central region to such an extent that the arrangement cannot be explained by flexibility of the molecules between the head and rod regions. Several myosin rods can be seen to bend away from the filament backbone suggesting a flexible 'joint' within the rod region. In certain cases, the distance between the heads and such a flexible point could be measured and it was found to be 35–50 nm. Thus if there are flexible joints in the non-muscle myosin molecules responsible for the filament structure, they must be situated approximately 45 nm from the heads. The presence of a flexible joint in such a position is supported by the observation of adrenal medulla myosin filaments with twisted bare central regions (figure 1, c); such a twist must reflect a flexible part of the rod region of the myosin molecules. Skeletal muscle myosin has been suggested to possess 2 flexible joints[2], one between the heads and the rod region and another in the rod region 43 nm from the heads, and in addition cardiac myosin has been shown to possess flexibility in the rod region[12]. Our results suggest that a flexible joint comparable to that found in the rod of skeletal muscle myosin[2] is also present in non-muscle myosins, and that it may be responsible for the variation in the disposition of the heads found in non-muscle myosin bipolar filaments[6,9]. The heads appear as compact or diffuse structures and the necessary flexibility involved probably derives from a flexible joint in the myosin rod as shown in figure 2. Models of bipolar filament structure should take into account such flexibility in the rod region of the molecule.

The twisted filament in figure 1, c exhibits a 2nd interesting feature, namely a bare central region which is split into 4 separate 'rods'. The existence of such split backbones was common in the adrenal medulla myosin preparations and it suggests that head-head interactions are important, as well as rod-rod interactions, in the formation and conservation of the bipolar filament structure. Head-head interactions have been previously suggested to be important in controlling the length of non-muscle myosin bipolar filaments[6] and in the formation of large assemblies of bipolar filaments[8,10].

The filaments of retinal myosin (figure 1, a and b) show a 3rd feature, the presence of faint transverse striations in the bare central region; in some cases, but not all, they traverse the width of the filaments. The distance between the striations varies from 6 to 20 nm, the average separation being 15 nm and there is some evidence for a regular arrangement. At present, the structural basis of the striations is not clear but they may represent the arrangement of the ends of the myosin rods composing the filament backbone. Perhaps this reflects some specificity in the interactions of the myosin rods during filament formation.

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The effect of bisamidines of 2,6-diaminoanthraquinone on *Entamoeba histolytica* infections in rats and hamsters

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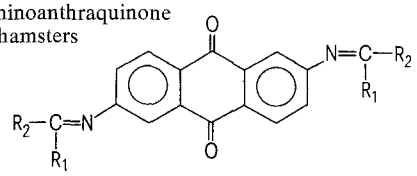
Summary. Bisamidines of 2,6-diaminoanthraquinone have demonstrated potent activity against cecal and hepatic *Entamoeba histolytica* infections in rats and hamsters, respectively. A number of these compounds compared favorably, in overall drug efficacy, with metronidazole and other standard agents.

A variety of drugs for the treatment of *Entamoeba histolytica* infections is available but indications and effectiveness differ considerably depending upon the severity of the disease. The organism may be present in the bowel lumen, the bowel well, extraintestinal tissues (primarily in the liver), or in both intestinal and extraintestinal sites. The modes of action and principal sites of action of various drugs differ. Asymptomatic intestinal infections can usually be treated successfully with halogenated hydroxyquinolines, however, optic atrophy and loss of vision have caused these drugs to be withdrawn from use in many parts of the world[3,4].

More severe intestinal infections have been treated with metronidazole or with varying sequential or concomitant treatment including the diloxanide furoate and metronidazole[5]. Hepatic abscesses were treated with metronidazole or sometimes by metronidazole followed by other therapy diiodohydroxyquin, or by dehydrometine or emetine followed by chloroquine and/or diiodohydroxyquin. Costs, mode of action, degree of toxicity and regional preferences among drugs with similar modes of action have been instrumental in governing the selection of the drug or combination of drugs used for treating amebiasis in different parts of the world. The nitroimidazoles are now generally recognized as the principal drugs of choice for amebiasis since they are effective against infections in all sites and are usually well tolerated. However, some have been reported carcinogenic in animals and a well tolerated product with at least equivalent efficacy and with no carcinogenic potential should provide a highly competitive substitute.

