

PM. ACR 786
796
6. 225

FOURTH PROGRESS REPORT FOR THE PERIOD
FEBRUARY 1, 1989 THROUGH JULY 31, 1989

GRANT No. DPE-5542-G-SS-7033-00
PROJECT No. 936-5542

"Analysis of Immunogenic Proteins of Microfilariae,
Adult Worms and Excretory/Secretory Products
of Onchocerca volvulus"

PROGRAM DESCRIPTION

A.1 Purpose of the Grant:

The purpose of the grant is to develop a new highly sensitive diagnostic test for onchocerciasis.

A.2 Specific Objectives:

A.2.(a) To identify immunogenic proteins associated with microfilariae, adult worms, and excretory/secretory products of Onchocerca volvulus for use in the development of a more highly sensitive and specific diagnostic test for onchocerciasis.

A.2.(b) To attempt to correlate the presence of individual immunogenic proteins with the severity of onchocercal disease, thereby providing insight into the host-parasite interaction.

Rec'd in SOL DEC 21 1989

A.3 Implementation: Technical Work Plan

A.3.(a) Phase I

A.3.(a).i Measurement of microfilarial skin densities and examination for onchocercal nodules in residents from the study communities in follow-up visits to the study communities; collection of serum samples and nodules.

According to the protocol established in the Grant, at the end of year one, the surveys of microfilariae in skin biopsies and palpation of nodules were repeated in all participating residents of the study communities. The time schedule for visits for Fincas Buena Vista, Las Delicias and Santa Margarita was done during February, 1989; Fincas La Torre, Mirandilla and Costa Rica, during March; and Fincas Panamá and Santa Adelaida, in April. As it was done previously, informed approval was obtained from each Finca owner before a visit was done.

Only a total of 446 (31.2%) of the 1,431 persons who were examined in the previous year, participated in the second examination. There has been an increasing reluctance on part of the people and refuse repeated examinations for skin microfilariae and nodules. A total of 236 different persons, however, were examined for the first time.

The data for age, sex, occupation, nodule rate and microfilarial skin positivity is being calculated now and will be presented in the next Progress Report.

Based on the microfilarial skin densities per milligram of skin (MFD), which was calculated on the persons examined during the first visit, the levels of endemicity for the Fincas has been adjusted, to the following: Fincas Buena Vista, Las Delicias, Santa Margarita, and Costa Rica are located in an onchocerciasis hyperendemic area (prevalence rates >65% positivity); Fincas La Torre, Mirandilla, and Panamá, in a mesoendemic area (prevalence rates between 35-65% positivity). This indicates that the levels of infected individuals in Finca Panamá, which was identified before as belonging to a hypoendemic area (prevalence rate <35% positivity) and Finca Costa Rica to a mesoendemic area, have changed over the year.

A total of 398 (27.8%) of the persons examined during the first visit to the onchocerciasis areas, agreed voluntarily to provide a blood sample for serum. The serum samples have been aliquoted and stored at -85C until further analysis is required.

An estimated number of at least 57 (4.0%) persons, from which information could be obtained, had either left the Fincas or have died during the interval between the third and fourth visits (a period of five to six months). This makes at least 141 (12.4%) persons which cannot be followed from the first visit.

Onchocerca nodules for adult filarial worms to prepare whole worm somatic antigens have been obtained from 62 positive persons in the two endemic areas, after nodulectomies performed by the paramedic brigade team of the Onchocerciasis Department of the National Malaria Service. The nodules were transported under a liquid nitrogen atmosphere to the laboratory and kept at -85C. The nodules have been digested with collagenase and male and female worms have been maintained at -85C until preparation of antigen for the ELISA and EITB tests.

In addition, 94 nodules have been collected and immediately placed in transport media (RPMI 1640 medium supplemented with antibiotics) to be taken, at room temperature, to the laboratory at Universidad del Valle de Guatemala. These nodules were processed and digested with collagenase (0.1 %) to obtain live female worms. We have been successful, however, in recovering only 14 intact females which were maintained alive in vitro at 37 C, for a period of two to four days. We are planning in obtaining more fresh nodules to isolate live worms and to prepare excretory/secretory antigens as well as microfilariae released in culture.

A.3.(a).ii Preparation of Onchocerca whole worm somatic antigens for ELISA test.

Onchocerca whole worm somatic antigens for ELISA (enzyme-linked immuno-sorbent assay) were prepared as described in the Grant according to the procedures of Luján et al. (1983, Am. J. Trop. Med. Hyg., 32:747). Briefly, 12.7 gm (wet weight) of adult O. volvulus males and females were macerated in extraction buffer (PBS 10 mM) containing protease inhibitors (EDTA 5 mM, PMSF 1 mM, TPCK 50 ug/mL). After six cycles of sonication at 4 C, the insoluble material was sedimented at 100,000 g at 4 C for 1 hour. The sediment was processed as described before, the supernatants were combined and dialyzed at 4 C for 24 h against eight changes of 1 L each of 10 mM PBS buffer, pH 7.2. Protein concentration (336 ug/mL) was determined by the Bio-Rad method and the antigen solution was stored in small aliquots at -80 C until used.

