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Evaluation of isoniazid-associated hepatitis by immunological tests

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SUMMARY

In a retrospective study of patients developing hepatitis or persistent serum glutamic oxaloacetic transaminase (SGOT) elevations while receiving isoniazid, it was found that the lymphocyte transformation test (LTT) was positive in nineteen cases (95%) in response to stimulation by isoniazid, isonicotinic acid and conjugates of these compounds with human serum albumin. However, no significant amount of antibody against isoniazid was detected in the sera of these patients by a sensitive radioimmunoassay. By contrast, no positive LTT was seen in normal controls or in patients receiving isoniazid without evidence of liver damage, while in patients with transient SGOT abnormalities, the LTT was positive only at the time of liver dysfunction. There was no correlation between the degree of lymphocyte transformation and the severity of liver damage. However, there were differences in the patterns of response to the four stimulatory preparations used. Thus patients with overt hepatitis most frequently responded to isoniazid, while individuals with only SGOT abnormalities showed stimulation in the LTT more often with a conjugate of isonicotinic acid and human serum albumin. It appears, therefore, that the presence of isoniazid-induced liver damage is associated with the presence of cellular hypersensitivity to the drug. The differences in lymphocyte reactivity in the two groups might indicate a potential means of predicting which individuals are at increased risk of developing overt hepatitis when exhibiting evidence of minor liver dysfunction while receiving isoniazid.

INTRODUCTION

It has been reported that evidence of liver dysfunction occurs in 18–20% of patients receiving isoniazid (INH), but, in the majority of cases, the mild elevation of serum glutamic oxaloacetic transaminase (SGOT) returns to normal with continuation of the drug (Bailey *et al.*, 1973, 1974; Beaudry *et al.*, 1974). However, in about 1% of patients, administration of INH results in the development of overt hepatitis (Garibaldi *et al.*, 1972; Maddrey & Boitnott, 1973; Tuberculosis Advisory Committee, 1974). At the present time, there are no means of predicting if a patient with a slightly elevated SGOT will progress to develop frank hepatitis with continuation of the INH. Clinical features are extremely variable, ranging from gastrointestinal symptoms, a viral-like illness or insidious jaundice, to a true hypersensitivity-like reaction with fever, rash and eosinophilia. The high mortality (12·3%) reported recently from the U.S. Public Health Service (Black *et al.*, 1975) suggests that clinical monitoring may be ineffective in predicting serious hepatic injury. It has been suggested that the whole policy of chemoprophylaxis with INH should be re-examined in the light of the serious risks of severe liver damage secondary to the use of this drug (Black, 1974; Comstock & Edwards, 1975; Moulding, 1975). There is, therefore, an urgent need to develop predictive tests of impending liver damage of significant severity, particularly in individuals with only a minimal evidence of liver dysfunction induced by INH.

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Mitchell & Jollows (1975a) have suggested an association between the occurrence of liver damage in patients receiving INH and their ability to rapidly acetylate it, although more recent evidence throws doubt upon this relationship (Dickinson, Bailey & Hirschowitz, 1977). It appears that metabolites of the drug, such as acetylisoniazid and acetylhydrazine (Snodgrass *et al.*, 1974; Mitchell *et al.*, 1975a, 1976), are capable of inducing liver damage in experimental animals. These metabolites are more likely to be produced in fast acetylators (Yard & McKenns, 1962; Peters, Miller & Brown, 1965; Mitchell *et al.*, 1975b) and might be responsible for inducing liver damage, either directly or via immunologically mediated hypersensitivity reactions following an interaction of the metabolites with liver-specific macromolecules.

To explore the latter possibility, the immunological responses to INH and its derivatives were examined in patients with hepatitis induced by INH, in patients receiving INH without complications and in normal subjects not taking this drug. The detection of an immune response to the drug in patients with INH-induced hepatitis might indicate a possible pathogenesis for this condition and suggest a potential predictive test.

