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Research Report # 3

from

Liliana Cardemil

Project # 936-5542

Grant # DPE-5542-G-SS-8073-00

Obligation # 8361137

Project # 7.068 (Off.Sc.Adv.)

Research Report # 3

Grant # : DPE-5542-G-SS-8073-00

Period covered: August 31, 1989
to
February 28. 1990

Preliminary Remarks.-

During the first year of research, we have written two reports. In the first report, from the period August 31, 1988 to February 28, 1989, we requested the amount of $ 13,820 for travel, salaries and reagents. That amount was deposited in the bank account of the University of Chile by September 27 of 1988. In our second report, from the period February 28 to August 30, 1989, we requested the amount of $ 16.250 for salaries, travel and reagents. The amount was deposited on November 26 , 1989.

During the past semester, we have spent most of the funds of the item "other costs" which were originally considered in our budget. However, we have spent less in salaries and therefore, we have requested to AID in Washington, to buy a computer for processing the data we are obtaining from the field research. The computer is for a total cost of $ 2,600. Since the Office of the Science Advisor replied possitively to this request (I am enclosing a copy from Dr. John Daly, Program Coordinator), we have rellocated $2,600 from the grant budget from the item for salaries to the

item for equipment.

This report is for the period August 31, 1989 to February 28, 1990. A detailed report of the expenses made during this period, is enclosed. The results obtained from August 31, 1989 to February 28, 1990, are described in this report.

Disbursements of Budget Grant# DPE-5542-G-SS-8073-00

Cumulative Detailed Report From:

August 31, 1989

to

February 28, 1990

	US $
Salaries	5,958
Equipment	2, 655
Travel	1,852
Reagents	1,506
Other Costs	6,146
Total	18,117

List of Equipment Purchased

PCS 286 Personal Computer with external floppy disk unit from Olivetti, with a cost of US $ 1420

DM 100 Printer from Olivetti, with a cost of US $ 235

Total: US $ 2,655.

Total Disbursements of Budget

Cumulative Report up to February 28, 1990 (First year + 6 months of Second year)

	From: August 30, 1988 to May 5, 1989	From: May 5, 1989 to August 30, 1989	From: August 31, 1989 to February 28,1990	Total
	US $	US $	US $	US $
Salaries	1,440	2,424	5,958	9,822
Equipment	45,755	1,431	2,655	49,841
Travel	5,876	1,794	1,852	9,522
Reagents	4,190	4,174	1,506	9,870
Other Costs	1,230	12, 473	6,146	19, 849
Total	58,491	22,296	18.117	98,904

A. Temperature Stress Experiments.

Previous Results Under AID Grant On *Araucaria araucana* And *Prosopis chilensis*.

Kinetics experiments of appearance of heat shock proteins (hsp) in seedlings of both systems revealed that as soon as 30 minutes after heat shock begins, the new proteins are present. A maximun accumulation of hsp occur after 3-4 hours of exposure to 36° C, in the *Araucaria araucana* case and to 45° C,in the *Prosopis chilensis* case.

The heat shock proteins are nine in seedlings of *Prosopis chilensis*. One of the hsp is very prominent, with a molecular weight of 62 KD. Another prominent protein increases considerably under exposure to 40-45° C, with a molecular weight of 71 KD.

The optimal temperatures for synthesis and accumulation of hsp were 36° C, for *Araucaria araucana* seedlings and 45° C, for *Prosopis chilensis* seedlings.

In all these experiments the seedlings were incubated,at the appropriate treatment of temperature,in the presence of ^{35}S-methionine used as precursor of protein synthesis.

The accumulation of mRNA for two hsp was studied by Northern hybridization analysis in Araucaria araucana seedlings,using probes containing the genetic information for these proteins. They are,the ubiquitine and the universal hsp 70 KD. Both proteins are highly conserved among the living organisms.

The results show the optimal accumulation for ubiquitine mRNA occurs at 36° C with a 6 fold increase of mRNA in the embryo axis which is the tissue that shows the best response. The mRNAs for the 70 KD protein, are almost not present at 28° and 32° C. The synthesis of mRNA for this protein turns on at 36° C. The results also demonstrated that there are two populations of mRNAs for the 70 KD protein.

Results Obtained On Heat Shock Expression During This Period.

Our laboratory owns antibodies against ubiquitine and against the 70 KD heat shock protein. The antibodies against ubiquitine are polyclonal of two kinds: One was raised in rabbits against the conjugated ubiquitine, therefore, against ubiquitine-protein complexes. The other was also raised in rabbits but against free ubiquitine. Both antibodies were kindly provided by Dr. Aaron Ciechanover from the Medical School at Washington University, St. Louis, Missouri.

The antibody raised against hsp 70 KD is a monoclonal antibody obtained against the purified hsp 72 KD from HeLa Cells. It was purchased to Amersham Chem.Co.

Seedlings of 48 hours after the start of imbibition of Prosopis chilensis and germinated at 35° C, were subjected to two hours at 35°,40°, 45° and 50° C. After the treatment, the proteins were extracted and electrophoresed in SDS- polyacrylamide. Half of the gel was stained for proteins and half was electrotransferred to nitrocellulose membranes overnight. The membranes were incubated with a first antibody (rabbit's IgG anti conjugated-ubiquitine) in a dilution of of 1/250 and the with a second antibody (goat's anti IgG conjugated to alkaline phosphatase) in a dilution of 1/15,000. The results of the Western analysis are shown on Figure 1, where it is seen several bands of high and low molecular weights that cross react with the antibody. The proteins obtained from seedlings heat shocked at 45° and 50° C, are the ones showing higher reactivity. Table I is a summary of the proteins that give possitive reaction with the conjugated-ubiquitine antibody. The intensity of the cross reactivity is given by the number of crosses, (+).

Figure 2 shows the results of a Western analysis performed with seedlings of Prosopis chilensis treated as before, using the polyclonal antibody raised against free ubiquitine (rabbit's IgG anti ubiquitine) in a dilution of 1/250 and next incubated with a second antiboby (goat's anti IgG conjugated to alkaline phosphatase) in a dilution of 1/15,000. Protein

extracts from seedlings heat shocked at 45° and 50° C show a band of 9 KD which is the reported molecular weight for ubiquitine, (Vierstra, 1987). The band cross reacts with the antibody. Extracts of seedlings treated at 50° C show three other very faint bands of 70,57 and 51 KD. Table II is a summary of these results.

Heat Shock Expression And Field Research

Several environmental parameters were measured in the natural population of Prosopis chilensis located at Quilapilun Station in Peldehue (Central Chile). The measurements were performed for a period of 24 hours on January 27-28 of the present year. Figure 3 is the soil temperature fluctuation measured at different distances from the soil: +10 cm; -2 cm, under Prosopis shadow and -2 cm under sunshine. Figure 4 is the environmental temperature fluctuation in 24 hours measured under Prosopis shadow. Figure 5 is the total radiation measured under shadow and under sunshine in the same period of 24 hours. Figure 6 and 7 are the fluctuations on humidity and evaporation respectively, during the same period.

The higher temperatures of the day took place at 5.00 PM with a maximun of 35° C when measured under Prosopis shadow; with a maximun of 60° C when measured under sunshine, Figures 4 and 3. Therefore, the maximun evaporation occurred at 5.00 PM, Figure 7. The maximun radiation took place at 3.00 PM, Figure 5, and the maximun humidity occurred between 2.00-9.00 AM under Prosopis shadow, Figure 6.

Future Work Planned On Heat Shock Expression.

We expect to perform Western analysis of the heat shock proteins on seedlings from Araucaria araucana subjected to heat shock, using the ubiquitin and 70 KD,antibodies. At the present we are performing Western analysis with the 70 KD antibody with Prosopis chilensis seedlings germinated at 35° C and heat shocked at 35°, 40°, 45° and 50° C.

The Northern hybridization analysis for mRNA extracted from Prosopis chilensis with probes containing the genetic information for these two proteins,is underway.

The field research will be continue by doing temperature measurements of Prosopis leaves under shadow and under sunlight. Western analysis of proteins extracted from leaves will be performed taking samples at different hours of the day for a period of 24 hours. The Western analysis will be done using the antibodies available in our Lab.

References. Vierstra, R.D. 1987. Ubiquitin, a key component in the degradation of plants proteins. Physiol. Plantarum 70: 103-106.

B. Injury Stress Experiments

Previous Results Under AID Grant On *Araucaria araucana* System.

We had purified two of the cell wall proteins from *Araucaria araucana* seedlings which cross react with polyclonal antibodies raised against carrot and soybean seed coats, extensins,by chromatofocusing using a PBE 118 gel purchased to Pharmacia Fine Chemicals. The two proteins come together in the same fracction collected from the column because both have similar isoelectric points of 10.6 and 10.4.

A further purification and characterization of the proteins are described for this reporting period.

Results Under AID Grant Obtained On *Araucaria araucana* System, For This Reporting Period.

A further purification and characterization of these proteins was performed at Dr. Joseph Varner's Laboratory, at Washington University, St. Louis Missouri. For this purpose,Alejandro Riquelme,a graduate student working on the project and Dra. Liliana Cardemil, the principal investigator of the project, visited Dr. Varner's Lab for a period of 2 and 1/2 months , from August 20 to November 1º,1989.

Figure 8 is an elution profile of the cell wall proteins extracted from purified cell walls of seedlings of 48 hours of imbibition. In the figure is shown that the proteins run in a pH gradient between 11 and 3.5. The solid line represents the amount of proteins present in the fractions. The dashed line is the Hydroxyproline content in each fraction. The fractions collected were those showing a higher content of proteins and Hydroxyproline and corresponded to the fractions 3-15 of Figure 3.

Figure 9 is a profile of a density gradient centrifugation in CsCl performed with the proteins eluted from fractions 3-15 in the chromatofocusing procedure. The diagonal dashed line is the density gradient, run between 1.7-1.3 (g/ml). The solid line represent the proteins of each fraction and the dashed line the Hydroxyproline of each fraction. The fractions collected were those with a higher content of Hydroxyproline and corresponded to fractions 28-35 of Figure 9.

SDS-polyacrylamide electrophoresis (SDS-PAGE) and native cationic gel electrophoresis showed that the two proteins can not be separated by density gradient centrifugation because they have a similar density of 1.35 and 1.32 (g/ml). The two proteins have a molecular weight of 100 and 60 KD as estimated by SDS-PAGE. Figure 10 is a summary chart where is shown the movilities of these proteins in SDS-PAGE and cationic neutral gel electrophoresis.

Due to the problems of separation of these proteins, we decided to perform a preparative purification of them using SDS-PAGE in Tris Tricine running buffer, instead of Tris Glycine, to avoid Glycine contamination. After

the electrophoresis the proteins were electrotransferred to Polyvinylidene Diflouride membranes, also known as Immobilon membranes. The membranes are plastics and allows the bands of proteins, after transfer to be stained with Coomassie Blue and therefore, to visualize the proteins on the membrane. It is easy then to cut the paper at the band place and to analyze the protein.

The pieces of the membranes containing the proteins were subjected to hydrolysis in 6N HCl for 24 hours at 110° C.The free amino acids were extracted from the membranes with 70% methanol. The amino acids were analyzed in an automatic HPLC analyzer. Table III shows the amino acid composition of the three bands of proteins which cross react with the extensin antibodies. It can be seen that the three proteins have a very similar amino acid composition since all of them have a high content of serine, between 14.6-20.3 % and they are extremely rich in glycine, between 16.5-31.8 %. Hydroxyproline was not detected at all. The high content of glycine is not a glycine contamination from the buffer since the SDS-PAGE was run in a buffer containing Tricine and not Glycine.

