# DIETHYLCARBAMAZINE

A REVIEW OF THE LITERATURE WITH SPECIAL REFERENCE TO ITS PHARMACODYNAMICS, TOXICITY, AND USE IN THE THERAPY OF ONCHOCERCIASIS AND OTHER FILARIAL INFECTIONS

by

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**Recommendations for research on diethylcarbamazine**

1. Mode of action of DEC upon microfilariae
2. *Mansonella ozzardi*
3. Skin test for onchocerciasis with DEC
4. Radioactive diethylcarbamazine
5. Action of DEC on adult worms
6. Therapeutic so-called allergic reactions to DEC in patients with onchocerca or other filarial infections
7. Local application of DEC to the eyes
8. Methods of mass administration
9. Toxicity of arsenical compounds
INTRODUCTION

Difficulties encountered from toxic reactions during treatment of onchocerciasis patients with diethylcarbamazine and suramin - the only two drugs at present considered suitable for treatment - necessitated a thorough search of the literature in an attempt to throw light on the pharmacodynamics and toxicity of these drugs, both of which are used experimentally in other fields of pharmacology, biochemistry, etc.

A literature search was done on Medline and with the assistance of Professor Lammler (suramin). It was stimulated as a result of recommendations of a Scientific Advisory Panel Working Group on the Chemotherapy of Onchocerciasis, Drug Efficacy and Toxicity (Geneva, 28-29 November 1974), and of the Scientific and Technical Advisory Committee of the Onchocerciasis Control Programme in the Volta River Basin (OCP) at its second and third sessions (Geneva, 3-5 June 1975 and 3-4 March 1976), and was financed by OCP (UNDP Project No. RAF/74/CO4).

The literature revealed by the search was studied and retrieved by Dr F. Hawking in two papers, one dealing with diethylcarbamazine and the other with suramin. This led to recommendations for future investigations; all possible sources of information in the literature to date have now been tapped.

1. CHEMISTRY

1.1 Chemical and physical properties

Name

1-diethylcarbamyl-4-methyl-piperazine

Formula

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{N}^{(4)} \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{O} & \quad \text{N} - \text{C} - \text{N} \\
\text{CH}_2 & \quad \text{CH}_2^{(1)} \\
\text{C}_2\text{H}_5 & \\
\text{C}_2\text{H}_5
\end{align*}
\]

Synonyms

Hetrazan, Banocide, Notézine, Caricide, Carbilazine, Supatonin, 3799 R.P.

Diethylcarbamazine was first issued as the chloride, but is now produced as the dihydrogen citrate which contains only half its weight as base. In reporting doses it is important to indicate whether the doses refer to a specific salt or to the base; unless otherwise stated, it can generally be assumed that the dose refers to the citrate. Diethylcarbamazine is a white powder, freely soluble in water with a slightly unpleasant sweetish taste. It is stable under all ordinary laboratory conditions and is fully stable to autoclaving, when mixed with diet.

1.2 Relation of structure to activity

The piperazine ring is of fundamental importance as was shown during the original investigations of Hewitt et al. (1948) although piperazine itself has no antifilarial activity. Piperazine is, however, active against Ascaris and other round worms in the intestine, and its ring structure may have specific action on parasitic nematodes in general. Various compounds with slightly different rings, e.g. N, N-diethyl-4-methyl-1, 4-diazacycloheptane-1-carbamide hydrochloride (JCS-110), have some antifilarial activity, but diethylcarbamazine remains the most effective known antifilarial agent (Reinertson & Thompson, 1955). In the piperazine series, a carbethoxy radical in position 1 of the ring, with various substitutions in position 4, produced high antimicrofilarial activity; but as the alkyl chain was increased the
toxicity became greater and the activity less. If the alkyl group is greater than -C₄H₉, activity is lost. The alkyl group in position 4 is not particularly significant, however, since high activity continues after it is removed. The only compounds lacking the carbethoxy group, which show marked activity against microfilariae, contain an ethyl, diisopropyl, dimethyl, or diethyl carbamyl group in position 1. A compound with the formula:

\[
\text{C}_2\text{H}_5 - \text{O}_2\text{C} - \text{N} \quad \quad \text{(4)} \quad \text{N} - \text{CH}_2 \text{CH}_2 - \text{N} \quad .2\text{HCl}
\]

is also active (Patra et al., 1969).

Apparently for the above compounds to be effective there must be two aliphatic amines connected by a saturated carbon framework; one amine must be basic and the other must be modified by some type of carbonyl function. The spatial separation of these amines in diethylcarbamazine is optimal; compounds with wider separation are less active.

A modification of the diethylcarbamazine structure has been described by Saxena et al. (1970) and studied further by Thompson et al. (1973) and by Sturm et al. (1974). It is named "compound II" for convenience, and will be described in more detail below. In this compound, the terminal carbon of one ethyl group is connected to the piperazine ring at the 2 carbon position as follows:

\[
\text{H}_3\text{C} - \text{N} \quad \text{(4)} \quad \text{CH}_2 - \text{CH}_2 - \text{N} - \text{C} - \text{N} - \text{CH}_2 - \text{CH}_3
\]

The essential structure is similar to that of diethylcarbamazine but the change makes the molecular structure rigid and locks the urea moiety into a single rotational conformation. In contrast, the diethylcarbamyl group of diethylcarbamazine can rotate on all its single bonds. The new compound is approximately planar with the carbon-oxygen bond of the carbamyl and all the nitrogens lying in the same plane. There are 2.8Å between N₁ and N₄ of the piperazine ring and 3Å between N₄ and N₂. Since the compound is as active or perhaps more active than diethylcarbamazine, this configuration is apparently optimum.

In addition to this modification, various linkages across the piperazine ring by -CH₂ - CH₂ - have been investigated by Sturm et al. (1974). Linkage between positions 3 and 5 on the piperazine ring does not diminish activity. Other linkages between positions 1 and 6 and 2 and 6 diminish activity moderately.

1.3 Analogues of diethylcarbamazine

1.3.1 Compound II (Centperazine)

This compound was first reported by Saxena et al. (1970); a brief description as well as its chemical formula has been given above. Its action is almost identical to that of diethylcarbamazine. When tested by Saxena et al. (1970) and by Thompson et al. (1973) in cotton rats and jirds infected with Litomosoides, the antifilarial response was not closely proportional to the dose, a situation which made accurate comparison difficult. Saxena et al. claimed that it was five times more active than diethylcarbamazine and Thompson et al. agreed that it was as active or slightly more active than diethylcarbamazine upon the microfilariae. Like diethylcarbamazine it had no action on the adult worms of Litomosoides. The acute toxicity in mice
was slightly less than diethylcarbamazine. This compound should be investigated further and tested in man against *Wuchereria bancrofti* and against *Onchocerca volvulus*. Although similar in behaviour to diethylcarbamazine in cotton rats and jirds, it is possible that in man it might present some advantages of greater activity on adult worms. It had no action against *Hymenolepis nana* or *Nippostrongylus* or against *Chandlerella hawkingi* in crows. Like diethylcarbamazine it showed anti-inflammatory action in rats and inhibited passive cutaneous anaphylaxis (Saxena et al., 1970).

### 1.3.2 Hoechst 33258

\[
2-\sqrt{2} \cdot (4\text{-hydroxyphenol}) - 6 \cdot \text{benzimidazolyl} - 6\cdot (1\text{-methyl-4 piperazyl}) \text{ benzimidazole - tri HCl.}
\]

![Chemical structure of Hoechst 33258](image)

This was described by Raether & Lämmler (1971). It is fluorescent. The hydrochloride is water soluble, but the diphosphate is sparingly soluble and is thus only slowly absorbed after oral and parenteral administration. In cotton rats infected with *Litomosoides* its action resembled that of diethylcarbamazine but was slower, more complete and more prolonged. The phosphate, given as 8 mg/kg i.p. on five consecutive days, caused all the microfilariae to disappear with 14-67 days; only very few had returned after 111-297 days. In half of the rats the adult female worms were dead but, in the other half, females were alive and producing microfilariae.

The maximum tolerated dose of the hydrochloride for mice was 250 mg/kg i.p. or 625 mg/kg s.c. The prolonged action is probably due to a depot effect since the compound remains in the tissues for a long period of time. When administered to mice, the nuclei of the liver and kidney still showed fluorescence after 37 days (Lämmler & Schütze, 1969). The compound binds to DNA, but whether such binding has a positive or negative value seems to depend upon which nuclei of the host or of the parasite are preferentially involved.

It has been tested by Duke (personal communication) on chimpanzees infected with human *O. volvulus*. One animal was given 5 mg/kg i.m. on five consecutive days. Four weeks later the skin had been completely cleared of microfilariae, but 10 days after this the chimpanzee was found dead. There were large sterile abscesses at the sites of injection. Adult worms were alive and contained both live and moribund microfilariae. Two other chimpanzees were treated with an improved pharmaceutical preparation, 4 mg/kg i.m. twice weekly for two to four weeks. This preparation did not cause abscesses. One lightly infected animal was completely cleared of microfilariae for over 10 months. In the other case, the microfilariae were reduced to near zero but after the sixth dose the animal became very weak, relapsed into coma and died. Most of the adult worms were dead, but a few were still alive although the microfilariae in them were deformed and mostly immobile. These worms were probably moribund. It appears that the compound kills the microfilariae of *O. volvulus*, and many of the adult worms, but it may be dangerously toxic. Unless the danger of toxicity can be removed, further trials seem inadvisable.

A further modification of Compound E has been introduced by Friedheim (1974) in which two molecules of Hoechst 33258 are combined with a molecule of an arsenical compound (P151) closely related to melarsoprol. When this was given intramuscularly to dogs infected with *Dirofilaria immitis* (dose, 3-41.5 mg/kg one to four doses at two- to four-day intervals), it killed the
microfilariae and the adult worms. This combination was tested against *O. volvulus* by Duke (personal communication). One infected chimpanzee was given four intramuscular doses of 6 mg/kg at three- to four-day intervals and another was given 3 mg/kg weekly. Both animals showed anorexia, lassitude and loss of weight. In both animals the microfilariae and the macrofilariae appeared to have been killed. One heavily infected man was then given four weekly doses of 1 mg/kg. The treatment was well tolerated; but the microfilarial count was little altered and three weeks after the last dose an excised nodule showed healthy adult worms. Six weeks after the last dose, the patient developed a large vitreous haemorrhage in his right eye producing blindness in that eye. After some months, the haemorrhage cleared and a degree of useful vision was restored. Another patient received four weekly doses of 2 mg/kg and after the last injection suffered anorexia, lassitude and pain in the knee joints. His microfilarial count fell dramatically to 3% of its initial value during the four weeks, and an excised nodule showed no living worms. Approximately six weeks later, however, he died suddenly of what appeared to be a diabetic coma. In view of these two mishaps, further trials were discontinued.

1.3.3 Hoechst 28637a

Cyclohexane carboxylic acid-M-methyl piperazine citrate. It is closely similar in structure and activity to diethylcarbamazine when tested on *L. carinii* in *Mastomys natalensis*, but its toxicity is lower (Lummer et al., 1971).

\[
\begin{align*}
\text{HOE 28637a} \\
N - CH_3\text{-citrate}
\end{align*}
\]

1.3.4 Hoechst 29691a

Tetrahydropyran carboxylic acid-N-methylpiperazine citrate. This is another compound closely related to diethylcarbamazine. It is slightly more active than diethylcarbamazine against the microfilariae of *L. carinii* in *M. natalensis*, and slightly less toxic (Lummer et al., 1971).

\[
\begin{align*}
\text{HOE 29691a} \\
N - CH_3\text{-citrate}
\end{align*}
\]

1.3.5 Hoechst 37598

This belongs to the bis benzimidazole series and has a long-acting repository effect. It was given by Duke (personal communication) to a chimpanzee infected with *O. volvulus*, at a dose of 6 mg/kg intramuscularly daily for five days. There was temporary diminution of microfilariae but they built up again in two months. The animal was then treated with 10 mg/kg twice weekly for seven doses but there was no change in the microfilarial count during the next 12 weeks. After 24 weeks of treatment, however, there were two large abscesses at the sites of injection.

1.4 Chemical estimation of diethylcarbamazine

Estimation in blood and urine can be made by a colorimetric method described by Lubran (1950). In this method the body fluid is made alkaline and extracted with ethylene dichloride. The addition of one drop of carbon disulfide to urine helps remove primary and secondary amines which otherwise give a higher blank value. The ethylene dichloride is then shaken with an aqueous solution of bromthymol blue, so that a coloured salt of diethylcarbamazine is formed which passes into the solvent, while the dye itself remains in the aqueous phase. Plasma or serum should not be deproteinized before extraction. Amounts can be measured as low as the normal blank value which is about 1 mcg/ml for serum, and 3-10 mcg/ml for urine. A similar
A modification using bromphenol blue has been described by Rao & Subrahmanyam (1970). Another method of estimation by picric acid has been reported by Ramachandran (1973). Methods for pharmaceutical estimation depending upon reineckates have been described by Sheng et al. (1963) and using bromocresol green by Vadodaria et al. (1968). When diethylcarbamazine has been added to the food of animals, its concentration can be measured by colorimetry, or by gas-liquid chromatography but not by polarography (Allen & Beckman, 1964).

Diethylcarbamazine has also been estimated with radioactive techniques by Bangham (1955a, b), using samples labelled with C\textsuperscript{14} in the piperazine ring or in the methyl group, and by Faulkner & Smith (1972).

2. ABSORPTION, EXCRETION, DISTRIBUTION AND METABOLISM

2.1 Absorption, excretion and distribution

When diethylcarbamazine is administered orally to either animals or man, it is rapidly absorbed from the alimentary canal. One dose of 10 mg base per kg produces a peak blood level of 4-5 mcg/ml in three hours, the time when toxic symptoms are most prominent. The level then gradually falls to zero within about 48 hours. Most of the excretion in the urine occurs in the first 24 hours, during which time 10-26% of the dose may be recovered as diethylcarbamazine. When 3 mg base per kg is taken twice daily for four-and-a-half days, the blood concentration of diethylcarbamazine reaches 4-5 mcg/ml by the third day and then falls even though the dosage is continued. Diethylcarbamazine apparently penetrates readily into hydrocele fluid, and presumably into other body fluids (Hawking, 1950; Lubran, 1950).

The distribution of tritium-labelled diethylcarbamazine in mice, after intraperitoneal injection, has been studied by autoradiography (Sakuma et al., 1967). The compound was rapidly distributed, and 20 minutes after injection the radioactivity in the liver, kidney, adrenal gland, muscle and gastrointestinal tract reached its highest density; by six hours it had diminished greatly. It accumulated in the brain at 20 minutes and diminished after one hour. Specially high accumulations occurred after one to three hours in the salivary gland, adrenal medulla, putuitary gland and lymph nodes. The compound accumulated in and was excreted from the kidney, from the glandular portion of the stomach wall, and from the liver into the bile.

