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.

Table 1. Comparative activity of compound 77-6 and standard drugs against *A. ceylanicum* in hamsters

Dose mg/kg	Group	Animals treated / Animals cleared	Percent cure	Worms recovered (mean with range)	Percent worm reduction
Compound 77-6					
70 and above × 1	Exp. (7)*	20/20	100	–	100
	Control	22/0	–	24 (10–42)	–
50 × 1	Exp. (9)	30/16	53.3	2 (0–8)	93.3
	Control	25/0	–	30 (10–42)	–
35 × 1	Exp. (3)	15/0	0	12 (5–22)	61.3
	Control	11/0	–	31 (22–42)	–
25 × 1	Exp. (2)	8/0	0	16 (7–30)	36.0
	Control	7/0	–	25 (10–42)	–
12.5 × 1	Exp. (1)	3/0	0	10 (6–14)	23.0
	Control	3/0	–	13 (10–14)	–
50 × 3	Exp. (1)	6/6	100	–	100
	Control	3/0	–	29 (24–35)	–
25 × 3	Exp. (3)	12/6	50.0	2 (0–9)	92.6
	Control	10/0	–	27 (22–35)	–
12.5 × 3	Exp. (2)	6/0	0	20 (6–31)	31.0
	Control	7/0	–	29 (22–35)	–
Mebendazole					
2.5 and above × 1	Exp. (3)	9/9	100	–	100
	Control	9/0	–	13 (10–14)	–
1.0 and above × 3	Exp. (1)	3/2	66.6	1 (0–2)	95.0
	Control	4/0	–	20 (16–23)	–
Tetramisole					
30 × 1	Exp. (1)	3/2	66.6	1 (0–2)	95.0
	Control	3/0	–	19 (18–21)	–
20 × 1	Exp. (2)	6/3	50.0	3 (0–4)	84.2
	Control	6/0	–	19 (18–41)	–
10 × 1	Exp. (2)	6/0	0	11 (5–21)	50.0
	Control	7/0	–	22 (16–25)	–
Levamisole					
30 × 1	Exp. (1)	3/3	100	–	100
	Control	3/0	–	19 (18–21)	–
20 × 1	Exp. (3)	8/2	25.0	7 (0–22)	72.0
	Control	9/0	–	25 (18–41)	–
10 × 1	Exp. (2)	5/0	0	6 (1–13)	68.4
	Control	7/0	–	19 (16–25)	–
Thiabendazole					
150 × 3	Exp. (1)	3/3	100	–	100
	Control	4/0	–	20 (16–23)	–
100 × 3	Exp. (1)	3/1	33.3	2 (0–4)	93.7
	Control	4/0	–	32 (26–40)	–
50 × 3	Exp. (1)	3/0	0	5 (1–9)	79
	Control	3/0	–	24 (21–25)	–
25 × 3	Exp. (1)	3/1	33.3	15 (0–31)	31.8
	Control	3/0	–	22 (12–24)	–

*No. of experiments.

Table 2. Activity of compound 77-6 against natural infection of *Syphacia obvelata* in mice

Dose mg/kg	Group	Mice treated / Mice cleared	Percent response	Worms recovered (mean with range)	ED_{50}
100 × 1	Exp. (3)*	11/11	100	–	Between 12.5 and 25 mg/kg (approximately)
	Control	7/0	–	10 (2–30)	
70 × 1	Exp. (3)	11/11	100	–	
	Control	7/0	–	10 (2–30)	
50 × 1	Exp. (3)	14/11	78.5	7 (2–15)	
	Control	11/0	–	11 (2–30)	
25 × 1	Exp. (3)	13/10	76.9	5 (2–10)	
	Control	11/0	–	11 (2–30)	
12.5 × 1	Exp. (2)	9/3	33.3	7 (2–10)	
	Control	7/0	–	10 (2–30)	
50 × 3	Exp. (2)	6/6	100	–	
	Control	10/0	–	23 (8–100)	
25 × 3	Exp. (3)	11/9	81.8	40 (5–75)	
	Control	15/0	–	20 (8–100)	
12.5 × 3	Exp. (2)	6/2	33.3	28 (2–80)	
	Control	10/0	–	23 (8–100)	

*No. of experiments.

Clean pups (2–4 kg) were experimentally infected with 4000±200 infective larvae of *A. ceylanicum*. 3 weeks later on faecal examination along with *A. ceylanicum*, eggs of *Toxocara canis* were also noticed (prenatal infection?). The infected animals were administered compound 77-6 in dose of 50 mg/kg for 3 consecutive days. The ascarids voided were picked up and the hookworms separated by decantation. This continued till the worm expulsion ceased when the infection was checked by ovoscopic examination.

Results. The comparative antihookworm efficacy of compound 77-6 and other drugs in hamsters has been presented in table 1. Compound 77-6, in repeated trials, was found to be 100% effective at a single oral dose of 70 mg/kg. A 50 mg/kg dose deparasitized half of the treated animals. Based on host clearance, the effective dose 50% (ED_{50}) was found at 50 mg/kg×1 and on worm reduction between 35 and 25 mg/kg b.wt. Multiple dose therapy did not provide any added advantage.

