Biochemical Pharmacology, Vol. 32, No. 18, pp. 2733–2738, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + 0.00

IMMUNE RESPONSES TO CHLORPROMAZINE IN RATS

DETECTION AND RELATION TO HEPATOTOXICITY

BARBARA M. MULLOCK,* DEREK E. HALL,† LYNNE J. SHAW and RICHARD H. HINTON
Robens Institute of Industrial and Environmental Health and Safety, and †Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH, U.K.

(*Received* 15 *March* 1983; *accepted* 21 *April* 1983)

Abstract—It has frequently been suggested that the jaundice which occurs in a small percentage of human patients following treatment with chlorpromazine is due to a hypersensitivity reaction. It has, however, proved impossible to obtain an animal model for this condition. We now show that oral administration of chlorpromazine at 25 mg/kg per day to Wistar albino rats results in formation of both humoral and secretory antibodies to chlorpromazine. We also demonstrate that the severity of the hepatic changes observed in chlorpromazine-fed animals (periportal glycogen loss and centrilobular fatty change) is enhanced by preimmunization of the rats via the gut-associated lymphoid tissue with a chloropramizine–protein conjugate. There was, however, no correlation between the titre of either serum or biliary antibodies in individual animals and the degree of liver damage. Our results therefore suggest than an immune mechanism is indeed implicated in chlorpromazine toxicity but show clearly that toxic symptoms are not a simple consequence of the formation of anti-chlorpromazine antibodies.

Jaundice, with the clinical signs of cholestasis, is a side effect regularly seen in a small proportion of human subjects treated with a number of structurally unrelated therapeutic agents [1]. No reliable animal models are yet available to evaluate which drugs have cholestatic potential [2]. A widely recognized, commonly used drug which produces cholestasis in man is chlorpromazine (2-chloro-*N*,*N*-dimethyl-10H-phenothiazine-10-propan-amine). Cholestatic jaundice is observed in between 0.18 and 1.9% of patients undergoing treatment [3] normally 2–4 weeks after commencement of therapy. Liver biopsy specimens typically show a marked cellular reaction in the portal zone but only slight changes in the hepatocytes [4]. It is generally believed that this type of jaundice is due to a hypersensitivity reaction [1] as the reaction is not dose-dependent [5] and desensitization may be achieved by repeated administration in individuals who have exhibited jaundice [6].

If the cholestasis observed in some human patients is indeed due to a hypersensitivity reaction, the failure to obtain a cholestatic response in experimental animals may be due either to animals not forming antibodies to chlorpromazine or to differences in the formation of antibody–antigen complexes explicable by, for example, interspecies differences in biliary excretion or in patterns of metabolism of the drug. In order to test the first hypothesis, that the lack of cholestatic response in rats treated with chlorpromazine is due to the known poor immune response [7] of this species to dietary allergens, we have now examined whether antichlorpromazine antibodies are present in the blood and bile of rats fed chlorpromazine, and whether pre-immunization with chlorpromazine affects the response of animals to the drug. In the latter experiment the animals were immunized by injection into Peyer's patches, a route which maximizes the IgA‡ element in the response [8]. This approach was chosen because the most pronounced histological changes showing hypersensitity to chlorpromazine are in the biliary tracts [4], and IgA is the principal biliary immunoglobulin in both rats and humans [9, 10].

MATERIALS AND METHODS

Induction of a biliary immune response to chlorpromazine. Male Wistar albino rats of the University of Surrey strain were used in all experiments. Chlorpromazine hydrochloride was obtained from Sigma London Ltd. (Poole, Dorset, U.K.). For conjugation to protein chlorpromazine hydrochloride was converted to 7-[or 8-](3-carboxypropionyl)-chlorpromazine and coupled to the desired protein as described by Hubbard *et al.* [11]. Chloropromazine thus coupled to *Limulus polyphemus* haemocyanin (Sigma London Ltd.) was used for most immunizations. Approximately 3 mg of the chlorpromazine–haemocyanin conjugate, precipitated with 2% aluminium hydroxide gel, was injected into the Peyer's patches of the small intestines of rats [8] anaesthetized with Sagatal (May and Baker, Dagenham, U.K.). Priming of the animals with haemocyanin (or in one experiment with the haemocyanin–chlorpromazine conjugate) was carried out by intraperitoneal injection of approximately 2 mg dissolved in physiological buffered saline 9–15 days before the Peyer's patch injections.

