers of bile plugs in canaliculi, and at 10 days (Boyer and Rouff, 1971) a liver biopsy showed necrosis, regeneration, minimal bile stasis, and moderate infiltration by chronic inflammatory cells.

The changes described thus far parallel reasonably closely the sequence of events seen in my series. Changes reported in longer surviving cases are of considerable interest but have also been somewhat confused. McLean et al., (Case 4) found centrilobular cloudy swelling and active regeneration at 17 days, and Toghill et al., at 18 days found acute hepatic necrosis, and generalised collapse of the reticulin pattern due to complete necrosis

of cells around central veins. The remaining cells showed degenerative changes - fatty infiltration, hydropic change, and cloudy swelling. Cholestasis was pronounced and there was no evidence of cell regeneration. It is difficult to understand how continuing degenerative changes can be related to the toxic effects of a rapidly metabolised and eliminated drug 17 and 18 days after ingestion. Even more puzzling is the finding in a liver biopsy taken 5 weeks after overdose (Benson and Boleyn, Case 2) of "massive hepatic necrosis with regeneration, micronodular cirrhosis and definite evidence of cholestasis". Cholestasis is mentioned by several authors but I have not seen any evidence of canalicular bile retention in the present material or in animal studies. The finding of cirrhosis after paracetamol hepatotoxicity is distinctly unusual. Only one of 17 survivors whose initial biopsy showed the pattern of interlobular bridging necrosis had appreciable residual fibrosis in a follow-up biopsy taken after 1 yr (Portmann et al, 1975). It would therefore appear that the risk of developing post-necrotic cirrhosis is small, a conclusion supported by the earlier experimental work.

The quantitative studies on this series provide an objective guide to the extent of necrosis but their value is diminished by the absence of other data with which to make comparisons. It was very surprising to find that in only 3 cases had adequate liver function tests been carried out. This is partly explicable on the grounds that in some

cases the nature of the overdose was not known by the clinicians and in the earlier cases (1969-70) they were probably not aware of the drug's hepatotoxicity. In the 3 cases with results, the peak enzyme value is apparently related to the extent of necrosis found at P.M. (Table 10D) but the number is, of course, too small to justify any firm conclusion.

Comparing these results with the quantitation performed on 26 fatal cases by Portmann et al., they found that all but 3 had viable hepatocyte volume fractions (HVF) of less than 40%. This figure is equivalent to a percentage necrosis by my method of greater than 53%. They found that 3 patients who survived had a level of necrosis above this figure, however.

In a series of 80 cases dying with fulminant hepatic failure (from a variety of agents) seen in King's College Hospital, Gazzard et al., 1975 found that although the HVF was below 55% in all cases (that is greater than 35% of the parenchyma necrotic), the lowest values (HVF < 12%) were found in cases in whom hepatic failure was the only cause of death, whereas higher HVF values (i.e. 35-85% necrosis) were found in those in whom death was in part due to infection, cerebral oedema, or haemorrhage. They suggest that patients in the latter category might have recovered had the complications been successfully treated. It is interesting to note that of those patients with hepatic necrosis in the present series 9 have less than 85% necrosis. Two of the three patients who had greater than 85% necrosis (10,11) were not considered to have died

TABLE 10D: LIVER FUNCTION TESTS and EXTENT OF NECROSIS

CASE No.	Prothrombin ratio	Maximum serum bilirubin mg%	Maximum S.G.P.T.	Total protein	Albumin	% Necrosis at P.M.
15	7.9	6.3	>1,000	7.5	3.4	68.1
17	4.6	9.3	7,600	4.8	2.9	72.4
18	8.3	6.0	11,900	7.0	4.2	89.8

in hepatic failure although both had gone into coma. One had a cardiac arrest, the other had an extensive haemorrhagic bronchopneumonia, 42 and 48 hrs after overdosage of paracetamol alone.

Two further points of interest have emerged from this study of liver histology concerning lipofuscin pigment and fibrin.

In a paper given to the Pathological Society in January 1975, Oliver, Watson and Williams described the liver histology in seven cases of death from unknown causes in which blood levels of paracetamol were found in amounts around and above the expected therapeutic level of the substance". They did not indicate what these levels were. According to the authors the liver contained large amounts of pigment which they did not characterise but from their photographs had the appearance and staining reactions of lipofuscin. They asserted that the presence of the pigment together with "hepato-cellular abnormalties not amounting to acute necrosis ..... in an otherwise unexplained death should invoke the suspicion of a paracetamol-related cause." In the present series lipofuscin was absent in some cases and when present, was usually found in amounts consistent with the patient's age. Three cases (7, 15, and 18) did show a minor increase but not approaching the amount illustrated by the Glasgow study. Ceroid pigment (a form of lipofuscin) is seen in macrophages in later stages, but this results from the phagocytosis of lipid membranes

derived from necrotic hepatocytes and their subsequent oxidation. It is possible, however, that intra-hepatocyte lipofuscin is a product of sub-lethal cell-injury, and accumulates over the first few days after such injury. This would account for its sparsity in livers showing widespread lethal cell-injury from patients dying up to 4 days after the overdose.

An increased content of lipofuscin has been used as evidence of the mildest form of paracetamol-induced liver injury. James et al., (1975) defined Grade I liver damage as, "Excess of lipofuscin in centrilobular hepatocytes where inappropriate for the age of the patient, focal hyperplasia of Kupffer cells, not more than two foci of hepatocytolytic necrosis". Histological grading was performed on needle liver biopsies taken four days after overdosage or as soon thereafter as the patient's condition permitted. Further support for lipofuscin accumulation being a consequence of sub-lethal cell injury is given by the finding that 18 out of 23 patients showing Grade I changes had normal serum transaminase levels throughout the period of hospitalisation.

The second additional point of interest was the failure to find fibrin in the post-mortem histology. The presence or absence of fibrin is of importance in regard to the coagulation defect complicating paracetamol-induced hepatic necrosis. Massive necrosis from any cause is frequently complicated by bleeding which in the main results from a

failure to synthesise clotting factors in the liver (Cook and Sherlock, 1965). An additional contribution to the bleeding diathesis may be intravascular coagulation which probably develops as a further consequence of hepatic necrosis (Rake, Flute, Panell and Williams, 1970). Williams (1972) proposed that microthrombosis might be expected to occur within the liver in relation to necrotic cells. The presence of fibrin thrombi within hepatic sinusoids has been documented in toxic hepatic necrosis by Popper and Franklin (1948) and acute hepatic failure of differing aetiology by Hillenbrand et al., (1974). Stainable fibrin was not seen, however, in any of the present cases. This was not unexpected as fibrin was not demonstrated in any of the experimental animals. Rake et al., (1973) in a study of CCl_4-induced hepatic necrosis using 119 test rats found that fibrin "was seldom evident by histological techniques although in a few animals ... small numbers of thrombi were seen in the liver sinusoids. However, the fibrin c ould have been removed by fibinolysis, which is known to be particularly active in the microcirculation".

Gazzard et al (1975) examined serial sections of liver kidney, lung, and brain from patients dying in hepatic failure and found two or more microthrombi in the livers of 12 out of 31 patients examined. The sections were stained with haematoxylin and eosin, however, and little if any reliance can be placed on the identification of microthrombi by this method.

On the basis of the more specific MSB staining method for fibrin used in my study, I conclude that microthrombosis within the liver does not seem to account for a consumptive coagulopathy in paracetamol hepatotoxicity.

2. KIDNEYS

One or more sections of kidney were available in 15 cases, 6 cases showed only variable post-mortem autolysis and/or congestion.

Two cases (9 and 13) show moderate to marked hydropic vacuolation in the lining epithelium of the proximal tubules. The vacuoles are for the most part indistinct giving the epithelium a granular appearance but are more prominent in the basal part of the cell, that is towards the basement membrane (fig. 10.15).

The remaining seven cases (10,12,14,15,16,17 and 18) show variable acute tubular necrosis (ATN). Necrosis of tubular epithelium is sometimes difficult to distinguish from autolysis and other features assist in the diagnosis. Filling of lumina in the affected segments by debris, and in the collecting tubules by amorphous granular casts, are part of the histological picture (figs. 10.16, 17, 18). The casts sometimes show slight brown pigmentation. Further features are associated degenerative changes such as hydropic vacuolation, nuclear pyknosis, and separation of cells. Some tubules are lined by regenerative epithelium showing occasional mitotic figures. One case (12) shows a few examples of so-called "haemopoiesis" in the vasa recta, (fig. 10.19).

In most cases with ATN the changes are patchy both in terms of the areas of cortex involved and the segments of the nephron affected. There appears to be no special

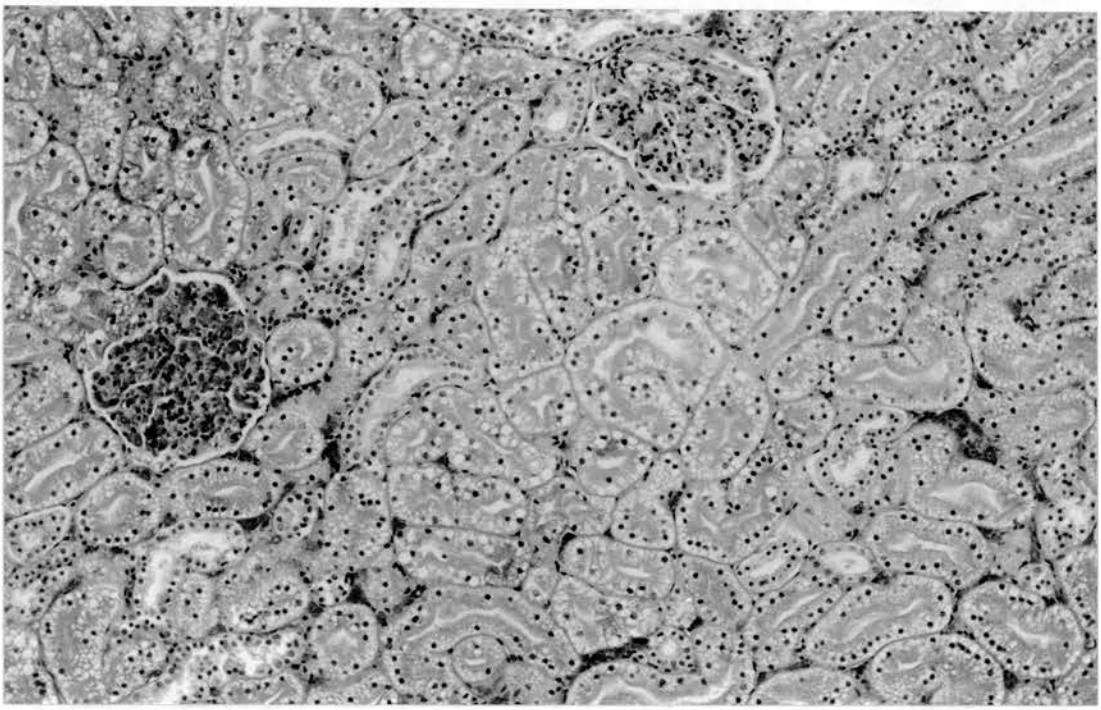

Fig.10.15 Case 9. Kidney. Hydropic vacuolation affecting principally proximal tubular lining cells. HE. X100

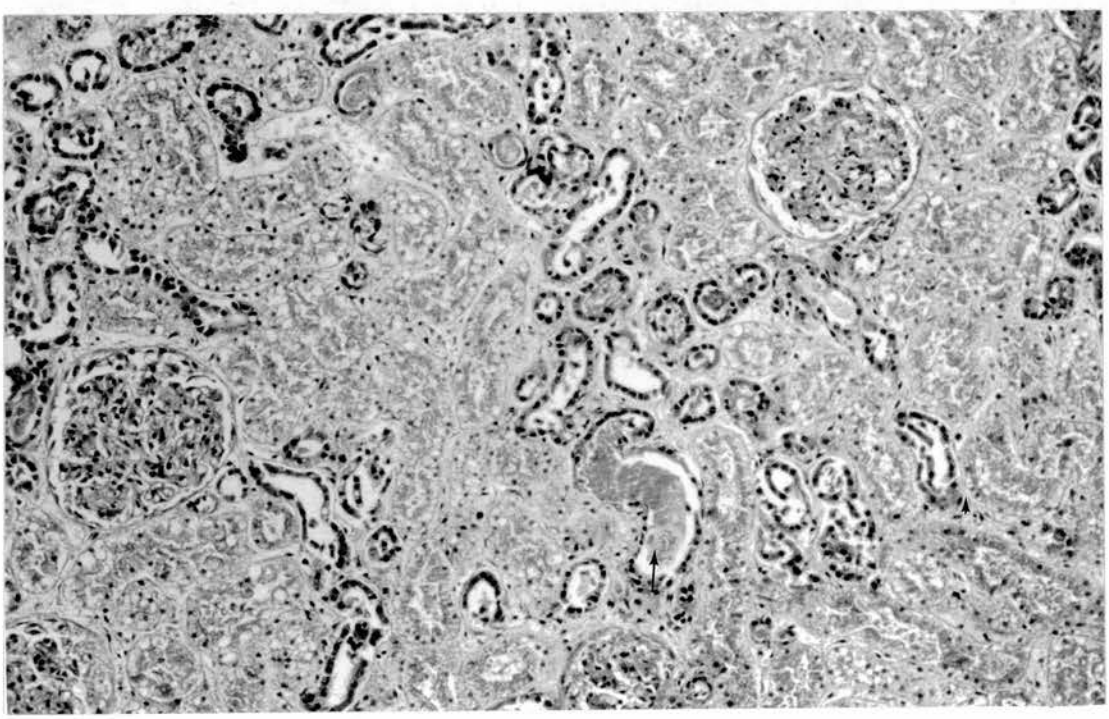

Fig.10.16 Kidney. Vacuolation and necrosis of proximal tubule cells together with a granular cast in a collecting tubule (arrowed). HE. X100

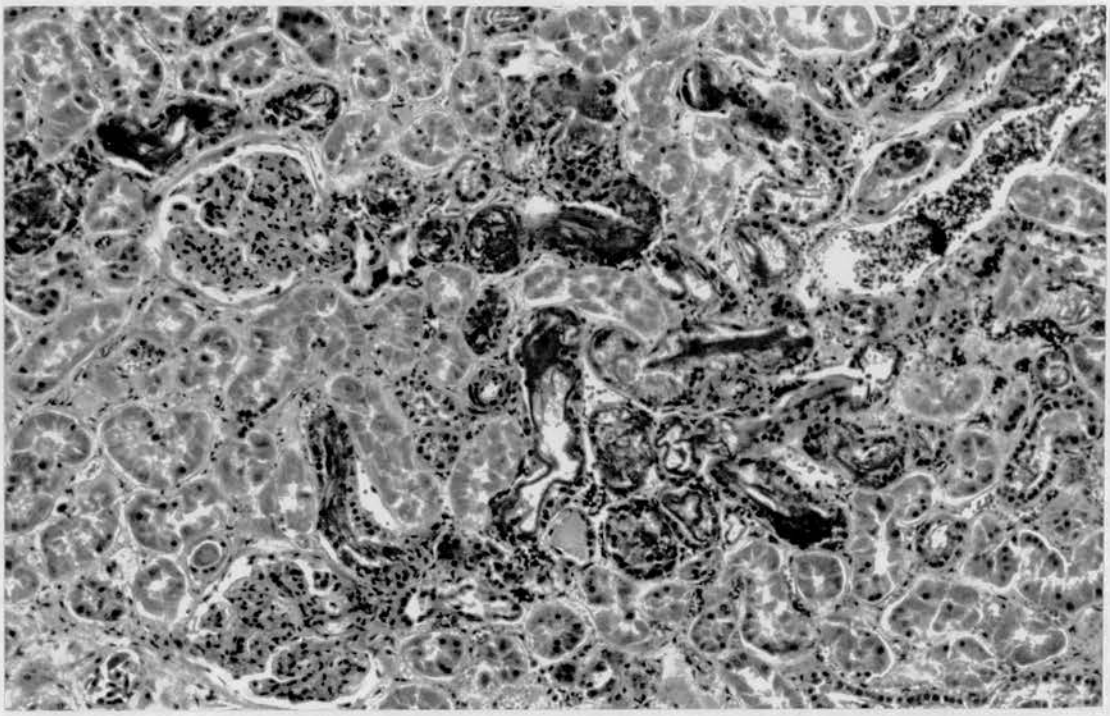

Fig.10.17 Case 15. Kidney. Necrosis of tubule lining cells with dark staining casts in distal tubules. HE. X100

Fig.10.18 Case 18. Kidney. Collecting tubules within the medulla contain desquamated cells and granular debris. HE. X100

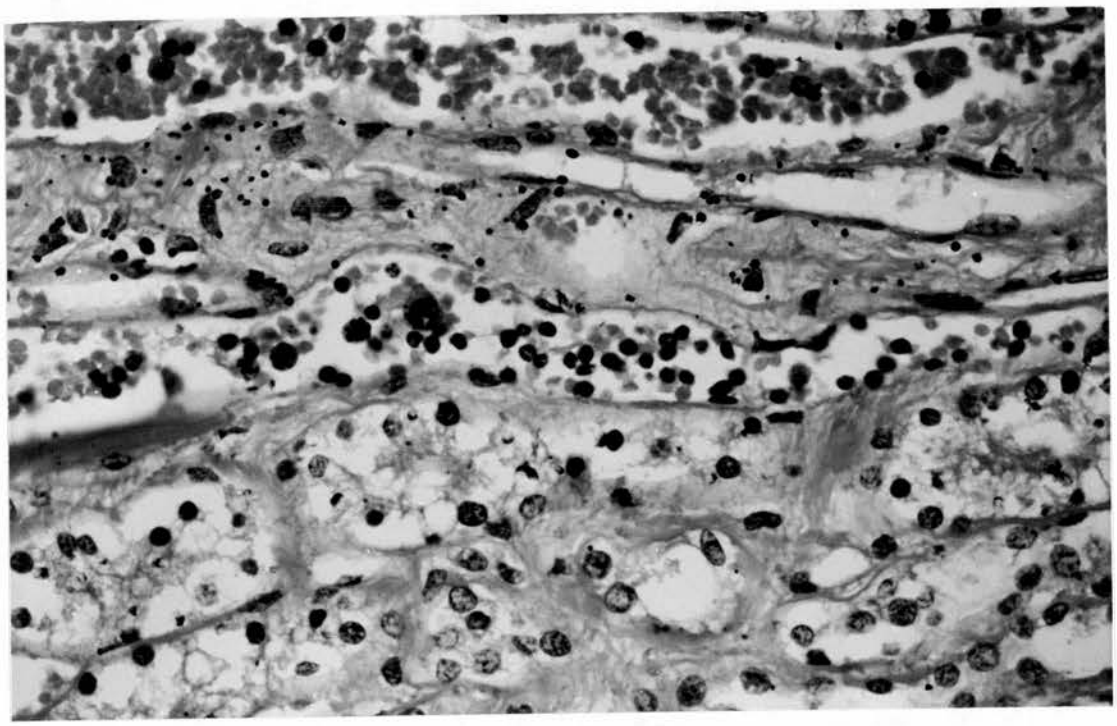

Fig.10.19 Case 12. Kidney. Increased numbers of small cells with deeply staining nuclei in a dilated vasa recta of the medulla - so-called "haemopoiesis". HE. X400

tendency for proximal tubules to be involved. Case 14 and 18 show extensive bilateral ATN which in the former is superimposed on a pre-existing acute pyelonephritis.

