IMMUNOLOGICAL DIAGNOSIS OF ONCHOCERCIASIS IN GUATEMALA

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Immunological technique is one of the most useful tools for diagnosis of parasite infections and has been widely used for prevalence study. In our project, the indirect haemaggulutination test, the double diffution test and the immediate type skin test have been employed, and the usefulness of this technique has been evaluated from two aspects, i.e., specificity and sensitivity.

a) IHA test. The antigen used was an extract of *O. volvulus* adult with PBS, and the blood samples to be tested were collected from the ear lobe of subject by using filter paper. This method of collecting blood on filter papers had various advantages over the more sophisticated methods; since no differences in the IHA titers were observed from those taken by other methods, and the antibody titers were not affected by the long-term storage. With this technique, 119 out of 137 mf positive subjects as well as only 4 out of 50 subjects from onchocerciasis free area showed positive IHA reaction. This result indicated IHA test had high sensitivity and specificity.

b) DD test. Double diffution test by Ouchterlony's method was employed with an antigen extracted from *O. volvulus* adult with PBS. By this technique, 50 out of 58 mf positive subjects and none of 291 subjects from onchocerciasis free areas showed positive reaction. This result also indicated DD test had high level of sensitivity and specificity. However, several disadvantages still exist in this technique for the use in epidemiological surveys, such as requirement of a large amount of test serum and antigen, and the difficulty of the storage of serum in the ambient condition.

c) Skin test. Three sorts of antigens prepared from homologous and heterologous materials were used. An antigen prepared from *D. immitis* was found to be unsatisfactory in terms of the level of sensitivity, since out of 237 cases of onchocerciasis proven by skin biopsy and/or nodule palpation, as many as 70 cases showed negative reaction. Subsequently, by using an antigen extracted from *O. volvulus* adults with VBS, 243 out of 246 mf positive subjects showed positive reaction, whereas as many as 116 out of 291 subjects from onchocerciasis free areas

showed presumable false positive reaction. Thus it is presumed that this antigen may be sensitive enough but lacks in specificity. In addition, an antigen extracted from mf of *O. volvulus* was tested, and the preliminary results showed high levels of sensitivity and specificity. However, in order to use this antigen practically in a large scale, the problems such as the difficulty in collecting sufficient volume of mf and the possible contamination with self antigens remain to be solved.

PROCESAMIENTO DE NODULOS EN EL PROGRAMA DE CONTROL DE LA ONCOCERCOSIS
PRESERVACION Y EXTRACCION DE ANTIGENOS DE *O. VOLVULUS*

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Introducción

El diagnóstico de la oncocercosis puede realizarse por métodos parasitológicos, inmunológicos o por sus manifestaciones clínicas. Los métodos inmunológicos que se han empleado son las pruebas de fijación de complemento, introdermorreacción, hemaglutinación, inmunofluorescencia y pruebas de precipitación. En vista de que todas las pruebas están condicionadas a la naturaleza y calidad de los antígenos de que se pueda disponer los metodos de obtención de antígenos son de primordial importancia.

La obtención de antígenos de *O. volvulus* es difícil, ya que la principal fuente son los nódulos de los pacientes, especialmente si se toma en cuenta que los pacientes con oncocercosis radican en comunidades dispersas con medios de comunicación deficientes y sin recursos de la boratorio.

El Programa de Control de la Oncocercosis visita frecuentemente dichas comunidades y lleva un control de los enfermos conocidos, de donde se obtienen nódulos u oncocercomas que se conservan en formol al 3%.

Con el propósito de optimizar la preservación de los nódulos y la obtención de antígenos describiré a continuación los experimentos llevados a cabo por el Instituto de Investigaciones Biomédicas y el Centro de Investigaciones Ecológicas del Sureste.

A continuación se describen los resultados obtenidos al valorar el mejor método de conservación de nódulos de *O. volvulus* obtenidos de humanos y mantenidos en formol, Glicerol, ó sulfato de amonio, así como el método de extracción de antígenos más adecuado, ya sea KCl 3M o Urea 8M.

Material y Métodos

Se empleó Formol al 3% ó Glicerol al 66% ó Sulfato de Amonio en solución saturada; las brigadas colocaron los nódulos superficiales extirpados de los enfermos de oncocercosis. Los nódulos así preservaods, en el laboratorio fueron homogenizados mecánicamente en una solución de KCℓ 3M o en una de Urea 8M y se sonicaron; se incubaron durante 18 horas en frío con agitación para extraer sus componentes; finalmente los extractos fueron centrifugados para eliminar el material particulado. Los sobrenadantes fueron analizados en cuanto a su contenido total de proteínas y al número de proteínas de distinto peso molecular por electroforesis en gel de acrilamida con mercaptoetanol y dodecil sulfato de sodio y en cuanto a su contenido de antígenos, por inmunoelectroforesis contra suero hiperinmune de conejo inmunizado con un extracto total y contra el suero de 5 pacientes oncocercosos.

En el gel de acrilamida se observan varias bandas de proteínas en los extractos preservados con glicerol o sulfato de amonio y extraídos con KCℓ ó Urea (carriles B y C); de los nódulos conservados en formol no fué posible solubilizar proteínas (carril A).

En inmunoelectroforesis se obtuvieron de 1 a 6 bandas de precipitación: en estas placas se ven las reacciones de los antígenos preservados en glicerol con los que se formaron 6 bandas frente a

los sueros de los conejos hiperinmunes.

Frente a los sueros de pacientes se formaron menos bandas de preipitación y exclusivamente con los antígenos conservados en glicerol y extraídos con urea.

En el cuadro se describen los resultados de la extracción de proteínas de nódulos preservados por los distintos métodos y se expresande tres maneras: % de rendimiento de proteínas con respecto al peso seco del nódulo, número de proteínas diferentes obtenidas, valoradas por electroforesis en gel de acrilamida, y número mínimo de antígenos presentes en cada extracto valorados por inmuno-electroforesis frente al suero de dos conejos hiperinmunes y de 5 pacientes oncocercosos. Como se puede observar la preservación en formol no permitió solubilizar proteínas por lo que todos los resultados fueron negativos. Con sulfato de amonio se obtienen 10 bandas de proteínas pero estas son muy poco antigénicas. Con glicerol se obtiene un mayor número de proteínas (13 y 20) y estas reaccionan con los sueros hiperinmunes dando 5 a 6 bandas, sin embargo con los sueros de pacientes solo el extracto obtenido con urea reacciona, los antígenos obtenidos por estos métodos contienen componentes del hospedero, los que habrán de ser eliminados por un proceso ulterior de purificación.