The relations between dosage, blood levels and microfilaricidal effect have been studied in patients infected with W. bancrofti by Hujimaki (1958). Treatment was given by him for 10-14 days. With daily oral doses of 0.3-0.6 mg/kg citrate given in three administrations, the morning blood level was less than 0.65 mcg/ml and there was no therapeutic effect. With daily doses of 1.5 mg/kg, the blood level was 0.5-1.1 mcg/ml in the morning and over 1.0 mcg/ml later in the day, and microfilariae were eventually all exterminated. After a single daily dose, e.g. 6 mg/kg, there was a peak blood level of 3.0 mcg/ml, which fell rapidly within several hours. On the other hand, if the same amount was divided into three or six doses, the blood level remained steady throughout the day. The minimum effective concentration in the blood seemed to be 0.8-1.0 mcg/ml. For the treatment of bancroftian filariasis, Hujimaki recommended a daily dose of 6 mg/kg divided into six administrations, for 14 days. This should give a blood level of 1.8-2.6 with an average of 2.1 mcg/ml in the morning and probably a higher level during the day.

2.2 Metabolism

A more specific analysis of the metabolism and distribution of DEC in rats and monkeys was made by Bangham (1955a, b), who worked with the drug labelled with C\textsuperscript{14} in the piperazine ring. An oral dose is rapidly absorbed, and about 70% of the piperazine metabolites are excreted in the urine within 24 hours. When an intravenous dose of 2.25 mg/kg is administered, 95% is excreted within 30 hours; 10-20% of this amount appearing as unchanged drug. This varies slightly, however, depending on the dose level given. Metabolism is very rapid and the drug is excreted in four different forms, in all of which the piperazine ring remains intact. Diethylcarbamylpiperazine accounts for 5-15%, methylpiperazine for 2-5%,
FIG. 1. BREAKDOWN OF THE METABOLITES EXCRETED IN THE URINE OF RATS AFTER AN ORAL ADMINISTRATION OF 20 mg/kg OF $^{14}$C-LABELLED DIETHYLCARBAMAZINE

Diethylcarbamazine-N-oxide (major) 50%

1-Ethylcarbamyl-4-methylpiperazin (major) 23%

Diethylcarbamazine
Reported by Bangham, 1955

Piperazine (minor) 5%

N-methylpiperazine (minor) 2-5%

Diethylcarbamylmethylpiperazin (minor) 5-15%

---

1 From Faulkner & Smith (1972).
and piperazine for 1-6% of the 95% excreted. The fourth form was an unstable basic compound which was not identified by Bangham. This metabolite could be demonstrated in the blood within two minutes of an intravenous injection.

The metabolism has been further investigated in rats by Faulkner & Smith (1972) using diethylcarbamazine labelled with 14C in the 3,5 positions of the piperazine ring. They found that the main metabolite, not identified by Bangham, was diethylcarbamazine-N-oxide (see Fig. 1). A second major metabolite was 1-ethyl carbamyl-4-methyl piperazine accounting for 23% of the compound excreted in the urine, while unchanged drug accounted for 15% of the urinary radioactivity. Thus when 20 mg/kg were given by mouth to rats, the radioactivity was excreted in the urine as follows:

- Unchanged drug 10-20% of dose
- Methyl piperazine 2-5%
- Piperazine 1-6%
- Diethylcarbamylpiperazine - i.e. methyl group split off 5-10%
- 1-ethyl carbamyl-4-methyl piperazine - i.e. one ethyl group split off 23%
- Diethylcarbamazine-N-oxide 50%

(See Fig. 1.)

Although metabolism is rapid and extensive, no metabolite thus far isolated appears to have an effect sufficiently profound to account for the extraordinary speed of action observed on the circulating microfilariae. Accordingly, this action is probably due to diethylcarbamazine itself. Studies of the distribution of the radioactive preparation, show that diethylcarbamazine soon equilibrates with all organs, blood cells and tissues (except fat), and that neither the microfilariae nor the adult worms of D. immitis concentrate it to any pronounced extent as compared with the surrounding tissues or fluids. Nearly all is excreted in the urine; the faeces contain only small amounts of piperazine metabolites.

2.3 Local absorption into eye

When diethylcarbamazine solutions are applied locally to the eyes of rabbits, the compound enters the aqueous humour in high concentration; no toxic effect was determined after an eight week trial (Lazar et al., 1968). The matter will be discussed further in section 6.1.3. A general account of the penetration of many types of drugs into the eye after local application is given by Benson (1974).

3. PHARMACOLOGY

3.1 General

When examined by the techniques of classical pharmacology, diethylcarbamazine is only slightly active. Reactions vary somewhat with different species and they cannot be assumed to be necessarily the same in man.

3.1.1 Smooth muscle

On rabbit intestine, 10 μg/ml produces relaxation. On the isolated uterus of rabbit and rat there was no action at concentrations of 1/100 000 (10 μg/ml), but with virgin guinea-pig uterus this concentration caused weak contractions (Harned et al., 1948). Subbu & Biswas (1971) reported that 20 μg/ml caused contraction of the uterus in the guinea-pig and rat and concluded that diethylcarbamazine might cause abortion in women. This has, however, not been reported clinically in spite of widespread use of diethylcarbamazine throughout the world. The question was extensively investigated by Fraser (1972) who found that large doses (100-200 mg/kg) given by mouth daily to pregnant rats and rabbits had no abortifacient action and no harmful effect on the foetuses. Sareen et al. (1961), while testing many substances as antifertility agents, found that diethylcarbamazine given to breeding mice at 200 mg/kg daily for 10 days, produced 50% infertility; many other compounds, however, were more active.
3.1.2 Blood pressure

When injected intravenously into anaesthetized dogs, 2.5-10 mg/kg of diethylcarbamazine causes a rapid rise of blood pressure, followed after about one minute by a moderate fall. Both these reactions are blocked by hexamethonium, a substance which blocks action on ganglia. The action of diethylcarbamazine is similar to that of nicotine. Apparently diethylcarbamazine stimulates sympathetic ganglia causing vasoconstriction, causes the adrenal medulla to release adrenaline, and stimulates the respiratory chemoreceptors in the aortic and carotid bodies (Forbes, 1972). These effects are of no clinical significance. In rats and calves, the intravenous injection of 20 mg/kg of diethylcarbamazine causes changes in the arterial blood pressure similar to those produced by the injection of histamine. In rats, diethylcarbamazine causes the release of histamine from the lungs, probably from the mast cells (Deline et al., 1973).

3.1.3 Respiration

Intravenous injection of diethylcarbamazine into anaesthetized dogs produces deeper and more rapid respiration. This may be due to direct action on the respiratory centre and also to action on the chemoreceptors just mentioned (Harned et al., 1948; Forbes, 1972).

3.1.4 Central nervous system

Parenteral injection of 25 mg/kg in cats and dogs often causes vomiting, apparently by stimulation of the vomiting centre. Oral administration to dogs of 50 mg/kg also causes vomiting, which is most likely due to direct action on the stomach. Larger doses may cause sleepiness (Harned et al., 1948). Toxic doses, e.g. 560 mg/kg administered orally to mice, cause convulsions. This convulsive action is probably due to stimulation of the cerebral cortex since the same convulsions have been seen in cases of piperazine citrate poisoning in children (Savage, 1967; Miller & Carpenter, 1967; Martindale, 1967). These children suffered from headache, incoordination, myoclonic jerks and coma, but recovered within 24 hours. The dose required for producing an effect is very large (1.5 g daily for two to three days in small children); such convulsions have never been seen with normal doses of diethylcarbamazine. Nevertheless, the headache, sleepiness and vomiting, which may occur after the administration of clinical doses of diethylcarbamazine, are probably due to the action of the compound on the central nervous system and on the stomach.

3.2 Anti-inflammatory effect

Diethylcarbamazine has an anti-inflammatory or blocking action in many anaphylactic reactions. These reactions are very complex and during the last 10 years they have been studied by many workers in a great variety of animals and organs. The usual procedure has been to provoke some systemic or localized anaphylactic reaction and to see whether the reaction is inhibited by a long series of blocking agents, of which diethylcarbamazine is one. Thus diethylcarbamazine has been used as an imperfectly understood tool for elucidating reactions which are also not yet understood. Furthermore, the results obtained by one worker in one set of experimental conditions do not agree with those of other workers in other conditions and results with one species of animal often differ from those with another species. Consequently, it is difficult to give a coherent account of these actions of diethylcarbamazine or to relate them to a logical system of underlying physiological events.

During anaphylaxis and other reactions in sensitized tissues, antibodies (mainly IgE) adhere to sites on tissue mast cells, basophil leucocytes and similar cells and thus sensitize them to the antigen. When an antigen comes into contact and reacts with them, these cells release various pharmacological agents called mediators, e.g. histamine, serotonin, slow reacting substance of anaphylaxis (SRS-A), prostaglandins, kinins and others yet unknown. The release of these mediators from the cells requires enzymes, calcium ions, and probably also the breakdown of cyclic adenosine monophosphate. When the mediators have been released, they stimulate smooth muscles (arterioles or bronchioles) to contract, capillaries to dilate and to become more permeable, and leucocytes of various kinds to congregate. Thus the phenomena of anaphylaxis and/or of localized inflammation are produced. These anaphylactic reactions may
be partially inhibited by a great variety of blocking compounds which can act in two ways. They may directly antagonize the pharmacological reaction of histamine or serotonin or one of the other active substances, perhaps by blocking the receptor sites on the muscle or other cells sensitive to the substances. This is called direct neutralization of histamine etc. Alternatively, they may act on the cells, e.g. mast cells, which release histamine or other substances, inhibiting one or more of the several stages which lead to such release, so that one or more of the mediators are not liberated.

It is believed that diethylcarbamazine usually does not neutralize histamine or serotonin directly, but that its main action is to block the release of SRS-A, an unsaturated hydroxy acid with a low molecular weight of around 400, and with biological activity at one nanogram or less. Sometimes, however, the action of diethylcarbamazine seems to be non-specific and in some circumstances it may antagonize the effects of histamine and serotonin directly (Burka & Eyre, 1974a). Furthermore, diethylcarbamazine must normally be supplied in extremely high concentrations to produce its effects. Thus during in vitro experiments, concentrations of 0.4 or 4.0 mg/ml have been employed (Burka & Eyre, 1974b) and for in vivo experiments on anaphylaxis in calves, intravenous doses of 20 mg/kg have been supplemented by constant infusion to maintain the blood level. These concentrations are much higher than those of other inhibitory compounds and much higher than would be obtained by therapeutic doses in man. Other important inhibitory compounds with which diethylcarbamazine is compared are as follows.

(a) Cromoglycate (FPL 670, INTAL) is a complex molecule.

![](image1)

It is believed to inhibit the release of histamine from cells, especially mast cells, started by reagin type antibodies (Cox, 1967). Possibly it stabilizes the membranes of the mast cells.

(b) Meclofenamate (Winder et al., 1965) is an "anti-inflammatory agent"

![image2]

which inhibits the action of SRS-A and antigenic bronchospasm of guinea-pigs. It also inhibits prostaglandin synthesis.

(c) Methysergide is an antagonist for serotonin (5-hydroxytryptamine, 5 HT).

(d) Isoproterenol (Isoprenaline)

![image3]

is a sympathomimetic amine which acts almost exclusively on beta receptors. It inhibits the in vitro allergic response in which antigen acts on sensitized leucocytes to cause a release of histamine (Lichtenstein & De Bernardo, 1971).

These compounds have no structural similarity to diethylcarbamazine.

The evidence for the above account is derived from numerous experiments by many workers; the results and the interpretations are therefore sometimes controversial. As described
above, the usual procedure is to provoke some kind of antigen-antibody reaction which may be
generalized or local, in vivo or in vitro. An attempt is then made to analyze this by
studying the inhibitory effect of the various blocking agents mentioned above. Since the
reactions are manifold, highly complex, and still imperfectly understood, it is impossible at
this stage to present a clear, consistent picture; the main pieces of experimental evidence
are, however, explained below.

3.2.1 Anaphylaxis

3.2.1.1 In calves

Calves are sensitized by injections of horse serum after which anaphylactic shock is
produced by intravenous injection of additional horse serum. In calves the main reaction
takes place in the lungs, where both the arterioles and the bronchioles are stimulated to
contract. As described above, the combination of antigen with antibody stimulates the release
of histamine, serotonin and SRS-A (Eyre, 1971). Eyre et al. (1973) studied the agents which,
when administered previously, inhibited this anaphylactic shock in the intact animal. They
found that the most effective inhibition was given by meclofenamate at 1.3 mg/kg, or by a
combination of cromoglycate and diethylcarbamazine at 20 mg/kg. This combination inhibited
almost all the symptoms of anaphylaxis such as contraction of bronchioles and pulmonary
arterioles, haemoconcentration, and hyperkalaemia, but did not inhibit the leucopenia.

Diethylcarbamazine alone produced 50% inhibition of the changes in systemic arterial blood
pressure, but it did not inhibit changes in pulmonary arterial blood pressure or in respiration
(Burka & Eyre, 1974a). Cromoglycate alone was inactive. It is concluded that in calves,
anaphylactic shock is due mainly to the release of SRS-A, and that diethylcarbamazine blocks
the release of SRS-A or antagonizes its action (Wells et al., 1973; Eyre et al., 1973;
Wray & Tomlinson, 1974; Burka & Eyre, 1974a). It might also block the release of histamine,
serotonin or prostaglandins.

If strips of pulmonary vein from sensitized calves are studied in vitro, they can be seen
to contract when brought into contact with antigens. This reaction is inhibited by anti-
histamines and by methysergide (antiserotonin). It is also 50% inhibited by diethylcarbamazine
or by cromoglycate and is totally inhibited by the two combined (Eyre, 1971). It can be
concluded from this that the reaction of sensitized calf pulmonary vein is a complex one of
histamine, serotonin, SRS-A, prostaglandins and possibly other agents.

3.2.1.2 In rats and monkeys

In rats which were first sensitized by chick ovalbumin, and then shocked with albumin
administered intravenously, diethylcarbamazine in doses of up to 40 mg/kg did not modify the
cardiovascular collapse caused by the shock. One can therefore conclude that in rats as
opposed to calves the shock is not due to the liberation of SRS-A (Lecomte & Salmon, 1972).

When serotonin or histamine was perfused through rat lungs, spasmogens (e.g. prostaglandins,
SRS etc.) were released, but this release was blocked by diethylcarbamazine at 1 mg/ml or by
indomethacin at 10 µg/ml. There is a marked difference in lungs among different species of
animals (Bakhle & Smith, 1972).

If sensitized monkey lung is exposed to antigen in vitro, diethylcarbamazine inhibits the
release of SRS-A and also of histamine (Ishizaka et al., 1971). In this reaction it is
synergic with isoproterenol.

