Cirrhosis in children from peanut meal contaminated by aflatoxin[1]

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The most toxic of the metabolites of *Aspergillus flavus* known to grow on peanuts is aflatoxin B_1 (1). It affects the young of many species of mammals and causes hepatic necrosis followed by bile duct proliferation (2, 3). There is, however, no information reported so far on the toxic manifestations of this compound in human beings. With a peak incidence at 3 years, Indian childhood cirrhosis occurs widely on this subcontinent, causing vague gastrointestinal symptoms with anorexia followed by firm hepatomegaly with a characteristic leafy border that may proceed to jaundice, ascites, and hepatic coma (4). In a separate communication from this laboratory (5), the presence of aflatoxin in 7% of the urine samples of cirrhotic children as seen on thin-layer chromatoplates was reported (6). Chromatographic identification was confirmed by the chemical test (Andrellos and Reid (7)).

Crude peanut oil and parboiled rice obtained from the homes of these children and random market surveys that we did revealed significant aflatoxin contamination of 50 to 500 µg per kg [illegible] data).

Based on these [illegible], the possibility of involvement [illegible] in the etiology of Indian childhood cirrhosis [illegible] suggested.

Materials and methods

In January 1969, [illegible] three male children [illegible] respectively, and [illegible] supplemented with [illegible] flour, as has been [illegible] [illegible] [illegible] [illegible] [illegible] abdominal [illegible] were observed [illegible] [illegible]

[illegible] tent was found to be 300 µg per kg. With this information, all the children who were known to have consumed the contaminated peanut meal were selected for the study. The study group comprised 20 children over 1.5 years to 5 years, all of whom had consumed 1 to 2 oz of the toxic material daily for periods varying from [illegible] days to 1 month. All were cases of protein calorie malnutrition with the exception of one normal child and one who had nephrosis. Detailed dietary history was obtained with particular reference to crude peanut oil, peanuts, and parboiled rice. There was no history of the consumption of these food items, as these children belonged to the lowest income group and their diet was comprised mainly of ragi (*Eleusine coracana*), a little milk, and vegetables. In our weekly clinic, the children were carefully examined, and records of hepatomegaly and other laboratory investigations were maintained. Repeated liver biopsies were taken every 2 to 3 months, and it is the practice in this clinic to take a liver biopsy after the first examination. In many children, three to four liver biopsies were done at 2- to 3-month intervals. In others, because they failed to attend the clinic, it was possible to do only two biopsies within the first 6 months. The liver biopsies were fixed in 10% [illegible] and processed for paraffin section. The sections were stained with hematoxylin and eosin and for reticulin fibers. Gastrocnemius muscle biopsies were performed on all 20 children initially and repeated after 6 to 9 months in eight.

Results

None of the 20 children displayed frank jaundice at any stage, although a subicteric tinge was noted in 13 cases. All the chil-

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dren, with the exception of four cases in whom the liver was not palpable initially, had soft hepatomegaly typical of protein malnutrition. This increased after 2 months and the consistency became firm. At the end of 1 year, 12 children had gross hepatomegaly and in 3 it was less, but the edges of the liver were sharp and scaly. The normal child in the series who consumed the toxic material for 1 month before she reported at the clinic still has characteristic but moderate hepatomegaly at this date (2 years later). Liver biopsy was performed after 1 month but was consistently refused later. In the child with nephrosis who consumed the toxic material for 5 days only, hepatomegaly had regressed to a just palpable liver with a sharp border after 2 years follow-up, as was also the case with another child who had consumed the toxic meal for 1 week. Three children died after 1.5 years in their respective villages after having been in hepatic coma. After 2 years, six children, including a pair of siblings, still have enlarged firm livers (3 to 5 cm). Liver biopsies were refused by the parents. Of the remaining eight cases, there is no follow-up at this state.

Histology of the liver

The first liver biopsy was taken approximately 1 to 2 months after consumption of the toxic meal, when the majority of the children reported to the clinic. It revealed moderate to severe fatty infiltration without any changes in the lobular architecture and the presence of an inflammatory exudate extending into the hepatic parenchyma (Fig. 1). In four cases, liver biopsies showed severe fatty infiltration and an entirely undisturbed lobular pattern. In two cases, hepatic cells were severely damaged and often showed no nuclear staining with ballooning of cells. Focal necrosis and inflammatory reaction were frequently seen. Biopsy taken after 4 months

