

IN VITRO ACTION OF ANTIFILARIALS ON SYNTHESIS OF MACROMOLECULES BY *SETARIA CERVI* ADULTS

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ABSTRACT

Setaria cervi, the filarial parasite of Indian water buffalo, is sensitive to diethylcarbamazine (DEC) and its analogue centperazine. Both these filaricides depress protein and RNA synthesising capacity in adult worms although to a varying degree. Centperazine was more active than DEC in inhibiting macromolecular synthesis.

INTRODUCTION

DIETHYLCARBAMAZINE (DEC)^{1,2} and centperazine (developed in CDRI Lucknow)^{3,4} are being used for controlling human and animal filariasis. Although these filaricides are effective against microfilariae (mf) *in vivo*, a marked difference in opinion exists about the *in vitro* activity of DEC on filarial worms; and a marked *in vitro* effect, on microfilariae of *Wuchereria bancrofti*⁵, *Dipetalonema viteae*⁶ and *Onchocerca volvulus*⁷, has been noted. The *in vitro* effect of DEC on adult filarial worms has been studied and this seems to depend on the species and/or the incubation method used⁸⁻¹⁰. The usefulness of *Setaria cervi*, a bovine filariid for detection and investigation of antifilarial activity has been mentioned^{11,12}. The present communication describes the *in vitro* effect of DEC and centperazine on the synthesis of macromolecules by *S. cervi* adults.

MATERIALS AND METHODS

Adult, motile *S. cervi* females were collected from the peritoneal folds of freshly slaughtered buffaloes at a local abattoir, freed from contaminants and preserved in salt solutions¹³ until used.

The effect of DEC and centperazine on the synthesis of protein, DNA and RNA were studied by incubating aerobically motile worms in Kreb's Ringer bicarbonate buffer (KRB), pH 7.4 with different concentrations of the filaricides for 6 hr at 37°C. The drug treated worms were then transferred in incubation mixture which in a total volume of 10 ml had: KRB buffer (pH 7.4), glucose (5.5 mM) and ¹⁴C-leucine (U) (1 mM)/2-¹⁴C-uracil (5 mM)/³H-thymidine (G) (10 mM). The specific activities of the above radiochemicals (all in mCi/mMole) were 30, 50 and 8-10, respectively. The reaction was terminated by chilling the worms in ice and washing with saline containing non radio active precursors and homogenizing in ice cold distilled water. Various macromolecular constit-

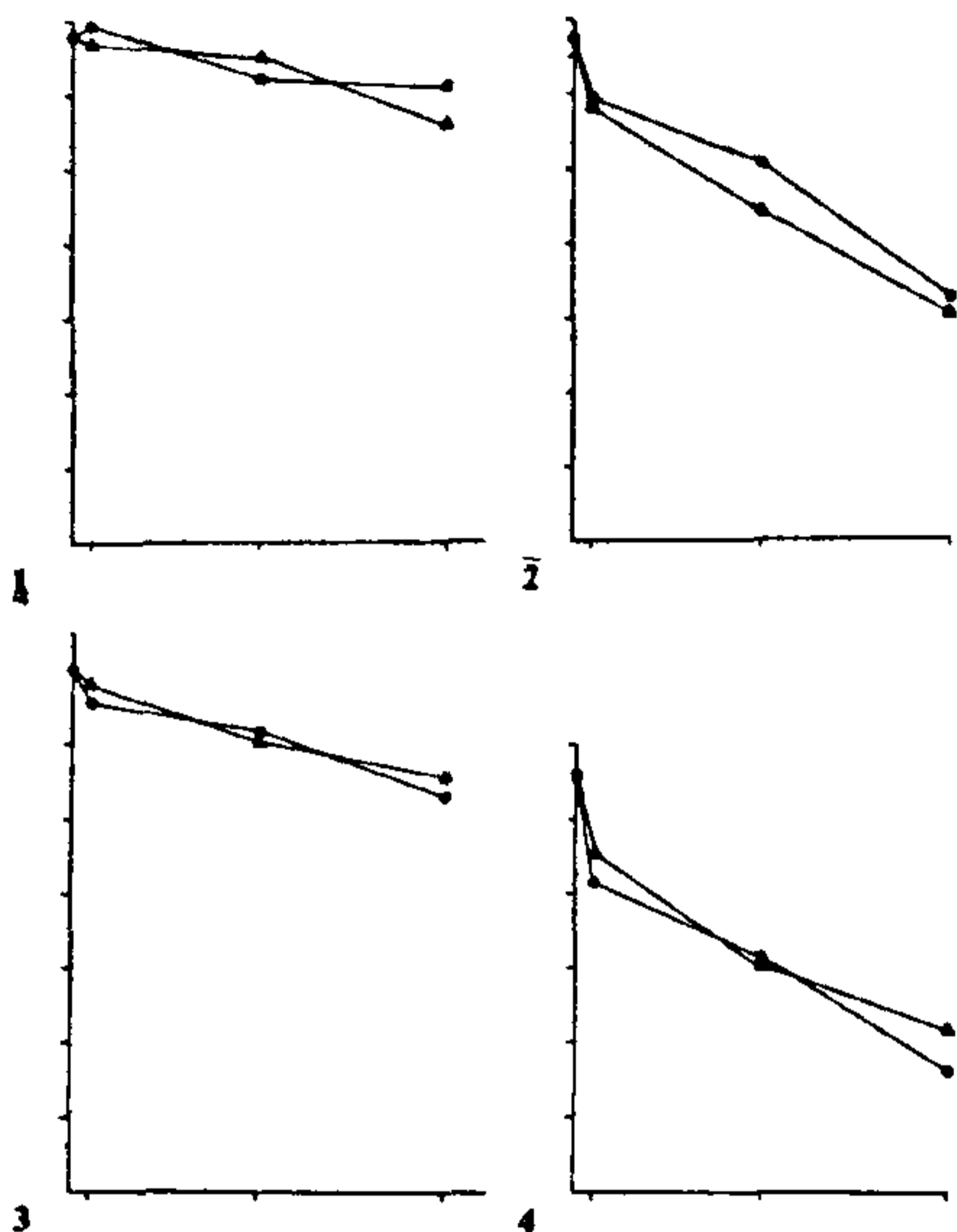
uents (protein/ DNA/ RNA) were isolated according to standardized procedures¹⁴.