3.2.1.3 Cutaneous anaphylaxis

Many studies have been made on cutaneous anaphylaxis in rats and other animals. The
results vary depending on the technique by which the reaction is provoked and also on the
species of animal used. Active cutaneous anaphylaxis is produced by immunizing the rat with
antigen (albumin) and later injecting the same antigen intradermally. Passive cutaneous
anaphylaxis is produced by intradermal injection of antiserum from an immunized animal and by
injecting the antigen intravenously 48 hours or more afterward. Both of these reactions are
inhibited by diethylcarbamazine and by antihistamines, but not by colchicine (Harada et al., 1971). Passive cutaneous anaphylaxis in calves was inhibited by diethylcarbamazine at 20 mg/kg; this inhibition was increased by cromoglicate (Eyre, 1971; Wells & Eyre, 1972). Harada et al. (1971) found that diethylcarbamazine at 250 mg/kg i.p., also inhibited the reactions to intradermal injections of histamine or serotonin in rats, i.e. in this experimental model, diethylcarbamazine neutralized histamine and serotonin directly. Pelczarska (1974), however, did not find this effect. In mice, passive cutaneous anaphylaxis was not prevented by diethylcarbamazine or cromoglicate, but specific antagonists for serotonin or histamine did prevent it (Casey & Tokuda, 1973).

3.2.2 Rat peritoneal cells

When peritoneal cells from sensitized rats are stimulated in vitro by antigen, SRS-A is released from the polymorphs; this release is inhibited by diethylcarbamazine. By contrast, histamine is released from mast cells and the release caused by antigen is not inhibited by diethylcarbamazine (Orange et al., 1968). On the other hand, if the basophil cells are stimulated by concanavalin A, so that they release histamine, this histamine release is inhibited by diethylcarbamazine (Siraganian & Siraganian, 1974). As a further complication, if the rat peritoneal cells are stimulated by corticotrophin or compound 48/80 to release histamine from the mast cell granules, this release is inhibited by diethylcarbamazine citrate, which is used by most experimenters, but not by diethylcarbamazine HCL. Thus, some of the observed effects may be due to citrate rather than to diethylcarbamazine base (Ruegg & Jaques, 1974).

3.2.3 Experimental eosinophilia

Repeated injection of Trichinella antigen into the footpads of guinea-pigs provokes eosinophilia in the corresponding lymph nodes. This effect is inhibited by diethylcarbamazine, provided it is administered 5-180 minutes before the injection of antigen (Thevathasan & Litt, 1971). Probably this is again due to the inhibition of the release of SRS-A. Diethylcarbamazine does not modify the eosinophilia occurring during anaphylaxis in rats or in guinea-pigs or in rats by repeated injections of histamine or 48/80. It does, however, prevent the rise of eosinophils which takes place between three and seven days after feeding guinea-pigs with ova or larvae of Ascaris. Perhaps this action may be due to the removal of the parasites (Sanyal, 1961; Sanyal & Sinha, 1962). Diethylcarbamazine is effective in the treatment of tropical eosinophilia; this is discussed in section 6.3.

3.2.4 Prostaglandine

Other substances which are formed and released locally by enzymes during inflammation include prostaglandins, which are highly active unsaturated fatty acids with a 20 carbon atom structure and many different pharmacological reactions which vary depending on the particular compound (Leopold, 1974). Their stimulatory or relaxant actions on ox pulmonary vein strips in vitro may be antagonized by diethylcarbamazine in high concentrations (0.4 and 4 mg/ml) and also, more powerfully, by phloretin or by SC-19220. This action of diethylcarbamazine seems to be non-specific (Burka & Eyre, 1974b).

3.2.5 Effect on skin tests

If patients were given diethylcarbamazine at doses of 1.5 g/day for five days and then skin tests were performed by intradermal injection of antigen from infected larvae of W. bancrofti, the resulting reaction was much diminished or even suppressed (Katiyar et al., 1974). This is probably another example of the anti-inflammatory reaction described above.

3.2.6 Summary of anti-inflammatory action

The liberation of pharmacologically active agents such as histamine and SRS-A, during inflammation and during antigen-antibody reactions is still poorly understood, and it differs in different experimental models and in different species of animals. Diethylcarbamazine is only one of a dozen compounds which interfere in these series of reactions, and there is no clear chemical similarity among them. It seems clear that diethylcarbamazine has a general tendency to inhibit the release of SRS-A, and sometimes other agents, which takes place...
following antigen-antibody reaction or in inflammation; this may explain the palliative action in bronchial asthma. Unfortunately, the exact details differ greatly in different experimental circumstances. Usually the concentration required in vitro of up to 1 mg/ml, is much higher than that of other inhibitors. It is not clear whether this anti-inflammatory reaction is always due to the same basic mechanism, or whether multiple mechanisms are involved. Similarly, it is not clear whether this action is due to the same structural configuration as the antifilarial action, or whether piperazine might have a somewhat similar action; or even whether the action sometimes depends on the citrate part of the molecule (Rüegg & Jaques, 1974).

The chief clinical effect of diethylcarbamazine in onchocerciasis is to cause inflammatory reactions in the skin, presumably due to release of microfilarial antigens in sensitized tissues; this contrasts sharply with the anti-inflammatory actions reported above. As is described below in section 4.1, the action of diethylcarbamazine upon microfilariae seems to be to cause them to release or to expose antigens inside a sensitized host. The relation between this action and the anti-inflammatory action reported above is not clear. Perhaps the two reactions are unrelated.

3.3 Bronchial asthma

Bronchial asthma is a complicated anaphylactic-like response in which some antigen-antibody reaction takes place releasing active substances, including SRS-A, which then provoke the characteristic symptoms. Probably there are multiple causes and reactions (Orange, 1973). In 1965, Salazar Mallén reported that diethylcarbamazine at daily doses of 10 mg/kg was effective in the treatment of asthma. This was confirmed by many workers (e.g. Srinivas & Antani, 1971; Sly, 1974; Thiruvengadam et al., 1974). Relief of subjective symptoms was usually greater than objective change in vital measurements. On the other hand, Benner & Lowell (1970), and Koivikko & Rantanen (1971) found no significant improvement in their patients. Good reports tend to come from geographical areas where human parasites are common; this may explain the differences reported (Koivikko, 1973).

4. ANTIFILARIAL ACTIVITY

The action of diethylcarbamazine on microfilariae and on adult worms must be described separately. Moreover, the effectiveness differs according to the different species of worms involved.

4.1 Action on microfilariae

4.1.1 In vitro

In contrast to its powerful action in vivo, diethylcarbamazine has no action in vitro upon either microfilariae or adult worms, and they can survive in relatively high concentrations of it for several days. Furthermore, serum from animals treated with diethylcarbamazine is not microfilaricidal in vitro, i.e. there is no conversion into an active metabolite (Hawking et al., 1950; Kobayashi et al., 1969).

Cavier et al. (1971) have stated that diethylcarbamazine at a concentration of 1/1000 at 28°C immobilized the microfilariae of Dipetalonema viteae in 24 hours. The pH was not controlled, however, and in any case, diethylcarbamazine does not act upon the microfilariae of D. viteae in vivo. Gonzalez Barranco et al. (1962) reported that diethylcarbamazine at concentrations ranging from 1/100 000 to 1/1000 at room temperature killed 82% of the microfilariae of Onchocerca in one day. This work probably has little relevance to what happens in the body, however, since 50% of the controls were killed, pH was not controlled, and there was no correlation between the concentration of the drug and the percentage killed.

Natarajan et al. (1973b) state that the microfilariae of Brugia sergenti from the slow loris, are immobilized after 12 hours by concentrations of 1/1000 or 1/10 000 at 28 or 37°C. In their experiments, the pH was stabilized at 7.2 and non-specific substances of similar structure viz. sodium benzoate, and methyl-piperazine HCl had no effect. All the same, the concentrations which they used are far higher than those reached in vivo, and the entire course of the reaction is so different in vivo that the weak in vitro action is probably not significant.
Hewitt et al. (1947) studied microfilariae in the frog filaria Folyella dolichoptera placed in solutions of diethylcarbamazine HCl at concentrations of from 1/100 to 1/10 000 which may have been acidic. These microfilariae are very large and have a narrow whiplike anterior end and a thicker posterior one. Immediately upon contact with the drug, the anterior end contracted into a tight coil and violent jerky movements occurred. Then the microfilariae straightened out and became motionless within 5-15 minutes. This behaviour probably has nothing to do with the way diethylcarbamazine kills microfilariae in vivo, but it may be a manifestation of its action upon acetylcholine and cholinesterases in the microfilariae.

4.1.2 In vivo

When diethylcarbamazine is administered to infected animals or humans by any of the usual routes, microfilariae rapidly disappear from the circulation. Single intraperitoneal doses of 50 mg of diethylcarbamazine citrate per kg given to cotton rats, usually cause a marked diminution in the microfilarial count which passes off after four to eight days. Daily doses as low as 1-5 mg/kg given for six days, exert a recognizable action. Large doses of up to 1100 mg/kg in nine hours, do not remove all microfilariae from the blood. After these treatments, numerous living microfilariae can be found post mortem in the pleural cavities of cotton rats infested with L. carinii (Hawking et al., 1950).

If diethylcarbamazine at 60 mg/kg is injected intravenously for rapid absorption into cotton rats, 80% of the microfilariae disappear in one minute, which is an astonishingly rapid disappearance; the same occurs with W. bancrofti in man. By contrast, however, a few microfilariae often persist for days in cotton rats or in man, even if large doses of diethylcarbamazine have been given (Hawking et al., 1950). In cats infected with B. malayi and B. pahangi and treated with doses of diethylcarbamazine which kill all the adult worms (as shown by autopsy), some microfilariae still persist in the blood (Edeson & Laing, 1959). The reason for this persistence has not been elucidated. It may be that persistent microfilariae are biologically different from the others, either new born or very old, or that there is some reservoir outside the circulation from which the microfilariae continually enter the blood. The latter is a probable explanation, since diethylcarbamazine does not affect microfilariae found outside the circulation, e.g. those of L. carinii in the pleural cavity of a cotton rat, those of W. bancrofti in a hydrocele, or those of O. volvulus in a fibrous nodule (Hawking, 1950, 1952). Another plausible explanation is that the microfilariae which persist are in a different immunological state from the others. As regards the sensitivity of the different species, diethylcarbamazine is effective against most species of microfilariae including those of O. volvulus and Dipetalonema streptocerca when they are in the skin.

There is surprisingly little information about the microfilariae of Manzonella ozzardi. Mazzotti (1948) reported that diethylcarbamazine had no effect against these microfilariae in Mexico. Montestruc et al. (1950) treated five patients in Martinique with 400 mg of diethylcarbamazine daily for 10 days and stated that the microfilariae disappeared from the blood in a few days. Botero et al. (1965) refer to one patient who was treated previously by Restrepo et al. (1962) without any effect on the microfilariae. Dr F. Biagi in a personal communication states that Mazzotti made extensive trials in Yucatan and found that 10 mg/kg of diethylcarbamazine per day was ineffective, but that 30 mg/kg per day for 15 days easily achieved total cure. This treatment was used successfully as mass chemotherapy in heavily infected areas of Yucatan, but unfortunately Mazzotti died before writing a final report on the subject. See also Biagi (1973).

Diethylcarbamazine is less effective, or even inactive, against the microfilariae of Dipetalonema perstans (Hawking, 1950), of D. viteae of jirds, and of Edesonfilaria malayensis from Thailand. It has little action upon the microfilariae of D. repens (Singh, 1962); two dogs were treated with 3.8 and 5.6 mg/kg, respectively, daily for five days, but there was no reduction of the microfilarial count; the microfilariae also developed normally in mosquitos which were allowed to feed during this treatment. The compound acts on the microfilariae of Icosiella neglecta of frogs (Minning & Ding, 1951), and upon those of Setaria equina in horses (Lapeyra & Zabala, 1970) and of S. cervi transplanted into rats or dogs (Singhal et al., 1972a,b).
4.1.3 Mode of action on microfilariae

4.1.3.1 Experimental evidence

When a search is made for the microfilariae of L. carinii which disappear from the circulation of cotton rats after treatment with diethylcarbamazine, they are often found in the liver, and to a lesser extent in the spleen and bone marrow. Within an hour after administration of the drug, phagocytes congregate around trapped microfilariae, and within 18 hours most of them have been destroyed (Hawking et al., 1950). This trapping of microfilariae has been studied in the living liver by Taylor (1960), and by Schaddein et al. (1968) with the electron microscope. Before the administration of diethylcarbamazine, the microfilariae could be seen freely circulating through the liver capillaries. Within five minutes of intravenous injection of diethylcarbamazine, most of the microfilariae had become adherent to the walls of the capillaries, usually by their tails. They remained like this, wriggling, for 4-60 minutes. Sometimes a leucocyte became attached to the tail of a stationary microfilariae; occasionally the microfilariae became so numerous that they blocked the capillary. In the electron microscope studies, before the administration of diethylcarbamazine, the liver contained few microfilariae; those present were normal and enclosed in a sheath. Twenty minutes after the drug had been administered, there were many microfilariae in the liver. Some were free in the sinusoids; an attachment by the tail would not, however, be seen under the electron microscope. They appeared similar to untreated microfilariae except that no sheath was visible. The microfilariae were surrounded by a clear space. There were occasional microfilariae inside hepatocytes which otherwise showed no localized cellular reaction or destruction. The microfilariae were partitioned from the cytoplasm by a clear space. Four hours after the administration of diethylcarbamazine, the microfilariae were less numerous in the liver, and many of those in the sinusoids were undergoing lysis. The adjacent Kupffer cells and hepatocytes contained many lysosomes and there were foci of inflammatory cells, mostly polymorphs, around the microfilariae which were undergoing lysis. The collection and destruction of the microfilariae in the liver is probably a function of the reticuloendothelial system, which is concentrated in the liver of rodents more than in other organs. It seems to depend upon the large, fixed macrophage of the tissues.

Destruction of the microfilariae of Loa loa has also been shown to occur in the liver of man (Woodruff, 1951). In a drill infected with L. loa which was treated with diethylcarbamazine and killed six hours later, many microfilariae were found to be undergoing destruction by the reticuloendothelial cells of the liver, but few or none were being destroyed in the spleen (Duke, 1960), although in the spontaneous immune reaction of the drill against L. loa, most of the microfilariae are destroyed in special nodules in the spleen, and none are destroyed in the liver. The part played by the bone marrow has not been properly investigated. Presumably, the microfilariae of W. bancrofti are destroyed in the same way as those of L. loa and Litomosoides carinii.

According to Kobayashi et al. (1969), who studied L. carinii in cotton rats, diethylcarbamazine is not effective in removing microfilariae from the blood unless antibodies are present. Thus, if microfilariae or adult worms are transplanted into a clean host which is then treated with diethylcarbamazine, the microfilaricidal action of the drug is or nearly so absent. If microfilariae are soaked in diethylcarbamazine at 1500 µg/ml in vitro, and then transfused into a clean host, they are not destroyed. When cotton rats containing inoculated microfilariae were passively immunized by injecting infected cotton rat serum, diethylcarbamazine had an immediate but transient effect in reducing the number of microfilariae in the blood. Kobayashi's conclusion is disputed by Zahner et al. (1977), but the tables given by Kobayashi et al. (1969) seem convincing.