Paracetamol and the kidneys

Impaired renal function was a frequent finding in the longer surviving cases in this series and 7 of the 10 patients surviving more than 36 hr after ingestion showed varying degrees of acute tubular necrosis on histology. Two other cases revealed tubular hydropic vacuolation and whilst clinical evidence of acute renal failure was not rec orded, the biochemical results were distinctly abnormal (Table 10E).

The present findings parallel closely the renal involvement described in the early reports, 9 of the 11 early cases in which PM details are given (Table 10A) showed renal changes - usually tubular necrosis. The consistent association between hepatic and renal tubular necrosis in paracetamol overdosage led to an impression that the tubular injury also resulted from a direct toxic effect of the drug. There is little or no evidence to support this conclusion. The results of experimental animal studies have usually failed to demonstrate significant acute nephrotoxicity but Chenery, Fisher and McLean (1976) have described tubular damage when rats were fed a diet containing a high proportion of fat and treated with phenobarbitone prior to the administration of a hepatotoxic dose of paracetamol. The development of chronic renal failure after long-term con-

TABLE 10E: CASES with RENAL TUBULAR ABNORMALITIES

CASE	HISTOLOGY	SERUM ELECTROLYTES mmol/l				Urea mg%
		Na^+	K^+	Cl^-	HCO_3^-	
9	HV	140	7.0	-	10	100
13	HV	141	6.1	115	6	58
10	ATN	151	5.5	111	-	30
12	ATN	159	7.9	-	-	60
14	ATN	140	3.6	-	25	110
15	ATN	129			18.9	81
16	ATN	146	4.6	100	30	133
17	ATN	151	2.8	100	22	101
18	ATN	139	3.5		21	72

NORMAL RANGE 135-145; 3.6-5.0; 98-107; 21-28; 15-40

HV = Hydropic vacuolation

ATN = Acute tubular necrosis

sumption of paracetamol appears to be a very rare event (Krikler, 1967; Edwards et al., 1971) and even these cases are controversial.

Wilkinson et al., (1977) analysed the frequency of acute renal failure in 160 patients with fulminant hepatic failure. They found that the frequency was not significantly higher in patients who had taken an overdose of paracetamol when compared with those with hepatic failure due to other causes. The same was found in another group of patients with less severe hepatic damage. In both groups, the development of renal failure was closely related to the occurrence of endotoxaemia as detected by the Limulus lysate assay, and the authors suggest that this is due to failure of the liver to filter endotoxins normally absorbed from the bowel into the portal venous blood. Their findings do not support the concept that an overdose of paracetamol has a specific toxic effect on the kidney.

Whilst their finding of endotoxaemia is of considerable interest and a logical explanation for the renal failure of the "hepato-renal syndrome", it does not necessarily explain the pathogenesis of renal injury in all cases. The authors specifically excluded from their study patients in whom renal failure had developed in the last 24 hours of life, in association with cardiac or respiratory arrest or severe terminal hypotension, yet these are the patients in whom renal failure may be prevented by careful management. It would be interesting to know how many patients fell into

this category and whether or not these complications were more common in the paracetamol group.

Analysis of the clinical records of the 7 cases in the present series showing acute tubular necrosis disclosed severe hypotension and/or cardiac arrests in all but one (Table 10F). It is obvious that renal failure complicating hepatic failure is a multifactorial problem. Some cases follow severe hypotension or cardiac arrests and represent the classical ischaemic tubular injury. Many others, on the basis of Wilkinson's findings, develop endotoxaemia which gives rise to renal injury by direct renal vasoconstriction and intravascular coagulation. It may be indeed that hypotension and cardiac arrests are further consequences of endotoxic shock so that endotoxaemia becomes the common denominator in the pathogenesis of all cases of renal failure complicating hepatic injury.

TABLE 10F: Cases with Acute Tubular Necrosis

CASE	TIME	
10	42 hrs	Profound hypotension for 24 hrs prior to death
12	60 hrs	Hypotension. Cardiac arrest 20 hrs before death
14	72 hrs	Blood pressure unobtainable. Unconscious. Hepatic failure
15	75 hrs	BP 110/65. Hepatic failure
16	84 hrs	Cardiac arrest x2, 48 hrs before death Remained hypotensive
17	96 hrs	Hypotensive (<80 mm systolic) for 24 hrs prior to death. Hepatic failure
18	5 days	Prolonged hypotension. Hepatic failure

3. Heart

Sections of the left ventricular myocardium were available from 12 cases. These were stained with HE and phosphotungstic acid-haematoxylin (PTAH) to demonstrate myofibrils.

Only one case (10) shows any abno rmality of the myocardium, namely an overall slight increase in interstitial cells which appear to be macrophages, and a few small collections of polymorphs found alongside fibres showing increased eosinophila (figs 10.20, 21). The PTAH shows patchy loss of striations but no clumping of myofibrils.

Paracetamol and the heart

Some controversy surrounds the effects, if any, of paracetamol on the heart. In two of the early fatal cases acute myocardial necrosis was attributed to paracetamol toxicity. Pinstone and Uys reported that "the myocardium showed evidence of diffuse damage. The fibres throughout were abnormal, appearing thin, wispy and poorly stained. Notable interstitial oedema was present and interstitial cells were prominent, many showing the cytological details of Anitschow myocytes. Beneath the endocardium these changes were more severe and here band-like foci of necrosis were noted". The authors considered it possible that the cardiac lesion precipitated circulatory collapse and materially contributed to the patients death "as liver histology showed only centrilobular necrosis with regeneration of cells already well established".

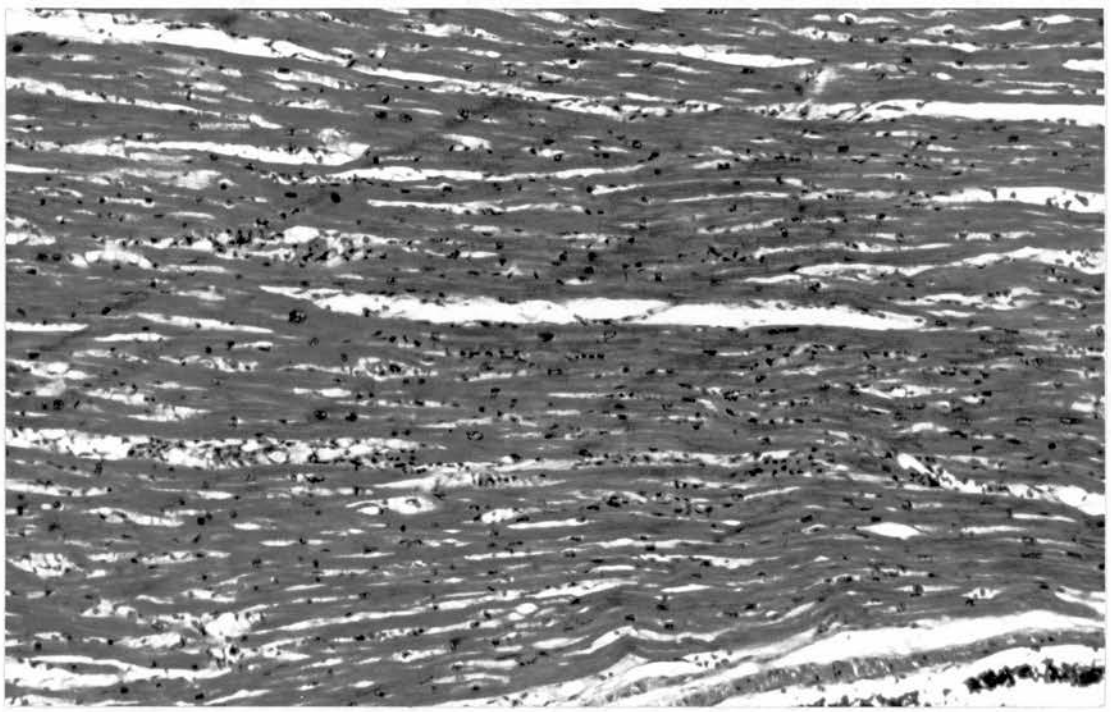

Fig.10.20 Case 10. Heart. Mild increase in interstitial cells in the myocardium. HE. X100

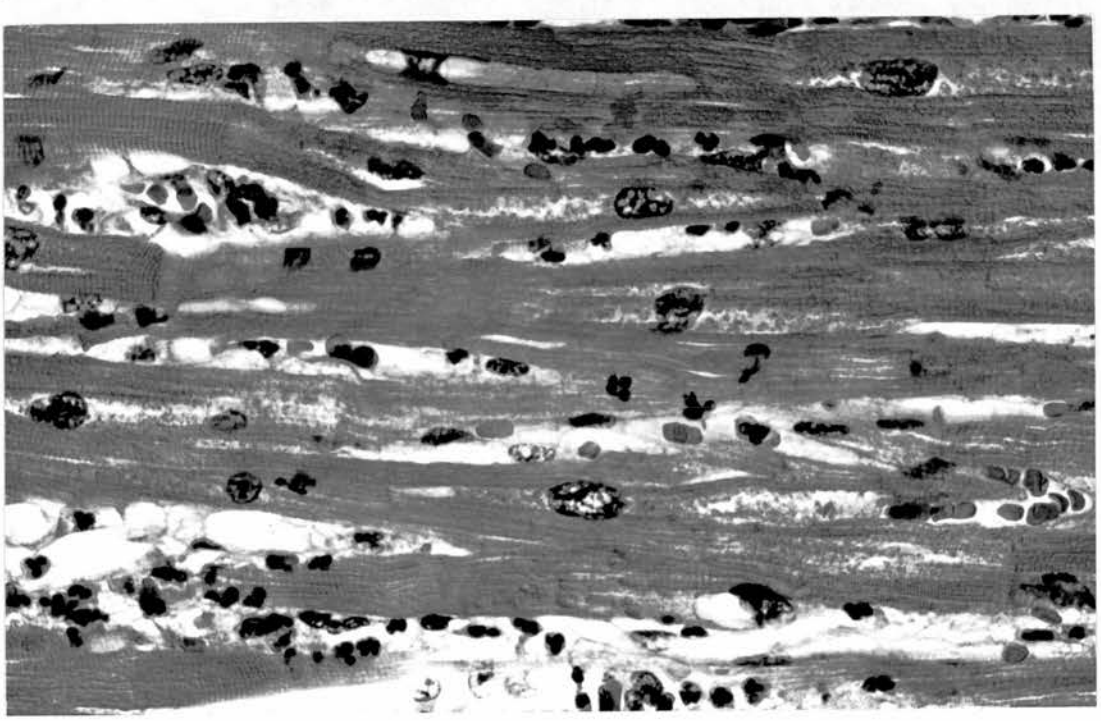

Fig.10.21 Case 10. Heart. Higher power view reveals polymorphs and macrophages adjacent to fibres showing patchy loss of cross-striations. HE. X400

Sanerkin (1971) reported that "the myocardium showed mild to moderate fatty degeneration and confluent early focal necrosis (sic), with irregular loss of staining and focal clumping of myofibrils in PTAH stains". On the basis of these findings he states, "The maximum hepatotoxic effect of paracetamol is known to occur between two and four days from ingestion. The cardiotoxic effect must be similarly delayed; the myocardial necrosis in this case was only 12-24 hours old".

Gazzard et al., (1975) in their study of causes of death in hepatic failure found 5 cases out of 96 patients autopsied with a pale flabby myocardium, probably due to fatty change. All five had died after paracetamol overdosage.

Weston and Williams (1976) reported that cardiac arrhythmias were found in most patients with hepatic failure. Asystolic arrests were common, and three patients arrested while still rousable, two after paracetamol overdosage and one with virus hepatitis. Thus, in their series of selected patients it was not possible to attribute the cardiac abnormalities either to the liver failure or the aetiological agent.

The case reported by Will and Tomkins (1971) is of further interest because serial ECGs performed on a girl of 19 yrs who had taken 30-50 paracetamol tablets revealed significant abnormalities on day 2 and day 4 although she did not go into hepatic failure. There was biochemical evidence of liver damage, however, at the time of the ECG

abnormalities.

Four patients in the present series died from cardiac arrest (4, 10, 11, and 12), and the latter three cases had severe hepatic necrosis at autopsy. Case 4 had a cardiac arrest 13 hrs after taking paracetamol alone, but no histology was available from this case. Only one case (10) showed any histological abnormalities in the heart and these were slight. Furthermore, some of the changes are explicable on the basis of cardiac massage which was performed following a cardiac arrest earlier in the morning of her death.

4. Brain

Sections from various parts of the brain were available in nine case.

In four cases (1, 6, 9 and 12) apart from minor congestion of meningeal vessels and post-mortem changes, the appearances are normal.

Two cases (10 and 15) show minor degrees of oedema and features of arterial disease, such as perivascular gliosis, or early anoxic damage such as satellitosis of neurones.

The brain in case 11 contains multiple purpuric haemorrhages around the third ventricle and in the substantia nigra, and a small area in the cortex reveals pigmented Gitter cells and astrocytic reaction. The latter changes are probably related to trauma some weeks prior to death.

In case 16 there is haemorrhagic softening in the pons and medulla, the cortex shows extensive loss of neurones, and the cerebellum shows degeneration and loss of Purkinje cells with widespread necrosis necrosis of the granular layer.

Case 14 shows occasional, mild Alzheimer Type II change in astrocytes but no neuronal damage.

Case 18 shows extensive small cell ischaemic degeneration (homogenising cell change) and in ammons horn larger neurones are involved. Astrocytes show type II change mainly in grey matter but also in white (figs 10.22,23).

Paracetamol and the brain

Cerebral oedema is a frequent finding in patients who

die in hepatic failure and was present in 36 of 92 patients examined at autopsy in the series of Gazzard et al. (1975). This high incidence was found despite treatment in 23 patients designed to combat the development of cerebral oedema.

The brain was examined in 17 of the present cases and found to be oedematous in at least 8 (see Table 10G). One brain was described as normal and yet weighed 1,600 g which is well above the upper limit of normal (Case 6).

On histological examination cases of hepatic failure with encephalopathy may show a diffuse increase in the size and number of protoplasmic astrocytes, some with clear nuclei and a well-defined nuclear membrane (Alzheimer Type II cells), and a patchy vacuolar degeneration and necrosis of the nerve cells in the deeper layers of the cerebral cortex, in the basal nuclei, and in the cerebellum. Alzheimer's Type II change was seen in two cases (14,18) but the neuronal damage present in 18 was probably attributable to hypoxia or hypoglycaemia rather than the toxic effects of hepatic encephalopathy.

The remainder of the histology was in keeping with the naked-eye appearances, and examination of the brain from case 6 did not shed any light on the cause of death. Unfortunately histology was available on only 2 of the 9 early cases, that is dying up to 36 hours after overdosage.

Recently, Alam et al., (1977) have suggested a possible mechanism of cerebral oedema in hepatic failure. These

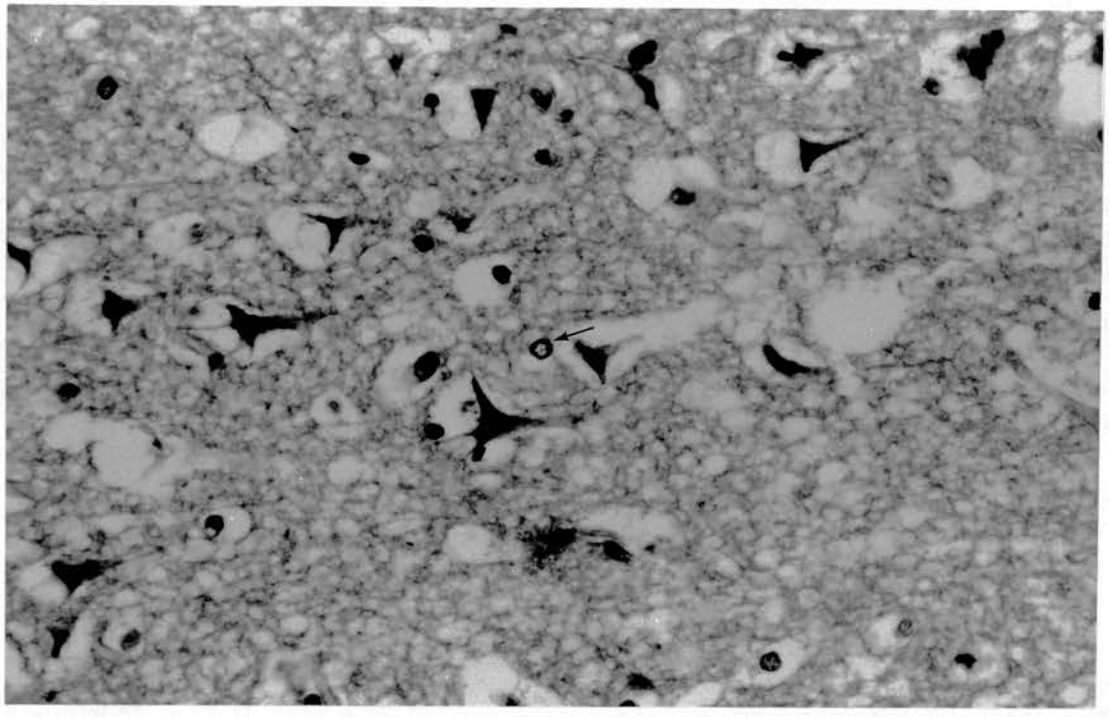

Fig.10.22 Case 18. Brain.

