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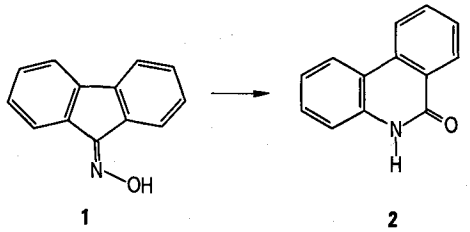
Summary. Fluorenone oxime is metabolized in vivo in the rat to phenanthridinone which is excreted in the urine. The structure of the metabolite has been determined by comparison of chromatographic and spectral properties of the metabolites with authentic phenanthridinone.

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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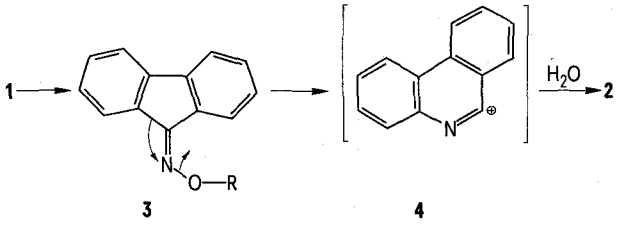
formation of the amide. Furthermore, such processes are generally concerted[10], so that if the metabolic reaction proceeds via a similar mechanism, electrophilic intermediates such as those generated in reactions following arylhydroxylamine esterification[9] and considered responsible for eliciting toxic or carcinogenic responses would not be expected to be formed.



3 4

Where R may = PO_3^{2-}
glucuronide
$-SO_3$
O
||
R′–C–

Pan and Fletcher[11] have reported that substituted fluorenone oximes exhibit anti-neoplastic activity, as indicated by their effect on Walker carcinosarcoma 256 and L-1210 leukemia. Parallel activity was found with the corresponding phenanthridinones, synthesized from the oxime by reaction with polyphosphoric acid. It now appears that this parallel activity between these structural isomers may not be fortuitous, but rather results from metabolic rearrangement of the oxime. This possibility is being investigated.

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Table 1. TLC Identification of phenanthridinone as a metabolite of fluorenone oxime

Stationary support	Solvent system	$R_{f\,oxime}$[a]	$R_{f\,amide}$[b]
Silica gel[c]	Ethyl acetate:methanol: ammonia [85:10:5]	0.88	0.80
Alumina[d]	Chloroform:methanol [9:1]	0.05	0.29
Polyamide[d]	Chloroform:methanol [9:1]	0.60	0.70

a) Fluorenone oxime detected fluorometrically. b) Phenanthridinone detected fluorometrically. c) E. Merck, Darmstadt, W. Germany. d) Eastman Chemical Co., Rochester, N.Y.

Table 2. Spectral properties of metabolites of fluorenone oxime

Property	Characteristics
UV[a,b]	λ_{max} 337 nm and 323 nm
Fluorescence[a,b]	λ_{ex} 323 nm; λ_{em} 360 nm λ_{ex} 337 nm; λ_{em} 360 nm
Mass spectrum[c]	Major bands: m/e 149 (6.3)[d] 135 (26.7) 120 (1.3) 93 (100)

a) All spectral characteristics of the metabolite corresponded to the properties of authentic phenanthridinone. b) Spectra recorded in chloroform:methanol (9:1) solution. c) All spectral bands with intensity equal to or greater than 1% of the base peak corresponded to the spectrum of authentic phenanthridinone which had been similarly chromatographed. d) % of relative abundance of base peak in parentheses.

The effect of clomiphene citrate and estradiol on body weight, vaginal cornification, and uterine weight after chronic treatment of ovariectomized rats[1]

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Summary. Clomiphene reduced the body weight gain of ovariectomized rats to a much greater degree than extradiol did. Estradiol had a more pronounced effect on vaginal cornification and uterine weight than clomiphene did.

Clomiphene citrate (Clomid®) is one of many antiestrogens which possess weak estrogenic potency[3,16,17] and have various effects on the female reproductive system[3,4,14,15]. One of the problems in evaluating the mechanism of action of these compounds is comparing them to the effect and potency of the natural estrogens. Depending on the parameter and dose, these antiestrogens may be more, less, or as effective as estradiol[4,5]. In addition, some of the antiestrogens appear to have effects which are quite different from those of estradiol[5,6]. One effect of estradiol is to reduce the b.wt gain observed in ovariectomized rats[7]. One series of antiestrogens was reported to be far more effective than estradiol in reducing rat b.wt gain despite its weak uterotrophic activity[8].

We have previously reported that clomiphene was far more effective than estradiol in increasing luminal epithelial glycogenesis in rat uterus[6]. One dose of clomiphene was less effective than estradiol in increasing glycogen concentration but 3 doses of the drug were as effective[9]. Because of the marked difference in the effects of clomiphene and estradiol on one uterine system, it was of interest to investigate the effects of chronic treatment on b.wt gain and 2 other sensitive indices of estrogenic potency-vaginal cornification and uterine wt. The experiment was conducted in order to investigate further the comparative estrogenicity of clomiphene and estradiol, and to determine if clomiphene followed the pattern of other antiestrogens which have a greater effect on b.wt than does estradiol.

Materials and methods. Virgin female Holtzman rats were housed 3 per cage under controlled lighting (14 h light) and temperature (22 °C) conditions and allowed free access