Study of an epidemic of venoocclusive disease in India

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SUMMARY   Twenty-five cases of rapidly developing ascites occurring in an epidemic form were observed in a tribal district in Central India during August 1972-May 1973. Eleven of the patients died. Six patients were brought to hospital and studied for periods of two to 17 months. Necropsy was performed on one patient who died. The clinical features suggested an outflow tract obstruction such as a Budd-Chiari-like syndrome or venoocclusive disease. Radiographic and haemodynamic studies demonstrated a combination of post and perisinusoidal blocks. Liver dysfunction was indicated by the presence of a marked bromsulphthalein retention and mild to moderate hypoalbuminaemia. Histological examination of the liver biopsies showed changes that ranged from centrizonal haemorrhagic necrosis to an extensive centrilobular fibrosis associated with central vein occlusion. The disease was apparently caused by a food toxin, and the possible nature of this is discussed.

A minor epidemic of liver disease characterised by rapidly progressing symptoms suggestive of portal hypertension with ascites occurred in a tribal district in Central India and was brought to our notice in May 1973. The affected area is a partly rocky terrain situated at an altitude of 1200 metres above sea level and having an annual rainfall of about 175 cm. It is a poorly developed region lacking modern amenities for communication and agriculture and is inhabited by tribal people of poor socioeconomic status. A team of epidemiologists visited the area in June 1973. A detailed report of their findings is to be published separately. Their observations generally suggested that the disease was confined to a small group of villages and was characterised by a rapidly developing ascites associated with debility and death in a high percentage of the patients. The epidemic afflicted 25 people out of the total population of 350 between August 1972 and May 1973. Several patients were known to have shared a common source of food. Of the 25 cases, 11 had died before the arrival of the investigating team, four cases were not traceable; three cases had completely recovered, and the remaining seven were still ill. All the seven were

brought to our hospital for investigation and treatment. One of them was found to have associated bilateral pulmonary tuberculosis with sputum positive for acid fast bacilli. She was transferred to a tuberculosis hospital and was therefore not available for further study. Clinical, laboratory, haemodynamic, and histopathological studies of liver biopsies performed on the remaining six patients (Fig. 1), and a necropsy study of one fatal case form the basis for the present communication.

Methods

A detailed clinical history and physical examination data were recorded on each of the six cases studied in detail. Special attention was given to the mode of onset, dietary habits, history of alcoholic intake, and ingestion of known hepatotoxic agents and herbal medicines.

Laboratory investigations included haemogram, routine liver functions, bromsuphthalein retention, hepatitis-B antigen (Alter *et al.*, 1971), and alpha-feto protein (Nayak *et al.*, 1975). Ascitic fluid was examined for proteins, cells, and bacteria. Radiological studies included a barium swallow, splenoportovenogram, and hepatovenogram. Oesophagoscopy was done when varices were not seen on barium study. Intrasplenic, free, and wedged hepatic vein pressures were recorded by the standard techniques

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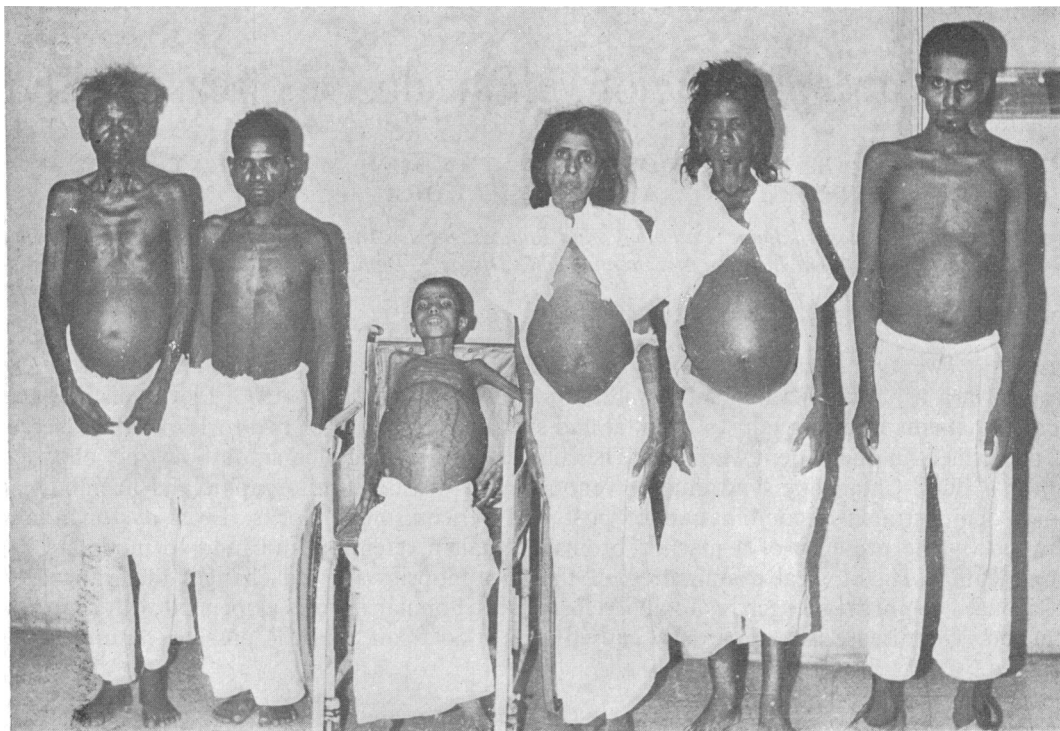
Fig. 1   *The six patients studied.*