Conclusiones

Los resultados indican claramente que el método óptimo para la preservación de nódulos de oncocercosos es el glicerol al 66% y que para la extracción de antígenos se debe utilizar urea 8M en solución salina fostatos 0.15M pH 7.4; y que aunque hay componentes del hospedero en el extracto antigénico la reactividad con los sueros de los oncocercosos atestigua fehacientemente de la presencia de antígenos de *O. volvulus*.

SINTOMAS OCULARES DE LA ONCOCERCIASIS EN GUATEMALA

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Introducción:

En Guatemala la población oncocercosa es de más o menos de 30,000 pacientes, y probablemente 0.3–1% son ciegos como resultado de la infección, la cual es una de las más importantes causas de ceguera en Guatemala.

El tratamiento, y control de la enfermedad es difícil en Guatemala, debido a la complejidad topográfica de las zonas oncocercosas. Estudio y temprana detección de casos con alto riesgo de manifestaciones oculares para el estudio, diagnóstico y tratamiento, es una seria responsabilidad del Ministerio de Salud Pública, especialmente, del Departamento de Oncocercosis del SNEM y de sus técnicos.

Esta presentación es el resultado, de cinco años de investigación en el campo oftalmológico de la enfermedad, bajo la dirección del Departamento de oncocercosis del SNEM, y la Agencia de Cooperación Internacional de Japón (JICA), que ha venido funcionando desde el año 1975.

El primer exámen oftalmológico de este programa fué realizado en 1977 por Yamada, encontrando en esa oportunidad que el índice de ceguera por oncocercosis era de 0.3–1% en las áreas endémicas de San Vicente Pacaya. Un segundo y tercer examen fueron realizados en 1978 y 1979 respectivamente, por el mismo autor; el cuarto exámen fue realizado por Méndez en la misma área piloto y alrededores.

Para los fines de la conferencia, presentamos 902 pacientes registrados por nombre, edad, sexo y localización habitacional, que fueron examinados en el área piloto de San Vicente Pacaya (Tabla 1). Se incluyeron a todas las personas de más de un año de edad, habitantes de 6 áreas en San Vicente Pacaya y 5 fincas afuera de la misma. Palín, Departamento de Escuintla, y Finca Buena Vista, Acatenango fueron incluidos para efectuar estudios especiales.

Biopsia de piel, dermatológico y/o exámen oftalmológico fueron preformados. El exámen oftalmológico fué realizado en cuarto obscuro y los signos oftalmológicos fueron escritos en una hoja de trabajo designado por los autores. La microfilaria en la camara anterior, fue examinada por medio de lámpara de endidura después de masaje ocular y/o con la cabeza dirigida hacia abajo por 5 minutos. Examen de fondo de ojo fue realizado por medio de oftalmología directa o indirecta, y con dilatación o sin dilatación de la pupila.

Resultados:

Las manifestaciones de los cambios de piel y los nódulos subcutáneos serán presentados por otros colegas. Generalmente, es dicho que cuando la densidad de microfilaria (Mf) aumenta alrededor del globo ocular, tienden a invadir la conjuntiva. La evidencia de la existencia de Mf en varios tejidos oculares ha sido probada, y la Mf en el nervio óptico y corioretina puede ser invadido de los tejidos por debajo del globo ocular. La mayor parte de pacientes en Guatemala se quejan de inyección conjuntival, irritación y fotofobia. Después de un tiempo Queratitis punteada, Queratitis esclerosante e Iritis agúda son encontradas. Microfilarias vivas son comunmente encontradas en cámara anterior. En etapa tardía coroidoretinitis, degeneración de coroidoretina, coroiditis exuda-

tiva, y atrofia del nervio óptico son generalmente acompañadas de serias opacificaciones de cornea, cataratas complicadas y glaucoma secundario introducen al paciente a ceguera total. Sin embargo en C.A. las lesiones del polo posterior son raras.

En áreas de S.V.P., exceptuando Bejucal que no es una área endémica y cuyos habitantes no están infectados, la prevalencia de lesiones de Córnea, Mf positiva en cámara anterior (AC), positividad de microfilaria y/o nódulos en el cuerpo, se muestra como sigue: 95/155 (61%) en hombres y 61/145 (42%) en jujeres, 26/155 (18%) en hombres y 7/141 (5%) en mujeres, y 121/160 (76%) en hombres y 87/151 (58%) en mujeres (Tabla 1). Tracoma fue comunmente encontrado y en Finca Santa Margarita la prevalencia fue de 27% en ambos sexos.

No ha sido establecido como la Mf juega la parte de patología ocular en oncocercosis, pero existe una obvia relación entre la densidad y distribución de Mf y la intensidad de defectos oculares. Para el estudio se tomaron biopsia en conjuntiva, canto externo, retroa-uricula, cuello, escapula, cintura y pantorrilla, con el objeto de determinar el índice clínico con relación al potencial de peligro para el tejido ocular.

Veintidos pacientes en Palín con positividad de MFAC fueron escogidos en previo exámen. Estos fueron divididos en positivos (13 pacientes) y negativos (9 pacientes), las siguientes lesiones oculares fueron encontradas en asociación infección por Mf; Queratitis esclerosante, Queratitis punteada, Mf en cornea, MFAC, Iritis, Catarata, Opacidades de vitrio, anormalidades de corioretina.

