PN-ABB-104

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 9/14/88

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. 6.3/5
PR 6th Ed 8/10/88

Attachment

UNIVERSIDAD PERUANA CAYETANO HEREDIA
INSTITUTO DE MEDICINA TROPICAL
"ALEXANDER VON HUMBOLDT"
AP. 5045 - LIMA 100, PERU - ☎ 815111

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USAID/LIMA

August 17, 1988

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Mr. Gerardo Arabe
Interim Chief of Health and
Nutrition Division
Agency for International Development (AID)

Dear Mr. Arabe :

If is a pleasure to report to you the progress of our project on natural remedies, diarrheal disease and parasitoses.

In section A you can find all what has been done on parasites.

When the extracts of plants were given by enema in mice with Aspiculurus (Oxiurus), all extracts seemed to be effective, specially papaya latex, ojè and paico.

When the same was done in mice with Trichuris muris (Trychocephalus), ojè seemed to be very effective, papaya latex less efective and paico juice ineffective.

Papaya latex and ojè by enema cause inflammation and probably even perforation of the colon (ojè). We are keeping the colon specimens taken during autopsy to study them histologically. In this way, we will understand better the deleterous effect of papaya latex and ojè on the colon.

In vitro, papaya seeds and latex first paralyzed and afterwards killed Oxiurus.

Pure extract and 50% solution of Allium sativum (ajo) paralyzed and killed Hymenolepis nana in vitro. However in vivo, in mice with Hymenolepis, that plant seemed to be ineffective.

In graphic (cuadro) 1 you can find the general development of the project.

In graphic 2 the toxic effect of the plants appears depicted. Zapallo and papaya seeds and paico juice and infusion were very safe even at doses which were 100 x and 1000 x the usual ones. On the contrary, papaya latex was

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very toxic in doses 15 x or more than 15 x the usual one: and ojè, in pure extract and in 1:1 solution in alcohol (pisco), was toxic in 20 x doses and very toxic in 100 x doses.

In graphic 3 it is shown how extract of Zapallo seed kill in vitro Trichocephalus, Oxiurus and Hymenolepis. The same is gotten with papaya seeds (graphic 4), papaya latex (graphic 5) (although Trichocephalus tends to resist up to 180 minutes of exposure), ojè latex pure (graphic 6) and in 1:1 solution in pisco (graphic 7), paico juice (graphic 8) and infusion of paico (graphic 9) (although with this infusion Trichocephalus and Oxiurus again seem to resist up to 180 minutes).

In graphic 10, the in vivo effectiveness of the various plant extracts in mice infected with Trichophalus, Hymenolepis and Oxiurus is shown. Only ojè in alcohol and dubiously paico juice seemed to have some effect on Trichocephalus. But ojè in alcohol caused at the same time high mortality in the mice.

Because of the discrepancy between the in vitro and the in vivo effectiveness of the various plant extracts, we had the idea of using the extracts in the form of enemas. The results gotten following this line have been very rewarding. There is only the problem of possible toxicity when high concentrations are used.

It is very probable that when the extracts are given intragastrically, they are digested or absorbed in the upper gastrointestinal tract of the mice. If it is true, better results could be gotten preparing mice or rats with small bowel fistulas to the surface of the body, infecting the animals with parasites, and treating the parasitoses giving the extracts through the fistula. Please, tell us if you consider this possibility interesting and worth of being pursued.

It has been very difficult till now to get helecho macho, but Dr. Hu has informed me that he will get it very soon. When we have helecho macho we will try its effectiveness on parasitoses.

In section B you can find what has been done on diarrheal disease.

Shigella and filtered supernatant of broth where Shigella was cultured were inocculated into rabbit intestine. Both the bacteria and supernatant cause accumulation of fluid and inflammation in the intestine. Contrarily, they did not affect the bowel in the infant mouse assay.

The best assay for Shigella is going to be determined; and when we get this, effects of Tara and Granada will be tested.