4.1.3.2 Theoretical conclusions

Since the action of diethylcarbamazine depends on a specific chemical configuration, it would seem that there must be an attachment to the microfilaria. No such fixation of the compound on microfilariae has yet been demonstrated, but it would be desirable to reinvestigate the subject with a radioactive, labelled compound. On the basis of experimental evidence it appears that, after fixation, diethylcarbamazine modifies the microfilariae in the following two ways, which may be independent of each other.
1. Effect on neuro-muscular system. Microfilariae are in a constant state of muscular activity (wriggling); waves of contraction pass down them from head to tail producing forward movement, and less frequently from tail to head producing backward movement. By this means, microfilariae can move towards more favourable locations, or back away from unfavourable ones. Most important in the case of *W. bancrofti*, *Loa loa* and other periodic microfilariae in the blood, these reverse movements enable the microfilariae to hold themselves in the small arterioles of the lungs (Hawking, 1967). Microfilariae contain acetylcholine (Mellanby, 1955) and cholinesterase (Bueding, 1952) which doubtless play a part in their neuro-muscular activity. Diethylcarbamazine potentiates the action of acetylcholine in causing the contraction of nerve-muscle preparations of *Ascaris*, such potentiation being shown in dilutions as high as those of eserine, i.e. about 50 nmol/ml at 27°C (Natarajan et al., 1973a). Such interference with the acetylcholine mechanism might well disturb the normal waves of contraction in microfilariae perhaps blocking the power to reverse. Clinically this would explain why: (a) The liberation of microfilariae of *W. bancrofti* from the lungs into the blood during the daytime is caused by provocative doses of diethylcarbamazine (see below). (b) One of the first reactions of the microfilariae of *Onchocerca* to the compound is that many of them pass from the dermis to the epidermis which is an unfavourable environment in which they are not normally found. (c) Similarly, the number of *Onchocerca* microfilariae in the urine, blood and sputum is increased. (d) After administration of diethylcarbamazine, some microfilariae of *Litomosoides* have penetrated into the hepatocytes of the liver, where they are never normally found (Schardein et al., 1968). Admittedly no obvious effect of diethylcarbamazine upon the mobility of microfilariae in vitro has yet been reported, but the usual technique of inspection under a coverslip would not reveal the more subtle changes of reversal of waves of contraction. This should be studied by cinematographic technique with the microfilariae compressed on an agar pad (Hawking & Clark, 1967).

This action of diethylcarbamazine in deranging the muscular activity of microfilariae might depend upon the piperazine ring part of the molecule rather than upon the microfilaricidal structure (see section 4.2.3). It would be interesting to investigate whether piperazine alone can liberate microfilariae into the blood stream. In any case this deranging action, although interesting, may not contribute much to the ultimate destruction of the microfilariae (see also 4.1.4).

Apparently organophosphorus compounds, particularly haloxon, have a microfilaricidal action very similar to that of diethylcarbamazine, i.e. when tested on *L. carinii* in Mastomys they cause a rapid reduction in the number of microfilariae in the blood, which does not last more than three days. Reduction by 86% takes one hour after an oral dose of haloxon at 100 mg/kg, or 10 minutes after an oral dose of diethylcarbamazine at 500 mg/kg (Lammier & Grüner, 1975). Organophosphorus compounds are well known to inhibit cholinesterases of worms and of mammals, but such inhibition has not been demonstrated for diethylcarbamazine (except as discussed above. It would be interesting to investigate this similarity of action further with particular reference to the following questions.

(a) Does haloxon cause microfilariae of *L. carinii* to be accumulated and destroyed in the liver, as diethylcarbamazine does?

(b) Does the microfilaricidal action of haloxon require the presence of antibodies in the host?

(c) Does haloxon or diethylcarbamazine alter the waves of contraction/relaxation which pass up and down microfilariae? This should be examined by cinematography on an agar pad as mentioned previously.

2. Effect on the surface layers of microfilariae. As described above, after diethylcarbamazine is given in vivo, microfilariae are seized by phagocytes and destroyed, but the finer mechanism of this reaction is not yet completely understood. Within five minutes of injecting diethylcarbamazine, microfilariae of *L. carinii* begin to adhere by their tails to the walls of liver sinusoids (Taylor, 1960). According to electron microscopy, the microfilariae of *L. carinii* had lost their sheath in less than 20 minutes (Schardein et al., 1968). In the case of the microfilariae of *Onchocerca* which do not have a sheath but which have a five layer cuticle, Rougemont et al. (1974) reported that when examined after 12 hours, the cuticle
had disappeared and the different layers could no longer be differentiated. In more recent work, Gibson et al. (1976) report that the earliest change occurs from 3.5 to 18 hours after injection of diethylcarbamazine and consists of an irregular enlargement of the middle layer of the cuticle which they called the "electro-lucent" zone. This observation is confirmed by Rougemont (personal communication). They also describe deposits of granular substance which could be antigen-antibody complexes, on the surface of the cuticle Rougemont did not, however, see these.

When the surface coat of the microfilariae has been removed or deranged by diethylcarbamazine, antigens would become exposed. The exposed antigens would immediately react with the antibodies present in the plasma, forming antigen-antibody complexes. Such complexes are known to attract and activate eosinophils. The eosinophils and other phagocytes would then attach and destroy the microfilariae as described above. If there were no antibodies present, as contrived experimentally by Kobayashi et al. (1969), there would, according to this hypothesis, be no destruction of microfilariae.

Further study should be made by electronmicroscopy to detect the earliest changes in the surface layers of microfilariae after exposure to diethylcarbamazine in vivo; also studies should be made by immunofluorescence techniques to detect host immunoglobulins on the surface of microfilariae after diethylcarbamazine treatment.

4.1.4 Mobilization of microfilariae by diethylcarbamazine

4.1.4.1 W. bancrofti

When diethylcarbamazine is injected intravenously into dogs infected with Dirofilaria immitis or into patients infected with W. bancrofti, during the daytime when the microfilarial count is relatively or absolutely low, the microfilarial count is much increased. There is a peak after two minutes and a return to normal by 30 minutes (Fukamachi, 1960; Hawking & Adams, 1964). This is in marked contrast to the rapid fall which results when the compound is injected intravenously into patients with W. bancrofti at night, or into cotton rats with L. carinii as described above. The initial rise of the count is presumably due to liberation of microfilariae from the capillaries of the lung where they accumulate during the daytime. Probably the compound interferes with the mechanism by which the microfilariae hold themselves in the lungs, although they appear quite motile when examined microscopically under a coverslip at this time. The subsequent fall of the count is presumably due to the capture of the microfilariae by fixed phagocytes under the influence of the drug. If the intravenous injection is repeated on successive days, the rise of the microfilarial count becomes less on each occasion, probably because many microfilariae have been destroyed by previous injections. Fukamachi (1960) reported that this rise of the count of D. immitis was inhibited by the previous administration of atropine, which would implicate acetylcholine in the reaction; but Hawking & Adams (1964), working with W. bancrofti, found that atropine made no difference.

This action of diethylcarbamazine has recently been investigated by many workers as a possible means of conducting filarial surveys. Blood samples can then be collected by day rather than by night. The general procedure has been to give a dose of diethylcarbamazine by mouth at about 10.00 and to take blood slides 30 to 60 minutes later and examine them for microfilariae (Sullivan & Hembree, 1970).

Iwamoto (1971) found that if 0.1 mg/kg of diethylcarbamazine was given by day, microfilariae often appeared in the circulation within five minutes and their numbers were maximal after 15 minutes. Manson-Bahr & Wijers (1972) reported that an oral dose of 100 mg given during the day increased the microfilarial count to one-third of that found by night. Katiyar et al. (1973) gave 4 mg/kg by mouth to 10 carriers at 18.00 when the microfilarial count was negligible; two hours later the average count had risen to 6.7% of the midnight "maximum". Rajapakse (1974) gave 5 mg/kg by mouth at 10.00. Before the administration of diethylcarbamazine only 2.5% of the night-positive persons were positive, but 20 minutes later 40% of the night positives could be detected by this method. Russel et al. (1975) gave the compound to carriers of W. bancrofti and B. malayi and found that 60-93% of the night positives
could be detected by day sampling. For *W. bancrofti* they recommended that the dose should be 4-6 mg/kg with examination 30-60 minutes later, and for *B. malayi* the dose should be 2 mg/kg with examination after 90 minutes.

To summarize, it may be said that this provocative administration of diethylcarbamazine would be sufficient to distinguish villages heavily infected with *W. bancrofti* from those lightly infected, but it would not detect a high enough percentage of carriers. If daytime surveys for *W. bancrofti* are desired, the membrane filtration method of Desowitz & Southgate (1973) might be more accurate if it were acceptable; it requires venous blood rather than finger blood.

4.1.4.2  *O. volvulus*

A somewhat similar effect occurs in onchocerciasis. In some patients, microfilariae become more common in the blood, urine, sputum and cerebrospinal fluid when they are first treated with diethylcarbamazine.

The occasional presence of microfilariae in the urine of untreated onchocerciasis patients was first emphasized by Buck et al. (1969), who found that in the Chad area this might happen in 11.4% of patients. In later work Buck et al. (1971) showed that the microfilariae probably travelled from the kidney or renal pelvis, but that many died during their transit of the bladder. However, in patients treated with diethylcarbamazine, microfilariae are more often found in the urine (Mazzotti & Osorio, 1949). Roux & Picq (1974) found microfilariae in the urine of 40 out of 42 patients during a treatment with diethylcarbamazine. Fuglsang & Anderson (1973) found a 12-fold increase in the number of microfilariae in the urine within 24 hours of the first treatment. Some of their patients showed respiratory distress after the first tablet and microfilariae were found in their sputum. Mazzotti (1959) has shown that after diethylcarbamazine *O. volvulus* microfilariae are more commonly found in the blood and in the cerebrospinal fluid. Other workers have shown that diethylcarbamazine causes mobilization of microfilariae in the epidermis (Rougemont et al., 1974) and in the cornea (Anderson & Fuglsang, 1973). Estimations of the total number of microfilariae in the various body fluids during treatment with diethylcarbamazine and suramin have been made by Duke et al. (1976a). Naturally these estimations can only be approximate. Microfilariae are thought to pass from the skin through the lymphatics into the blood stream. From the blood some of them pass through the capillary walls of the glomeruli (not readily) into the urine, through the pulmonary alveoli (not readily) into the sputum, and through the choroid plexuses (fairly easily) into the cerebrospinal fluid. Microfilariae in the anterior chamber of the eye appear to come, not from the blood, but from the uveal tissues; when diethylcarbamazine is given, they are not usually destroyed in the aqueous humour; their numbers fall, however, because their source of replenishment has been cut off. After diethylcarbamazine has been given, the number of microfilariae in the blood and the urine rises rapidly on the 2nd and 3rd day of treatment and may remain high for one to two weeks. In five heavily infected patients studied by Duke et al. (1976a), the mean total number of microfilariae in the skin was about $28 \times 10^6$; the total number in the blood was about $40 \times 10^3$, i.e. 0.14% of those in the skin; and the total number excreted in the urine during the first week of treatment was 460 which represents 1.2% of the total load in the blood. The total number passing from the blood into the urine, sputum and cerebrospinal fluid was relatively small so that apparently over 97% of those in the blood, killed by diethylcarbamazine treatment, were destroyed elsewhere, presumably in the liver.

This mobilization of *Onchocerca* microfilariae is interesting, but as regards the blood, urine and sputum, does not seem to have much practical significance. Mobilization of microfilariae into the cerebrospinal fluid is more dangerous, however, and if the number in the fluid exceeds 3 microfilaria/ml, diethylcarbamazine treatment may provoke vertigo and other neurological symptoms (Duke et al., 1975) (see section 5.3.1.1).

With both *W. bancrofti* and *O. volvulus* the early provocative action of diethylcarbamazine may be interpreted as a disturbance, probably a partial paralysis of the muscular mechanisms by which microfilariae normally hold themselves in their preferred positions that is, in the lung capillaries for *W. bancrofti*, or in the skin, in the case of *O. volvulus*.  

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4.1.5 Other actions of diethylcarbamazine upon microfilariae

Attempts to interfere with the in vivo action of diethylcarbamazine by previous treatment with compounds of somewhat similar chemical structure, e.g. diethylurea, nicotinamide, nicotinic acid, nikethamide, quinine or lucanthone, were unsuccessful (Hawking et al., 1950).

If microfilariae of *L. carinii*, but not of *Wuchereria* or *Dirofilaria*, are placed in an electrophoretic field, they orient their heads toward the anode and then their wriggling gradually carries them in that direction. There is no true electrophoretic transportation of these, or other microfilariae, by electrophoretic force. Addition of diethylcarbamazine to the medium does not alter this state of affairs. Consequently, there is no sign that the compound affects the electric charges of microfilariae.

Ortiz et al. (1962) reported that microfilariae of *O. volvulus* had a proteolytic action upon human serum in vitro, setting amino acids free, and that this action was increased by diethylcarbamazine 1/6250 at 37°C. This observation has never been confirmed.

Jaffe & Doremus (1970) made an exhaustive study of the metabolism of glucose by microfilariae. They found considerable utilization of radioactive glucose and incorporation into many vital compounds. This metabolism was not affected by diethylcarbamazine, apart from slight inhibition of incorporation of glucose into the lipid fraction. The significance of this work is that it shows that the compound has no direct toxic action upon microfilariae.

Mohan (1973, 1974) reports that in the white rat infected with *Lithomosoides* and treated with diethylcarbamazine, he did not find that phagocytes destroyed microfilariae located in the liver. He considers that the main action of diethylcarbamazine is stimulation of eosinophils, which show excess lobation of the nucleus. This view, however, seems to be based on a single infected rat treated with diethylcarbamazine and killed six hours later; more evidence is required before this concept can be properly evaluated.

4.2 Action on adult worms

4.2.1 In vitro

In vitro, diethylcarbamazine has no action at 37°C on the adult worms of *L. carinii* or *Dipetalonema viteae* in concentrations 10 times greater than those obtainable in vivo, nor does diethylcarbamazine act on these species in vivo. When adult worms of *B. sergenti* from slow lorises, were exposed to 1/1000 concentrations of diethylcarbamazine for seven hours at 28°C, they became elongated and sluggish. When they were examined in vitro attached to a recording lever, 0.3 µmole (approximately 1/9000) caused a reduction of tone and of spontaneous contractions and 1.0 µmole (1/3000) caused loss of tone and paralysis (Natarajan et al., 1973b). But it is doubtful if this action has any significance for the therapeutic action in vivo.

