IgG deposits and Disse's space pathology in human schistosomal liver[1]

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Summary. IgG deposits were identified in Disse's spaces of patients with advanced Symmers' fibrosis consequent to schistosomal infection.

In chronic human schistosomiasis, the hepatic lobule is always involved in pathological modifications. The pathology of the Disse's space, recently described[4], corresponds to the capillarization of hepatic sinusoids[5]. It is characterized by modification of the vascular pole of hepatocytes including the loss of regular microvilli, the deposit of cell debris in the Disse's space, together with an amorphous or fibrillar deposit and an abnormal quantity of collagen bundles. The sinusoidal endothelium is often pluristratified. A fine fibrillar layer resembling a basement membrane is found beneath the endothelial cells. All these modifications correspond to the formation of an abnormal barrier between hepatocytes and the blood circulation and to the alteration of hepato-vascular exchanges (figure 1).

The etiology of this modification seems to be related to the portal blood, the pressure and the quality of which are progressively changed during the evolution of the disease. It is known that the progressive arterialization of hepatic blood circulation leads to a substantial increase of intrasinusoidal pressure[6]. This fact was supposed by Iber[7] to be able to cause laying down of collagen in the space of Disse. At the same time, numerous substances are secreted by worms into the portal blood, substances which can be inert, toxic or antigenic[8–10].

The present study was undertaken to elucidate if, besides the increase of native collagen and cell debris, exogenous substances participate in the filling of the Disse's space, and particularly if substances involved in immunological reactions can be identified. Whilst different deposits in pathological modifications of renal glomeruli were ex-

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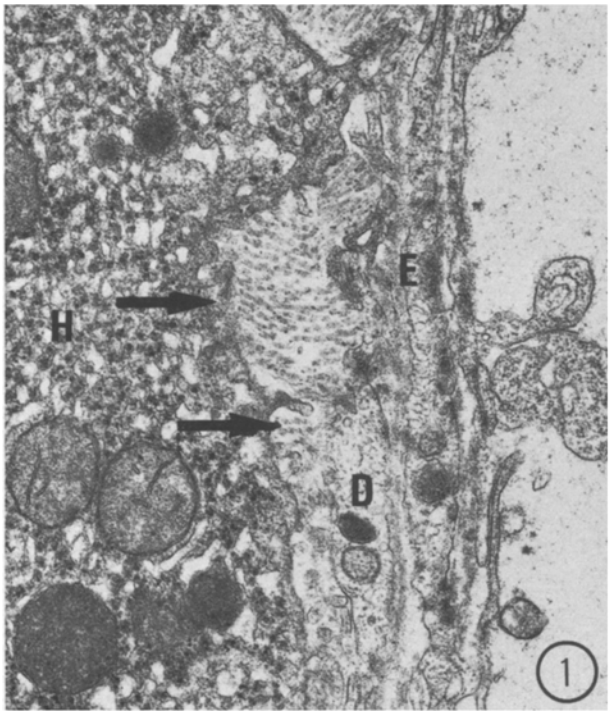

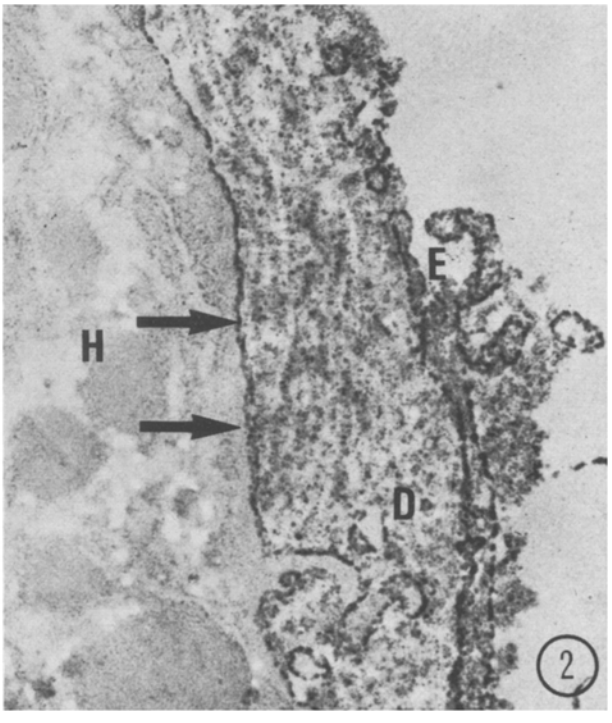

Fig. 1. Pathological Disse's space in chronic human schistosomiasis. H, Hepatocyte; E, Endothelium; D, Disse's space; →, Flattened vascular pole of hepatocyte. ×19,500.

Fig. 2. IgG deposits shown by anti-IgG peroxidase conjugate. H, Hepatocyte; E, Endothelium; D, Disse's space; →, Flattened vascular pole of hepatocyte. ×17,200.

tensively studied, such substances observed in the Disse's space were not described.

