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Evaluation of Paracetamol-Induced Damage in Liver Biopsies

Acute Changes and Follow-Up Findings

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Summary. In 100 patients who had taken an acute overdose of paracetamol the liver was biopsied percutaneously on the 4th day or as soon thereafter as recovery of the clotting mechanism allowed. A system for grading the histological changes in the liver is described. The hallmark of severe (grade III) damage is centrizonal necrosis, for which there is probably a dosage threshold. Consequent changes include phagocytosis of cell debris, reticulin collapse, and an inflammatory infiltration; acidophil degeneration of hepatocytes is sometimes prominent; regenerative growth is already evident after 4 days. Mild (grade I) damage is characterized by excess of lipofuscin pigment in centrizonal hepatocytes, sparse focal necrosis of liver cells, and some phagocytosis of lipofuscin. The findings in moderate (grade II) damage are similar but more pronounced. Five patients died in acute hepatic failure, and 22/100 showed severe (grade III) changes but survived. Forty-nine patients representing all three grades of liver damage were rebiopsied after 3 months. Central necrotic zones were found to have been completely reconstituted leaving only minor abnormalities except for one patient who showed mild centrilobular fibrosis and scarring of a nonprogressive nature.

Key words: Paracetamol (acetaminophen) poisoning — Liver biopsy histopathology — Centrilobular liver necrosis — Liver regeneration — Liver failure.

Paracetamol (4-hydroxyacetanilide, N-acetyl-p-aminophenol, acetaminophen) is a potent analgesic and antipyretic agent which has come to be regarded as remarkably nontoxic and largely free from harmful side effects when properly administered in therapeutic doses. Nevertheless a single large overdose can result in fulminant liver necrosis and death, as first reported in Britain by Davidson and Eastham (1966). At the present time the drug is widely used in the U. K. and is freely available to the public without prescription, so it is not surprising to find that it is often taken in acute overdose for the purpose of suicide (Prescott et al., 1971; Clark et al., 1973; James et al., 1975; Portmann et al., 1975). The number of hospital admissions in England and Wales attributable to paracetamol overdose has recently been estimated as about 1,000 per year (James et al., 1975), though it is notable that this experience has not as yet been paralleled in the U.S.A. In this paper we give an account of the histological findings in liver biopsy specimens obtained from 100 such patients in the early acute stages and from 49 of these who were rebiopsied after three months.

Material and Methods

Acute Study. Percutaneous liver biopsy samples were obtained from 100 patients who were referred to the North-East Regional Paracetamol Overdose Service (James et al., 1975) at the Royal Victoria Infirmary over a period of some 12 months. All of these patients had

recently ingested a single large dose of paracetamol; the amount consumed ranged from 5 to 50 g. Some patients are included who had also taken alcohol with the overdose, but we have excluded from this study known chronic alcoholics and those known to have taken other hepatotoxic drugs with the paracetamol overdose. The patients' ages ranged from13 to 65 years, but 40% were less than 20 years old and only 11% were more than 40 years old. Females greatly outnumbered males (79/100).

Follow-Up Study. Forty-nine patients had a second liver biopsy after an interval of 3 months; 10 of these 49 had sustained severe liver damage (see below) in the acute stage.

Clinical Management and Biochemical Investigations. A full account of these aspects of the study has already been given alsewhere (James et al., 1975; Hamlyn et al., 1976). Briefly, all patients were treated with intraveneous 5% dextrose, vitamins, and appropriate amounts of potassium for at least 4 days after admission to hospital. Nothing but limited clear fluids was taken by mouth during this period. If signs or symptoms of hepatic encephalopathy developed the full conventional treatment for acute hepatic failure was carried out. Daily estimations of conventional liver function tests (bilirubin, alkaline phosphatase, aspartate aminotransferase, and prothrombin ratio) were performed together with serum bile acid levels (estimated spectrofluorometrically). The plasma potassium level was estimated on admission by a spectrophotometric method.

Patients selected for follow-up liver biopsy were admitted to a programmed investigation unit for full assessment of all liver function tests to coincide with the taking of the biopsy specimen.