Because the densities of these proteins are similar to the densities reported for peroxidases, we run the two purified proteins in native cationic gel electrophoresis. After the run the gel was incubated in a solution of 0,05 g of o-fenilenediamide, 200 ul of 3% H_2O_2 and 50 ml of 0.1 M Sodium Citrate ph 4.5. The two proteins gave a positive reaction for peroxidases.

Future Work Planned On Injury Stress With *Araucaria araucana* Seedlings (Cell Walls Proteins).

We are planning to perform a bidimensional gel electrophoresis of the wall peroxidases to detect how many isoforms of the enzymes are present in each of the bands observed by unidimentional gel electrophoresis.

We expect to perform a sequence analysis of the NH_2 terminal group for the three cell wall proteins purified by preparative SDS-PAGE.

Results Under AID Grant On Injury Stress In *Prosopis chilensis* System, For This Reporting Period.

Quantification of the extensin proteins were performed in cell walls of seedlings of *Prosopis chilensis*, using the Bradford's methods for quantitative analysis of proteins and Drozdz's methods for quantitative analysis of Hydroxyproline. The seedlings were taken at 48 hours after imbibition starts, and separated in two groups: one group was wounded with a razor blade making cross cuts on cotyledons, hypocotyls and radicles; the other group was the control group without wounding. The first group was denominated "D" for dammaged and the control group as "ND" for non dammaged. The first group that was dammaged was further separated into two other groups. One was wounded for a period of 12 hours and the other for

a period of 36 hours. After the treatment, the tissues were homogenized and the cell walls were obtained and purified by the methods described by Cassab et. al., 1985. Proteins were extracted from the cell walls with $CaCl_2$ and quantified by Bradfod's method. The Hydroxyproline content of the proteins was determined by Drozdz's method. The Figure 11 shows the amount of ug of hydroxyproline per gram of fresh weight (gfw) of cotyledons, hypocotyls and radicles. Figure 12 shows the amount of ug of Hydroxyproline per ug of protein in the three tissues. Both figures show that the best response to wounding is given by seedlings dammaged for a period of 12 hours. Therefore, the amount of Hydroxyproline content, either if quantified as Hyp/gfw or as Hyp/ug of proteins increase in all wounded tissues with a greater response given by the hypocotyls. SDS-PAGE of the proteins extracted from cotyledons shows that there is a new protein present in the walls of cotyledons that were wounded for 36 hours. Therefore, this extra protein is absent in the non wounded cotyledons, Figure 13. Western analysis of cationic neutral gel electrophoresis of the native proteins from cell walls of cotyledons, shows that there is one band of protein in the non dammaged tissue and three bands of proteins in the dammaged tissue which cross react with the soybean seed coat extensin antibody, Figure 14.

Future Work Planned On Injury Stress With *Prosopis chilensis* Seedlings (Cell Wall Proteins).

We are planning to purify and further characterize the cell wall proteins of *Prosopis chilensis* seedlings, expressed under injury stress.

References. Cassab, G., Nieto Sotelo, J., Cooper, J., Van Holst, G.J., and Varner, J.E. 1985. A developmentally regulated hydroxyproline-rich glycoprorein from the cell walls of soybean seed coats.Plant Physiol. 77: 532-535.

Enclosures

1.- Fourteen figures and three tables

2.- Summries of the presentations to Sociedad Iberoamericana de Biología Celular, 42 Congreso and to Sociedad de Biología de Chile, 32º Reunión Anual.November 21-25, Viña del Mar, 1989.

3.- Copy of Dr. John Daly's letter.

Figure Legends

Figure 1.- Western blot analysis of the proteins extracted from *Prosopis chilensis* seedlings, using polyclonal antibodies raised against cojugated-ubiquitine. The seedlings were grown from seeds germinated at 35° C and then subjected to heat stress at temperatures of 35°, 40°, 45° and 50° C, for two hours. The proteins were extracted and run in SDS-PAGE and electrotransferred to nitrocellulose membranes. The membranes were incubated with the first antibody (IgG anti conjugated-ubiquitine) and then with a second antibody conjugated to alkaline phosphatase. PM are molecular weight markers, which have been prestained.

Figure 2.- Western blot analysis of the proteins extracted from *Prosopis chilensis* seedlings, using polyclonal antibodies raised against free ubiquitine. The seedlings were grown from seeds germinated at 35° C and then subjected to heat stress at temperatures of 35°, 40°, 45° and 50° C, for two hours. The proteins were extracted and run in SDS-PAGE and electrotransferred to nitrocellulose membranes. The membranes were incubated with the first antibody (IgG anti free ubiquitine) and then with a second antibody conjugated to alkaline phosphatase.

Figure 3.- Temperature fluctuation of the soil in a period of 24 hours, on January 27-28, 1990 at Quilapilun Station in Peldehue. The temperature was measured with a Thermistor under *Prosopis chilensis* shadow at: 10 cm over the soil surface, -10 cm under the soil surface, -2 cm under the soil surface and under sunshine at: -2 cm under the soil surface. Each point is the average of two independent measurements

10 cm over the soil surface : -□-

10 cm under the soil surface: -+-+

2 cm under the soil surface (shadow): -◇-

2 cm under the soil surface (sunshine): -△-

Figure 4.- Environmental temperature fluctuation in a period of 24 hours on January 27-28, 1990 at Quilapilun Station in Peldehue. The temperature was measured with a Thermohydrograph 10 cm over the soil surface under the shadow of Prosopis chilensis. Each point is the average of two independent measurements.

Figure 5.- Radiation fluctuation in a period of 24 hours on January 27-28, 1990 at Quilapilun station in Peldehue. The radiation was measured at sunshine (-+-+-) and under the shadow of Prosopis chilensis (-□-□-), with a Pyr Heliograph. Each point is the average of two independent measurements.

Figure 6.- Environmental relative humidity fluctuation in a period of 24 hours on January 27-28,1990 at Quilapilun Station in Peldehue. The humidity was measured under the shadow of Prosopis chilensis with a Thermohydrograph. Each point is the average of two independent measurements.

Figure 7.- Environmental evaporation fluctuation in a period of 24 hours on January 27-28, 1990 at Quilapilun Station in Peldehue. The evaporation was measured with an Evaporimeter of Piche. Each point is the average of two independent measurements, done under the shadow of Prosopis chilensis.

Figure 8.- Elution profile of the chromatofocusing of the cell wall proteins extracted from Araucaria araucana seedlings, performed with the PBE 118 gel from Pharmacia Fine Chemicals Co. The dashed diagonal line (-.-.-) is the pH gradient of the column. The solid line (——) represents the amount of proteins of the fractions and the dashed line (- - -) represents the amount of Hydroxyproline of the fractions. The fractions with a higher content in proteins and Hydroxyproline are those eluted from # 8-15.

Figure 9.- Density gradient centrifugation in CsCl of the cell wall proteins from A. araucana seedlings which were eluted from fractions 8-15 in the chromatofocusing porcedure. The dashed diagonal line (-.-.-) is the density gradient. The solid line (——) is the amount of proteins of the fractions . The dashed line (- - -) is the amount of Hydroxyproline of the fractions. The higher content of Hydroxyproline is coming in fractions 28-35.

Figure 10.-Summary figure of the movilities of the cell wall proteins of Araucaria araucana seedling in SDS-PAGE and cationic neutral gel electrophoresis of the native proteins. The three proteins which cross react with the extensin antibodies are denominated as B1, B2 and B3. The molecular weights of these proteins were estimated by SDS-PAGE running molecular weight markers in the same gel.

Figure 11.-It shows the Hydroxyproline content in micrograms of Hydroxyproline / gram of fresh tissue,(microgramos Hyp/gpf) in three tissues of Prosopis chilensis seedlings of 48 hours of imbibition. The seedlings were divided in two groups before the analysis: one was dammaged with a razor blade 12 hours before and the other was dammaged 36 hours before. The seedlings were dissected in cotyledons, hypocotyls and radicles, the cell walls prepared, the proteins extracted and the hydroxyproline quantified by Drozdz' method in the protein extract."D"means dammaged ;"ND"means non dammaged. A, hypocotyls. B, cotyledons. C, radicle.

Figure 12.-It shows the Hydroxyproline content in micrograms of Hydroxyproline / micrograms of protein (microgrs. Hyp/microgrs.Prot.) in three tissues of Prosopis chilensis seedlings of 48 hours of imbibition. The seedlings were divided in two groups before the analysis: one was dammaged with a razor blade 12 hours before and the other was dammaged 36 hours before. The seedlings were dissected in cotyledons, hypocotyls and radicles, the cell walls prepared, the proteins extracted and quantified by Bradford's method, and the Hydroxyproline quantified by Drozdz's method in the protein extract. "D" means dammaged; "ND" means non dammaged.A, hypocotyls. B,cotyledons. C,radicles.

Figure 13.-SDS-PAGE of the cell wall proteins of seedling cotyledons of Prosopis chilensis imbibed for 48 hours. After electrophoresis the gel was stained with Coomasie Blue stain.

Channels:

a.- Molecular weight markers

b.- Cotyledons wounded (dammaged) 12 hours before the analysis.

c.- Cotyledons non dammaged, control , mantained for 12 hours in identical conditions as b, except for dammage.

d.- Cotyledons dammaged 36 hours before the analysis.

e.- Cotyledons non dammaged, control, mantained for 36 hours in identical conditions as d, except for dammage.

f and g are repeats of d and e.

Arrows indicate the new bands of proteins.

Figure 14.-Western analysis of the cell wall proteins of seedling cotyledons of Prosopis chilensis imbibed for for 48 hours. The cotyledons were dammaged for 36 hours before the analysis. The analysis was performed also with cell wall proteins of non dammged tissue (control). For the analysis, the proteins were run in cationic neutral gel electrophoresis and electrotransferred to nitrocellulose membranes to be incubated with a first antibody raised against extensin from soybean seed coats. After the first antibody,the membrane was incubated with goat anti IgG alkaline phosphatase conjugated. "D" means dammaged; "ND" means non dammaged; Ext. is a positive control run with 1 ug of extensin from soybean seed coats. Arrows point the bands of proteins showing positive reaction with the antibody.

Table I. Summary table of the proteins extracted from seedlings of *Prosopis chilensis* which cross react with polyclonal antibodies raised against conjugated ubiquitine. The seedlings were subjected to heat shock at different temperatures for two hours, prior to the protein extraction. The number of crosses gives the intensity of the cross reactivity. MW, means Molecular Weight in KD.

