THE LANCET

Paracetamol Hepatotoxicity

SERIOUS toxicity is almost unheard of when paracetamol (4-hydroxyacetanilide, N-acetyl-*p*-aminophenol, acetaminophen) is used sensibly in therapeutic doses. However, this apparent safety is deceptive since in overdosage it causes acute centrilobular hepatic necrosis which may be fatal,[1–4] and paracetamol poisoning is now one of the commonest causes of hepatic failure in Britain.[5] Apart from pallor, nausea, and vomiting there are no abnormal physical signs in the first 12–24 hours after overdosage, and the true gravity of the situation may not become apparent until fulminant hepatic failure supervenes 4–6 days after ingestion. In patients who develop liver damage and recover there is often an initial mild metabolic acidosis which is followed by mild transient jaundice, prolongation of the prothrombin-time, and striking increases in plasma aspartate and alanine aminotransferase activity (G.O.T., G.P.T.) with little or no rise in alkaline phosphatase. The G.P.T. and G.O.T. may go as high as 10 000 units/l, presumably reflecting the release of enzymes from a large mass of acutely damaged and necrotic liver cells. Liver-function tests usually return to normal in 1–2 weeks with full recovery, but the prognosis is very poor if hepatic failure occurs. In one series of 60 selected patients admitted to a liver unit the mortality-rate was 20%.[4] On histological examination, liver-biopsy specimens show acute hepatic necrosis with collapse of the reticulin framework[4 5] but no subsequent evidence of cirrhosis.

Conventional tests of liver function are of limited prognostic value during the first 48 hours after overdosage because the greatest changes are delayed for 3–5 days. The outlook is said to be poor if the bilirubin and prothrombin-time ratio exceed 4 mg/dl and 2·2, respectively, by the 3rd–5th day,[4] and in one report G.O.T. values above 400 units/l were associated with severe histological liver damage.[5] The quantity of paracetamol said to have been taken by the patient is often wildly inaccurate and there is only one reliable way to assess the severity of intoxication when the patient is first seen—namely, to measure the plasma-paracetamol concentration.[2 6 7] The level on admission can be interpreted only if the time of ingestion is known, but the plasma-paracetamol half-life can be used as a built-in liver-function test to determine the prognosis within a few hours of admission. Although there is great individual variation in susceptibility to the hepatotoxicity of paracetamol,[3] plasma concentrations above 250–300 μg/ml at 4 hours and above 50 μg/ml at 12 hours after ingestion, or half-life values exceeding 4 hours, are usually associated with liver damage.[2 6 7]

Until lately, the mechanisms of paracetamol-induced hepatic necrosis were unknown and there was no effective treatment other than removal of unabsorbed drug by gastric lavage. Administration of corticosteroids, antihistamines,[8] and heparin,[9] forced diuresis,[10] hæmodialysis,[11] and charcoal-column hæmoperfusion[12] have all been tried but none of these measures prevent liver damage in severely poisoned patients. Now that MITCHELL and his colleagues[13–17] have elucidated the biochemical basis of paracetamol hepatotoxicity the position has changed radically. In a series of ingenious experiments they have shown that, although most of a dose of paracetamol is conjugated with glucuronide and sulphate, a small fraction is converted by liver microsomal mixed-function oxidase to a highly reactive intermediate alkylating metabolite. With a therapeutic dose this potentially toxic metabolite is rendered harmless within the hepatocyte by preferential conjugation with glutathione and is subsequently excreted in the urine as cysteine and mercapturic-acid conjugates. With a large toxic dose, however, hepatic glutathione is depleted and the excess metabolite is free to combine irreversibly with vital cell constituents, causing cell damage and death. All the observed facts fit together surprisingly well. The extent of irreversible (covalent) binding of paracetamol to liver-cell proteins is directly related to the degree of hepatic necrosis,[14 15] stimulation of hepatic microsomal enzymes with phenobarbitone or 3-methylcholanthrene enhances paracetamol hepatotoxicity in most species while inhibition with piperonyl butoxide protects,[13] and previous depletion of hepatic glutathione with diethylmaleate[16] or feeding a low-protein diet[18] greatly potentiates hepatotoxicity. Furthermore, the striking species differences in susceptibility to