MATERIALS AND METHODS

Subjects. All patients in this series had been seen during the period January 1972 to January 1976 at the Health Sciences Centre, Winnipeg, Manitoba, and had been receiving INH chemoprophylaxis for positive conversion of the tuberculin skin test or recent exposure to tuberculosis. The majority of the patients with INH-induced hepatitis (fifteen out of twenty) were found by a retrospective survey of patients' records. Individuals with overt hepatitis had developed an increase in serum bilirubin, alkaline phosphatase and SGOT levels. A second group of patients had had their INH discontinued because of the development of a persistent elevation of the SGOT of more than 100 units on two consecutive occasions, or an elevation of the SGOT to greater than 200 units on one occasion (Bailey *et al.*, 1973). All patients with overt hepatitis or persistently elevated SGOT were hepatitis B antigen (HBsAg) negative by reverse passive haemagglutination assay. Liver biopsy was carried out on only two patients, both of whom showed a patchy subacute hepatic necrosis. In all patients who developed hepatitis while on INH, the abnormal liver function tests rapidly returned to normal when the drug was discontinued. This fact, and the lack of any other demonstrable cause for elevation of the SGOT, alkaline phosphatase or bilirubin, makes it most likely that the hepatic dysfunction was INH-induced. Individuals receiving other potentially hepatotoxic drugs, immunosuppressive drugs or with a history of excessive ethanol intake were excluded from this series. In patients with overt INH-induced hepatitis, because of the retrospective nature of the survey, tests were carried out at varying periods from several weeks up to 2 years after the episode. Most patients were tested on only one occasion. A third group of subjects consisted of patients developing a mild (less than 100 iu) elevation of the SGOT during INH therapy that returned to normal with continuation of the drug. These patients were tested at the time of the development of liver dysfunction, and again 3 months after the return of the liver function to normal.

Three control groups were included: (a) normal controls who had never been given INH; (b) those receiving INH without evidence of liver enzyme changes; and (c) those who had received INH without adverse effects in the recent past. In addition, a small number of patients with HBsAg-positive hepatitis were tested.

Lymphocyte transformation test (LTT). Blood was taken by venipuncture into heparinized syringes (10 units sodium heparin per ml of blood). Mononuclear cells were separated by Ficoll–Isopaque (Lympho-prep, sp. gr. 1·072 g/ml, Nyegaard & Company, Oslo), according to the method of Böyum (1968), and after washing were re-suspended at a cell concentration of $0{\cdot}5 \times 10^6$ cells per ml of RMP I1640 (Gibco, Grand Island, New York) in 2 ml cultures containing 10% AB serum. The lymphocyte cultures were stimulated with (a) INH at 10 μg, 100 μg and 500 μg/ml; (b) isonicotinic acid (INA, the major metabolite of INH) at 10 μg, 100 μg, and 200 μg/ml; and (c) INH–human serum albumin (HSA) conjugates containing thirteen haptenic groups per mol of HSA, prepared as described by Tse *et al.* (1976), at 10 μg, 100 μg and 500 μg/ml. In addition, the response to phytohaemagglutinin (PHA Difco Laboratories, Detroit, Michigan) was assessed using 0·1 ml of a 1:100 dilution of a stock solution per ml of culture. Cultures were incubated in triplicate at 37°C in 5% CO_2 and air for 72 hr for mitogen-stimulated cultures and for 120 hr for antigen-stimulated cultures. At the end of this period, 1·0 μCi of [^{3}H]thymidine (sp. act. 2 Ci/mmol, Amersham–Searle Corporation, Oakville, Ontario) in 0·01 ml was added to each ml of culture and incubation was continued for a further 18 hr.

Cells were harvested on glass fibre filters (Whatman GF/C, Reeve Angel, Clifton, New Jersey) by saline washing followed by 5% trichloracetic acid precipitation. Precipitates were dried after a methanol wash and the radioactivity was assessed by liquid scintillation counting in a Beckman LS 335 counter, using 10 ml of scintillator (3800 ml of toluene with 19 g of 2,5-diphenyloxazole and 0·38 g of *p-bis*-(5-phenyloxazolyl-benzene) with an internal standard. The stimulatory index (SI) is defined as the ratio of counts from mitogen or antigen-stimulated cultures to non-stimulated cultures.