MW	Temperatures ^{o} C			
KD	35°	40°	45°	50°
190	-	-	++	++
110	+	+	+	++
95	++	++	++	++
69	++	++	++	+++
59	++	++	++	+++
52	+	+	+	++
48	-	-	-	+
46	-	-	-	+
41	+	+	+	-
28	-	-	+	++
19	-	-	+	++
9	+	+	+	+

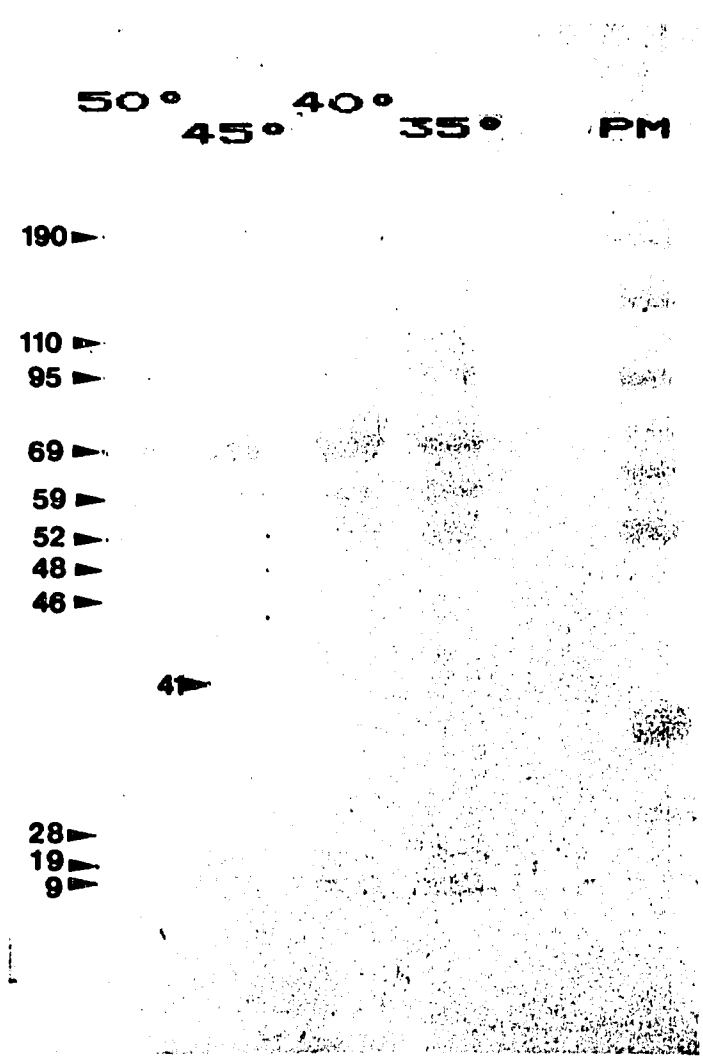
Table II. Summary table of the proteins extracted from seedlings of *Prosopis chilensis* which cross react with polyclonal antibodies raised against free ubiquitine. The seedlings were subjected to heat shock at different temperatures for two hours, proir to the protein extraction. The number of crosses gives the intensity of the cross reactivity. MW, means Molecular Weight in KD.

MW	Temperatures ^{o} C			
KD	35°	40°	45°	50°
71	-	-	+	++
59	-	-	+	++
52	-	-	++	++
50	-	-	++	++
9	-	-	+++	+++

Table III. Amino Acid composition of the three proteins purified by preparative SDS-PAGE and Western analysis on Inmobilon membranes. After purification, the proteins were subjected to hydrolysis with 6N HCl for 24 hours and analyzed for amino acid composition by High Liquid Preasure Chromatography.

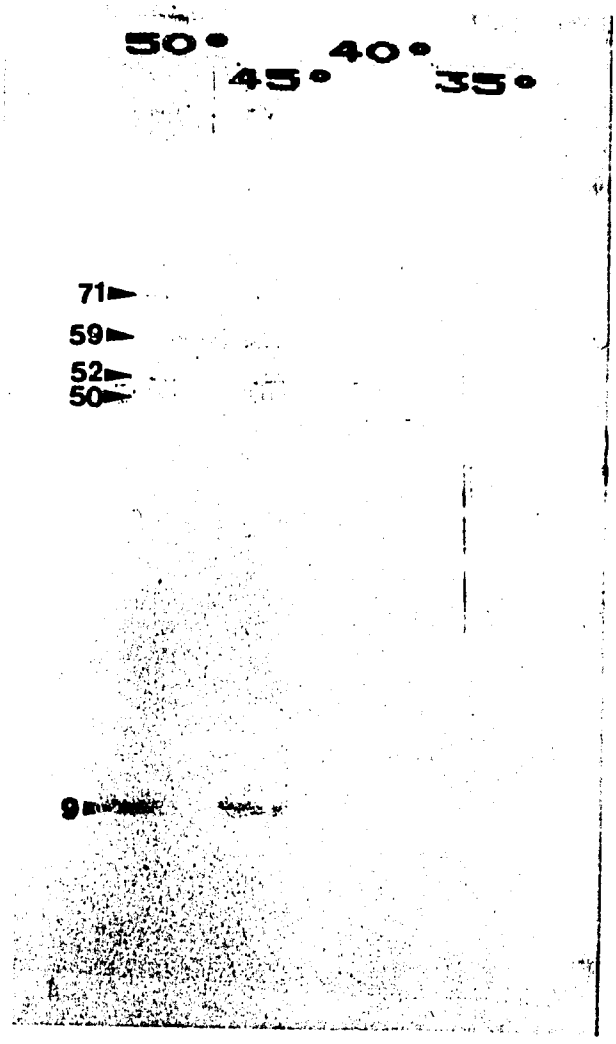
Amino Acid	B1 Band 1		B2 Band 2		B3 Band 3	
	p molar	p mole %	p molar	p mole %	p molar	p mole %
Hyp	0.00	0.00	0.00	0.00	0.00	0.00
Asx	119.00	5.63	102.23	4.93	175.89	5.18
Thr	106.60	5.04	100.75	4.86	156.51	4.61
Ser	361.59	17.12	419.93	20.26	496.09	14.60
Glx	199.43	9.44	220.44	10.63	291.64	8.58
Pro	94.48	4.47	38.46	1.86	154.54	4.55
Gly	559.60	26.50	594.59	28.68	1079.73	31.78
Ala	189.44	8.97	173.87	8.39	255.41	7.52
Cys	0.00	0.00	0.00	0.00	0.00	0.00
Val	30.03	1.42	31.71	1.53	77.50	2.28
Met	0.00	0.00	0.00	0.00	0.00	0.00
Ile	52.11	2.46	49.87	2.41	95.51	2.81
Leu	106.41	5.03	85.80	4.14	174.32	5.13
Tyr	12.85	0.60	0.00	0.00	23.67	0.70
Phe	25.14	1.20	17.57	0.85	42.04	1.24
His	69.77	3.30	68.56	3.31	97.73	2.88
Lys	96.05	4.50	95.17	4.59	153.65	4.52
Arg	89.24	4.22	74.19	3.58	122.89	3.62