A.3.(a).iii ELISA test.

The enzyme-linked immunosorbent assay (ELISA) test for measuring human IgG antibodies to O. volvulus has been developed, as described in the Grant according to the procedures of Luján et al. (1983, Am. J. Trop. Med. Hyg., 32:747). Briefly, polystyrene microtiter plates (Immulon 2) were coated

with 0.06 ug/well of antigen solution in 0.05 mL of carbonate/bicarbonate buffer (pH 9.6). After an incubation period of three hours at 37 C, the plates were washed five times with PBS containing 0.5% Tween 20 for three minutes each time. To reduce non-specific binding, wells were blocked with a solution of 10% (w/v) of nonfat dry milk in PBS for 1 h. Washing was repeated as described before, and serum samples (0.05 mL/well) were added at an initial dilution of 1:80 in dilution buffer (PBS buffer containing 0.5% bovine serum albumin, 0.5% casein and 0.1% Tween 20), diluting two-fold to a final dilution of 1:10,240. After an incubation period of 30 minutes at 37 C and a cycle of five washes, the conjugate (goat anti-human IgG horseradish peroxidase-labelled, IgG-HRPD) was added at an optimal dilution of 1:4,000, in 0.05 mL of dilution buffer. After a similar period of incubation and washes, the substrate was added (ortho-phenylene diamine in acetate buffer pH 5.4 containing hydrogen peroxide) and the plates were incubated in the dark at room temperature for 15 minutes. The enzyme reaction was stopped with 8 N sulfuric acid and the optical density of the wells read in a Multiskan MCC ELISA plate reader (Flow Laboratories, Inc.) at 492 nm.

The results of the reference standard curve for IgG antibodies to Onchocerca volvulus are illustrated in Figure 1. To determine the cutoff O.D. value of each unknown sample, the resulting O.D. values are compared to the mean plus-two and

-three standard deviations of the mean for the curve derived from 37 normal human sera obtained from healthy Guatemalan donors without evidence of intestinal parasites and onchocerciasis. The mean of five human sera from patients with onchocerciasis is also illustrated. Serum samples of patients, with and without onchocerciasis, from the visits to the Fincas are being processed and the results will be presented in the next Progress Report.

A.3.(b) Phase II

A.3.(b).i Trans-blot ELISA (EITB).

The EITB procedure has been applied in the laboratory at Universidad del Valle de Guatemala after the training received at CDC with Dr. Victor Tsang. Several modifications have been made, however, to adjust to the equipment (Bio-Rad) available here. Linear gels of 12.5% acrylamide and gradient gels from 5-22.5% acrylamide have been used to resolve the O. volvulus adult worm extract proteins obtained, as described in the Third Progress Report. In addition, comparisons of the Tsang's method with the Laemmli system have also been made, as well as processing of the samples under native versus denaturing conditions. Results of the different molecular weight antigen profiles recognized by sera

selected for blotting, will be presented in the next Progress Report. According to the Protocol in the Grant, patients with different parasitological conditions due to onchocerciasis were to be obtained. Several groups of individuals, ranging from 0 to >100 MFD and those presenting microfilariae in the eyes, will be compared as well as their antibody responses to specific molecular weight antigens. Nevertheless, we have obtained 306 sequential serum samples for EITB analysis, as described in Table I. Three sequential serum samples, at five to seven month intervals, have been obtained from 231 patients; in addition, from 75 patients at one year interval. In both instances, however, two skin biopsies for microfilariae have been performed at one year interval.

A.4 Comparisons of actual accomplishments with goals established for the period.

The goals established for this period have been achieved as initially proposed: The follow-up visit to the communities in the two endemic areas have been completed at the end of year one, the surveys of microfilariae in skin biopsies and palpation of nodules were repeated in participating residents of the study communities, and serum samples and nodules have been collected. The EITB and ELISA tests for evaluation of antibody responses to adult worm Onchocerca

volvulus antigens have also been established. A data base to store and analyze the results of each of the examinations has been created using DBASE III+.

Three problems, however, have still been encountered namely, the participation of persons during the follow-up visits, patients treated for onchocerciasis, and collection of nodules for antigen preparation.

