decreased with in 2 min to about 3 contractions/min at 8 °C in aquarium A, and stopped altogether in aquarium B (6 °C). In both cases the jellyfish sank immediately and stayed near the bottom without any further displacement (table). Some motility began when the temperature exceeded about 11 °C: it was clearly observed at that point that the animals were moving following the temperature gradient towards the surface and towards the heat source (point 3). Once in the warmer corner of the tank, they did not depart from its surroundings until the temperature in the aquarium reached a uniform value of 15 °C: only then did the animals begin to swim randomly throughout the basin.

The inverse experiment, i.e. to observe the effects of lowering the temperature, has not been performed completely owing to practical difficulties and to the lack of fresh specimens. We could, however, observe that some specimens of *Pelagia noctiluca,* left unattended in a small tank filled with sea water at the external temperature (11 °C), corresponding to the conditions of their capture, reduced their activity from about 54 to 4–6 contractions/min after 12 h at 8 °C, when they were found to be staying at the bottom.

The effect of thermal shock is evident from the figure: specimens in the aquarium B ($t_0 = 6$ °C) present a reduced activity with respect to those in aquarium A ($t_0 = 8$ °C) at all temperatures. In both cases, however, 11 °C seems to be the temperature (table) at which *Pelagia noctiluca* starts to move showing a positive thermotropism for temperature gradients greater than about 0.01 °C/cm.

A correlation between water temperature and the pulsating rate of the umbrella of *Pelagia noctiluca* was found by Skramlik in the Gulf of Napoli[7]; in that case, frequencies in the range from 24 contractions/min at 5 °C to 66 contractions/min at 20 °C were observed. Our experimental data confirm the existence of such a correlation, but show that the contractions in the young specimens collected in the Gulf of Trieste are almost completely inhibited at 6 °C. Furthermore, according to our experience, *Pelagia noctiluca* can be reasonably considered as having a positive thermotropism.

A logical explanation, based on the above observations, of the disappearing of *Pelagia noctiluca* from the surface layer in the northern Adriatic during winter is that these animals reduce their activity and sink when the temperature of 11 °C is approached. These animals can then survive in the bottom layer during the cold season, when the water temperature is generally vertically uniform and not less than 6–8 °C. In springtime, when the seasonal thermocline is set up and the temperature rises to about 11 °C in an intermediate-deep layer[8], the now mature specimens of *Pelagia noctiluca* begin to move again, reach the warmer surface layer, and continue their biological cycle.

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Restriction enzyme studies on human highly repeated DNAs[1]

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Summary. Various restriction enzymes digest human highly repeated homogeneous DNA to discrete fragments, some of which are present in the male and absent in the female. The male specific 2.4 kilobase HaeIII fragment corresponds to human male satellite DNA IV.

Human satellite DNAs I, II, III and IV and an additional highly repeated fraction, called homogeneous DNA, identified within the light side of the main band DNA in an Ag^+-Cs_2SO_4 gradient have been extensively studied[3–9]. Restriction enzyme digestion studies have recently been carried out on human highly repeated DNAs[10–15]. We report here on restriction patterns of human satellite and homogeneous DNAs.

DNA extraction procedures from human placenta, DNA analytical ultracentrifugation and preparative fractionation in CsCl, Ag^+-Cs_2SO_4 and Hg^{++}-Cs_2SO_4 equilibrium density gradients were as previously described[3,4]. All DNA fractions were dialyzed against 0.1 M ammonium carbonate and then evaporated by air insufflation at 37 °C and resuspended in a small volume of 10 mM Tris-HCl, pH 7.5, 1 mM EDTA.

DNA fractions were then digested by several restriction enzymes (commercial preparations purchased from Bethesda Research Laboratories, Bethesda, MD) at a DNA-to-enzyme ratio of 1 µg-DNA to 3 units of enzymes according to the conditions indicated by the vendor. The restriction enzymes used were the following: HaeIII, EcoRI, EcoRII, XbaI, AluI, HindIII, HinfI, BamHI, HpaI, HpaII, BglII, and HhaI.

Assay buffers contained Tris-HCl, $MgCl_2$, 2-mercaptoethanol or dithiothreitol, and NaCl at various molar concentration according to the enzyme, as indicated by the vendor's catalogue, and digestion was carried out to completion for 5–7 h at 37 °C.