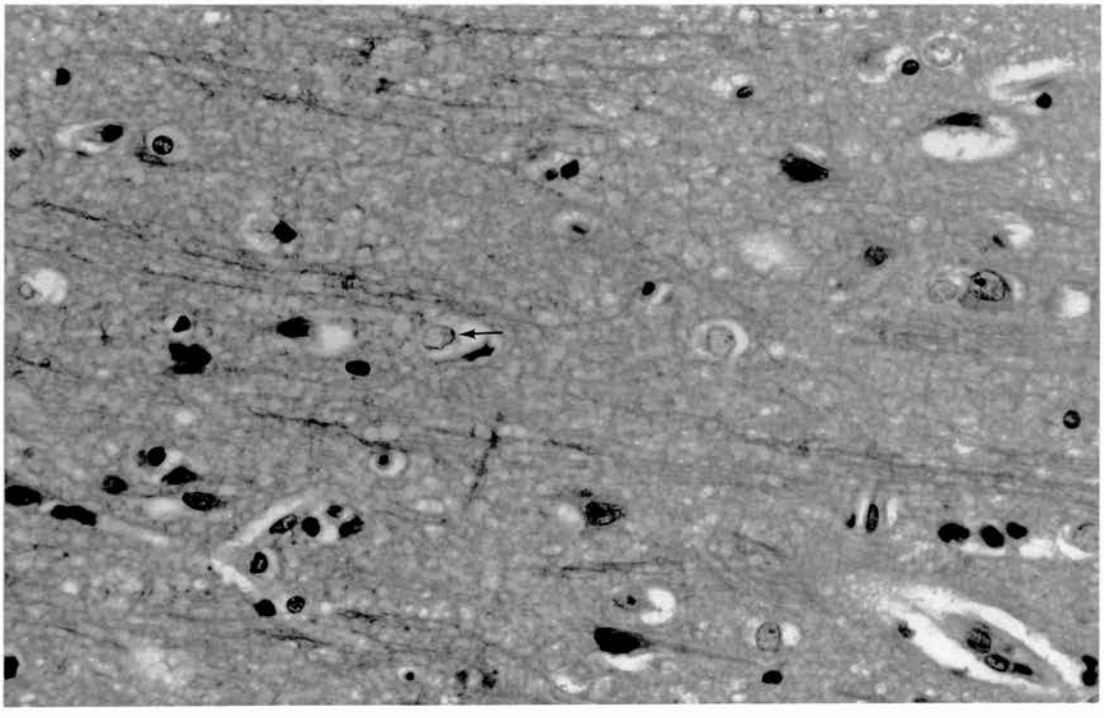

Fig.10.23 Case 18. Brain. In addition to interstitial oedema and variable shrinkage of neurones, the figures contain occasional astrocytes showing Alzheimer's Type II change (arrowed). HE. X400

TABLE 10G

Gross appearances of the brain

Case	1.	Congested but otherwise normal	
	2.	Nil of note	
	3.	Normal	1290 g
	4.	Old haemorrhage in right temporal lobe but elsewhere normal	
	5.	Congested and swollen	
	6.	Normal (?)	1600 g
	7.	Normal	
	8.	Generalised oedema	1400 g
	9.	Normal	1430 g
	10.	-	
	11.	Soft and slightly congested	1300 g
	12.	Slight oedema	1250 g
	13.	Considerable oedema	1380 g
	14.	Normal	1295 g
	15.	Very oedematous with 'coning'	1320 g
	16.	Soft with haemorrhagic changes	1400 g
	17.	Oedema; 'marked flattening of convolutions'	
	18.	Mild oedema	1250 g

authors found a relationship between cerebral oedema and a raised leucocyte water content and proposed that changes in intracellular electrolyte composition similar to those in leucocytes may account for the swelling of brain cells. They further suggest that cell swelling in other tissues by compressing capillary blood flow, might also contribute to dysfunction in other organs, including kidney and heart, as well as contributing to further hepatocyte damage.

5. Lungs

Sections of one or both lungs were available in 15 cases.

In 2 cases (1,8) the lungs were intensely congested with areas of collapse associated with fatal inhalation of vomit.

In 5 cases there is a variable degree of broncho-pneumonia, which in 3 instances was sufficiently severe to be the immediate cause of death (cases 5, 11 and 13). In 3 less severe cases (9, 10, 17) the presence of amorphous debris in alveoli and bronchioles indicates that the bronchopneumonia has followed inhalation of vomit (figs 10, 24, 25).

In the remaining cases the lungs are either normal (3) or show variable congestion (7) and oedema (6, 10, 14, 15, 18).

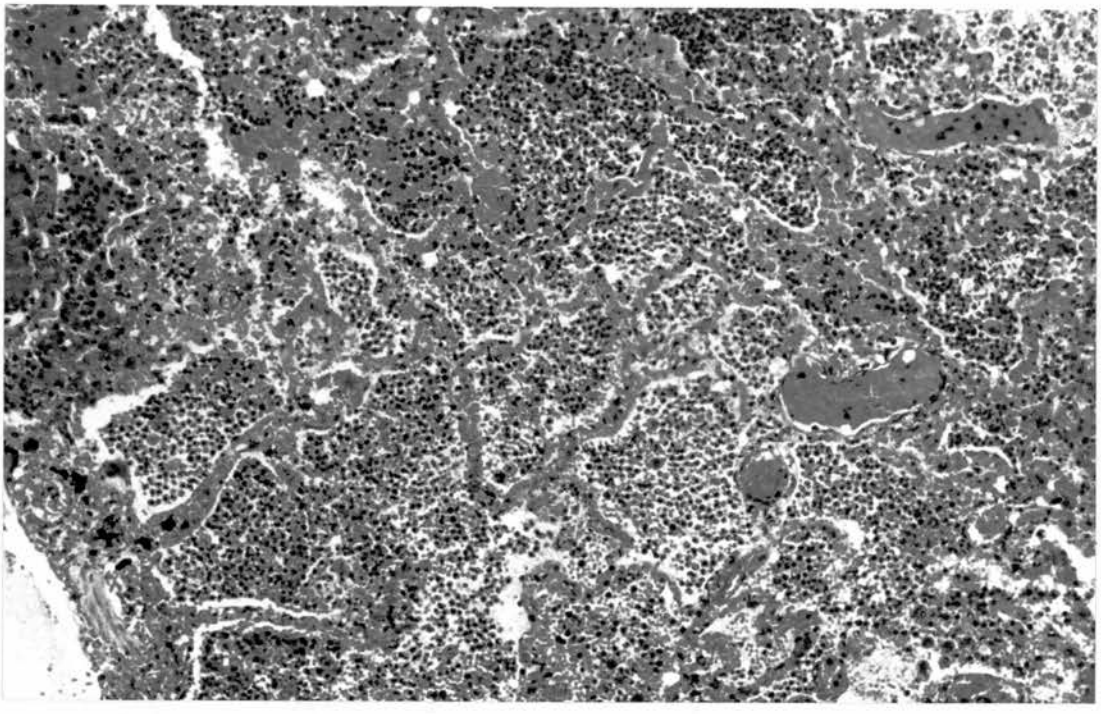

Fig.10.24 Case 9. Lung. Alveoli are packed with neutrophil polymorphs in the consolidated areas confirming the presence of bronchopneumonia. HE. X100

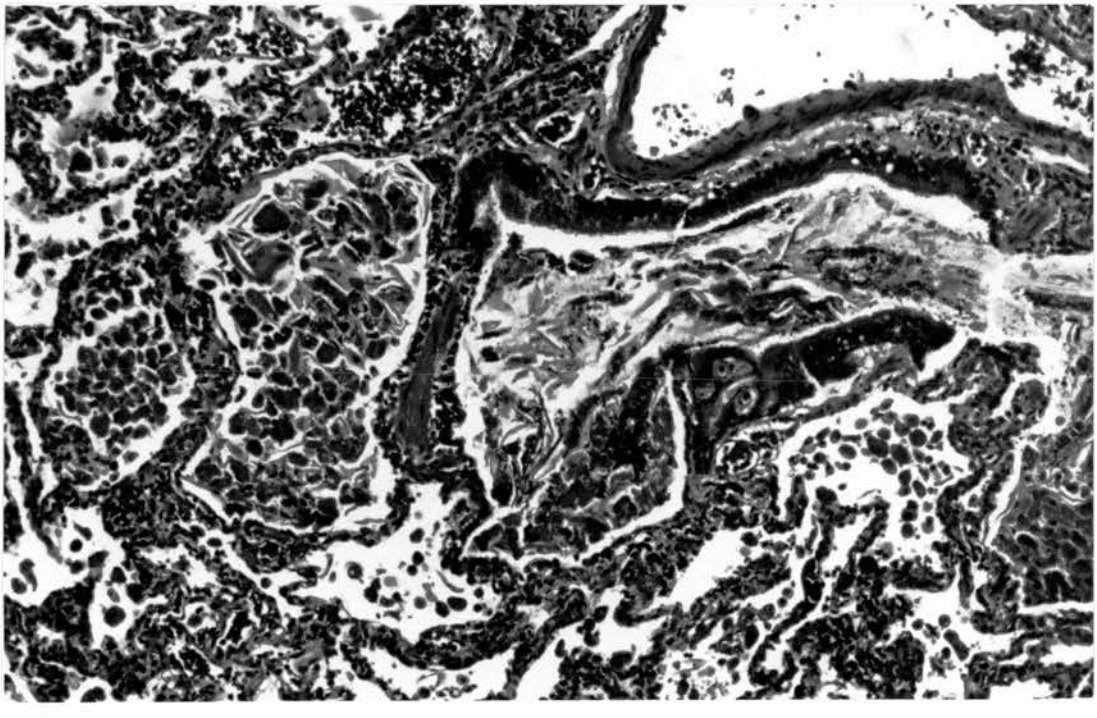

Fig.10.25 Case 10. Lung. Bronchioles and adjacent alveoli contain aspirated material. HE. X400

6. Other organs

The pancreas (from 6 cases) shows variable autolytic changes but no evidence of a vital reaction suggesting ante-mortem inflammation.

The adrenals (6 cases) show focal or diffuse lipid depletion, and intense congestion of the zona reticularis compatible with a 'stress' response.

The uterus was examined in 6 cases, one of which shows the changes of pregnancy, but otherwise show no notable abnormality.

The spleen (5 cases), thyroid (4 cases) oesophagus (2 cases), ovaries (3 cases) and bronchi and stomach (1 case) show no changes which could be interpreted to a toxic effect of paracetamol.

Reference to the possible effects of paracetamol on organs other than liver, kidneys and brain are completely lacking in the literature.

Dr A. N. Hamlyn (personal communication, 1977) claims that 22 per cent of 201 cases of paracetamol overdosage seen in Newcastle had a raised serum amylase although none had clinical pancreatitis. No pancreatic lesion was demonstrable in the 6 cases in which histology was available in the present series.

It would appear from the incomplete selection of tissues represented in this autopsy series that there are no abnormalities which cannot be explained on the basis

of secondary lesions consequent upon the overdosage. There is nothing to suggest that other organs are directly involved by paracetamol toxicity.

POST-MORTEM TOXICOLOGY

All of the cases in this series were confirmed as paracetamol overdosage by P.M. toxicology. Free paracetamol was measured in blood (usually taken from the leg veins) and a variety of other samples; liver, brain, stomach contents, urine and in a few cases, bile. Up to 1974 the levels of free paracetamol were determined by the method of Gwilt et al., (1963) and thereafter by the method of Willson, Thompson and Williams (1973). The levels found in blood and liver are listed in Table 10H.

There is considerable variation in the levels of paracetamol found in fatal cases as they obviously reflect the variation in time between ingestion and death as well as the initial dose. It appears from clinical reports that levels in excess of 15 mg/100 ml at 12 hrs are associated with potentially fatal liver damage and the P.M. results are in general accordance with this figure. The one anomalous result in the early cases is case 4, where 13 hrs after ingestion a blood level of 3 mg% was obtained. It can be seen that the liver level (which usually runs parallel to blood levels and is of the same order of magnitude) in this case is 19 mg% which indicates a substantial overdosage. The discrepancy may result from dilution of the blood sample during collection or some technical error in the estimation. There was no evidence of another drug being implicated.

TABLE 10H

Blood paracetamol figures: Fatal cases

Case	> Time Ingestion Death	Blood level mg% (free paracetamol)	Liver mg/100 g
1	< 12 hrs	20.5	32.2
2	< 12 hrs	102	65.0
3	12 hrs	20.0	29.0
4	13 hrs	3.0	19.0
5	16 hrs	21.6	-
6	< 24 hrs	19.0	26.0
7	24 hrs	46.0	36.0
8	28 hrs	22.0	31.0
9	36 hrs	1.2	1.3
10	42 hrs	2.0 (14.8 at 24h)	-
11	48 hrs	4.0	15.0
12	60 hrs	40.0	52.0
13	70 hrs	1.0	0
14	72 hrs	1.4	2.5
15	75 hrs	3.4	trace
16	84 hrs	1.6	-
17	96 hrs	5.9	2.0
18	5 days	0.61 (30 at 9 h)	2.5

The levels found after 36 hrs are, as one would expect much lower. In two cases ante-mortem levels give a clearer indication of the size of the overdose. In case 12 dying at 60 hrs the blood level of 40.0 mg% is exceptionally high and may reflect early and very severe hepatic necrosis potentiated by alcohol which has greatly prolonged the paracetamol half-life. This patient took 100 x 500 mg tablets.

Ten of the 18 patients had taken alcohol or other drugs along with paracetamol. The additional toxicological findings are listed in Table 10I.

Four patients took large amounts of alcohol immediately prior to the paracetamol overdose. In two early deaths (1, 6) the ethanol levels of 307 mg% and 296 mg% indicate that acute alcohol toxicity may well have contributed to death. In cases 12 and 16 levels were not available. A further case (14) had a history of chronic alcoholism but there was no evidence of alcohol ingestion around the time of overdosage.

Alcohol is frequently taken immediately before a drug overdosage. Amongst 207 male patients admitted in 1971 to the Glasgow Western Infirmary, heavy drinking preceded an overdose of drugs in about 70% of cases, and in them the mean blood-alcohol concentration on admission was 146 mg%; of 313 female cases about 40% had taken alcohol before the overdose, and their mean blood concentration was 102 mg%, (Patel et al. 1972). In conjunction with paracetamol, alcohol is particularly hazardous. It will not only

TABLE 10I

Toxicology: Alcohol and other drugs present

Case	1.	307 mg/100 ml Ethanol
	3.	Therapeutic dose of Librium (0.1 mg/100 g in liver)
	5.	Small amount of Oxazepam detected
	6.	296 mg/100 ml Ethanol
	8.	0.2 mg% Methaqualone hydrochloride
	12.	1.2 mg% Tuinal (Alcohol)
	13.	'positive reaction' for glutethimide
	16.	(Alcohol)
	17.	2.5 mg% Methaqualone
	18.	12.mg% salicylate (at 9 hrs)

exacerbate the acute liver injury by its own direct hepatotoxicity but prior consumption will have had an inducing effect on microsomal enzymes and accelerate paracetamol metabolism. Such potentiation by alcohol has been described by Wright and Prescott (1973).

With regard to other drugs, in cases 3 and 5 the taking of a therapeutic dose of librium and a small quantity of Oxazepam (which is relatively non-toxic) are of dubious significance. The 'positive reaction for glutethimide' in case 13 (70 hrs), probably indicates more than a therapeutic dose. Glutethimide (Doriden) is a non-barbiturate hypnotic which in overdosage may lead to unconsciousness, apnoea or hypotension.

In case 8 the finding of 0.2 mg% of methaqualone at 28 hrs is of doubtful significance as therapeutic levels may be up to 0.5 mg%, but in case 17 (96 hrs) the methaqualone level indicates an overdosage. In case 12, an overdose of Tuinal has been taken as, in normal therapy, levels would not be expected to exceed 0.5 mg%. This would result in C.N.S. and respiratory depression. Finally, in case 18 the salicylate level is much higher than a therapeutic level.

The finding of multiple overdosage in 10 of the 18 patients (55%) is not unexpected. Widdop (1974) found more than one drug in over 60% of urine samples from overdose patients analysed by the Guy's Hospital Poisons Unit in 1972, and Ghodse (1977) reported that 45% of 949 cases of self-poisoning had taken more than one drug. In view of

these findings, reports on large series of patients with acute paracetamol overdosage which do not mention coincident ingestion of alcohol and other drugs, such as the 280 cases seen by the Newcastle Unit, must be viewed with some scepticism.

CAUSES OF DEATH

The clinical progress of a typical fatal case of paracetamol overdosage is that following ingestion there is a latent period of 12-24 hrs during which there may be little, if any, constitutional upset, thereafter the patient develops progressive jaundice and dies some days later in hepatic failure. There are two main points of distinction between the present cases and the typical case. Firstly, the inclusion of early deaths encountered in Forensic practice, and secondly the absence of the classical clinico-pathological syndrome of hepatic failure in many of the post-24 hr deaths (Table 10J)

With regard to the early deaths, 7 occurred before (or at) 24 hrs after ingestion. Four cases were found dead at home and a fifth was discovered moribund in some public toilets and died minutes after reaching hospital. In case 7 (24 hrs) the liver showed early necrotic changes involving 33% of the hepatic parenchyma, but this relatively low figure and the short time between ingestion and death argue against hepatic necrosis as the cause of death. No other explanation was discovered. The cases dying after 24 hrs all showed severe hepatic necrosis but in many the immediate cause of death was apparently a non-hepatic complication of the overdosage. Only four cases had the usually accepted clinical and pathological features of hepatic failure, namely jaundice, coma, and a bleeding tendency. This is not to say that the hepatic necrosis did not make a major contribution to death

TABLE 10J

Cause of death

Case	Time ingestion/death	
1	<12 hrs	Inhalation of vomit
2*	<12 hrs	Inhalation of vomit
3	12 hrs	Not known
4*	13 hrs	Cardiac arrest
5	16 hrs	Bronchopneumonia
6	<24 hrs	Not known
7*	24 hrs	Not known
8	28 hrs	Inhalation of vomit
9*	35 hrs	Cardiac arrest
10*	42 hrs	Cardiac arrest
11*	48 hrs	Bronchopneumonia
12	60 hrs	Cardiac arrest Bronchopneumonia Renal failure
13	70 hrs	Bronchopneumonia Cerebral oedema
14*	72 hrs	Hepatic failure
15*	75 hrs	Cerebral oedema Hepatic failure
16	84 hrs	Cerebral ischaemia Cardiac arrests Bronchopneumonia Renal failure
17	96 hrs	Cerebral oedema Renal failure Hepatic failure
18	5 days	Hepatic failure Renal failure

* Paracetamol alone

and it is likely that most of the patients would ultimately have died a typical "liver-death" if the fatal complications had not intervened. It is possible, however, that the cases with the lowest levels of necrosis (e.g. 9 and 13) might have been saved if the complications had been successfully managed.

Goulding et al., (1976) investigated 6 fatal cases in whom hepatic necrosis was not reported to be the cause of death and found that all had taken other drugs in addition to paracetamol. Whilst multiple overdose has probably contributed to the deaths of 8 of the 14 patients who did not appear clinically to be in liver failure. This still leaves 6 patients who took paracetamol alone, 2 of whom died before hepatic necrosis could have developed.

Sudden death after paracetamol overdosage is a controversial topic. Its occurrence in two patients who on the basis of a full toxicological analysis were shown to have taken paracetamol alone, and two others who had taken in addition only therapeutic doses of librium and oxazepam, has not been reported by previous authors. In experimental work, however, early death of animals without significant hepatic injury is encountered from time to time. Indeed in the toxicity study of Boyd and Bereczky only 10% of the rats dying after a massive dose of paracetamol showed hepatic necrosis, and 4 out of 6 dogs given 750 mg/kg of paracetamol died before the development of extensive hepatic damage, 2 within 24 hrs and 2 more by 48 hrs

(Maxwell et al., 1975).

Looking at the 8 paracetamol-alone cases, in two the immediate cause of death was inhalation of vomit and bronchopneumonia which can complicate any drug overdose. Three cases died as a result of cardiac arrest which in one instance (9) may have been a consequence of hyperkalaemia due to renal impairment. Renal tubular abnormalities were present in 3 other cases and may also have contributed to their deaths. Two patients died in typical hepatic failure and in one of these the immediate cause of death appeared to be cerebral oedema. In the remaining case (7) the cause of death was not ascertainable.

The causes of death in this series have important implications as regards management since some of the complications are avoidable and their prevention or successful treatment may be life-saving. That they were not so treated is to a large extent an indictment of poisons management in this region. In the absence of a specialised unit with the appropriate expertise, patients are at the mercy of a completely random admission system. The 13 patients who received hospital treatment did so in 8 different hospitals scattered around the region, and in some the basic tenets of overdose management were neglected.