Chlorpromazine feeding experiments. In each experiment a group of preimmunized rats fed chlorpromazine hydrochloride (25 mg/kg per day) mixed into the powdered diet were compared with a

* To whom correspondence should be addressed.

‡ Abbreviations: IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; anti-α, antiserum specific for IgA; anti-γ, antiserum specific for IgG; anti-μ, antiserum specific for IgM.

group of untreated animals received the same chlorpromazine-containing diet and with a group of normal animals receiving the powdered diet with no additions. Preimmunized rats were primed with haemocyanin and received haemocyanin–chloropromazine conjugate injections via the Peyer's patches 12 days later. Access to the chlorpromazine-containing diet was begun 2 or 7 days after the Peyer's patch injection as noted in the Results. Tail blood samples were taken at intervals for measurement of plasma IgA and free secretory component by rocket immunoelectrophoresis. The plasmas were also examined for anti-chlorpromazine activity by solid phase radioimmunoassay [8] using a bovine serum albumin–chlorpromazine conjugate and a ^{125}I-labelled anti-(rat) F(ab')$_2$ to detect bound antibody.

Three days before killing rats were returned to normal diet to reduce the amounts of chlorpromazine and metabolites in the bile as these would interfere with the assay of anti-chlorpromazine antibodies. Bile collected in the first 30 min after cannulation was used for radioimmunoassay. After at most 2 hr from the establishment of biliary cannulation, blood samples were taken by heart puncture and samples of liver, and in some cases other organs, were removed, fixed in 10% buffered formalin and stained by standard procedures (H and E, PAS and Oil Red O) for histological examination. Bile and serum samples were examined for anti-chlorpromazine activity as for tail blood samples but using ^{125}I-labelled anti-(rat α), anti-(rat γ) and anti-(rat μ) to detect respectively IgA, IgG and IgM bound to the immobilized chlorpromazine.

RESULTS

Rats fed chlorpromazine for 65–90 days were slightly subdued as compared with controls but gained weight smoothly and showed no evidence of ill health. During the course of feeding, the overall titre of antichlorpromazine antibodies in plasma showed no significant change. At killing, five out of nine animals showed some immune response to chlorpromazine (Table 1). Both IgA and IgG response were detected and antibodies were found in both serum and bile. It is, therefore, clear that prolonged feeding of chlorpromazine can result in the formation of anti-chlorpromazine antibodies in rats.

Immunization of rats via Peyer's patches results in an immune response with a strong involvement of IgA which can readily be detected in bile [8]. Since this is probably the best available model of a response to orally-administered allergens, we attempted to produce an immune response against chlorpromazine using this technique. In initial