Liver biopsies of patients infected with Schistosoma mansoni were collected in Brazil and in Egypt. Materials and methods of histological and ultrastructural studies were given in an earlier publication[8]. For immuno-enzymatic electron microscopic study, the tissue was fixed with 4% paraformaldehyde in cacodylate buffer (0.1 M; pH 7.4) with 7.5% sucrose, for 12 h at 4 °C. After washing in a solution of 0.1 M cacodylate and 0.2 M sucrose, 6 µm cryotome sections were obtained. They were incubated with the anti-IgG peroxidase conjugated serum (Institut Pasteur), diluted 1:10 in a 0.1 M phosphate buffer (pH 7.4), for 1 h at 20 °C, and rinsed in the same buffer. Endogenous peroxidase and anti-IgG peroxidase conjugate were demonstrated by diaminobenzidine[11]. Postfixation was achieved with osmium tetroxide (1%) in 0.5 cacodylate buffer (pH 7.4). After dehydration with ethanol and impregnation in Epoxy-resin, tissues were studied by electron microscope.

The specificity of the immuno-enzymatic reaction was controlled by: 1. detection of endogenous peroxidase in sections not incubated with the anti-IgG peroxidase conjugate; 2. incubation of sections with a serum with no specific anti-IgG reactivity; 3. inhibition of endogenous peroxidase[12].

As demonstrated on figure 2, electron dense deposit corresponding to peroxidase-labelled anti-IgG serum was observed: 1. in membranes of the vascular pole of hepatocytes; 2. inside the broadened Disse's space loaded with amorphous of fibrillar deposits; 3. on both sides of endothelial cells; 4. on the flocular deposits of the sinusoidal side of endothelium.

The demonstration of the IgG deposits in the abnormal Disse's space in human schistosomiasis shows the participation of immunological reactions to this pathological alteration. Pathological deposits of immune complexes were demonstrated in the kidney of patients with chronic schistosomiasis[13], and specific immune complexes were demonstrated in the blood in experimental and in human schistosomiasis[9]. The IgG deposit in the Disse's space may be another aspect of the same phenomenon.

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Metabolic rearrangement of fluorenone oxime to phenanthridinone[1]

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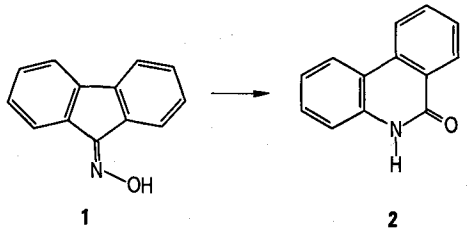
Summary. Fluorenone oxime is metabolized in vivo in the rat to phenanthridinone which is excreted in the urine. The structure of the metabolite has been determined by comparison of chromatographic and spectral properties of the metabolites with authentic phenanthridinone.

Oximes are formed by mammalian liver systems as metabolic products of primary aliphatic amines[2-4]. The oximes have been characterized as being metabolically inert, although they are susceptible to non-enzymatic hydrolysis, forming the corresponding ketone[2,5]. In vitro studies with liver homogenates indicated that these functionalities are reduced by hepatic cytochrome P-450-dependent reductases to yield hydroxylamines and amines, although this appears to be a very inefficient biotransformation pathway[6,7].

We wish to describe a new route for oxime detoxication involving its rearrangement to an amide. Fluorenone oxime, **1**, was administered i.p. (600 mg/kg) to rats and urine collected over sodium fluoride (present as a preservative) for 48 h. Urine samples were evaporated to dryness and extracted with chloroform:methanol (9:1). The extract was evaporated, the concentrates spotted onto TLC plates, and separation of components achieved in selected solvent systems (table 1). Phenanthridinone, **2**, was identified as a metabolite of **1**, by comparison of the TLC behavior of the urinary extract component with an authentic sample of **2**, prepared by established methods[8]. R_f values of the metabolite were identical to those of pure **2**, as determined using 3 different solvent systems and 3 different stationary supports (table 1). To further establish the metabolite structure, the areas with spots were then scraped off, eluted with a small volume of chloroform:methanol (9:1) and subjected to further analysis. The identity of the metabolite was corroborated by determining its UV, fluorescence and mass spectra which were identical to authentic phenanthridinone (table 2) which had been similarly chromatographed.



1 2

The reaction, exemplified by the conversion of fluorenone oxime to phenanthridinone, is well established in chemical systems, and is an example of the Beckman rearrangement. If a parallel is drawn between the chemical and metabolic reaction, it may be postulated that the metabolic process is initiated by conjugation (esterification) of the oxime (forming **3**)[8]. Enzymatic esterification of N-hydroxy compounds has been well-documented[9]. In chemical systems, such esterification is known to facilitate loss of the oximino hydroxyl group, triggering migration of carbon (generating intermediate **4**), and eventual

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