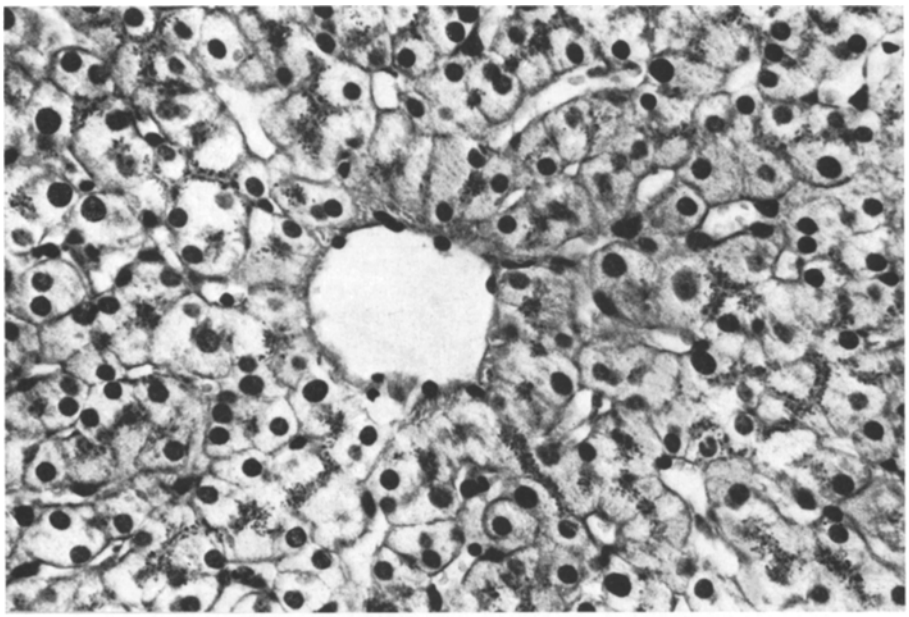
Liver Biopsy. In all instances the patients or their relatives gave informed consent to the taking of a liver biopsy specimen. The samples of liver tissue were obtained by means of the Menghini needle on the fourth day following paracetamol overdose or as soon after that as the patient's general condition and the prothrombin ratio allowed. In 5 fatal cases samples had to be taken immediately after death. The tissue was fixed in neutral buffered 10% formol saline and postfixed in mercuric chloride solution. Serial paraffin sections were stained routinely by haematoxylin and eosin, the picro-Mallory trichrome method, haematoxylin van Gieson, the periodic acid-Schiff method with and without amylase predigestion, Foot's silver impregnation method for reticulin and Perls' prussian blue method for iron. When specimens from 54 patients had been accumulated the histopathological changes were reassessed independently by two observers (ML; AJW), all abnormalities being graded subjectively for severity on a one to three plus scale. This was carried out without knowledge of the clinical and biochemical findings. Interspersed randomly amongst the sections from the paracetamol overdose patients were sections of biopsies previously obtained from normal livers. Finally a single inclusive grade of severity was assigned: Grade 0 denoted normal appearances while grades I, II and III signified mild, moderate, or severe changes (see below). The criteria so established were applied to the assessment of liver damage in a further 46 patients.

Results

Acute Histopathological Findings

Grade I (mild) damage was expressed mainly by an abundance of lipofuscin pigment granules in the centrilobular hepatocytes (Fig. 1), the amount present being especially inappropriate for such a predominantly youthful group. Sometimes lipofuscin granules were present in the midzonal hepatocytes, though less plentifully. Occasional prominent Kupffer cells containing amylase-resistant PAS positive granules were another feature. The presence of not more than two foci of hepatocytolytic necrosis ("spotty" necrosis) was allowable in this grade.

Grade II (moderate) damage comprised changes similar to those described above but "spotty" necrosis was more widespread; these foci were infiltrated by inflammatory cells (Fig. 2) and marked by a localised condensation of reticulin



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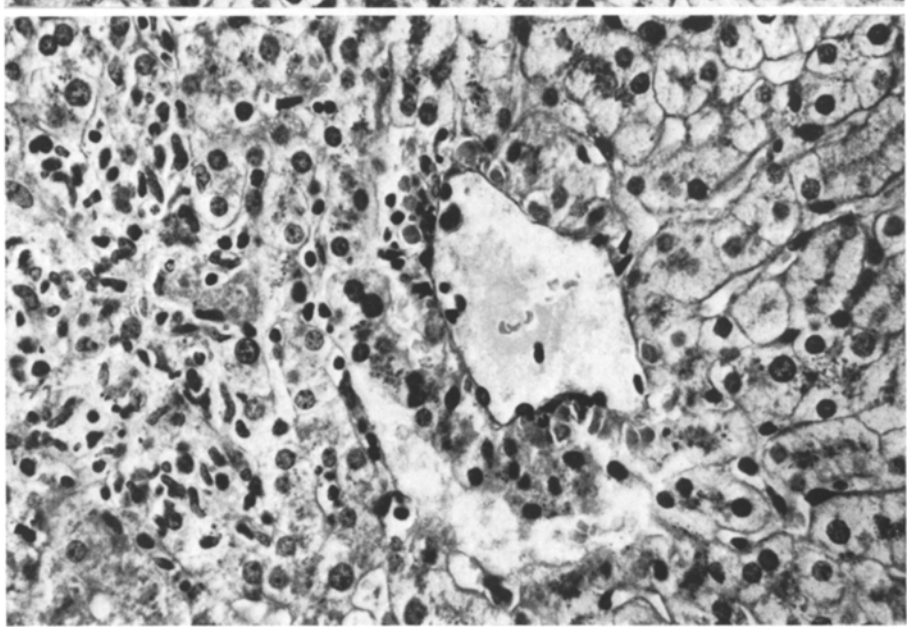