Radioimmunoassay for antibodies to INH. The radioimmunoassay for anti-INH antibodies was done according to the method described by Schwenk *et al.* (1976). Samples were first dialysed against 2 litres of 6 M urea for 24 hr in order to dissociate any antibody–drug complexes that may have been present in the serum as a result of the patients' taking INH at the time

when the serum sample was collected. Further dialysis against phosphate-buffered saline (PBS) was continued for 5 days. Following centrifugation at 7000 rev/min for 30 min to remove any precipitates, 0·2 ml of each serum sample were mixed with 0·2 ml of PBS containing [^{3}H]INH with approximately 3800 ct/min (sp. act. = 1·0 Ci/mmol). All determinations were done in duplicate. After incubation at room temperature for 2 hr, the gamma globulin fraction in each sample was precipitated by the addition of an equal volume of saturated ammonium sulphate. The precipitates were then washed and counted in a scintillation counter. Normal human sera were used as the negative controls, and rabbit anti-INH serum (Tse *et al.*, 1976) was used as positive standard.

RESULTS

Lymphocyte transformation in control subjects

As can be seen in Table 1, there was no significant difference between the maximum SI for control groups 1 and 2 when the lymphocytes of normal individuals not taking INH or of patients taking INH without adverse reaction were exposed *in vitro* to varying concentrations of the INH or INA preparations. In none of the cultures was stimulation by INH, INA or their HSA conjugates (used at different concentrations) demonstrated, although a normal response to PHA was seen. The lymphocytes from patients who had recently discontinued INH (control group 3) showed an overall lower level of stimulation to INH, INA or to HSA conjugates as compared to groups 1 and 2.

TABLE 1. Mean maximum SI for control groups

Control group	Number of subjects	Mean SI (±s.d.)			
		INH	INA	INH–HSA	INA–HSA
Normal (a)	6	1·3±0·23	1·22±0·21	1·25±0·15	1·41±0·28
INH controls					
(b)	8	1·26±0·49	1·09±0·25	1·21±0·43	1·37±0·33
(c)	5	0·79±0·33	0·69±0·45	1·01±0·58	1·1±0·44
Overall mean SI ±s.d.	19	1·13±0·44	1·05±0·34	1·17±0·4	1·32±0·35

(b) Patients taking INH without adverse reaction; (c) patients who recently discontinued INH.

As a criterion for stimulation in the hepatitis group, a stimulatory index of 3 or greater was taken as indicating a positive result (Fig. 1). This is more than four standard deviations (s.d.) from the mean maximum SI for the control subject group.

Lymphocyte transformation in hepatitis controls

In patients with viral hepatitis, i.e. whose sera were positive for hepatitis B antigen, *in vitro* lymphocyte stimulation did not occur with any of the drug preparations used in these experiments (Table 2).

Lymphocyte transformation in patients with INH-induced liver damage

In Table 3, the highest stimulatory index for PHA and for each antigenic and haptenic preparation is given for the lymphocyte cultures of patients with frank hepatitis (group A) or with persistent SGOT elevation (group B). It can be seen that significant stimulation, i.e. more than 4 s.d. from the mean for INH controls, occurred with at least one of these preparations in nineteen out of twenty patients. Stimulation was seen most often with INH (six out of twenty), INH–HSA (five out of twenty) and INA–HSA (nine out of twenty) when these preparations were used at concentrations of 100 μg/ml or greater. There was a significant difference in the levels of lymphocyte stimulation seen in the INH-induced hepatitis and control groups for each antigen or hapten preparation used (for INH, $P<0·01$; INA, $P<0·05$; INH–HSA, $P<0·001$; INA–HSA, $P<0·01$ by the Student's t-test). The variation in ct/min for stimulated triplicate cultures was ±15%. Although the results are expressed as SI, there was no significant difference between the levels of isotope incorporation in the unstimulated cultures in the