Mrs. Finch who does the microbiological part of our studies recently delivered a baby, and this is why the progress was slow. However, we are now retaking vigorously the microbiological work.

Please, make any suggestions and criticism which you judge pertinent.

In the mean time, as always, receive our best regards, gratitude, and sincere appreciation,

Dr. Raúl León-Barúa
Professor of Medicine
Co-Principal Investigator
Project "Diarrhea and Nutrition"

/cm

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INFORME DE MEDICINA TRADICIONAL

1.- Esquema general de desarrollo del proyecto: CUADRO No 1

2.- Pruebas de toxicidad de plantas: Cuadro No 2

3.- Resultados in vitro de la acción antihelmíntica de las plantas: Cuadros Nos: 3, 4, 5, 6, 7, 8, 9.

4.- Resultados in vivo de la acción antihelmíntica de las plantas: Cuadro No 10.

RESULTADOS DE NUEVOS PROTOCOLOS DE TRABAJO

A.- Tratamiento de Ascaridiasis con extractos de plantas por vía rectal.

Material y métodos: Fueron inoculados 5 ratones para cada planta y se utilizó 1 ratón control por planta.

Procedimiento: Las cremas fueron aplicadas durante 3 días seguidos, por las mañanas, previamente se les privó de la comida y tomaron poca agua.
A los controles se les aplicó agua destilada.
Al cuarto día fueron sacrificados.

NOTA: A todos se les aplicó por vía rectal 0.8 ml de la dosis óptima.

RESULTADOS

PLANTA	DOS/NOR	DOS/OP	No/A/I	No/A/CON	X/G/RI	No/G/RC
ZAPALLO	5.5g/Kg	100X	5	1	18.2	42
PAPAYA-L	1ml/Kg	10x	5	1	5.5	34
PAPAYA-P	0.7g/Kg	100x	5	1	13.0	54
SUE PERO	1ml/Kg	30x	5	1	5.3	27
PAICO-Z	0.015ml/Kg	1000X	5	1	13.0	71

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B1.- Aplicacion de enemas de extractos de plantas con aplicacion de lavados con SF antes y despues de aplicado el extracto.

Material y metodos: Se utilizaron 9 ratones tratados y 4 ratones control para cada planta.

Procedimiento: Se coloco enema previo de 1ml de SF una hora antes de la aplicacion del extracto, luego se aplico el enema del extracto 0.8 ml por tres dias consecutivos despues de una hora de la ultima dosis se aplico el segundo lavado de SF.

RESULTADOS

PLANTA	DOS/NOR	DOS/OP	N/AI	X/G/AI	N/AC	X/G/AC
ZAPALLO	0.9g/Kg	100X	9	20	4	44
PAPAYA-L	1ML/Kg	10X	9	1	4	33
OJE FURO	1ML/Kg	30X	9	1	4	33
PAICO-Z	0.015ML/Kg	1000X	9	14	4	37
PAPAYA-P	0.7g/Kg	100X	9	15	4	38

B2.- Experimentacion in vivo: Latex de papaya .Via oral.

PLANTA	DOS/NOR	DOS/OP	N/AI	X/G/AI	N/AC	X/G/AC
PAPAYA-L	1ml/Kg	10X	9	9	4	35

(A)

INFORME DE MEDICINA TRADICIONAL

1.- Esquema general de desarrollo del proyecto: CUADRO No 1

2.- Pruebas de toxicidad de plantas: Cuadro No 2

3.- Resultados in vitro de la acción antihelmíntica de las plantas: Cuadros Nos: 3, 4, 5, 6, 7, 8, 9.

4.- Resultados in vivo de la acción antihelmíntica de las plantas: Cuadro No 10.

RESULTADOS DE NUEVOS PROTOCOLOS DE TRABAJO

A.- Tratamiento de Aspiculuris con extractos de plantas por via rectal.
Material y metodos: Fueron inoculados 5 ratones para cada planta y se utilizó 4 ratones control.