After digestion sucrose was added to 6% and the samples were applied to the wells of a horizontal 17 cm × 13 cm × 0.3 cm agarose gel. Agarose concentration was 1% for all enzyme experiments except for HaeIII and XbaI digests in which cases 2.5% and 1.5% agarose concentrations were used respectively. The slab gels were made up in 40 mM Tris-HCl, 20 mM sodium acetate and 2 mM sodium EDTA at pH 8.3, and run at 20 mA, 40 V for 17 h. The gels were stained with ethidium bromide (0.5 µg · ml^{-1}) and were photographed under UV-light with a Kodak 23A filter with a Polaroid MP-4 land camera and 665 Polaroid slides. Phage λ DNA digested with HaeIII or HindIII was used as size marker.

Figure 1A shows a fractionation experiment of high molecular weight (10 million daltons) male human placenta

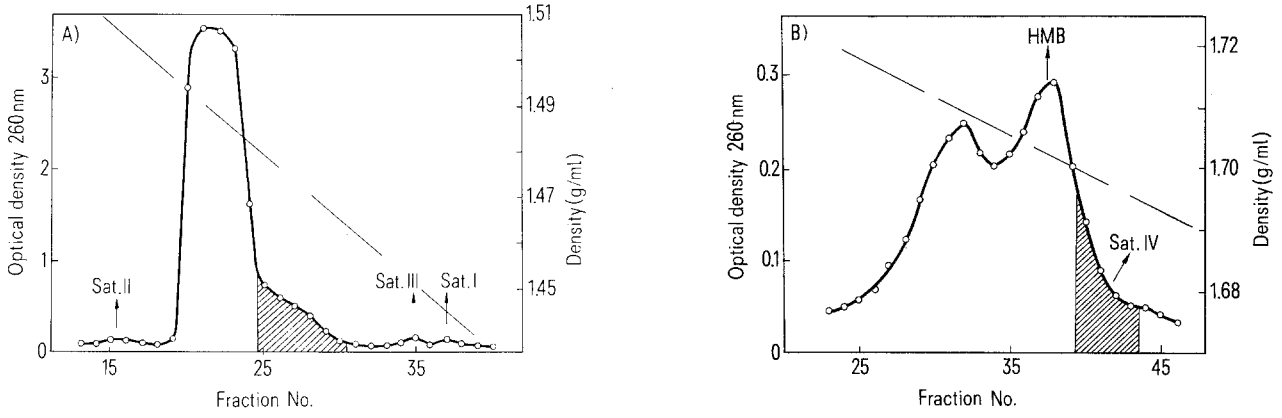

Figure 1. *A* Male human placenta total native DNA, centrifuged to equilibrium in an Ag^+-Cs_2SO_4 density gradient (Ag^+/DNA-P = 0.27) Sat. I, II, III, human satellite DNAs I, II, III. *B* DNA from the hatched region of the gradient of figure 1A centrifuged to equilibrium in CsCl after exhaustive dialyses against 5 M NaCl. HMB, human homogeneous main band DNA; Sat. IV, human satellite DNA IV.

native DNA in a preparative Ag^+-Cs_2SO_4 gradient. The hatched fractions in this figure were pooled and centrifuged in a preparative CsCl gradient (fig. 1B). The light peak of figure 1B corresponds to the homogeneous DNA[3,4].

A DNA fraction isolated from the light side of the homogeneous DNA in CsCl (hatched region of fig. 1B) is digested by HaeIII to a fragment of 2.4 kilobases (fig. 2). Such a fraction appears to correspond to human satellite DNA IV according to its position in the Ag^+-Cs_2SO_4 gradient and to its CsCl density[5].

The 2.4 kilobase fragment obtained by HaeIII digestion is specific to the male sex and is absent in the female sex. It corresponds to the 1.6 million dalton fragment originally identified by Cooke[11] in total human male DNA. In figure 2 the digestion patterns of male satellite DNA IV using EcoRII and AluI are also reported. Satellite IV is digested by AluI to 3 fragments of 2.4, 2.1 and 1.8 kilobases in size and by EcoRII to 2 fragments of 3.4 and 1.8 kilobases in size.

In the following figures the digestion patterns of male and female human homogeneous DNA with different restriction enzymes are reported. Figure 3 shows the distribution of sizes of fragments obtained by AluI digestion of human male and female homogeneous DNA. In the female samples 4 bands are present; besides those, 2 additional bands of 2.6 and 2.0 kilobases sizes are present in the male sample. As also seen in figure 3 EcoRI digestion of human homogeneous DNA gives rise to a series of fragments of different sizes, the majority of which are present either in male and female EcoRI digests; however, a few fragments, of 3.5, 3.0 and 2.0 kilobase sizes respectively, appear to be present only in the male, while fragments from 1.6 to 0.8 kilobase sizes are contained in greater relative amounts in the female than in the male and might therefore be located at least in part on the X chromosomes.