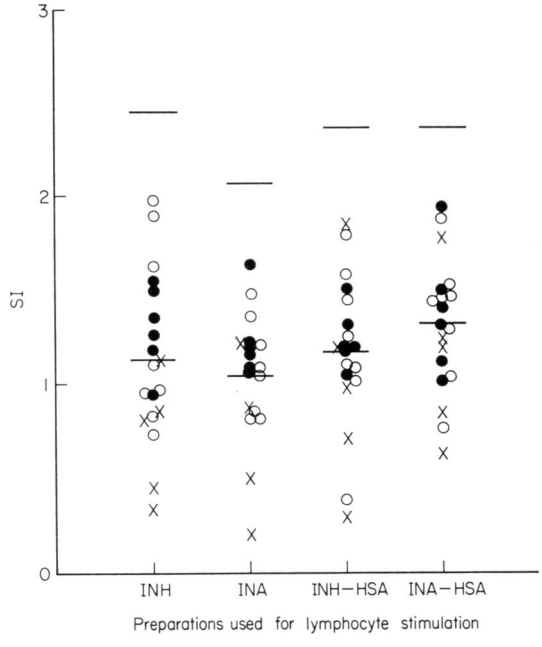

FIG. 1. Results of the lymphocyte transformation test in control subjects using INH, INA, INH–HSA and INA–HSA. Maximum SI and the mean ± 3 s.d. from the mean for each preparation used for lymphocyte stimulation is indicated. Values for normal controls (●) controls receiving INH (○) and controls who recently discontinued INH (×) are shown (horizontal lines show range of mean ± 3 s.d.).

TABLE 2. SI

Patient	Preparations used for lymphocyte stimulation				
	PHA	INH	INA	INH–HSA	INA–HSA
(1)	274	1·8	1·3	< 1·0	< 1·0
(2)	92	1·3	—	< 1·0	—
(3)	70	< 1·0	< 1·0	< 1·0	< 1·0

control and hepatitis groups. In all cases, stimulation was seen most frequently with the highest concentration of antigen or hapten preparation. Individuals tested on more than one occasion retained the same pattern and level of response. In addition, no association existed between the length of time elapsed since the episode of hepatitis and the degree of stimulation in the LTT. Thus patients Nos 1 to 4 and Nos 9 to 15 were tested within 3 months of discontinuing the INH, Nos 5, 6 and 16 to 19 were studied at 6–12 months and Nos 7, 8 and 20 were tested 18–24 months after the INH-induced reaction.

In Table 4, the highest SI for each antigenic and haptenic preparation is given for the lymphocyte cultures of patients with transient SGOT abnormalities, both at the time of liver dysfunction and 3 months after the episode. Significant stimulation occurred with INA–HSA in five out of six patients at the time of the reaction but in none out of five patients at 3 months. In patients with overt hepatitis or persistent SGOT abnormalities (groups A and B), the lymphocyte stimulation in response to INH or INA antigenic and haptenic preparations was detectable up to 2 years after the episode of hepatitis.

Although there appeared to be no differences in the degree of stimulation of patients with frank hepatitis as compared to that of individuals with persistent or transient SGOT elevation, there was a difference in the patterns of response to the four stimulatory preparations used. The former group responded in

TABLE 3. Maximum SI for patients with INH-induced liver damage

Patient	Preparations used for lymphocyte stimulation				
	PHA	INH	INA	INH–HSA	INA–HSA
Group (A)					
(1)	65	4·4*	1·8	1·9	1·3
(2)	131	2·5	1·5	1·4	1·0
(3)	20	3·2*	1·1	1·1	1·0
(4)	66	1·4	< 1·0	< 1·0	3·5*
(5)	108	3·4*	2·1	2·0	1·6
(6)	74	1·4	1·5	1·9	9·1*
(7)	65	8·6*	1·3	4·0*	2·9
(8)	255	8·9*	2·0	1·6	1·2
Group (B)					
(9)	75	5·1*	1·0	1·7	1·8
(10)	6	1·4	< 1·0	2·6	3·4*
(11)	—	1·5	1·3	2·1	6·9*
(12)	43	< 1·0	1·5	3·0*	1·3
(13)	126	2·0	8·4*	3·3*	2·5
(14)	102	1·2	1·1	3·4*	1·2
(15)	10	1·1	1·2	< 1·0	4·6*
(16)	146	2·6	4·0*	2·7	13·8*
(17)	25	1·8	1·9	1·7	6·7*
(18)	105	1·3	3·2*	1·4	1·1
(19)	121	2·5	1·7	2·7	3·1*
(20)	129	1·4	1·5	3·3*	4·0*

Group (A) patients with definite hepatitis; group (B) patients with persistent SGOT elevation.

* SI greater than 4 s.d. from mean for control group.