1. Davidson, D. G. D., Eastham, W. N. *Br. med. J.* 1966, iii, 497.
2. Prescott, L. F., Wright, N., Roscoe, P., Brown, S. S. *Lancet*, 1971, i, 519.
3. Prescott, L. F., Wright, N. *Br. J. Pharmac.* 1973, **49**, 602.
4. Clark, R., Thompson, R. P. H., Borirakchanyavat, V., Widdop, B., Davidson, A. R., Goulding, R., Williams, R. *Lancet*, 1973, i, 66.
5. James, O., Lesna, M., Roberts, S. H., Pulman, L., Douglas, A. P., Smith, P. A. Watson, A. J. *ibid.* 1975, ii, 579.
6. Prescott, L. F., Newton, R. W., Swainson, C. P., Wright, N., Forrest, A. R. W., Matthew, H. *ibid.* 1974, i, 588.
7. Stewart, M. J., Simpson, E. *Ann. clin. Biochem.* 1973, **10**, 173.
8. Maclean, D., Peters, T. J., Brown, R. A. G., McCathie, M., Baines, G. F., Robertson, P. G. C. *Lancet*, 1968, ii, 849.
9. Gazzard, B. G., Clark, R., Borirakchanyavat, V., Williams, R. *Gut*, 1974, **15**, 89.
10. Rose, P. G. *Br. med. J.* 1969, i, 381.
11. Farid, N. R., Glynn, J. P., Kerr, D. N. S. *Lancet*, 1972, ii, 396.
12. Gazzard, B. G., Willson, R. A., Weston, M. J., Thompson, R. P. H., Williams, R. *Br. J. clin. Pharmac.* 1974, **1**, 271.
13. Mitchell, J. R., Jollow, D. J., Potter, W. Z., Davis, D. C., Gillette, J. R., Brodie, B. B. *J. Pharmac. exp. Ther.* 1973, **187**, 185.
14. Jollow, D. J., Mitchell, J. R., Potter, W. Z., Davis, D. C., Gillette, J. R., Brodie, B. B. *ibid.* p. 195.
15. Potter, W. Z., Davis, D. C., Mitchell, J. R., Jollow, D. J., Gillette, J. R., Brodie, B. B. *ibid.* p. 203.
16. Mitchell, J. R., Jollow, D. J., Potter, W. Z., Gillette, J. R., Brodie, B. B. *ibid.* p. 211.
17. Mitchell, J. R., Thorgeirsson, S. S., Potter, W. Z., Jollow, D. J., Keiser, H. *Clin. Pharmac. Ther.* 1974, **16**, 676.
18. McLean, A. E., Day, P. A. *Biochem. Pharmac.* 1975, **24**, 37.

paracetamol correlate exactly with the activity of the metabolic pathway which forms the mercapturic-acid conjugate. Thus, highly resistant species such as the rat convert a relatively small proportion of a low dose of paracetamol to the mercapturic-acid conjugate, whereas the highest conversion was observed in mice and hamsters—the most susceptible species.[19] This mechanism probably explains why chronic alcoholics and patients who have been taking microsomal inducing drugs (e.g., epileptics) are particularly vulnerable to the hepatotoxicity of paracetamol.[20] Diet is another very important factor, as shown by a thirteen-fold decrease in the L.D.$_{50}$ of paracetamol in rats after a change in diet.[18]

Any form of treatment which selectively inhibited the oxidation of paracetamol to the toxic metabolite, prevented glutathione depletion, or acted as an alternative nucleophile would undoubtedly be effective in the treatment of overdosage. Administration of glutathione itself might seem an obvious solution, but glutathione does not enter cells readily and has no protective action in animals[21] unless given in very large doses.[22] However, precursors such as L-cysteine and L-methionine are very effective in protecting against paracetamol hepatotoxicity in animals,[16 18 23] and L-cysteine rapidly restores depleted glutathione stores to normal.[23] Both these aminoacids are probably effective in man. Cysteamine (β-mercaptoethylamine) is another SH-containing compound which is effective in animals,[17 21 23] and the successful treatment of severe paracetamol poisoning in man with intravenous cysteamine has been reported by PRESCOTT and his colleagues.[6] Liver damage was absent or trivial in patients with very high plasma-paracetamol concentrations who would otherwise have been expected to develop severe liver damage, but the treatment caused nausea, vomiting, and drowsiness. To be effective, cysteamine must be given within 8–10 hours of ingestion of paracetamol and some liver damage may still occur in highly susceptible patients such as alcoholics with very severe poisoning who are treated late.[24] Not only is administration of cysteamine and the S-containing aminoacids later than 10–12 hours after overdosage useless, but it is also fraught with danger since they cannot be metabolised by a damaged liver and may precipitate hepatic coma. Treatment must therefore be restricted to patients seen early with plasma-paracetamol concentrations high enough to put them at risk of moderate to severe liver damage.[6]