As indicated in previous Progress Reports, we obtained a poor response and compliance from the individuals in the study communities for providing additional serum samples (only 398 of 1,431 [27.8%]). In spite of these problems, however, the principal objective of the study, which is to obtain 30-40 sequential serum samples has been achieved. We have tried to motivate the communities by offering physical examinations and providing basic health care attention. To this extent, we have included a complete ophthalmological examination (Annex I) performed by Ophthalmologists of Roosevelt Hospital, a hematologic examination including blood group, hematocrit and leukocyte counts (Annex II), dermatological examination (Annex III) performed by a Dermatologist of the Institute of Dermatology, and a parasitological examination (Annex IV) for intestinal parasites. Medicines for eye infections, diarrheal diseases and vitamins were provided as well as an anthelmintic (Levamisole) for those persons requiring them. We feel, however, that better compliance could be obtained if a social

worker could motivate the communities on a continuous basis and if a drug for onchocerciasis could be provided. Many individuals refuse to be examined for onchocerciasis since they have never received a medicine for it, in spite of yearly examinations.

Patients treated for onchocerciasis by the Rodolfo Robles Hospital or the Guatemalan Ministry of Health have not been obtained since no treatment is offered in the country at this time. Next year, however, the Rodolfo Robles Hospital will initiate treatment of onchocerciasis in the Yepocapa District starting in April, 1990. We have been requested to participate in such program, especially in evaluating the effectiveness of treatment program using serological conversions of the treated individuals.

Nodules for isolating live worms will be obtained by the Department of Onchocerciasis paramedic brigades until February and March of 1990, where we expect to culture female worms to collect excretory/secretory products and microfilariae.

Finally, Dr. Victor Tsang from CDC or an assistant from his laboratory will be unable to visit Guatemala during this year, due to commitments in other projects. We have been able, however, to obtain technical assistance from other groups in Guatemala and Mexico. A student technician in the Project at Universidad del Valle de Guatemala received training at the Institute of Biotechnology and Molecular Biology in Cuernavaca,

Mexico, sponsored by the Guatemalan Biotechnology Commission, on chromatography and electrophoresis techniques. Therefore, we believe that we can continue with the procedures at the laboratories in Universidad del Valle, hoping that in a near future we can obtain assistance of a scientist from CDC when he/she travels to Guatemala.

A.5 Other activities.

On May 15, 1989 we sent 28 aliquots of serum (400-500 uL each) samples to Dr. Gary J. Weil from the Division of Infectious Diseases, The Jewish Hospital of St. Louis, St. Louis, MO. This was done in response to a request by Dr. Weil to test Guatemalan sera from onchocerciasis patients, especially for an antigen detection assay which they are developing. Interestingly, however, the sera was also tested for IgG₄ antibodies to Onchocerca, demonstrating a higher specificity. We are, therefore, also developing an ELISA assay to determine these antibodies in the serum samples collected.

TABLE I

Distribution of sequential serum samples obtained from Guatemalan individuals examined for onchocerciasis according to nodules and microfilarial skin densities from January 1988 to April 1989 for the study

"Analysis of Immunogenic Proteins of Adult Worms, Microfilariae and Excretory/Secretory Products of Onchocerca volvulus," Project No. 6.225

Onchocerciasis Group	Number of sequential serum samples		
	Two ^{a)}	Three ^{b)}	Total
Skin MFD - ^{c)} Nodule +	1 ^{d)}	4	5
Skin MFD - Nodule -	28	62	90
Skin MFD + ≤ 1 - 10	30	97	127
Skin MFD + 11 - 50	12	42	54
Skin MFD + 51 - 100	3	14	17
Skin MFD + > 100	1	12	13
TOTAL	75	231	306
Eye MF + ^{e)}	17	62	79

- a) Samples obtained at a twelve month interval
- b) Samples obtained at five to seven month intervals
- c) MDF = microfilarial skin density, mf/mg, in four biopsies/individual
- d) Number of individuals
- e) Microfilariae in anterior chamber of the eye in individuals from any of the previous groups