OVERDOSAGE WITH COMPOUND TABLETS

A wide variety of compound analgesic tablets containing paracetamol is available. The total consumption of such preparations can only be roughly estimated but more accurate figures can be given for compound tablets obtained on prescription. The following figures are millions of tablets (equivalent to 500 mg paracetamol) prescribed by general practitioners in 1974 (from Spooner and Harvey, 1976):

Paracetamol alone	293
Paracetamol + codeine	150
Paracetamol + dextropropoxyphene	272
Paracetamol + dihydrocodeine	89
Other combinations	118
	922

Since the combination with dextropropoxyphene (Distalgesic) contains only 325 mg of paracetamol, the actual number of tablets of this combination prescribed is about 400 million tablets and is thus the most widely prescribed single preparation containing paracetamol.

I have traced 16 fatal overdosage cases occuring between 1972 and early 1976 in which compound preparations were incriminated. These are listed in Table 10K.

In all but two cases, those taking Distalgesic died before, or very soon after, reaching hospital. The two longer survivors died from cerebral anoxic injury following a cardiac arrest or inhalation of vomit which occurred shortly after taking the overdose.

Dextropropoxyphene is both structurally and pharmaco-

TABLE 10K

Overdosage with compound tablets

	Age/ Sex	Overdose	Time Ingestion to Death	Cause of Death (where known)
1.	29F	Distalgesic Salicylate	30 mins	-
2.	69F	Distalgesic Nortriptyline	3 hrs	-
3.	20M	Distalgesic	7 hrs	Inhalation of vomit
4.	60F	Distalgesic Alcohol	8 hrs	-
5.	22M	Distalgesic Alcohol	<12 hrs	-
6.	62F	Distalgesic	<12 hrs	-
7.	30M	Distalgesic	<12 hrs	-
8.	83F	Distalgesic	<12 hrs	-
9.	78F	Panadeine Co.	22 hrs	-
10.	51F	Distalgesic	<24 hrs	-
11.	47M	Distalgesic	<24 hrs	-
12.	54M	Distalgesic	?	-
13.	56M	Benorylate	3 days	Pontine haemorrhage (liver necrosis)
14.	35M	Panadeine Co.	3 days	Cardiac arrest Hepatic failure
15.	23M	Distalgesic	9 days	Cerebral infarction following cardiac arrest
16.	24M	Distalgesic	24 days	Cerebral infarction Bronchopneumonia following inhalation of vomit

TABLE 10L: Overdosage with compound tablets - blood levels

Case	Time ingestion to death	Time to analysis	Blood Paracetamol mg%	Other drug(s)
1	30 mins	PM	56	Dextropropoxyphene 0.18 mg% Salicylic Acid
2	3 hrs	PM	69	Dextropropoxyphene Nortriptyline
3	7 hrs	PM	18	Dextropropoxyphene (1.1 mg%)
4	8 hrs	PM	21	Dextropropoxyphene 48 mg% Alcohol
5	<12 hrs	PM	11	Dextropropoxyphene 228 mg% Alcohol Sparine, Dalmane, Lentizol
6	<12 hrs	PM	34	Dextropropoxyphene
7	<12 hrs	PM	17.1	Dextropropoxyphene
8	<12 hrs	PM	11	Dextropropoxyphene 69 mg% Alcohol
9	22 hrs	PM	88	Codeine
10	<24 hrs	PM	27	Dextropropoxyphene
11	<24 hrs	PM	22	Dextropropoxyphene

Table 10L - cont.

Case	Time ingestion to death	Time to analysis	Blood Paracetamol mg%	Other drug(s)
12	?	PM	42.4	Dextropropoxyphene 95mg% Alcohol
13	3 days	PM	0.85	54 mg% Salicyclic Acid (24 hrs)
14	3 days	32 hrs	10	Codeine, Phenelzine
15	9 days	18 hrs	2.4	Dextropropoxyphene
16	24 days	10 hrs	3.9	Dextropropoxyphene

logically related to methadone and the effects of overdosage are similar to those of morphine. Clinical features include nausea and vomiting, constricted pupils, loss of consciousness, convulsions, respiratory depression and cardio-vascular collapse. In most of the present series, encountered in Forensic practice, there were no morbid anatomical findings to explain death but cardio-respiratory arrest is the most likely cause. In these cases the rapidity of death and the known acute toxicity of dextropropoxyphene indicate that this drug and not paracetamol is responsible. Although it is a theoretical possibility that a patient who receives successful treatment for the acute respiratory-depressant effects of dextropropoxyphene, may later succumb to hepatic necrosis induced by paracetamol, I know of no such sequence of events. In the two long-survivors in this series, one dying at 9 days showed no evidence of previous liver damage, but the other (24 days) showed minor irregularity of the reticulin pattern around central veins, prominent Kupffer cells containing ceroid pigment, and increased numbers of regenerative hepatocytes, indicative of earlier liver necrosis. Liver function tests confirmed a moderate degree of hepatic necrosis with peak values (at 3 days) of SGOT = 400 i.u./l, SGPT = 74 i.u./l, Bilirubin = 16 μmol/l and Alkaline phosphatase = 9 K.A. units.

Despite the fact that in this region the Coroner's records contained almost as many fatalities from Distalgesic as were attributable to paracetamol, dextropropoxyphene

poisoning has received relatively little attention. Indeed the first report of a series of cases in the United Kingdom did not appear until April 1977, when Carson and Carson described 30 fatal cases occurring in Northern Ireland. Although a few reports of successful treatment with nalorphine or noloxone have appeared (Hunt, 1973; Tarala and Forrest, 1973; Lovejoy et al.; 1974) most deaths occur before the patient reaches hospital or is seen by a doctor.

In 1974, in England and Wales, there were 39 deaths attributed to dextropropoxyphene, alone or in combination with other drugs, but the series from N. Ireland and the present study would suggest that this is a serious understatement and that the number is increasing rapidly. Carson and Carson estimate that 1,000 people may have died from dextropropoxyphene poisoning over the past 3 years in the U.K.

There is a general lack of awareness amongst physicians of the dangers of this drug and there are strong arguments for stricter controls on its availability. A recent leader in the Lancet (1977) drew attention to the problem, but in covering the treatment of dextropropoxyphene poisoning it perhaps did not lay enough stress on the potential rapidity of death and ways of preventing overdosage by education and controls.

Three of the 16 cases, took compounds other than Distalgesic. Two took Panadeine Co., (paracetamoland codeine phosphate) and one died at 22 hrs possibly due to respiratory

depression, but the other developed classical hepatic failure. The third patient took an overdose of Benorylate, a paracetamol and salicylate mixture, and died from pontine haemorrhage resulting from a severe coagulation disorder. At P.M. he had evidence of widespread hepatic necrosis.

CHAPTER XI

The treatment of paracetamol overdosage

The most logical approach to the problem of paracetamol overdosage would be to incorporate a non-toxic antidote into the tablets and prevent any possibility of hepatotoxicity. With this in mind, we have suggested incorporation of α-tocopherol, and McLean (1974) has proposed L-methionine or L-cystine. It has to be admitted, however, that the effects of simultaneous oral administration of these antidotes on the absorption of paracetamol in man and, more importantly, on its analgesic properties have not been studied, nor has patient acceptability of a combined preparation been tested. What is more pertinent is that production and marketing of a drug with a built-in antidote is probably not considered to be desirable from a stri ctly commercial viewpoint. In the light of this, paracetamol overdosage is likely to be

an increasing clinical problem demanding prompt and effective treatment.

Early attempts at treatment were empirical and based on experience with other drugs or other forms of liver injury. The sequence of events, ingestion, absorption, circulation, metabolism, and excretion is common to most drugs and provided the basis for the early therapautic measures, but more rational approaches to treatment had to await the elucidation of the cellular mechanisms of paracetamol hepatotoxicity.

Ingestion

Little can be done to limit the ingestion of a drug that is freely available to the public. Child-resistant packaging may prevent accidental child poisoning (Silbert, Craft and Jackson, 1977) but this is a very small part of the paracetamol problem. The adoption of packages containing only small numbers of tablets (strip or "blister" packs) might make a minor contribution, but the inclusion of more specific warnings on labels could create difficulties for the normal user, with no influence on the person intent on self-poisoning (Poore, 1976).

Absorption

As with any drug overdosage, efficient gastric lavage through a wide-bore tube is mandatory in the initial management of paracetamol overdosage. Before any specific treatment became available, the King's Group showed that those patients who have a stomach wash-out within 6 hrs of

overdosage have a very significantly lower mortality than patients who have not been lavaged by this time (reported by Gazzard, 1976).

Various means of delaying or preventing absorption have been tried. Administration of activated charcoal has long been advocated for the binding of toxic substances in the stomach and intestine. Although the absorption of small oral doses has been markedly reduced by the simultaneous oral administration of charcoal (Levy and Gwilt, 1972) and cholestyramine, absorption was only slightly reduced when these adsorbents were given 60 minutes after paracetamol (Dordoni, Willson, Thompson, and Williams, 1973). The administration of such agents therefore appears to be of little value in the treatment of overdosage. Other means of slowing absorption have included the use of antispasmodic drugs such as propantheline which slows gastric emptying and so lessens absorption (Nimmo et al., 1973).

Variations in gastric emptying time and intestinal absorption do not explain the marked individual variation in susceptibility to paracetamol overdosage. We have shown in the rat that considerable variation in hepatic damage occurs even when paracetamol is administered by portal venous infusion (Kelleher et al., 1977). It therefore seems unlikely that attempts to slow the rate of absorption, even if successful, will prevent the development of hepatic necrosis in susceptible individuals.

Circulation

Attempts at lowering circulating levels of paracetamol have included haemodialysis and haemoperfusion. Treatment by haemodialysis has been advocated by Farid, Glynn and Kerr (1972) but although plasma paracetamol half-life was shortened in the 7 cases dialysed, this had little effect in the three patients who, judging by their plasma paracetamol concentrations, were at risk of hepatotoxicity. As far as haemoperfusion is concerned, initial experiments in the pig using polyhydroxyethylmethacrylate (polyhema) -coated charcoal, were encouraging (Willson et al., 1973) but a subsequent study in man was inconclusive (Gazzard et al., 1974 a). In this study eight patients were treated as part of a prospective controlled trial. The plasma clearance of paracetamol by the charcoal column was variable and low (4 - 119 ml/minute) and the cumulative amounts removed were also small (0.2 - 5.2 g, mean 1.4 g). The practical value of haemoperfusion and dialysis seems very limited. Paracetamol is rapidly absorbed and has a high hepatic clearance (Auty and Branch, 1973) so that in the great majority of patients a potentially necrogenic dose will have been taken up by the liver before these attempts at removal could be instituted.

Another possible therapeutic approach is that modification of blood flow through the liver might alter the uptake of paracetamol and slow the production of a toxic metabolite. Auty and Branch (1973) attributed the beneficial effects of

β-blockers on experimental paracetamol hepatotoxicity to a decrease in hepatic blood flow. They suggested that liver blood-flow is a rate-limiting factor in the production of the toxic metabolite and that slowing of production allows glutathione synthesis to keep pace with the toxic metabolism and permit complete conjugation. Rosner, Romero-Ferret, and Mottot (1973) studied the effects of treatment with the β-blockers propanolol, alprenolol, practolol and prindolol, on mice given large doses of paracetamol. Whilst the first 3 drugs significantly lowered mortality, no protection was afforded by prindolol, thus suggesting that the protective mechanism might not be β-blockade per se. These experiments did not lead to any practical advances in treatment.

Excretion

Just as methods aimed at accelerating the clearance of paracetamol from the circulation failed in clinical practice, so efforts to enhance urinary excretion of the drug have not proved beneficial. The renal clearance of unchanged, but not conjugated paracetamol is related to urine flow, and forced diuresis has been tried to hasten excretion (Rose, 1969). This measure, however, has not brought about any substantial increase in paracetamol clearance and is of little clinical value (Prescott and Wright, 1973), indeed it can be hazardous in precipitating acute pulmonary oedema (Wright, 1974).

Metabolism

The work of Mitchell and his colleagues on paracetamol

metabolism paved the way for a rational approach to the treatment of overdosage. Having shown that detoxification was brought about by glutathione conjugation with formation of mercapturic acid, they went on to demonstrate that pre-treatment with the glutathione precursor cystine, and other nucleophilic sulphydryl compounds such as cysteamine, could prevent hepatic necrosis. Furthermore, they demonstrated that intra-venous cysteamine protected against necrosis when given after paracetamol overdosage provided it was administered before maximal glutathione depletion and covalent binding had occurred, (Mitchell et al; 1973b, 1973c, 1974).

Some clinical experience on the intravenous use of cysteamine in man had been gained from studies aimed at protecting against irradiation injury (Bacq, 1965) and it was this compound which was chosen as the first rational attempt to prevent the development of hepatic necrosis in human overdose cases, (Prescott et al., 1974).

In their first trial, Prescott and his colleagues administered cysteamine on 10 occasions (1 patient received it for 4 episodes of overdosage) where the 4 hr paracetamol level ranged from 218-542 µg/ml. Previous experience indicated that at these paracetamol levels hepatic necrosis would have been inevitable. For example, 11 patients with 4 hr levels over 300 µg/ml all developed liver damage with a mean maximum SGOT of 5180 i.u./l, and 2 died in hepatic failure. When the 4 hr level was in the range 250-300 µg/ml, 7 of 10 patients developed necrosis with a mean maximum SGOT

of 2380 i.u./l but there were no deaths. Cysteamine treatment resulted in a dramatic improvement. In 6 of the 10 episodes there was no clinical or biochemical evidence of hepatic necrosis and on the remaining 4 occasions there was only mild transient liver damage (maximum GOT levels were 160, 180, 149 and 85 i.u./l). Subsequent experience with cysteamine has emphasised its efficacy (Prescott, Park and Proudfoot, 1976) but there remains a critical time after which treatment will be ineffective and these authors have stressed that cysteamine must be administered within 10 hours of paracetamol ingestion.

Although the rationale underlying the use of cysteamine was to increase the supply of sulphydryl groups for conjugation and neutralisation of the toxic metabolite, Prescott et al., were aware that cysteamine can inhibit steroid β-hydroxylation (Fleming, Gierhass and Seydewite, 1973) and suggested that it may act by inhibiting the formation of the toxic metabolite. This alternative action is supported by the findings of Harvey and Goulding (1974) who showed that in perfused liver preparations the rates of disappearance of phenazone and acetanilide, two compounds extensively metabolised by microsomal oxidation, were drastically reduced by the addition of cysteamine. They had previously demonstrated in a study of paracetamol metabolites that cysteamine resulted in a 33% reduction in thio-ether-conjugated excretion (that is, conjugation between the

toxic metabolite and glutathione or cysteamine) and a 70% increase in glucuronide excretion. The authors concluded that the protective action of cysteamine results primarily from its ability to block formation of the toxic metabolite, rather than inactivating the metabolite by conjugation to thio-ether compounds.

Following the report of Prescott's early experience with cysteamine, other compounds were suggested as possible antidotes on the basis of animal experiments. McLean (1974) administered methionine simultaneously with paracetamol to phenobarbitone-pretreated rats and greatly reduced the expected mortality, and we demonstrated the protective effect of α-tocopherol (Kelleher et al., 1974). Strubelt, Siegers, and Schült (1974) investigated 9 thio-compounds with a view to identifiying the most effective antidote. They found that cysteamine, cysteine, and dithiocarb given 1 hr after adminstration of paracetamol to mice reduced the death rate from 67% in controls to 10, 15, and 10% respectively. Some reduction was found with glutathione and thiazolidone carboxylic acid, but penicillamine, thioctic acid, silymarin and dimercaprol were ineffective.

In spite of Prescott's convincing results with cysteamine, there appeared to be resistance to its use in some centres. Treatment with this drug does have its problems. It was difficult to prepare in a stable, sterile solution for injection and it had very unpleasant side-effects, mainly nausea, vomiting, and depressant effects on the

central nervous system. Presumably with some of these reservations in mind, Douglas, Hamlyn, and James (1976) reported on a "controlled-trial" of cysteamine in acute paracetamol poisoning, in which they claimed that "there was no advantage of cysteamine in preventing biochemical abnormalities of liver function except for aspartate aminotransferase and serum ferritin levels which were significantly less after cysteamine therapy". In fact, only 6 of their patients were treated within 10 hrs and if these patients were compared with the 7 early cases not treated with cysteamine, then on biochemical and histological grounds significant differences were obtained and almost complete protection against hepatotoxicity seen in the cysteamine group. In pooling the 6 "early" and 12 "late" patients for most of their comparisons, the Newcastle workers were obscuring the value of cysteamine in that the "late" group were admitted and treated far too late for cysteamine to be effective. Prescott, Park and Proudfoot argued against the Newcastle workers' interpretation and expressed concern at the ethics of controlled trials of cysteamine in this situation when there was the likelihood of fatalities if serious paracetamol overdosage was treated by other methods. The King's group reported their experience with cysteamine shortly after publication of Prescott"s letter emphasising the drugs usefulness, and their results were very similar if allowance was made for the lower dose

of cysteamine employed (Hughes, Trewby, and Williams, 1976). In the King's series of 13 patients treated within 10 hr of ingestion none developed hepatic encephalopathy or renal damage, no patient died, and subsequent liver histology did not show evidence of severe damage, despite high plasma paracetamol levels (mean level at 4 hr = 348 µg/ml. They concluded, with apparent reticence, that "taking all available evidence into account we would suggest that cysteamine may be effective in protecting against paracetamol-induced hepatic necrosis, although without a large scale controlled trial one cannot be certain. Because of this, and in view of the known toxic effects of cysteamine, we feel it should only be used in patients with high plasma-paracetamol concentration who are at risk from hepatic damage, and certainly not more than 12 hr after the overdose".

This line of management posed certain problems. Which patients are at risk? Do patients with lower paracetamol levels receive no specific treatment? If speed is of the essence, can accurate paracetamol levels be obtained in the emergency situation? It was with these and other questions in mind that a symposium on paracetamol overdosage and its management was held at the Royal College of Physicians, London in February 1976, and it provided sufficient answers for guidelines on management to be issued, (Journal of International Medical Research, supplement 4, 1976) as follows;-

The assessment of patients at risk is facilitated by

plotting the plasma paracetamol level on a graph showing blood paracetamol levels on a log scale against time. Reference is made to a line joining the point at 200 µg/ml at 4 hrs with the point at 70 µg/ml at 10 hours. If the initial paracetamol level is above the line there is a possibility of hepatic damage occurring, with an increasing probability of damage the further above the line the level lies. Such patients should be given cysteamine provided the administration can commence within 10 hrs of ingestion of the overdose. If the cysteamine is not available oral methionine should be given. In addition to cysteamine or methionine, appropriate supportive therapy should include fluid replacement with 5% dextrose, vitamin K for a prolonged prothrombin time and maintainence of electrolytes.

If the paracetamol blood-level falls just below the line significant hepatic damage is unlikely. However, the blood-level estimation should be repeated after an interval of at least 2 hours (if within 10 hrs of the overdose) and liver function tests performed at least 6 hrs after the alleged overdose. The prothrombin time is the most useful test. If the repeat paracetamol-level is above the line then cysteamine treatment should be instituted.

If the initial level falls well below the line any paracetamol taken is very unlikely to have a significant toxic effect.

If more than 10 hrs have elapsed since ingestion of the alleged overdose, liver function tests should be

performed and management of a supportive nature instituted, based on the extent of any hepatic damage.