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Fig. 1 Grade I liver damage in a young woman. Centrizonal hepatocytes contain excess lipofuscin pigment. PAS after amylase digestion, × 350

Fig. 2. Grade II liver damage. Focus of hepatocytolytic necrosis with leukocytic infiltration adjacent to efferent venule. PAS after amylase digestion, × 350

Table 1. Distribution of different grades of acute liver damage

Grade of liver damage	Number of patients
0	2
I	50
II	21
III	22
Subtotal lobular necrosis	5

fibres. Often there was evidence of early regenerative activity in hepatocytes, including occasional mitoses. Kupffer cells containing lipofuscin were sometimes present in small clusters.

Grade III (severe) damage was characterised by one essential diagnostic feature viz. centrizonal necrosis of hepatocytes (Fig. 3). As the biopsy was taken 4 or more days after ingestion of paracetamol the necrotic cells were usually found to have been removed by phagocytosis leaving the reticulin framework in the centrizonal area intact but partially collapsed (Fig. 4). In some instances central necrotic zones in adjacent lobules were in continuity, and the resultant collapse of the reticulin framework led to interlobular bridging by passive septa (Fig. 5). Some of these severely damaged livers (6/22) showed a distinctive appearance in the central necrotic zones resulting from the presence of variable numbers of altered hepatocytes which still retained their individual identity. These cells were near normal in size, with increased acidophilia of the cytoplasm and deeply stained or even pyknotic nuclei (Fig. 8). Occasional acidophil bodies were also seen (Fig. 8) but this form of "shrinkage necrosis" (Kerr, 1971) seemed distinct from the zonal acidophil degeneration described above. In the more usual form of centrizonal necrosis Kupffer cells in the necrotic zones were thrown into prominence by the disappearance of hepatocytes, possibly by focal hyperplasia, and by their content of strongly PAS positive amylase resistant granules resulting from phagocytosis of cell debris (Fig. 6). Surviving hepatocytes bordering these zones usually displayed prominent cytoplasmic vacuolation whereas the more peripheral hepatocytes often appeared undamaged and retained a high glycogen content. The surviving cells commonly showed evidence of brisk regenerative activity with numbers of cells undergoing mitotic division (Fig. 7). Morphological evidence of cholestasis was rarely seen.

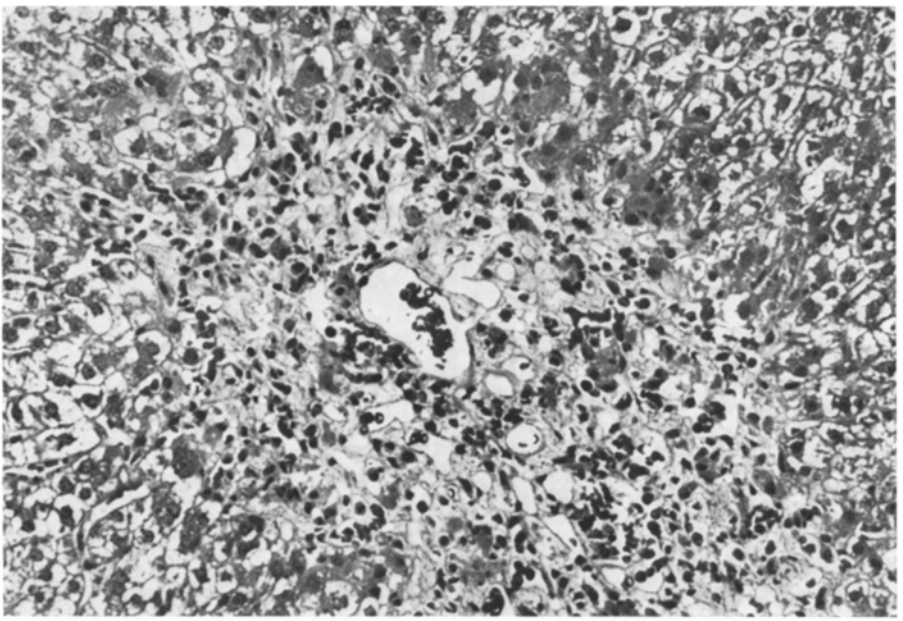
The number of cases falling into each grade of severity is shown in Table 1

Liver Findings in Fatal Cases

Five patients died in acute hepatic failure. All showed subtotal lobular necrosis with only small islets of altered hepatocytes surviving in the immediate periportal region (Fig. 9).