En el grupo con positividad de MFAC, la prevalencia de Mf en conjuntiva fue de 92.3% y en el otro grupo de 33.3%. Existe una gran diferencia de prevalencia entre los dos, (P=0.025). De la misma manera una gran diferencia de prevalencia en canto externo entre los dos (P=0.05). En la asociación con aparición de microfilaria en cornea (MFC); el crecimiento positivo fue de 50% y de 27.8% en el grupo negativo no hay una diferencia significante (P=0.1). La asociación entre cada uno de los grupos y cada una de las lesiones oculares se muestran en tabla 3. Cada una de las diferencias significantes se muestran así: Queratitis esclerosante (SK); (P=0.005), Queratitis punteada (PK) (P=0.10). Iridocilitis (IC) (P=0.05), en opacidades de vitreo y Corioretina no hubo diferencia.

El estudio de pacientes oncocercosos con fluoresceina angiográfica, puede ser de gran ayuda en la deteción de la patogenesis de las lesiones del foco posterior. Este estudio ya fue realizado por Bird (1976), Trojan (1975) y Quarcoopone. Este estudio fue realizado por nosotros en 1978 en veintiún pacientes con microfilaria en cámara anterior, defectos pigmentarios de corrioretina similares a Toxoplasmosis y lesiones corioretineanas oncocercosas, los cuales parecen sugerir que el primer daño de tejidos pigmentario de la retina en la región temporal, progresa a severos cambios coriocapílares, y finalmente lesiones atroficas (tipo degerativo) circunscritas de la retina con vasos coroidales visibles.

De acuerdo con 4 años de investigación en San Vicente Pacaya. Las prevalencias de las lesiones oculares más importantes son así: Queratitis punteada varió entre 8.4–51.8% en ambos masculino y fermenino. Prevalencia de microfilaria en cornea varia entre 0.5–22.2%.Prevalencia de Queratitis esclerosante varia entre 6.7–55.9%. Prevalencia de microfilaria en la camara anterior varia entre 0.6–17%. Prevalencia de lesiones del polo posterior varia entre 1.0–8.8%.

En pacientes con más de 50 años de edad hombres y mujeres; se encuentran signos de Queratitis esclerosante en el 80%. La prevalencia de queratitis punteada alcanza su máximo a la edad de 29 años. Iritis aguda o torpida son más comunes y aumentan con los grupos de edad avanzada. La prevalencia de MFAC parece corelacionarse con la de Queratitis punteada.

Como en Guatemala la mayor parte de lesiones oculares ocurren en el polo anterior del ojo. Es de suponer que los criterios para detección de casos con riesgo de ceguera deben buscarse en el mismo por lo que hemos puesto énfasis en comportamiento y conveniencia de las diferentes formas de Queratitis en distintas áreas oncocercosas.

Fernandez de Castro

En México la prevalencia de problemas oculares severos es por lo menos 20 veces menor (en 1965 solo teníamos alrededor de 50 ciegos para una población de oncocercosos muy similar 30,000 en Chiapas). Como hay simulitud ecológica, etnica, parasitologica entre México y Guatemala, cabe pensar que hay otras variables que deben estudiarse.

Rivas Alacala

¿Los datos estadísticos sobre queratitis punteada son basados sobre pacientes positivos a biopsia y nódulos o de toda la población?

Méndez

Estudiamos a pacientes de más de un año de edad en las zonas mencionadas.

Fernandez de Castro

In Mexico the prevalence of servere ocular problems is at least 20 times less (in 1965 we only had around 50 blinds in an onchocercotic population of around 30,000 in Chiapas). As there is an ecological, ethnical, parasitological similarity between Mexico and Guatemala, perhaps other variables should be studied.

Rivas Alacala

Are the statistical data on punctate keratitis based on patients positive to a biopsy and nodules or on the whole population?

Méndez

We studied patients over one year of age in the mentioned areas.

STATISTICAL ANALYSIS OF ASSOCIATION BETWEEN OCULAR SYMPTOMS AND HEAD NODULES IN ONCHOCERCIASIS IN GUATEMALA

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The results of our previous studies[1)-4)] conducted in 1977 and 1978 have indicated that the main ocular manifestations of onchocerciasis encountered in the endemic areas in Guatemala are the corneal changes frequently associated with iritis, such as reported by Monjusiau et al.[5)] In our previous surveys, corneal changes were noted in 52% (155/296) of the inhabitants examined in our pilot study area and in 60% (275/459) of those in other endemic areas.[3)] The prevalence of sclerosing keratitis increases with advancing age, while punctate keratitis of "fluffy opacities" is more frequently seen in age-groups with longer duration of outdoor labor, or in the group of 19 to 39 years of age. The occurrence of microfilariae (mf) in the anterior chamber has shown profound relation to the density of mf infestation in the skin as well as to the age of the patients. Typical cases of exudative fibrous iridocyclitis, described by Pacheo-Luna[6)] and subsequently incriminated as the primary cause of blindness are rare in onchocerciasis in Guatemala but the incidence of the so-called inactive iritis is noticeably high and rises with the increase of age along with complicated cataract.[3), 4), 7)] The previous surveys also revealed that such conditions as secondary glaucoma, chorioretinal changes and optic neuropathy were rare.

The present study was conducted in order to accumulate data with which comparison could be made from epidemiologic and ophthalmologic points of view with the findings reported by Strong,[8)] Woodruff,[9)] Monjusiau et al.[5)] and Brandling-Benett et al.[10)] Special attention was paid to find out the interrelationship between the occurrence of head nodules and eye lesions.

The present surveys were carried out in 1979 in the three fincas, Pacayal, Santa Emilia and Buena Vista, where onchocerciasis is more or less highly endemic. A total of 369 males and 226 females were examined in these fincas. As shown in Table 1, the rate of persons who had head nodules either in the past or at the time of present survey was 87.4% (277 of 317) in males and 84.4% (152 of 180) in females in the three fincas, namely, 91.8% (45 of 49) in male and 83.8% (31 of 37) in female of Buena Vista, 88.9% (176 of 198) in males and 81.4% (79 of 97) in females of El Pacayal, and 80.0% (56 of 70) in males and 91.3% (42 of 46) in females of Santa Emilia. These rates were not much different among the three fincas. However, the rate of persons with head nodules at the time of the present survey was significantly higher in Buena Vista (40.4% or 21 of 52 in males and 23.2% or 10 of 43 in females), where the regular nodulectomy service had not been done for about 2 years, than in El Pacayal (11.4% or 27 of 237 in males and 9.4% 12 of 128 in females) and in Santa Emilia (12.3% or 10 of 81 in males and 5.3% or 3 of 57 in females), where the nodulectomy service had been performed regularly (Table 2). On the other hand, the prevalence of various ocular manifestations were mostly higher in Buena Vista than in Santa Emilia and El Pacayal. The statistical significance of the difference among the three fincas in rates of various ocular manifestations as estimated from Chi-square test were also worked out, and the detailed figures are being published in Tokai Medical Journal. The results observed in Santa Emilia, where nodulectomy had been done regularly, are virtually consistent with those reported by Brandling-Benett et al, as in Table 1.