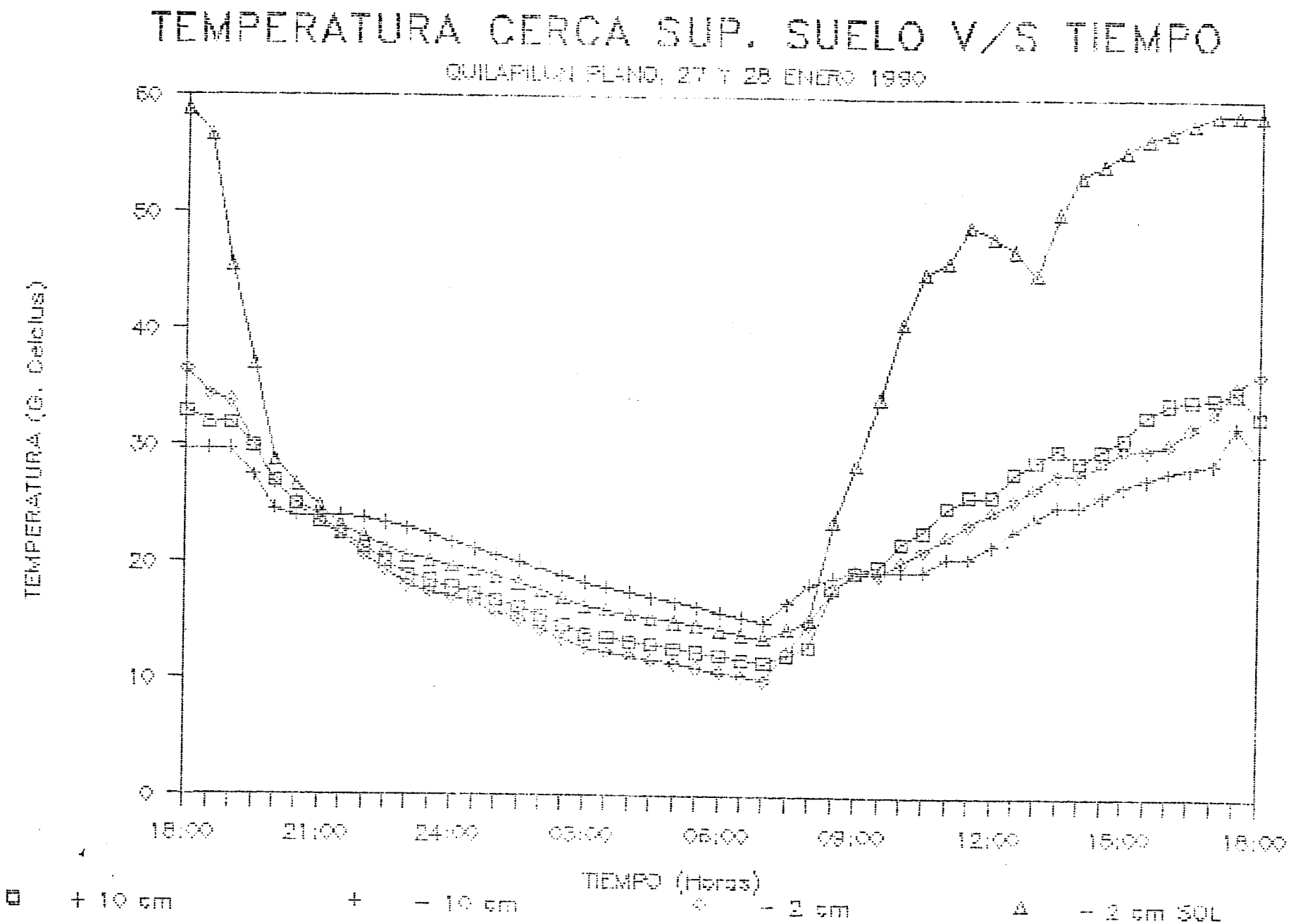
Figure 1



24

Figure 2




TEMPERATURA CERCA SUP. SUELO V/S TIEMPO
QUILAPILUN PLANO, 27 Y 28 ENERO 1990
TEMPERATURA (G. Celcius)
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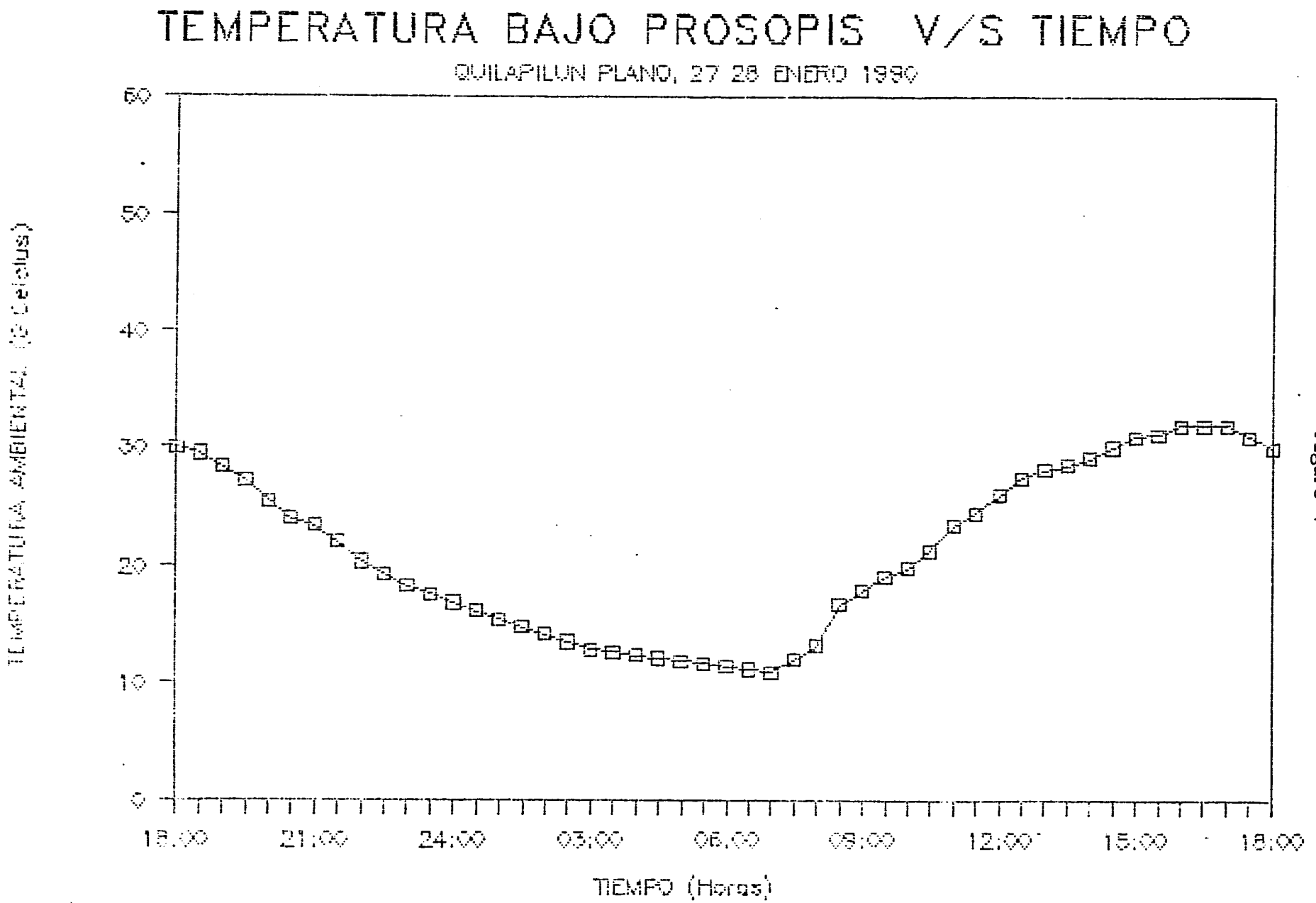
10
20
30
40
50
60
18:00
21:00
24:00
03:00
06:00
09:00
12:00
15:00
18:00
TIEMPO (Horas)
+ 10 cm
- 10 cm
- 2 cm
- 2 cm SOL

Figure 3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QUILAPILUN PLANO, 27 28 ENERO 1990
TEMPERATURA AMBIENTAL (Grados Celsius)
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50
40
30
20
10
0
18:00
21:00
24:00
03:00
06:00
09:00
12:00
15:00
18:00
TIEMPO (Horas)

Figure 4

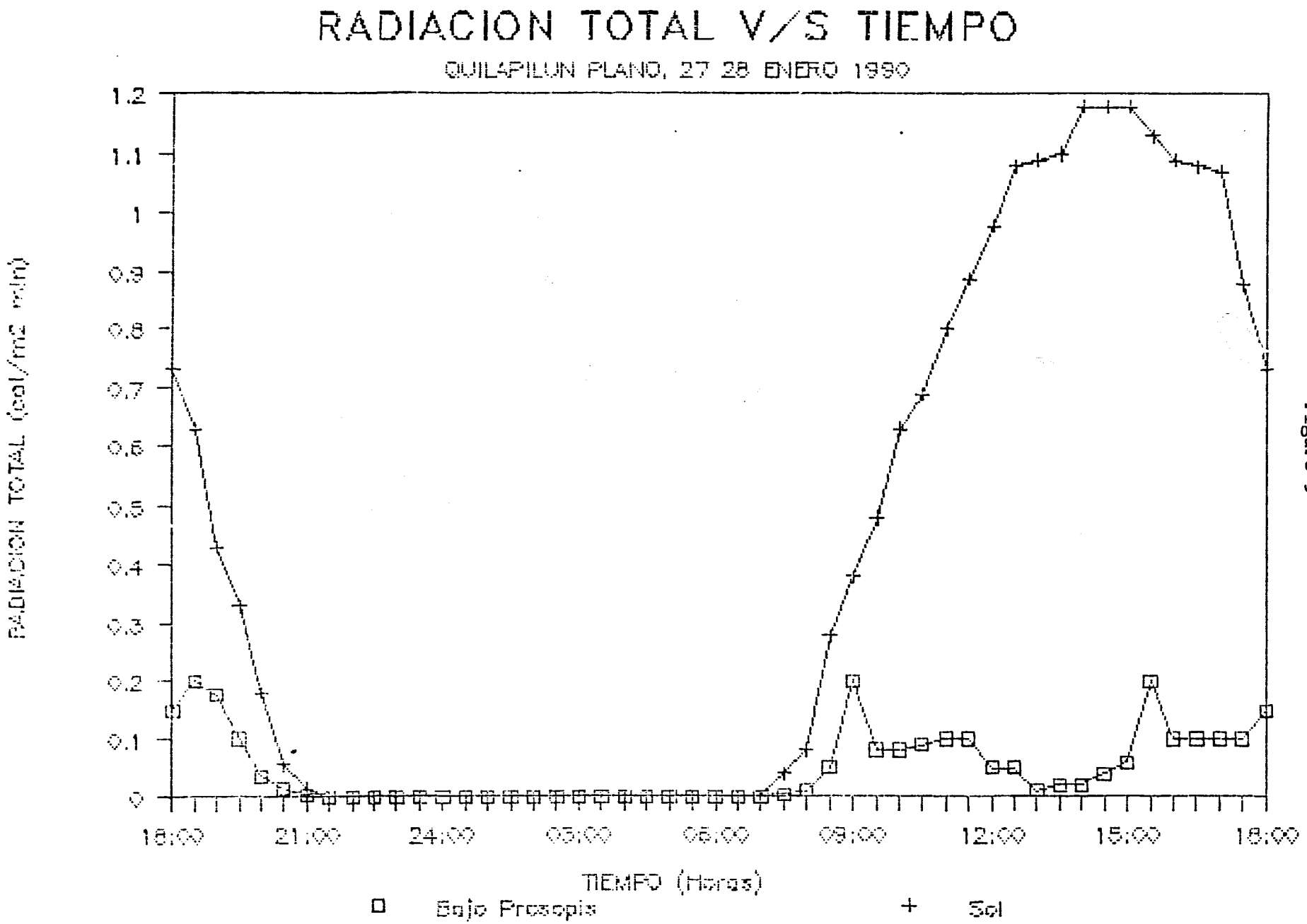

RADIACION TOTAL V/S TIEMPO
QUILAPILUN PLANO, 27 28 ENERO 1990
RADIACION TOTAL (cal/m2 min)
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
18:00
21:00
24:00
03:00
06:00
09:00
12:00
15:00
18:00
TIEMPO (Horas)
Bajo Prosopis
Sol

Figure 5

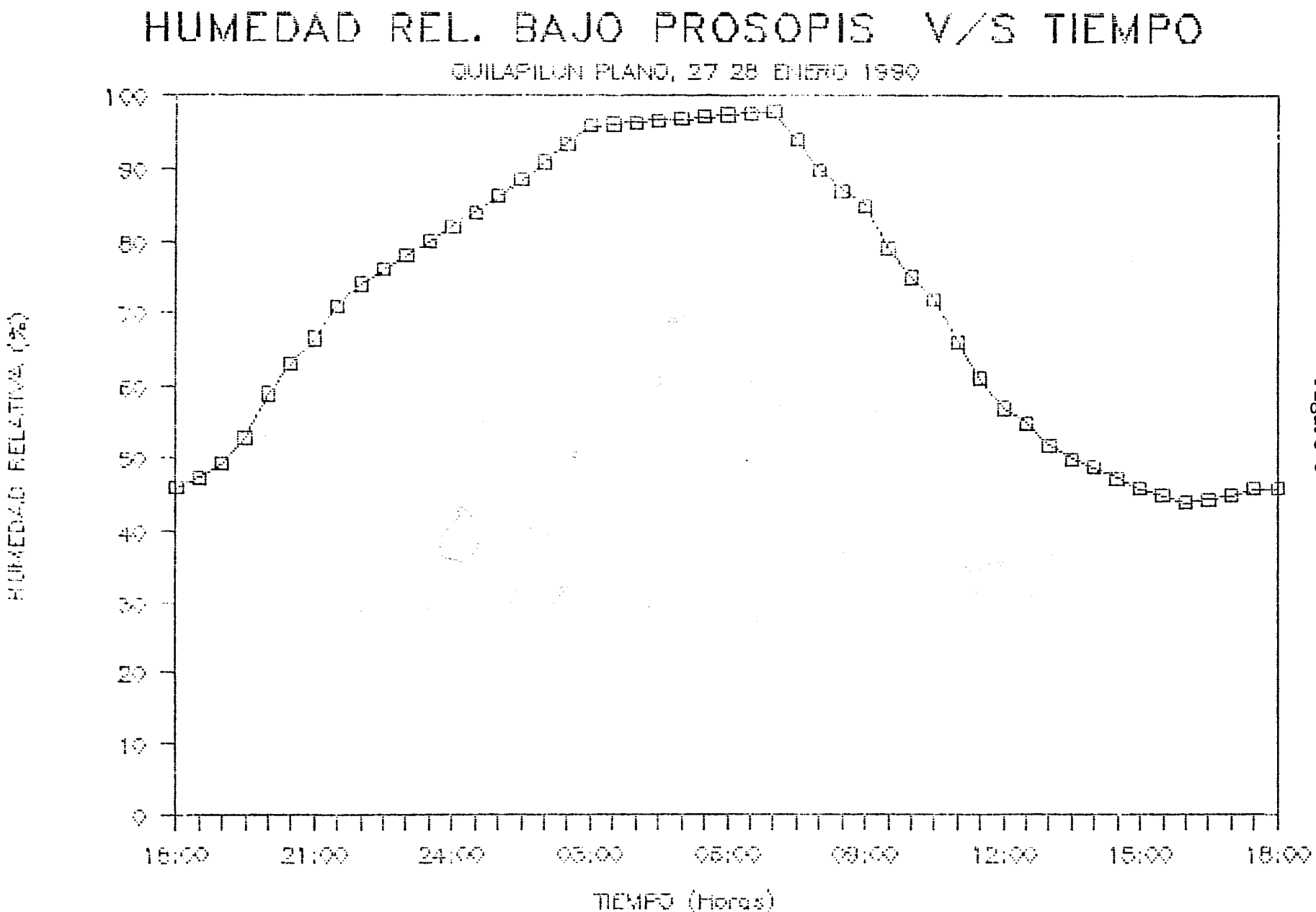

HUMEDAD REL. BAJO PROSOPIS V/S TIEMPO
QUILAPILUN PLANO, 27 28 ENERO 1990
HUMEDAD RELATIVA (%)
100
90
80
70
60
50
40
30
20
10
0
18:00
21:00
24:00
03:00
06:00
09:00
12:00
15:00
18:00
TIEMPO (Horas)