Liver Findings after 3 Months

Residual histopathological abnormalities were present in all of the previously damaged livers. Those patients who initially had grade I or II changes still showed an excess of lipofuscin in centrilobular hepatocytes, occasional Kupffer



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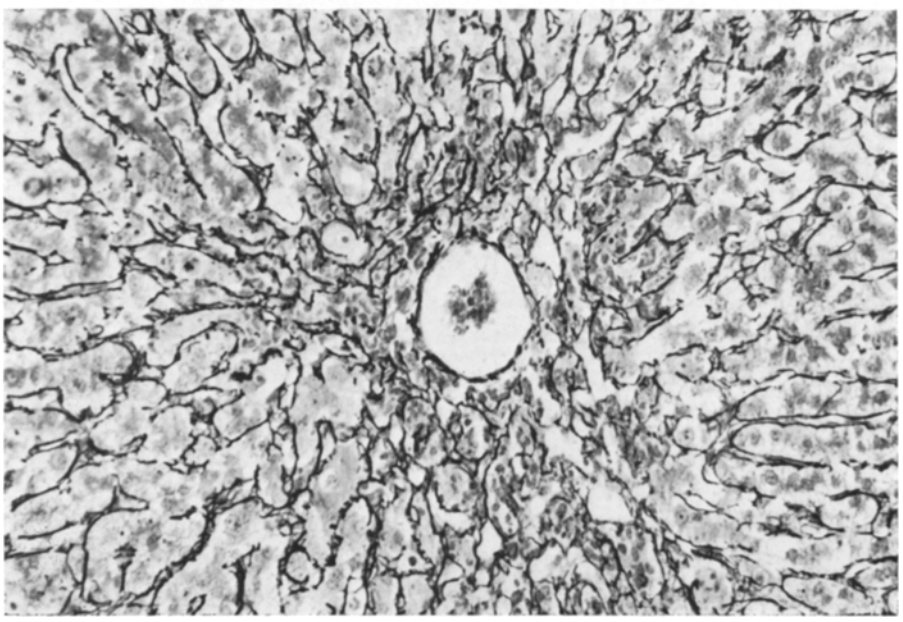

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Fig. 3. Grade III liver damage: centrizonal necrosis after 4 days; most of the necrotic hepatocytes have been removed. H and E, × 225

Fig. 4. Grade III liver damage showing partial collapse of reticulin framework following loss of centrizonal hepatocytes. Foot's silver impregnation, × 225

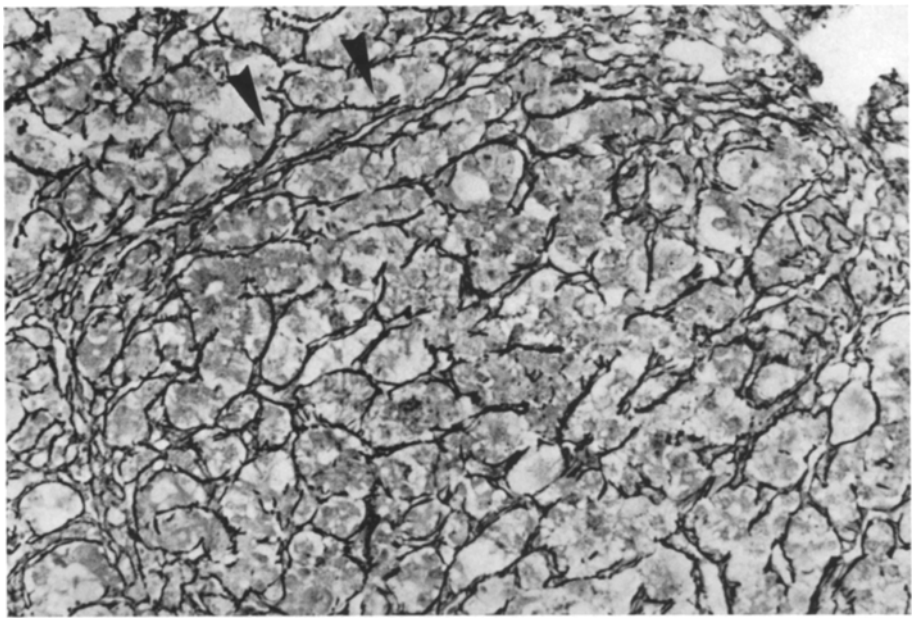


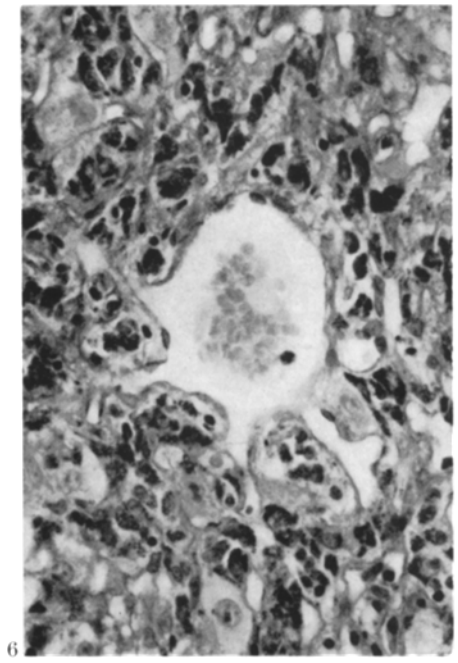
Fig. 5. A passive septum (arrowed) forming a bridge between adjacent centrilobular necrotic zones. Foot's silver impregnation, × 245