In figure 4 EcoRII digestion patterns of male and female human homogeneous DNA are reported. A 4 kilobase fragment is evident only in the male DNA: another fragment, of 2 kilobase size, is greater in amount in male DNA; while other fragments, of 3, 2.3, 1.7 and 1.4 kilobases are greater in relative amount in the female homogeneous DNA. XbaI digestion patterns of male and female homogeneous DNA are also shown in figure 4. Several bands are present corresponding to fragments of sizes from 0.4 to 2.2 kilobases. Some fragments are greater than others in relative amount. There are not evident differences between male and female XbaI digests. 6 fragments having sizes of 1.05, 0.7, 0.56, 0.4, 0.32 and 0.25 kilobases are obtained by

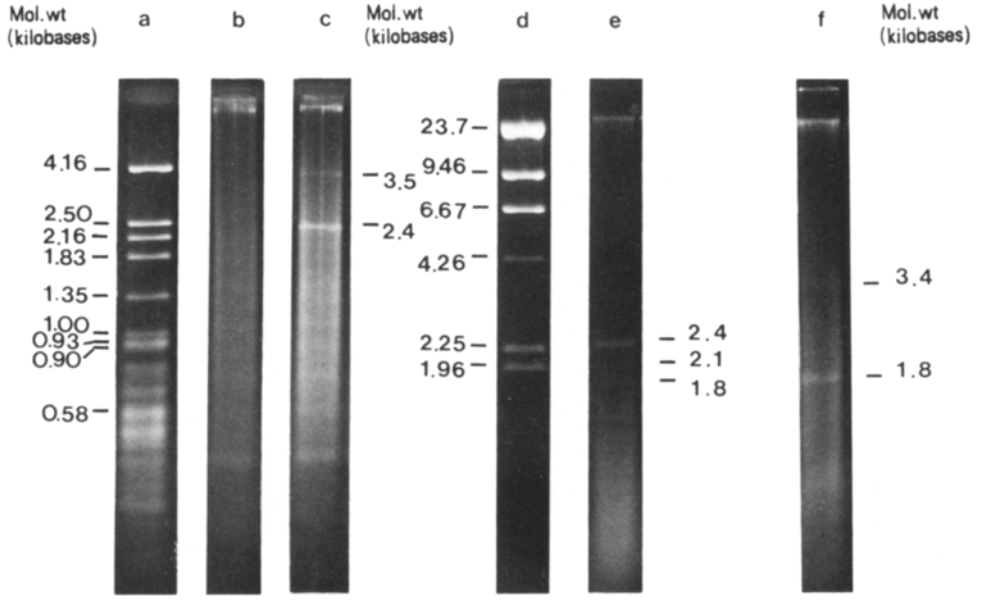

Figure 2. Digestion of λ phage DNA with HaeIII (a) and with HindIII (d) as size markers, of human female satellite DNA IV with HaeIII (b) and of human male satellite DNA IV with HaeIII (c), with AluI (e) and with EcoRII (f).

digestion of homogeneous DNA with HinfI. No consistent differences were evident between digestion patterns in the 2 sexes. Human homogeneous DNA was digested by HindIII to a fragment of 1.9 kilobases size and by BamHI to a fragment of 2.2 kilobases size. No discrete bands were obtained by digestion of human homogeneous DNA with the following enzymes: HpaI, HpaII, BglII and HhaI. Human satellite DNA III was not digested to any discrete fragments by HindIII, BamHI, XbaI, HhaI, HpaI, HpaII, EcoRI, and EcoRII. A slight smear with no discrete fragments was the digestion pattern of satellite III with BglII, AluI, and HinfI. In some experiments with HaeIII digestion of satellite III, extensively purified and the properties of which had been checked by renaturation kinetics and by the separation of the complementary strands in an alkaline CsCl gradient[4], we failed to obtain the usual pattern of fragments[12]: all the DNA remained undigested or just a rudiment of the typical satellite III HaeIII digestion pattern was obtained. On the contrary, homogeneous DNA was constantly digested by HaeIII to the usual pattern of fragments identical to that commonly observed with satellite III. Human satellite DNA II was not digested to discrete fragments by EcoRII, BamHI, AluI, BglII. Also human satellite DNA I was not digested to discrete fragments by EcoRI, EcoRII, AluI, BamHI, HpaII, XbaI, BglII, HhaI and HindIII.