4.2.2 In vivo

In experimental animals, the action on adult worms can easily be observed at post-mortem examination. In man, on the other hand, it must usually be inferred from indirect evidence. Different species of worm vary greatly in their susceptibility. The adult worms of *L. carinii* are insensitive both in vitro and in vivo. After prolonged treatment of cotton rats, e.g. 250 mg/kg i.p. twice daily for 28 doses, most of the adult worms can be found alive and containing active microfilariae (Taylor & Terry, 1960). Similarly the adult worms of *Dirofilaria immitis* or *D. repens* of dogs and of *Dipetalonema viteae* of jirds are also resistant. The adult worms of *O. volvulus* are also insensitive, and after intensive treatment of man with diethylcarbamazine, live worms can be found in the nodules. These nodules, even in untreated patients, usually contain some dead worms, so that it is impossible to say whether a few may or may not have been damaged by the drug.

On the other hand, with *Loa loa* the compound seems to kill many of the adult worms. Soon after treatment, small elongated wheals may appear under the skin, and on biopsy these
wheals are found to contain dead worms. Furthermore, the microfilariae disappear from the
blood and do not reappear even during prolonged follow-up observations.

In the same way, the adult worms of D. streptocerca which live under the skin, are killed
by diethylcarbamazine. When it was given to a patient, papules of 1.0-2.5 cm in size appeared
in the skin; if these were excised 24 hours after the first dose of diethylcarbamazine, dead
worms were found. The worms were coiled up and were sometimes surrounded by exudate containing
degenerating eosinophils and a few polymorphs. There were fractures in the cuticle of some
of the worms (Meyers et al., 1972).

With W. bancrofti and B. malayi, it has seldom been possible to excise and examine the
adult worms, but there is indirect evidence as follows. (1) After adequate treatment, the
microfilariae of most patients disappear quickly and do not reappear even after 12 months by
which time any surviving worms could presumably have recovered from any temporary damage.
(2) In perhaps 5% of the patients small areas of acute inflammation persist for about a week
in the groin, spermatic cord, etc., where adult worms are probably situated.

Moreover, direct evidence on the subject has been supplied by Ch'en (1964). When
patients with bancroftian or malayan filariasis were treated in China with single large
doses of diethylcarbamazine, it was found that small nodules often developed on the lymphatics
of the thigh, spermatic cord or axilla. When these nodules were excised, they were found to
contain dying or dead adult worms surrounded by degenerated cells. From this evidence, it
is concluded that adult female worms are killed or permanently sterilized by adequate treat-
ment with diethylcarbamazine.

In cats infected with B. malayi or B. pahangi, adequate doses of diethylcarbamazine,
e.g. more than 5 mg/base/kg i.p. daily for seven days, killed all adult worms as was shown
by subsequent autopsy (Edeson & Laing, 1959). Curiously, microfilariae still persisted in
diminished numbers in the blood of these cats after the adult worms had been killed.

In the case of Setaria digitata, in the anterior chamber of the eye of horses,
diethylcarbamazine given orally at 80 mg/kg body weight, was apparently successful in
removing the worms in two out of four horses (Ahmed & Gupta, 1965).

The above discussion may be summarized by saying that diethylcarbamazine kills the adult
worms of L. loa, W. bancrofti, B. malayi, D. streptocerca and S. digitata, but not those of
O. volvulus, Dirofilaria immitis, D. repens, Litomosoides carinii or Dipetalonema viteae.
There is possibly a slight lethal or sterilizing action on D. perstans (McGregor et al.,
1952).

The mechanism by which diethylcarbamazine kills adult worms of Loa and other human
filariae, is not clear. It is difficult to investigate the problem since the adult worms of
experimental filarial infections (Litosomoides and Dirofilaria) are not affected by diethyl-
carbamazine in vitro or in vivo. It is probable that phagocytes are concerned, and certainly
dead worms of Loa are surrounded by such phagocytes, but it is not clear whether the phago-
cytosis is the cause of the worms' death or the result.

4.2.3 Action of piperazine upon Ascaris

Piperazine and derivatives of piperazine including diethylcarbamazine cause paralysis of
the muscles of intestinal nematodes such as Ascaris, and this action often leads to the
expulsion of the Ascaris through the anus.

In vitro the stimulating action of acetylcholine upon fragments of Ascaris is blocked by
piperazine (Norton & De Beer, 1957). The action of piperazine upon muscle cells of Ascaris
has been further studied by Del Castillo et al. (1964) using electrophysiological techniques.
It was found that piperazine produces hyperpolarization of the muscle cell membrane around the
neuro-muscular synapse, which leads to the inhibition of the muscle cell's function. The
hyperpolarization depends on an increase in the permeability of the membrane to chloride ions
and to volatile fatty acids. In this way piperazine acts as a pharmacological analogue of a
natural inhibitory neuro-hormone. In view of all this, it is possible that the above interference of diethylcarbamazine with the neuro-muscular activity of microfilariae may be due to the piperazine ring part of the molecule rather than to the specific filaricidal configuration. Incubation of Ascaris with piperazine greatly reduces the production of succinic acid (Bueding et al., 1959) which might have something to do with the increase of cell permeability to volatile fatty acids mentioned above.

4.3 Action on forms in the insect vector

The developing forms of D. repens in the mosquito are resistant to diethylcarbamazine (Hawking et al., 1950).

Microfilariae of Litomosoides carinii are not prevented from developing in the vector mites if the host has been treated with diethylcarbamazine just before the vectors suck blood. Similarly, W. bancrofti and D. immitis are not prevented from developing in mosquitoes (Kanda et al., 1967b). Apparently, diethylcarbamazine is not active in the arthropod vector.

4.4 Action on infective larvae and immature worms

The infective larvae of L. carinii seem to be destroyed by diethylcarbamazine if given at 500 mg/kg daily for 12 days during exposure to infective mites, since the subsequent development of microfilariae is prevented. Given for six days, one week or two weeks later, the compound is much less effective. It seems able to prevent the male infective larvae from developing, but whether it also kills the female larvae is less clear (Hawking et al., 1950). There is no action on infective larvae of W. bancrofti in vitro (Moreau & Pichon, 1972; Jordan, 1958) and the action in vivo is not known. The prophylactic action on B. malayi in cats has been investigated by Ewert & Emerson (1975). Cats were infected by the injection of infective larvae and diethylcarbamazine was given from the day of infection for seven successive days. The cats were killed 14 days after infection and a search was made in the appropriate tissues for developing larvae. Larvae were found in all 37 control cats. Diethylcarbamazine at 1 mg/kg had no prophylactic action. After 2 mg/kg a few moribund larvae were found in 8 out of 10 cats; after 5 mg/kg in 2 out of 5 cats; after 10 mg/kg in 1 out of 5 cats; and after 25-100 mg/kg in none out of 17 cats. Apparently diethylcarbamazine at 25 mg/kg is effective in preventing the development of the infective larvae of B. malayi and B. pahangi. Extensive studies of early forms of Loa loa have been carried out by Duke (1961, 1963). Infective stages of L. loa were obtained from the insect vector Chrysops, and injected subcutaneously into young monkeys (drills); these were killed three to six months later and the number of adult worms which had developed under the skin was counted. In untreated controls about a third of the injected worms established themselves. Other groups of monkeys were treated with diethylcarbamazine given as 4-20 oral doses of 150 mg/kg or various smaller amounts. The minimum dose which produced complete prophylaxis in 90% of the animals was 5 mg/kg given from two days before until 14 days after infection. The work was then extended to human volunteers and it was found that the minimum effective dose was 5 mg/kg given for at least three days; this was effective if given within one month after infection. When the invading worms were killed by this treatment, they caused small papules in the skin at the sites of their destruction. Consequently, by infecting a man and then administering treatment after a suitable number of days, a picture could be obtained of the migration of the worms from their portal of entry. The infective and early developing forms of Loa are more susceptible than the adult worms. For practical prophylaxis of man against infection by Loa, a regimen of 200 mg per man twice daily for three days every month is recommended. Possibly the same procedure might be effective as prophylaxis against B. malayi or W. bancrofti but this needs investigation. In the case of Wuchereria infections, it might be easier to administer a curative course once yearly, rather than monthly prophylactic ones. As a public health measure, it would be better if the entire population could be treated thus eliminating the reservoir of microfilariae for re-infection. Prolonged studies of early forms of D. immitis have been carried out by Kume et al. (1962, 1964, 1967). With this worm, the early stages develop under the skin, but after 85-120 days they migrate to the right ventricle of the heart. Their environment is obviously quite different during these two phases. Infective larvae were injected subcutaneously into dogs; 130-165 days later the dogs were killed and the number of worms in the heart was counted. Diethylcarbamazine was given to the dogs according
to various schedules. When given by mouth at 220 mg/kg daily for 5 consecutive days beginning 1, 30 or 60 days after inoculation it did not prevent infection (Kume et al., 1962). When 11 mg/kg were given daily, however, beginning two months before and seven months after inoculation, this dosage prevented infection completely. It is now recommended by Kume et al. that dogs exposed to infection should be given 5.5 mg/kg daily beginning just before the season of infection and continued for four weeks afterwards. This dosage is non-toxic and will completely prevent infection. Apparently diethylcarbamazine at 45 mg/kg for three days every three months, has been used successfully for prophylaxis in northern Australia by Aubrey (1964). This action of diethylcarbamazine upon the immature forms of D. immitis is interesting because the compound has little action on the adult worms in the heart. Perhaps the subcutaneous position of the worms makes them more susceptible, although this is not the case with Dipetalonema viteae where neither microfilariae nor adults are susceptible to diethylcarbamazine. More probably, the metabolism or cuticle of immature Dirofilaria immitis differ from those of the adult. The prophylactic action on Onchocerca has been investigated by Duke (1968) in two chimpanzees given respectively 5 and 23 mg/kg of diethylcarbamazine daily for 18-24 days following inoculation of infective larvae and in two human volunteers given 10 mg/kg daily for five and 16 days respectively. No prophylactic action against the development of the infective larvae could be found. Thus diethylcarbamazine prevents the development of infective larvae and immature worms in the case of B. malayi, B. pahangi, L. loa, D. immitis and Litomosoides carinii but not in the case of O. volvulus.

4.5 Development of drug resistance

According to modern conceptions, drug resistance develops from mutants which appear during the reproduction of an organism and which are then selected out for propagation by exposure to the drug. The reproductive cycle of filariae of from three months to several years, is so long compared with that of bacteria or protozoa, that it seems theoretically unlikely that acquired drug resistance to diethylcarbamazine, or to any other drug, will become important in connexion with these worms. This conclusion is supported by investigations by Hawking et al. (1950) on the effect of prolonged treatment. Two groups of cotton rats infected with O. carinii were treated with 10 mg/kg and 100 mg/kg of diethylcarbamazine, respectively, by mouth daily for 108 days. This treatment caused most but not all of the microfilariae to disappear from the blood; they gradually reappeared, however, when treatment stopped. There was no evidence of drug resistance during this period.

4.6 Action on other worms

Diethylcarbamazine is also active against various other worms, a fact which might give some clue to the nature of its action on filariae.

Setaria. When adult worms of Setaria are taken from the peritoneum of cattle at the slaughter house and transplanted intraperitoneally into rats or dogs, microfilariae appear in the blood of the new host and persist for many days. These microfilariae are sensitive to doses of diethylcarbamazine, and such artificially infected rats can be used for screening antifilarial drugs (Singhal et al., 1972a,b).

Four horses with S. digitata (Filaria oculi) in the anterior chamber of the eye were treated by oral doses of diethylcarbamazine at 80 mg/kg and the worms were destroyed in two of them (Ahmed & Gupta, 1965). During in vivo tests on S. digitata (host not stated in abstract of article), Kono (1965) found that diethylcarbamazine reduced the number of microfilariae in the blood, but had no obvious effect on the adult worms.

Four horses near Seville infected with S. equina, were treated with diethylcarbamazine at 200 mg/kg for three days, repeated after 15 days. The microfilariae disappeared from three of them (Lapeyra & Zabala, 1970). Sheep in Iran, suffering from lumbar paralysis, probably due to infection of the spinal cord by Setaria, were successfully treated with diethylcarbamazine (Baharsefat et al., 1973).

Guinea worm (Dracunculus medinensis). It was reported by Roussset (1952) that diethylcarbamazine had a prophylactic action in man preventing the development of immature
worms and killing the adults. But this treatment for guinea worm has now been replaced by niridazole, metronidazole and thiabendazole.

Lung worms (Dictyocaulus viviparus) in calves. Diethylcarbamazine is most active against the adult forms in the bronchi. Accordingly, treatment should be started at the first sign of respiratory distress that might be "husk". The dose is 22 mg/kg given i.m. on three successive days. Diethylcarbamazine is also active against D. filaria in sheep. It has been recommended for the cat lung worm Aleurostrongylus abstrusus (Connan & Zurbourg, 1966) and for Metastrongylus apiropis of pigs (Kashinskii, 1963). Nishimura (1965) tested it upon the rat lung worm Angiostrongylus cantonensis, which sometimes causes eosinophilic meningitis in man in South-East Asia and the Pacific. He found that diethylcarbamazine was not effective.

Toxocara canis. Several workers have reported that diethylcarbamazine is active against Toxocara canis infections in mice. It is most effective if given while the larvae are migrating through the viscera and before they have established themselves in the brain and skeletal muscle (Burren, 1968; Dafalla, 1972; Pike, 1960; Wiseman et al., 1971).

Strongyloides. Nwokolo & Imohiosen (1973) reported a human case of strongyloidiasis of the respiratory tract which resembled asthma; but there were larvae and ova in the sputum. The patient improved when diethylcarbamazine was given at 12 mg/kg daily for 18 days, but relapsed eight weeks later and was treated with thiabendazole. This action of diethylcarbamazine may be due to the piperazine ring.

Ascaris, Cooperia, Ancylostoma. Diethylcarbamazine is active in removing Ascaris lumbricoides in man. This action is due to the piperazine ring and it is exerted more powerfully by piperazine citrate and similar compounds. The activity appears to depend upon temporarily paralysing the muscular movements of the Ascaris by which it maintains its normal position in the human intestine. Consequently, the worm is expelled by the anus (Cavier & Hawking, 1973). Diethylcarbamazine has also been found active in the treatment of dog ascarids and of dog hookworm often in combination with styril pyridinium (Berger et al., 1969; Casey et al., 1971) and in the treatment of Cooperia infections in calves (Cornwell et al., 1972). Diethylcarbamazine in full doses has been recommended to kill the larvae of Ancylostoma caninum which cause creeping eruption in the skin of man; but it is probably inferior to thiabendazole.

Thelazia. T. gulosa and T. skrjabin infections of cattle are said to be cured by subcutaneous injection at 14 mg/kg of diethylcarbamazine in the upper third of the body (Gorodovich, 1971).

Trichinella spiralis. In large doses, diethylcarbamazine can kill the adult worms of T. spiralis when they develop in the intestine during experimental infections, but it has little effect on encysted larvae.

Cysticercus. Against C. celluloseae in young pigs, subtoxic doses of diethylcarbamazine at 10-25 mg/kg daily gave promising results (Baretto & de Siqueira, 1963); but in four steers infected with C. bovis, Urquhart (1960) found no action.

In 13 patients in Iran, diethylcarbamazine seemed to be successful in suppressing epileptic seizures (Tumada & Margona, 1973); but this action might be anti-inflammatory rather than anthelmintic.