The results obtained from surveys of the three fincas indicate that the prevalence of various ocular manifestations in onchocerciasis cases are highly correleted with that of the occurrence or head nodules, and strongly suggests that performance of nodulectomy services at intervals of 6 months is effective in the prevention of developments of various eye lesions.

Table 1. Comparable manifestations between Brandling-Benett *et al*, and present study in Santa Emilia

	No. of case	MFS	Nodules	MFC/ corneal change	MFAC	Bilateral blindness
Brandling-Benett in 1977	100	91 (91%)	87 (87%)	59 (59%)	32 (32%)	4 (4%)
Yamada in 1979	138	97 (70.3%)	116 (84.1%)	70 (50.7%)	20 (14.5%)	1 (0.72%)

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Duke

1. ¿Tiene un estimado de las personas en Guatemala que están ciegas en términos económicos, como resultado de la oncocercosis?
2. ¿Cuál es la frecuencia relativa de la ceguera oncocercósica debido a lesiones del segmento anterior, en comparación con la ceguera oncocercósica debido a lesiones del segmento posterior?
3. ¿Qué proporción de la ceguera del segmento anterior en la oncocerciasis se debe a endociclitis o secuela de la misma, o qué proporción se debe a queratitis esclerosant?
4. ¿Hasta que alcance está disponible el DEC o se usa en pacientes oncocercósicos, y existe alguna evidencia de que el tratamiento con DEC esté produciendo lesiones coroidoretinales.

Duke

1. Do you have an estimate of the number of persons in Guatemala who are economically blind as a result of onchocerciasis?
2. What is the relative frequency of onchocercal blindness due to lesions of the anterior segment compared to onchocercal blindness due to lesions of the posterior segment?
3. What proportion of anterior segment blindness in onchocerciasis is due to iridocyclitis of its sequelae or what proportion is due to sclerosing keratitis?
4. To what extent is DEC available or used by onchocerciasis patients, and is there any evidence that DEC treatment is producing optic neuritis or choroidoretinal lesion?

Yamada

1. Se cree que en el área de San Vicente Pacaya la tasa de ceguera es de 0.3-0.5%. En otras áreas endémicas la tasa es similar a la de San Vicente Pacaya. Así es que parece ser que más o menos 50 pacientes ciegos debido a la oncocerciasis fueron detectados por oftalmólogos, de las 2000 personas examinadas.
2. La queratitis esclerosante es común, pero nunca se ha visto ceguera debido a la misma. La principal causa de la ceguera oncocercósica en Guatemala se debe a complicaciones del segmento anterior del ojo, v.g. cataratas complicadas, bloque de la pubila, y después a iridociclitis.
3. Las lesiones posteriores oncocercósicas en Guatemala son muy pocas. La iridociclitis (inactiva o activa) aumenta con la edad.

   Esta tendencia posiblemente revelará no solo cambios debido a la edad sino una positividad en la cámara anterior.
4. El tratamiento con DEC está disponible, según cero, y el Dr. Zea ya inició el tratamiento de pacientes hospitalizados en el Hospital de Amatitlán. Por seguir investigación de los pacientes en Amatitlán, no hemos encontrado el caso de atrafia optica después de tratamiento.

Yamada

1. In San Vicente Pacaya area, the blindness rate is thought 0.3-0.5%. In other endemic area, the rate is similar to that in San Vicente Pacaya. So it seems to be more or less 50 blind patients due to onchocerciasis detected by ophthalmologists, out of 2,000 persons examined.
2. The sclerosing keratitis is common, but blindness is never seen due to it. Main cause of onchocercal blindness in Guatemala is complications of anterior segment of the eye, e.g. complicated cataract, pupillary block, after iridocyclitis.
3. Onchocercal ocular posterior lesions are very few in Guatemala. Iridocyclitis (inactive or active) increases with age. This tendency is possibly related not only to age change, but to mf positivity in the anterior chamber.
4. DEC treatment is available, I believe, and Dr. Zea starts the treatment to hospitalized partients in Amatitlán. And from our follow-up examination of the patients after treatment, until now, we have never seen optic atrophy cases.

DERMATOLOGICAL SURVEY OF ONCHOCERCIASIS IN GUATEMALA

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A number of skin changes have been reported as clinical manifestations due to onchocerciasis, such as onchocercoma, onchocercal dermatitis, pretibial depigmentation, lymphadenopathy, elephantiasis, and an eruption called "erisipela de la costa". Although a large number of reports have been made concerning the skin lesions associated with onchocerciasis in Africa, little is known about the dermatological characteristics of the disease in Central America. In view of the evidence that considerable differences exist in the clinical and epidemiological features of onchocerciasis among different regions of the Americas and Africa, a series of studies were carried out from 1977 to 1979 in our pilot study area of San Vicente Pacaya, as well as in the surrounding endemic areas in Guatemala on the dermatological aspects of the disease manifestations. The purpose of the present surveys were: 1) to elucidate the characteristics of dermatological manifestations of onchocerciasis in Guatemala, 2) to see whether the results of dermatological examinations would offer useful information in the dynamics of transmission and epidemiology of the disease, especially in connection with the progress of control of the vector, and 3) to see the relationship between the skin manifestations and other signs of onchocerca infection, including the specific immunoglobulin levels.