(Myers and Taylor, 1951; Turner *et al.*, 1957). Estimated hepatic blood flow (EHBF) was determined in four patients by the Indocyanine Green single injection technique (Dobson *et al.*, 1953).

Liver function tests were repeated at six to eight weeks intervals during the follow-up period. Two or more serial percutaneous needle biopsies of liver were obtained in each patient for histopathological study, after four weeks to 10 months of illness. A full necropsy was performed on one patient who died one year and five months after the onset of the disease.

CLINICAL OBSERVATIONS

There were three males and three females aged from 12 to 38 years. The onset of the illness was characterised by mild continuous pain in the right upper quadrant and epigastrium. This was followed within seven to 15 days by a drop in urinary output and rapidly filling ascites. None gave a history of jaundice or gastrointestinal bleeding. All patients had ascites as the most prominent and presenting feature of their illness (Fig. 1). Ascites was graded as massive in four patients and mild to moderate in two patients. Pedal oedema was present in three patients. Distended prominent veins over the abdomen with

flow away from the umbilicus were observed in five patients. Hepatic enlargement of 2·5 to 5 cm and splenomegaly of 5-7 cm below the costal margin were detected in four and three patients, respectively, when ascites was relieved. None had icterus or endocrinal manifestations known to be associated with chronic liver disease.

There was significant anaemia with haemoglobin less than 7 g/dl in two patients, both of whom had a microcytic hypochromic peripheral blood picture. Total and differential white cell counts were normal. Erythrocyte sedimentation rate (Wintrobe) was more than 50 mm for the first hour in two, between 20 and 50 mm in two, and less than 20 mm in one. Platelet and reticulocyte counts were normal in all the patients.

Liver function tests carried out initially within two weeks of hospitalisation disclosed only minor alterations (Fig. 2). Serum bilirubin levels were slightly raised in three patients. Alkaline phosphatase was normal. SGOT and SGPT were mildly raised in three and two patients, respectively. Prothrombin time was significantly increased in four patients; this was not corrected after the administration of parenteral vitamin K. Bromsulphthalein retention was normal in only one patient. In others it was sig-
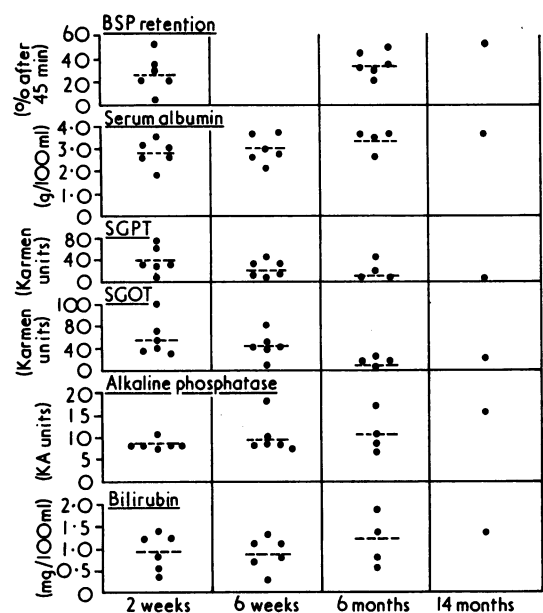
Fig. 2 *Liver function tests at different periods from the time of hospitalisation. The horizontal dotted lines indicate mean values.*
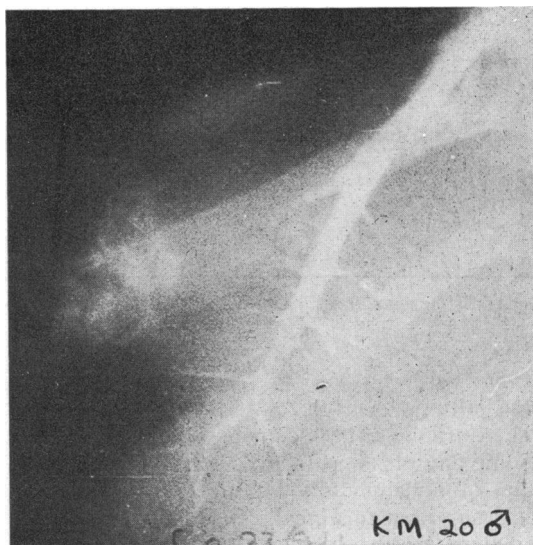