CONTROL CURVE ONCHOCERCIASIS ELISA

IgG - HRPO

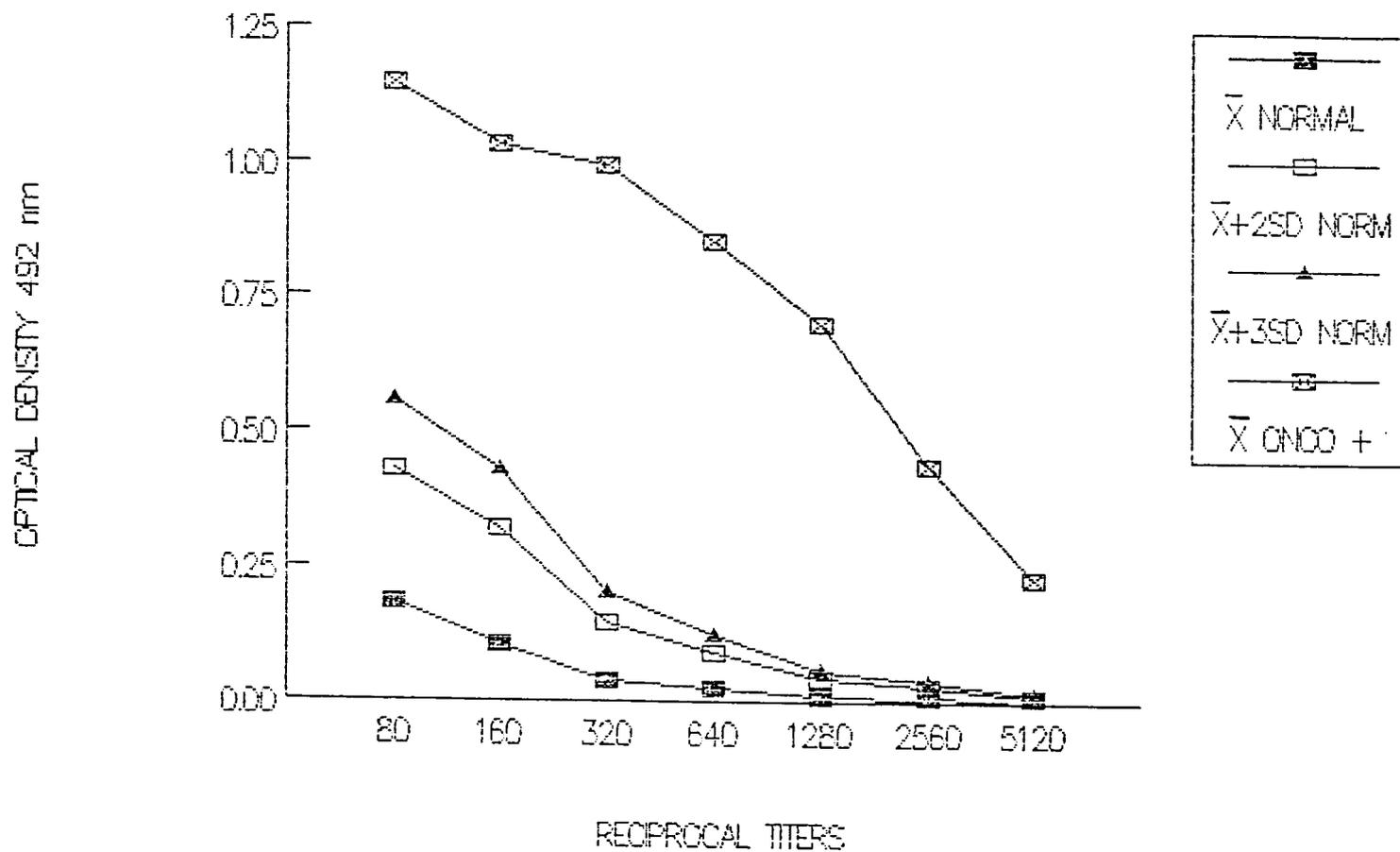


FIGURE 1

BEST AVAILABLE COPY

ANNEX I
ONCOCERCOSIS

Formulario 2. Examen oftalmológico.

Fecha: _____ / _____ / _____ (día) (mes) (año)	I.D. #: <u>ONCHO</u> - _____ - _____ - _____ 07 08 09 10 11 12 13
Nombre: _____	
1er nombre	2do nombre
1er apellido	2do apellido

	OJO DERECHO	OJO SINIESTRO
<u>Agudeza visual.</u>		
sin corrección:	NE _____ 14	NE _____ 15
con corrección:	NE _____ 16	NE _____ 17
cuenta dedos 3 m:	NE NO SI _____ 18	NE NO SI _____ 19
cuenta dedos 1 m:	NE NO SI _____ 20	NE NO SI _____ 21
percibe luz:	NE NO SI _____ 22	NE NO SI _____ 23

	OJO DERECHO	OJO SINIESTRO
<u>Signos y síntomas.</u>		
Enrojecimiento:	NE NO SI _____ 24	NE NO SI _____ 25
Prurito:	NE NO SI _____ 26	NE NO SI _____ 27
Lagrimeo:	NE NO SI _____ 28	NE NO SI _____ 29
Fotofobia:	NE NO SI _____ 30	NE NO SI _____ 31
Ceguera nocturna:	NE NO SI _____ 32	NE NO SI _____ 33

<u>Párpados.</u>		
Edema:	NE NO SI _____ 34	NE NO SI _____ 35
Blefarospasmo:	NE NO SI _____ 36	NE NO SI _____ 37
Triquiasis:	NE NO SI _____ 38	NE NO SI _____ 39
Entropión:	NE NO SI _____ 40	NE NO SI _____ 41
Ectropión:	NE NO SI _____ 42	NE NO SI _____ 43