Procedimiento: Los enemas fueron aplicados durante 3 dias seguidos, por las mañanas, previamente se les privó de la comida y bebieron poca agua.
A los controles se les aplicó agua destilada.
Al cuarto dia fueron sacrificados.

NOTA: A todos se les aplicó por via rectal 0.3 ml de la dosis óptima.

RESULTADOS

PLANTA	DOS/NOR	DOS/OP	No/A/I	No/A/CON	X/G/RI	No/G/RC
ZAPALLO	0.8g/kg	100X	5	1	18.2	42
PAPAYA-L	1ml/kg	10x	5	1	6.5	34
PAPAYA-P	0.7g/Kg	100x	5	1	13.0	39
OJE PURO	1ml/Kg	30x	5	1	6.3	27
PAICO-Z	0.018ml/Kg	1000X	5	1	13.0	71

B.- Tratamiento de Trichuris muris por via rectal
Los materiales y métodos así como los procedimientos fueron realizados en la misma forma que el parásito anterior.

RESULTADOS

PLANTA	DOS/NOR	DOS/OF	No/A/I	No/A/CON	X/G/RI	No/G/RC
PAPAYA-L	0.6g/Kg	100x	6	6	74	148
OJE PURO	1ml/Kg	30x	6	6	0	75
PAICO-Z	0.016ml/kg	1000x	6	6	107	112

OBSERVACIONES:

En el caso de la aplicación del latex de papaya se observó que hay un sangrado rectal,una inflamación en la abertura anal.
Se observó que los parásitos eran eliminados con las heces unos minutos después de la puesta del enema.

Algunos de los animales inoculados ,especialmente con oje se murieron con el intestino reventado despues de la segunda dosis.

C.- Inactivacion de las muestras de pepas y de latex de papaya.

Las muestras fueron calentadas a 56oC por 30'

Se experimentó in vitro con Aspiculuris tetraptera

RESULTADOS.

Pepas de papaya

30'	12	gusanos móviles
60'	12	" "
90	12	" semi-móviles
120	12	" paralizados
150	12	" "
180	12	" muertos, sin destrucción de tegumento.

Latex de papaya

30'	12	gusanos móviles.
60'	12	" "
90'	12	" paralizados
120'	12	" "
150'	12	" muertos, sin destrucción de tegumento.

D.- Actividad antihelmíntica del ajo Allium sativum

Experimentación in vitro: Hymenolepis nana
Se trabajó con estracto puro de bulbos frescos, en solución al 50% en PBS y el grupo control se trató con PBS.

RESULTADOS

TIEMPO/M'	PBS	Est. 100%	Est./50%
5'	10*	10**	10**
30'	10*	10***	10***
60'	10*	10***	10***

* Gusanos con movimientos normales.
** Guzanos paralizados, no se recuperan al colocarlos en PBS.
*** Gusanos paralizados con tegumento destruido progresivamente en relación al tiempo de exposición.

Experimentación in vivo: Hymenolepis nana.

Se trabajaron 12 ratones de 18 gr de peso, infectados con 100 huevos del parásito.
La dosis utilizada fue de 0.15 ml de estracto de ajos, diluido en una proporción de 1:1 con aceite en dosis diarias por 6 dias.
A los 2 dias de terminado el tratamiento los ratones fueron sacrificados.

RESULTADOS

PLANTA	DOS/OP	No/A/I	No/A/CON	X/G/RI	No/G/RC
ajos	0.15ml	4	2	50	101
		4	2	56.75	60

CUADRO 1

EXPERIMENTACIÓN IN VITRO DE ALGUNAS PLANTAS CONSIDERADAS ANTIHELMÍNTICAS

1. PLANTAS UTILIZADAS:

Cucurbita maxima	"zapallo"	pepas
Carica papaya	"papaya"	pepas - Latex
Ficus antihelmíntica	"ojé"	Latex (pH/OH)
Chenopodium ambrosoides	"paico"	infusion y zumo

2. MODELOS DE LOS PARÁSITOS EN ESTUDIO HECHOS EN RATONES SWISS:

Trichuris muris Aspiculuris Tetraptera Hymenolepis nana.