Radioactivity in the sample was counted according to the following procedure. The alkali solution of protein or the aqueous fraction of TCA free DNA or RNA was spread with a clinipette on Whatmann 3 mm strips (2 × 2 cm), dried thoroughly in a stream of hot air and placed individually in low K-vial containing scintillation fluid made up of 2,5 diphenyl oxazole, (0.4%) and 1,4 bis-2-(4 methyl-5 phenyloxazolyl)-benzine (0.01%) in freshly distilled toluene. Counting was done in a Packard Tricarb liquid scintillation counter. The efficiency of the counter was 80-90% for ¹⁴C and 40-50% for ³H under the conditions employed.

RESULTS AND DISCUSSION

The results presented in figure 1 indicate that incorporation of ³H-thymidine into DNA fraction was not significantly altered in DEC or centperazine treated parasite. However, under the influence of these filaricides, the incorporation of 2-¹⁴C-uracil into DNA and RNA fraction of adult parasite (figures 2 and 3) revealed that at higher concentration (10 mM) RNA synthesis was lowered by about 50% by both the compound while incorporation into DNA remained unaffected. The inhibitory effect was dependent on the concentration of drugs employed. Incorporation of ¹⁴C-leucine (U)-into protein fraction was significantly inhibited in DEC as well as centperazine treated worms (figure 4). On a molar basis, centperazine was more effective than DEC in inhibiting protein synthesis under *in vitro* conditions.

Earlier reports from this laboratory have indicated differential sensitivity of these two filaricides on glucose uptake, certain enzymes of carbohydrate metabolism, loss of motility and liberation of microfilariae by adult females *S. cervi*¹⁵. Microfilariae of *Litomoxdies carinii* (a cotton rat filariid) and *S. cervi* also



Figures 1-4 *In vitro* effect of diethylcarbamazine (closed triangle) and centperazine (closed circle) upon the ability of *S. cervi* to incorporate. 1. ^3H -thymidine into DNA, 2. $2\text{-}^{14}\text{C}$ -uracil into DNA. 3. $2\text{-}^{14}\text{C}$ -uracil into RNA. 4. ^{14}C -leucine (U) into protein.

exhibited similar pattern¹⁵. This difference may possibly be due to their altered penetration rate across the surface membranes of intact adult worm.

Rapid incorporation of ^{14}C -leucine (U) into protein fraction as observed in the present study during its *in vitro* maintenance in KRB medium suggests that rapid protein synthesis is essential for survival and viability of the filarial worms.

From the above results it can be concluded that DEC and its analogue centperazine behave more or less in similar fashion in inhibiting synthesis of macromolecular constituents like RNA and protein which are essential for rapid growth of microfilariae inside the

uteri. Centperazine was more active than DEC, the conventional drug.

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