cells with ingested lipofuscin, and sparse foci of reticulin condensation. In all 10 rebiopsied patients who had originally sustained severe (grade III) liver damage the central zones and the interzonal bridges were found to have been reconstituted, but all showed some minor residual changes (Fig. 10). Only one patient showed mild centrilobular fibrosis and reticulin scarring, but there was nothing to suggest that this process was progressive (Fig. 11).

Discussion

Pathogenesis of Liver Damage

Liver-cell necrosis after paracetamol overdose is mediated by a chemically active intermediate metabolite of the drug, which combines irreversibly with hepatic macromolecules. After therapeutic doses of paracetamol (0.5 to 1 g orally up to five times daily) the metabolite is conjugated with hepatic glutathione to form nontoxic mercapturates. When the dose is sufficiently large to deplete the reserves of hepatic glutathione, the excess metabolite remains free to exert harmful effects leading to cell death (Jollow et al., 1973; Mitchell and Jollow, 1975). Further studies by these workers on experimental animals showed a dose threshold for paracetamol-induced centrilobular necrosis which correlated well with the dose required to deplete hepatic glutathione. This group also studied the kinetic aspects of the relationship between the rate of formation of the metabolite and the capacity of the liver cells to detoxify it. In healthy human subjects



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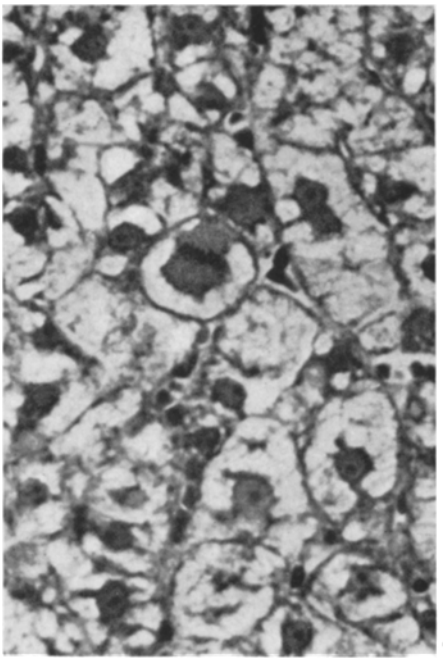

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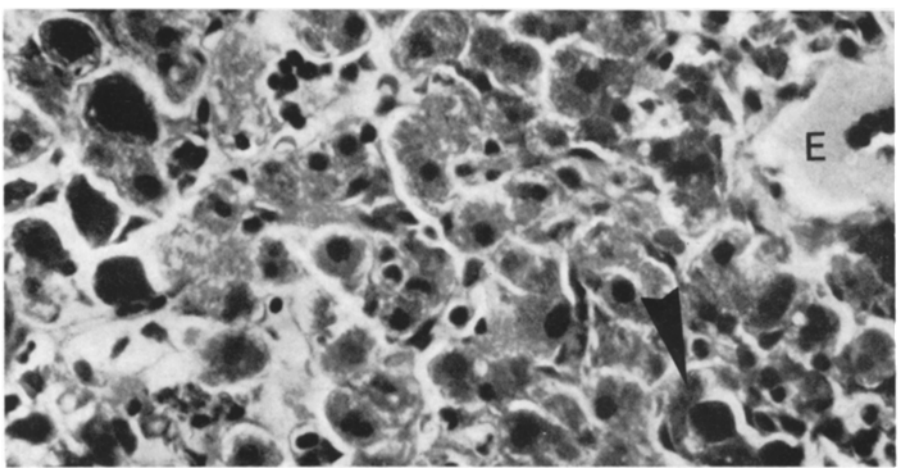

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Fig. 6. Pigment filled Kupffer cells in centrilobular necrotic zone. PAS after amylase digestion, × 365

Fig. 7. Vacuolation of surviving hepatocytes in liver with grade III damage. Note dividing cell in metaphase. PAS after amylase digestion, × 545

Fig. 8. Variant of centrizonal necrosis with irreversibly damaged hepatocytes still retaining their individual identity; deeply eosinophilic cytoplasm contains yellowish granules; nuclei becoming pyknotic. Some cells (top left) undergoing shrinkage necrosis to form acidophil bodies (example arrowed). Efferent venule (*E*) H and E, × 455