The results reported here indicate that satellite III is not digested to discrete fragments by any one of the restriction enzymes here tested, while homogeneous DNA is digested to a series of discrete fragments by several of the same enzymes. Homogeneous DNA appears to constitute the bulk of the discrete fragments identified in total human DNA after digestion with restriction enzymes.

Satellite DNAs I and II are in general not at all cut by restriction enzymes.

Since highly repeated DNAs might have the function of controlling chromosome structure and organization, they might be changed in conditions in which chromosome

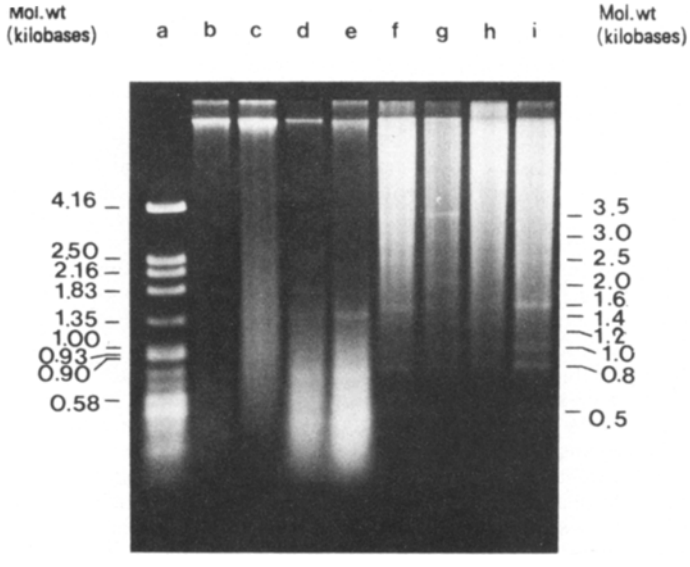

Figure 3. Digestion of λ phage DNA with HaeIII (a) as size marker, of human male satellite DNA I (b) and satellite DNA III (c) with AluI, of human male (d) and female (e) homogeneous DNA with AluI, and of human male (f and g) and female (h and i) homogeneous DNA with EcoRI.

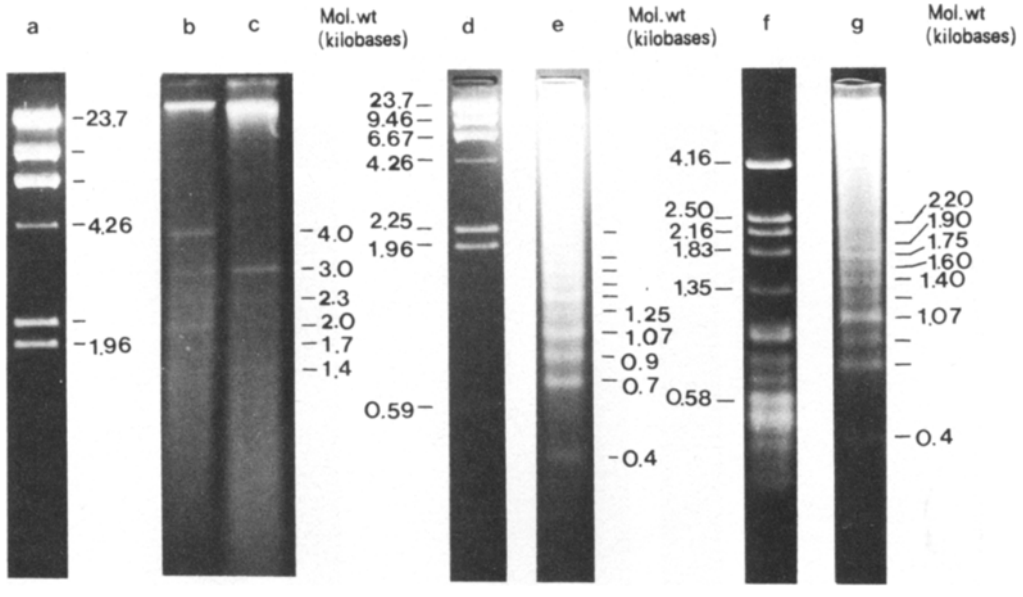

Figure 4. Digestion of λ phage DNA with HindIII (a and d) and with HaeIII (f) as size markers, of male (b) and female (c) human homogeneous DNA with EcoRII, and of human male (e) and female (g) homogeneous DNA with XbaI.

anomalies occur with high frequency as in neoplasias. The study of restriction patterns of highly repeated DNAs might help in understanding the molecular basis of some chromosome anomalies.

