

PD-AAX-384
54791

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE:

1/21/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18
FROM: AID/SCI, Victoria Ose *VO*
SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 4. 577

Attachment

misc

file: 4.577 (



UNIVERSIDAD PERUANA CAYETANO HEREDIA
INSTITUTO DE MEDICINA TROPICAL
"ALEXANDER VON HUMBOLDT"

Handwritten initials

USAID / LIMA
MAR 29 1985
RECEIVED

AP. 5045 - LIMA 100 - TELF. 817551

CAR-IMT-109-85

Marzo 28, 1985.

See date 4/10

ACTION COPY	
ACTION TAKEN:	_____
DATE:	_____
INITIALS:	_____

Miss Linda Lou Kelley
Agency for International Development
Av. España 386
L i m a /

De mi consideración:

Me permito poner a su disposición el informe científico y los documentos del movimiento económico del Project AID-PSMC 936-5542, Obligation Nº 364-1297, para dar cumplimiento a lo estipulado en la ejecución del proyecto mencionado.

Agradeciendo su atención, lo saludo.

Atentamente,

Handwritten signature
Dr. Hugo Lumblerás
Director

ACTION:	<i>Hen. D. J. J.</i>
Info:	<i>HNE</i>
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SCIENTIFIC REPORT

(PERIOD - JULY 1984 - DECEMBER 1984)

PROJECT AID - PSTC 936 - 5542
OBLIGATION No. 364 - 1297

" PROTEOLYTIC ENZYMES OF FASCIOLA HEPATICA AS MARKERS OF HUMAN AND ANIMAL INFECTION IN CATTLE - RAISING AREAS OF PERU "

INITIAL EXPERIMENTS

1.- Set - up the life cycle of Fasciola to obtain significant amount of juvenile flukes :

A room in the Animal house of Instituto de Medicina Tropical "Daniel A. Carrión" has been implemented with tables, aquaria and a supply of de deionized water. The snails (Lymnaea viatrix = viator) have been brought from an endemic area for Fascioliasis (Mantaro Valley, Central Highlands). They continue their growth and reproduction to be infected by miracidia obtained from eggs of Fasciola hepatica hatched in the laboratory.

2.- Assay of activity of proteolytic enzymes :

2.1. Mounting of the Azocoll Method:

The substrate Azocoll was used with a known protease (Nagarse) following chavira et al. (Analytical Biochemistry 136 : 446 - 450, 1984). A linear correlation was found between the hydrolytic activity and the concentration of enzyme (2.7 to 21.88 MG of protein/tube). For the same amount of enzyme there was a linear response from 20 to 60 minutes. After the incubation, the absorbance spectrum of the hydrolyzes Azocoll showed two peaks, a large one between 518 - 520 nm, and a small one between 404 - 410 nm.

2.2. Assay with Fasciola hepatica adults :

It was performed with fresh, homogenized, and fractionated parts of the adult flukes. A assay with the suspension liquid of the adults collected was done.

2.2.1. Assay with the entire fresh fluke: the adults of Fasciola hepatica.

Were obtained from livers of cattle killed in local abattoirs. This....



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fresh material was homogenized in glass teflon homogenizers, with about 10 strokes of. The teflon pestle in diverse buffers (see below). Using the homogenized fluke material as the enzyme source, assays were performed with different buffers, and at varying pH values, as follows: One unit of activity was established to be equal to the absorbance at 520 nm x mg protein⁻¹ x minute⁻¹.

<u>BUFFER</u>	<u>pH</u>	<u>ACTIVITY (UNITS)</u>
50 mM Fris, 1mM Ca Cl ₂	7.8	7 x 10 ⁻⁶
0.2 M Sodium Citrate	4.0	1.44 x 10 ⁻⁴
0.2 M Sodium Citrate	5.0	1.47 x 10 ⁻⁴
0.2 M Sodium Citrate	6.0	9.4 x 10 ⁻⁵

2.2.2. Assay with fractionated parts of the fluke:

Using 0.2 M Sodium citrate, pH 4.0, the parasites were homogenized as described above and centrifuged at 1,500 G. The supernatant showed 1/3 of the total activity.

Using 50 mM tris, 1mM Ca Cl₂, pH 7.8, a supernatant obtained from homogenized parasites in mortar, and after 40,000 G. of centrifugation, showed traces of hydrolytic activity. This material was lyophilized.

2.2.3. Assay with suspension liquid of the adults of Fasciola hepatica:

Aleve adults of Fasciola hepatica were collected in saline (0.9% - Na Cl). After an hour, a dark material stains it, and when the proteolytic activity was assayed, the results were:

<u>BUFFER</u>	<u>pH</u>	<u>ACTIVITY (UNITS)</u>
50mM Tris, 1mM Ca Cl ₂	6.0	1.54 x 10 ⁻⁴
0.2 M Sodium Citrate	4.0	3.30 x 10 ⁻⁴

3.- Collection of sera from people and cattle infected by Fasciola hepatica :

Sera from infected people is being obtained from patients came to the Instituto de Medicina Tropical "Daniel A. Carrión" and Instituto de Medicina Tropical "Alexander Von Humboldt".



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Sera from infected cattle is being collected in the local Abattoirs.

