PN-ABA-564

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON D.C. 20523

DATE: 4/5/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 7. 875
PR 7/87 - 12/87

Attachment

1 cy.

7.075

SCIENTIFIC REPORT

PROTEOLYTIC ENZYMES : EVALUATION AS PURIFIED ANTIGENS IN DIAGNOSIS OF FASCIOLIASIS IN PERU

Project No. 936-5542

Covered Period : 7/87- 12/87

1. Fasciola hepatica metacercaria:

In having this evolutive stage of the parasite is essential to be confirm our previous results showing proteolytic activity in the extract of the metacercaria.

Our snail colony of Lymnaea viatrix is increasing with new generations of snails brought them from Mantaro Valley; presently, we are infecting snails with miracidia from eggs incubated in the Laboratory.

We are collecting metacercaria and they are frozen for further studies.

2. Factors involved in the obtention of excreted-secreted (E/S) material from adults of Fasciola hepatica .

2.1. Transport medium: The following media were studied:

a) Saline (Na Cl 0.9%) .
b) Buffer Phosphate pH 7.4
c) Deionized water .

2.1.1. Collection of Fasciola hepatica adults: The adults were collected in the abattoirs of Lima. The parasites were taken from the biliary tract of the infected cattle and transferred to the transport medium.

2.1.2. Obtention of E/S material: When the parasites arrived to the laboratory were rinsed twice in deionized water before transferring to the containers with cold distilled water. They were kept them in refrigeration at 4oC. After this cold shock, the parasites started excretion of the digestive content.

APR

In some occasions, after 15 minutes, the distilled water was replaced by fresh water. After t hours, all the E/S was filtered and the proteolytic enzime activity was assayed, using the azocoll method as is described : Azocoll was used as substrate (5 mg./ml) , plus dithiothreitol 0.1 mM and 0.1 ml. of the sample. The final volume was 4 ml.. Absorbance was read at 520 nm.

The results showed that the Saline transport medium was better than the others.since it allowed greater yields of excreted enzyme.

2.2. Time of the cold shock:

The following times were observed : 1, 2, 3, 4, 5, 6, and 24 hours.

The collection of Fasciola hepatica adults and the transportation of the adults were as described under 2.1.1.

The parasites transferred to cold distilled water were maintained at 4oC for the times mentioned.

The filtered material was used to check the proteolytic activity, using the azocoll method and the protein concentration was determined by the Lowry method.

The results showed a good amount of specific activity at 2 hours and some increasing in the following hours (3-4). After 5 hours,the specific activity started decreasing; after 24 hours hours, the difference is large, as is showen in Fig. 1.

On the another hand the amount of E/S protein is higher at 4 hours. The difference between 3 and 4 hours is close to 20%. We believe that 4 hours of cold shock would be enough to obtain a larger amount of proteolytic enzyme, keeping in mind that we need a large amount of enzyme for its further purification.

In some experiments the E/S material was dialyzed, but this condition did not improve our results.

In the same way, the change of distilled water after 60 minutes of the cold shock do not increase the yield.

2.3. Relationship betweeen the number of the adult parasites with the volume of cold distilled water to obtain the E/S material:

We assayed different proportions ,but the best result was obtained when that proportion was 12 parasites for each 10 ml of volume.

3. Purification of the Proteolytic enzymes:

3.1. Column chromatography with Sephadex G-100.

A column 2.5 x 50 cms. of Sephadex G-100 (Pharmacia) was prepared.

The material of E/S (~ 250 mg.) was suspended in 5-6 ml. of Citrate buffer (0.2 M Na Citrate , pH 4.9) and homogenized at 4oC with Teflon-pestle Potter Elvejihm. The preparation was clarified with 1-2 centrifugations at 20,000 g. at 4oC for 10 minutes. The clarified material was loaded at the top of the columnn. The elution buffer was the same Citrate buffer. The elution fractions were collected in test tubes (13 x 100 mm) with 2 ml in each tube. Around 200 ml of elution buffer was used to elute all the protein content. The profile of the eluted material at 280 nm is shown in Fig. 2. As it may be seen, the proteolytic activity was present only in fractions 55-85. All these fractions were pooled and kept at -20oC for the lyophilization and further studies.

3.2. Electrophoresis:

The material after elution from the column and lyophilization was subjeted to SDS acrylamide gel electrophoresis.