Musich et al.[16] have recently identified some families of highly repeated nucleotide sequences in human DNA and have called them human alphoid DNAs for similarity to the α component by them previously isolated from African green monkey DNA. At least the 1.9 kilobase fragment identified in the HindIII restriction pattern of alphoid sequences is present also in the human homogeneous DNA here studied. Therefore we believe that some of the repeated sequences in human alphoid DNA and in human homogeneous DNA correspond.

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3,5-Dibromo-2′-chloro-4′-isothiocyanatosalicylanilide, a potent anthelmintic

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Summary. The compound, 3,5-dibromo-2′-chloro-4′-isothiocyanatosalicylanilide, has been tested against various nematode and cestode parasites in experimental and domestic animals. It shoved 100% activity against *Ancylostoma ceylanicum, A. tubaeformis, Syphacia obvelata, Ascaridia galli, Toxocara* spp., *Toxascaris* sp., *Gnathostoma spinigerum, Hymenolepis nana, Raillietina* spp. and *Taenia* spp. in doses 25–70 mg/kg given in single or multiple administrations.

Among the various helminthic infections, the incidence of intestinal helminthiases is alarmingly high in tropical and subtropical countries[2,3], mainly due to poor sanitation and standard of living. Although during the last 2 decades a number of effective anthelmintics have been developed, none is capable of eliminating the majority of the intestinal helminths occurring concurrently. Mebendazole, though effective against a variety of helminths, is contraindicated during pregnancy because of teratogenic and embryotoxic effects[4]. Further, the drug is not indicated in children below 2 years of age[5]. The highly prevalent tapeworm, *Hymenolepis nana* is insensitive to mebendazole[6-9]. Hence there is a need to develop a drug capable of eliminating most, if not all, of the enteric helminth parasites.

In a programme to develop effective anthelmintics, compounds of different chemical series were tested against a number of experimental helminth parasites. In this communication, we wish to report the broad spectrum anthelmintic activity of a new salicylanilide derivative, 3,5-dibromo-2′-chloro-4′-isothiocyanatosalicylanilide (CDRI compound 77-6). Its cestodicidal efficacy and wide safety has already been reported elsewhere[10].

Materials and methods. A) In vivo screening. The experimental animals were sacrificed under deep chloroform anesthesia.

1. *Ancylostoma ceylanicum.* Hamsters of either sex (40–60 g) were infected orally with 60 3rd stage larvae of *A. ceylanicum.* On day 17–20 post-inoculation, the infection was checked by ovoscopic examination. Hamsters found positive were treated with test compound or standard antihookworm drugs[11] in groups of 3–5 animals in each dose schedule. The efficacy was expressed in terms of absolute clearance of the host and percent worm reduction.

2. Dual infection of *A. ceylanicum* and *H. nana.* To obtain mixed infection, the infective larvae of *A. ceylanicum* were first administered and 2 h later 200 eggs of *H. nana* were fed to each hamster. Both the parasites matured in about 17–20 days' time when the efficacy was evaluated[10,11].

3. *Syphacia obvelata.* Adult mice of either sex, 9–10 months old (30–40 g) harbouring a natural infection with *S. obvelata,* were treated with the compound 77-6[12] in groups of 5–10 animals each at different dose levels. The mice freed from worms, as observed on autopsy, formed the basis of drug effectivity.

4. *Nippostrongylus brasiliensis* and *Nematospiroides dubius.* Drug testing against *N. brasiliensis* was carried out according to Katiyar and Sen[13]. In the case of *N. dubius,* the method of Misra et al.[14] was adopted.

B) In vitro screening. Experiments were conducted as per Sen and Hawking[15]. Worms which appeared to be dead or paralyzed were re-examined for revival of activity after incubation in saline for 1 h.

C) Clinical evaluation in fowls, cats and dogs. Adult fowls, obtained from a local market, naturally infected with tapeworms and roundworms, were treated with compound 77-6 in single or multiple doses. Droppings were examined for worms or worm parts eliminated. When the worm expulsion ceased, birds were sacrificed and the intestine was examined for any retained worm.

Stray cats obtained locally showing helminth eggs in their faeces, were treated with compound 77-6 and the efficacy assessed as described above.