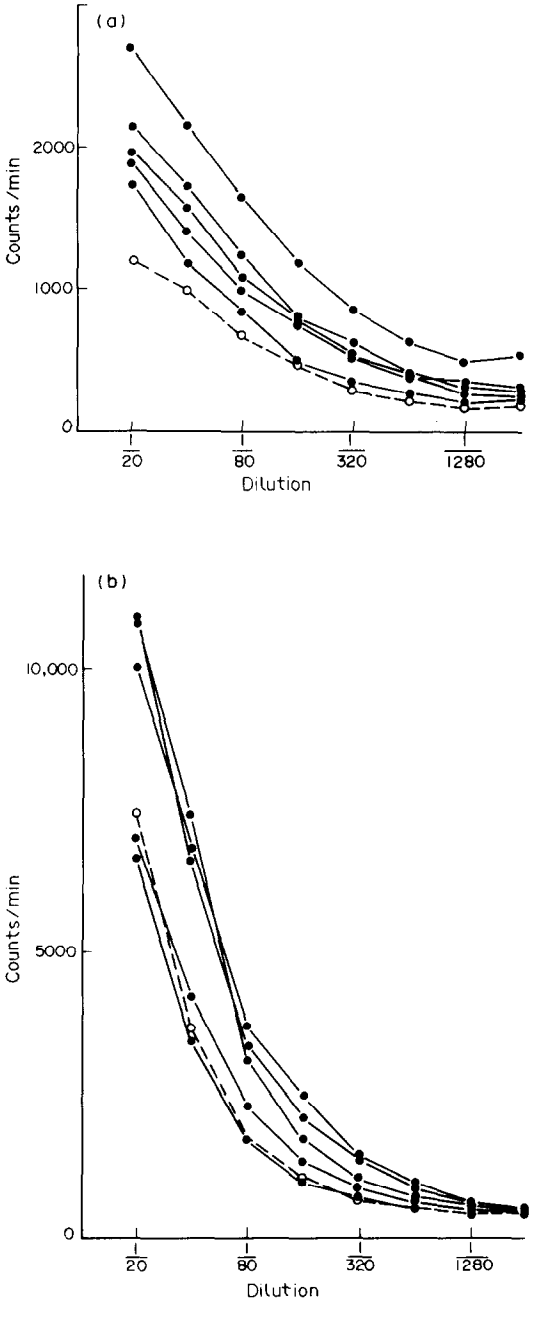


Fig. 1. IgA class anti-chlorpromazine activity in the bile of rats primed with (a) haemocyanin and (b) haemocyanin–chlorpromazine conjugate before Peyer's patch injection of haemocyanin–chlorpromazine conjugate. Bile was collected for radioimmunoassay 5–6 days after Peyer's patch injections. ●——●, Each line is a single experimental rat; ○– –○ are each the mean of three control rats.

Table 1. Anti-chlorpromazine activity—No. of animals higher than highest control

	Serum γ	Bile γ	Serum α	Bile α
Chlorpromazine feeding 65 days	0/2	0/2	1/2	0/2
Chlorpromazine feeding 75 days	1/5	1/5	1/5	1/5
Chlorpromazine feeding 90 days	2/2	1/2	1/2	1/2

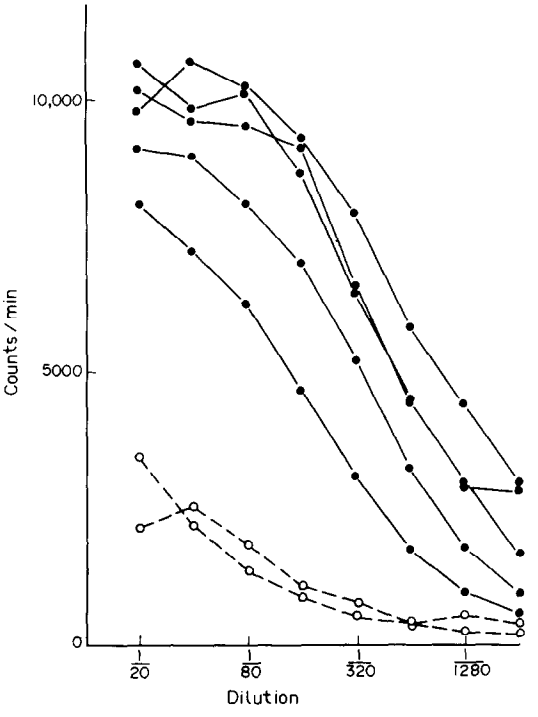


Fig. 2. Anti-chlorpromazine activity in the serum of rats 6 days after Peyer's patch injection of haemocyanin–chlorpromazine conjugate following priming with haemocyanin–chlorpromazine conjugate. Each line represents a single rat. ●——●, Experimental; ○- - -○, control.