<u>Conjuntiva.</u>		
Congestión:	NE NO L M S _____ 44	NE NO L M S _____ 45
Edema palpebral:	NE NO L M S _____ 46	NE NO L M S _____ 47
Hiperpigmentación:	NE NO L M S _____ 48	NE NO L M S _____ 49
Hipertrofia papilar:	NE NO SI _____ 50	NE NO SI _____ 51
Herber pits:	NE NO SI _____ 52	NE NO SI _____ 53
Xerosis conjuntival:	NE NO SI _____ 54	NE NO SI _____ 55
Cicatrices:	NE NO SI _____ 56	NE NO SI _____ 57
Folículos:	NE NO #: _____ 58 59	NE NO #: _____ 60 61

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ANNEX I

Fecha: 01/02/03 04/05/06 I.D. #: 0NCHO - 07 08 - 09 10 11 12 - 13

	OJO DERECHO		OJO SINISTRO	
Córnea.				
Queratitis punctata:	NE NO #:	<u>62</u> <u>63</u>	NE NO #:	<u>64</u> <u>65</u>
		<u>66</u> <u>67</u>		<u>68</u> <u>69</u>
		<u>70</u> <u>71</u>		<u>72</u> <u>73</u>
		<u>74</u> <u>75</u>		<u>76</u> <u>77</u>
		<u>78</u> <u>79</u>		<u>80</u> <u>81</u>
Queratitis esclerosante:	NE NO #:	<u>82</u> <u>83</u>	NE NO #:	<u>84</u> <u>85</u>
		<u>86</u> <u>87</u>		<u>88</u> <u>89</u>
		<u>90</u> <u>91</u>		<u>92</u> <u>93</u>
		<u>94</u> <u>95</u>		<u>96</u> <u>97</u>
		<u>98</u> <u>99</u>		<u>100</u> <u>101</u>
Microfilarias vivas:	NE NO #:	<u>102</u> <u>103</u>	NE NO #:	<u>104</u> <u>105</u>
		<u>106</u> <u>107</u>		<u>108</u> <u>109</u>
		<u>110</u> <u>111</u>		<u>112</u> <u>113</u>
		<u>114</u> <u>115</u>		<u>116</u> <u>117</u>
		<u>118</u> <u>119</u>		<u>120</u> <u>121</u>
Microfilarias muertas:	NE NO #:	<u>122</u> <u>123</u>	NE NO #:	<u>124</u> <u>125</u>
		<u>126</u> <u>127</u>		<u>128</u> <u>129</u>
		<u>130</u> <u>131</u>		<u>132</u> <u>133</u>
		<u>134</u> <u>135</u>		<u>136</u> <u>137</u>
		<u>138</u> <u>139</u>		<u>140</u> <u>141</u>
Limbal haze:	NE NO SI	<u>142</u>	NE NO SI	<u>143</u>
Pigmento endotelial:	NE NO SI	<u>144</u>	NE NO SI	<u>145</u>
Panus:	NE NO SI	<u>146</u>	NE NO SI	<u>147</u>
Queratosis:	NE NO SI	<u>148</u>	NE NO SI	<u>149</u>
Xerosis corneal:	NE NO SI	<u>150</u>	NE NO SI	<u>151</u>
Ulceración corneal:	NE NO SI	<u>152</u>	NE NO SI	<u>153</u>
Necrosis corneal:	NE NO SI	<u>154</u>	NE NO SI	<u>155</u>

ANNEX I

Fecha: 01 / 02 / 03 / 04 / 05 / 06 I.D. #: ONCHO - 07 / 08 / 09 / 10 / 11 / 12 / 13

	OJO DERECHO			OJO SINIESTRO		
<u>Segmento anterior.</u>						
Microfilarias vivas:	NE	NO	#:	156	157	
						158 159
Microfilarias muertas:	NE	NO	#:	160	161	
						162 163
Células:	NE	NO	SI			164 165
Sinequia anterior:	NE	NO	SI			166 167
Sinequia posterior:	NE	NO	SI			168 169
Cataratas:	NE	NO	SI			170 171
Episcleritis:	NE	NO	SI			172 173
Iritis:	NE	NO	SI			174 175
Limbitis:	NE	NO	SI			176 177
Uveitis:	NE	NO	SI			178 179
Iridociclitis:	NE	NO	SI			180 181
<u>Segmento posterior.</u>						
Corioretinitis:	NE	NO	SI			182 183
<u>Disco óptico.</u>						
Neuritis:	NE	NO	SI			184 185
Atrofia:	NE	NO	SI			186 187
Mácula afectada:	NE	NO	SI			188 189
Presión intraocular:	NE			190	191	192
						193 194 195
<u>Otros.</u>						
						196 197
						198 199
						200 201
<u>Tratamiento.</u>						
						202 203
						204 205
						206 207
<u>Comentarios:</u>						

<u>Médico examinador:</u>						

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ANNEX III
ONCOCERCOSIS

Formulario 4. Examen dermatológico.