A number of bisamidines of 2,6-diaminoanthraquinone have displayed antiamebic activity against experimental *E. histolytica* infections in test animals[6]. These novel non-nitro compounds were non-mutagenic when tested in the Ames test and the Dominant-lethal test. Metronidazole, the leading marketed agent has been shown to be mutagenic and carcinogenic in test animals[7]. The compounds de-

Table 1. Activity of bisamidines of 2,6-diaminoanthraquinone against experimental amebiasis in rats and hamsters



	R_1	R_2	Maximum non-lethal/minimal effective dose mg/kg/day (safety margin) Cecal amebiasis in rats		Hepatic amebiasis in hamsters	
1a	H	$N(CH_3)_2$	1000/20	(50)	500/10	(50)
1b	CH_3	$N(C_2H_5)_2$	250/10	(25)	500/2.5	(200)
1c	CH_3	$N(CH_3)_2$	100/2.5-5	(20-40)	250/2.5	(100)
1d	C_2H_5	$N(CH_3)_2$	500/10-20	(25-50)	500/2.5	(200)
1e	CH_3	$NH(C_2H_5)$	250/10	(25)	500/1	(500)
1f	p-ClC_6H_4	$N(CH_3)_2$	1000/20	(50)	200/10	(20)
1g	$CH_2CH(CH_3)_2$	$N(C_2H_5)_2$	500/10	(50)	ND*	
Metronidazole			2000/10	(200)	200/10	(20)
Nitrimidazine**			500/20	(25)	500/100	(5)
Tinidazole**			2000/5	(400)	2000/25	(80)

* Not determined. ** Marketed outside the US.

scribed here were active in treating cecal and hepatic amebic infections in rodents.

Materials and methods. The organism used to induce cecal and hepatic infections in rats and hamsters was the National Institute of Health 200 μ strain of *Entamoeba histolytica* cultured in the presence of an unidentified fecal flora in Cleveland-Collier Medium; a liver infusion agar base overlaid with a horse serum: saline mixture (1:6) to which was added a few mg of sterile rice powder. The amebas were maintained in culture at 37 °C by transfer to fresh medium twice weekly.

Cecal infections in female albino Wistar rats. Pooled overlays (0.25 ml) containing large numbers of amebas were injected into the cecums of anesthetized weanling rats during laparotomy. Treatment was begun the day after inoculation. The compounds were dissolved or suspended in 0.2% aqueous agar and administered once daily, by gavage, for 5 consecutive days. 6 days after inoculation of the amebas, the rats were sacrificed and a scraping from the cecal wall of each rat was mixed with a drop of 0.85% saline and examined microscopically for amebas. The clearance rate (number cleared of ameba/number treated) for each regimen was calculated and corrected for non-specific clearance observed in the untreated infected controls. The minimal effective dose, expressed in terms of mg/kg/day, was the lowest dose which cleared at least 50% of the rats treated. The maximum non-lethal dose wa the highest dose which produced no signs of toxicity in normal healthy rats.

Hepatic infections in female golden hamsters. A piece of ameba-laden absorbable sponge, about 25 mm^2, was inserted between the middle lobes of the livers of anesthetized hamsters during laparotomy. Untreated hamsters usually died from the resulting infections about 7 days after inoculation. Treatment was started on the day of inoculation, as soon as the hamsters had recovered from the surgical anesthetic. The test compounds were dissolved or suspended in 0.2% aqueous agar and administered once daily by gavage for 5 consecutive days. Effective drugs prevented mortality. Survival ratios were corrected for non-specific survival observed in untreated groups. The minimal effective dose expressed in mg/kg/day, was the lowest dose which protected at least 50% of the hamsters as evidenced by survival 14 days after inoculation of amebas. The maximum non-lethal dose was the highest dose of drug which produced no mortality in normal healthy hamsters.

Ames and Dominant-lethal tests. The Ames test is essentially that described in the literature[8]. The drug was tested at 1000 μg per disc and per plate with and without activation against 5 bacteria (TA-98, TA-100, TA-1535, TA-1537 and WP-2uvrA$^-$). Positive and negative control drugs were used as references. In the Dominant-lethal test[9], the drug was administered by gavage to male rats at 20, 50 and 100 mg/kg/day for 5 days. Treated male rats were mated with untreated virgin females for 8 weeks, each week with a different female. The drug had no effect at any of the doses tested on the pregnancy rate, the total number of implantations or on the early and late postimplantation deaths. All fetuses were morphologically normal on gross examination.

Results. A number of bisamidines of 2,6-diaminoanthraquinone were about as potent as metronidazole against experimental cecal amebiasis in rats. The safety margins were somewhat lower, but since the drugs did not contain a nitro-moiety, they were sufficiently interesting to warrant further consideration. Against experimental hepatic amebiasis in hamsters, these drugs were, in general more potent and has a greater margin of safety than metronidazole (table 1).

From this group, **1b** (2,6-anthraquinonylene)bis[N′,N‴-diethylacetamidine] was selected for additional studies. This drug was shown to be nonmutagenic when tested in the Ames and Dominant-lethal tests. The drug was compared to metronidazole in experimental *E. histolytica* in rats and hamsters. The data, which appear in table 2, show it to be a highly effective drug for the treatment of experimental amebiasis in rats and hamsters. The safety margins compare favorably to those for metronidazole. The absence of a

Table 2. Comparison of (1b), (2,6-anthraquinonylene)bis[N′,N‴-diethylacetamide and metronidazole in experimental Amebiasis

Drug	Cecal amebiasis in rats ED_{50} (mg/kg/day)*	LD_{50} (mg/kg/day)*	Approximate safety margin	Hepatic amebiasis in hamsters ED_{50} (mg/kg/day)*	LD_{50} (mg/kg/day)*	Approximate safety margin
1b	5.6 (4.2-7.0)**	416 (268-1237)**	70	1.0 (0-7-2.3)**	850	850
Metronidazole	5.9 (4.6-7.3)**	2200	370	7.3 (5.5-9.2)**	200	30

* Dose of drug administered by gavage once daily for 5 days. ** 95% confidence limits.

nitro group in the molecule, the lack of mutagenicity in our tests and the high degree of activity make this series interesting as antiamebic agents and further evaluation is in progress.