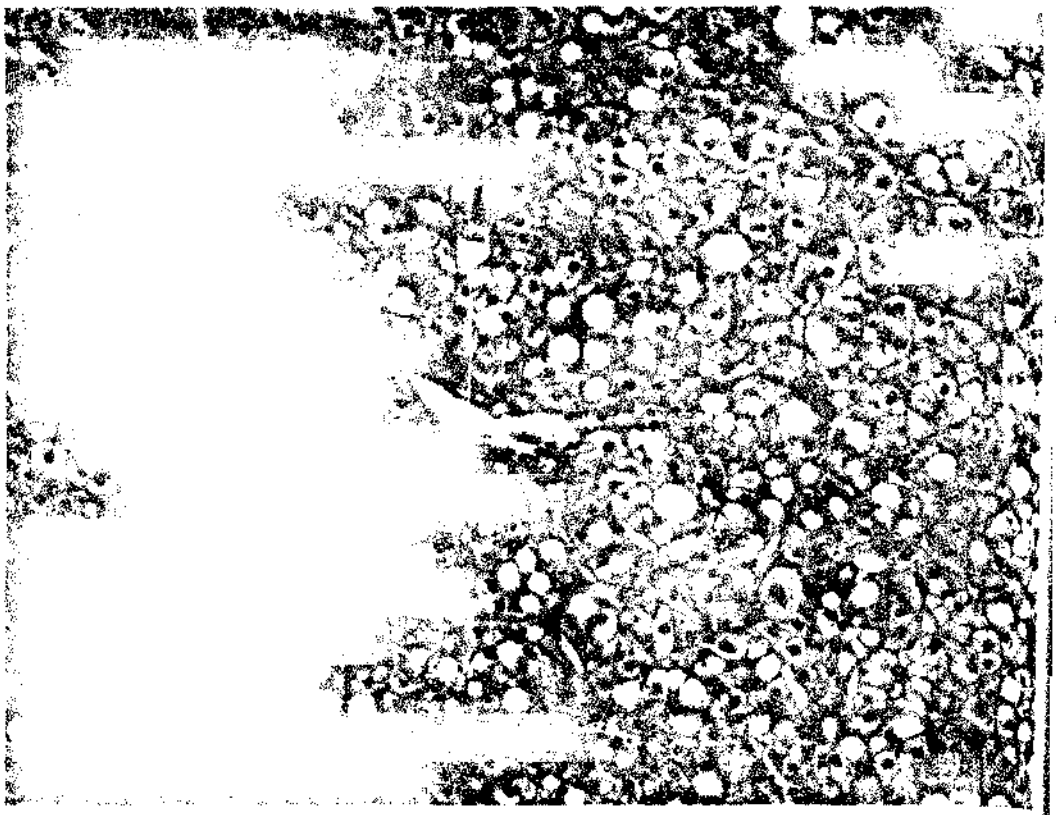

FIG. 1. Shows severe fatty infiltration of the liver. H&E × 200.

FIG. 2. Shows strand-like inflammatory exudate associated with fibrosis of liver. H&E ×200.

owed fibrous tissue appearing from the tal tract and infiltrating into surrounding enchyma followed by severe cellular in- ation in which perilobular fibrosis was quently seen. In two cases, septa extended the parenchyma, but the lobule was not ected. Focal necrosis was frequently seen. e portal tract often contained inflamma- y cells. Fine connective tissue membranes e seen extending throughout the paren- yma. In some areas, the connective tissue mbranes were seen extending throughout parenchyma. Also in some areas, the con- ective tissue membranes condensed to form ta, producing fibrosis with strand-like in- mmatory exudate (Fig. 2). Biopsies taken m children between 10 months and 1 year ealed a complete abolition of the lobular hitecture with dissection of septa of vary- thicknesses. The typical regenerative dules were not seen. In some cases, the patic cells appeared unaltered and showed p basophilia of the persisting cytoplasm. many cases, focal coagulation occurred in containing cells. Focal necrosis was fre- ntly observed and bile duct proliferation was increased. The fibrous tissue septum surrounding the lobule contained variable numbers of inflammatory cells with moderate ductular proliferation and fat-containing hepatic cells (Fig. 3). The section stained for reticulin fibers showed dissection of lobular architecture by septums of varying thicknesses (Fig. 4). Muscle biopsies done on all cases after 1 to 2 months revealed acute fragmentation with loss of striations and cellular infiltration. In the repeat biopsies done after 6 to 8 months, fragmented muscle bundles with interstitial fibrosis, fatty infiltration, and chronic inflammatory cells were seen (Fig. 5).