Table 3. Efficacy of compound 77-6 against intestinal helminths in fowls and cats

Animal	Parasite	Dose mg/kg	Worms expelled	Autopsy results
Fowl	*Raillietina* sp.	250×1	21	No worm (4)*
		100×1	40	No worm (3)
		50×1	30	No worm (2)
		50×1	10	No worm (3)
	Ascaridia galli	250×1	7	No worm (4)
		100×1	Nil	45 worms (3)
		100×1	Nil	8 worms (3)
		50×1	Nil	5 worms (3)
		25×1	Nil	5 worms (2)
		100×2	3	No worm (3)
		50×3	3	No worm (8)
Cat	*Taenia* spp.	50×1	30	5 scolices (9) found attached to the intestinal villi
		50×1	60	No worm (4)
		25×1	1	No worm (4)
		50×2	Innumerable worms	No worm (8)
	Toxocara sp. and *Toxascaris* sp.	100×1	3	4 worms (5)
		50×2	12	No worm (9)
		50×2	26	1 worm (8)
		50×2	15	No worm (4)
		25×3	2	No worm (4)
	Ancylostoma tubaeformis	50×1	46	No worm (2)
	Gnathostoma spinigerum	50×2	7	No worm (8)

*Day of autopsy.

Table 4. Efficacy of compound 77-6 against mixed infection of *A. ceylanicum* and *Toxocara canis* in dog

Animal No.	Dose mg/kg	Worms recovered Hookworms	Ascarids	Results (based on ovoscopic examination)
1	50×3	2047	10	No egg (4)*
2	50×3	1477	5	No egg (5) Pup died on day 7. 4 *A. ceylanicum* (2♂+2♀) were recovered
3	50×3	1713	9	No egg (5)

*Day of examination.

In identical experiments, 95–100% clearance of *A. ceylanicum* was obtained with thiabendazole, mebendazole, tetramisole and levamisole at doses 150 mg/kg×3, 2.5 mg/kg×1, 30 mg/kg×1 and 30 mg/kg×1 respectively.

Against mixed infection of *A. ceylanicum* and *H. nana* the compound 77-6 at a dose of 50 mg/kg×1 removed all *H. nana* and 93.7% *A. ceylanicum*. At this dose thiabendazole was ineffective against both the parasites. Levamisole and tetramisole removed most of *A. ceylanicum* but not *H. nana* and mebendazole expelled *A. ceylanicum* from all treated hamsters and *H. nana* from majority of them (not shown in table).

In vitro, the compound killed *A. ceylanicum* at 1 µg/ml concentration.

Against *S. obvelata* a 70 mg/kg dose cleared all the treated mice (table 2). 25 mg/kg×1 exhibited 77% activity. In vitro the compound killed oxyurids at 0.1 µg/ml concentration.

Compound 77-6 was found inactive against *N. brasiliensis* and *N. dubius*, even at the 250 mg/kg×3 dose level.

In fowls infected with *Raillietina* spp., doses of 50 mg/kg and above removed all worms (table 3). The affected worms were dead, since washing and incubation failed to reactivate them. On autopsy, no parasites, fragments or scolex were seen. *Ascaridia galli* were eliminated at 250×1, 100×2 or 50 mg/kg×3 of compound 77-6. Other doses attempted were ineffective.

In cats, a single administration of 25 mg/kg and above removed *Taenia* spp. within 48 h of treatment from the majority of animals (table 3). In 1 cat, harbouring heavy infection and treated with 50 mg/kg (single dose), when sacrificed, 5 worms (scolices with some immature segments) were found attached to the intestinal wall. Doses of 50 mg/kg×2 and 25 mg/kg×3 removed ascarids from all treated cats, except from one which on autopsy exhibited one *Toxocara* sp. Against hookworms *(Ancylostoma tubaeformis)* a single dose of 50 mg/kg was 100% effective. Spiruroid infection *(Gnathostoma spinigerum)* observed in 1 cat was cleared following 50 mg/kg×2 of the compound.

From pups, the majority of *A. ceylanicum* and *T. canis* were expelled in the 1st 2 days of treatment (50 mg/kg×3) (table 4). However, the worm expulsion continued for 3–4 days. Ovoscopic examination of treated pups on day 4 and 5 revealed no helminth eggs. 1 pup (No. 2), which died on day 7 following drug administration, had 4 *A. ceylanicum*.