Fig. 3 *Free hepatogram showing paucity and narrowing of secondary and tertiary hepatic vein radicles.*
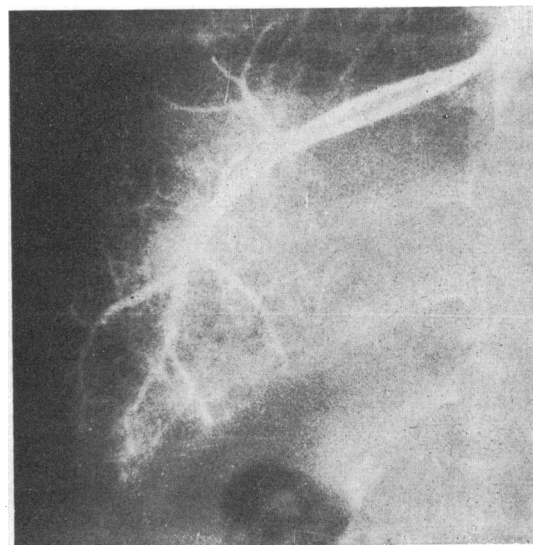

Fig. 4 *Wedged hepatogram showing irregular margins of the hepatic vein radicles with paucity and irregularity of the secondary branches, and patchy filling of the hepatogram.*

nificantly abnormal. Serum albumin levels were low in all the patients. The albumin/globulin ratio was altered with a relative increase in globulins. Serum protein electrophoresis showed a significant increase in gamma globulins and a decrease of albumin in all the patients studied. Ascitic fluid was a transudate in each of the four patients tested. No acid-fast or pyogenic organisms were grown in any. Hepatitis-B antigen and alpha-feto protein were not detected in the serum of any case.

Radiographs of the chest showed no abnormality. Oesophageal and/or gastric varices were demonstrated in three patients by barium swallow and in two by oesophagoscopy. Only one patient was not found to have varices by either procedure. Splenoportovenograms done in three patients showed no evidence of obstruction in the splenic or portal vein. Collateral veins were seen in each of them but tributaries of the intrahepatic portal vein were normal. Hepatograms done in three patients revealed narrowing and irregularity of hepatic vein radicles, paucity of smaller branches, and patchy distribution of the dye in the hepatogram phase (Figs. 3 and 4). Wedged hepatic vein pressure (WHVP) was raised in all the four patients tested (Table). Intrasplenic pressure was also increased in all the three patients tested. Estimated hepatic blood flow (EHBF) ranged from 2565 to 3810 ml/min/M^2 of the body surface in con-

trast with the mean of 1211 ml/min/M^2 in the normal healthy subjects. EHBF was 54 and 37·7% of the cardiac output in two patients and 25·4% in one patient as compared with the average of 32·5% in the control subjects (Table).

Table   *Results of haemodynamic studies*

Subjects	Intrasplenic pressure (mmHg)	Hepatic vein pressure		Estimated hepatic blood flow (EHBF) (ml/min/M²)	EHBF as % of cardiac output
		Free (mmHg)	Wedged (mmHg)		
Control (5)*	8-10	0-5	6-8	1211	32·5
Patients (4):					
HAN	18	4	9	3131	54
KAM	—	5	23	3810	—
LAL	40	7	21	2726	25·4
BAD	20	3	9	2565	37·7

*From one of our earlier studies (Sama *et al.*, 1971).