TABLE 4. SI for patients with INH-induced transient liver dysfunction

Patient	SGOT*	Preparations used for lymphocyte stimulation			
		INH	INA	INH–HSA	INA–HSA
(1)	60	2·9	2·0	1·8	3·2†
	21	0·9	1·1	0·7	1·6
(2)	100	2·0	1·8	2·6	4·8†
	24	1·3	1·9	1·3	2·7
(3)	91	0·8	2·8	0·8	7·4†
	27	n.t.	n.t.	n.t.	n.t.
(4)	49	0·6	1·0	0·7	2·4
	15	0·4	0·8	0·7	1·2
(5)	53	1·1	1·8	1·3	3·0†
	33	0·6	1·2	1·1	1·3
(6)	52	0·8	0·9	1·1	3·6†
	30	0·7	0·7	0·4	2·7

n.t. = Not tested.

* SGOT in iu at time of reaction and at 3 months post-reaction.

† SI greater than 4 s.d. from mean control group.

62·5% of the cases to stimulation with INH, whereas only 5·5% of the latter group showed stimulation with this hapten. In contrast, 66·6% of the patients with only SGOT abnormalities responded to INA–HSA, while 25% of subjects with overt hepatitis reacted to the conjugate.

Radioimmunoassay for specific antibodies against INH

The standard rabbit anti-INH serum used as positive control in the experiment (containing 2·7 mg of antibodies per ml) was able to bind 30% of the total radioactivity of the added [^{3}H]INH at a dilution of 1:2000 and bound 10% of the radioactivity at a dilution of 1:8000. A 1:2000 dilution of the antiserum corresponded to the mid-point of the titration curve for the tracer dose of [^{3}H]INH used in the radioimmunoassay. However, none of the undiluted human serum samples bound more than 9% of the total radioactivity of the added [^{3}H]INH and, therefore, it may be concluded that none of these sera had an appreciable level of anti-INH antibodies as determined by the radioimmunoassay.

DISCUSSION

Although a clear relationship has now been demonstrated between the administration of INH and the development of hepatitis, the mechanisms by which this drug induces liver damage are unclear at present.

It has been shown that INH is metabolized primarily by acetylation (Yard & McKenns, 1962; Peters *et al.*, 1965) and in fast acetylators 94% of the INH is excreted as acetylisoniazid, as compared to 63% in slow acetylators (Snodgrass *et al.*, 1974). In experiments in rats, Snodgrass *et al.* (Snodgrass *et al.*, 1974; Mitchell *et al.*, 1975a) have demonstrated that phenobarbital pre-treatment, which increases the activity of the drug-metabolizing enzymes, potentiates the liver necrosis induced by isoniazid, acetylisoniazid and its metabolite acetylhydrazine. The necrosis is prevented by pretreatment with cobalt chloride, which inhibits the synthesis of cytochrome P-450, the microsomal enzyme system involved in drug metabolism. Similarly, the covalent binding of acetylhydrazine and acetylisoniazid that occurs in liver tissue is enhanced by pre-treatment with phenobarbital and decreased by cobalt chloride pretreatment (Nelson, Snodgrass & Mitchell, 1975). Thus, in part, the liver damage may be induced by INH as a result of the production of toxic metabolites in susceptible individuals such as fast acetylators, although it is unclear why only certain individuals develop this complication.

There are several reports of patients with suspected INH-induced hepatitis who were re-challenged with the drug and rapidly developed a further episode of hepatitis, with associated systemic symptoms suggestive of a hypersensitivity reaction (Merritt & Fetter, 1950; Haber & Osborne, 1959; Cohen, Kaiser & Thomson, 1961; Maddrey & Boitnott, 1973), although this is not a common feature of isoniazid-associated hepatitis. Therefore, the possibility exists that following the induction of transient liver dysfunction by toxic mechanisms, immunogenic moieties consisting of conjugates of INH, or its metabolites, with tissue macromolecules that are capable of sensitizing susceptible individuals might be formed. The pathological findings of INH-induced hepatitis frequently resemble the changes seen in acute viral hepatitis (Moss, Lewis & Knauer, 1972; Maddrey & Boitnott, 1973) or, in the chronic form, those of chronic active hepatitis (Moss *et al.*, 1972). In HBsAg-positive, acute and chronic hepatitis, viral antigens have been shown to appear on or in hepatocytes (Alberti *et al.*, 1975) and circulating cytotoxic lymphocytes can be detected that are capable of destroying autologous and homologous hepatocytes (Thomson *et al.*, 1974; Paronetto & Vernace, 1975; Guebel *et al.*, 1975). It is therefore conceivable that the hepatitis associated with ingestion of INH is also caused by an immunological attack by sensitized lymphocytes upon the liver cells associated with drug conjugates. The fact that hepatotoxic lymphocytes may also be found in other liver diseases (Paronetto & Vernace, 1975) does not argue against this potential mechanism in INH-associated hepatitis.