Figure 6

29

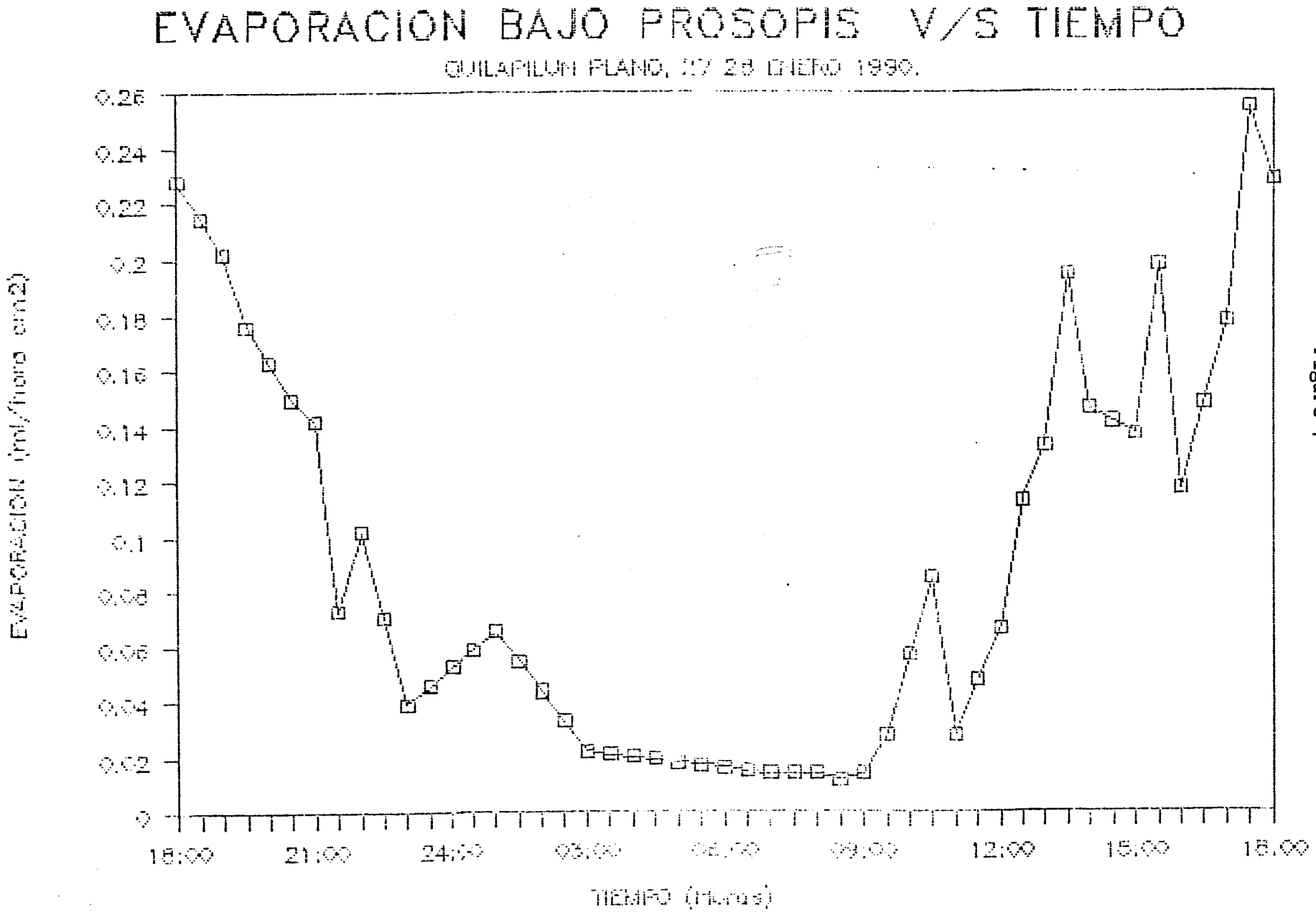

EVAPORACION BAJO PROSOPIS V/S TIEMPO
QUILAPILUN PLANO, 27 28 ENERO 1990.
EVAPORACION (ml/hora cm2)
0.26
0.24
0.22
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
18:00
21:00
24:00
03:00
06:00
09:00
12:00
15:00
18:00
TIEMPO (Horas)

Figure 7

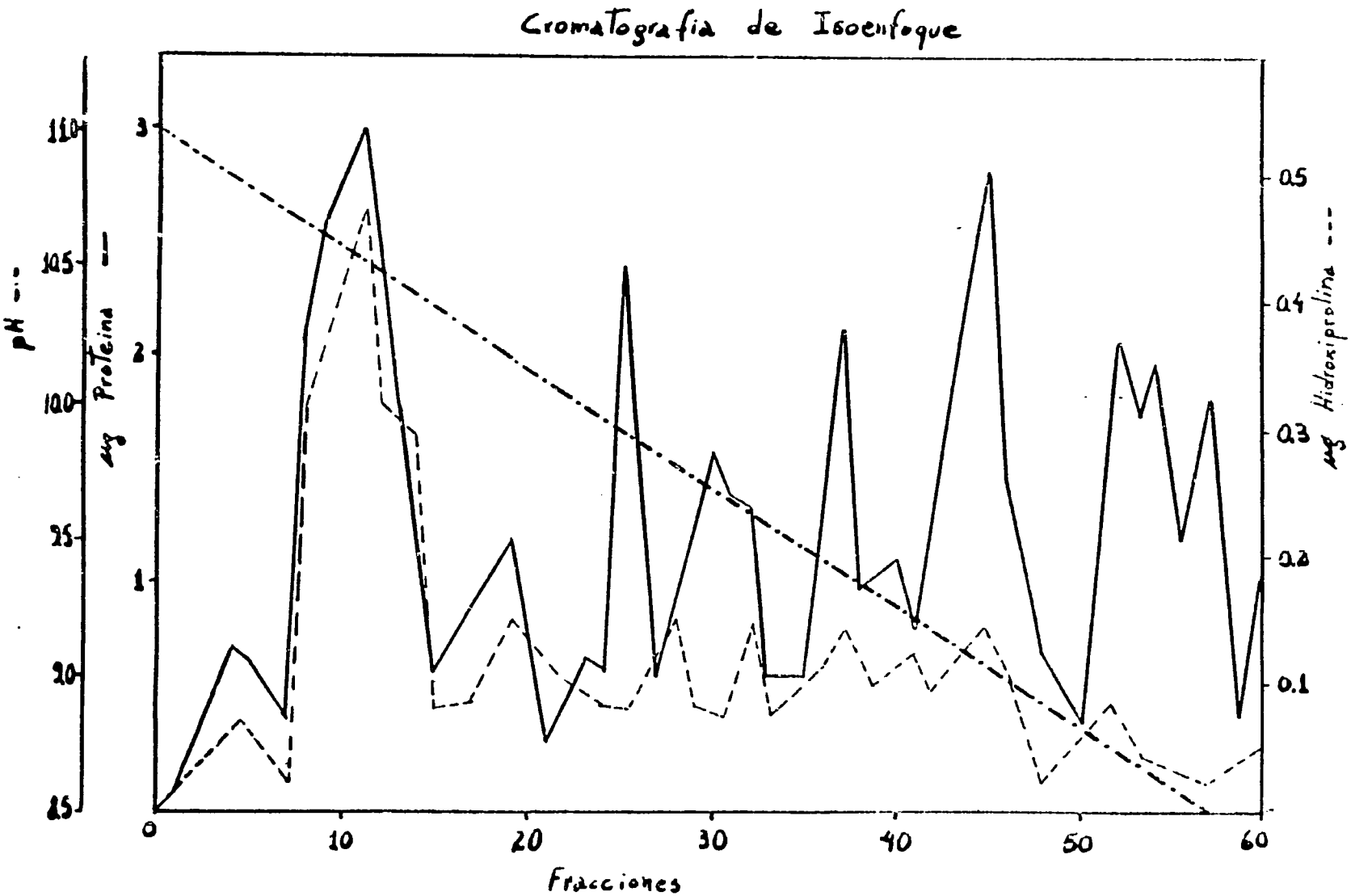

Cromatografia de Isoenfoque
pH ---
mg Proteina —
mg Hidroxiprolina ---
11.0
10.5
10.0
9.5
9.0
8.5
3
2
1
0.5
0.4
0.3
0.2
0.1
0
10
20
30
40
50
60
Fracciones

Figure 3

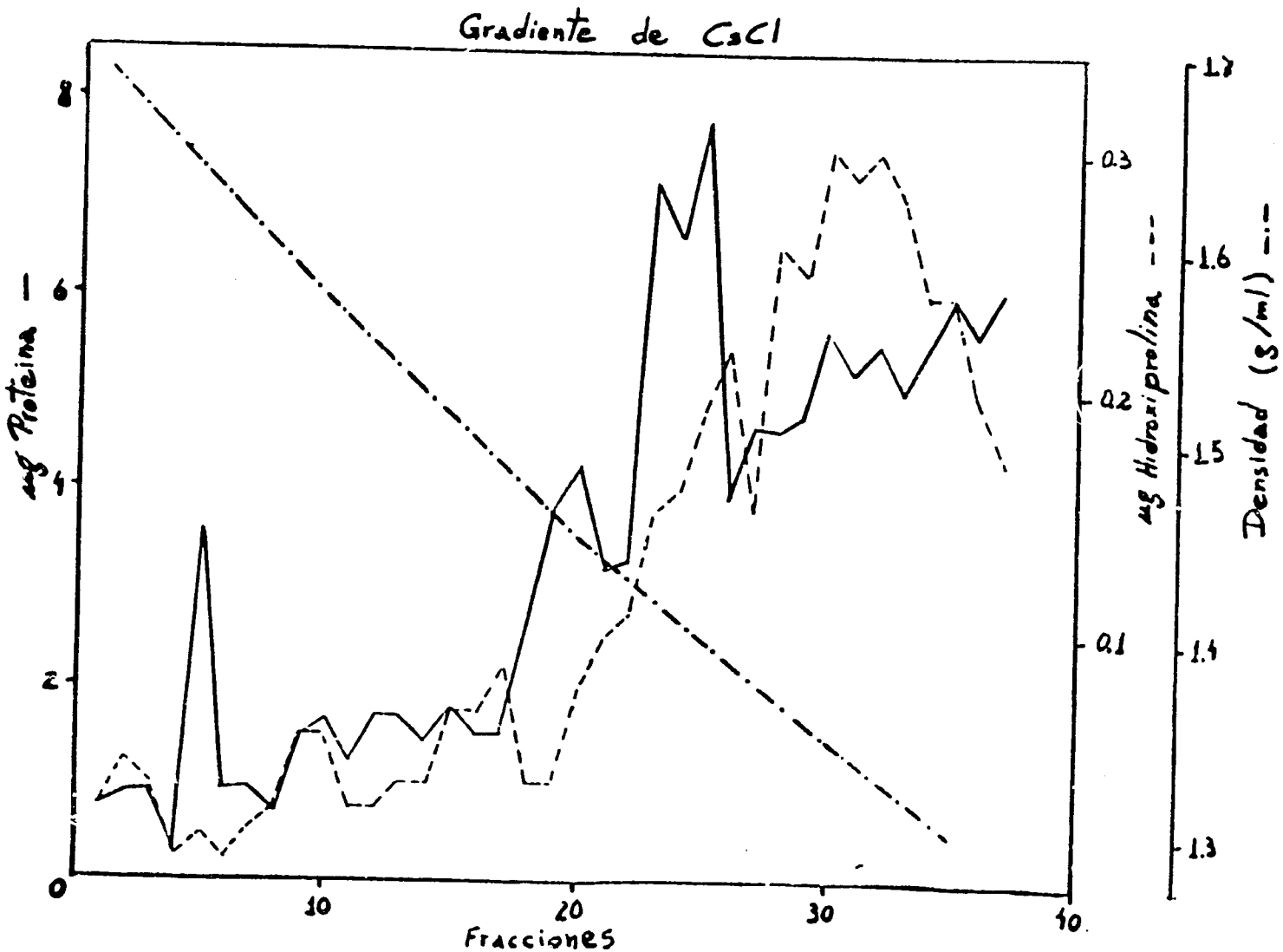
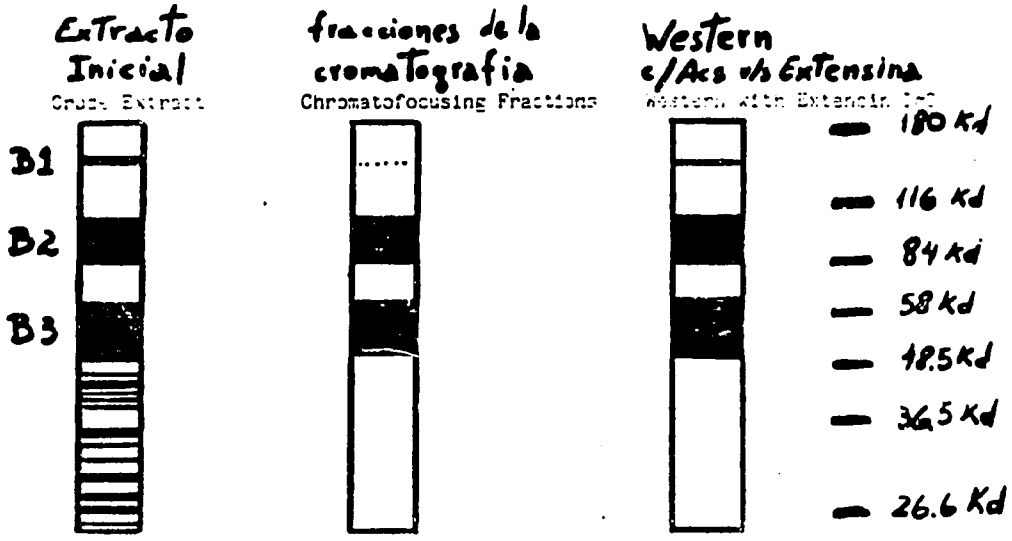

Gradiente de CsCl
µg Proteina
µg Hidroxiprolina
Densidad (g/ml)
Fracciones
0
2
4
6
8
10
20
30
40
0.1
0.2
0.3
1.3
1.4
1.5
1.6
1.7

Figure 9

Figure 10

SDS-PAGE

PAGE-SDS.