Discussion

The fact that kwashiorkor rarely progresses to cirrhosis is well documented (8, 9). The etiology of Indian childhood cirrhosis is obscure (4). Since 1961, 3,500 children with protein-calorie malnutrition have been treated by us on an outpatient and inpatient basis with protein-rich peanut meal. In more than 70%, the follow-up has been for more

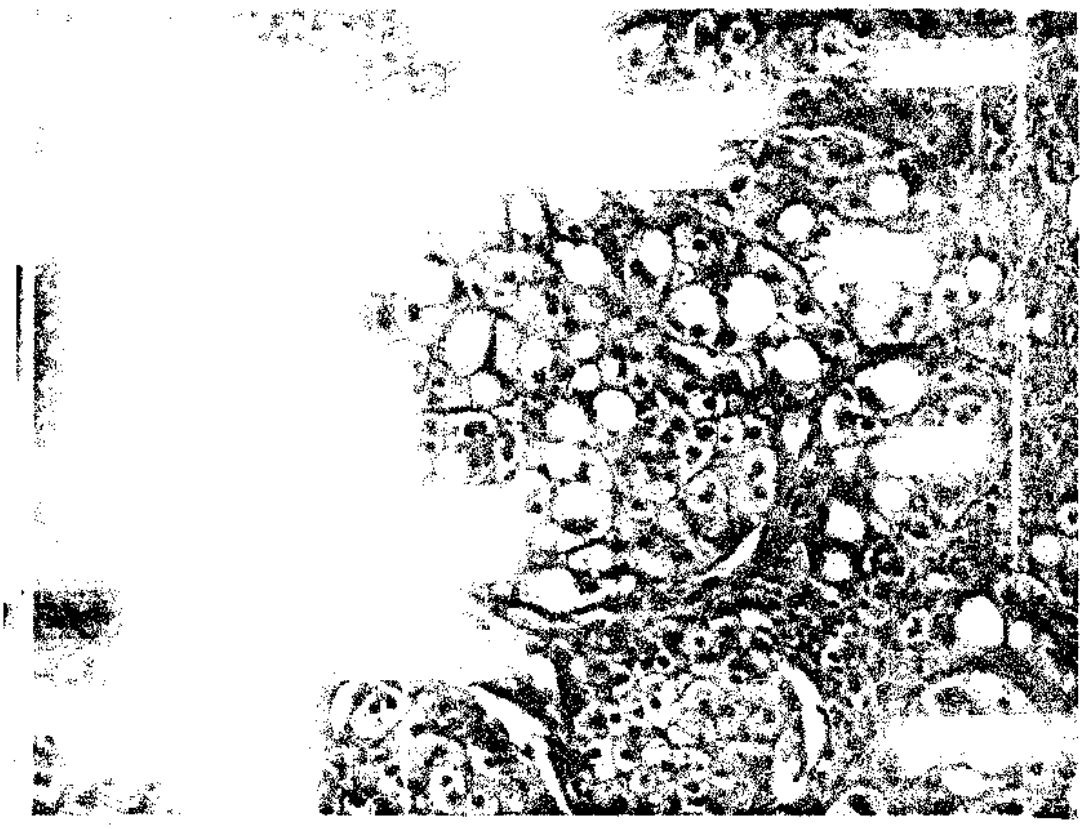

FIG. 3. Shows fibrous tissue septum surrounding the lobule. H&E. × 200.

than 1 year and in several cases for more than 3 years. It was customary to perform a liver biopsy on admission, then 1 month after disappearance of edema with subsequent weight gain, and when the albumin globulin reversal came to normal. We found that fatty metamorphosis of the liver rapidly yielded to dietary protein supplements and the livers became normal within 6 weeks. In no case has cirrhosis of the liver been noted clinically or on liver biopsy material. We present this observation as a control factor in the absence of a controlled study. In contrast, the children who consumed peanut protein contaminated with aflatoxin showed a gradual transition from an increased central and periportal fat of the liver to formation of fatty cysts to fibrosis and cirrhosis over a period of 1 year. Repeated analyses of these protein-rich foods over the years reveal the aflatoxin content to be less than 30 μg per kg. The urine of cases reported here were consistently negative for aflatoxin. In our animal experiments, it was noted that aflatoxin B was excreted in the urine in small amounts within 24 hr. In these 20 children who consumed contaminated meal, the earliest signs (as is usual with Indian childhood cirrhosis also) were gastrointestinal, at which stage the toxic meal was discontinued. Urine was collected for aflatoxin content after a fortnight, when increased hepatomegaly was obvious, and the toxic meal was analyzed. We could in no way correlate the severity of liver pathology with aflatoxin found in urine of 7% of the 250 cirrhotic children studied (5). We have also found aflatoxin in the urine of 10% of normal children (5). The explanation we put forward on the basis of our experience with these kwashiorkor cases is that the exact period of toxic injury is long past by the time characteristic hepat-