3. PRUEBAS DE TOXICIDAD DE LAS PLANTAS.
OBTENCION DE DOSIS ÓPTIMA.
Animales de experimentación: ratones cepa swiss.

4. PRUEBAS DE EFECTIVIDAD EN PLANTAS: IN VIVO
Animales de experimentación: ratones, cepas swiss.

N → número
A → animales
V → vivos
M → muertos.

CUADRO 2

Prueba de Toxicidad de plantas antihelmínticas.

* por procedimiento de Técnica, no se pudo realizar mayor concentración

Plantas / Dosis	Zapallo			Papaya						C J E.						Palco					
	Hojas			Pepas			Latex			Polvo			Polvo / OH (1 : 1)			Zumo			Infusión		
Dos	0.5 gr/Kg			0.5 gr/Kg			1 ml/Kg			1 ml/Kg						0.01 ml/Kg			5 gr/LT		
	N/A/Tot	N/A/V	N/A/M	N/A/Tot	N/A/V	N/A/M	N/A/Tot	N/A/V	N/A/M	N/A/Tot	N/A/V	N/A/M	N/A/Tot	N/A/V	N/A/M	N/A/Tot	N/A/V	N/A/M	N/A/Tot	N/A/V	N/A/M
1 X	6	6	0	6	6	0	9	9	0	9	9	0	9	9	0	9	9	0	9	9	0
10 X	6	6	0	6	6	0	9	8	1	9	9	0				9	9	0	9	9	0
15 X							9	1	8												
20 X							9	2	7	9	9	0	9	7	2						
25 X							9	1	8												
30 X										9	8	1	9	6	3						
40 X										9	7	2							9	9	0
* 60 X										9	6	3									
* 100 X	6	6	0	6	6	0				9	0	9				9	9	0			
1.000 X																B	B	A			

CUADRO 3

Resultados de la acción antihelmíntica in vitro de Cucurbita máxima "ZAPALLO".

macerado de pepas

Acc/planta/T'	T. muris	A. Tetraptera	H. nana
30'	12 .	12 .	12 .
60'	12 .	12 ..	12 ..
90'	12 .	12	12 ...
120'	12 .		12 ...
150'	12 .		12 ...
180'	12		12 ....

Concentración de la planta: 0.05 gr/grp.
Nº gusanos parásitos experimentales = 12

. Movimientos lentos
.. Alteración de la estructura interna
... Destrucción de tegumento
.... Muerte comprobada (por incubación en PBS a 37ºC)

CUADRO 4

RESULTADO DE LA ACCIÓN ANTIHELMÍNTICA IN VITRO DE

CARICA PAPAYA "PAPAYA"

macerado de pepas

Acc/planta/T'	T. mucis	A. Tetrodera	H. nana
30'	12.	12.	12.
60'	12.	12..	12..
90'	12.	12....	12...
120'	12.		12...
150'	12....		12...
180'			12....

Concentración de la planta: 0.08 gr/grp
Nº de gusanos parásitos experimentales = 12

. Movimientos lentos, Totalmente inmóviles
.. Alteración de la estructura interna
... Destrucción de Tegumentos.
.... Muerte comprobada (por incubación en PBS a 37°C) Destrucción Total.

CUADRO 5

Resultado de la acción antihelmíntica in vitro de

Carica papaya "papaya"

Latex

Acc/planta/T'	T. muris	A. tetraptera	H. nana
30'	12 .	12 .	12 ..
60'	12 .	12 ..	12 ...
90'	12 .	12	12 ....
120'	12 .		
150'	12 .		
180'	12 .		