experiments, we used chicken IgG conjugates of chlorpromazine aggregated by immune precipitation. Injection of approximately 3 mg of this conjugate into the Peyer's patches resulted in a definite IgA-mediated anti-chlorpromazine response in only one out of ten rats. Since the chicken IgG appeared to conjugate only small amounts of the chlorpromazine derivative, we tried immunization with conjugates to *Limulus polyphemus* haemocyanin, a protein which appeared to couple more chlorpromazine. Immunization with this conjugate gave a significant titre of IgA antibodies in the bile of one out of four animals. If, however, rats were primed by prior intraperitoneal injection of haemocyanin or haemocyanin–chlorpromazine conjugates 9–15 days before immunization of the chlorpromazine conjugate into Peyer's patches, significant titres of IgA antibodies were found in the bile of seven out of ten animals; no difference was observed between animals primed simply with haemocyanin and those primed with haemocyanin–chlorpromazine conjugates (Fig. 1). Anti-chlorpromazine antibodies were also observed in serum (Fig. 2), although in this case we did not investigate the class of antibody formed.

To investigate the effects of preimmunization on the toxicity of chlorpromazine in rats, groups of animals, primed by intraperitoneal injection of haemocyanin, were immunized by injection into Peyer's patches of chlorpromazine conjugated to haemocyanin and then fed diets containing chlorpromazine. The preimmunized animals were compared both with animals simply fed the chlorpromazine-containing diet and with rats fed control diet. Tail blood samples were assayed for IgA and free secretory component which we have found to be sensitive indicators of cholestasis [12]. One preimmunized animal (E7) had some secretory component in plasma after 10 weeks and elevated serum IgA. A second animal (CPZ8) which had simply been fed chlorpromazine showed a trace of secretory component in plasma after 10 weeks but in this case there was no elevation of plasma IgA. None of the animals showed any evi-

Table 2. Changes following chlorpromazine feeding in normal (CPZC) and preimmunized (E) rats

Animal	Days between immunization and start of feeding	Days of feeding	Anti-chlorpromazine antibodies						Liver histology		
			Serum			Bile					
			IgG	IgM	IgA	IgG	IgM	IgA	Periportal glycogen loss	Fatty changes	Increased cellularity of portal tract
CPZC1	—	65	±	±	+	+	−	±	−	−	−
CPZC2	—	65	−	−	−	−	−	−	+	±	+
CPZC3	—	75	−	ND	−	−	ND	−	+	+	+
CPZC4	—	75	−	ND	−	−	ND	−	−	−	+
CPZC5	—	75	−	ND	−	−	ND	−	−	+	+
CPZC6	—	75	−	ND	−	±	ND	−	++	+	+
CPZC7	—	75	+	ND	+	−	ND	+	+	±	−
CPZC8	—	90	+	ND	−	−	ND	−	+	+	−
CPZC9	—	90	+	ND	+	−	ND	+	+	±	−
E1	2	65	+	+	−	−	−	−	+	+	+
E2	2	65	+	+	±	±	−	−	+	−	−
E3	2	65	+	−	−	−	−	−	+	−	−
E4	2	65	+	−	−	−	−	−	++	−	−
E5	7	75	−	ND	−	−	ND	−	−	−	−
E6	7	75	−	ND	−	±	ND	−	±	+	±
E7*	7	75	−	ND	−	+	ND	+	+++†	+	+
E8	7	75	+	ND	−	+	ND	+	+++	++	+
E9	7	75	−	ND	−	+	ND	−	+	±	−

* This animal showed elevated levels of IgA and secretory component in plasma.
† Loss from all areas of liver.