The previously published evidence for an immunological response to INH or related antigens in INH-induced hepatitis has been inconclusive. There was no evidence that circulating antibodies to INH occur in this condition (Mitchell *et al.*, 1975a, 1976) and, in this respect, similar negative findings were obtained in this investigation using a sensitive radioimmunoassay. The technique used was capable

of detecting very low concentrations of antibodies to INH and INA in experimental animals. However, the possibility remains that antibodies specific for some metabolite of INH for the corresponding conjugate with tissue macromolecules, possessing an antigenic specificity different from that of INH or INA, may be produced by patients with INH-induced hepatitis.

An *in vitro* lymphocyte transformation response to INH has been previously reported in INH-induced hepatitis by Assem *et al.* (1969), raising the possibility that cell-mediated immunity might be responsible for the INH-induced liver damage. However, attempts to confirm this finding in a relatively large series of patients by Dove, Chaperos & Hedrick (1972), using INH as the stimulating antigen, at a number of concentrations, were unsuccessful.

In the studies reported here, lymphocyte stimulation *in vitro* was demonstrated in response to INH, INA or conjugates of these agents with HSA in 95% of patients with INH-induced liver damage, whereas no significant stimulation was found in normal subjects, patients receiving INH without evidence of liver dysfunction and in patients with HBsAg-positive hepatitis. A major problem in the detection of an immune response to a low molecular weight drug is that it may induce an *in vitro* immunological manifestation only in the form of a conjugate with an appropriate carrier molecule, which may be tissue-specific. *In vitro* immunological responses to haptens such as INH or INA are probably dependent upon the interaction of these molecules with carrier proteins during the incubation period. Janeway and associates (Janeway, 1976) have shown that *in vitro* lymphocyte responses may occur to an antigen coupled to a number of unrelated carriers, possibly because of structural similarities between these carriers in the region adjacent to the hapten. It is therefore possible that the relatively low levels of stimulation seen reflect the use of inappropriate hapten–carrier conjugates having structural similarities to the true sensitizing antigens. Lymphocyte responsiveness varied between groups with regard to the preparation inducing stimulation, although in all individuals proliferation occurred most frequently at higher concentrations. In the present series of patients, significant stimulation was seen in six out of twenty patients (30%) with INH alone. However, 62·5% of patients with overt hepatitis demonstrated stimulation with this hapten. In contrast, 5·5% of all patients with only SGOT abnormalities (either persistent or transient) showed stimulation with INH, while in 66·6% of this group, proliferation occurred in response to INA–HSA. Taking an overall view of the results, one may conclude that there was a clear correlation in nineteen out of twenty patients between, on the one hand, the development of definite hepatitis or a persistent or marked elevation of the SGOT while receiving INH and, on the other, the *in vitro* lymphocyte transformation response to INH, INA or their HSA conjugates. Proliferation in response to INH itself occurred rarely in patients manifesting transient or persistent SGOT abnormalities rather than overt hepatitis. However, there was no correlation between the degree of lymphocyte stimulation *in vitro* and the severity of the liver cell damage induced by the drug. Certainly, it should be stressed that the existence of a lymphocyte transformation response to INH and related compounds in these patients indicates only that an immunological recognition of the drug is occurring, i.e. the response is not necessarily an indication of impending hepatitis. Mathews, Pan & Wells (1972) have shown that *in vitro* stimulation of lymphocytes by INH may occur in patients with drug-induced rashes or fever only. Similarly, a positive lymphocyte transformation response to INH in patients with other forms of allergic reactions to INH was demonstrated in this laboratory. Hence, in INH-induced hepatitis, some other factor such as that related to the handling of the drug by the liver must be involved in the pathogenesis. It requires a clear demonstration of the existence of drug-specific hepatotoxic lymphocytes in patients with INH-induced hepatitis to exclude the possibility that the lymphocyte transformation response to INH is merely a secondary phenomenon. However, it remains possible that the pattern of antigenic response, e.g. whether to INH alone or to INA–HSA, may be of predictive value in determining the patients at risk of developing significant liver damage.