Liver biopsies

In the initial stages, there was a marked strictly zonal, centrilobular haemorrhagic necrosis. The surrounding sinusoids were dilated. There was a variable degree of inflammatory infiltration of a nonspecific nature in this area. The hepatocytes in the outer third of the lobules showed a variety of degenerative changes including the presence of hyaline but no fat. The reticulin framework was collapsed in the necrotic zone (Fig. 5). The sinusoids continued to be dilated for several weeks after the onset of the disease.

Subsequent biopsies showed a progression of changes following a sequence of events. The centrizonal areas of necrosis were replaced by radiating scars which tended to link with their neighbouring counterparts producing a picture of reversed lobulation (Fig. 5). Hepatic veins were mostly not recognised but the scars were strewn with secondary vascular channels representing dilated sinusoids and numerous tubular structures apparently derived from attenuated hepatocytes. Regenerative activity was generally mild, but occasional unusually large nuclei with prominent nucleoli were seen. Degenerative
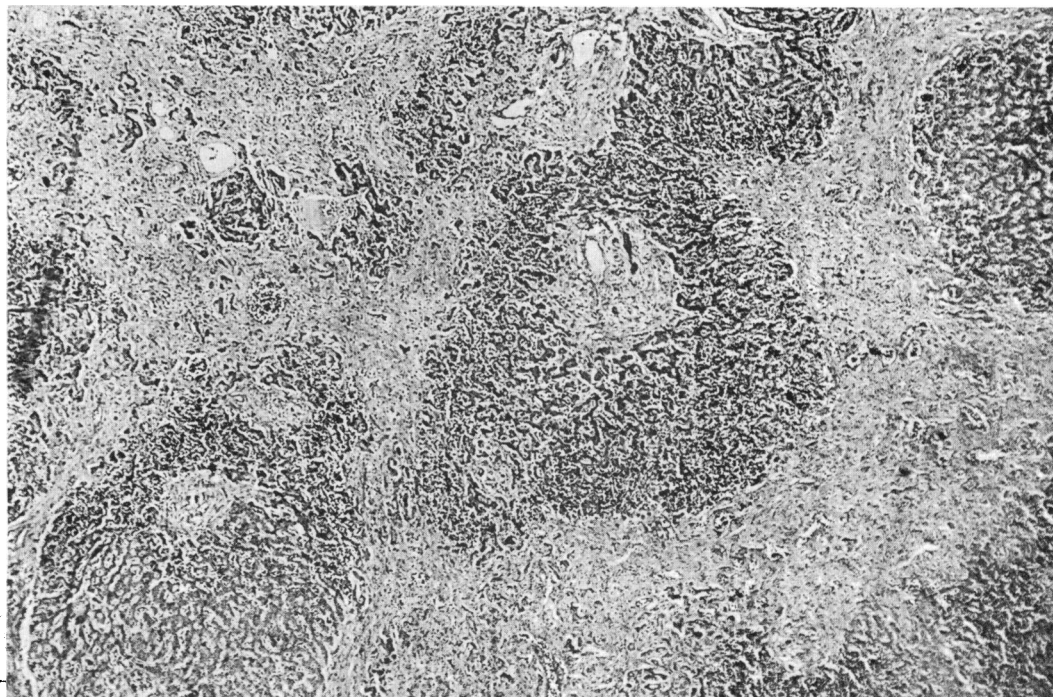