Paragonimus westermani. Infections with this worm in children have been treated with diethylcarbamazine by Gutman et al. (1969). This caused a reduction of egg-output but did not produce a radical cure as bithionol did.

In experimental infections of rabbits with Fasciola hepatica, diethylcarbamazine plus bis(2-hydroxy-3,5-dichlorophenyl) sulphone was effective against the immature flukes (Kimura & Ono, 1971).
Summary. The action of diethylcarbamazine on worms in the alimentary canal seems to be mainly due to the piperazine ring rather than to the specific diethylcarbamazine structure. It apparently depends upon paralysis of the muscles of the worms. The other actions, e.g. on lung worms, seem to be more specific, but they give little indication of the mechanism by which this helminthicidal action is produced.

5. TOXICITY

5.1 Animals

The toxicity of diethylcarbamazine is very low. In mice, the acute LD₅₀ by intraperitoneal injection is 240 mg/kg and by oral administration 560 mg/kg. In rats the oral LD₅₀ is 395 mg/kg. There is little accumulation of the compound in albino rats given repeated doses (Harned et al., 1948). Chronic toxicity does not occur even at doses as high as 170 mg/kg given intraperitoneally to cotton rats twice daily for over 12 doses, all doses as base. Young rats fed for nine weeks on a diet yielding them 9 mg/kg body weight per day gained weight nearly as fast as the controls. Breeding pairs of mice placed for four months on a diet yielding 15 mg/kg body weight per day, bore as many, or slightly more, baby mice than the controls on a normal diet; and all the offspring were normal. There was no sterility, male or female, and no teratogenic effect (Hawking & Marques, 1967). Fraser (1972) found that large doses of 100-200 mg/kg given daily to pregnant rats and rabbits had no abortifacient action and no harmful effects on the foetuses (see section 3.1).

5.2 Man - uninfected

When given to man, the compound is remarkably safe. Although hundreds of thousands of people have been treated, no case of death proved to be due to diethylcarbamazine has been reported. The untoward reactions in man, when they occur, may be annoying but they are seldom dangerous. Deaths which have been attributed to diethylcarbamazine will be discussed below.

In uninfected persons, large oral doses of the compound, e.g. 10-20 mg base/kg, may cause gastrointestinal disturbances, i.e. anorexia, nausea and vomiting, which come on in two to four hours. Headache and sleepiness have also been noted. These symptoms are probably due to the direct action of the drug upon the patient. In China, single doses of 1.0-1.5 g per person have been given to large numbers of people during mass campaigns. Apparently many persons vomited, and it is recommended that the dose be administered in the evening; but otherwise, no ill effects were reported (Ch'en, 1964).

5.3 Man - infected with filariae

With infected persons, different and more severe reactions may occur which vary according to the type of infection. They are most marked with W. volvulus and less marked with W. bancrofti.

5.3.1 W. volvulus

5.3.1.1 Clinical

In patients with onchocerciasis, a specific reaction occurs which is so constant that it can be used as a convenient diagnostic test (Mazzotti's reaction). It is obviously due to the destruction of many microfilariae in a sensitized subject. There is often premonitory itching which begins 15-30 minutes after the first dose, and which may not last very long. After a few hours there is usually a strong reaction which is well marked in 16 hours. It includes swelling and oedema of the skin, especially of the buttocks, thighs and genitals (the areas where the highest concentrations of microfilariae usually occur), intense pruritus, enlargement and tenderness of the inguinal lymph nodes, sometimes a fine papular rash, hyperpyrexia up to 39°C (102°F), tachycardia, headache, etc. There may occasionally be a fall of blood pressure and there may be respiratory distress. These symptoms persist for three to seven days and then subside, after which doses as high as 12 mg/kg/day can be tolerated without further reaction. The severity of the reaction is proportional to the number of microfilariae
initially present in the skin and only partially to the size of the initial doses. Particularly severe symptoms have been reported by Fuglsang & Anderson (1974) after the administration of 50 mg to 15 heavily infected patients in north Cameroon; two to four hours later most of them were prostrated. One man of 30 years collapsed and seemed unconscious for 10 minutes. His breathing was shallow and rapid with scanty frothy sputum and his pulse was 135/minute and weak. After two hours he recovered somewhat, and after two days he was normal again. Six other patients also developed severe or moderate respiratory distress.

Similar severe reactions have been reported by Rougemont et al. (1975) who treated 290 persons in Bamako, Mali. The initial dose was 25 mg twice daily rising to 200 mg. Children were free from reactions except for puritus, but some of the older people from 15-45 years old were severely affected. Thirty-six hours after the first dose, 20 people were prostrated. In particular, three women who tried to continue working were found lying on the ground semi-conscious, polypnoeic and with a systolic blood pressure of 70-80 mm Hg. They were treated with 60 mg methylprednisolone intravenously or intramuscularly and improved in a few hours. The reactions to diethylcarbamazine, and to suramin as well, seem to be much more severe in the savanna zone of West Africa than in other parts of Africa or America.

Many heavily infected patients complain of vertigo during the first week of diethylcarbamazine treatment, and Duke et al. (1976) have suggested that this may be due to passage of increased numbers of microfilariae into the cerebrospinal fluid under the stimulation of the compound (see section 4.1.4.2). Such vertigo usually begins on the second or third day of treatment and may continue for five to 16 days. Sometimes it stops before the treatment stops. The vertigo often causes prostration and incapacitation. It may be accompanied by headache, nausea and vomiting. In one of Duke's patients there was a syndrome like Parkinsonism which lasted for seven days. The vertigo is believed to be due to the action, which is possibly allergic, of microfilariae upon the cerebellum rather than upon the labyrinth. It occurs when the microfilariae in the cerebrospinal fluid exceed 3 mg/ml. Eventually the symptoms disappear without leaving permanent damage and a second course of treatment can be given without incident. Fuglsang & Anderson (1974a) emphasize that, with patients in heavily infected foci of onchocerciasis, great care is needed in the administration of diethylcarbamazine, and that corticosteroids should be given to prevent or diminish the reactions.

5.3.1.2 Histology of diethylcarbamazine reaction in onchocerciasis

The histology of the cutaneous reaction in onchocerciasis has been described by Hawking (1952), Martínez Baez (1960), Rodger (1962), Connor et al. (1970) and more recently and in greater detail by Rougemont et al. (1974). According to Rougemont et al., before treatment with diethylcarbamazine there is usually a mild and variable inflammation of both epidermis and dermis. Microfilariae are present, but there is usually no reaction around them. When treatment has been given, changes begin in a few hours and are maximal on the second and third days. The epidermis becomes swollen with fluid and infiltrated by some eosinophils. Microfilariae actively penetrate the epidermis, head to surface, but their structure is not altered in this position. Apparently, the normal reactions of the microfilariae have been deranged by the compound. In the papillary dermis there is inflammation. Lymphocytes and polymorphs, especially eosinophils, accumulate in foci, and the eosinophils often cause micro abscesses. The microfilariae first stain more faintly and then disappear in small granulomata. In some patients the reaction is intense, with fibrinoid necrosis of collagen fibres and of the walls of blood vessels, together with infiltration by eosinophils. In the middle dermis the pilosebaceous follicles are surrounded with inflammatory cells, especially polymorphs. Microfilariae are few. They are often located between the fibres of the erector muscles and they are lysed. The deep dermis is relatively unaffected. Connor et al. (1970) reported that one hour after giving diethylcarbamazine, microfilariae penetrated into the epidermis. Simultaneously other microfilariae began disintegrating in the dermis, and eosinophils collected around them. Rougemont et al. (1974) did not find eosinophils numerous in the dermis until after one to two days. After four to eight days of treatment the inflammation subsided; eosinophils became fewer, lymphocytes and plasma cells predominated, and microfilariae disappeared.

When preliminary examinations were made by electron microscopy, it was seen that before treatment the microfilariae of *O. volvulus* had a cuticle consisting of three dense layers and
two clear zones, under which there was a fibrillar locomotor system. Twelve hours after the first dose of diethylcarbamazine, the cuticle had disappeared and the different layers could not be differentiated (Rougemont et al., 1974) (see section 4.1.3.2). More electron microscope studies would be desirable.

Therapeutic shock

This response to treatment which Salazar Mallén named "therapeutic shock", is obviously a kind of allergic reaction to the sudden destruction of microfilariae and the liberation of filarial antigens in the skin and other sensitized tissues. It differs from anaphylactic shock in various ways. True urticaria or angioneurotic oedema are not seen, blood pressure generally remains normal, there is usually no respiratory obstruction, and pyrexia and prostration are marked (Salazar Mallén et al., 1962). Blood histamine and complement are not changed during the reaction, but serotonin increases significantly in the venous (jugular) blood and a "reactive-protein C" also appears in the blood. During "therapeutic shock" the mast cells of the conjunctiva become degranulated, possibly due to the liberation of histamine or serotonin, with diffusion of a fluorescent substance (Salazar Mallén & Chevez Zamora, 1965).

Salazar Mallén et al. (1962) suggested that an endopeptidase is liberated from the disintegrating microfilariae and acts as a toxic factor; but no further support has come for this hypothesis.

Much work has been devoted to finding drugs which diminish the reaction of "therapeutic shock" both for practical purposes and for investigating the causation of the reaction. The results have been reviewed by Aranda-Villamayor (1970). Antihistamine drugs have usually proved to be valueless. Antagonists of serotonin such as methysergide and cyproheptadine have been recommended, but they have been disappointing. Salazar Mallén et al. (1962) found that cyproheptadine alone was ineffective, but that cyproheptadine and dexamethasone together reduced all the reactions; pruritus reduced the least. Lagraulet et al. (1964) found that the effect of methysergide in Upper Volta was barely significant and considered that its use was not worthwhile. Aranda-Villamayor (1970), in a study of 200 patients treated with methysergide and indomethacin in Mexico, found that although methysergide might alter the relative frequency of symptoms somewhat, the total number of reactions was not diminished. Moreover there were some harmful side effects, and the antimicrofilarial action was reduced somewhat. He concluded that methysergide should be banned from field use.

On the other hand, corticosteroids in some form have usually been found to be beneficial. Some of them may be too expensive for field use, however, or they may weaken the antifilarial action. Prednisone was used by Schofield & Rowley (1961) for patients with W. bancrofti in Papua. They found that three to four times more microfilariae persisted in patients after prednisone than in patients with diethylcarbamazine alone. Sasa et al. (1963) found that the febrile reaction (W. bancrofti) was diminished by paramethasone and chlorpromazine. Triamcinolone plus methdilazine was found beneficial in onchocerciasis patients by Torroella (1964). Bernhard et al. (1964) found that triamcinolone given before starting diethylcarbamazine reduced the reactions to O. volvulus significantly. This treatment, however, was expensive and the same authors (Garcia Manzo et al., 1965) later recommended betamethasone beginning 12 hours before the first dose of diethylcarbamazine. This reduced most of the symptoms except the pruritus. Duke & Anderson (1972) recommend betamethasone when severe reactions are expected.

Summary

Specific antagonists of histamine, or of serotonin, have proved disappointing for the reactions in onchocerciasis and should be avoided. Corticosteroids, especially betamethasone, diminish most of the reactions which occur in patients with O. volvulus or other filariae. Presumably this is due to a general anti-inflammatory action. It must be remembered that corticosteroids and serotonin antagonists may also diminish the antifilarial action slightly.

5.3.2 Other filariae

In patients infected with B. malayi (Wilson, 1950) or with Loa loa, there are often similar but milder general symptoms, without the local swelling and inflammation of the skin. In patients infected with W. bancrofti symptoms are often absent, but 25% of the patients may suffer from headaches, nausea, vomiting, anorexia, cough and pain in the chest, pains in
muscles or joints, general malaise and pyrexia, or rarely a papular rash. The reactions are proportional to the number of microfilariae originally present in the blood. They are more common in older persons than in younger persons. After a few days, these symptoms subside and treatment can be continued, the same or higher dose being given without any reaction occurring. In the absence of heavy onchocerciasis or of encephalitis due to L. loa, none of these symptoms is ever so severe as to endanger life or to cause anxiety. However, they are of great practical importance because they often render mass administration of the compound unpopular and unacceptable. They may thus prevent the use of diethylcarbamazine to eradicate filariasis on a community basis.

5.3.3 Local reactions

In addition to the above general symptoms, small focal reactions of pain, tenderness and inflammation sometimes occur in the groins or thighs of persons infected with W. bancrofti or B. malayi, and small nodules may develop at these sites. They subside in a few days and are probably due to a local reaction around a dying adult worm (Ch'en, 1964). In patients with L. loa small wheals may appear in the skin due to dying worms, and in patients with Dipetalonema streptocerca there may be flat papules in the skin due to the same cause. In onchocerciasis, severe pain in the hip may be caused by the death of adult worms in the capsule of this joint.

5.4 Deaths reported as due to diethylcarbamazine treatment

5.4.1 Discussion of evidence

Although deaths during diethylcarbamazine treatment have been reported, the evidence that they were in fact due to such treatment is usually slight. Oomen (1969) reported that he had treated 327 hospital patients for onchocerciasis and seven had died. All those who died were in poor condition before treatment; but 49 other patients who were also in poor condition reacted favourably to the treatment. In the case of the seven patients who died, there had been no clinical reaction to the diethylcarbamazine. The same was true, however, in the case of 65 other patients. The patients who died relapsed into coma after taking 225-900 mg of diethylcarbamazine between day three and day eight. Death occurred in four to 12 days after the initial dose. The evidence that these deaths were actually caused by diethylcarbamazine is slight, but nevertheless, if patients are in poor general condition it would be wise to give the compound cautiously.

Jones (1970) reported the case of a woman in Nepal who had many microfilariae of W. bancrofti in her blood and who was given two doses of 100 mg diethylcarbamazine at four-and-a-half hour intervals. One hour after the second dose she was found pulseless and dying. The cause of death is obscure and there is little evidence that it was due to the compound, particularly in view of the fact that almost a million other people have taken similar doses without serious reaction.

5.4.2 Encephalitis

Encephalitis occurring during infection with L. loa, with microfilariae present in the central nervous system and in the cerebrospinal fluid, is a very grave condition. Over 20 cases have been reported in the literature. Cauchie et al. (1965) give a good review of the literature and report a case which was apparently made worse by treatment with diethylcarbamazine. In spite of prednisone given prophylactically, the patient went into coma and died. Downie (1966) reviews 12 cases of encephalitis. All were said to have been due to filariasis, especially loa, although no microfilariae were found in the cerebrospinal fluid of many of these patients. Five were treated with diethylcarbamazine alone, four of them died. Four were treated with diethylcarbamazine plus steroids and all recovered, often with neurological lesions. Apparently, encephalitis believed to be due to loiasis should certainly be treated with corticosteroids, but the decision about diethylcarbamazine treatment is more difficult. Since the condition of encephalitis indicates inflammatory reactions already proceeding in the brain, and since these allergic reactions will certainly be exacerbated by diethylcarbamazine, it would seem advisable to postpone diethylcarbamazine treatment in the hope that the encephalitis will subside under corticosteroid therapy. If this happens, diethylcarbamazine may be
given with great caution at a later date. The compound per se is not toxic, but, if a
dangerous allergic condition is already present, it might possibly exacerbate it.