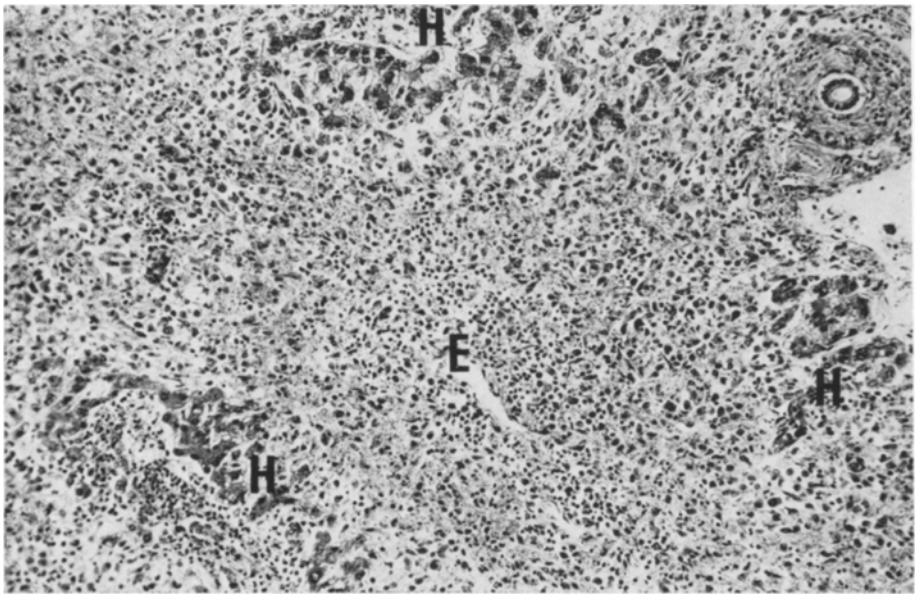

Fig. 9. Subtotal lobular necrosis of liver in a fatal case. Efferent venule (*E*); altered surviving hepatocytes (*H*); heavy infiltration of inflammatory cells in necrotic region. H and E, × 100

paracetamol is rapidly absorbed from the gastrointestinal tract and peak plasma levels are attained in half to one hour. Within seven hours of ingestion of a nontoxic dose paracetamol is normally cleared from the circulation.

Dose-Damage Relationship

We have been unable to establish any close relationship between the severity of the histopathological changes in the liver and the stated or estimated size of the paracetamol overdose. We do not exclude the possibility that there may be a relationship and there are several factors which could cause it to be obscured. Information from the patients as to the number of tablets consumed may be inaccurate or unreliable and in any event the size of the swallowed dose may have been reduced by vomiting. Because only one estimation of plasma paracetamol level was made, and because the blood sample was taken at times which ranged from 2 to 48 hours after ingestion (depending on the speed of admission to hospital) it was not possible to extrapolate or interpolate the results to a standard time interval (James et al., 1975). The attempt by Prescott et al. (1971) to correlate the plasma-paracetamol half-life with the extent of hepatic necrosis has little practical clinical application. Nevertheless we have previously shown (James et al., 1975) that levels above an arbitrary line ranging from 400 g/l at 2 h to 30 g/l at 18 h are very frequently associated with severe (grade III) damage whereas blood levels on admission below 120 g/l at 2 h to 20 g/l at 18 h are usually associated with mild (grade I) damage and never with severe damage.

