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ISOLATION AND CHARACTERIZATION OF THE MOST IMMUNOGENIC FRACTION OF *TRICHOSTRONGYLUS COLUBRIFORMIS*

BY

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INTRODUCTION

Gastrointestinal nematodes are among the important common parasites infesting sheep in Egypt. Control of these parasites is directed toward the treatment of infected animals, beside , application of some hygienic measures. However immunological control was lacking.

Ozerol and Silverman (1970), Cripps and Adams (1978), Adams et al. (1980), Wedrychowicz (1984) and Ramadan et al. (1989) studied the immunogenic activity of the fractions of *T. colubriformis*, *Haemonchus contortus*, and *ostertagia circumcincta*.

THe present work aim at the extraction of antigen that had highest immunogenicity so that it might be used in vaccination against *T. colubriforimis*.

MATERIALS AND METHODS

1) Preperation of the antigen:

For preperation of *Trichostrongylus colubriformis* extract, five rabbits were infected orally with

20,000 *T. colubriformis* infective larvae. The adult worms were collected one month later and washed several times with 0.05 M. phosphate buffer saline (pH 7.0). The worms were chopped into short pieces and then homogenized thoroughly in p b s. The whole worm antigen was homogenized in phosphate buffer 3 times and centrifuged at 10,000 r.p.m. for 30 minutes. The supernatant was pooled together after each centrifugation and the total protein content (mg/test & mg/ml) was determined according to Lowry et al. (1951).

2) Isolation of the immunogenic fractions of T. colubriformis:

This was carried out using column chromatography adopted by Ogunba (1972). The column used was 90 cm in length x2.5 cm in diameter (Pharmacia Co., Sweden). It was packed with sephadex G 200 (Pharmacia Co. Sweden).

Five ml of whole *T. colubriformis* worm antigen with a total protein concenteration of at least 6 mg/ml were passed through the column. Phosphate buffer saline (pH. 7.0) was used as eluting buffer, and the flow rate was adjusted to 3 ml/30 minutes. The produced fractions were collected by means of an LKB fraction collector. Ultra-violet adsorption of the fractions was determined at 280 nm, the reading of each tube was recorded and the values (mg/ml protein for each tube) were represented in curves. The fractions of each peak were tested against *T. colubriformis* rabbit antisera by gel-diffusion test (Ouchterlony, 1953) and Indirect haemagglutination test (Kessel et al., 1965).

3. Determination of the total protein of each peak:

The protein content of the different peaks was measured colorimetrically using the modified-Lowry et al. method (1951).

4. Concenteration of pooled fractions:

Concentration was performed using dialysis bag (sigma Co. U.S.A.). The bags were filled with each of the pooled fractions and then exposed to polyethyleneglycol over night at 4°C.

RESULTS

Results of gel filtration of *T. colubriformis* on sephadex G 200:

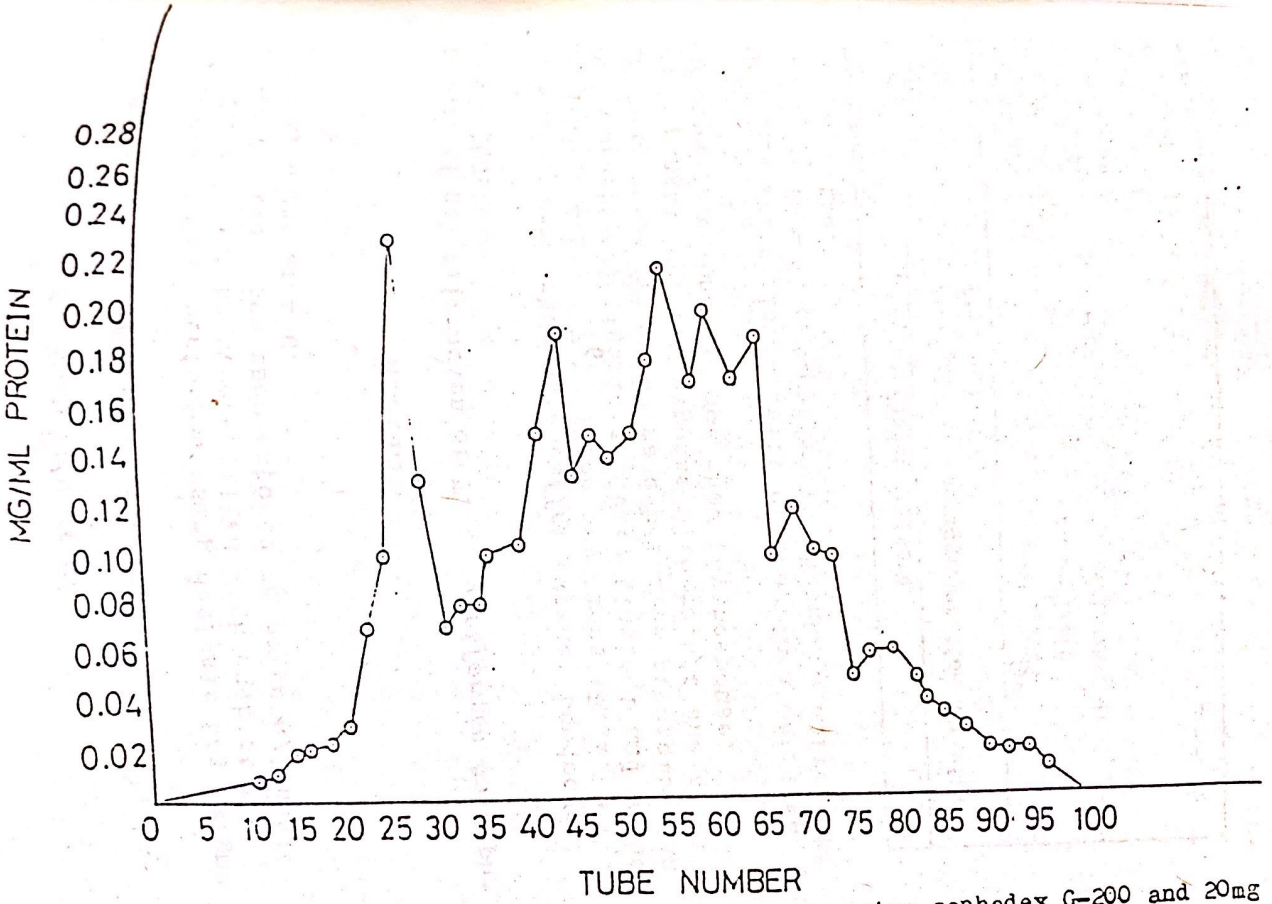
T. colubriformis phosphate buffer extract produced six fractions (F_1 - F_6). Three fractions (F_1,F_2 & F_3) showed specific antigenic activity (Table 1 and curve 1). These three fractions gave positive precipitating and haemagglutinating response with rabbit antisera. The other fractions did not show any response.

Table (1): Antigenic activity of fractions obtained from *T. colubriformis* phosphate buffer extract

Antigen	Rabbit antisera	
	Agar gel diffusion test	Haemagglutination activity
Fraction No. 1	+	+ 1/1024
Fraction No. 2	+	+ 1/256
Fraction No. 3	+	+ 1/128
Fraction No. 4	-	-
Fraction No. 5	-	-
Fraction No. 6	-	-

DISCUSSION

The present work mentions the preliminary fractionation of *T. colubriformis* extracts which contain a



Curve 1 : T.colubriformis gel-filtrotiom curve using sephadex G-200 and 20mg total protein

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high level of worm antigens. Six fractions (F_1-F_6) were seperated from *T. colubriformis* extract, and three of them were found to be most immunogenic using agar gel-diffusion and passive haemagglutination tests. The present results were similar to those obtained by Ramadan et al. (1989) who seperated 5 fractions (F_1 - F_5) from *H. contortus* phosphate buffer extract through column chromatography using sephadex G 200. They found only 2 fractions (F_4 & F_5)to be antigenically specific in precipitating and haemagglutinating antibody responses. Also Wedrychowicz (1984) seperated 6 fractions (F_1 - F_6) from the fourth *Ostertagia circumcincta* larval somatic extract that were chromatographed on sephadex G 200 column. Three fractions (F_2, F_3 & F_4) showed positive precipitating and haemagglutinating antibodies against rabbit antisera. The same author obtained 5 fractions (F_1 - F_5) from *O. circumcincta* fifth stage larvae. Fractions 1,2 & 4 showed positive precipitating and haemagglutinating activities. Moreover, Ozerol and Silverman (1970) obtained, two fractions from third and fourth larval antigen of *Haemanchus contortus*.

SUMMARY

Six fractions (F_1 - F_6) were observed from *Trichostrongylus colubriformis* phosphate buffer extract separated by column chromatography using Sephadex G 200. Only three fractions (F_1,F_2&F_3) were antigenically specific in precipitating and haemagglutinating antibody responses.

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