Since so many filarial patients all over the world have been given diethylcarbamazine it
would be surprising if some deaths had not occurred during such treatment. Nevertheless, it
has not been possible to find any specific case, apart possibly from encephalitis as described
above, in which the death could be clearly proven to have been due to diethylcarbamazine.

6. CLINICAL USE

In describing the clinical use of diethylcarbamazine, a distinction must be made as to
whether the aim is to improve the condition of single patients, or whether it is to suppress
or eradicate the infection in a whole community.

6.1 Individual patients

In the case of individual patients, microfilariae and adult worms of _W. bancrofti_,
_B. malayi_ and _L. loa_ can certainly be destroyed by adequate courses of treatment. After the
worms have been removed, further damage due to them will cease once the reaction to the
disintegration products has subsided; but the damage already caused, e.g. lymphostasis or
elephantiasis, will not be reversed. In the case of _O. volvulus_ the microfilariae can be
destroyed, but not the adult worms, so that after a few months the microfilariae gradually
reaccumulate in the skin.

6.1.1 Patients with _W. bancrofti_

With _W. bancrofti_, a patient of average weight (60-70 kg) should be given 100 mg of
diethylcarbamazine citrate by mouth three times a day for 10 days. If preferred, the course
can be extended to 21 days, but it is wiser to examine the blood a few nights after the end
of the first course. If the microfilariae have not completely disappeared, a second course
can be given after an interval of several weeks. The drug can also be given as a single dose
of 200-300 mg daily; this is simpler to administer, but it is more likely to cause gastro-
intestinal disturbance. Hujimaki (1958) recommends 6 mg/kg per day, divided into six doses,
for 14 days, in order to maintain a constant high level of drug in the blood (see section 5.2).
There is no evidence as to whether a constant high level is more, or less, effective than a
succession of high peaks.

Patients may be classified into several categories. (1) The symptomless carrier with
microfilariae in the blood should be treated; although the worms may seem to cause no harm,
they serve no positive function, and are a source of infection to others. (2) The patient
with attacks of lymphangitis, with or without microfilariae, should be treated, preferably in
a quiescent period between attacks; although the treatment may not immediately stop all
further attacks, it will probably diminish their number and severity. (3) For the patient
with advanced hydrocele, elephantiasis, chyluria, etc., unless microfilariae are present,
treatment is hardly worthwhile, since it cannot reverse these chronic lesions. (4) The
patient with tropical eosinophilia can also be treated effectively (see section 6.3).

6.1.2 Patients with _B. malayi_ and _L. loa_

These patients should be treated similarly to those with _W. bancrofti_. Treatment is
usually very effective, but "allergic" reactions are often greater than with _W. bancrofti_.
Dosage should be started at 50 mg of the citrate by mouth three times a day for three days,
and then 100 mg three times a day for seven days. In patients infected with _L. loa_, wheals,
which mark the sites of dead or dying adult worms, may appear under the skin.

6.1.3 Patients with _O. volvulus_

6.1.3.1 Treatment of light infections

Duke & Anderson (1972) recommend that light or moderate infections should be initially
treated with diethylcarbamazine to kill the existing load of microfilariae. On the first day,
50 mg diethylcarbamazine citrate should be given orally, on the second day 100 mg after morning and evening meals, and on the next five days 200 mg twice daily. For persons under 40 kg, these doses should be reduced in proportion to their weight. These authors consider that symptomatic relief for the symptoms of the reaction may be obtained with antihistamines, e.g. 100 mg antazoline HCl (Antistin) every six hours for an adult, as well as with antipyritics or analgesics. If the reaction is expected to be severe, it may be reduced by betamethasome without interfering greatly with the destruction of microfilariae. One milligram of betamethasone three times daily should be given orally one day before starting diethylcarbamazine, and be continued for three to five days; after that the dose is gradually reduced to zero over the next four days. Later a course of suramin should be given to kill the adult worms since radical cure of onchocerciasis is never obtained with diethylcarbamazine alone. If treatment with suramin is not acceptable, treatment with diethylcarbamazine may be repeated at intervals of from three to six months, or 100 mg may be taken weekly to maintain the suppression of the microfilariae. During these subsequent treatments, the allergic reactions will probably be less severe and possibly absent.

6.1.3.2 Treatment of ocular onchocerciasis

Anderson et al. (1976) treated 39 patients with ocular lesions in north Cameroon. The dose was 50 mg of diethylcarbamazine twice daily for the first day; 100 mg x 2 during the second day; and 150-200 mg x 2 for the next 10 days. Betamethasone at 1.0-1.5 mg x 2 was also given on the day before treatment and continued for five days. General reactions were severe and acceptability was low. Patients could not be persuaded to take weekly suppressive doses afterwards. The numbers of microfilariae in the eye were temporarily reduced and the lesions of the anterior segment were temporarily improved, but both soon relapsed when treatment ceased. Lesions in the posterior segment were not improved. These authors concluded that in heavily infected cases with ocular involvement, the administration of diethylcarbamazine should be carefully handled. Its main use would be as an emergency drug together with betamethasome in acute onchocerciasis of the anterior segment, or to reduce the microfilariae in the eye before giving suramin. There is not sufficient benefit for it to be given after suramin. Possibly it might also be used to supplement the excision of all head nodules.

6.1.3.3 Local application for ocular onchocerciasis

The local application of diethylcarbamazine to the conjunctiva was first suggested by Lazar et al. (1968, 1970). They showed that if the compound was instilled into the conjunctival sac of rabbits, high concentrations appeared in the aqueous humor. No local irritation occurred. Lazar et al. (1969) produced experimental uveitis in the eyes of rabbits by injection of bovine serum albumin and found that the local application of diethylcarbamazine had no anti-inflammatory action, although 6-mercaptopurine, chloromycetin and antilymphatic serum were anti-inflammatory. Ben-sira et al. (1970) treated 10 blind patients in Malawi with 5% diethylcarbamazine citrate neutralized to pH 7, at a dosage of two drops four times daily for two weeks. The treatment was well tolerated but there was a small reaction due to the death of microfilariae. It consisted of moderate oedema of eyelids and slight congestion of the conjunctiva which subsided during the second week of treatment and disappeared when treatment ceased. The microfilariae, as studied by slit lamp, disappeared from the anterior chamber within 48 hours after the onset of treatment, but reappeared in their original numbers 48 hours after the cessation of treatment. Anderson & Fuglsang (1973) treated eight patients in Cameroon, instilling 3% diethylcarbamazine into the conjunctival sac at a dosage of two drops four times daily for nine days. This was well tolerated by a control patient without microfilariae in the eye, but in heavily infected patients, it provoked severe anterior uveitis, causing treatment to be stopped in three out of six such subjects. In the anterior chamber, microfilariae were somewhat reduced in number but they were not eliminated and they reappeared quickly when treatment stopped. Microfilariae were initially stimulated to invade the cornea but afterwards most of them died. Microfilariae reappeared quickly when treatment stopped. These workers consider that in heavily infected patients there is no subjective improvement following the local application of diethylcarbamazine to eyes, and that reactions which may occur, require adequate ophthalmological supervision. The suppression of microfilariae in the anterior chamber is only temporary. Accordingly, local therapy is not recommended for general use. The greatest benefit from either topical or systemic diethylcarbamazine would be expected if therapy were begun before the eyes were heavily invaded.
6.2 Mass therapy

6.2.1 Bancroftian and malayan filariasis

6.2.1.1 Mass treatment with diethylcarbamazine

Although diethylcarbamazine is very effective and satisfactory for the treatment of individual patients, the most promising use lies in its administration to all the infected persons in a district so as to suppress the infection on a public health basis. There is no known animal reservoir of infection for W. bancrofti, and even with B. malayi such a reservoir existing in monkeys, occurs only in limited parts of Malaya. The only important source of infection for man is man himself. Therefore, in theory, if all persons infected with W. bancrofti (or B. malayi or L. loa) were adequately treated, all the worms would be destroyed, there would be no microfilariae left to be transmitted by mosquitoes to new patients, and the disease would die out. Such a procedure would have the advantage over the public health alternative, viz. suppression of the mosquito vectors or Chrysops by insecticides, that the destruction of the worms would be immediate. With vector control, on the other hand, it would be 10 years or more before the worms would die out. Moreover, control of culicine mosquitoes by insecticides is usually difficult, and eradication has been impossible.

In practice, however, large-scale or mass diethylcarbamazine therapy is not so easy and has encountered many difficulties. These difficulties are proportional to the size of the population to be treated. Where the procedure was adequately applied, as was first done in Tahiti by Kessel (1957) and his colleagues, or in pilot trials in single villages and small islands, the results have been good and filarial infection has been reduced to an insignificant level. Elsewhere the minor toxic and allergic reactions described above have often made treatment unpopular and unacceptable to the populations which need treatment. There is a large amount of literature published on the subject.

Briefly, four main types of dosage schedule have been employed. The first three are administered to all the population without blood examination:

1. one dose, e.g. 4 mg of diethylcarbamazine citrate/kg, daily for five to seven days. This is the easiest schedule to administer, and it is the minimum which is likely to have any effect. It has been used in India, Japan, Africa and Brazil;

2. an interrupted dosage schedule of 5 mg/kg monthly or weekly for six to 12 doses. The total dose should be 72 mg/kg for W. bancrofti and 40 mg/kg for B. malayi. This is more effective and less toxic than other schedules, but it is more laborious to administer. If administrative problems can be overcome, it is probably the best to employ. It has been employed particularly in the Pacific and Malaya;

3. a large single dose, e.g. 1.0-1.5 g. This is somewhat heroic since many patients vomit; but if the population can be persuaded or compelled to accept it, the simplicity of administration makes it attractive. This method has been employed in China (Ch'en, 1964);

4. treatment of microfilariae carriers, detected by systematic surveys, e.g. 100 mg per person, thrice daily for seven days. This is usually acceptable and may reduce the level of infection considerably; but it involves laborious blood surveys and theoretically it cannot eradicate the infection, since many latent cases will be missed. In practice, moreover, the procedure is often vitiated further by taking samples of blood which are too small for proper examination, so that only the carriers with large numbers of microfilariae are detected. This method has been used in Ceylon and north-east Brazil.

Experience has shown that all these schedules have been effective in greatly reducing the level of infection, provided that people can be persuaded to take them. Unfortunately there is often difficulty over this point. Persuasion is easier when the incidence of elephantiasis is high, causing people to be afraid of infection, and when the population is small making personal influence easier. Thus it has been successful in Tahiti with a population of 20,000,
and in many pilot trials involving some hundreds of persons. On the other hand, it has proved impossible in India, where populations amounting to millions have to be treated.

6.2.1.2 Review of mass therapy

The attempts to control filariasis (bancroftian and malayan) have differed in their success in different parts of the world.

In the Pacific area, treatment has usually been given to the whole population of an island once weekly or monthly for 12 doses. It has usually been possible to obtain the cooperation of the population concerned and the results have been very successful in reducing the microfilarial rate, as measured by blood films, to a low level, even though little or no mosquito control has been carried out. In fact, except for Tonga where a mass campaign is now being planned, filariasis has been greatly reduced over most of the area. Thus in American Samoa, where mass treatments began in 1963, the microfilarial rate was 21% before 1963, 3.1% in 1965, and 0.36% in 1967 (Kessel et al., 1970a, b).

However, it has recently been shown by Desowitz & Southgate (1973) that filtration techniques for detecting very low levels of microfilaraemia, e.g. one microfilaria in 5 ml of blood, reveal that a few microfilariae will persist in many (23%) of the previous carriers after such mass therapy. Furthermore, such microfilariae are capable of developing in mosquitoes and of being transmitted. This finding might seem to cast doubt on the permanent value of such control measures, but the doubt is unjustified. The aim of these mass campaigns is "control" and not "eradication". If the reservoir of infection is reduced to a small fraction of its original level, it will take a long time for slowly multiplying parasites like the filarial worms to build up to their previous level, and they might even die out from the low probability of the two sexes of worms meeting each other in sufficient quantities to maintain the next generation. In any case, the practical results of chemotherapeutic control in Tahiti and Samoa are most striking. In Tahiti in 1955, before control started, the microfilarial rate was over 40%, and filarial fever and elephantiasis were common. Now, after 20 years of control, the microfilarial rate has been reduced to a low level, 5.6% in 1967, and clinical symptoms of filariasis are negligible.

In Western Samoa there was mass administration of diethylcarbamazine during 1965/1966. Before treatment, the infective rate among Aedes polynesiensis mosquitoes, the main vector, was 2.95%. After treatment, only three infective mosquitoes could be found during four years, the rate being 0.071% (Suzuki & Sone, 1975). In these instances, filariasis may not have been eradicated, but it has been reduced to a level where it is no longer a serious health problem.

In Japan and the adjacent islands a national filariasis control programme has been conducted since 1962, mainly by examination of the population at risk, and treatment of detected microfilariae carriers. As a result, the mean microfilarial rate in the infected areas fell from 2.8% in 1962 to 0.5% in 1969 (Ishimaru, 1972; Sasa, 1974). In South-East Asia, in Indonesia, and in Papua New Guinea, no large-scale control programmes have yet been possible.

In India a large national filariasis control programme was started in 1955 based particularly on a five- to seven-day course of 4 mg/kg per day for all at risk. Unfortunately there was insufficient popular cooperation, and it proved impossible to persuade the vast populations concerned to accept and swallow the tablets which were offered them. Consequently, the campaign had to be abandoned. Since then antifilarial measures have been restricted to a few small pilot trials of diethylcarbamazine and to attempted mosquito control.

In Malaya, diethylcarbamazine was given weekly for six weeks, and between 1956 and 1958, 112 700 persons were treated. In one typical area (Burkit Meriam in Kedah) infected with B. malayi, the microfilarial rate fell from 26% in 1957 to 0.7% in 1966.

In Sri Lanka, mosquito control and treatment of carriers with diethylcarbamazine has been applied. Since 1969, mass treatment has been given in some areas. A great reduction has been effected in the microfilarial rate.
In Brazil, control has been attempted by the detection and treatment of carriers with 6 mg/kg of diethylcarbamazine daily for seven days. In Belem the microfilarial rate fell from 9.8% in 1952 to 2.0% in 1966, and in Recife from 6.9% to 1.8% over the same period.

Summary

Mass chemotherapy, often with little or no effort at mosquito control, has reduced bancroftian and malayan filariasis to a low level over most of the Pacific area, Malay peninsula and Japan. In Sri Lanka, there has been great reduction, with recent recrudescence. In Brazil, there has been reduction in many parts, but filariasis was less prevalent here than in Asia. Elsewhere, the level of filariasis has probably remained unchanged except for small pilot trials.