A comprehensive survey of onchocerciasis was performed on 1,259 inhabitants in San Vicente Pacaya and the surrounding areas. Examination of the microfilarial density was carried out by the skin snip method. There were 610 microfilarial positives (50.4%) out of 1,211 persons examined with 448 males (60.9%) and 162 females (34.1%). Nodules were examined by palpation, there were 404 (32.8%) positives, out of 1,232 persons, with 299 males (40.3%) and 105 females (21.4%). Eczematous dermatitis was seen in 149 persons, the generalized type in 13, and other types in 136. Out of 1,159 indivuduals examined, depigmentation on the lower extremities was seen in 290 persons. The severity was graded from 0 to 4, grade 1 depigmentation was seen in 91, grade 2 in 143, grade 3 in 52, and grade 4 in 4 person. The positives for lymphadenopathy (above grade 2) were 464 (41.2%) out of 1,127 individuals. Itching was seen in 129 (18.3%) of 705 individuals examined by the inquiry card method. Hanging groin, elephantiasis and "erisipela de la costa" were not seen in this endemic area, and the degree of skin changes were slight, as compared with that reported from Africa.

In 1978 and 1979, another dermatological surveys was performed on 1,185 inhabitants in the same areas. There were 259 microfilarial positives (41.6%) out of 623 males, and 97 (17.3%) out of 562 females. The positive rate of nodules was 20.1% in males, and 6.8% in females. The depigmentation on the lower extremities was seen in 30 (4.8%) out of 623 males, and 36 (6.4%) out of 562 females. Five cases presented lesions agreeing with the so called "pretibial depigmentation", and all of them were males. On the other hand, the traumatic scars on the lower extremities were seen in 195 (31.3%) out of 623 males, and in 159 (28.3%) out of 562 females. In order to see the relation between the blackfly bites and the skin changes, the frequency of blackfly bites was

recorded on face, upper extremities and lower extremities of the inhabitants. The positive rates of blackfly bites on face and upper extremities were similar to the microfialria positive rate. There was a higher frequency of blackfly bites on face and upper extremities in the microfilaria positive group, than in the negative group. From those results, it is suspected that the skin changes of onchocerciasis are caused not only by *Onchocerca volvulus*, but also by other factors.

The serum levels of the four immunoglobulins, IgG, IgA, IgM and IgE, were determined quantitatively by the laser immunoassay system or the radioimmunosorbent test in Guatemalan onchocerciasis patients. The IgG and IgE levels in the Guatemala onchocerciasis cases with positive *Onchocerca volvulus* microfilariae were significantly higher than the levels in the Guatemalan controls with negative microfilariae and in the Japanese healthy controls. However, no diffenence in the levels of IgA and IgM was observed between the Guatemalan onchocerciasis patients group and the two control groups. Furthermore, a study with ELISA method for specific detection of IgG and IgE antibodies against *Onchocerca volvulus* antigen was performed. One of the interesting findings in this study was that IgG- and IgE-OC-ELISA values were clearly elevated in onchocerciasis patients who were microfilariae positive in the skin snip examination, and a good correlation was found between the levels of IgG and IgE antibodies.

Ojordan

Nosotros también hemos medido los niveles de anticuerpos IgE en pacientes oncocercósicos y hemos encontrado que eran bastante altos que es común en una infección parasítica. Pero además, hemos encontrado un lato nivel de complejos circulantes de IgE. ¿Es posible que sus resultados se deban a estos últimos factores (complejos) y no necesariamente a un fenómenos específico del antigeno del parásito?

Yoshimura

Los valores de IgG-OC-ELISA y los valores de IgE-OC-ELISA que están mostrados eran todos especificos contra antigeno de *O. volvulus.*

Ojordan

We have also measured the IgE antibodies level in Onchocerciasis patients, and have found they were very high, which is common in a parasitic infection. But in addition, we have found a high level of circulating IgE complexes. Could your results be due to these last factors (complexes) and not necessarily to a specific phenomenon to the parasite antigen?

Yoshimura

IgG-OC-ELISA values and IgE-OC-ELISA values shown here were all specific against *Onchocerca volvulus* antigen.

UN ENSAYO DE QUIMIOTERAPIA PARA ONCOCERCIASIS EN GUATEMALA

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Una de las principales quejas que a diario presentan las personas que habitan áreas oncocercosas, es derivado del hecho de que frecuentemente se les examina dentro de las actividades parasitológicas de las campañas de investigación de la enfermedad y que no se les proporciona ningún tipo de tratamiento a sus afecciones.

Practicamente la totalidad de los estudios que se hacen para control y erradicación de la oncocerciasis, van encaminados exclusivamente al control del vector, lo cual ha ido dejando en el olvido a las personas ya infectadas y con alto riesgo de ceguera. No se han desarollado nuevas drogas para tratamiento. Mucho se ha descrito sobre las reacciones secundarias de medicamentos "tradicionales" como la dietilcarbamazina, las cuales se ha intentado usar como tratamiento en masa dentro de las poblaciones afectadas. El presente trabajo pretende hacer una correlación clínica de los hallazgos de la enfermedad con lo cual se puede hablar de un "síndrome oncocercoso", y fijar así las normas para la búsqueda de nuevas drogas efectivas contra la enfermedad y libres de reacciones secundarias para el paciente. Dentro del aspecto "clínico" de la oncocerciasis es poco lo que hemos podido avanzar debido a carencia de recursos para implementarlo. Sin embargo, está ya contemplado el efectuar estudios histo-patológicos y exámenes serológicos a los pacientes oncocercosos.

Se ha iniciado recientemente un estudio electroencefalográfico de los pacientes oncocercosos, dado que hemos podido observar un número considerable de pacientes con "síndrome convulsivo" dentro de la población de áreas de alta endimicidad.

Para los fines de este ensayo de quimioterapia, estudiamos a 252 personas adultas, predominantemente del sexo masculino, provinientes de Pochuta, Chimaltenango, Guatemala, a los cuales se les practicó biopsia de piel en las regiones escapular e ilíaca izquierdas, mediante el sclero corneal punch tipo Holth, habiéndose encontrado 238 personas positivas y 14 negativas; a éstas últimas se les hizo diagnóstico mediante la prueba de Mazzotti. Del total de los pacientes tratados, 93 (36.9%) manifestaron presentar molestias oftalmológicas de las cuales predominó la disminución de la agudeza visual y fotofobia (72.04%).