Concentración de la planta: puro + mick (1:1).
nº gusanos parásitos experimentales = 12.

. Movimientos lentos o totalmente inmóviles
.. Alteración de la estructura interna.
... Destrucción de tegumento
.... Muerte comprobada (por incubación en PBS a 37°C ó destrucción total)

CUADRO 6

RESULTADO DE LA ACCIÓN ANTIHELMÍNTICA IN VITRO DE

FICUS ANTIHELMÍNTICA "ojé"

Latex de Corteza

Hca/planta/T'	T. mucis	A. Tetraptera	H. nana
30'	12...	12.	12..
60'	12...	12...	12....
90'	12...	12....	
120'	12...		
150'	12....		
180'			

Concentración de la planta : extracto puro
Nº gusanos parásitos experimentales = 12

. Movimientos lentos ó totalmente inmóviles
.. Alteración de la estructura interna
... Destrucción de Tegumento
.... Muerte Comprobada (por incubación en PBS a 37°C ó destrucción total)

CUADRO 7

Resultado de la acción antihelmíntica in vitro de

Ficus antihelmíntica "ojé"

Latex de Corteza

Acc./planta/T'	T. muris	A. tetraptera	H. nana
30'	12.....	12 .	12 ...
60'		12	12 ....
90'			
120'			
150'			
180'			

Concentración de la planta: Latex puro + pisco (1:1)
Nº gusanos parásitos experimentales = 12

. Movimientos lentos ó totalmente inmóviles
... Destrucción de Tegumento
.... Muerte Comprobada (por incubación en PBS a 37° C) ó destrucción total.

CUADRO 8

Resultado de la acción antihelmíntica in vitro de Chenopodium ambrosoides "paico"

Zumo

Acc/planta/T'	T. muris	A. Tetraptera	H. nana
30'	12.	12.	12.
60'	12.	12..	12..
90'	12.	12..	12...
120'	12.	12....	12....
150'	12.		
180'	12....		

Concentración de la planta: zumo puro.
Nº gusanos parásitos experimentales = 12

. Movimientos lentos
.. Alteración de la estructura interna..
... Destrucción de Tegumento
.... Muerte Comprobada (por incubación en PBS a 37°C.)

CUADRO 9

RESULTADO DE LA ACCIÓN ANTIHELMÍNTICA IN VITRO DE *Chenopodium ambrosoides* "PAICO"

Infusión

Acc/planta / T'	T. muris	A. Tetraptera	H. nana
30'	12 .	12 .	12 .
60'	12 .	12 .	12 ..
90'	12 .	12 .	12 ...
120'	12 .	12 .	12 ....
150'	12 .	12 .	
180'	12 .	12 .	

Concentración de la planta: infusión de 1gr./mL
Nº gusanos parásitos experimentales = 12

. Movimientos lentos
.. Alteración de la estructura interna
... Destrucción de Tegumento
.... Muerte Comprobada (por incubación en PBS a 37°C)