The lyophilized material was suspended in distilled water and treated with mercaptoethanol and SDS.

The gel was a 15% of polyacrylamide. Electrophoresis was performed at 25 mAmp. When the run was finished, the gel was stained with Coomassie Blue. We identified two bands: one at 29,000 M.W. and the other at 14,000 M.W.

The band at 29.000 M.W is larger than of 14000 M.W. (Fig. 3).

We are going to perform electroelution of these bands to used them as antigens in "in vivo" and "in vitro" models, including experiments with monoclonal antibodies.

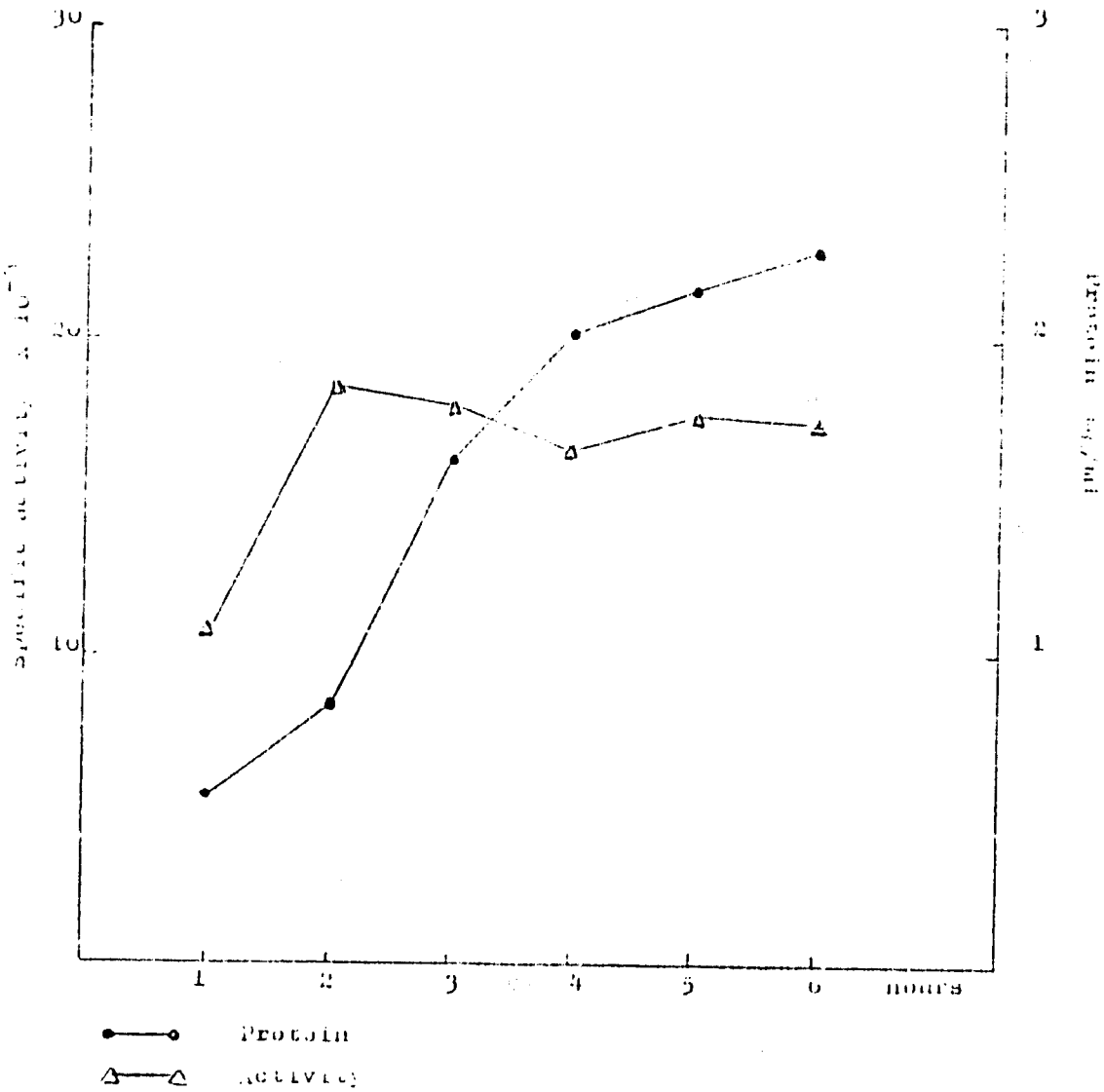

Fig. 1 TIME OF COLD SHOCK

Fig. 2 . Profile of G-100 Sephadex Column

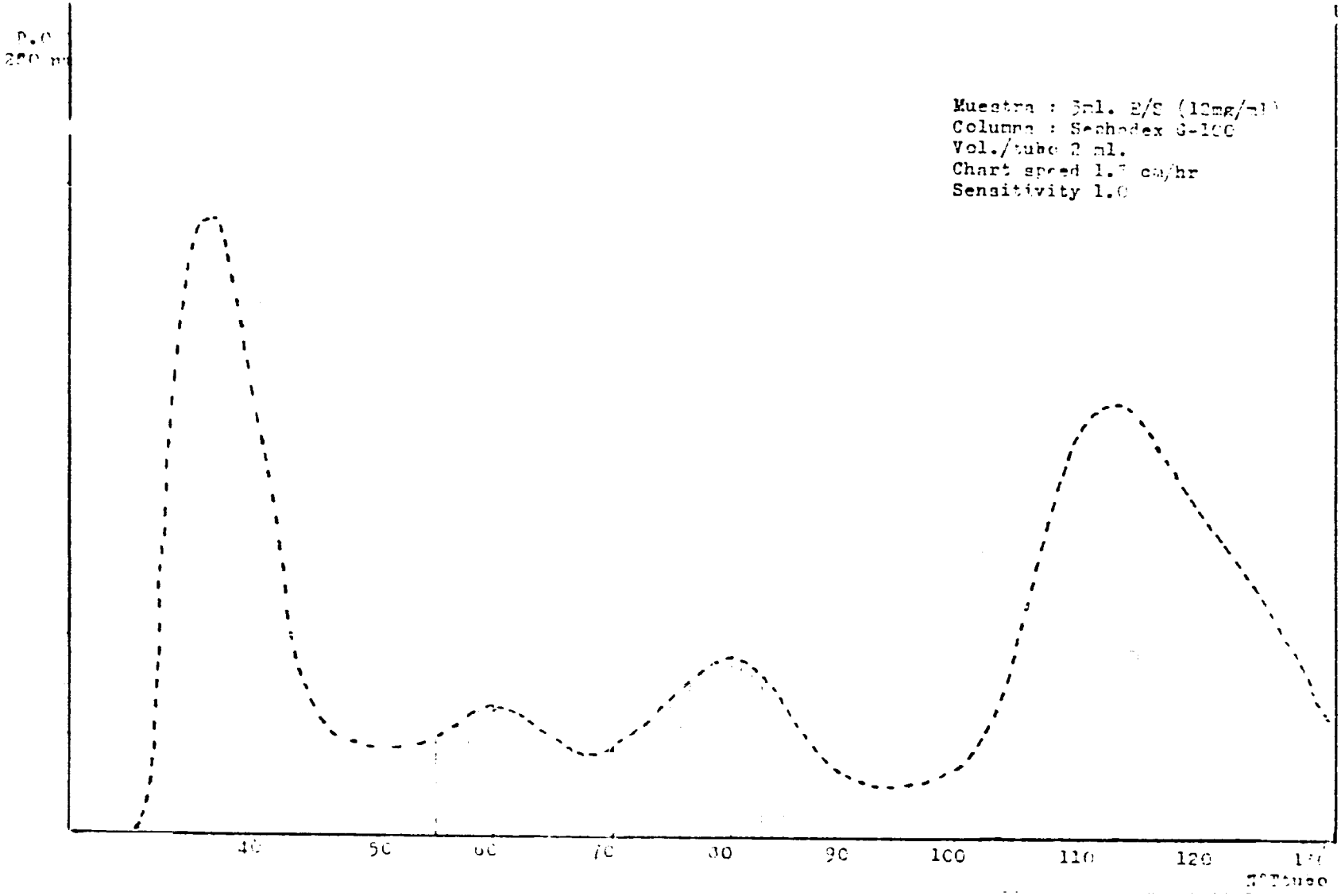

Fig. 3 S-D-S-Acrylamide Electrophoresis of E/S and Elution from Sephadex G-100.

1-2. E/S material (different dilutions)
3-6. Elution from Sephadex G-100 (different dilutions)
7. M.W. markers.