Fecha: <u> </u> / <u> </u> / <u> </u> (día) (mes) (año)	I.D. #: <u>ONCHO</u> - <u> </u> / <u> </u> / <u> </u> - <u> </u> / <u> </u> / <u> </u> - <u> </u> / <u> </u> / <u> </u> - <u> </u> / <u> </u> / <u> </u>
Nombre: <u> </u>	<u> </u> <u> </u> <u> </u> <u> </u>
	1er nombre 2do nombre 1er apellido 2do apellido

Signos y síntomas actuales:			
	1. no examinado 2. ausente 3. leve	4. moderado 5. severo 6. otro	Localización corporal (ver atlas anatómico):
Prurito:	<u> </u> 14	<u> </u> / <u> </u>	<u> </u> <u> </u> <u> </u> <u> </u> 15 16 17 18
Edema:	<u> </u> 19	<u> </u> / <u> </u>	<u> </u> <u> </u> <u> </u> <u> </u> 20 21 22 23
Eritema edematoso:	<u> </u> 24	<u> </u> / <u> </u>	<u> </u> <u> </u> <u> </u> <u> </u> 25 26 27 28
Rash:	<u> </u> 29	<u> </u> / <u> </u>	<u> </u> <u> </u> <u> </u> <u> </u> 30 31 32 33
Pápulas:	<u> </u> 34	<u> </u> / <u> </u>	<u> </u> <u> </u> <u> </u> <u> </u> 35 36 37 38
Excoriación:	<u> </u> 39	<u> </u> / <u> </u>	<u> </u> <u> </u> <u> </u> <u> </u> 40 41 42 43
Liquenificación:	<u> </u> 44	<u> </u> / <u> </u>	<u> </u> <u> </u> <u> </u> <u> </u> 45 46 47 48
Despigmentación:	<u> </u> 49	<u> </u> / <u> </u>	<u> </u> <u> </u> <u> </u> <u> </u> 50 51 52 53
Hiperpigmentación:	<u> </u> 54	<u> </u> / <u> </u>	<u> </u> <u> </u> <u> </u> <u> </u> 55 56 57 58
Hiperqueratosis:	<u> </u> 59	<u> </u> / <u> </u>	<u> </u> <u> </u> <u> </u> <u> </u> 60 61 62 63
Ictiosis:	<u> </u> 64	<u> </u> / <u> </u>	<u> </u> <u> </u> <u> </u> <u> </u> 65 66 67 68
Atrofia dérmica:	<u> </u> 69	<u> </u> / <u> </u>	<u> </u> <u> </u> <u> </u> <u> </u> 70 71 72 73
Fascies leonina:	<u> </u> 74	<u> </u> / <u> </u>	<u> </u> <u> </u> <u> </u> <u> </u> 75 76 77 78
Erisipela de la costa:	<u> </u> 79	<u> </u> / <u> </u>	<u> </u> <u> </u> <u> </u> <u> </u> 80 81 82 83
		(F/D/L) (D/I) (localización)	

Linfadenopatía:			
	1. no examinado 2. no palpable 3. ≤ tamaño frijol 4. ≥ punta dedo índice	5. ≥ dos nódulos 6. ausente 7. presente 8. otro	Localización corporal (ver atlas anatómico):
Axilar:	<u> </u> 84	<u> </u> / <u> </u>	<u> </u> <u> </u> <u> </u> <u> </u> 85 86 87 88
Dolor:	<u> </u> 89	<u> </u> / <u> </u>	<u> </u> <u> </u> <u> </u> <u> </u> 90 91 92 93
Inguino-femoral:	<u> </u> 94	<u> </u> / <u> </u>	<u> </u> <u> </u> <u> </u> <u> </u> 95 96 97 98
Dolor:	<u> </u> 99	<u> </u> / <u> </u>	<u> </u> <u> </u> <u> </u> <u> </u> 100 101 102 103

ANNEX III

Fecha: 01 / 02 / 03 / 04 / 05 / 06
 (día) (mes) (año)

I.D. #: 0 N C H O - 07 08 - 09 10 11 12 13

Región inguinal:		Localización corporal (ver atlas anatómico):			
1. no examinado					
2. ausente					
3. presente					
Hernia:	_____ / _____ / _____ / _____ 104	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	105 106 107 108
"Hanging groin":	_____ / _____ / _____ / _____ 109	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	110 111 112 113
Hidrocele:	_____ / _____ / _____ / _____ 114	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	115 116 117 118

Otros hallazgos dermatológicos.