How does cysteamine work? Unlike L-cysteine and L-methionine it is not a physiological precursor of glutathione, and it does not seem to react directly with the toxic paracetamol metabolite to form a cysteamine conjugate. It increases hepatic glutathione after experimental paracetamol poisoning but is not as effective in this respect as L-cysteine.[23] Could cysteamine inhibit the oxidation of paracetamol to the toxic metabolite? This is a possibility since it inhibits the 11β-hydroxylation of steroids[25] and the oxidation of hexobarbitone[26] and isolated-rat-liver perfusion studies suggest that it reduces the oxidation of paracetamol.[27] Cysteamine is readily oxidised to cystamine (with which it is probably in equilibrium in vivo) and both compounds strongly inhibit cytochrome P-450 reductase[28 29]—the enzyme which is thought to limit the rate of microsomal drug oxidation. Vitamin E (α-tocopherol), another antioxidant, also reduces the severity of paracetamol-induced liver damage in rats.[30] Cysteamine and cystamine are radioprotective agents,[31] and other thiols (including L-cysteine) protect against the effects of ionising radiation and radiomimetic alkylating agents.[32] They may act by trapping free radicals which might otherwise attack vulnerable sulphydryl groups and disulphide bonds of essential proteins and enzymes. Furthermore, it has been known for years that the toxicity of many heavy metals is related to their great affinity for SH groups and that thiols such as glutathione, L-cysteine, and dimercaprol can protect against arsenic, mercury, and lead poisoning. It begins to look as though some of the pieces of the puzzle might fit together to give a common mechanism for some types of cell damage caused by ionising radiation, alkylating agents, heavy metals, paracetamol, and many other compounds which are converted to highly reactive metabolites, and a common mechanism of protection by thiols. The following observations are relevant to this hypothesis:

1. Cysteamine and cystamine protect against carbon-tetrachloride hepatotoxicity and this action is thought to be mediated by its microsomal oxidation to the free radical CCl_3.[28 29 33]
2. Diethyldithiocarbamate, an antidote for poisoning with heavy metals such as nickel and thallium, is also a radioprotective agent and protects against carbon-tetrachloride[34] and paracetamol hepatotoxicity.[23]

19. Jollow, D. J., Thorgeirsson, S. S., Potter, W. Z., Hashimoto, M., Mitchell, J. R. *Pharmacology*, 1974, **12**, 251.
20. Wright, N., Prescott, L. F. *Scott. med. J.* 1973, **18**, 56.
21. Gazzard, B. G., Hughes, R. D., Portmann, B., Dordoni, B., Williams, R. *Br. J. exp. Path.* 1974, **55**, 601.
22. Benedetti, M. S., Louis, A., Malnoe, A., Schneider, M., Lam, R., Kreber, L., Smith, R. L. *J. Pharm. Pharmac.* 1975, **27**, 632.
23. Strubelt, O., Siegers, C. P., Schutt, A. *Archs. Toxicol.* 1974, **33**, 55.
24. Scott, C. R., Stewart, M. J. *Lancet*, 1975, i, 452.
25. Flemming, K., Heierhass, B., Seydewitz, V. *Biochem. Pharmac.* 1973, **22**, 1241.
26. Liebecq., C., Bacq, Z. M., Thomou, A. *ibid.* 1964, **13**, 51.
27. Harvey, F., Goulding, R. *Lancet*, 1974, ii, 1082.
28. Castro, J. A., de Ferreyra, E. C., de Castro, C. R., Gomez, M. I. D., D'Acosta, N., de Fenos, O. M. *Tox. appl. Pharmac.* 1973, **24**, 1.
29. Castro, J. A., de Castro, C. R., de Fenos, O. M., de Ferreyra, E. C., Gomez, M. I. D., D'Acosta, N. *Pharmac. Res. Comm.* 1972, **4**, 185.
30. Walker, B. E., Kelleher, J., Dixon, M. F., Losowsky, M. S. *Clin. Sci. mol. Med.* 1974, **47**, 449.
31. Straube, R. L., Patt, H. M. *A. Rev. Pharmac.* 1963, **3**, 293.
32. Connors, T. A., Jeney, A., Jones, M. *Biochem. Pharmac.* 1964, **13**, 1545.
33. Castro, J. A., Cignoli, E. V., de Castro, C. R., de Fenos, O. M. *ibid.* 1972, **21**, 49.
34. Lutz, L. M., Glende, E. A. Jr., Recknagel, R. O. *ibid.* 1973, **22**, 1729.

3. Dimercaprol, the more familiar antidote for arsenic and mercury poisoning, has some protective action against paracetamol-induced liver damage.[17]

Perhaps not surprisingly there are discrepancies. D-penicillamine and thioctic acid have great affinity for "sulphydryl seekers" such as mercury and arsenic, yet they do not prevent experimental paracetamol hepatotoxicity.[21 23] Whatever the mechanisms involved, cysteamine, L-cysteine, and L-methionine are probably all effective antidotes for paracetamol poisoning if give early enough, and clinical trials with these and other agents are in progress in several centres.

