No mortality or significant weight loss occurred in any animals during the experiment. The effects of asbestos on DNA synthesis in various organs are summarized in the Table; each value represents the mean ± SE of 3 replicate analyses. The data presented for all organs, except the pancreas, are based on only 2 specified segments, as these were representative of values in other segments of the same organ; for the pancreas, data on each individual segment and their composite means are also presented.

Levels of thymidine incorporation in all organs were relatively low, in contrast to previous studies in rodents in which comparable doses of tritiated thymidine were used (Table[23,24]). As can be seen from the standard errors, variability in thymidine incorporation between animals in the same test or control group was relatively high. In general, incorporation was similar in all organ segments of control and test animals (Table). A marked increase, approximately twice control values, in thymidine incorporation was consistently found in all segments, head, body, and tail, of the pancreas 9 days following administration of asbestos. Incorporation in the pancreas at other intervals, and at all intervals in all other segments of all other organs was comparable to control values. Histopathological examination of the pancreas and other organs assayed for thymidine incorporation, failed to reveal any differences between test and control animals.

While the relatively small numbers of animals used in these preliminary studies limits formal statistical analysis of the data, the marked increase in DNA synthesis, which was consistent in all segments of the pancreas, 9 days following asbestos administration is of particular interest. These findings are in contrast with data from the rat, in which asbestos induced elevated thymidine incorporation in DNA of the stomach, duodenum, and jejunum, but not in DNA of the colon or pancreas over a 3-day period following administration of chrysotile in a dose range of 5 to 100 mg/kg[23]; transient increases in DNA synthesis were noted at other intervals in the GI tract of the rat following 100 mg/kg dosage[22].

The small number of primates tested over restricted intervals following oral administration of asbestos limits generalization from these preliminary findings. While the evidence of increased DNA synthesis in the pancreas 9 days following gavage is consistent with data on the widespread dissemination of asbestos fibres in various organs of experimental animals following its ingestion[15–17], failure to demonstrate such effects in the GI tract of primates may be consistent with findings of recent studies in which asbestos fibres could not be identified in the gastric mucosa of baboons following gavage with asbestos[24]; it has been suggested that the high level of gastric mucus in monkeys may limit mucosal penetration of asbestos fibres[25].

Increased synthesis of pancreatic DNA in primates and similar effects in the GI tract of rats at relatively short intervals following the ingestion of asbestos may reflect DNA replication following asbestos-induced cytotoxicity or a direct stimulation of DNA replication. Further study of such early effects of asbestos on DNA synthesis may provide information on basic mechanisms of asbestos carcinogenesis.

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Antischistosomal and Some Toxicological Properties of a Nitrodiphenylaminoisothiocyanate (C 9333-Go/CGP 4540)

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Summary. A procedure to enhance the schistosomicidal effectiveness in vivo of an isothiocyanate derivative and some of its antischistosomal properties are reported. Determinations of the effects of this compound on tissue thiol levels and on highly sensitive bacterial tester strains have indicated that its mutagenic potential is of a low order and that the latter is decreased further after reduction of the host's intestinal bacterial flora.

Studies of STRIEBEL[2] have revealed that 4-isothiocyanato-4′-nitrodiphenylamine (C9333-Go/CGP4540) has high antischistosomal activity when administered as a single oral dose to animals experimentally infected with schistosomes. In order to be effective, a high oral dose (200 to 300 mg/kg) of moderately large particle sizes (average diameter: 30 μm)of this compound was required. The effectiveness of this isothiocyanate derivative was greatly enhanced when its particle size was reduced to an average of 0.5 μm in diameter by ball mill treatment for 14 days of a suspension of the compound in 1% Cremophor EL (BASF) and 25% glycerol. When this formulation (subsequently referred to as 'formulated' compound) was administered to mice infected with *Schistosoma mansoni*, parasitological cures in 23% of the mice were observed with a single dose as low as 5 mg/kg; when this dose was doubled, 95% of the mice were free of worms (Table). On the basis of this steep dose response, there were only slight variations in the susceptibility of the entire worm population to the antischistosomal effects of this compound. There were few, if any, differences in the susceptibility of 4 geographic strains of *S. mansoni* (3 strains from Puerto Rico, 2 strains from Liberia, and 1 strain each from Brazil and St. Lucia); furthermore, a strain resistant to another antischistosomal compound, hycanthone[3,4], proved susceptible to the isothiocyanate derivative. In