Despite the number of questions remaining unanswered with regard to the pathogenesis of INH-induced hepatitis, the present study does indicate that an immunological response to INH and related antigens occurs in this condition and suggests a number of future avenues for investigation, both into the mechanisms involved in the production of liver cell damage by INH and into the means of predicting impending hepatitis before significant liver damage occurs.

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REFERENCES

ALBERTI, A., REALDI, G., TEMOLADA, F. & CADROBBI, P. (1975) HBAg on liver cells surface in viral hepatitis. *Lancet*, **i**, 346.

ASSEM, E.S.K., NDOPING, N., NICHOLSON, H. & WADE, J.R. (1969) Liver damage and isoniazid allergy. *Clin. exp. Immunol.* **5**, 439.

BAILEY, W.C., TAYLOR, S.L., DASCOMB, H.E., GREENBERG, H.B. & ZISKIND, M.M. (1973) Disturbed hepatic function, during isoniazid chemoprophylaxis. *Am. Rev. resp. Dis.* **107**, 523.

BAILEY, W.C., WEILL, H., DEKOVEN, T.A., ZISKIND, M.M., JACKSON, H.A. & GREENBERG, H.B. (1974) The effect of isoniazid on transaminase levels. *Ann. int. Med.* **81**, 200.

BEAUDRY, H.P., BRICKMAN, H.F., WISE, M.B. & MACDOUGALL, D. (1974) Liver enzyme disturbances during isoniazid chemoprophylaxis in children. *Am. Rev. resp. Dis.* **110**, 581.

BLACK, M. (1974) Isoniazid and the liver. *Am. Rev. resp. Dis.* **110**, 1.

BLACK, M., MITCHELL, J.R., ZIMMERMAN, H.J., ISHAK, K.G. & EPLER, G.R. (1975) Isoniazid-associated hepatitis in 114 patients. *Gastroenterology*, **69**, 289.

BÖYUM, A. (1968) Separation of leucocytes from blood and bone marrow. *Scand. J. clin. Lab. Invest.* **21**, Suppl. 97, 77.

COHEN, R., KAISER, M.H. & THOMSON, R.V. (1961) Fatal hepatic necrosis secondary to isoniazid therapy. *J. Am. med. Ass.* **176**, 877.

COMSTOCK, G.W. & EDWARDS, P.W. (1975) The competing risks of tuberculosis and hepatitis for adult tuberculin reactors. *Am. Rev. resp. Dis.* **111**, 573.

DICKINSON, D.S., BAILEY, W.C.E. & HIRSCHOWITZ, B.I. (1977) The effect of acetylation status on isoniazid (INH) hepatitis. *Am. Rev. resp. Dis.* **115**, Abst, 395.

DOVE, J.T., CHAPEROS, S.D. & HEDRICK, S.R. (1972) Failure to demonstrate transformation of lymphocytes of patients with isoniazid-associated hepatitis. *Am. Rev. resp. Dis.* **106**, 485.

GARIBALDI, R.A., DRUSIN, R.E., FEREBEE, S.H. & GREGG, M.B. (1972) Isoniazid- associated hepatitis: report of an outbreak. *Am. Rev. resp. Dis.* **110**, 581.

GUEBEL, A.P., KELLER, R.H., SUMMERSKILL, W.H.J., STOTO, J.D., TOMASI, T.B. & SHORTER, R.G. (1975) Cytotoxicity of autologous and normal lymphocytes against isolated liver cells from patients with chronic active liver disease. *Gastroenterology*, **68**, 1084.

HABER, E. & OSBORNE, R.K. (1959) Icterus and febrile reactions in response to isonicotinic acid and hydrazine. *New Engl. J. Med.* **260**, 417.