CUADRO 10

PRUEBAS DE EFECTIVIDAD DE PLANTAS: IN VIVO

Parasito / Planta	Trichuris Muris							Hymenolepis Nana							Aspiculuris Tetraptera						
	dp	d/d/nd	Nº/A/+	Nº/A/−	Nº/A/M	Nº/A/I	Nº/A/C	dp	d/d/nd	Nº/A/+	Nº/A/−	Nº/A/M	Nº/A/I	Nº/A/C	dp	d/d/nd	Nº/A/+	Nº/A/−	Nº/A/M	Nº/A/I	Nº/A/C
Jaguillo Dx: 0.4 g/Kg 0.8 g/Kg	100X	1/1/3	3	0	0	3	3	100X	1/1/1	6	0	0	6	6	100X	1/1/5	6	0	1	6	6
Papayo Papas Dx: 0.4 g/Kg	100X	1/1/1	6	0	0	6	6	100X	1/1/1	6	0	0	6	6	100X	1/1/5	6	0	0	6	6
Papaya Latex Dx: 1 ml/Kg															10X (tratamiento)						
OJE Fosc Dx: 1 ml/Kg	40X	1/1/3	6	0	6	6	6	10X	1/1/3	9	0	0	9	4	50X	1/1/3	5	0	2	5	4
OJE OH Dx: 1 ml/Kg	40X	1/1/3	2	4	3	6	6	20X	1/1/3	4	0	0	4	4	20X	1/1/3	8	0	1	8	4
0.06 g/Kg Paico Zumo Dx: 0.06 g/Kg	1000X	1/1/3	17	1	0	18	18	1000X	1/1/3	18	0	0	8	6	1000X	1/1/5	6	0	0	6	6
Paico / Infusión Dx: 0.4 g/LT	50X	1/1/5	6	0	0	6	6	50X	1/1/5	5	0	0	5	5	50X	1/1/5	6	0	0	6	3

Dx/cp : dosis optima.
d/d/nd : dosis / día / nº días
Nº/A/+ : Nº animales positivos
Nº/A/− : " " " negativos
Nº/A/N : " " " muertos

Nº/A/I : Nº animales inoculados
Nº/A/C : " " " controles

B

July 17, 1988

To: Dr. Raul Leon Barua
From: Dory Finch

PROGRESS REPORT - MICROBIOLOGY SECTION

I. Effect of Colonization of Rabbit Intestines by *Shigella dysenteriae 1*

A. Source of *Shigella*

The strain of *Shigella dysenteriae 1* used in the rabbit ileal loop was obtained from the Microbiology Laboratory, Instituto Peruano Cayetano Heredia, isolated from a human stool. The bacteria was grown on McConkey Agar for purification. Then the bacteria was grown on trypticase soy broth and was stored with 50% glycerol at -70C for further use. The *Shigella dysenteriae 1* was identified again by Gram stain, API 5 strip and serology.

B. Preparation of *Shigella*

The bacteria was grown on trypticase soy broth supplemented with 0.6% yeast extract. One or two colonies are inoculated in the broth and incubated in a 37C water-bath with agitation at 180 rpm. After 18 hours of growth, the broth is centrifuged in a Sorvall at 6,000 rpm for 30 minutes at 4C. The broth is then saved for further use and the bacterial pellet used for the live challenges in the rabbit ileal loop assay.

C. Inoculation of *Shigella* in the Rabbit Intestine

The bacterial pellet obtained in the preparation was washed twice and reconstituted with PBS using the 0.5 McFarland standard. Then starting with this concentration, the solution was further diluted with PBS in two-fold dilutions, ~~[illegible]~~ using dilutions of 1/100, 1/10000 and 1/1000000. One milliliter of the standard preparation and each of the dilutions was injected in the rabbit ileal loops in duplicate and placed at random. PBS+0.1% gelatin was used as negative control. Four rabbits were used.

After the rabbits were sacrificed, the intestines were removed and the volume and length of each loop were measured. The ratio, volume over length was calculated for each loop.

D. Inoculation of *Shigella* supernatant in the Rabbit Intestine

The supernatant derived from the bacterial growth was filter-sterilized using a Gelman 0.2 um membrane. The supernata-

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nt was diluted 1/10, 1/100, 1/1000 with PBS + 0.1% gelatin and inoculated in like manner with the bacterial suspension.

E. Effects of the Shigella suspension and supernatant in rabbit ileal loop assay.

As can be seen from the scattergram the Shigella live challenge appears somewhat to cause an accumulation of fluid in the rabbit ileal loop. The fluid was noted to be bloody, and very viscous with mucous. The fluid accumulation has a very small trend in that the effect decreases as the dilution of the Shigella suspension increases. The effect noted here however, is not remarkable when compared with the effect seen with the cholera toxin challenge. It is premature at this time to deduce any conclusive information from this effect but it appears that colonization has taken placed. Further work is needed to qualify and standardize the methodology for the Shigella assay. One suggestion was to use the KIAMD model for this type of bacterial colonization assay.