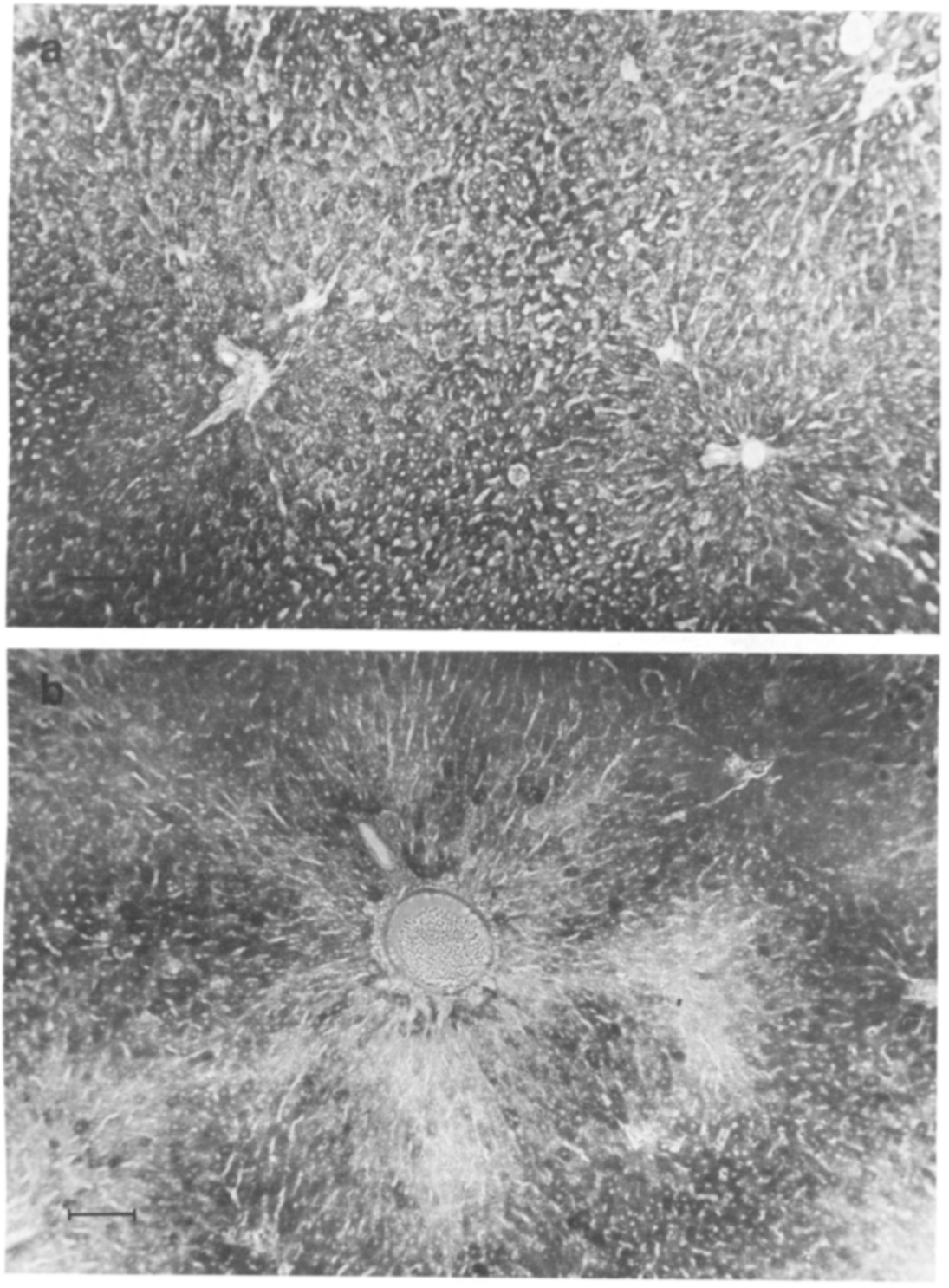
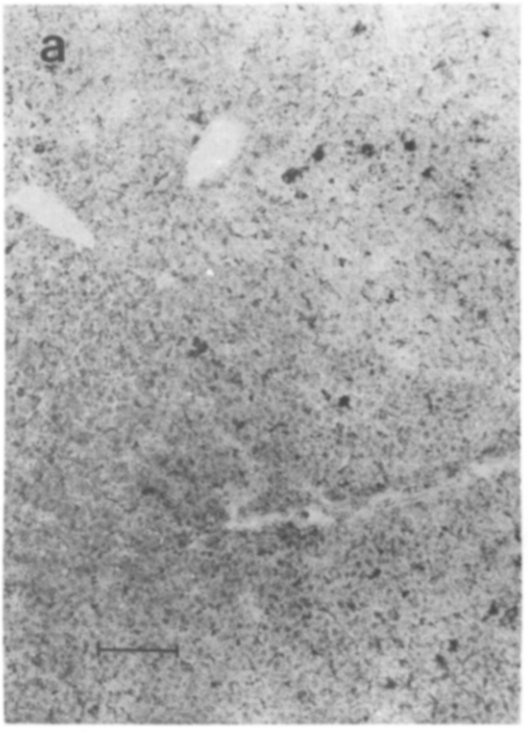