Ciento veinticinco (125) pacientes (49.6%) manifestaron encontrarse asitomáticos al momento de iniciar este estudio. Se palparon y extrajeron 136 nódulos de los cuales 98 (72.05%) se encontraron en la cabeza. Se efectuó un conteo de glóbulos blancos antes del tratamiento y tres días después encontrando un aumento considerable en número, principalmente a expensas de eosinófilos. Previo al tratamiento se efectuaron electrocardiogramas a los pacientes, no encontrando ningún cambio atribuible a esta enfermedad.

Luego de estos exámenes preliminares, se les inició tratamiento con dietilcarbamazina en dosis entre 3 y 10 mg/kg de peso corporal y las reacciones secundarias presentadas predominantes fueron:

1o. Prurito: en 176 pacientes (69.84%), de los cuales fue de leve a moderado en su mayoría.
2o. Fiebre: en 112 pacientes (44.44%) de los cuales fué leve (37°–38°C) en su mayoría.
3o. Eritema: en 41 pacientes (16.27%).
4o. Edema: en 31 de ellos (12.3%) de los cuales el edema facial fué predominante (38.71%).

Del total de pacientes tratados las reacciones se presentaron severas en 7 de ellos (2.78%) a quienes hubo de suspendérseles el tratamiento.

Conforme iban terminando el tratamiento y con una periodicidad de quince (15) días después de terminado el mismo, los pacientes eran examinados de nuevo en sus áreas de residencia. De los 252 pacintes clasificados como positivos, 139 (55.15%) se encontraron negativos y 103 (40.88%) permanecieron positivos aunque se notó una disminución en la densidad de microfilarias en piel. No fué posible examinar a 10 pacientes (3.97%).

Para minimizar los efectos gástricos secundarios de la dietilcarbamazina, se proporcionó a los pacientes, antes del tratamiento, Pamoato de Pyrantel (Combantrin Compuesto* Pfizer). Actualmente estamos trabajando en un grupo de pacientes sin darles este medicamento ya que llama la atención que las molestias gastro-intestinales fueron "cero". Para contrarestar el edema y el prurito se utilizó el antihistaminico Prometazina (Fenergan* Specia) y para control de fiebre y dolor se administró aspirina.

Con los resultados expuestos, considero que la administración de dietilcarbamazina en dosis "no peligrosas" es posible en pacientes hospitalizados como agente microfilaricida. Deben apoyarse los estudios encaminados a dilucidar el comportamiento clínico de la enfermedad.

El equipo oftalmológico del Programa Guatemala-Japón, sobre la Enfermedad de Robles, hizo una evaluación de los habitantes de las áreas de donde provienen los pacientes y se encontró que las lesiones fluffy disminuyeron hasta en un 81.3%. La microfilaria en Cámara Anterior desapareció en el 100%. Los cambios efectivos de Cornea Precipitada se vieron en 59.3% de los casos estudiados.

Fernandez de Castro

En México hemos logrado buena aceptación de la DEC (es decir, no es ese el problema). El fracaso, es que no hemos logrado abatir la transmisión porque la DEC no negativa totalmente al paciente y sólo ocurre en un 40% la negativización.

Pero estamos temiendo mucho problema con los grupos religiosos que recomiendan no tratamientos médicos de ninguna clase.

Rivas Alacalá

¿Cuánto tiempo posterior a la administración de DEC fue hecha la evaluación oftalmológica?

Zea

La evaluación se efectuó 3 meses después del tratamiento.

Ruben Dario Martinez

¿Dosis de DEC? ¿Y por cuantos días?

Zea

La dosis fue variable entre 3 y 10 mg/kg peso corporal durante 10 días.

Fernandez de Castro

In Mexico we have been able to have good acceptance of DEC (that is, that is not the problem). Its failure is that we cannot interrupt transmission as DEC does not make all the patients negative. This ocurrs only in 40% of the patients.

But we fear that we will have many problems with religious groups who recommend no medical treatments of any kind.

Rivas Alcalá

How long after the administration of DEC was ophthalmological evaluation made?

Zea

The evaluation was carried out three months after the treatment.

Ruben Dario Martinez

What was the dosage of DEC and how many days?

Zea

The dosage fluctuated between 3--10 mg/kg of body weight for 10 days.

Ruben Dario Martinez

Hay que utilizar la DEC en diferentes dosis según el nivel de endemicidad de las localidades, igualmente durante diferente tiempo.

Es posible negativizar de mf en piel de los oncocercosos si se continúa con el tratamiento trimestral en 3 ocasiones efectivas.

Ruben Dario Martinez

DEC has to be used at different dosages according to the level of endemicity of the localities, and likewise, for different periods of time.

It is possible to make mf negative in the skin of onchocercotic patients if quarterly treatment is continued on three effective ocassions.