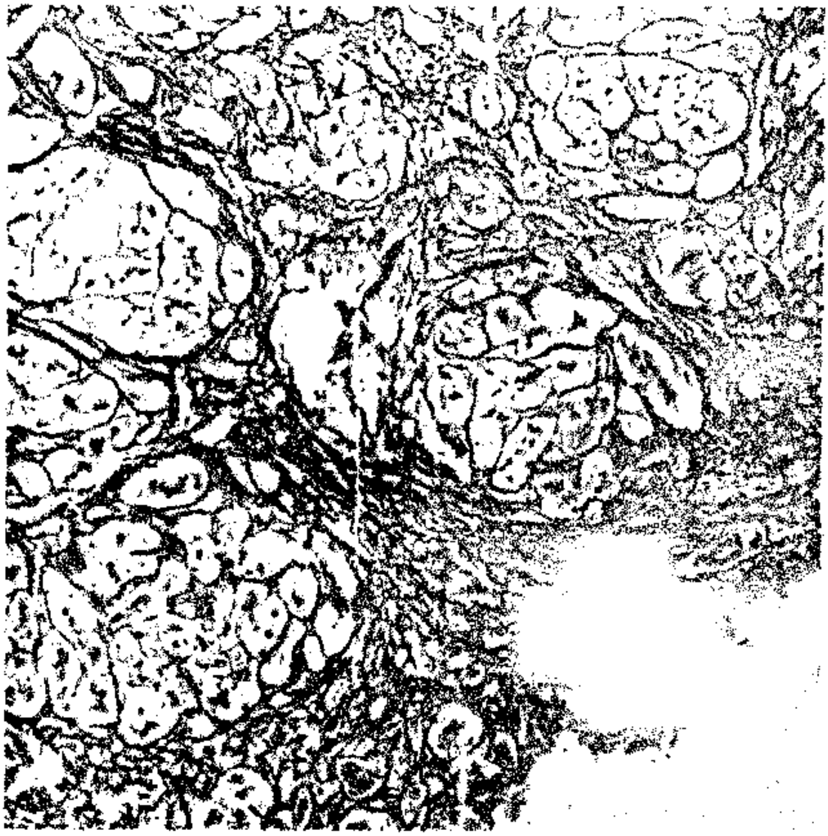

FIG. 4. Shows dissection of lobular architecture by septa; reticulum stain × 200.

FIG. 5. Muscle section showing fragmentation and fibrosis. H&E × [illegible]

[illegible] occurs, and when the diagnosis of Indian childhood cirrhosis [illegible] made. In these children, it took [illegible] 1 year for characteristic clinical and histopathological lesions to occur in the liver. The presence of aflatoxin in the urine of cirrhotic and noncirrhotic children only confirms the on-and-on consumption of toxin containing materials (peanut oil and parboiled rice) by these children. Children with fatty liver seem to be more susceptible to toxic effects. It may be possible that both dietary and toxic components like aflatoxin [illegible] cirrhosis, as Madhavan et al. (10) have reported that protein depletion in weanling rats enhanced the toxic effects of aflatoxin on the liver. The histological pictures of the livers of the cases reported here are identical to those of Indian childhood cirrhosis (4). The histopathological changes noted by us had a direct correlation with the duration of toxin ingestion. The child with nephrosis and one other child who had ingested the toxic material for less than a week had mild histopathological changes that regressed considerably over a period of 1 year. The normal child and others who had consumed the contaminated meal for 1 month displayed moderately severe histological changes that were found progressive in the cases in which [illegible] biopsies were possible. It is of interest that characteristic skeletal muscle changes noted by us in cirrhotic children (11) and in toxin-fed rats (unpublished data) were also noted in the children who consumed the toxic meal. Serious attention [illegible] to be given to the possible hazards to human health from the consumption of aflatoxin-contaminated food. Some of these children are still under observation, and liver biopsies will be taken from time to time to study the progression of the lesion. These results will be published at a later date.

Summary

Children [illegible] varying degrees of protein [illegible] malnutrition had accidentally consumed [illegible] aflatoxin-contaminated, low fat [illegible] peanut protein [illegible] for periods ranging from 5 days to 4 weeks. [illegible] hepatic lesions showed a gradual [illegible] increase in central and [illegible] cells and cirrhosis which [illegible] treated kwashiorkor. The lesions are identical to Indian childhood cirrhosis. ■

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