JANEWAY, C.A. (1976) The specificity of T lymphocyte responses to chemically defined antigens. *Transplant. Rev.* **29**, 164.

MADDREY, W.C. & BOITNOTT, J.K. (1973) Isoniazid hepatitis. *Ann. int. Med.* **79**, 1.

MATTHEWS, K.P., PAN, P.M. & WELLS, J.H. (1972) Experience with lymphocyte transformation tests in evaluating allergy to aminosalicyclic acid, isoniazid and streptomycin. *Int. Arch. Allergy appl. Immunol.* **42**, 653.

MERRITT, A.D. & FETTER, B.F. (1950) Toxic hepatic necrosis (hepatitis) due to isoniazid: report of a case with cirrhosis and death due to hemorrhage from esophageal varices. *Ann. int. Med.* **50**, 804.

MITCHELL, J.R. & JOLLOWS D.J. (1975a) Progress in Hepatology. Metabolic activation of drugs to toxic substances. *Gastroenterology*, **68**, 392.

MITCHELL, J.R., THORGEIRSSON, V.P., BLACK, M., TIMBRELL, J.A., SNODGRASS, W.R., POTTER, W.Z., JOLLOW, D.J. & KEISER, H.R. (1975b) Increased incidence of isoniazid hepatitis in rapid acetylators: possible relation to hydrazine metabolites. *Clin. Pharmac. Ther.* **18**, 70.

MITCHELL, J.R., ZIMMERMAN, H.J., ISHAK, K.G., THORGEIRSSON, V.P., TIMBRELL, J.A., SNODGRASS, W.R. & NELSON, S.D. (1976) Isoniazid liver injury: clinical spectrum, pathology and probable pathogenesis. *Ann. int. Med.* **84**, 181.

MOSS, J.D., LEWIS, J.E. & KNAUER, C.M. (1972) Isoniazid-associated hepatitis. *Am. Rev. resp. Dis.* **106**, 849.

MOULDING, T.S. (1975) The competing risks of tuberculosis and hepatitis for adult tuberculin reactors. *Am. Rev. resp. Dis.* **112**, 581.

NELSON, S.D., SNODGRASS, W.R. & MITCHELL, J.R. (1975) Metabolic activation of hydrazines to reactive intermediates: mechanistic implications for isoniazid and isoniazid hepatitis. *Fed. Proc.* **34**, 784.

PARONETTO, F. & VERNACE, S. (1975) Immunological studies in patients with chronic active hepatitis. Cytotoxic activity of lymphocytes to autochthonous liver cells grown in tissue culture. *Clin. exp. Immunol.* **19**, 99.

PETERS, J.H., MILLER, K.S. & BROWN, P. (1965) Studies on the metabolic basis for the genetically determined capacities for isoniazid inactivation in man. *J. Pharmac. exp. Ther.* **150**, 298.

SCHWENK, R., KELLY, K., TSE, K.S. & SEHON, A.H. (1976) A radioimmunoassay for isoniazid. *Clin. Chem.* **21**, 1059.

SNODGRASS, W.R., POTTER, W.Z., TIMBRELL, J., JOLLOW, D.J. & MITCHELL, J.R. (1974) Possible mechanism of isoniazid-related hepatic injury. *Clin. Res.* **22**, 323.

THOMSON, A.D., COCHRANE, M.A.G., MCFARLANE, I.G., EDDLESTONE, A.L.W.F. & WILLIAMS, R. (1974) Lymphocyte cytotoxicity to isolated hepatocytes in chronic active hepatitis. *Nature (Lond.)*, **252**, 721.

TUBERCULOSIS ADVISORY COMMITTEE, CENTRE FOR DISEASE CONTROL, DEPARTMENT OF HEALTH, EDUCATION AND WELFARE. (1974) Isoniazid-associated hepatitis (summary). *Morbid Mortal Weekly Report*, **23**, 97.

TSE, K.S., VIJAY, H., ATTALLAH, N. & SEHON, A.H. (1976) Antibodies specific to isoniazid and isonicotinic acid. *J. Immunol.* **116**, 965.

YARD, A.S. & MCKENNS, H., JR (1962) Aspects of the metabolism of isoniazid and acetylisoniazid in the human and the dog. *J. medul. pharm. Chem.* **5**, 169.