TRABAJOS ADICIONALES

RESOURCE PAPERS

SEASONAL PREVALENCE, DIURNAL BITING ACTIVITY AND THE BEHAVIOUR OF ONCHOCERCIASIS VECTORS IN GUATEMALA

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In order to see the seasonal prevalence of the vector species, collections on human bait were made for two hours, from 8:30 to 10:30 hours every 10 days in 1976 and 1977. Two sampling stations, Guachipilín and Injerto, were chosen from the northern part of the pilot control area, and another two stations, Finca Hamburgo and Finca San Nicolás, from the southern part. Many females of *Simulium ochraceum* were collected from the northern stations but they were rather scarce in the southern stations. At the station Guachipilín the female density of *S. ochraceum* was high in the dry season from November to April, while in the rainy season it was low. At the station Injerto, no clear tendency such as seen in Guachipilín was observed, and the female density kept a high level through-out the year, with the highest density of the year in June. The female density of *S. ochraceum* seems to depend on the condition of the breeding sites of immature stages in the streams near the station. In the rainy season, frequent flushes of large amount of water seemed to wash away most of the simuliid larvae. However, the rainfalls are not uniform even in the pilot area, often restricted to certain valleys while in other valleys the streams are kept constant for two or three weeks without flushes, thus allowing the breeding of the vectors. In such cases the density of flies suddenly increases. *Simulium metallicum* showed a different pattern of seasonal prevalence in the four stations. In Guachipilín the female density maintained high level throughout the year. In Injerto it was similar to that of Guachipilín but the density was higher in the rainy season than in the dry season. In Hamburgo and San Nicolás, the density was high in the dry season. During the rainy season the low density continued in San Nicolás, while in Hamburgo a sudden increase was observed in "canícula" when the rain stopped for several weeks. For the species such as *S. ochraceum* and *S. metallicum* which breed in both the temporal and the permanent streams, the seasonal prevalence is highly affected by the rain fall near the station. *Simulium callidum* breeds in streams with relatively large volume of water discharge, and showed the same pattern of seasonal prevalence in the four stations; the female density was higher in the dry season than in the rainy season, showing slight increase when the rain stops for a few weeks.

The investigation on the daily biting rhythms of female flies were carried out for three days in the first half of the dry season in and outside of the pilot area. In each day collections were made at two different sites in coffee plantations of the same area. A total of 6 observations were repeated.

For the first half of each hour the flies landed on human baits were collected, and for the latter half the collection was suspended. The biting activity of *S. ochraceum* commenced at dawn at 6:00 hours and ceased towards evening at 17:30 or 18:30 hours of the day, but the daily activity patterns were variable by the days and the sites. In two cases there were two clear peaks, one in the morning and the other in the afternoon. The morning peak occurred at 9:00 or 11:00 hours, and the afternoon peak occurred at 15:00 hours. In the rest cases no marked peaks were observed; the biting density increased till 8:00–9:30 hours, then somewhat fluctuated, and after 16:00 hours it began to decrease toward evening. The activity of *S. metallicum* began at 6:00

hours and ended at 18:30 hours, same as in *S. ochraceum*; it showed a large and a small peak; if the peak in the morning was large, the peak in the afternoon was small; in the contrary, if the peak in the morning was small, then the peak in the afternoon was large. The morning and the afternoon peaks of *S. metallicum* occurred earlier than those of *S. ochraceum*, and appeared in 6:00–8:30 hours and in 14:00–15:30 hours, respectively. Such patterns of diurnal bitting activity seemed to change according to environmental and meterological factors, such as temperature, humidity, light intensity, etc.

In order to see the biting behaviour, two kinds of collections using human and animal baits were carried out, and at the same time observations were made on the flight activity of the females attracted to the bait.

In the first experiment the flies attracted to human baits sitting on a chair or on a stone were collected separately according to the body parts of their landing, whether on the upper half (trunk, head and arms) or on the lower half (legs). In *S. ochraceum*, 65% were captured from the upper body parts, while 80–90% of *S. metallicum* and more than 90% of *S. callidum* were collected from legs. In another experiment, differences in bitting densities among man and some animals commonly found in the pilot area were investigated; at a distance of about 5 m, man, cattle, horse, dog and goat were placed on a circle and flies attracted to them were captured.

S. ochraceum were found to be attracted more on man than on cattle, horse, and dog, but fairly large number of *S. ochraceum* were collected on goat.

Furthermore, it was observed that females of *S. ochraceum* came to bite man usually landed directly on the skin and immediately started to suck blood without hesitation, and even when the collector disturbed their blood sucking, they soon came back after a short flying. On the other hand, females of *S. ochraceum* attracted to an animal bait were flying around on the animal back, and could not easily land; even when they landed, this was mostly on the back where long and dense hairs prevented them from reaching the skin to suck blood; they were mostly struggling in the hairs for a while and then flew away. Such an observation suggests that actual blood-sucking of *S. ochraceum* on animals is very rare. On the other hand, many females of *S. metallicum* and *S. callidum* were found to be flying around legs of human bait but could not easily land. Even when they landed, they were very nervous and by a slight distrubance they flew away from the skin before commencement of the blood sucking. However, females of *S. metallicum* and *S. callidum* behaved in a quite different manner on the animals; they readily landed on animals belly where hairs are short and thin, without spending a long time flying around the animals, and easily reached the skin, where they could easily engorge with the blood.

LABORATORY OBSERVATIONS OF GONOTROPHIC CYCLE OF *SIMULIUM OCHRACEUM*

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In order to determine the gonotrophic cycle of *Simulium ochraceum* in Guatemala, flies newly emerged from wild collected pupae and blood engorged flies caught on human bait in the field were reared in the laboratory. They were dissected day by day to observe their ovariole developments. From the results obtained, the gonotrophic cycle was estimated.

The parous rate as well as the rate of flies with sac-like relics in the female collected in the afternoon were higher than those in the morning. These facts indicate that the majority of parous flies come to bite man soon after oviposition. In other words, the flies with large sac-like relics captured in the afternoon seem to have laid eggs in the morning on the same day, and the flies with smaller sac-like relics captured in the morning seem to have laid eggs in the afternoon the day before.

Most of *S. ochraceum* coming to bite man in the field had follicles at stage I (including IN, Ia and Ib) with some exceptions in some areas.

In the female flies kept at 22°C immediately after emergence, the follicle developed to stage Ib took place on day 2, and remained at the same stage until day 9.

The flies which showed the fastest ovariole development were estimated to mate and take the first blood meal on the day of the emergence. At least four days were required for the maturation of ovarium in the engorged female collected in the field and kept in incubator at 22°C, and the deposition of eggs took place on the next day. The 2nd blood meal is taken probably on the same day. Thus, one gonotrophic cycle at 22°C seems to be 5 days in the shortest case. Garms and Ochoa (1979) reported that the gonotrophic cycle of this species was 2 to 4 days, but if the day of oviposition is added as we did, it becomes 3 to 5 days.

Assuming that the average atomosphelic temperature in the endemic areas in Guatemala is 20 to 25°C, 8 days seem to be needed for the microfilariae to become infective larvae under the field conditions. This means that *S. ochraceum* can transmit the infective larvae of *O. volvulus* at the 3rd or the later blood meal, or 9 or more days after taking the infective blood meal.