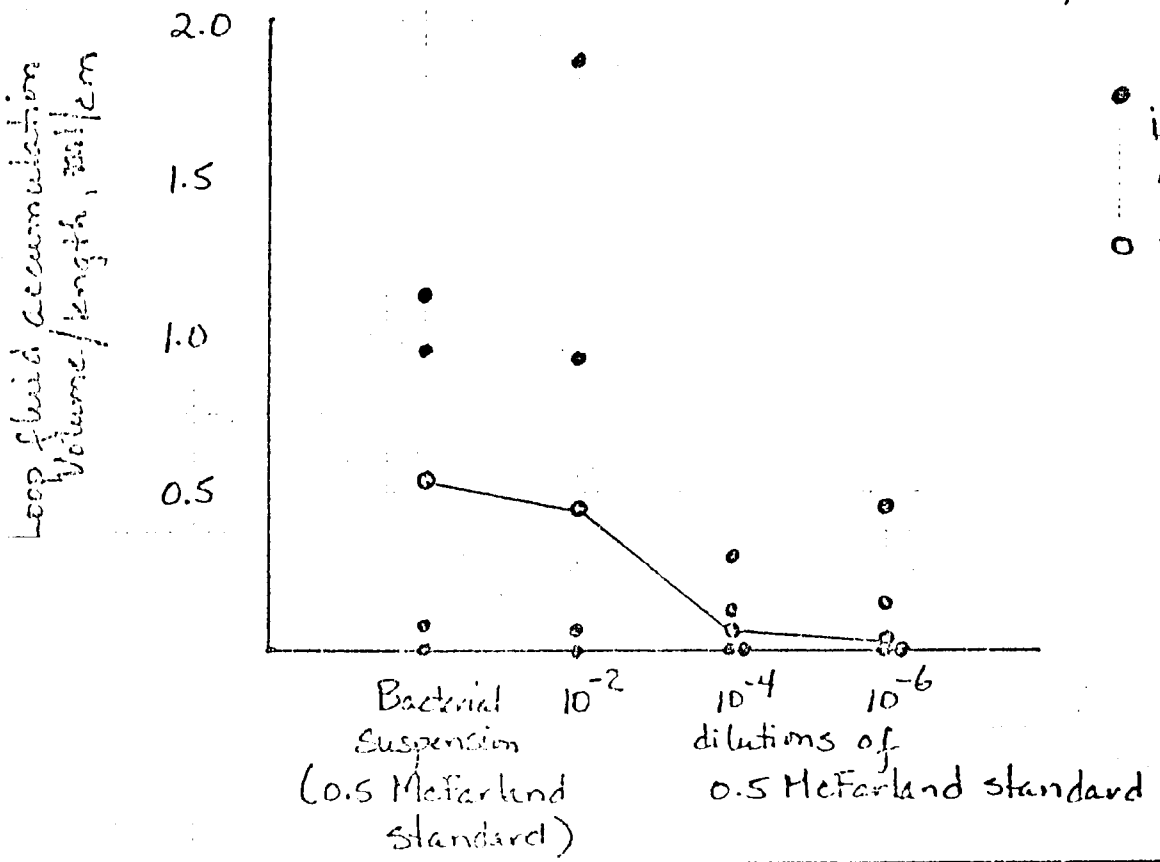
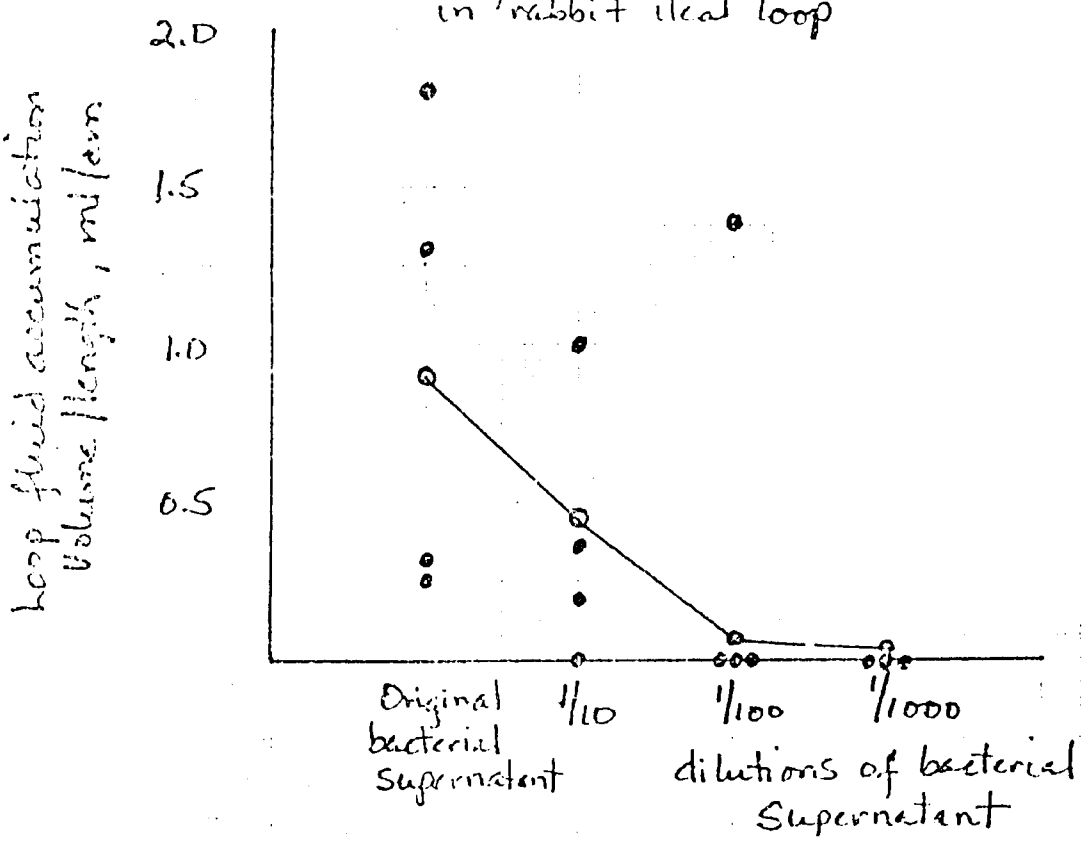
In like manner, the Shigella supernatant was inoculated in the rabbit ileal loop. The supernatant challenge also appears somewhat to cause an accumulation of fluid in the loops. The fluid accumulation appeared also to be bloody and very viscous with mucous. Further testing is needed to ascertain the extent of these effects in the rabbit ileal loop assay.

We have also tested the Shigella supernatant in the infant mouse assay. We have used 7 infant mice (3 days old) for controls and 20 infant mice to test the toxicity of the supernatant. One milliliter of the supernatant preparation was inoculated in each of the test mice. Then the mice were sacrificed after four hours. The gut/remaining body ratio were calculated. The result show no difference from the control mice. Both control and test mice had ratios less than 0.080. Ratio above 0.083 is considered positive in this assay. We therefore conclude that the infant mouse assay is not a useful test to determine the toxicity of the Shigella supernatant.

F. Future Work on the Traditional Plants

We will test the Granada and Jara against live challenges of Shigella dysenteriae 1 and its supernatant as soon as we determine the best animal assay to utilize for this bacteria.

Effect of Shigella dysenteriae suspension inoculated in rabbit ileal loops



Effect of Shigella supernatant inoculated in rabbit ileal loop