Fig. 5   *Extensive centrilobular scarring linked by fibrous tissue producing reversed lobulation. One portal tract can be seen completely encircled by fibrous septa, at necropsy 17 months after onset of disease. H and E, × 128.*
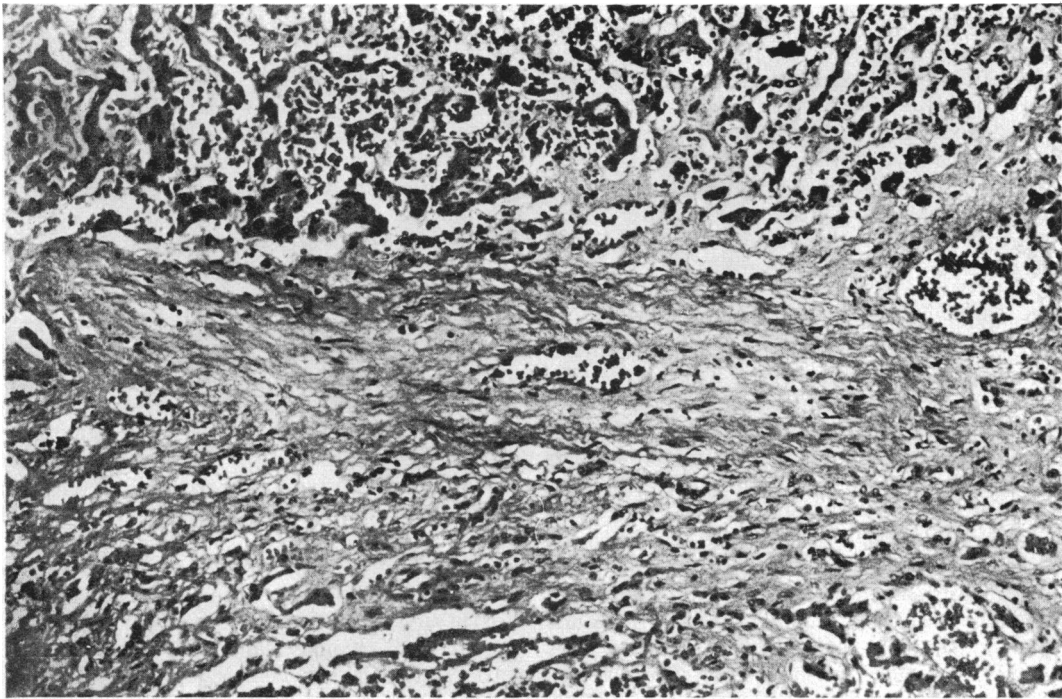
Fig. 6 *Same case as in Fig. 5. The central vein is markedly narrowed owing to proliferation of subintimal connective tissue. The neighbouring sinusoids continue to be markedly dilated. H and E, × 512.*

changes in the hepatocytes continued for several months though to a lesser degree.

CLINICAL COURSE

The condition of five patients greatly improved within three to four months on symptomatic treatment. Ascites and oedema were significantly reduced. A steady rise in the mean value of serum albumin and fall in transaminases was noted (Fig. 2). However, bromsulphthalein retention remained abnormal. At the time of discharge each of these five patients was much improved, though three still showed mild ascites.

An 18-year-old patient who remained in the hospital for 17 months showed improvement during the initial six months but thereafter his condition deteriorated progressively. Ascites worsened and his liver function deteriorated, hepatic encephalopathy developed, and he finally died.

Necropsy

There was bilateral gynecomastia and marked ascites. The liver was shrunken and showed a diffuse fine granularity. Lobular markings were accentuated. It weighed 800 g. Major blood vessels—for example, inferior vena cava, hepatic and portal veins, and hepatic artery were patent. Microscopically, the most conspicuous feature was severe centrizonal scarring associated with variable thickening and occlusion of the hepatic veins (Fig. 6). The lumina of the veins were filled with oedematous connective tissue and many contained several endothelial lined vascular channels. Fibrous tissue was also seen linking the neighbouring centrizonal scars producing a prominent reversed lobulation. Some portal tracts were also involved in the scars (Fig. 5). Marked sinusoidal dilatation persisted at the periphery of centrizonal scars. The spleen was congested and weighed 200 g. Other significant lesions present were glomerulosclerosis and testicular atrophy.

Discussion

This is the report of a very unusual epidemic of acutely developing portal hypertension with severe or moderately severe ascites and nearly 50% fatality rate. Prodromal and icteric phases suggestive of viral hepatitis were absent. The clinical features were highly suggestive of an outflow tract obstruction either due to Budd-Chiari-like syndrome or venoocclusive disease (VOD). Rapidly developing massive ascites with only a mild hepatomegaly would

clinically be more consistent with VOD (Bras, 1973).

Haemodynamic and radiological studies excluded obstruction of the hepatic or the portal veins and favoured the possibility of the pathological process in and around the central veins and the sinusoids. Increased hepatic wedged pressure would support post-sinusoidal and perisinusoidal blocks. Unfortunately, there is no account of haemodynamic studies in venoocclusive disease of the liver in the literature. It is unlikely that slightly increased EHBF contributed to the portal hypertension (Sherlock, 1974).