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Effect of a single oral dose of formulated 4-isothiocyanato-4'-nitrodiphenylamine in mice infected with mature *S. mansoni* and *S. japonicum*

Schistosome species	Single oral dose (mg/kg)	Number of mice	Reduction in number of worms (%)	Mice with parasitological cures [a] (%)
S. mansoni	2.5	24	52	0
	5	82	80	23
	7	216	92	84
	10	196	99	95
	20	141	100	100
S. japonicum	5	22 (Philippine strain)	100	100
		15 (Japanese strain)	87	67
		14 (Chinese strain)	91	75
	10	20 (Philippine strain)	100	100
		12 (Japanese strain)	100	100
		36 (Chinese strain)	100	100
	20	15 (Philippine strain)	100	100
		22 (Japanese strain)	100	100
		18 (Chinese strain)	100	100

[a] Percentage of mice in which not a single live worm was recovered.

view of the notorious differences in drug susceptibilities of various strains of schistosomes, their uniform response to this drug formulation is noteworthy. Experiments with hamsters infected with *S. mansoni* revealed that this drug formulation is equally effective in the latter host species.

Another unusual feature is provided by the observation that, in contrast to any other known antischistosomal compound, 3 geographic strains of *Schistosoma japonicum* were at least as, if not more, susceptible than *S. mansoni* to this compound (Table).

The susceptibility in vivo of *S. mansoni* to this formulation of the isothiocyanate derivative varied in relation to the age of the parasite. There was no difference when the age of the infection was 55 days or older; however, 1.5 to 3 times higher doses of the formulated compound were required to produce parasitological cures in infections whose age varied between 22 and 51 days. Worms 18 days or younger were even less susceptible. On the other hand, larger doses administered either orally or parenterally proved to have prophylactic activity, i.e., they prevented the development of adult worms following exposure (by tail immersion) of the mouse host to 100 cercariae of *S. mansoni*.

Within one to several hours after the oral administration of a curative dose of formulated CGP4540, there was a complete shift of *S. mansoni* from the mesenteric veins to the liver sinuses. This hepatic shift was associated with a reduction of muscular activity, especially in the acetabulum, and with a decreased length of the worms[5]. While all the worms initially shifted to the liver, many of those surviving a 2- to 3-week posttreatment period returned to the mesenteric veins, but were eliminated eventually after an additional 20 to 30 days. Apparently at least two mechanisms are involved in the effect of this isothiocyanate derivative, one resulting in an almost immediate hepatic shift, and another leading to the eventual destruction of the worms. This is indicated also because some marked biochemical changes, such as glycogen loss and pronounced reductions in the activities of glycogen phosphorylase phosphatase[6] and of a specific globinase[7] were observed only after the hepatic shift had occurred[8].

Another method to reduce the average particle size to approximately 0.5 μm consisted of milling the isothiocyanate derivative with crystalline sucrose in light mineral oil for 6 to 8 h[8]. This yielded a product whose schistosomicidal effectiveness approached that obtained by ball milling in Cremophor EL and glycerol.

Studies of the mutagenic and carcinogenic potential of the compound have been initiated. Administration of carcinogenic azo dyes to mice and rats results in an increase in acid soluble tissue thiols, while noncarcinogenic analogs of these compounds fail to have this effect[9–11], which has been ascribed to an interaction of the electrophilic carcinogens with nucleophilic thiols and to the resulting removal of a feedback mechanism, bringing about an overproduction of sulfhydryl compounds[12]. As in the case of azo dyes, administration of nitroaromatic carcinogens, such as nitrofurans, nitrothiazoles, and nitrophenyl derivatives, was followed by an elevation of mouse tissue thiol levels. By contrast, these levels remained unchanged after the administration of high doses (200 to 300 mg/kg) of the formulated isothiocyanate derivative.