6.2.2 Diethylcarbamazine in cooking salt

Since most of the difficulties of mass administration of diethylcarbamazine are difficulties of persuasion, it has been suggested that these might be circumvented by incorporating diethylcarbamazine in some article of common diet, such as cooking salt. Alternatively, it might be incorporated in some popular food such as the Japanese miso soup or orangeade (Kanda et al., 1967a). Incorporation of a drug in salt has been widely employed in Brazil, where chloroquine was so used in order to prevent malaria. The technical and administrative problems involved are thus well understood. The conditions for diethylcarbamazine in salt against filariasis are much more favourable than they were for chloroquine against malaria. Further, diethylcarbamazine is stable to cooking with food. It is not destroyed during cooking and it does not develop harmful by-products. It is well tolerated by growing rats and pregnant mice. Furthermore, this procedure of incorporating diethylcarbamazine in the food has been employed in veterinary practice to protect dogs against infection with Dirofilaria immitis, e.g. Abadie et al. (1969) used diethylcarbamazine in dog food to protect 33 dogs for 31 months.

As regards human therapy, a number of pilot trials have now been conducted. In Brazil, it was given to two closed communities totally 2300 adults. Diethylcarbamazine was added to the salt in concentrations of 0.2 or 0.4% (w/w) giving a calculated intake of 40 or 80 mg per head per day. Administration was continued at the lower level of 0.1% for a whole year. These concentrations were completely acceptable to the men, and no adverse comments about taste were received. There were no untoward reactions. After the first six weeks, 70% of the carriers no longer showed microfilariae in the blood and the others showed only single microfilariae in 40 mm$^3$ samples (Hawking & Marques, 1967).

In another trial, carried out in East Africa by Davis & Bailey (1969), medicated salt containing 0.1% diethylcarbamazine was supplied to a closed community of 700 adult men for six months. Tolerance of the drug/salt mixture was extremely good. The mean microfilarial densities fell steadily, being reduced by 90% after six months. In this trial: 0.1% was too low; and a concentration of 0.2 or 0.3% would have been better.

Several trials have been carried out in India under village conditions by Raghavan et al. (1968) and by Basu et al. (1971) employing 0.1% diethylcarbamazine for eight to 12 weeks and more recently by Sen et al. (1974) who gave 0.26% diethylcarbamazine for 11 weeks. In all these trials acceptance was good, there were no significant allergic reactions, and the microfilarial counts were greatly reduced although not always to zero. Judging by these Indian trials, the drug concentration should be 0.25-0.3% and the medication should be continued for four months or more in order to obtain optimal results.

A very successful trial has been carried out in the Kinmen (Quemoy) Islands, Taiwan, by Fan et al. (1975). Medicated salt containing 0.33% diethylcarbamazine was supplied to 7128 persons in 26 villages for six months. This is equivalent to 42 mg three times daily. The salt was completely acceptable and no side effects were recorded. The microfilarial rate fell from 9.6% to 0.4%; the mean microfilaraemia fell from 14.4 microfilariae per 20 mm$^3$ to 1.9, and the infective rate among Culex fatigans mosquitoes fell from 3.7% to 0.2%. These workers concluded that diethylcarbamazine-medicated salt was a very rapid and efficient agent for the control of filariasis and it was probably also the cheapest and most practical method for use in the future.
Note: In all campaigns for public health control, including diethylcarbamazine by whatever method of administration, it is essential to obtain the enthusiastic cooperation of the people concerned by suitable approach and propaganda. The public health work in the People's Republic of China is a striking illustration of the great results that can be obtained by enlisting the active cooperation of the people themselves.

6.2.3 Mass therapy of other filarial infections

Diethylcarbamazine can be used against B. malayi in the same way as against W. bancrofti, but the doses should be reduced, since the worms are more susceptible to treatment, and allergic reactions are more severe. Single doses of 1.5 g once yearly for several years have been recommended in China (Ch'en, 1964).

As regards L. loa a trial was carried out by Duke & Moore (1961) on 50,000 persons living on a rubber estate in Nigeria. All who contained microfilariae in the blood were offered treatment at 200 mg diethylcarbamazine citrate three times a day for 20 days. This treatment was well tolerated and the microfilarial reservoir of infection was reduced to 2-12% of its previous level in the persons who were treated. Unfortunately, one-third of the people did not cooperate, probably on account of apathy, and this failure to cooperate was a serious handicap to the control of filariasis by means of drugs.

Against O. volvulus, mass administration of diethylcarbamazine has not been acceptable, because of reactions, nor has it been efficacious, because the adult worms are not killed. In Mexico and Guatemala, the public health service endeavours to detect infected persons and to treat them, either by excision of nodules or by chemotherapy. The use of diethylcarbamazine is handicapped by the reactions which it produces, but Torroella (1964) recommends a course consisting of 8 mg methdilazine alone on the first day. This is repeated on the second, third and fourth days, half an hour before giving treatment with diethylcarbamazine at 600 mg/day plus triamcinolone 24 mg/day, combined as "filaricort". On the fifth to eleventh days, only diethylcarbamazine, with or without triamcinolone, is given. The course should be given two or three times a year to persons exposed to infection.

6.3 Treatment of tropical eosinophilia

Tropical eosinophilia is a condition characterized by eosinophilia, patches of consolidation in the lungs, and raised erythrocytic sedimentation rate. It occurs in many warm, moist parts of the world. There has been much speculation as to its etiology, but work by Beaver, Danaraj, and their associates, make it seem highly probable that it is a hypersensitive state due to infection with some filarial worm, usually W. bancrofti. Accordingly, the treatment consists of diethylcarbamazine. This has been described in detail by Danaraj (1958). He recommends large doses, i.e. 6 mg/kg three times daily for five days. Marked improvement in the symptoms occurs in two to four days, and cure should be completed in two weeks. In about 10% of his patients, severe bronchial spasm occurred. This should be treated with antispasmodics. Before giving treatment, the night blood should be examined for microfilariae. If these are present, smaller doses (3-4 mg/kg) should be given. In the rare cases in which there is no response to diethylcarbamazine, oxophenarsine or neoarsphenamine should be given intravenously in the usual doses as for syphilis, weekly for six to eight weeks; but the possibility of dangerous idiosyncrasy should be remembered.

7. RECOMMENDATIONS FOR RESEARCH ON DIETHYLCARBAMAZINE

(1) Mode of action of diethylcarbamazine upon microfilariae

(a) Electron microscope studies to see the effect of diethylcarbamazine on the surface layers of microfilariae, especially during the first 10 to 20 minutes, should be undertaken. It is believed that diethylcarbamazine alters the surface of microfilariae so that underlying antigens become exposed to the host's antibodies. Morphological evidence would be valuable. This might be studied with laboratory infections, Litomosoides and Brugia, and with human ones, Onchocerca and Wuchereria. Do any of these early morphological changes take place in vitro?

(b) The relationship between the action of diethylcarbamazine on microfilariae and the antibodies of the host should be determined.
(i) It needs to be confirmed that diethylcarbamazine does not act if the host is not sensitized to filariae.

(ii) Attempts should be made to detect host antibodies adhering to microfilariae which have recently (10 minutes) been exposed to diethylcarbamazine. Immunofluorescence might be helpful.

(iii) Investigations should be undertaken to determine which antibodies of the host react with microfilariae after diethylcarbamazine has been administered.

(iv) After in vivo treatment with diethylcarbamazine, 1-5% of the microfilariae often persist in the blood (see section 4.1.2). Are these microfilariae immunologically different from those originally present? Or is it an aging difference? This should be investigated in laboratory animals.

(c) When microfilariae are examined under a coverslip on an agar pad, waves of contraction can be seen usually passing from head to tail but sometimes conversely. It should be determined whether diethylcarbamazine alters or paralyses these waves, i.e. inhibits the reverse waves and thus prevent microfilariae from backing out of impassible positions (see section 4.1.3.2). Cinematographic techniques are advantageous for this problem.

(d) It should be determined whether diethylcarbamazine inhibit cholinesterase of microfilariae or interfere with their mechanisms depending on acetylcholine.

(e) The mode of action of haloxon, a potent inhibitor of cholinesterase, should be investigated for comparison and contrast with diethylcarbamazine (see section 4.1.3.2.1).

(2) Mansonella ozzardi. The action of diethylcarbamazine on microfilariae of Mansonella should be investigated. Biagi says that it is active, but requires large doses (see section 4.1.2). It should be remembered that there may be two species of worm confused under the name M. ozzardi, and that the microfilariae found in coastal districts of Central America and transmitted by Culicoides, may be different from those in the Amazon valley transmitted by Simulium.

(3) Skin test for onchocerciasis with diethylcarbamazine. It has been suggested that if diethylcarbamazine is injected intradermally into a patient with onchocerciasis, a local reaction might be provoked by localized destruction of microfilariae. This would be analogous to a skin test, producing a Mazzotti reaction on a local scale, which would be less deleterious than the generalized systemic reaction provoked by giving diethylcarbamazine by mouth. This suggestion would be worth investigating. Probably the diethylcarbamazine will diffuse away so rapidly that larger doses than expected may be needed.

(4) Radioactive diethylcarbamazine. A sample labelled with radioactive carbon on the piperazine ring should be obtained. This should be used to study the attachment of diethylcarbamazine to microfilariae and to adult worms which are susceptible to it, e.g. Loa, Wuchereria, or Brugia. The distribution of diethylcarbamazine in the body and its excretion could also be studied in this manner.

(5) Action of diethylcarbamazine on adult worms. This should be studied further with Wuchereria and with Brugia. If nodules develop along lymphatics in patients treated with diethylcarbamazine, they should be excised and examined to see if the adult worms are being killed by the compound.

(6) "Therapeutic shock" or allergic reactions to diethylcarbamazine in patients infected with Onchocerca volvulus or other filariae. These are obviously of the greatest importance and they should be studied in man whenever opportunities occur. It would be valuable to find drugs which prevent or relieve these reactions. Does betamethasone also diminish the therapeutic effect?

(7) Local application of diethylcarbamazine to the eyes (see section 6.1.3.3). This should be further investigated using due caution. It might be more beneficial when applied to lightly infected eyes than to heavily infected ones.
(8) **Methods of mass administration.** Investigation of methods for mass administration should be continued, e.g. improved dose schedules, and improved methods of propaganda and administration to enlist popular cooperation. These investigations should include the use of medicated salt (see section 6.2.2) or the incorporation of diethylcarbamazine in some other common article of the diet. The control of bancroftian filariasis by the systematic administration of diethylcarbamazine to children in the schools should also be studied.

(9) **Toxicity of arsenical compounds.** Arsenical compounds such as melarsoprol are very active in killing macrofilariae. Unfortunately, a small number of patients, e.g. one in 500 or one in 2000, may die after the administration of smaller doses than those which have been well tolerated by hundreds of other patients. The toxic effects usually occur in the liver in the form of acute yellow atrophy or necrosis or in the brain as encephalopathy. They seem to be due to a rare idiosyncrasy of these particular individuals to arsenicals. If some simple test, e.g. a skin test, could be devised by which individuals with this idiosyncrasy could be detected, these people could be excluded from the series. Then these powerful compounds could be safely used to kill macrofilariae in the rest of the population.

Accordingly, it is recommended that investigations should be made on the immunological reactions provoked by arsenical compounds combined with proteins or other antigens with a view to discovering such a test. For this work, cooperation between an immunologist, a toxicologist, and a chemist would be advantageous. This is a long-range project.

**RESUME**

Etant donné les problèmes posés par les réactions toxiques à la diéthylcarbamazine et à la suramine - seuls médicaments actuellement jugés acceptables pour le traitement de l'onchocercose - il a fallu dépouiller la littérature ayant trait à ces produits afin de tenter d'éclairer leur pharmacodynamie et leur toxicité. Conformément aux recommandations d'un Groupe consultatif scientifique sur la Chimiothérapie de l'Onchocercose, l'Efficacité des Médicaments et leur Toxicité (Genève, 1974) et du Comité consultatif scientifique et technique du Programme de Lutte contre l'Onchocercose dans le Bassin de la Volta (deuxième et troisième session - 1975 et 1976), on a utilisé à cette fin le système Medline. Toutes les informations ainsi obtenues ont été étudiées et réunies dans deux documents traitant respectivement de la diéthylcarbamazine et de la suramine. Le document sur la suramine paraîtra prochainement. Quant au présent document, il analyse la littérature traitant de la diéthylcarbamazine, et plus particulièrement de sa pharmacodynamie, de sa toxicité et de son emploi thérapeutique contre l'onchocercose et d'autres filariose.

Les divers points examinés dans ce document figurent sous les rubriques suivantes :

1) chimie de la diéthylcarbamazine, y compris ses propriétés chimiques et physiques, le rapport entre sa structure et son activité, ses produits analogues (par exemple la Centperazine et les composés Hoechst 32258 - 28637a - 29691a - 37598) et l'évaluation chimique du médicament;
2) absorption, excrétion, répartition et métabolisme de la diéthylcarbamazine; 3) pharmacologie de cette substance, en particulier ses effets anti-inflammatoires qui ont été étudiés sur des animaux et des organes très divers; 4) son activité microfilaricide et macrofilaricide in vitro et in vivo contre *Onchocerca volvulus*, *Wuchereria bancrofti* et d'autres espèces de filaires; 5) sa toxicité chez l'animal et chez l'homme, tant infectés que non infectés par *O. volvulus* et d'autres espèces de filaires; 6) son emploi clinique individuel chez des malades infectés par *W. bancrofti*, *Brugia malayi*, *Loa loa* et *O. volvulus*, en thérapie de masse contre la filariose à *W. bancrofti* et *Brugia malayi*, et d'autres infections filariennes; et dans le traitement de l'Éosinophilie tropicale.
Toutes les sources possibles d'information existant à ce jour sur la diéthylcarbamazine ont maintenant été exploitées et l'analyse s'achève sur un certain nombre de recommandations concernant les recherches à entreprendre. Celles-ci devraient notamment porter sur les sujets suivants : mode d'action de la diéthylcarbamazine sur les microfilaires; activité du médicament contre les microfilaires Mansonella ozzardi; mise au point d'une épreuve cutanée pour la recherche de l'onchocercose au moyen de la diéthylcarbamazine; emploi de diéthylcarbamazine radioactive pour étudier sa fixation sur les microfilaires et les vers adultes ainsi que sa répartition dans l'organisme et son excrétion; action du médicament sur les vers adultes Wuchereria et Brugia; réactions allergiques à la diéthylcarbamazine des malades infectés par O. volvulus et d'autres espèces de filaires; possibilités d'applications topiques sur les yeux des malades; et méthodes d'administration de masse. Sont également recommandées des recherches visant à neutraliser la toxicité des composés arsenicaux qui sont extrêmement actifs contre les microfilaires mais qu'il faudrait pouvoir employer sans risque.

La présente étude se complète de deux listes de références bibliographiques sur la diéthylcarbamazine, l'une indiquant les références citées dans le texte et l'autre les références non citées, fruits de la recherche documentaire entreprise.
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