Morphological studies of the binpsy and necropsy specimens of the liver were quite helpful in understanding the pathophysiology of the disease. The most conspicuous finding was the presence of centrizonal haemorrhagic necrosis and scarring that is characteristic of outflow tract obstruction such as observed in Budd-Chiari syndrome and VOD (Bras, 1973). Viral hepatitis and post-hepatic cirrhosis of the liver could easily be excluded. Budd-Chiari syndrome could also be excluded, as no thrombi or any other obstructive lesions were seen in the major hepatic venous channels. Obliterative lesions of the smallest centrilobular and sublobular hepatic veins diagnostic of well-established VOD (Bras, 1973) were not seen in the biopsies and were observed only at necropsy in one patient who died in the present series. Stirling *et al.* (1962), using histological and immunofluorescent techniques, have shown that, in early VOD, fibrin may immediately surround the hepatic vein radicles and apparently obstruct the inflow of blood from the sinusoids. The obliterative lesion could be secondary to an interruption of the blood supply (Rappaport *et al.*, 1969). It is thus conceiveable that patients with VOD in the early stages may have no venous occlusion demonstrable on light microscopy. A vasospastic process resulting from either circulating or fixed tissue toxin which affects both sinusoids and central veins so as to produce outflow tract obstruction at multiple sites has been suggested (Mendenhall *et al.*, 1974; Iber, 1969). Such a process may have been operative in our patients.

The aetiology of this epidemic remains unknown. The epidemiological data, which include the sharing by several cases of a common source of food and the clinical and haemodynamic data correlating with the liver biopsy findings are strongly suggestive of the epidemic being caused by a food toxin. Unfortunately, the food material taken by these patients before the onset of the disease was not available for study. Centrizonal haemorrhagic necrosis and a clinical picture of rapidly developing ascites due to portal hypertension, the two characteristic findings in the present group of patients, have been reported in toxic liver injury due to pyrrolizidine alkaloids

(Bras, 1973), dimethylnitrosamine (DMN) (McLean *et al.*, 1965; Butler and Hard, 1971), and mycotoxins (Kraybill, 1969).

Pyrrolizidine alkaloids derived from *Senecio* and *Crotalaria* species cause VOD along with centrizonal haemorrhagic necrosis. Classical changes of VOD were observed as a late feature in one patient of the present series. DMN is known to produce in animals histological changes of VOD except the occlusion of the veins (McLean *et al.*, 1965; Butler and Hard, 1971). DMN is not a recognised ingestant as such, although it is possible that a chemical substance with similar toxic property might occur in nature or could possibly be produced endogenously from other dietary components. Under certain conditions sodium nitrite reacts with dimethylamine in fish to form dimethylnitrosamine; such a reaction is considered responsible for the 1961 outbreak of severe disease in Norway among ruminants, sheep and mink that were fed herring preserved with sodium nitrite (Koppang, 1964).

Since the discovery of aflatoxins in 1960-61, several other mycotoxins have been recognised as hepatotoxic (Wogan, 1973). The mycotoxins are difficult to isolate and recognise and the general feeling of investigators in this field is that a variety of liver injuries may be produced by as yet unrecognised mycotoxins (Feuell, 1969; Wogan, 1973) and, therefore, such a possibility must be considered in our patients. Histological alterations caused by mycotoxins include centrizonal haemorrhagic necrosis, bile ductular cell proliferation, and, even, a VOD-like picture (Kraybill, 1969; Wogan, 1973). Bile ductular proliferation was not a significant feature in any of the liver biopsy specimens in the present series.

Pyrrolizidine alkaloids and mycotoxins can at best be discussed as possible aetiological agents but there is no proof to implicate either of them. It is interesting that in sequence we have encountered two other epidemics of liver disease with high mortality most likely related to food toxins—namely, mycotoxin and pyrrolizidine alkaloids. During October 1974 to March 1975 in two districts of Rajasthan (India) an epidemic of jaundice and ascites occurred; this is suspected to be due to contamination of maize with mycotoxin (Krishnamachari *et al.*, 1975). The other epidemic of VOD, very similar to the series reported here, was studied by us in the Gulran district of Afghanistan (Tandon and Tandon, 1975) and which has since been proved to be due to contamination of wheat with the seeds of a plant of the *Heliotropium* species which contained alkaloids of the pyrrolizidine series. As there are many similarities in the clinical features and the histopathology of the liver among the patients of the present series and those of the Afghanistan epidemic, it is likely that food con-

taminated with pyrrolizidine alkaloids was responsible for this epidemic in Central India.

Addendum

Another epidemic of a similar kind in the same region has recently been brought to our notice. An epidemiological study of this epidemic has been launched to find out its cause and means of prevention. Sixty-seven individuals are already affected in an estimated population of 487 and 40 have died. Eight of the affected individuals are currently undergoing treatment and investigation at the All-India Institute of Medical Sciences, New Delhi.

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