Rapid determination of the admission plasma paracetamol level has been made possible by the development of a simple estimation kit which enables a result to be obtained in less than 5 minutes (Kendal, Lloyd-Jones, and Smith, 1976). The assay is based on the production of a nitro-derivative of paracetamol, 2-nitroso-4-acetamido phenol, which in alkaline solution gives a characteristic yellow colour. The intensity of the yellow colour which develops within one minute is assessed using a calibrated colour transparency. The paracetamol concentration can be estimated within 50 µg/ml and an operator can readily distinguish between 50, 100, 200, and 300 µg/ml levels. The comparability of levels obtained using the kit and orthodox laboratory methods was examined by Widdop (1976) who showed that the kit gave sufficiently accurate results to base management on.

Although the guidelines for management had been drawn up by representatives of the major centres in Britain dealing with paracetamol overdosage, there remained a question-mark hanging over the use of cysteamine in view of its toxicity and the lack of a widely available stable solution. Trials and animal experiments were carried out on likely alternatives. Prescott et al., (1976) compared cysteamine with methionine and penicillamine, and confirmed the protection afforded by cysteamine given within 10 hrs but found it ineffective after 12 hrs or more. Methionine appeared to

be effective in most patients but 3 patients treated within 10 hrs suffered severe liver damage including 1 apparently not at great risk with a 4-hr plasma paracetamol concentration of only 252 µg/ml. Methionine was less toxic than cysteamine but its failure to protect these 3 patients cast doubt on its further use. Penicillamine was abandoned after treatment of only 5 patients. One patient sustained severe liver damage and acute renal tubular necrosis, and another with a relatively low 4 hr paracetamol concentration and insignificant liver danage developed renal damage.

Crome et al., (1976) reported their experience with early methione treatment in severe over-dose cases. Thirty patients received oral methionine within 10 hrs and of these 21 exhibited no signs of liver damage as judged by serial aspartate aminotransferase estimations; 6 patients suffered minor damage (AST 35-250 i.u./l) and 3 developed more severe liver damage (>1000 i.u./l). The authors concluded that "oral methionine may be effective in reducing the frequency and severity of paracetamol-induced liver damage". They found it well tolerated when given by mouth and that it could be given to unconscious patients.

Despite its therapeutic failings, the use of methionine, as opposed to cysteamine was further championed by McLean (1976). In a letter to the Lancet he suggested that "methionine is probably the treatment with the best cost/risk/benefit profile of the treatments so far devised, for any hospital other than a poisons centre with staff available

to measure paracetamol levels, and sterilise and watch cysteamine infusions . . . . . methionine given at 8 hours will certainly be better than waiting to give cysteamine at 11 hours, after the blood levels have been measured". McLean's reasoning is difficult to understand. Rapid paracetamol measurements are within the capabilities of even the smallest hospitals using the estimation kit, and should be considered mandatory in assessing the need for, and monitoring the efficacy of, specific treatment regimes. Furthermore, cysteamine can be prepared as a stable injection (Brouwers and Vermeij, 1976; Prior, 1977) so that the requirement for freshly made-up solutions injected through Millipore filters is removed.

The search for protective sulphydryl compounds continued and Hughes et al., (1977) reported their experience with dimercaprol in a controlled trial in which it was compaired with cysteamine. Dimercaprol had long been employed as a chelating agent in the treatment of heavy-metal poisoning and is given by deep (and painful) intramuscular injection. It was one of the many sulphydryl compounds tested in experimental paracetamol overdosage by Strubelt, Siegers, and Schütt but was found to be ineffective. Hughes et al., found that peak abnormalities in serum bilirubin concentrations and prothrombin times were significantly greater in patients treated with dimercaprol than in patients given cysteamine, and the severity of hepatic necrosis found on liver biopsy was

also greater in the dimercaprol group. Their results showed that although dimercaprol offered some protection against liver necrosis, greater protection was afforded by cysteamine, but they again commented on the high incidence of unpleasant side effects and inconvenience of cysteamine.

The most promising of the recently explored alternatives to cysteamine is N-acetylcysteine. This drug had been readily available as a sterile 20% aqueous solution for about 15 years and in 1974 Prescott and Matthew suggested its use as an alternative to cysteamine. Piperno and Berssenbruegge (1976) compared the effect on paracetamol overdose in mice of subsequent administration of N-acetylcysteine and cysteamine. Not only did they demonstrate a significant protective effect when N-acetylcysteine was given up to 4½ hrs after paracetamol, but found a much greater survival than in the cysteamine treated group. A single case, a 32 yr old man, treated with N-acetylcysteine 15 hrs after paracetamol overdose was reported in January 1977 by Lyons, Studdiford and Sommaripa. Although hepatic necrosis was not prevented, the overdose may well have had a fatal outcome, without N-acetylcystine treatment.

Prescott et al., (1977) treated 15 patients who were at risk of hepatic necrosis with N-acetylcysteine. Twelve patients were treated within 10 hrs of ingestion and liver function tests remained normal, or were only slightly disturbed, in all except one. This patient had taken other

unknown drugs and the prothrombin time was already prolonged on admission. Three patients were treated after 10 hrs and all sustained severe liver damage but without hepatic encephalopathy. The authors found that N-acetylcysteine was very well tolerated. About half of their patients had nausea and vomiting, mostly during the first hour or so of treatment, but these symptoms had usually been present before treatment. There was a minor transient rise in blood pressure in three patients, but no arrhythmias were observed during treatment. The drug had no obvious effects on the central nervous system.

The results obtained by Prescott and his colleagues are very encouraging. The fact that N-acetylcysteine is available as a sterile pharmaceutical preparation together with its ease of use and lack of side effects, suggest that this compound may become the treatment of choice for paracetamol poisoning.

POSTSCRIPT

The work recorded in this thesis covers a nine year period of research. I became interested in the effects of paracetamol overdosage in 1969 at a time when the problem was just coming into clinical prominence. During the past nine years a great deal of research in many centres has been undertaken into this subject, stimulated on the one hand by the magnitude and gravity of the clinical problem, and on the other by the information such research might yield on the general mechanisms of toxicity of wider interest to biochemists, pharmacologists, cell biologists, and others. It is also pertinent that this is in many ways an iatrogenic problem, an adverse effect to a drug which although taken in excessive amounts, is widely available and whose dangers were not known to those taking the overdose. Furthermore it is an adverse effect for which, initially, the medical profession could offer no satisfactory treatment. The sense of inadequacy and frustration provoked by this situation has been a major stimulus to research.

It has been particularly stimulating and satisfying to be able to look back over what is a relatively short time in the context of a major clinical problem, and to appreciate the progress that has been achieved. From the first well-intentioned but misguided attempts at treatment, we have advanced through a rational understanding of the toxic metabolism of paracetamol, to a point where effective antidotes can be given and guidelines for proper management

drawn up. Nevertheless important questions remain unanswered Among these are the nature of the toxic metabolite, the precise mode of action of the recognised antidotes, the feasibility of producing a non-hepatotoxic analogue, and many others.

Recently the possibility of long-term hepatotoxicity has been raised by a report of chronic hepatic inflammation and fibrosis allegedly due to low doses of paracetamol (Bonkowsky, Mudge, and McMurty, 1978) and this potential hazard demands careful monitoring.

A more disturbing aspect to emerge from my review of all aspects of paracetamol poisoning has been the large and rapidly increasing number of deaths attributable to the combined preparation with dextropropoxyphene. Overdosage with dextroprophene frequently leads to sudden death before medical attention can be summoned. These deaths have received precious little attention in the medical literature and despite a few warnings (see Breckenridge, 1978) the preparation (Distalgesic) continues to be the most widely prescribed analgesic in Britain. The frequency of fatal self-poisoning, the fact that dependency may develop, and its dubious superiority over other, much safer, mild analgesics, are facts which require a great deal more publicity, leading, one hopes, to the withdrawal of dextropropoxyphene from analgesic preparations.

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EXPERIMENTAL PARACETAMOL-INDUCED HEPATIC NECROSIS: A HISTOPATHOLOGICAL STUDY

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PLATES CXXV AND CXXVI

HEPATIC necrosis resulting from massive overdosage with paracetamol (N-acetyl-*p*-aminophenol, acetaminophen, APAP) was first reported in rats by Boyd and Bereczky in 1966. A report of this complication in man soon followed (Davidson and Eastham, 1966) and a total of 12 such cases have now been reported (Thomson and Prescott, 1966; Maclean *et al.*, 1968; Pimstone and Uys, 1968; Rose, 1969; Toghill *et al.*, 1969). Whilst Boyd and Bereczky were mainly concerned with acute oral toxicity, they summarised the histopathological findings in a large number of tissues and gave a brief description of the histological changes in the liver. There has not been a detailed histopathological study of paracetamol-induced liver necrosis, nor has the natural course of the lesion been adequately described. The main objective of the present study was to provide such information by examining the changes in the livers of rats killed at various intervals after administration of a single large dose of paracetamol.

MATERIALS AND METHODS

The experiments were performed in two stages. In the first, two dosages of paracetamol were used, the higher being close to the expected LD50 of the drug. Rats surviving 5 days were killed to determine whether or not all the treated animals showed hepatic necrosis. In the second stage a further group were given the higher dosage of paracetamol and examined after longer intervals.

Experiment 1. Twenty male albino Wistar rats weighing 250–400 g were divided into two equal groups; group 1 received paracetamol in a dosage of 2·5 g per kg and group 2 a dosage of 3·5 g per kg. Paracetamol (B.P.C.) was given by gavage, in a suspension of 200 mg per ml stabilised with 0·2 per cent. tragacanth. The rats were fed on a standard Spillers Autoclaved Diet for 2 wk before the experiment. Food was withdrawn 16 hr before paracetamol administration, but water was not restricted. Rats dying within 4 days of drug administration were examined as soon after death as possible. The surviving rats were killed on the 5th day by cervical dislocation.

Experiment 2. Paracetamol was administered to a further eight male albino Wistar rats under the same conditions as before. It was given as a 300 mg per ml suspension (with 0·2 per cent. tragacanth) by gavage in a dosage of 3·5 g per kg. Two rats died within 48 hr. Of the survivors, two were killed after 7 days, a further two after 14 days, one after 21 days and the last rat on the 28th day.

At necropsy the livers were excised and three representative " blocks " removed and fixed in formol-saline. Frozen sections were prepared from one block and stained for fat

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by the sudan IV method. The other two blocks were post-fixed in corrosive-formol solution for 24 hr and then processed and cut, and stained with haematoxylin and eosin (HE), and by silver impregnation for reticulin. Where indicated, further sections were stained by Best's carmine for glycogen.

RESULTS

Animals dying within 24 hr of paracetamol administration

Three rats given paracetamol at the dose-rate of 2·5 g per kg and two given 3·5 g per kg died during this period. There was evidence of hepatic damage in three animals only.

The earliest detectable change is chromatolysis, that is loss of the basophilic granules (granules of Berg), normally present in the cytoplasm of hepatocytes. These granules are composed of numerous cisternae of rough-surfaced endoplasmic reticulum covered by ribosomes. Chromatolysis is seen initially in the centrilobular cells, but later involves midzonal cells.

In parallel with this loss of basophilia, the cells also show evidence of aqueous swelling. The cytoplasm contains numerous small vacuoles which at low power impart a " ground-glass " appearance to the cells. Many centrilobular cells contain larger vacuoles, just visible at low power. The special stains indicate that these vacuoles do not contain fat or glycogen and that the appearances are those of hydropic vacuolation (fig. 1).

In addition to chromatolysis and hydropic vacuolation there is mild to moderate congestion. The periportal hepatocytes appear healthy. There is no inflammatory infiltration of portal tracts or Kupffer cell hyperplasia.

Animals dying 24–48 hr after paracetamol administration

Three rats given 2·5 g per kg and two given 3·5 g per kg died during this period.

The livers all show marked congestion, the central veins are dilated and the surrounding sinusoids are disrupted and packed with red blood cells. In addition, frank necrosis of hepatocytes is seen. Necrosis is evidenced by nuclear disintegration and increased eosinophilia of the cytoplasm. The nuclei in these dying cells at first show pyknosis and later karyorrhexis, the cells containing discrete fragments of nuclear material (fig. 2). At this stage the structure of the liver cell plates is largely preserved, but in some areas eosinophilic, anuclear cells have coalesced to form amorphous masses. The extent of necrosis varies from small foci surrounding the central veins to confluent bands involving centrilobular and midzonal cells (figs. 3 and 4). In these severely damaged livers even the periportal cells show marked hydropic vacuolation. Two animals show an extreme degree of necrosis, where only the portal tracts are discernible amidst an amorphous mass of necrotic hepatocytes and red blood cells (fig. 5). The necrotic zones contain variable numbers of neutrophil polymorphs, but there is no inflammatory cell reaction at the margins. The endothelial and Kupffer cells are largely preserved within the

PARACETAMOL-INDUCED HEPATIC NECROSIS

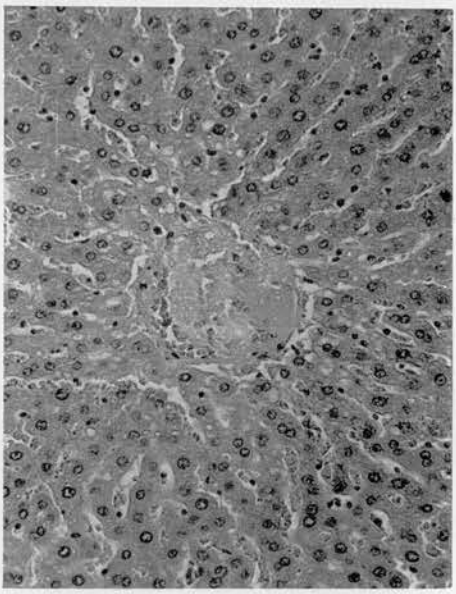

FIG. 1.—Rat liver, less than 24 hr after paracetamol. Early damage indicated by hydropic vacuolation affecting centrilobular hepatocytes. Haematoxylin and eosin (HE). ×200.

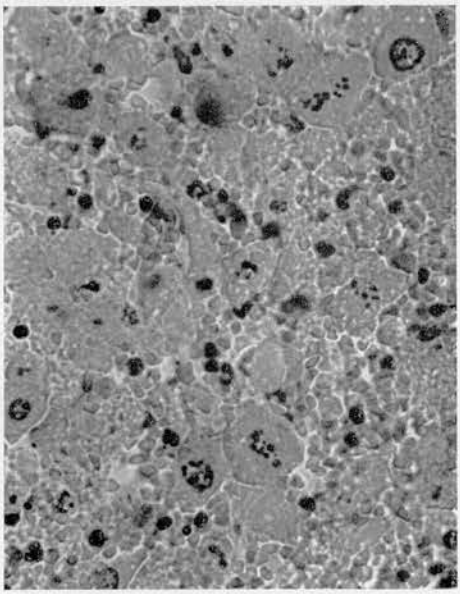

FIG. 2.—24 hr. There is marked congestion and necrosis of hepatocytes. Most cells show karyorrhexis, and contain discrete fragments of nuclear material. HE. ×440.

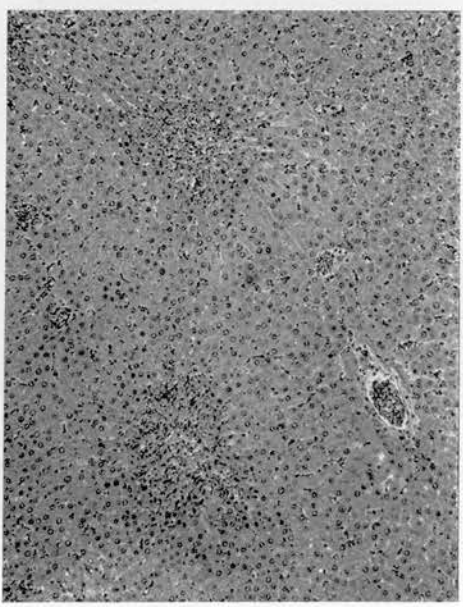

FIG. 3.—24 hr. Mild necrosis confined to centrilobular zones. HE. ×80.

FIG. 4.—36–48 hr. The liver shows considerable necrosis and marked congestion involving centrilobular and midzonal hepatocytes, so that areas of confluence are produced. HE. ×40.

PARACETAMOL-INDUCED HEPATIC NECROSIS

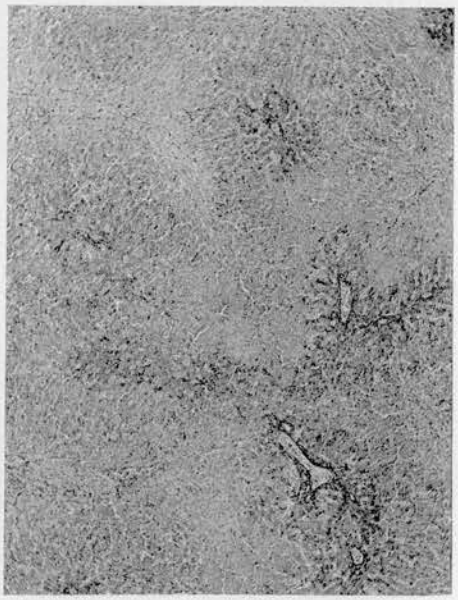

FIG. 5.—36–48 hr. Massive necrosis with only a narrow rim of surviving hepatocytes around portal tracts. Reticulin. ×40.

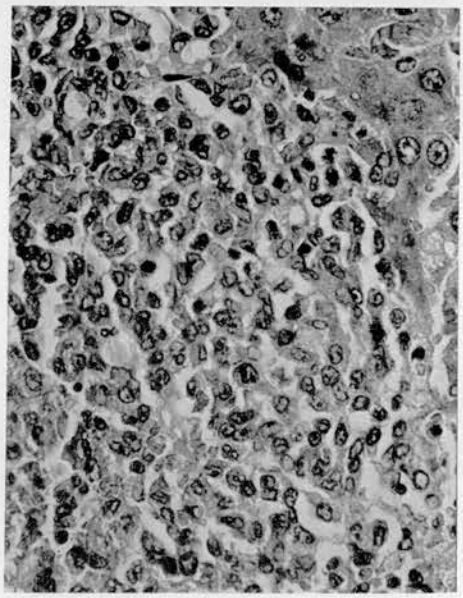

FIG. 6.—5 days. Cellular infiltrate consisting of plump cells with bulky granular cytoplasm and, in many instances, indented "monocytoid" nuclei. HE. ×360.

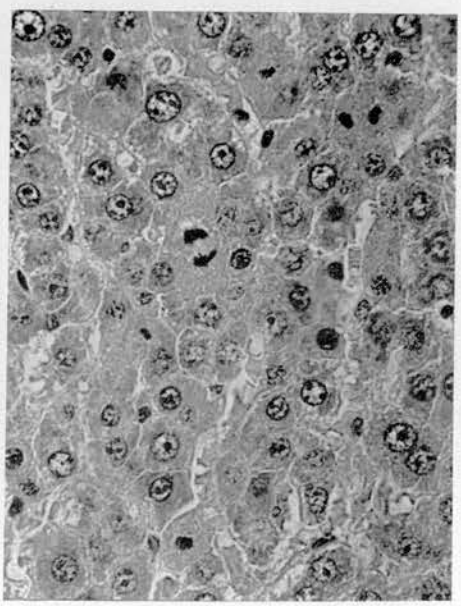

FIG. 7.—5 days. The islands of surviving hepatocytes show marked mitotic activity. HE. ×360.