Fig. 3. Periportal glycogen loss after chlorpromazine feeding of an immunized rat. Liver samples were fixed in Rossman's fixative and stained with periodic acid–Schiff. (a) Control; (b) immunized and chlorpromazine-fed animals. The bar represents 20 μm.

dence of ill health throughout the period of the experiment.

No significant gross pathology was observed in any animals at autopsy. Histological examination of kidney, spleen, pancreas, lungs and ileum (including Peyer's patches) from selected animals showed no treatment-related pathology. However, the livers of some animals fed chlorpromazine exhibited periportal loss of glycogen (Fig. 3). Other hepatic changes observed included focal fatty changes, frequently close to central veins (Fig. 4), and increased cellularity of the portal tracts (Fig. 5). The periportal

loss of glycogen was markedly greater in preimmunized animals than in animals simply fed chlorpromazine (Table 2). However, correlation of the results obtained in individual animals showed no significant association between the titre of antibodies to chlorpromazine in either serum or bile and the degree of liver damage (Table 2).



DISCUSSION

For reasons mentioned earlier it has frequently been stated that the cholestasis observed in a small proportion of patients treated with phenothiazine derivatives is due to a hypersensitivity reaction. We have now found that anti-chlorpromazine antibodies can be detected in the serum and bile of rats following feeding of chlorpromazine for 65–90 days. We have also observed that treatment of rats with chlorpromazine results in hepatic changes. The most pronounced change, periportal loss of glycogen, does not seem to have been reported elsewhere either for humans or for experimental animals. Changes in the portal tracts have been observed in human patients suffering from chlorpromazine-induced jaundice [13], although the changes which we observed in rats were much less severe than the changes found in human patients. Fatty changes similar to those which we report have also been observed in dogs and guinea pigs treated with chlorpromazine [3] and have occasionally been reported in human patients [13]. We did not, however, see any histological evidence of biliary stasis in rats although a single, preimmunized animal did show changes in serum IgA and free secretory component which we have found associated with partial biliary obstruction ([12] and unpublished experiments).

As a result of these experiments we conclude that the jaundice observed in a small proportion of patients following treatment with chlorpromazine is unlikely to be a simple consequence of the formation of anti-chlorpromazine antibodies. However, preimmunization of rats with chlorpromazine conjugates did appear to exacerbate some of the effects of