REMARKS

- 1.- The assays show proteolytic enzyme activity in adult fluke material.
- 2.- Material excreted from adult flukes show higher proteolytic enzyme activity than homogenized whole flukes.
- 3.- The limited experiments with variation of pH show greater activity at acid pH : 4 and 5
- 4.- The life cycle of Fasciola hepatica is being set up in the laboratory; Lymnaea viatrix snails are being harvested from the field and acclimatized in Aquaria for breeding.
They will be infected with laboratory hatched Fasciola hepatica eggs.

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SCIENTIFIC REPORT

(PERIOD JULY 1985 - DECEMBER 1985)

PROJECT AID - PSTC 936-5542
OBLIGATION No. 364-1297

"PROTEOLYTIC ENZYMES OF *Fasciola hepatica* AS MARKERS OF HUMAN AND ANIMAL INFECTION IN CATTLE-RAISING AREAS OF PERU"

1.- Raising of snails to maintain life cycle of *Fasciola hepatica*:

Lymnaea viatrix (viator) snails were collected several times from an area endemic for Fascioliasis (Mantaro Valley). They were brought to the Laboratory conditioned in the Instituto de Medicina Tropical "Daniel A. Carrión", where, after an adaptation period, they started their reproduction satisfactorily. They were used for supporting infection with miracidia from *Fasciola hepatica* eggs hatched in the laboratory.

During this period, it was possible to obtain cercaria and metacercaria in amounts sufficient for experimental infection of animals (sheep and rats) and to look for proteolytic enzyme activity in metacercaria. These results are shown later.

2.- Assay of the activity of Proteolytic Enzymes:

In our previous report, we showed the existence of at least two groups of proteolytic enzymes, one group with a high molecular weight (Arg-Arg proteinase) and the other group with a relatively low molecular weight (Phe-Arg Azocoll proteinase).

These enzymes were detected in fluids from homogenized adults and in excretory-secretory material (ESM).

2.1. Fractionation by precipitation with Ammonium Sulphate:

Assuming that fewer proteins other than the proteolytic enzymes would be present in the (ESM) of *Fasciola hepatica*, assays were performed using that material, although in a previous assay, the supernatant after precipitation at 30% Ammonium Sulphate of homogenized *Fasciola hepatica* adults showed more activity.

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Ammonium Sulphate at 30, 50, 60, 70 and 90% (final concentration) was added to 10 ml of ESM. Proteolytic activity (Azocoll Method) was assayed in the precipitated material after dialysis. The following were the results:

% of saturation	Specific Activity of Proteolytic enzyme(s) (Activity units/mg of protein/min.)	
	Without DTT	With DTT
30	8.4 x 10 ⁻³	34.1 x 10 ⁻³
50	35.4 x 10 ⁻³	57.9 x 10 ⁻³
60	28.6 x 10 ⁻³	49.2 x 10 ⁻³
70	23.3 x 10 ⁻³	40.7 x 10 ⁻³
90	17.1 x 10 ⁻³	24.5 x 10 ⁻³

As is shown 50% of saturation gives more precipitated proteins with proteolytic activity.

To continue the purification of this material, column chromatography will be performed.

2.2. "Regurgitant" test:

We wanted to collect as much of the ESM as possible, so that it could be used for enzyme purification. Adult *Fasciola hepatica* were collected from cattle in the local abattoirs and placed in saline. As soon as they arrived in the laboratory the saline was replaced with distilled water at 4°C for 4 and 22 hours.

These results at four hours show a good "regurgitant" effect. The specific activity of the proteolytic enzymes increased 3 to 4 times. At 24 hours the effect appears not to have been increased. We are going to perform more assays using shorter periods of time.

2.3. Temperature effect:

During the life cycle of *Fasciola hepatica* the parasite must develop in environments at different temperatures (mammals, snails, water), so we wanted to know if the activity of the proteolytic enzymes of *Fasciola hepatica* changed with the temperature.

Material from ESM and adults of *Fasciola hepatica* were put at 4°C, room temperature (24°C) and 37°C for 24 hours. ESM and the total homogenized material from adult worms were incubated as described. This material allowed the determination of proteolytic activity with the following results:

The mean of the 5 experiments show:

4°C	Specific activity (Activity units/mg. of protein/min)	
	ESM	Homogenized adults 1/4
4°C	10.2 x 10 ⁻³	35.6 x 10 ⁻³
Room temperature	32.0 x 10 ⁻³	36.2 x 10 ⁻³
37°C	11.0 x 10 ⁻³	14.4 x 10 ⁻³

2.4. Proteolytic activity in the juvenile forms (metacercaria):

Metacercaria of *Fasciola hepatica* obtained from the snails experimentally infected with miracidia hatched in the laboratory were used to assay proteolytic enzymes activity.

Using, at least one hundred metacercaria we were able to show the presence of enzymes with proteolytic activity.

In the next coming weeks more metacercaria will be submitted to the Tielens method to obtain juvenile worms to repeat the same assays.

3.- Experimental infection of animals:

Three month old sheep, free of *Fasciola hepatica* infection, were obtained and maintained in the Veterinary School of the San Marcos University. They were infected with 25 metacercaria using a stomach tube, and bled to obtain sera.

The infection of these animals will be followed for 5 months looking for eggs in their stools and the presence of known antibodies (Arc 2) in their sera.

The sera will be used as control to the presence of antibodies against the proteolytic enzymes isolated in Houston (Texas). Similar infections are to be performed in rats, mice and rabbits.