Pesos Moleculares

Molecular Weights

Banda 1 : 160.000 - 150.000 ⇒ 155.000 d

Banda 2 : 110.000 - 90.000 ⇒ 100.000 d

Banda 3 : 65.000 - 55.000 ⇒ 60.000 d

Cationic Neutral Gel Electrophoresis

Electroforesis Cationica

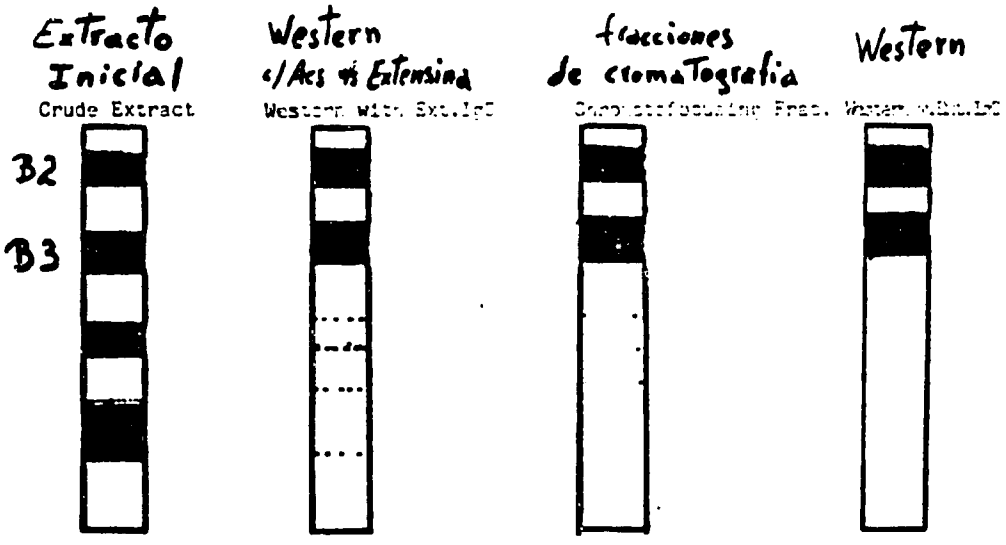


Figure 11

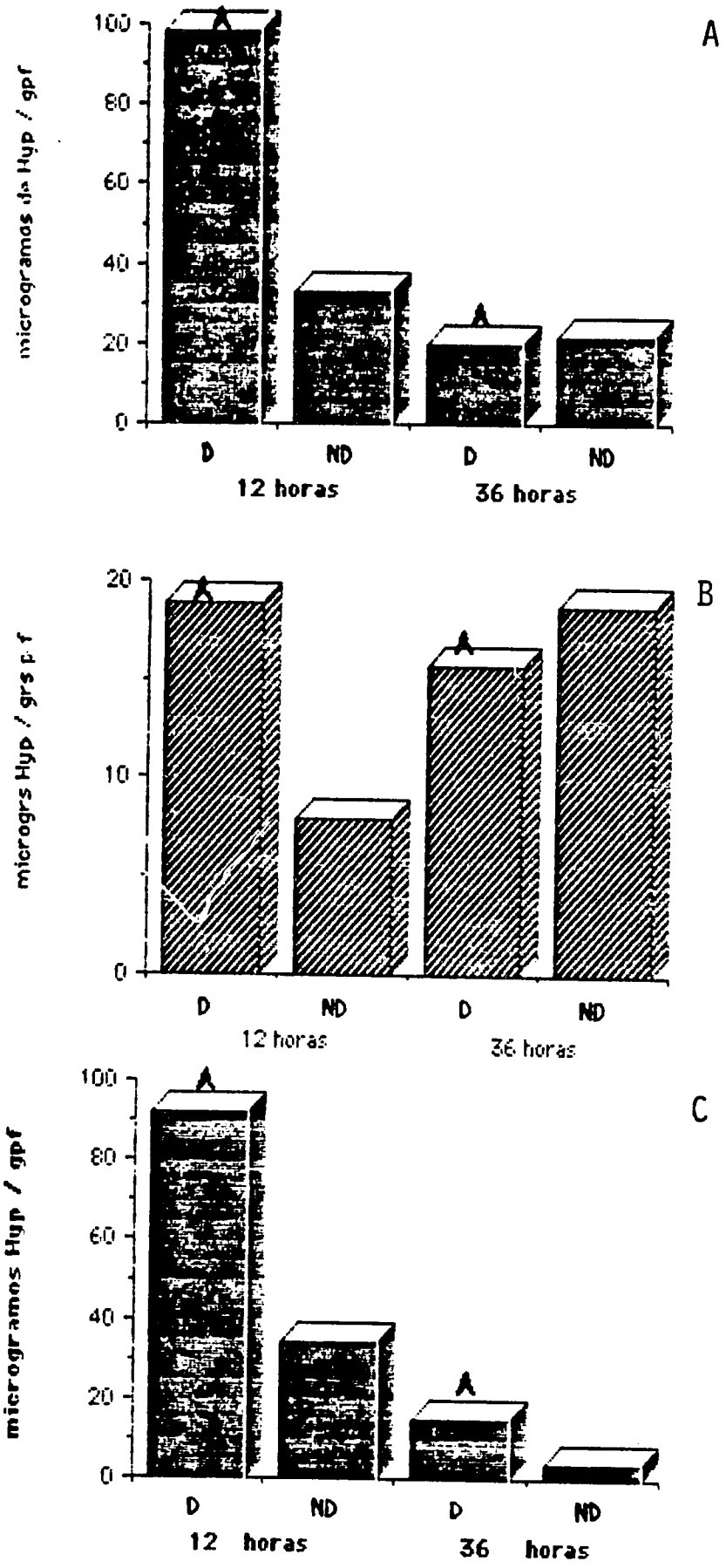
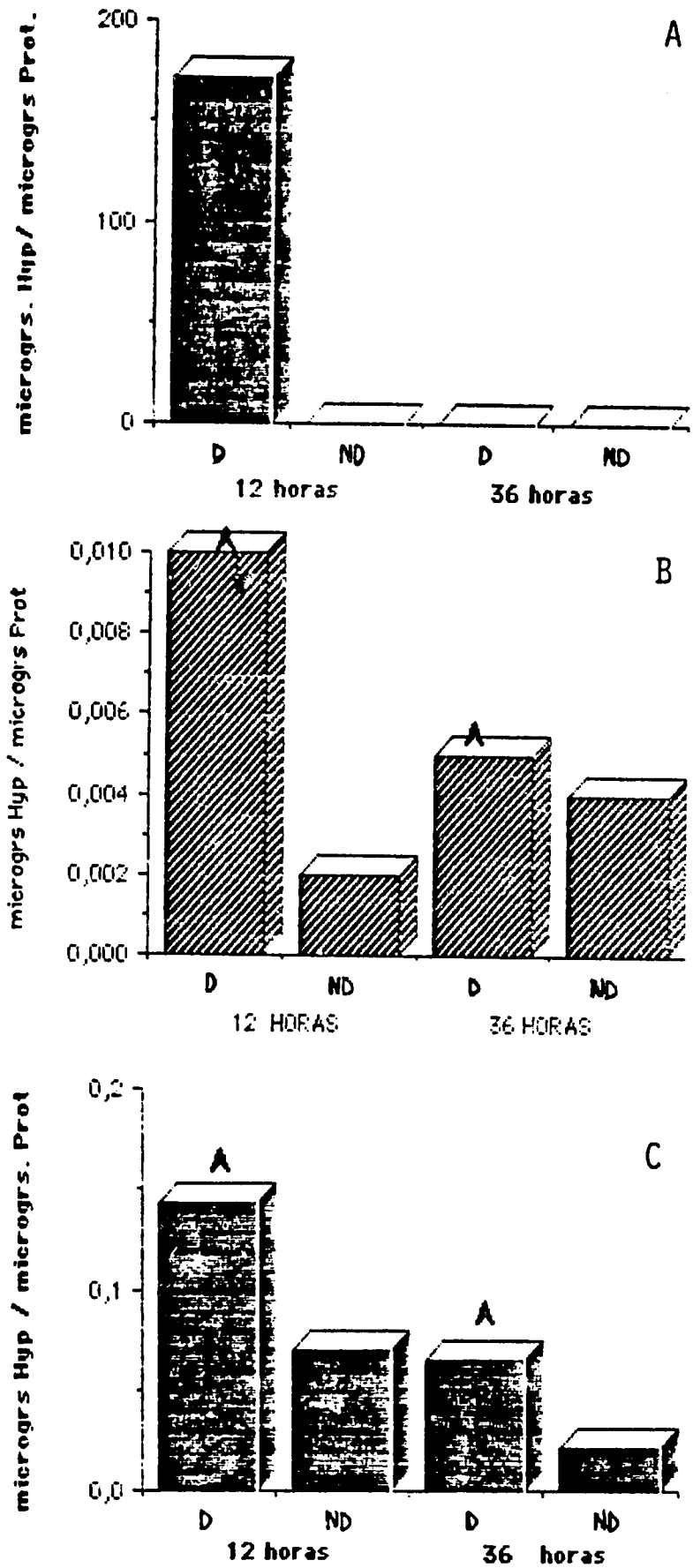

A
100
80
60
40
20
0
D
ND
D
ND
12 horas
36 horas
B
20
10
0
D
ND
D
ND
12 horas
36 horas
C
100
80
60
40
20
0
D
ND
D
ND
12 horas
36 horas

Figure 12


A
200
100
0
microgrs. Hyp/ microgrs Prot.
D
ND
D
ND
12 horas
36 horas
B
0,010
0,008
0,006
0,004
0,002
0,000
microgrs Hyp / microgrs Prot
A
A
D
ND
D
ND
12 HORAS
36 HORAS
C
0,2
0,1
0,0
microgrs Hyp / microgrs. Prot
A
A
D
ND
D
ND
12 horas
36 horas