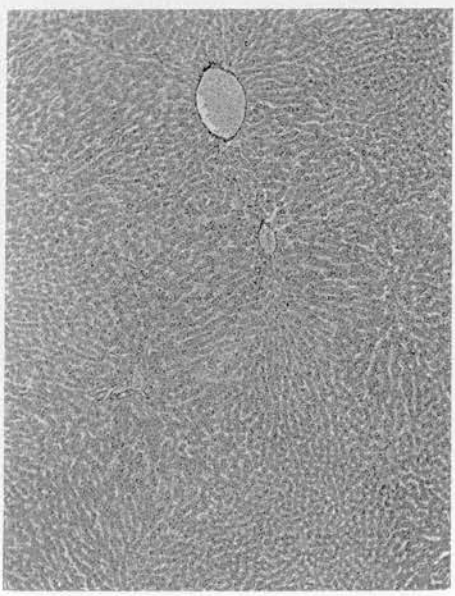

FIG. 8.—14 days. Low-power field showing restoration to normal. Reticulin. ×40.

necrotic zones, and reticulin stains show no collapse of the reticulin framework. Sudan stains reveal numerous small intracytoplasmic droplets of fat in cells surrounding the necrotic zones.

Animals dying or killed 3–5 days after paracetamol administration

This group comprises four rats given 2·5 g per kg and eight given 3·5 g per kg.

All animals show moderate to marked hepatic involvement. The centrilobular zones are now occupied by large numbers of plump cells possessing bulky granular cytoplasm and indented or " monocytoid " nuclei. The appearances are those of macrophage infiltration (fig. 6). Some animals show evidence of continuing necrosis but on a considerably reduced scale. Scanty residual necrotic hepatocytes together with occasional polymorphs are seen within the infiltrated areas. As expected, the degree of macrophage infiltration parallels the previous extent of necrosis and varies from small foci to confluent bands.

A further feature is the presence of regenerative activity. Many cells show increased cytoplasmic basophilia, hyperchromatic and double nuclei, and mitotic figures. The mitotic activity is variable, and in some sections as many as two to three figures are seen in each high-power field (fig. 7). There is no evidence of hydropic vacuolation or fatty change in the surviving hepatocytes. Whilst the HE-stained sections show widespread loss of hepatocytes, the silver stain reveals complete preservation of the reticulin framework.

Animals killed 7 days after paracetamol administration

There is no evidence of continuing necrosis in two rats given 3·5 g per kg. The hepatocytes show increased basophilia and hyperchromatic nuclei, but there are only occasional double nuclei and very scanty mitotic figures. Macrophage infiltration is considerably less evident than at 5 days and most centrilobular veins are surrounded by only a narrow zone of macrophages. The reticulin stains reveal the first evidence of condensation of fine reticulin fibres around a few central veins. The lobular architecture, however, is preserved.

Animals killed 14, 21 and 28 days after paracetamol administration

This group consisted of four rats given 3·5 g paracetamol per kg. Apart from a few widely scattered residual foci of collapsed reticulin, the appearances are normal. There is no evidence of fibrosis, inflammatory cell infiltration, or bile-duct proliferation (fig. 8).

Discussion

Boyd and Bereczky (1966) found centrilobular congestion and " pale staining " in the livers of rats dying up to 24 hr after paracetamol administration; rats dying 1–7 days after administration showed centrilobular and general hepatic necrosis. Animals were studied only up to 7 days, however, and the

further progress of the hepatic lesions in the surviving animals is not described. The effects of prolonged administration of paracetamol at high dosage were studied by Boyd and Hogan (1968). Rats dying after daily administration of doses from 0·5 to 1·1 g per kg up to 100 days showed hepatic congestion, fatty degeneration, and diffuse necrosis, whilst rats given larger daily doses (up to 4 g per kg) showed hepatic necrosis and *cirrhosis*.

Apart from these studies where the hepatic lesions have been described incidentally as one facet of paracetamol toxicity, there has not been a detailed histopathological investigation of this serious complication. Likewise, the long-term effects of a single massive dose that might occur in patients taking an overdose of paracetamol have not been elucidated. Self-poisoning with paracetamol is becoming increasingly common, and it is important to determine whether or not cirrhosis follows the hepatic damage resulting from a single massive dose of the drug.

The natural progress of the hepatic lesions in the rat appears to be centrilobular necrosis, followed by macrophage infiltration and regenerative activity, leading quickly to complete recovery. Although occasional foci of collapse were seen in the reticulin stains at 7 days, animals killed after this time showed complete preservation of the lobular architecture and cessation of regenerative activity. Furthermore, macrophage infiltration had disappeared and there was no evidence of fibrosis. These findings, albeit from a small group of animals, indicate that cirrhosis is unlikely to follow a single large dose of paracetamol in the rat.

Most of the reports of human cases of hepatic necrosis accompanying overdosage contain details of liver histology obtained at necropsy or by percutaneous liver biopsy. Cases in which hepatic necrosis has been diagnosed on the basis of biochemical changes alone have been reported by Thomson and Prescott (1966) and by Maclean *et al.* (1968) (two cases). In general the histological changes seen in these patients parallel those found in the rat. Although follow-up studies on the survivors have not been reported, it is reasonable to expect in cases of paracetamol-induced hepatic necrosis that if the patient can be supported through the acute phase then the ultimate prognosis is excellent.

Summary

A detailed histopathological study of paracetamol-induced hepatic necrosis in the rat is presented. The main events are hydropic vacuolation, centrilobular necrosis, macrophage infiltration and regenerative activity with rapid restoration to normal. There are only a few scattered condensations of the reticulin framework and a small group of animals studied up to 28 days after paracetamol administration showed no evidence of progression to cirrhosis.

The histological changes reported in human cases of paracetamol overdosage are essentially similar to those obtained in the rat.

Our thanks are due to Professor G. L. Montgomery; to Mrs S. Wilson, Miss D. Bell and Mr W. Robb for valuable technical assistance; to Miss Christine Archer for secretarial help; and to Mr J. Paul of the Department of Medical Photography.

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Effects of Mepyramine, Promethazine, and Hydrocortisone on Paracetamol-Induced Hepatic Necrosis in the Rat

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INTRODUCTION

Acute paracetamol [acetaminophen Tylenol] poisoning is now a common clinical problem and is of particular importance because of the risk of severe hepatic necrosis [1, 2]. Maclean et al. [3] recommend the administration of hydrocortisone and mepyramine in the treatment of paracetamol poisoning, and suggest that these drugs restrict the degree of liver necrosis. Although a number of antihistamines, including promethazine [4, 5], have been shown to protect against experimental hepatic necrosis induced by various agents, no controlled study of the influence of such drugs on paracetamol-induced liver necrosis has previously been reported. The effects of mepyramine, promethazine, and hydrocortisone were therefore studied in rats given single hepatotoxic doses of paracetamol.

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MATERIALS AND METHODS

Groups of male albino Wistar rats weighing 200 to 300 gm were maintained on Spiller's Autoclaved Diet. Food was withheld for 16 hr and either paracetamol (2.5 gm/kg as a suspension containing 200 mg/ml with 0.2% tragacanth) or an equivalent volume of suspending agent was given by gavage. At the same time mepyramine, promethazine, hydrocortisone, or 0.9% saline were injected intraperitoneally as detailed in Table 1. It was planned to give injections of these drugs twice daily for four days but with the higher doses of mepyramine and promethazine this was not possible because of the poor clinical condition of the animals. The number of doses given is shown in Table 1.

Autopsies were carried out as soon as possible on rats dying during the experiment. On the fourth day the survivors were killed by cervical dislocation. The livers were removed immediately and prepared for histological examination as described previously [6].

RESULTS

Mortality

None of the 25 control rats receiving paracetamol and saline died during the experiment. In contrast, there was a dose-related increase in mortality in the groups treated with paracetamol and mepyramine or promethazine (Table 2). All of the rats given paracetamol together with promethazine (75 and 150 mg/kg) died, and the corresponding mortality rate in those given 75 mg/kg of mepyramine was 40%. One rat receiving 150 mg/kg of promethazine alone died, and one receiving paracetamol and hydrocortisone died on the fourth day.

Liver Histology

A detailed histological study was made of the livers from rats receiving paracetamol with saline, mepyramine (20 mg/kg), promethazine (20 mg/kg) and hydrocortisone. Rats receiving higher doses of mepyramine and promethazine were excluded because early deaths prevented meaningful comparisons between groups [6].

TABLE 1. Schedule of Drugs and Dosages

Group	Treatment	No. of rats	Dose, mg/kg i.p.	Number of doses	Duration of treatment days
1	Paracetamol + Saline (0.4 ml i.p.)	25	--	8	4
2	Paracetamol + Mepyramine	12	20	8	4
		5	37.5	8	4
		10	75	2	1
3	Paracetamol + Promethazine	12	20	8	4
		5	75	1	1
		10	150	1	1
4	Paracetamol + Hydrocortisone	11	100	8	4
5	Suspending agent + Mepyramine	3	20	8	4
		3	75	2	1
	Suspending agent + Promethazine	3	20	8	4
		4	150	1	1
	Suspending agent + Hydrocortisone	4	100	8	4

TABLE 2. Mortality Rates

Group	Treatment	Dose, mg/kg	Number of rats	Mortality, %
1	Paracetamol + Saline		25	0
2	Paracetamol + Mepyramine	20	12	0
		37.5	5	20
		75	10	40
3	Paracetamol + Promethazine	20	12	33
		75	5	100
		150	10	100
4	Paracetamol + Hydrocortisone	100	11	9
5	Suspending agent + Mepyramine	20	3	0
		75	3	0
	Suspending agent + Promethazine	20	3	0
		150	4	25
	Suspending agent + Hydrocortisone	100	4	0

Sections for histological examination were coded and examined "blind" by one observer (M.F.D.). Hepatocellular damage was scored arbitrarily as follows:

(0) No damage to hepatocytes (some livers showed minor degrees of Kupffer cell hyperplasia and lymphocytic infiltration).
(1) Centrilobular hydropic vacuolation without necrosis.
(2) Small foci of centrilobular necrosis.
(3) Necrosis affecting all centrilobular zones with occasional areas of confluence.
(4) Confluent necrosis with large areas of viable parenchyma.
(5) Massive necrosis with only a narrow periportal zone of surviving hepatocytes.

As judged by the mean histological scores, mepyramine, promethazine, and hydrocortisone did not significantly reduce the degree of hepatic necrosis (Table 3). Although objective measurements were not made in animals receiving the higher doses of mepyramine and promethazine, their livers all showed gross hepatic lesions, and there was no evidence of protection against paracetamol toxicity.

DISCUSSION

Diphenhydramine, promethazine, triprolidine, and tripelennamine have been shown to protect rats and mice against experimental hepatic necrosis produced by thioacetamide, carbon tetrachloride, glutathione, and murine hepatitis virus [4, 5, 7, 8]. In order to be effective, the antihistamines had to be given before or at the same time as the hepatotoxic agent. While this is impossible in the clinical situation, it is not surprising that antihistamines should be given to patients with paracetamol poisoning in an attempt to prevent or reduce hepatic necrosis [3]. The use of prednisolone has been recommended in fulminating viral hepatitis, but its value has been disputed [9].

Maclean et al. [3] believed that mepyramine maleate and hydrocortisone restricted hepatic damage in patients with paracetamol poisoning and recommended the use of high doses of hydrocortisone and antihistamines in all such cases. However, they treated only two patients with these drugs, one of whom was apparently not poisoned with paracetamol at all since the plasma paracetamol concentration three hours after ingestion was only a small fraction of that expected following a therapeutic dose [2]. Even without the specific drug therapy proposed by Maclean et al. [3], serum transaminase and bilirubin levels return to normal within a few days in

TABLE 3. Hepatic Damage--Mean Histological Scores

Group	Treatment	No. of animals	Mean score, ± S.E.
1	Paracetamol + Saline	15	3.3 (± 0.2)
2	Paracetamol + Mepyramine (20 mg/kg)	10	3.0 (± 0.3)
3	Paracetamol + Promethazine (20 mg/kg)	6[a]	3.1 (± 0.4)
4	Paracetamol + Hydrocortisone (100 mg/kg)	10	3.5 (± 0.3)
5	Mepyramine, promethazine, or hydrocortisone	10	0

[a] Four rats dying within 48 hr were excluded.

the great majority of patients taking a hepatotoxic dose of paracetamol [1, 2].

In the present studies there was no evidence that mepyramine, promethazine, or hydrocortisone administered at the same time as a hepatoxic dose of paracetamol offered any significant protection against liver damage. Furthermore, there was an alarming dose-related increase in mortality when mepyramine or promethazine were given together with paracetamol.

Clinical studies [2] have failed to demonstrate any protective effect of mepyramine and hydrocortisone against paracetamol-induced hepatic necrosis. In view of the present findings, the use of antihistamines and hydrocortisone cannot be recommended for the treatment of paracetamol poisoning.

SUMMARY

Rats were given a single oral hepatotoxic dose of paracetamol together with saline, mepyramine, promethazine, or hydrocortisone by intraperitoneal injection. Not only did these drugs fail to protect against paracetamol-induced hepatic necrosis, but there was a marked dose-related increase in mortality in animals receiving promethazine or mepyramine. There seems to be no justification for the use of antihistamines or hydrocortisone in the treatment of paracetamol poisoning.

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EXPERIMENTAL PARACETAMOL-INDUCED HEPATIC NECROSIS: A LIGHT- AND ELECTRON-MICROSCOPE, AND HISTOCHEMICAL STUDY

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PLATES IV–VII

ACUTE overdosage with paracetamol (N-acetyl-p-aminophenol, acetaminophen) is becoming increasingly common and is of particular clinical importance because a significant proportion of such patients develop acute hepatic necrosis with occasional deaths from liver failure.

Paracetamol-induced hepatic necrosis was first described in rats in an experimental toxicological study by Boyd and Bereczky (1966). Their histological observations were supplemented by those of Dixon, Nimmo and Prescott (1971), who studied the evolution of the lesion up to 28 days after administration, and found hydropic vacuolation, centrilobular necrosis, macrophage infiltration and regenerative activity with a rapid restoration to normal.

A logical extension of this study is a more detailed analysis of the cellular events leading to necrosis and its immediate sequelae. With this objective, we have undertaken a light- and electron-microscope, and histochemical study of the livers of rats killed at various time intervals up to 48 hr after paracetamol overdosage.

MATERIALS AND METHODS

Thirty male albino Tuck-Wistar rats weighing 280–300 g were divided into six equal groups. They had been housed under identical conditions and maintained for 2 weeks prior to the experiment on Oxoid pasteurised diet and water *ad libitum*. Food was withdrawn 16 hr before paracetamol administration but water was not restricted. The test animals received paracetamol (B. P. C.) in a suspension of 300 mg per ml stabilised with 0·2 per cent. tragacanth. The drug was given by stomach tube without anaesthetic in a dosage of 3·0 g per kg. One or two animals in each group acted as controls and they were given a similar volume of a 0·2 per cent. solution of tragacanth in distilled water alone. After administration, the animals were again given access to food.

Groups of rats were killed by cervical dislocation 1½, 3, 6, 12, 24 and 48 hr after paracetamol administration. One of the rats in the 24-hr group became moribund and had to be killed at 22 hr. The abdominal cavities were opened quickly and the anterior part of the right lateral lobe of the liver was removed for histology and histochemistry.

The portal vein was then cannulated with a thin-walled 21-G needle and secured with a ligature. After cutting the hepatic veins, the liver was perfused with a solution of 2·5 per cent. glutaraldehyde in 0·1M phosphate buffer $pH = 7·4$, from an elevated reservoir at a pressure approximately equivalent to 10 cm of water. Perfusion was maintained for about 10 min. by which time the liver had become uniformly hard. It was then removed and random small (1 mm) cubes were taken into ice-cold 2·5 per cent. buffered glutaraldehyde for electron

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microscopy. After fixation for 1 hr, the cubes were washed in phosphate buffer, post-fixed in a 2 per cent. osmium tetroxide solution for a further 1 hr and embedded in Epon after dehydration in a graded series of alcohols. Semi-thin sections were cut with a Cambridge ultramicrotome and stained with methylene blue Azur II (Richardson, Jarett and Finke, 1960) for light microscopy. Selected areas of the blocks were trimmed and 50-nm thick sections cut on a LKB ultratome I: these were mounted on uncoated 400 mesh copper grids, stained with 8 per cent. uranyl acetate followed by lead citrate, and examined with Philips' EM 100 and EM 300 electron microscopes.

The unperfused tissue for light microscopy and histochemistry was dealt with in two ways:

1. Slices 2–3 mm thick were mounted on a cryostat chuck, quenched immediately in liquid nitrogen, and stored in the liquid nitrogen until required. 8 μm cryostat sections were then cut, picked up on gelatinised slides, air-dried, and fixed in Lillie's calcium acetate formalin (Lillie, 1965) at 4°C for 5 min. Sections were well washed and Gomori's method for acid phosphatase (Gomori, 1950) was carried out.

6 μm cryostat sections were picked up on coverslips, briefly dried, and rinsed in acetone at 4°C for 10 s to remove lipid artefacts. Succinate dehydrogenase (SHD) was demonstrated by the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT) method of Pearse (1960).

Other cryostat sections were stained with methyl-green pyronin (MGP); Sudan black B; oil-red O; and periodic acid-Schiff (PAS).

2. Slices 2–3 mm thick were fixed in Lillie's calcium acetate formalin and processed to paraffin. 5 μm paraffin sections were cut and stained by the following methods: Harris's haematoxylin and eosin (HE); periodic acid-Schiff; Gordon and Sweets' reticulin method; and the Martius, Scarlet, Blue method (MSB) (Lendrum *et al.*, 1962).

Results

Control animals

Whilst the normal appearances of the rat liver are well known, three features have to be stressed in regard to controls; these are vacuolation, glycogen content, and lipid globules.

All control livers show some degree of extremely fine peripheral vacuolation in hepatocytes. In some animals they are scanty and are only found in a few randomly distributed hepatocytes, in others they are seen in the majority of cells. On electron microscopy, the vacuoles range from 0·5 to 0·8 μm in diameter and have a single-membrane lining. They are considered to be pinocytotic in nature.

The glycogen content varies considerably in control animals, a feature probably resulting from the period of starvation prior to the experiment. The 1½- and 3-hr control animals, for example, show some loss of glycogen in all periportal areas so that even when depletion is exaggerated in test animals the finding must be interpreted with caution. After 6 hr, however, the controls have a relatively even distribution of glycogen with only occasional hepatocytes showing depletion.

Small lipid globules are seen in scattered hepatocytes in control livers. They are found mainly in periportal and midzonal cells, and although never conspicuous, are most prominent in the 48-hr control.

The methyl-green-pyronin stain demonstrates cytoplasmic ribonucleoprotein and all control animals show an even distribution of pyroninophilia throughout the parenchyma.

In the Gomori preparations, acid phosphatase activity is identified by lead deposition which appears as dense " granules " of variable size. The granules are predominantly aggregated towards the contiguous surfaces of the hepatocytes in relation to bile canaliculi, but other small deposits are scattered throughout the cytoplasm.