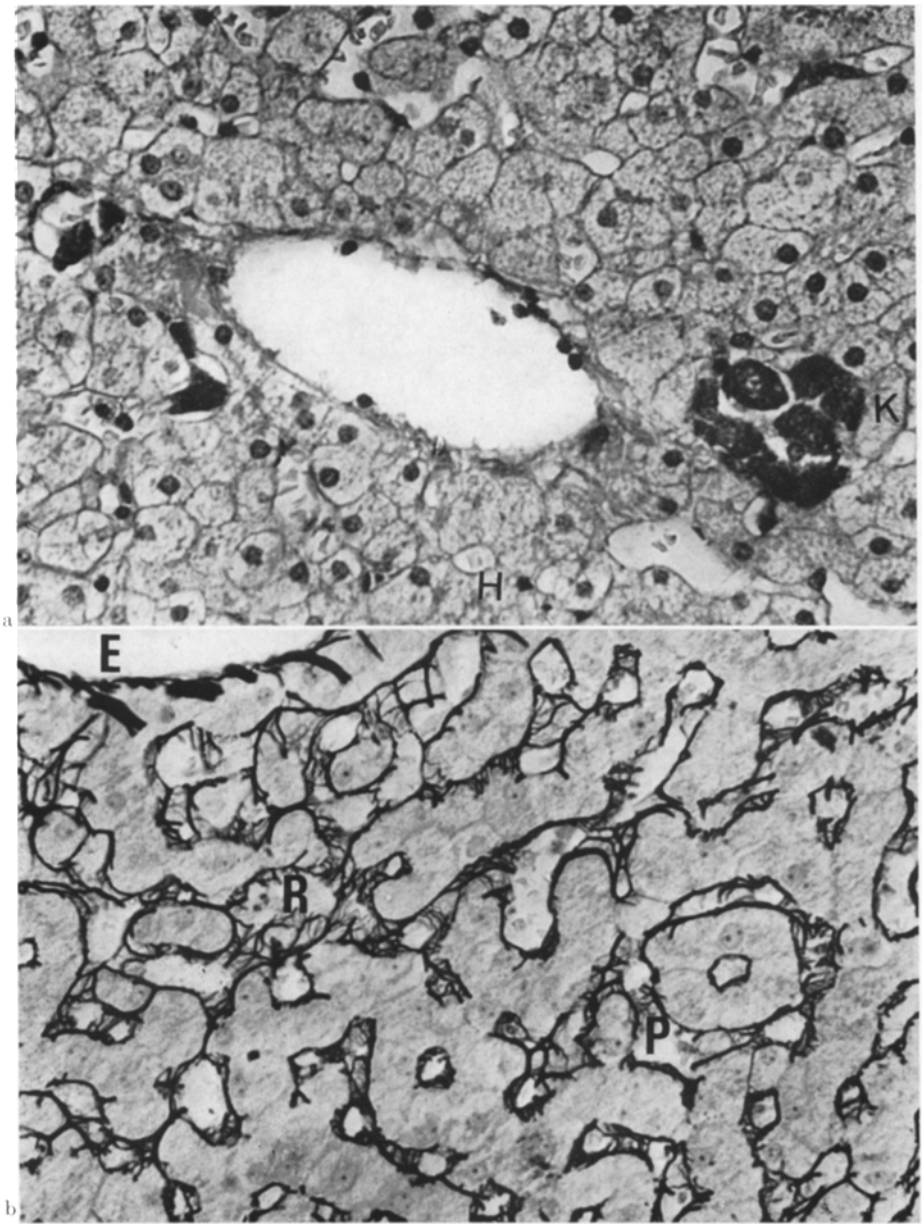

Fig. 10. Restoration of centrilobular necrotic zone with residual abnormalities after 3 months. Pigment filled Kupffer cells (*K*); regenerated hepatocytes devoid of lipofuscin (*H*); thickened liver-cell plates; pseudo-acinus (*P*); slight reticulin condensation (*R*). a PAS after amylase digestion, × 420; b Foot's silver impregnation × 420

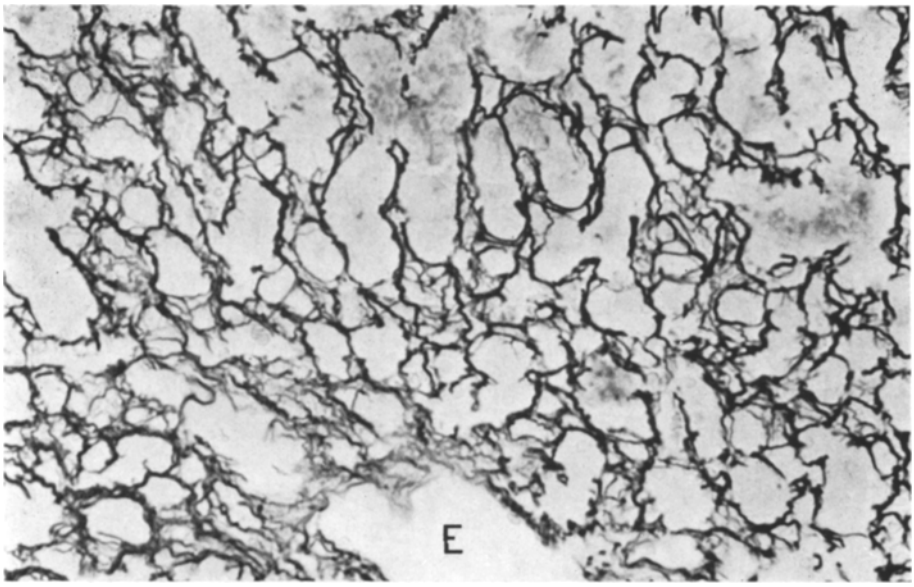

Fig. 11. More pronounced centrilobular fibrosis 3 months after recovery from grade III damage. Efferent venule (*E*). Foot's silver impregnation, × 400

In a clinico-pathological study of 60 patients Clark et al. (1973) concluded that a dose of 13 g of paracetamol is sufficient to cause appreciable liver damage and that 25 g may be fatal. Twenty-seven of our patients admitted to taking 25 g or more and 21 of these (including 5 fatal cases) sustained severe liver damage. On the other hand several patients who took a considerably larger overdose had only moderately severe (grade II) liver damage.