The antischistosomal isothiocyanate failed to exhibit any detectable mutagenic activity when tested in the absence or in the presence of liver microsome preparations with 2 highly sensitive his$^-$ *Salmonella* tester strains, TA-98 and TA-100, recently developed by Ames et al.[13,14]. This is of considerable interest because 2 presently widely

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used antischistosomal drugs, niridazole and hycanthone, are mutagens[15–17] and, as found recently, are also carcinogenic in mice[18,19]. Furthermore, an antischistosomal nitrofuran (furapromidium, F-30066), widely used in China for the treatment of patients infected with *S. japonicum*[20], has a mutagenic potency of the same order of magnitude as niridazole[21]. When a host-mediated assay system was used, some mutagenic effects on *Salmonella* strain TA-100 were detectable when these bacteria were injected i.p. 2 h after the administration of a dose of the formulated compound exceeding the curative dose by a factor of 25 (i.e. 250 mg/kg). 6 h thereafter the bacteria were washed out from the peritoneal cavity and plated on a histidine-deficient agar. Since the number of mutant colonies was more than twice as great as that of the controls, a mutagenic metabolite must have been produced in the host. This was confirmed by the finding that, following the oral administration of the formulated compound to mice, one of several urinary metabolites was found to be mutagenic for *Salmonella* strain TA-100; none was mutagenic for strain TA-98. A very marked decrease in this mutagenic urinary metabolite, and of the mutant colonies found in the host-mediated assay, was observed when the bacterial flora of the host's intestines was reduced by the oral administration of succinylsulfathiazole (1 g/kg once daily for 3 successive days), preceding the administration of formulated CGP4540. This finding suggests a role of intestinal bacteria in the formation of a mutagenic compound from CGP4540. Preliminary results indicate that reduction of the intestinal bacterial flora by the administration of succinylsulfathiazole or of a mixture of antibiotics does not eliminate the antischistosomal activity of the isothiocyanate derivative, but more studies are required to determine whether the schistosomicidal activity of this compound can be dissociated completely from its metabolism to a mutagen.

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Effects of Physalaemin, a Vaso-Active Peptide from Amphibian Skin, on the Excitability of an Identifiable Molluscan Giant Neurone of *Achatina fulica* Férussac

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Summary. We examined effects of several vasoactive peptides (substance P, physalaemin, neurotensin, bradykinin, angiotensin etc.) on the excitability of molluscan giant neurones identified in the subesophageal ganglia of *Achatina fulica Férussac.* Of these peptides, only physalaemin showed a remarkable excitatory effect on a giant tonically autoactive neurone.

We attempted to elucidate effects of vaso-active peptides, listed in the Table, on neuronal excitability, using 2 spontaneously firing giant neurones (the TAN, tonically autoactive neurone and the PON, periodically oscillating neurone)[2] identified in the subesophagealg anglia of an African giant snail (*Achatina fulica* Férussac). Of the examined peptides, only physalaemin[3], a hypotensive peptide extracted from the skin of a South-American amphibian (*Physalaemus fuscumaculatus*), showed any effect on TAN excitability. All peptides examined had no effect on PON excitability.

We implanted a microelectrode into the cell body of the identified neurone, recorded its biopotential with a pen-writing galvanometer, and counted the number of its spike discharges per min with a spike counter. We applied the peptides to be examined in 2 ways: a peptide dissolved in the physiological solution[4] was directly applied to the dissected ganglia (bath application); or a microdrop (100 ~ 150 μm diameter) of a peptide solution was formed at the tip of a micropipette containing the solution by oil pressure, and placed just on the surface of the identified neurone (microdrop application)[5].

Vaso-active peptides examined in the present study

No.	Substance	Amino acid sequence
1	Substance P[a]	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
2	Physalaemin[a]	Pyr-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH_2
3	Eledoisin-related peptide[a]	Lys-Phe-Ile-Gly-Leu-Met-NH_2
4	Neurotensin[a]	Pyr-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu
5	Xenopsin[b]	Pyr-Gly-Lys-Arg-Pro-Trp-Ile-Leu-OH
6	Bradykinin[a]	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
7	Lys-Bradykinin[a]	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
8	Met-Lys-bradykinin[a]	Met-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
9	Angiotensin I[a]	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu
10	Angiotensin II[a]	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe
11	Hypertensin[c]	Asn-Arg-Val-Tyr-Val-His-Pro-Phe
12	Angiotensin III[a]	Arg-Val-Tyr-Ile-His-Pro-Phe

[a] Product of Protein Research Foundation, Osaka; [b] donnated by Eisai Co. Ltd.[1]; [c] donnated by Ciba-Geigy Ltd.