The MTT method demonstrates mitochondrial succinate dehydrogenase activity by the production of small blue-black formazan dots. Control animals show an even distribution of such dots both within the cytoplasm of individual hepatocytes and in the hepatic parenchyma in general.

Test animals

1½-*hr group.* The semi-thin and HE appearances do not differ from those in controls. The PAS preparations, however, show a variable degree of glycogen depletion in excess of the controls but always in periportal or midzonal areas. There are no significant differences from the controls either on electron microscopy or in the histochemical preparations.

3-*hr group.* Again, the semi-thin and HE sections do not differ from controls. One animal, however, shows marked diffuse glycogen depletion (PAS) and the MTT method reveals a slight increase in the size of formazan dots in centrilobular zones. Despite this finding, the ultrastructural appearances are within normal limits. The other MTT and the acid phosphatase preparations are normal.

6-*hr group.* On electron microscopy the earliest change noted is slight exaggeration of the peripheral vacuolation seen in controls. The vacuoles are about 1·5 μm in diameter and a few show apparent detachment of the plasma-membrane lining.

In the semi-thin sections the centrilobular and midzonal areas are pale with loss of the normal cytoplasmic " granularity ", mainly as a result of loss of glycogen. In the HE sections, hepatocytes in these areas show loss of cytoplasmic basophilia, and parallel loss of pyroninophilia in the MGP stain.

The pallid areas show a variable degree of aqueous (cloudy) swelling and vacuolation. Aqueous swelling is seen predominantly in midzonal cells, in the HE sections as a uniform " ground-glass " appearance in the cytoplasm, and in the semi-thin sections as a fine, diffuse cytoplasmic " vesiculation " (fig. 1). Under the electron microscope, hepatocytes in the immediate vicinity of the central vein exhibit glycogen depletion and uniform cellular matrix swelling whilst midzonal cells show, in addition, dilatation of the rough and smooth endoplasmic reticulum and the Golgi apparatus (fig. 2). The swelling of rough surfaced endoplasmic reticulum is accompanied by detachment of ribosomes, which underlies the loss of cytoplasmic basophilia and pyrininophilia seen on light microscopy. Some cells appear to have a predominance of smooth membranes, a finding partly due to the loss of ribosomes from the rough membranes and partly to a uniform swelling of the entire canalicular system of the cell. These changes are seen in all animals in the 6-hr group but at different stages of development in adjacent cells (fig. 3).

One animal in this group shows a more advanced type of degeneration more commonly seen in the 12-hour group, revealing numerous large vacuoles in centrilobular and midzonal areas. These clear vacuoles, which do not contain lipid, appear to be formed by coalescence of vesicles in the smooth endoplasmic reticulum (fig. 4). In the MTT preparations of this liver, the centrilobular zones are pale due to loss of mitochondrial staining and hydropic vacuolation (fig. 5). There is also slight enlargement of the formazan dots in a narrow band of cells around a few of the damaged zones. On electron microscopy mitochondria show matrix swelling but there is good preservation of the overall architecture of the organelle.

12-*hr group*. These animals show considerable variation in the character and extent of damage.

One animal shows only a minor loss of glycogen. Another shows changes similar to those seen in the 6-hr group i.e. pallor and glycogen depletion with aqueous swelling and mild vacuolation in midzonal cells. Two animals, however, reveal much more advanced changes amounting to confluent centrilobular necrosis in the blocks examined (fig. 6). Such necrosis is expressed ultrastructurally as disintegration of the canalicular system with formation of small electron-clear vesicles, gross mitochondrial swelling accompanied by disruption of cristae, and breakdown of the plasma membrane which appears interrupted (fig. 7). Only a minority of necrotic cells show evidence of previous gross vacuolation. The nuclear changes vary from clumping of chromatin to pyknosis and karyorrhexis.

A few of the " surviving " hepatocytes contain ingested red blood cells (erythrophagocytosis), and hepatocytes immediately adjacent to the necrotic areas contain large clear vacuoles.

Congestion in sinusoids is prominent, especially towards the periphery of the necrotic areas. In addition to large numbers of red blood cells, the sinusoids contain membrane fragments, vesicular structures enclosing remnants of rough endoplasmic reticulum, and occasional small fibrin-like deposits.

There is diffuse loss of glycogen in the two severely affected livers involving even the apparently viable periportal cells, and a complete absence of pyroninophilia in the necrotic areas.

Histochemistry reveals a variety of appearances paralleling the morphological findings. In the MTT preparations, one of the animals appears normal, whilst in another there is patchy increase in size and density of formazan dots in centrilobular areas. The two necrotic livers showed widespread loss of enzyme activity with accentuated activity in the few surviving periportal cells.

The acid phosphatase preparations show no change from the control in the two animals with minor histological abnormalities, but in the other two, coarse clumping of granules in periportal cells and a progressive loss of activity towards the central veins in necrotic hepatocytes is seen.

Fat stains on the necrotic livers show an increase in small lipid globules in surviving hepatocytes. The other livers show no abnormality.

24-*hr group*. These animals have a more uniform appearance than the 12-hr group in regard to both the extent and progress of the lesion.

All show more advanced coagulative necrosis. The plasma membranes of affected hepatocytes are frequently absent and it is often difficult to distinguish individual cell borders. The sinusoids contain numerous multivesicular bodies. Nuclear degeneration is most apparent in this group and karyorrhexis is frequently seen. The necrotic areas are infiltrated by small numbers of neutrophil polymorphs and macrophages, many of which contain phagocytosed cytoplasmic debris.

A few hepatocytes bordering the necrotic areas have an acidophilic shrunken appearance with condensed pyknotic nuclei. On electron microscopy, in addition to condensation of the nucleus and swelling of the nuclear membrane, the cytoplasm contains numerous small vacuoles separated by a dense matrix. These distinctive degenerative changes, which do not seem to involve breakdown of the endoplasmic reticulum, represent the ultrastructural features of " shrinkage necrosis " a form of cell death resulting in the formation of spherical acidophilic (Councilman) bodies composed of compacted organelles and sometimes nuclear material (Kerr, 1971). Acidophil bodies are scanty but are much more readily found than in controls (fig. 8).

The necrotic zones are also bordered by dense cells either deeply indented by large extracellular vacuoles or apparently containing intracellular vacuoles which often impinge on the nucleus. The latter type contain loosely packed electron-dense amorphous material but, as determined by serial sections, are in continuity with the clear peripheral and extracellular vacuoles. The plasma membrane lining many of the vacuoles is clearly seen. The cytoplasm of these vacuolated cells is glycogen depleted but contains well formed, regular, rough-surfaced endoplasmic reticulum frequently surrounding mitochondria. The mitochondria are of normal size but have a very dense matrix and slightly irregular cristae (fig. 9). The microvilli are diminished in size and number.

In addition to vacuolated cells, other hepatocytes adjacent to the necrotic areas contain increased numbers of fine, dispersed lipid globules, and a few reveal large, pale, schiff-positive globules in the PAS stain.

The MTT preparations contain bands of extreme pallor indicative of almost complete loss of activity in the necrotic zones. These areas are surrounded by a narrow band of exaggerated activity, the bordering hepatocytes containing increased numbers of large formazan dots. The periportal cells remain normal.

Loss of acid phosphatase activity has progressed so that the majority of necrotic cells contain only a few coarse, dense granules in the paracanalicular areas and some cells show a complete loss of activity. In addition, macrophages contain discrete granules of variable size and some Kupffer cells show increased activity. Occasional heavily stained spherical " bodies " are seen within macrophages and these presumably represent heterolysosomes or ingested acidophil bodies.

48-*hr group*. Although necrosis is fully established in each liver there is moderate variability in its extent. The smaller necrotic areas contain numerous discrete deposits of eosinophilic debris apparently within the cytoplasm of macrophages. The larger areas show persistence of amorphous cellular debris, and infiltration by macrophages is less pronounced.

Electron microscopy reveals spherical collections of cytoplasmic debris in which degenerate mitochondria are the predominant recognisable particle. Such collections are found either within macrophages or in the process of engulfment by macrophages. Other particulate cellular debris is seen in macrophages or lying free in sinusoids. After phagocytosis, the collections of debris become aggregated and large, generally spherical, electron-dense inclusions are formed.

The necrotic areas are devoid of glycogen and show marked loss of pyroninophilia, surrounding hepatocytes show loss of glycogen but the midzonal and periportal cells have, in the main, a normal glycogen content.

Transitional forms between irregularly shaped, shrunken hepatocytes and spherical acidophil bodies are seen. The bodies are found either within macrophages or, more frequently, lying free in sinusoids around the margins of the necrotic zones. Electron microscopy shows that they are composed of compacted mitochondria, endoplasmic reticulum with ribosomes, and a variety of small clear vesicles (fig. 10). Nuclear remnants are not identifiable. In some instances, the intracellular bodies show evidence of continuing breakdown when the internal components are much more electron dense and less distinct.

In the fat stains some increase in lipid is seen in midzonal cells and a few globules are found in the necrotic areas and in macrophages. One liver contains abundant black-staining material within necrotic areas in the Sudan Black preparation, but stains much less impressively with oil red O, suggesting the presence of oxidised lipids in the hepatocyte debris.

The centrilobular loss of succinate dehydrogenase activity persists, but the peripheral exaggeration of activity seen at 24 hr is now inconsistent and not seen in one test animal.

The macrophage infiltrate reveals conspicuous acid phosphatase activity containing numerous discrete granules of variable size. Kupffer cells lining sinusoids close to the necrotic areas also show increased activity. Densely staining intracellular spherical " bodies " are more readily found than at 24 hr but are still scanty. The majority probably represent phosphatase activity associated with the ingestion of acidophil bodies.

The above features of resolution are accompanied by regeneration, evidenced by high mitotic activity and an increase in size of hepatocytes which show an increased nuclear diameter and new endoplasmic reticulum formation. Ribosomes are of normal size and are attached to regular parallel arrays of endoplasmic reticulum which, although distributed throughout the cytoplasm, are closely related to normal-looking mitochondria. Glycogen particles are reduced in number in many of these hepatocytes. The cell surfaces possess normal microvilli and new bile canaliculi are formed.

Discussion

The earliest morphological changes seen in the development of paracetamol-induced liver injury are loss of glycogen and cytoplasmic matrix swelling followed by loss of ribosomes and mitochondrial abnormalities. These changes are

entirely non-specific and are common to liver injury provoked by a wide variety of agents. Similar appearances have been described in ischaemia (Bassi and Bernelli-Zazzera, 1964), hypovolaemic shock (Blair *et al.*, 1968), and as a result of such diverse poisons as carbon tetrachloride (Smuckler and Arcasoy, 1969), tannic acid (Arhelger, Broom and Boler, 1965), galactosamine (Scharnbeck *et al.*, 1972), and the azo-dye 2-methyl-4-dimethylaminoazobenzene (La Fontaine and Allard, 1964).

In the centrilobular cells there appeared to be a rapid progression from these early changes to frank coagulative necrosis. This contrasted with cells in midzonal and occasional periportal areas where vesiculation of the endoplasmic reticulum was a conspicuous feature. In some cells this appeared to progress through gross hydropic vacuolation to cell-death, but the fact that at 48 hr necrosis was in general confined to centrilobular areas, suggests that in the majority of midzonal and periportal cells vesiculation represents a reversible form of injury. Indeed, although vesiculation of the endoplasmic reticulum is frequently seen in many types of liver injury, its previous interpretation as one of the sequence of events leading to necrosis has been disputed (Judah, Ahmed and McLean, 1965; Magee, 1966). Conclusive evidence of reversible injury is the finding of autophagosomes within recovering hepatocytes. Whilst these form a prominent feature of some types of liver injury, for example in that due to heliotrine (Kerr, 1969) and glucagon (Arstila and Trump, 1968), we found only a few autophagosomes in midzonal cells in the later groups, certainly fewer than would be expected if sublethal cell injury is an important feature of paracetamol hepatotoxicity.

Vacuolation was a conspicuous finding in this study and four distinct types could be recognised. Firstly, very small vacuoles indenting the sinusoidal margins of some hepatocytes were found in both control and test animals. These are interpreted as pinocytotic in nature, and have been previously described in control material in an electron-microscope study of anoxic changes in liver cells (Oudea, 1963). The exaggeration of these vacuoles in the 6-hr test animals may reflect early damage to the plasma membrane, but where the injury was more advanced and swelling of the cell had occurred, such pinocytotic vacuoles were not seen.

The second type of vacuolation was that found predominantly in midzonal cells in association with vesiculation of the endoplasmic reticulum. These vacuoles appear to arise by coalescence of smaller dilatations of the cisternae reflecting continued accumulation of fluid within the cells, and represent the ultrastructural counterpart of the " hydropic " vacuoles seen on light microscopy. In carbon tetrachloride hepatotoxicity, correlations between light microscopic changes and ultrastructural alterations have also established that hydropic vacuolation corresponds to an extensive dilatation of cisternae of the endoplasmic reticulum (Stenger, 1966, 1970). Swollen mitochondria have been claimed to contribute, albeit to a lesser degree, to the overall swelling of the injured cells (Ashworth *et al.*, 1963), but we found little or no evidence of mitochondrial swelling in hydropic cells. Mitochondrial changes were only conspicuous in central necrotic cells which remained remarkably free from

vacuolation. This failure of centrilobular cells to show hydropic swelling in contrast to the less severely injured cells in the midzone had been previously emphasised by Dixon and McCullagh (1957).

Large clear vacuoles were also found within hepatocytes bordering the necrotic areas in the 12- and 24-hr groups but they differed from the hydropic vacuoles just described in that apart from minor mitochondrial changes and loss of glycogen, the remainder of the cell appeared normal. Serial sections revealed that many of these vacuoles communicated with the extracellular space. The appearances therefore indicated that a mechanism other than the progressive intracellular accumulation of fluid was responsible. A minority of these large clear " bordering " vacuoles were apparently entirely extracellular, being situated between adjacent hepatocytes. The possibility that the latter two forms of vacuolation were artefacts produced, or exaggerated, by perfusion-fixation was considered, but their presence in parallel paraffin sections of unperfused liver, their absence in controls, and the consistency of their location around necrotic areas, argued against an artefactual origin. Similar vacuolation of liver cells has been described in a classical series of experiments by Trowell (1946–47), who showed that such " watery vacuoles " resulted from a combination of anoxia and congestion and that the entry of fluid into liver cells was due to the hydrostatic pressure in the sinusoids and not to osmotic absorption. More recent ultrastructural studies of the changes produced in liver cells by respiratory hypoxia have confirmed the presence of large clear vacuoles with a single-membrane lining which communicate with the space of Disse (Oudea) and other vacuoles which are entirely extracellular (Brewer and Heath, 1965). The centrilobular necrosis seen in this study was invariably accompanied by marked sinusoidal congestion. Thus the consistent presence of clear vacuoles around the necrotic and therefore congested zones suggested that stasis of blood leading to hypoxia and raised hydrostatic pressure might be responsible for their formation. Glynn and Himsworth (1948) were sufficiently impressed by changes in blood flow through the liver in carbon tetrachloride injury to suggest that the centrilobular necrosis was a result of the restriction of blood supply consequent upon swelling of the cells. Whilst such a mechanism is now considered improbable (Magee), the presence of morphological changes related to secondary vascular effects rather than to the primary hepatotoxin has been largely overlooked in many recent studies of liver injury. Furthermore, possible exaggeration of the injury by these secondary vascular changes might explain why a reduction in mortality after paracetamol overdosage in mice has been achieved by treatment with vasoactive compounds (Dixon, Dixon and Aparicio, 1973; Rosner, Romero-Ferret and Mottot, 1973).

Lipid accumulation is not a prominent feature of paracetamol liver injury, but a narrow band of midzonal cells bordering the necrotic areas showed fine lipid globulation at 24 and 48 hr. This may be indicative of a sub-lethal injury or a much slower form of cell-death, as the accumulation and aggregation of fat within cells requires continuing metabolic activity. It is also conceivable, however, that this fatty change is a further manifestation of secondary hypoxic damage.

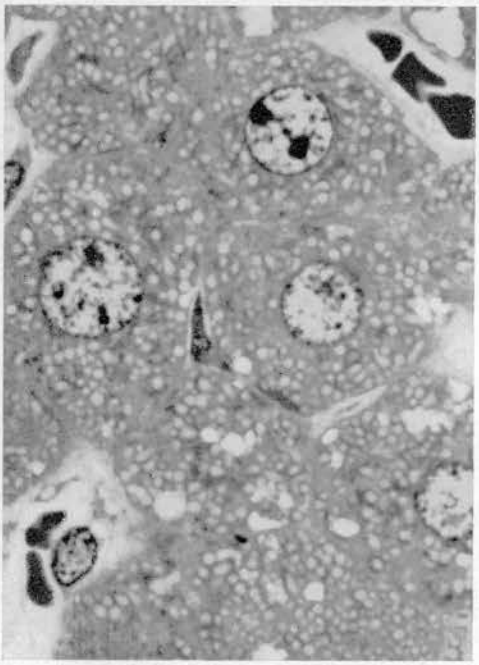

FIG. 1.—6 hr after paracetamol. Midzonal hepatocytes showing cytoplasmic vesiculation and scattered small clear vacuoles. Semi-thin section (STS). Methylene blue—Azur II (MBA). ×1600.

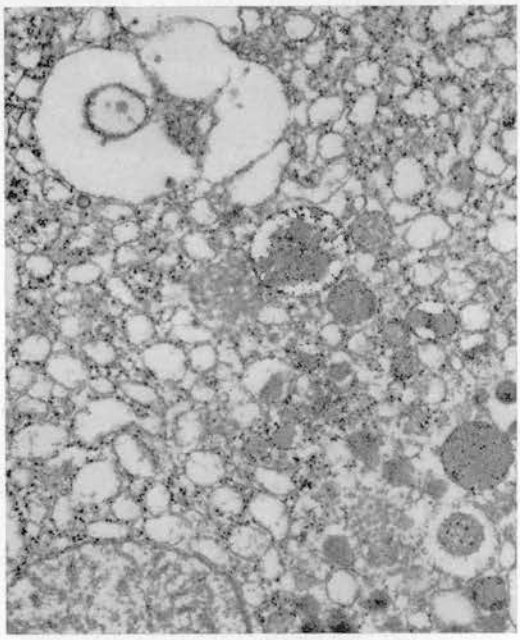

FIG. 2.—6 hr. Detail of a midozonal hepatocyte showing absence of glycogen, swelling of rough endoplasmic reticulum, and a focal accumulation of lysosomes. Electron micrograph (EM). ×20,000.