Dermatitis eczematosa:	_____ / _____ / _____ / _____ 119	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	120 121 122 123
1. localizada					
2. generalizada					
3. prurigo nodularis					
4. eczema infantum					
5. dermatitis atópica					
6. neurodermatitis					
7. eczema					
8. otros					
Impétigo contagioso:	_____ / _____ / _____ / _____ 124	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	125 126 127 128
Acne vulgaris:	_____ / _____ / _____ / _____ 129	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	130 131 132 133
Furúnculos:	_____ / _____ / _____ / _____ 134	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	135 136 137 138
Várices:	_____ / _____ / _____ / _____ 139	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	140 141 142 143
Escabiosis:	_____ / _____ / _____ / _____ 144	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	145 146 147 148
_____	_____ / _____ / _____ / _____ 149	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	_____ / _____ / _____ / _____	150 151 152 153
_____	_____ / _____ / _____ / _____ 154	(F/D/L) (D/I)	(localización)	_____ / _____ / _____ / _____	155 156 157 158

Tratamiento recomendado:	_____	159 160
	_____	161 162
	_____	163 164

Comentarios:	_____

Médico examinador:	_____

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APPENDIX 1

Project Title:

"Analysis of Immunogenic Proteins of Microfilariae, Adult Worms and Excretory/Secretory Products of Onchocerca volvulus," (Proposal 6.225)

Granting Agency:

Program in Science and Technology Cooperation (PSTC)
Office of the Science Advisor
Room 215, SA-18
Agency for International Development
Washington, D.C. 20523, U.S.A.

Project Office: S&T/SCI.

Specific Support Grant No.: DPE-5542-G-SS-7033-00

Innovative Scientific Research Project No.: 936-5542

Project Duration: July 28, 1987 - November 27, 1989

Principal Investigator:

Ricardo Luján, Ph.D.

Institution:

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SUMMARY OF THE PROJECT

Human onchocerciasis is a disease caused by Onchocerca volvulus, a filarial (nematode) parasite. It occurs in several African and Latin American countries, affecting well over 50 million people and causing blindness in about one million others (WHO Tech Rep Ser 1976, No. 597). For effective diagnosis, treatment, prevention, and control of onchocerciasis, however, more information on the biology of the host-parasite relationship and on its pathogenesis is needed (Henson et al., Bull WHO 1979, 57:667; OCP/OCT/83.1 Geneva: WHO 1983).

BEST AVAILABLE DOCUMENT

Diagnostic procedures for onchocerciasis are used for determining the prevalence and intensity of infection, identifying individuals requiring drug treatment, evaluating the success of treatment, and assessing the impact of control efforts (WHO Tech Rep Ser loc cit.; Sasa. Tokyo: Univ Tokyo Press 1978, 166, 687). Present parasitologic methods, however, miss on detecting parasites in the skin or eye because of the low sensitivity of the techniques, leading to an inaccurate and inefficient diagnosis. Alternative immunodiagnostic assays, which are more sensitive and specific for detecting antibodies to or antigenic fractions of O. volvulus in sera of infected individuals, should be tried.

The overall aims of the present study were:

- a) To identify immunogenic proteins associated with the different life cycle stages of the parasite for use in the development of a more highly sensitive and specific diagnostic test for onchocerciasis;
- b) To attempt to correlate the presence of individual immunogenic proteins with the severity of onchocercal disease, thereby providing insight into the host-parasite interaction.

PROJECT FINDINGS AND ACCOMPLISHMENTS

(For the period from July 28, 1987 to November 27, 1989)

Nearly 1,500 Guatemalans living in seven "Fincas" (coffee plantations) located in two areas of different endemicity for onchocerciasis, were examined in this Project. The examinations performed in all participating individuals included the following: Complete biographical information (name, age, sex, place of birth, occupation, length of time living at the community, and house number); ophthalmological examination (with indirect ophthalmoscope and slit lamp); body palpation for presence of superficial nodules and history of previous nodulectomies; four skin biopsies for microfilariae; dermatological examination; stool examination for intestinal parasites; blood group and hematocrit; and collection of serum samples for serology.

It was found that, in spite of limited but regular nodulectomy campaigns in the country, the disease remains as prevalent as many decades ago. For example, between 33.3% to 91.9% of the individuals examined at each "Finca" had onchocerciasis, with a prevalence rate of 20 to 50% in children five to nine years of age; prevalence rates in older individuals approached 95% in some places. Microfilariae were

found in the anterior segment of the eye in 23.3% of the people examined. Intestinal parasites, in particular Ascaris lumbricoides and Trichuris trichiura, were found in more than 80% of the people examined, regardless of age and sex.

Serum samples from 1,136 individuals were obtained in the first visit and an additional 398 to 636 serum samples were obtained in two follow-up visits, at intervals of five to seven months apart from each other. These sera are now being analyzed for IgG antibodies to O. volvulus. Initial findings indicate that significant differences are observed in the geometric mean titers (GMT) between infected and noninfected individuals (5780 vs. 1158). The GMT of control sera for individuals with intestinal parasites was 225, and for uninfected normal individuals, 22. So far, the sensitivity of the ELISA test, using adult soluble antigens, has been 90.5% and the specificity 83.4%. The remaining of the serum samples need to be examined.