35

Figure 13

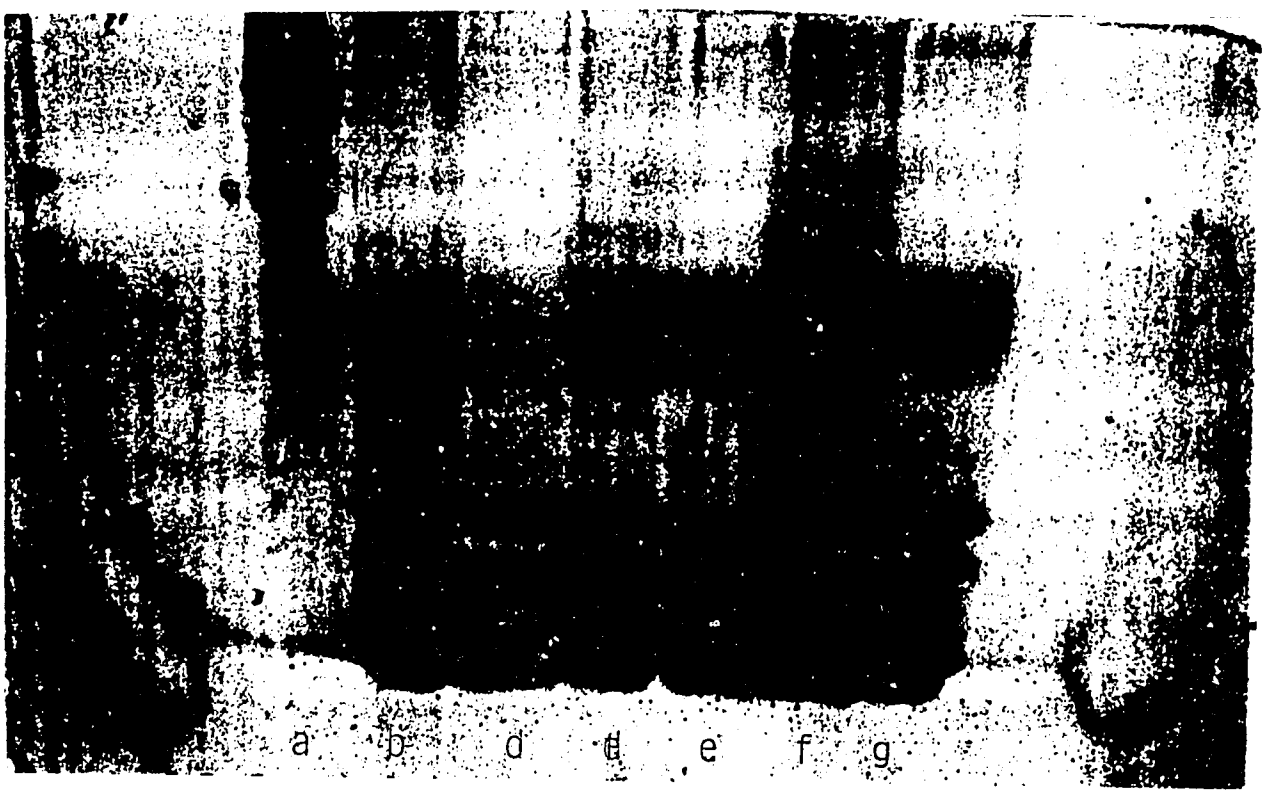
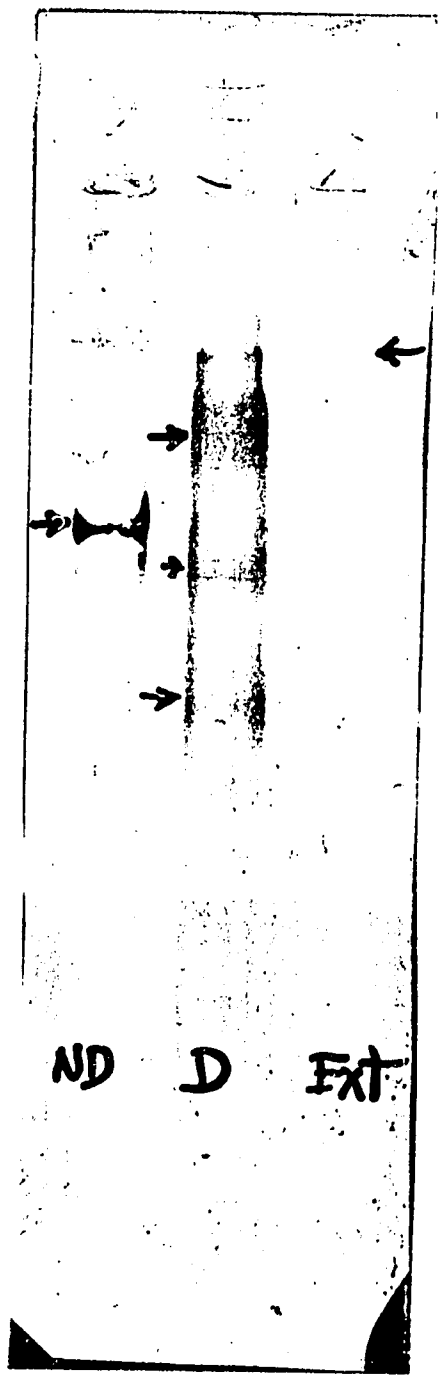


Figure 14



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VOL. 22 NOVIEMBRE 1989 Nº 3

SOCIEDAD IBEROAMERICANA DE BIOLOGIA CELULAR
4º. Congreso

SOCIEDAD DE BIOLOGIA DE CHILE
32ª. Reunión Anual

SOCIEDAD DE GENETICA DE CHILE
22ª. Reunión Anual

SOCIEDAD DE BIOQUIMICA DE CHILE
13ª. Reunión Anual

SOCIEDAD DE BIOLOGIA CELULAR DE CHILE
3ª. Reunión Anual

SOCIEDAD CHILENA DE REPRODUCCION Y DESARROLLO
2ª. Reunión Anual

Viña del Mar
21 al 25 de noviembre de 1989

Resúmenes de
Conferencias, Simposios y Comunicaciones

Sociedad de Biología de Chile

SIMPOSIO DIFERENCIACION CELULAR EN PLANTAS.

Coordinadora: *Gloria Montenegro*

Simposio: DIFERENCIACION CELULAR EN PLANTAS (Differentiation in plant cells). Montenegro, G. Coordinador. Laboratorio de Botánica, Departamento de Ecología, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile.

La importancia del conocimiento de la biología celular y molecular en plantas es hoy día indiscutible debido a la creciente neceridad de seleccionar plantas resistentes a patógenos y a herbicidas; propagar plantas resistentes a la sequía con fines de reforestación en zonas áridas y otras que generen recursos vegetales con potencial económico ya sea industrial. medicinal o alimenticio.

Durante el desarrollo del simposio se analizarán los últimos avances en aspectos de la estructura molecular de la pared celular y el rol en el crecimiento y diferenciación de la célula; cambios ultraestructurales de células sometidas a la falta de gravedad (experimentos recientes realizados a bordo de la Nave Espacial Columbia) y respuestas morfogénicas de plantas cultivadas in vitro correlacionadas con la manipulación de factores exógenos y de la totipotencialidad de la célula vegetal.

Proyecto FONDECYT 199/88

MIMICKING IN ULTRASTRUCTURAL EFFECTS OF MICROGRAVITY IN PLANT CELLS. Randy Moore and William Bowie, Department of Biological Sciences, Wright State University, Dayton, OH, USA and Krug International, Lyndon B. Johnson Space Center (MASA), Houston, TX, USA.

The objective of this research was to determine how effectively the actions of a classical clinostat and the Slow Turning Lateral Vessel (STLV) mimic ultrastructural effects of microgravity in plant cells. We accomplished the objective by comparing the ultrastructure of cells grown on clinostats and in the STLV with that of cells grown at 1g and in microgravity aboard the Space Shuttle Columbia. Cells of calli and intact seedling of corn and mustard spinach grown in microgravity and in the STLV contained significantly more lipid bodies, significantly less starch and fewer dictyosomes than comparable cells grown at 1g. Cells of seedlings grown on clinostats had significantly different ultrastructure than those grown in microgravity or in the STLV, indicating that clinostats do not mimic microgravity at the cellular level. Calli and seedlings grown in the STLV had ultrastructure similar to those grown in microgravity, indicating that the STLV effectively mimics microgravity at the cellular level.

REGULACION DE RESPUESTAS MORFOGENICAS DE PLANTAS IN VITRO. (In vitro regulation of Plant Morphogenesis). Jordán, M. Laboratorio de Botánica, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, Casilla 114-D, Santiago.

Mecanismos conducentes a la regeneración de plantas in vitro a partir de células y tejidos, implican revertir inicialmente programas ontogénicos pre-determinados gatillándose posteriormente diversos procesos de diferenciación, organogénesis y embriogénesis. En el caso de una reembrionalización de células parenquimáticas, de microsporas, o de tejidos, las respuestas morfogénicas en diferentes especies pueden ser gatilladas por la concentración y niveles entre varias fitohormonas utilizadas (androgénesis, embriogénesis somática, organogénesis caulinar y radicular), la especificidad de citocininas (variación somaclonal), el tiempo de inducción por auxinas (rizogénesis) y la densidad celular junto con condicionantes en medios de cultivo para suspensiones celulares y fusión de protoplastos. Las respuestas morfogénicas por factores endógenos dependen mayormente del tipo y condición del explante, de la edad del tejido u órgano, la evolución del metabolismo secundario, especialmente el grado de oxidación de compuestos fenólicos en plantas leñosas y, la situación de estabilidad metabólica y cromosomal en cultivos de larga permanencia in vitro. Se ilustran diferentes ejemplos de plantas estudiadas en nuestro laboratorio que responden específicamente ante las condiciones de cultivo indicadas.

Proyecto FONDECYT 0669/89, DIUC 85/86 y PNUD/ONUDI-RLA/83/003/PRB.

LAS PROTEINAS DE LA PARED CELULAR Y SU POSIBLE ROL EN EL CRECIMIENTO Y DIFERENCIACION DE LA CELULA VEGETAL. (The plant cell wall proteins and their possible role on the growth and differentiation of the plant cell).

Cardemil, L. Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

La pared celular de las células vegetales puede considerarse como una matriz extracelular constituida de polisacáridos neutros, ácidos, polímeros de resistencia, y de proteínas, entre las cuales la mas conocida es la extensina. La extensina es una proteína básica rica en hidroxiprolina y glicosilada en los residuos de hidroxiprolina y serina con arabinosa y galactosa respectivamente. La pared celular regula el crecimiento en longitud de la célula vegetal y le da su forma durante la diferenciación celular. Además protege a la celula vegetal.

De todos los componentes de la pared, es la extensina la que mas aumenta cuando la célula sufre heridas o algún daño mecánico. El aumento de extensina en la pared celular se correlaciona con una detención del crecimiento celular.

Las semillas y plántulas de Araucaria araucana, responden al estrés de heridas con un aumento de hasta 3 veces el nivel basal de proteínas de pared, en el caso del embrión y de 20 veces en el caso del megagametofito. Inmunoimpresión de los tejidos de semillas y plántulas, en papel de nitrocelulosa, usando anticuerpos policlonales obtenidos contra extensina de zanahoria o contra la extensina de cubiertas de semillas de soja, revela que la expresión de de la proteína depende del tiempo de desarrollo y del tejido dañado.

Las proteínas de pared celular de semillas y plántulas de A. araucana, se han caracterizado por electroforesis en geles SDS poliacrilamida y catiónica y su composición amino acídica se ha determinado. (FONDECYT 0160;#1D#OPE55A2C-SS-8073-00).