The hepatotoxicity of paracetamol remains a serious problem, and liver damage has been observed after absorption of as little as 6·2 g[3]—not much more than the recommended maximum daily dosage. If paracetamol was discovered today it would not be approved by the Committee on Safety of Medicines and it would certainly never be freely available without prescription. There have been suggestions that incorporation of vitamin E[30] or L-methionine[18] into the tablets would solve the problem, but this is not really the answer. The hepatotoxicity of paracetamol is abolished by minor structural changes which prevent its oxidation to the toxic metabolite, such as N-methylation or placing the hydroxyl group in the *ortho* rather than the *para* position, to give 2-hydroxyacetanilide instead of 4-hydroxyacetanilide.[19] Surely the time has come to replace paracetamol with an effective analogue which cannot cause liver damage.

e and anti-e

THE severity and chronicity of liver disease associated with HBsAg varies greatly, irrespective of the antigen subtype. In some patients the disease progresses to cirrhosis after HBsAg has disappeared from the serum; in others there is little liver damage despite a chronic HBsAg carrier state. Among suggested factors in this wide spectrum of disease are variations in host response and in virulence of the virus.[1] In 1972, MAGNIUS and ESPMARK[2] described a new antigen/antibody system, which they designated "e", in some HBsAg-positive sera. The e antigen appeared as a precipitin line between two HBsAg-positive sera and did not show a line of identity with HBsAg, indicating that the two antigens are immunologically distinct; they also have different physiochemical properties.[3]

Several investigations now suggest that persistence of e in serum may have prognostic implications in patients with hepatitis-B infection; and the antigen may also be related to infectivity. NIELSEN and others[4] detected e antigen significantly more frequently in patients with chronic hepatitis (58% positive) or cirrhosis (31% positive) than in those with acute hepatitis (10% positive). Furthermore, when present in acute hepatitis, e antigen indicated a poor prognosis, progression to chronic liver disease being common. Other differences were an increased proportion of anicteric cases and lower mean serum bilirubin and aspartate-transaminase levels in the e-positive patients, but a more aggressive histological picture with loss of the limiting plate and striking Kupffer-cell proliferation. MAGNIUS et al.[5] likewise found the e antigen more frequently in HBsAg carriers with the histological finding of chronic persistent or chronic aggressive hepatitis; and additional observations circumstantially link e antigen with infectivity. It was commonly present in patients undergoing maintenance hæmodialysis—a group notorious for their infectivity—and serial investigations on five patients with acute hepatitis showed that e antigen appeared in the serum during the early incubation period, at about the time of appearance of HBsAg and before the rise in serum-transaminase. Most of twelve healthy HBsAg carriers who had donated blood without producing clinical hepatitis possessed antibody to the antigen, which suggests that when anti-e is present the carriers may no longer be infectious.

Several subsequent investigations have confirmed the association of e antigen with chronic liver disease. EL SHEIKH and associates[6] found e antigen in 15 out of 28 patients with chronic aggressive hepatitis, but less frequently in chronic persistent hepatitis and not at all in healthy HBsAg carriers. Two articles in this issue extend these observations and include a more detailed assessment of the import of e antibody. Dr ELEFTHERIOU and associates (p. 1171) confirm that e antigen occurs only in patients with hepatitis-B infection. They could not find e antigen in patients with acute hepatitis who made an uneventful recovery, but it was often present in patients with chronic active liver disease. By contrast, e antigen was only rarely detected in chronic inactive liver disease or in carriers with minor histological abnormalities in the liver. Such patients, however, have a high incidence of antibody to e antigen. Similarly, Dr FEINMAN and others (p. 1173), studying seventy HBsAg-positive carriers, found that those with anti-e had only minor histological changes, whereas the two patients with detectable e antigen had the most severe disturbances of liver function and structure. Some patients, however, had persist-

1. *Lancet*, 1975, ii, 16.
2. Magnius, L. O., Espmark, J. A. *J. Immun.* 1972, **109**, 1017.
3. Magnius, L. O. *Clin. exp. Immun.* 1975, **20**, 209.
4. Nielsen, J. O., Dietrichson, O., Juhl, E. *Lancet*, 1974, ii, 913.
5. Magnius, L. O., Lindholm, A., Lundin, P., Iwarson, S. *J. Am. med. Ass.* 1975, **231**, 356.
6. El Sheikh, N., Woolf, I. L., Galbraith, R. M., Eddleston, A. L. W. F., Dymock, I. W., Williams, R. *Br. med. J.* 1975, iv, 252.