Centrizonal Necrosis

It should be pointed out that the type of centrizonal necrosis seen in severe paracetamol poisoning is entirely non-specific and is common to liver injury induced by a variety of agents (Beckert, 1975). Accounts of the earliest morphological changes in paracetamol-induced liver damage are mostly based on studies in rats (Dixon et al., 1975a) and other experimental animals. Hamsters and mice appear to be especially susceptible and the threshold dose for liver damage in hamsters has been established as 200 mg/kg (Potter et al., 1974). Above that threshold the incidence and severity of liver necrosis were dose dependent. The type and extent of liver damage in our series of patients is in keeping with the threshold theory of Jollow et al. (1973). The salient difference between grades I and II damage, and grade III damage is the occurrence of centrizonal necrosis. Why some cases with grade III damage should be distinguished by acidophilic degeneration of centrizonal hepatocytes instead of the more usual hepatocytolytic necrosis remains unexplained. The vulnerability of this central zone is doubtless a function of the particular enzymatic and metabolic activities of the centrilobular hepatocytes. We have not attempted to formally quantify the extent of the zonal necrosis. Dixon et al. (1975b) found that arbitrary grading of paracetamol-induced liver necrosis in the rat correlated well with a point counting technique

or with the use of an image-analysis computer, but in man significant comparisons could be made only if multiple biopsy samples were available from each patient.

Follow-Up Study

Only one of the twenty patients rebiopsied after three months had anything more than minor residual changes in the liver. The exception was a patient with initially severe damage who showed persistent centrizonal condensation of reticulin with scarring. A similar type of mild residual hepatic fibrosis was reported by Clark et al. (1973) in 2 out of 11 patients rebiopsied 3 months after the overdose. Even in such a highly selected group of very seriously ill patients as that reported by Portmann et al. (1975) the degree of histological recovery was remarkable.

All of the patients in our follow-up group appeared to have made a complete clinical recovery and liver function tests had reverted to normal except for the serum total bile acids (Hamlyn et al., 1976). Although the duration of the follow-up period must be extended, there is nothing in the findings so far to suggest that a single episode of acute paracetamol overdose is liable to initiate any form of severe lasting or progressive liver damage.

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References

Beckert, W.: Centrilobular parenchymal necrosis of the liver. Drug-induced lesions of the liver. Zbl. allg. Path. path. Anat. **117**, 219–226 (1975)

Clark, R., Thompson, R. P. H., Borirakchanyavat, V., Widdop, B., Davidson, A. R., Goulding R., Williams, R.: Hepatic damage and death from overdose of paracetamol. Lancet **1973 I**, 66–70

Davidson, D. G. D., Eastham, W. N.: Acute liver necrosis following overdosage of paracetamol. Brit. med. J. **1966 II**, 497–499

Dixon, M. F., Dixon, B., Aparicio, S. R., Loney, D. P.: (a) Experimental paracetamol-induced hepatic necrosis: A light-and-electron-microscope, and histochemical study. J. Path. **116**, 17–29 (1975)

Dixon, M. F., Fulker, M. J., Walker, B. E., Kelleher, J., Losowsky, M. S.: (b) Serum transaminase level after experimental paracetamol-induced hepatic necrosis. Gut **16**, 800–807 (1975)

Hamlyn, A. N., Douglas, A. P., James, O. F. W., Lesna, M., Watson, A. J.: Liver function and structure in survivors of paracetamol (acetaminophen) poisoning: a follow-up study of serum bile acids and liver histology. (In press)

James, O., Lesna, M., Roberts, S. H., Pulman, L., Douglas, A. P., Smith, P. A., Watson, A. J.: Liver damage after paracetamol overdose. Comparison of liver function tests, fasting serum bile acids, and liver histology. Lancet **1975II**, 579–587

Jollow, D. J., Thorgeirsson, S. S., Potter, W. Z., Hashimoto, M., Mitchell, J. R.: Acetaminophen-induced hepatic necrosis. VI. Metabolic disposition of toxic and non toxic doses of acetaminophen. Pharmacology **12**, 251–271 (1974)

Kerr, J. F. R.: Shrinkage necrosis: a distinct mode of cellular death. J. Path. **105**, 13–20 (1971)

Mitchell, J. R., Jollow, D. J.: Metabolic activation of drugs to toxic substances. Gastroenterology **68**, 392–410 (1975)

Portmann, B., Talbot, I. C., Day, D. W., Davidson, A. R., Murray-Lyon, I. M., Williams, R.: Histopathological changes in the liver following a paracetamol overdose: a correlation with clinical and biochemical parameters. J. Path. **117**, 169–183 (1975)

Potter, W. Z., Thorgeirsson, S. S., Jollow, D. J., Mitchell, J. R.: Acetaminophen-induced hepatic necrosis. V. Correlation of hepatic necrosis, covalent binding and glutathione depletion in hamsters. Pharmacology **12**, 129–143 (1974)

Prescott, L. F., Wright, N., Roscoe, P., Brown, S. S.: Plasma-paracetamol half-life and hepatic necrosis in patients with paracetamol overdose. Lancet **1971II**, 519–522

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