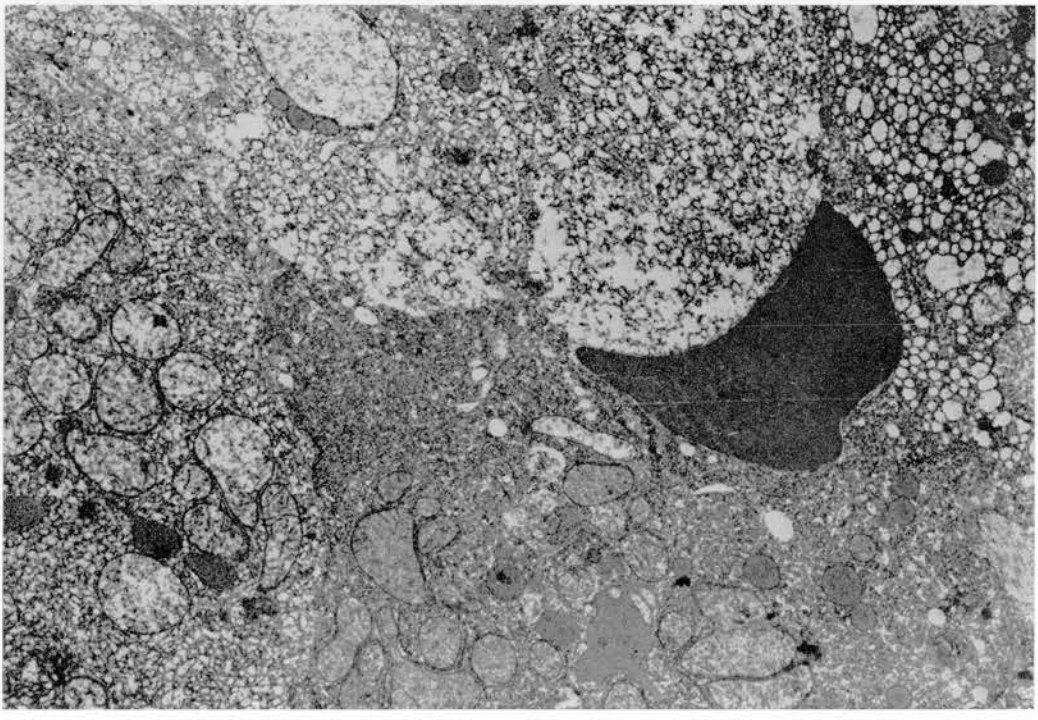

FIG. 3.—6 hr. Three midzonal hepatocytes showing glycogen depletion and varying degrees of dilatation of the canalicular system and mitochondria. This dilatation, together with an increase in cytoplasmic matrix, has led to narrowing of an intervening sinusoid which contains a distorted erythrocyte. EM. ×12,000.

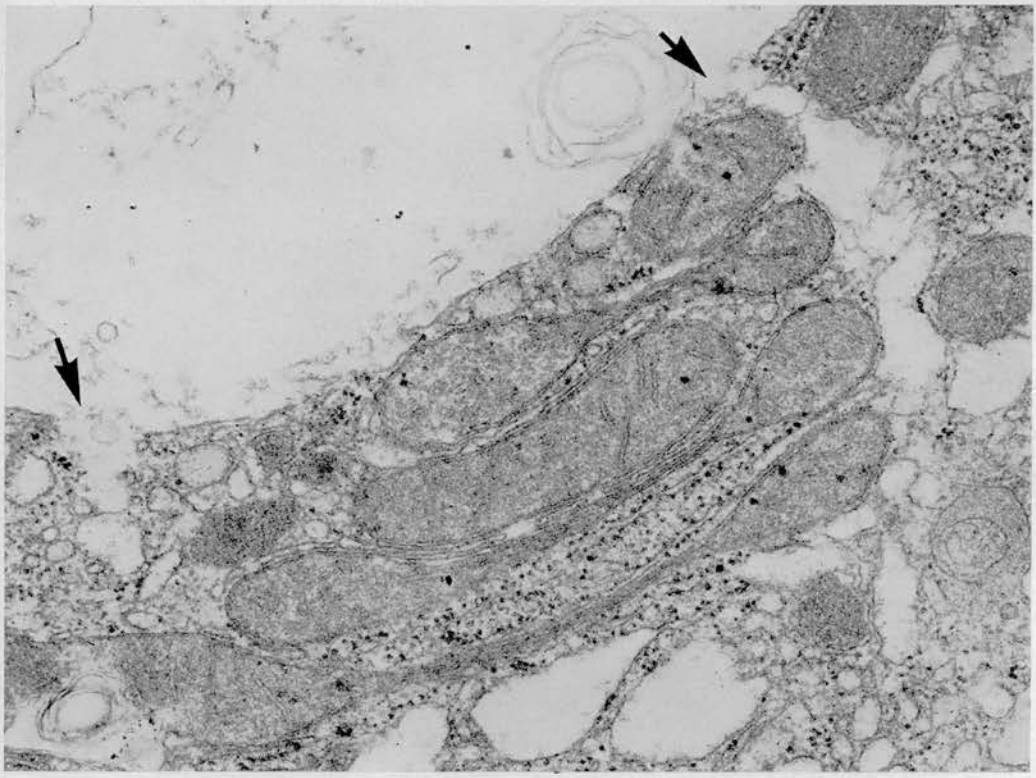

FIG. 4.—6 hr. Detail of a grossly vacuolated hepatocyte showing communications between the swollen canalicular system and a large intracellular vacuole (arrows). EM. ×39,000.

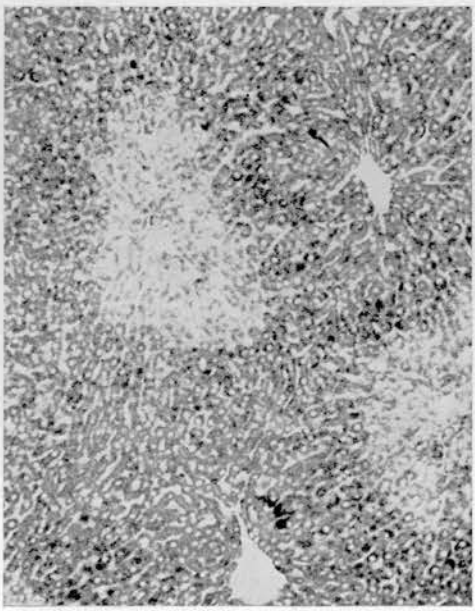

FIG. 5.—6 hr. Loss of succinate dehydrogenase activity in centrilobular zones. MTT. ×70.

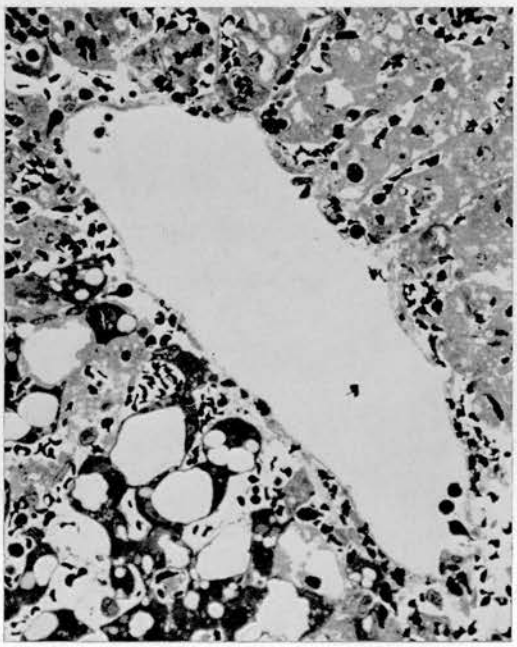

FIG. 6.—12 hr. Coagulative necrosis and gross vacuolation in centrilobular hepatocytes associated with marked sinusoidal congestion. STS, MBA. ×300.

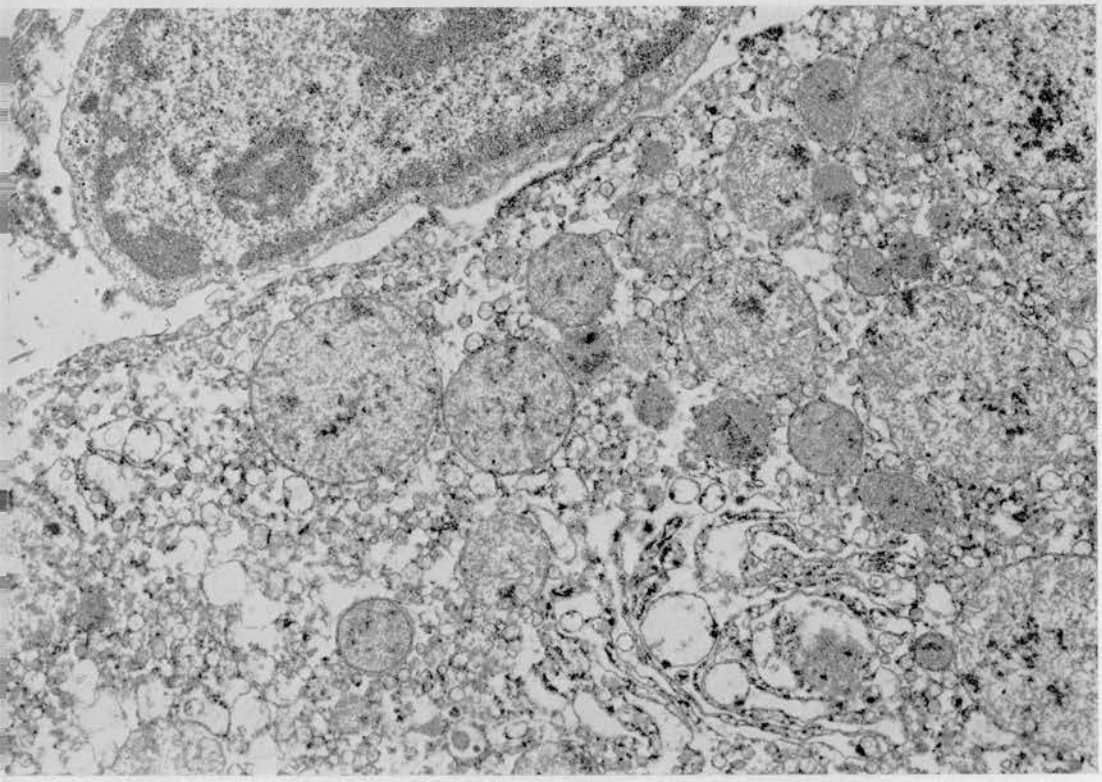

FIG. 7.—12 hr. Part of a centrilobular hepatocyte showing swelling of the matrix and mitochondria and fragmentation of the canalicular system with formation of discrete vesicles. Part of a small macrophage in the enlarged space of Disse is included. EM. ×8500.

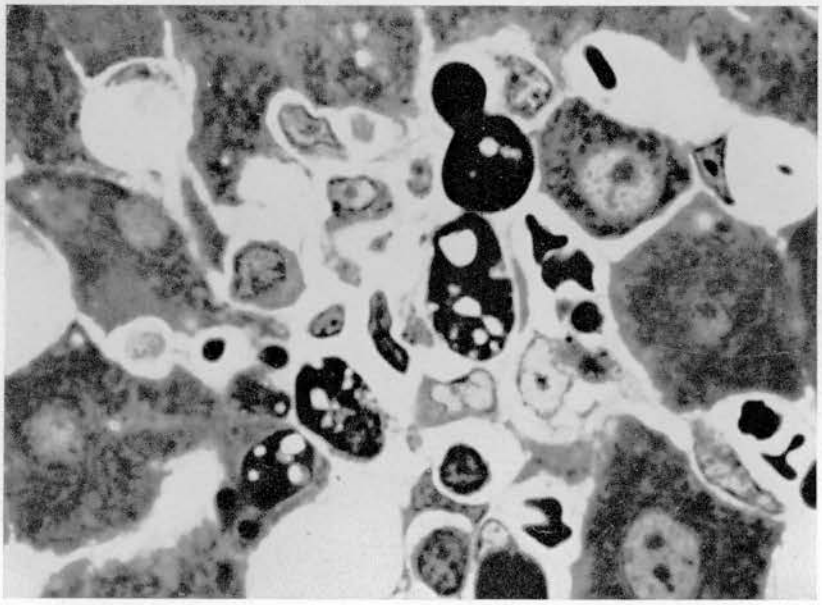

FIG. 8.—24 hr. A group of four Councilman bodies in an area of macrophage infiltration. The upper body shows apparent " budding ". STS, MBA. ×1600.

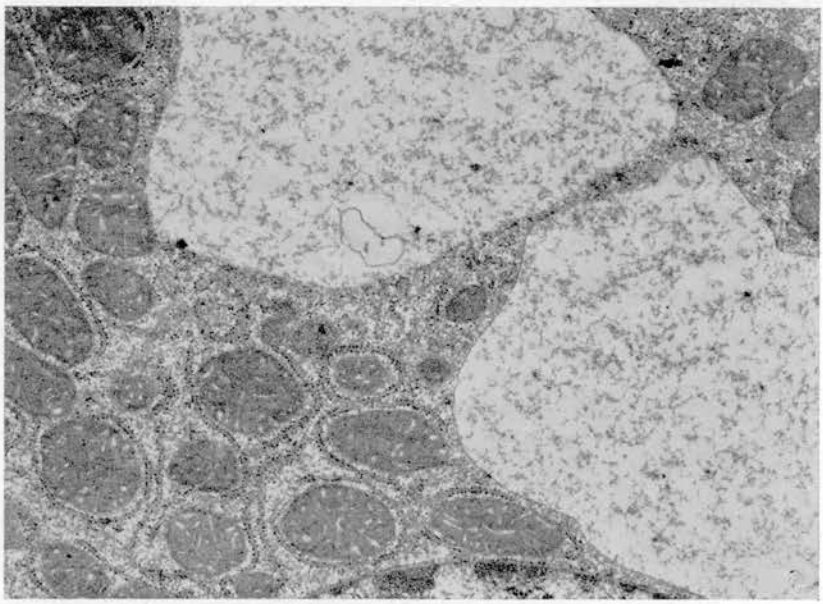

FIG. 9.—24 hr. Part of a vacuolated hepatocyte bordering the necrotic zone. The vacuoles contain electron-dense material and are lined by a single membrane. The cytoplasm shows glycogen depletion and increased mitochondrial matrix density but the rough endoplasmic reticulum is well preserved. EM. ×13,000.

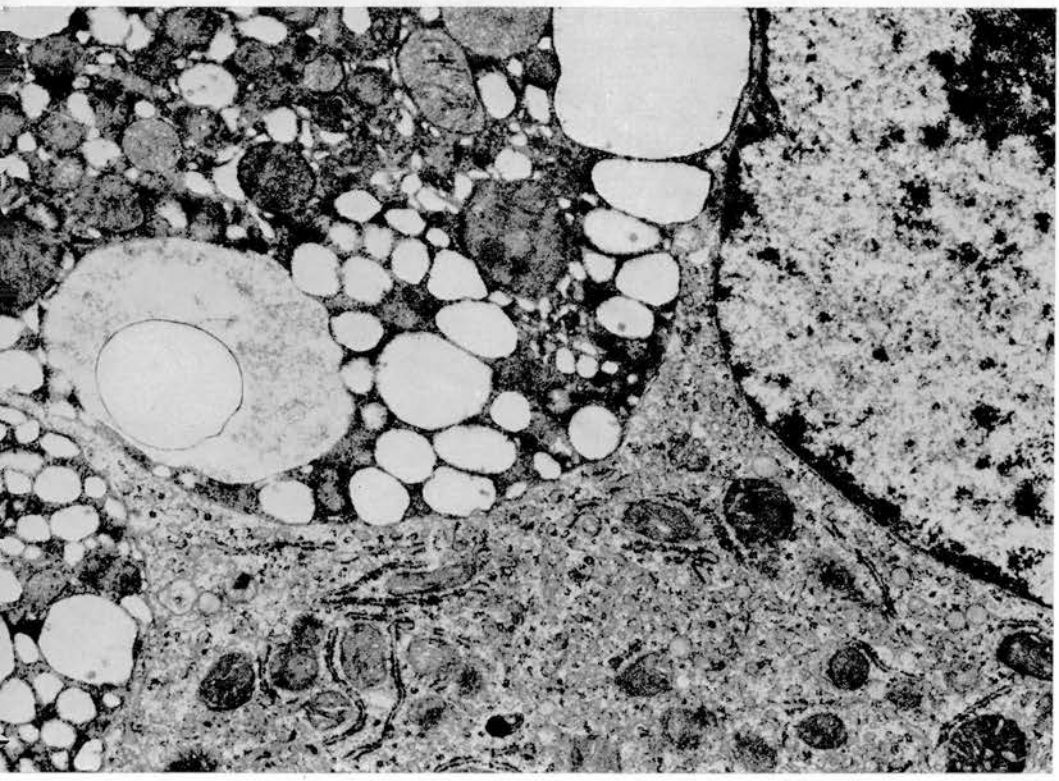

FIG. 10.—48 hr. Part of a hepatocyte containing two round membrane-bound inclusions composed of compacted mitochondria, endoplasmic reticulum and a variety of small vesicles. EM. ×13,000.

The principle changes found in the MTT stains for succinate dehydrogenase were an inconsistent rise in activity in centrilobular areas first seen at 6 hr followed by a uniform loss of activity in degenerating cells. At 24 hr the necrotic areas were surrounded by a narrow zone of increased activity. These changes are identical to those found in experimental carbon tetrachloride and thioacetamide intoxication by Smith and Coote (1963). The initial transient rise in activity has been interpreted by Kerr (1965) in a study of ischaemic liver injury, as reflecting increased accessibility of substrate to enzyme in damaged mitochondria. Whilst we found some increase in mitochondrial matrix density at this stage, there was good preservation of the architecture of the organelle with no evidence of enlargement such as that described as early as 1–2 hr after a single large dose of carbon tetrachloride (Reynolds, 1963). Loss of succinate dehydrogenase activity, however, was associated with mitochondrial swelling and disruption of cristae but was inevitably accompanied by advanced changes in the remainder of the cell amounting to frank necrosis. We conclude, therefore, that significant mitochondrial injury is a relatively late feature of paracetamol hepatotoxicity which follows an initial and more conspicuous injury to the endoplasmic reticulum. This interpretation is compatible with the finding that covalent binding of tritiated paracetamol *in vitro* is predominantly directed towards the microsomal fraction of the cell (Potter *et al.*, 1973).

The acid phosphatase preparations did not suggest a role for lysosomes in the early paracetamol injury; loss of activity was first seen at 12 hr and was only found in already necrotic cells. This parallels the findings of Slater (1969) in carbon tetrachloride hepatotoxicity and may account for the failure of drugs known to stabilise lysosomes, namely corticosteroids and antihistamines, to modify experimental paracetamol-induced necrosis (Nimmo, Dixon and Prescott, 1973).

Whilst the general sequence of events leading to necrosis appeared to be consistent, the time course and ultimate extent varied considerably. Most animals, however, showed the earliest evidence of damage at 6 hr and by 24 hr all showed frank centrilobular necrosis. The variation in the extent of necrosis between animals was not unexpected, as other studies on paracetamol-induced liver injury in the rat in which multiple blocks have been examined by light microscopy have revealed considerable variation in the extent of necrosis within the same liver (Walker *et al.*, 1973, 1974).

Thus far we have been mainly concerned with the events leading to necrosis, but detailed study of the later groups revealed some important aspects of healing. Between 24 and 48 hr there was a rapid accumulation of macrophages in the necrotic areas which showed marked acid phosphatase activity probably associated with the formation of heterolysosomes. Acidophil bodies, although numerous in certain types of liver injury, for example following ligation of the portal vein (Kerr, 1971), were scanty even at 48 hr. They are also removed by phagocytosis and degradation in macrophages, " fixed " Kupffer cells and hepatocytes. These findings indicate a very active phase of phagocytosis and absorption immediately following injury, but such processes do not operate in isolation and healing is largely achieved through a concurrent phase of rapid