Discussion. In tropical countries helminthiases occupy a very important position from the points of view both of nutrition and of morbidity. In the absence of any vaccine, chemotherapy remains the only choice to combat these infections. No single anthelmintic is effective against many helminths of economic importance. Obviously a drug with a broad spectrum of anthelmintic activity is the need of the day. Of the various synthetic compounds tested, compound 77-6 emerged as most potent and effective against many test organisms. Moreover its efficacy is not limited to enteric helminths only. It has been found to exhibit activity against the tissue-dwelling filarial parasites *Brugia maayi* in *Mastomys* (P. Kalpana Murthy, personal communication) and *Dipetalonema viteae* in *Mastomys* (R.K. Chatterjee, personal communication). The oral LD_{50} in mice has been found to be 2392 mg/kg. In general pharmacological investigations, the compound was devoid of any cardiovascular, respiratory and autonomic effects in cats and CNS effects in mice. On the isolated guinea-pig ileum, however, a mild, nonspecific antispasmodic action was noticed (B.N. Dhawan, personal communication).

The next step in drug development is to perform chronic toxicity studies followed by clinical pharmacology and limited field trials. Before taking up such costly and time-consuming studies, it is advisable to confirm the experimental results on target and allied parasites in small

domestic animals. The compound proved promising against different helminths of fowl, cat and dog. The high order of anthelmintic activity coupled with a wide margin of safety "acute toxicity and general pharmacology" calls for detailed investigations.

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Distribution of steroidal glycoalkaloids in reciprocal grafts of *Solanum tuberosum* L. and *Lycopersicon esculentum* Mill.

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Summary. TLC analyses of steroidal glycoalkaloids of the scion and stock of reciprocally-grafted potato and tomato plants and tracer studies involving administration of [^{14}C]-labeled alkaloid precursors to scion and stock suggest that alkaloid transport between root and shoot does not take place in these species.

Steroidal glycoalkaloids are particularly prominent in members of the Solanaceae but their function in the plant is still uncertain[2]. Because of their toxicity, particularly to fungi and insects, it is generally thought that they may play a protective role in plants. Their sites of biosynthesis and accumulation are reasonably well understood[3,4], but their mobility in the plant, knowledge of which could help clarify their function, has long been a subject of debate. Kern[5] found measurable quantities of alkaloid in sap exuded from decapitated tomato plants but later work[4,6] failed to confirm this finding and alkaloids have not been detected in liquid culture medium supporting the aseptic growth of excised roots of *Lycopersicon* spp.[4,7,8]. However, the extent to which excision and cultural conditions could have influenced results is not known. Nor would either of these methods provide information on transport from the shoot (which is the main region of alkaloid synthesis) to the root. More recently, Segal and Schlösser[9] proposed that the aglycone of steroidal glycoalkaloids is the physiologically-active moiety with the glycoside being the 'water-soluble transport form'.

In this study, root/shoot transport of the steroidal glycoalkaloids of potato (α-solanine [**I**] and α-chaconine [**II**]) and tomato (α-tomatine [**IV**]) has been investigated using reciprocal grafts between these plants. Phylogenetically-related species were chosen to help ensure biochemical compatability (the alkaloids are chemically similar, but chromatographically distinct) and graft formation. Use of grafted plants also overcomes a problem associated with normal plants viz. radioactivity appearing in metabolites of non-treated parts due to transport of a radioactive precursor, followed by synthesis.

Materials and methods. Tomato (cv. Suttons Potentate Best of All) and potato (cv. Majestic) plants were grown in John Innes No. 2 compost for approximately 8 weeks and 4 weeks respectively. Glasshouse temperature was 20–22 °C. 'Whip and tongue' grafts were made by cutting obliquely into the stem of intact plants 10 cm above soil level, upwards in the scion species and downwards in the stock species, and inserting the scion 'lip' into the stock groove. The graft region was then bound with aluminium foil and sellotape. Plants were grown in this condition for approximately 4 weeks after which stems were cut, 5 cm above the graft union in the stock and 5 cm below the union in the scion. Grafted plants were grown on for between 2 weeks and 3 months. Plants grown for 3 months on a tomato stock were re-potted after 4 weeks; those on a potato stock, after 4 and then 8 weeks.

2-week-old grafted plants (approximately 25–30 cm) were removed from their pots, their roots cleaned of compost and washed, and divided into stock and scion by cutting 2 cm above and below the graft union. The union itself was discarded. 3-month-old plants (approximately 60–70 cm with tomato as stock and approximately 130 cm with potato as stock) were cleaned, as above, but divided into a greater number of parts viz. a) leaf + petiole b) stem of scion excluding the 3 cm zone directly above the graft union c) the 3 cm of scion stem above the graft union d) the 3 cm of stock stem directly below the union e) remainder of the stock stem f) root system g) tubers (where appropriate). For each of the 4 groups of plants used (potato on tomato, 2 weeks and 3 months; tomato on potato, 2 weeks and 3 months) 3 individual plants were analyzed.

In radiotracer studies, 4-week-old grafted plants were used, with all side shoots removed. To 1 batch of plants, 37kBq of [4-^{14}C]-cholesterol (in 0.1 cm^3 acetone) were applied to each of 5 selected young leaves every second day for 10 days (i.e. a total application of 925kBq). In another batch, root systems were thoroughly washed then placed in aerated liquid nutrient medium[10] containing 925kBq of DL-[2-^{14}C]-mevalonic acid lactone in a darkened glass container. After 7 days, root solutions were renewed and left for a