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Association of intermediate filaments with other cell organelles in carcinoid tumor of the colon[1]

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Summary. Carcinoid tumor of the colon was studied in electron microscope. In cytoplasm, prominent intermediate-sized filaments were seen frequently attaching to nucleus and mitochondria. Direct contacts of intermediate filaments with secretory granules were not observed.

Intermediate filaments, microfilaments and microtubules form the cellular cytoskeleton[2-4]. The function of microfilaments and microtubules has been extensively studied[5,6] while the role of intermediate filaments is still incompletely known. They have been suggested to have mainly a skeletal, cell-supporting function[7] and we have recently shown that intermediate filaments are of major importance in nuclear anchorage[8]. However, there are also suggestions proposing a role for intermediate filaments in movement cell organelles[9], e.g. in neuronal cells[10], and in melanocytes[11].

Intermediate filaments seem to be increased in number in neoplastic cells[12], offering an opportunity to study their relationship to other cell organelles better than in normal

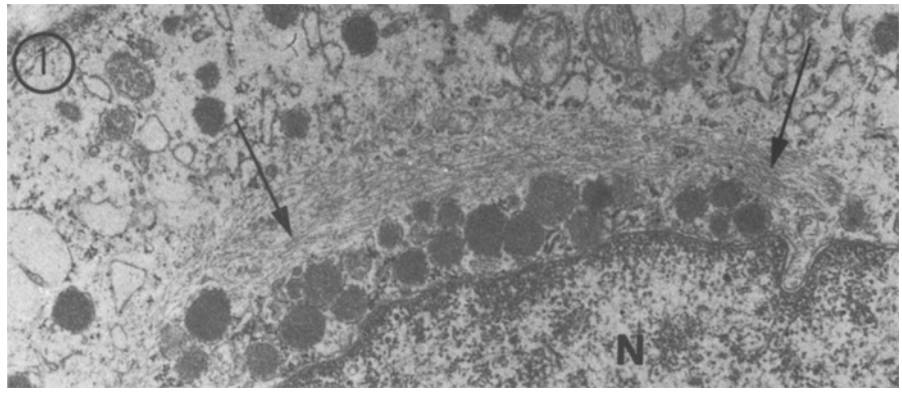

Fig. 1. Intermediate filaments form a prominent perinuclear bundle (arrows) which seems to displace the secretory granules. N, nucleus. × 18,500.

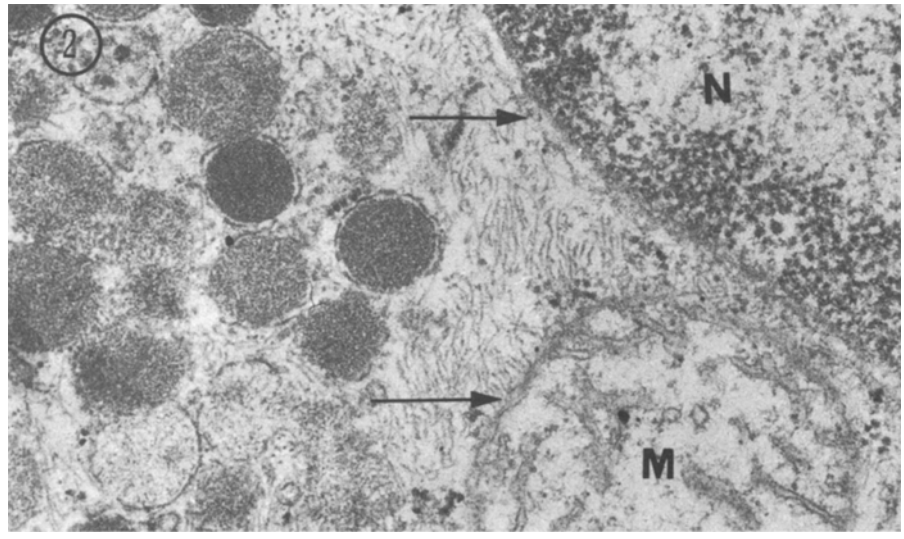

Fig. 2. Intermediate filaments are seen to attach to nucleus and mitochondrion. At the attachment sites, the membranes have a fuzzy appearance (arrows). N, nucleus; M, mitochondrion. × 46,000.