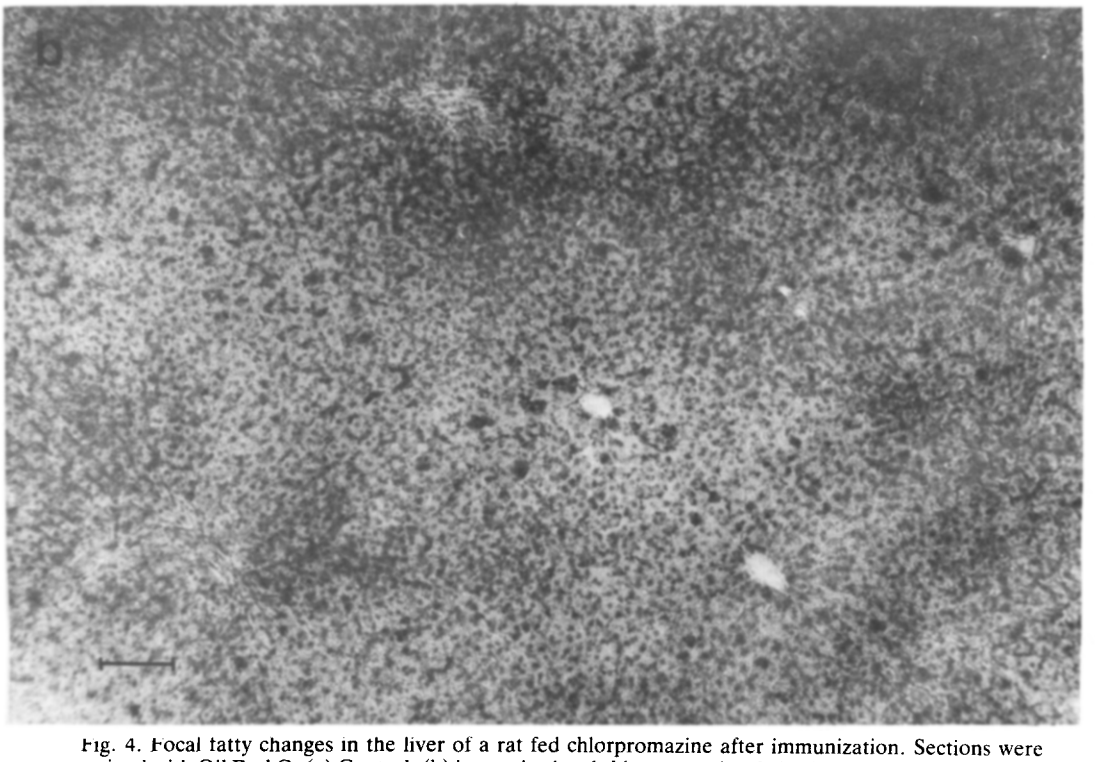


Fig. 4. Focal fatty changes in the liver of a rat fed chlorpromazine after immunization. Sections were stained with Oil Red O. (a) Control; (b) immunized and chlorpromazine-fed animals. The bar represents 20 μm.