Preliminary analysis of specific molecular weight antigens resolved by immunoblot techniques (EITB) in 5-22.5% acrylamide gels, indicates that bands of 200 Kd and 17 Kd are not recognized by patients without onchocerciasis; and antigens of 66 - 97 Kd are recognized by patients with onchocerciasis. One band (14 Kd) was recognized by all sera, including normal human sera. A total of 306 sequential serum samples were obtained from patients with different microfilarial skin loads, in follow-up visits at five to seven month intervals. These sera still needs to be analyzed and the results compared with the clinical conditions of each individual.

In addition, two important findings, not considered at first, have been derived from this Project. One, was the incorporation of migratory ("cuadrilla") population during the examinations at the "Fincas." This group of individuals travel each year, for agricultural purposes, from nonendemic to onchocerciasis-endemic areas at the times of greater transmission. These individuals, however, have never been included in the regular surveillance programs by the Onchocerciasis Department of the Guatemalan Ministry of Health. We found, to our surprise, that 26.3% of 58 individuals were positive for skin microfilariae, two persons had nodules, 10.5% had a history of previous nodulectomies, and 14% had microfilariae in the anterior chamber of the eye along with other signs of onchocercal pathology. The intensity of the infection, however, was significantly lower than in the permanent resident population at the "Fincas." Most of them had yearly migratory patterns which included areas of different endemicity where onchocerciasis is being transmitted. These findings are extremely important, specially if control

measurements by chemotherapy (e.g., Ivermectin) will be done in Guatemala. The "cuadrilla" will have to be included in a national program for onchocerciasis, but more information needs to be obtained before developing a comprehensive program. Immunologically speaking, these individuals represent a naive population which is exposed to onchocerciasis but to a lesser degree and with less frequency than the resident population. If infected, they could possibly serve as reservoirs for continual transmission of the disease, if not treated.

The second finding has been the preliminary analysis of IgG4 antibodies to O. volvulus. The sensitivity but, more over, the specificity of the ELISA test has been improved by the analysis of IgG4 antibodies. More serum samples will have to be examined to verify the validity of the assay.

Taking all of the above findings together, it is valid to assume that a greater geographical extent of the disease may occur in the country because the diagnostic methods routinely used are poorly sensitive. Therefore, the importance of this study is the potential development of a more sensitive method, which can be used for epidemiological surveillance of onchocerciasis in order to have a true idea of the distribution of the disease, specially in areas of low intensity of infection, and for the assessment of control measurements.

APPENDIX 2

"Analysis of Immunogenic Proteins of Microfilariae,
Adult Worm, and Excretory/Secretory Products
of Onchocerca volvulus,"

Proposal 6.225

TECHNICAL WORK PLAN

<u>Proposed Activities</u>	<u>Project Status</u>	
	<u>Accomplished</u> (July 28 '87 - November 27 '89)	<u>Pending</u> (November 28 '89- July 31 '90)

PHASE I.

A.		
a) Selection of communities with high, medium and low prevalence of onchocerciasis	Yes	
b) Initial census and mapping of study communities	Yes	
c) Identification of patients with onchocerciasis at Rodolfo Robles Hospital receiving treatment	No	Ivermectin Treatment will start April - October
B.		
a) Measurement of microfilarial skin densities and examination for onchocercal nodules in residents from the study communities; Idem. at one year interval	Yes Yes	
b) Collection of serum samples and nodules for first visit and follow-up (2nd & 3rd) visits	Yes	4th visit pending
c) Collection of serum samples from residents of Guatemala with and without intestinal parasites; serum samples from serum bank of CDC	Yes	

TECHNICAL WORK PLAN (cont.)

<u>Proposed Activities</u>	<u>Project Status</u>	
	<u>Accomplished</u> (July 28 '87 - November 27 '89)	<u>Pending</u> (November 28 '89- July 31 '90)
d) Processing of nodules for antigen preparation for ELISA and EITB tests	Yes	Microfilarial and E/S antigens will be prepared or cloned antigens will be obtained for EITB
C.		
a) Testing of serum samples (IgG) from patients with onchocerciasis by ELISA test	Yes	Sera of patients from 4 "Fincas"
b) Testing of serum samples (IgE) from patients with onchocerciasis by ELISA test	No	Sera of patients from 7 "Fincas"
<u>PHASE II.</u>		
a) Learn the EITB procedure in Atlanta and adapt to <u>O. volvulus</u>	Yes	
b) Apply EITB procedure in Guatemala	Yes	Need to process 306 sequential serum samples
c) Development of DOT-ELISA test	No	Need to develop
<u>PHASE III.</u>		
a) Data analysis	Yes	Need to complete follow-up visits
b) Data for publication	Presented six works at International Congress	Need to prepare manuscripts for publication
c) Technical Progress Reports	Four Semiannual submitted	Two Semiannual and Final pending