PEPTIDOS OPIOIDES Y ACTIVIDAD CITOLITICA NK. (Opioid peptides and cytolytic NK activity). Javier Puente Piccardo. Departamento de Bioquímica y Biología Molecular. Facultad de Ciencias Químicas y Farmacéuticas. Universidad de Chile.

La interrelación entre el sistema inmune y el sistema nervioso se basa en la directa inervación del parenquima de tejidos tales como timo, bazo, ganglios linfáticos, médula ósea y otros tejidos linfoides. Además la presencia en las células inmuno competentes de receptores para diversas hormonas y neurotransmisores, como también la propiedad de éstas células de producir múltiples señales, en muchos casos compartidas con el sistema neuroendocrino, han establecido claramente una vasta inter-comunicación que ha permitido avanzar en el entendimiento de diversas situaciones fisiológicas y patológicas.

Esta comunicación bidireccional ha sido estudiada a muchos niveles y, en el presente caso, nuestro interés se ha centrado en la actividad citolítica mediada por células NK y su modulación por péptidos opioides.

Hemos estudiado la regulación ejercida sobre esta actividad por los péptidos, metionina-encefalina, leucina-encefalina y β-endorfina, tratando de correlacionar la estimulación observada con la existencia y propiedad de los receptores para péptidos opioides tanto en muestras normales como patológicas. Es un hecho establecido que los péptidos opioides influyen en diversas funciones del sistema inmune, como la síntesis de anticuerpos, proliferación de linfocitos y citolisis NK. Sin embargo, las características de los receptores involucrados y el mecanismo general de acción de estos péptidos en el sistema inmune no han sido hasta ahora aclarados.

Proyecto Fondecyt 88-0874

INTERACCIONES ENTRE SISTEMA INMUNE Y NEUROENDOCRINO (Inmune - Neuroendocrine Network). Sepúlveda Cecilia
Departamento de Medicina Hospital Clínico Universidad de Chile.

Aunque el sistema inmune posee sus propios mecanismos de regulación, actualmente está bien establecida la inmunorregulación neuroendocrina. Esta tiene su base en una compleja red de interacciones bidireccional entre sistema inmune, sistema neuroendocrino, y estructuras autonómicas.

Las linfoquinas, monoquinas y Ac producidos durante la respuesta inmune afectan mecanismos neuroendocrinos. El sistema neuroendocrino puede o no responder emitiendo señales reguladoras que limiten su expansión excesiva, previniendo enfermedades autoinmunes y linfoproliferativas.

La sobreestimulación del sistema neuroendocrinopuede ser uno de los factores mediadores de la inmunosupresión que se observa en la fase aguda de enfermedades infecciosas, shock séptico, trauma, quemaduras, cáncer, y otras condiciones patológicas.

La inmunomodulación pueda contribuir a restablecer la función inmune en estos individuos

Proyecto Fondecyt 88-0874

SIMPOSIO GENOMA Y EVENTOS FISIOLOGICOS EN CELULAS VEGETALES

Coordinador: *Jorge Sans*

SIMPOSIO
GENOMA Y EVENTOS FISIOLOGICOS EN CELULAS VEGETALES (Genome and physiological events in vegetal cells).
Sans, J. (Coordinador).

Durante la vida celular ocurren una infinidad de eventos fisiológicos, que de una u otra manera, dependen de la expresión de genes en momentos, muy particulares.

Se han utilizado diversas estrategias experimentales en células vegetales mantenidas en un tejido in toto, para tratar de vincular eventos celulares a sectores del genoma. Una de estas estrategias ha sido la producción de células multinucleadas con núcleos homo y heteroploides para asociar eventos propios del ciclo celular con la presencia, en estos núcleos, de determinados cromosomas. Otra metodología utilizada con este mismo fin, ha sido el incorporar al DNA nucleótidos que bajo determinadas condiciones pueden alterar la expresión del genoma. De esta manera, la bromosustitución del genoma e irradiación posterior con luz de 313 nm., por una parte y la incorporación al DNA de un análogo de la citidina (5-azacitidina) que provocan hipometilación del DNA, por otra parte, han permitido asociar algunos eventos a sectores del genoma que replican en momentos particulares del período S.

Por último, mediante experimentos en que se utiliza el estrés térmico, se ha podido detectar sectores del genoma que confieren propiedades de termo tolerancia en semillas de árboles autóctonos chilenos.

En este Simposio se analizan estas estrategias y los logros con ellas alcanzados como así las perspectivas futuras de nuevos abordajes metodológicos en este tipo de estudios.

LA INDUCCION DE CELULAS MULTINUCLEADAS Y SU EMPLEO EN EL ESTUDIO DE EVENTOS DEL CICLO PROLIFERATIVO (The induction of multinucleated cells and its usefulness in the study of events in the proliferative cycle).
Giménez-Martín, G. Centro de Investigaciones Biológicas, CSIC, Velázquez 144, 28006-Madrid, España.

La inducción experimental de células homocariótizas poliploides 8n en Allium cepa L. puede llevarse a cabo por inhibición de dos citocinesis sucesivas. Así se pueden formar células binucleadas (4n-4n), trinucleadas (2n-4n-2n) o tetranucleadas (2n-2n-2n-2n), dependiendo de la proximidad de los grupos cromosómicos en metafase y de su inclusión o no en un único núcleo al cursar la telofase.

Si simultáneamente se emplea un agente multipolarizante, las células 8n pueden distribuir su genoma en varios núcleos de ploidías muy diferentes y decididamente aneuploides en su composición génica.

Tanto las primeras, homocariótica, como las segundas, heteroploides y aneuploides, se han utilizado para determinar el grado de autonomía de cada núcleo en la replicación y culminación de las etapas interfásicas y mitóticas, así como el papel de ciertos cromosomas (como el cromosoma NOR) en dicha autonomía nuclear, proporcionando una metodología útil para la disección cromosómica del ciclo celular.

40

GENOMA Y REGULACION DE EVENTOS DEL CICLO CELULAR (Genome and regulation of events the cell cycle).

De la Torre, C., Sans, J., Marcudich, D., Galanti, N., González-Fernández, A., Giménez-Abián, M. y Allar, P.
Centro de Investigaciones Biológicas, CSIC, Madrid, España y Departamento Biología Celular y Genética, Facultad de Medicina, Universidad de Chile.

La replicación secuencial del genoma permite alterar la funcionalidad de segmentos de éste por bromación durante fracciones del período S y posterior irradiación, en anoxia, de las células con su DNA parcialmente bromado.

Esta metodología ha permitido, en meristemos de la raíz de Allium cepa L., determinar el momento de replicación de secuencias del genoma relacionados con cada uno de los cuatro puntos de transición (TPs) donde se requiere síntesis de proteínas para la progresión de la célula a etapas más avanzadas de su ciclo celular. La replicación de las secuencias implicadas en los dos TPs para síntesis de proteínas situadas en el temprano y tardío G1 están en el temprano y tardío S respectivamente. Las secuencias implicadas en el TP situado en G2 lo hacen en el centro del S, mientras que secuencias dispersas por todo el genoma parecen ser responsables del TP situado en profase.

METILACION DEL DNA Y EVENTOS RELACIONADOS CON DIFERENCIACION Y DIVISION CELULAR. (DNA methylation and events retaled to cell differentiation and cell division).

Sans, J., Leyton, C. and Marcudich, D. Departamento Biología Celular y Genética, Facultad de Medicina, Universidad de Chile.

Se ha postulado que la metilación de citocina en secuencias dinucleotídicas C-G del DNA, sería un mecanismo mediante el cual se controlaría la expresión génica. Se ha comprobado, en una gran variedad de células, que la administración de 5-azacitidina (5-AzaC), análogo de la citidina, y poderoso agente hipometilante del DNA, produce activación génica.

En nuestro laboratorio, con el fin de asociar, desde un punto de vista morfológico, sectores del genoma con eventos celulares involucrados con el proceso de división o diferenciación celular hemos usado como estrategia experimental el efecto hipometilante del DNA DE 5-AzaC en raíces de Allium cepa L.

Nuestros resultados muestran que la administración de 5-AzaC provoca : a) un aumento significativo en la elongación celular que ocurre en la zona de diferenciación de la raíz, b) una aceleración de la reorganización nucleolar que se lleva a cabo al final de la mitosis y c) una alteración en el grado de condensación de la cromatina durante la mitosis. Los dos últimos eventos son particularmente evidentes cuando el análogo se administra durante la primera mitad del período replicativo.

Estos resultados sugerirían que los eventos estudiados estarían controlados por genes cuya expresión se regularía por el grado de metilación del DNA. (Proyecto FONDECYT 89/812).

EXPRESION DE GENES DE RESISTENCIA AL ESTRES TERMICO EN DOS ARBOLES NATIVOS DE CHILE, Araucaria araucana Y Prosopis chilensis. (Gene expression of resistance to heat shock in two native trees of Chile, Araucaria araucana and Prosopis chilensis).

Cardemil, L. Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

Araucaria araucana constituye bosques relictuales templados de alta montaña en la Región Austral de Chile, donde existen diferencias de temperatura entre día y noche y entre estaciones de 20ºC. Prosopis chilensis en cambio, es una leguminosa, fijadora de nitrógeno de la región árida y semi árida del Norte y Centro de Chile. En estas regiones la temperatura llega a mas de 60ºC en los días de Verano.

Hemos investigado como las semillas y plántulas de estos dos árboles responden a una exposición a altas temperatura, a través de experimentos de estrés térmico y como las semillas y plántulas adquieren termotolerancia, cuando son expuestas a temperaturas subletales.

En verdad, el principal objetivo de esta investigación es probar la hipótesis de que "Arboles nativos están adaptados a su medio natural porque ellos muestran un alto grado de expresión de los genes de resistencia para soportar condiciones extremas ambientales. El alto grado de expresión puede deberse ya sea a mecanismos de regulación de la funoión del gen, o a un mayor número de copia de los genes de resistencia".

Electroforesis en poliacrilamida SDS, de las proteínas sintetizadas durante el estrés de temperatura y en presencia de ^{35}S-metionina, como también hibridización Norte usando dos sondas de proteínas de estrés térmico, nos ha permitido evaluar esta hipótesis. PROYECTO FONDECYT, 0160/89; AID GRANT# DPE-5542-G-SS-8073-00; PROYECTO de Desarrollo, Universidad de Chile # 178501-4.

Agency for International Development
Washington, D.C. 20523

February 21, 1990

Dr. Liliana Cardomil
Department of Biology
Faculty of Sciences
University of Chile
Casilla 653
Santaigo, Chile

FAX (562) 2712983
phone: 2712983

Dear Dr. Cardomil,

Ref.: Project 7.068, Thermotolerance and Injury Resistance of *Prosopis chilensis* and *Araucana araucana*

With respect to your request to procure a computer, you may reallocate up to $2,600 from the grant budget for salaries to the item for equipment in order to purchase a computer.

Please refer to your grant instructions, G. Source and Origin of Goods and Services. It is expected that you will follow the letter and the spirit of the standard provisions for the procurement of computer. Geographic code 941 refers to countries in the selected free world, this includes North and Central America, Europe and Japan. You should prepare written specifications, including geographic source of U.S., and other free world, and submit it to a minimum of three suppliers, explaining that first priority would go to 1) the lowest cost response from a U.S. manufacturer or 2) from a supplier from the free world, and the bidders should give you a quotation for cost, insurance, freight, if the equipment is not available off the shelf. If a U.S. manufactured computer is not offered within your price range, you may select a non-U.S. manufactured piece of equipment. Please keep you document, and write a short memorandum to your files on the process of selection. You need not send it to us.

I hope these instructions will assist you in your procurement of a computer.

Sincerely,

John A. Daly, Ph.D.
Program Coordinator
Office of the Science Advisor

clearance: SCI, CGordon

S&T/FNR, MBenge