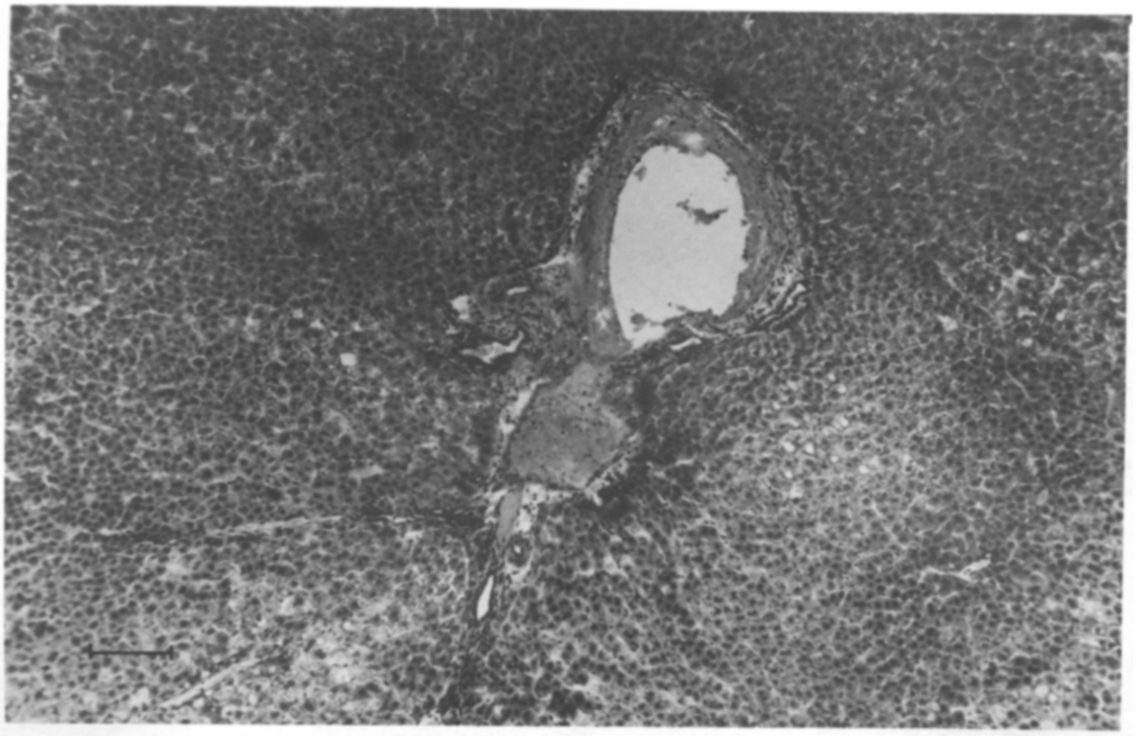


Fig. 5. Increased cellularity of the portal tracts in the liver of a rat fed chlorpromazine after immunization (E8). Haematoxylin and eosin. The bar represents 20 μm.

chlorpromazine and, as discussed in the Introduction, the syndrome in human patients [6] is strongly suggestive of a hypersensitivity reaction. Hence it seems likely that an immune response is implicated in the pathogenesis of chlorpromazine-induced cholestasis, although mere formation of antibodies to chlorpromazine does not automatically result in a cholestatic reaction.

Acknowledgements—We thank Professor J. W. Bridges for useful discussions, Miss. J. Howerth for skilled technical assistance and Dr. J. Peppard of the Chester Beatty Research Institute for much useful help and advice and for the supply of antisera. Financial support was provided by the Medical Research Council.

REFERENCES

1. H. Popper, *A. Rev. Med.* **19**, 39 (1968).
2. F. Leuschner, W. Neumann and R. Hempel, in *Handbook of Experimental Pharmacology* (Eds. G. V. R. Born, A. Farah, H. Herken and A. D. Welch), Vol. 55/1, pp. 225–265. Springer, Berlin (1980).
3. L. Julou, R. Ducrot, P. Ganter, R. Maral, R. Populaire, J. Durel, E. Hintree, J. Myon, S. Pascal and J. Pasquet, *Proc. Eur. Soc. Study Drug Toxicty* **9**, 11 (1968).
4. S. Sherlock and A. Ajdukiewiez, in *Mechanisms in Drug Allergy* (Eds. C. H. Dash and H. E. H. Jones), pp. 103–109. Churchill, London (1972).
5. L. E. Hollister, *Am. J. Med.* **23**, 870 (1957).
7. F. J. Ayd, Jr., *J. Neuropsychiat.* **3**, 177 (1982).
7. H. C. Thomas and D. Parrott, *Immunology* **27**, 631 (1974).
8. J. V. Peppard, E. Orlans, E. Andrews and A. W. R. Payne, *Immunology* **45**, 467 (1982).
9. T. B. Tomasi, *A. Rev. Med.* **21**, 281 (1970).
10. E. Orlans, J. V. Peppard, A. W. R. Payne, B. M. Fitzharris, B. M. Mullock, R. H. Hinton and J. G. Hall, *Ann. N.Y. Acad. Sci.* (in press).
11. J. W. Hubbard, K. K. Midha, I. J. McGilveray and J. R. Cooper, *J. pharm. Sci.* **11**, 1563 (1978).
12. J. Woolcy, B. M. Mullock and R. H. Hinton, *Clin. chim. Acta* **92**, 381 (1979).
13. A. E. Read, C. V. Harrison and S. Sherlock, *Am. J. Med.* **31**, 2249 (1961).