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Table Presumptive relationship between gonotrophic cycle of *Simulium ochraceum* and transmission of *Onchocerca volvulus* from laboratory experiments

	Simulium ochraceum					*Onchocerca volvulus*	
Days after emergence	Gonotrophic cycle (shortest case)	Survival rate after blood feeding*	Days after blood-feeding			Development of Mf. in S. *ochraceum* at 25°C**	30°C***
1	emergence						
2	mating?						
3	1st blood feeding	100%	0			Mf. taken by *S. ochraceum*	
4	1st	86.6	1			↓	↓
5	gonotrophic	75.0	2				develop to infective larvae
6	cycle (5 days)	64.9	3				↓
7		56.2	4				larvae migrate to head
8	1st oviposition and 2nd blood feeding	48.7	5	0		develop to infective larvae	become transmissible
9	2nd	42.2	6	1		↓	
10	gonot.	36.4	7	2			
11	cycle	31.5	8	3		larvae migrate to head	
12		27.3	9	4		↓	
13	2nd oviposition and 3rd blood feeding	23.7	10	5	0	become transmissible	
14	3rd	20.5	11	6	1		
15	gonot.	17.8	12	7	2		
16	cycle	15.4	13	8	3		
17		13.3	14	9	4		
18	3rd oviposition and 4th blood feeding	11.6	15	10	5		
19	4th	10.0	16	11	6		
20	gonot.	8.7	17	12	7		
21	cycle	7.5	18	13	8		
22		6.5	19	14	9		
23	4th oviposition and 5th blood feeding	5.6	20	15	10		

* From the Tables 1 and 2, the duration of one gonotrophic cycle of *S. ochraceum* is 5 days and average parous rate is 48.7%, hence the daily survival rate is 0.866 (Davidson, 1955).

** Matsuo *et al.* (1980) *** Collins (1977)

EXPERIMENTAL OBSERVATION OF DEVELOPMENTAL PERIOD OF *ONCHOCERCA VOLVULUS* IN THE GUATEMALAN BLACK FLY, *SIMULIUM OCHRACEUM*

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For the purpose of determining the exact rate of development of *Onchocerca volvulus* in the Guatemalan black fly, *Simulium ochraceum*, the female flies engorged on volunteers with microfilariae of *O. volvulus* were collected, and were reared in an incubater at 25°C and 20°C with the method of Matsuo et al. (1978).

At 25°C, a total of 133 living flies were dissected. Ninety-five 1st and 2nd stage larvae were found in 24 flies and 82 3rd stage larvae in 31 flies. Three days after ingestion, the parasites were first seen in the thoracic muscles of flies as 1st stage larvae, measuring 181–231 μm long, with a wide body and a definite tail. Five days later, 1st and 2nd stage larvae were seen in thorax. After six or seven days, the majority of larvae were seen in thorax as 2nd stage larvae, measuring 441–546 μm long, with esophagus and intestine. On the 8 day of infection, 3rd stage larvae were first seen in the head of flies. On the following days, majority of the 3rd stage larvae were seen in head, but some in thorax and abdomen. These larvae measured 558–683 μm long. The lengths of the anterior esophagus, posterior esophagus and intestine comprised 18–22%, 41–44% and 28–33% of the total body length, respectively, Measurements and morphological characters of these larvae were similar to those in the African black fly, *S. damnosum*, as described by Duke (1967, 1968).

At 20°C, a total of 49 living flies were dissected during 10–15 days after ingestion. Two 1st stage larvae were seen in the thorax of 2 flies on day 12, 2 on day 15. Five 2nd stage larvae were seen in the abdomen and thorax of 3 flies on day 15. One 3rd stage larva was seen in the abdomen of 1 fly on day 11, and measured 555 μm long. At this temperature, far smaller numbers of larvae were found in the flies as compared with those kept at 25°C, and the development of these larvae was more irregular. It seems that the lowest temperature to allow the full development of the *Onchocerca volvulus* larvae in the flies is close to 20°C.

Watanabe et al. (1979) studied the development of ovaries of the Guatemalan *S. ochraceum*, and estimated that the length of gonotrophic cycle was 5 days at 22°C. Therefore, assuming that *S. ochraceum* adults deposit eggs and take subsequent blood meal on the same day, it is estimated that the flies infected at their 1st blood meal are capable of transmitting the infective larvae when they take the 3rd or subsequent blood meal at about 25°C.

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Table 1. Development of larvae of *Onchocerca volvulus* in *Simulium ochraceum* maintained at 25°C

Days after ingestion of microfilariae		3	5	6	7	8	9	10	11	12	13	14
Stage of larvae found	Abdomen	I	I	II			I	III		III		III
	Thorax	I	I	I II	II		I III		III	III	I III	
	Head					III	III	III	III	III	III	III

I: first stage, II: second stage, III: third stage

EFFECTS OF INSECTICIDE TREATMENT OF STREAMS FOR THE CONTROL OF BLACKFLY LARVAE ON THE NON-TARGET ORGANISMS

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A series of studies were conducted with the mountain streams in Japan in the effects of insecticide applications for the control of blackfly larvae on non-target organisms. In general, treatments of the streams with some selected organophosphorous insecticides at doses sufficient for killing blackfly larvae caused more or less extensive deaths of insects and other related arthropods living in the same streams, but there were little toxic effects on algae, plants and lower or higher animal groups. Such an effect was found to vary greatly according to the kind of insecticides used, and their doses. After the insecticide application, massive growth of algae (especially diatomes) frequently accurred on the stream beds, due to absence of phytophagous insects. Then, after some time, unusual breeding of chironomid larvae feeding on these algae was usually observed.

In Guatemala, the effects of temephos application into the breeding streams of *S. ochraceum* were studied by Hasegawa. The insecticide was applied, as a rule, at a dose of 0.1 ppm per 60 minutes flow volume. Quantitative collections of bottom inhabiting organisms were made before and after the treatment. Animals flushed off by the insecticide were collected by drift nets. The insecticide treatments were carried out twice on a stream (No. La 4-1 of Los Lavaderos), 27th of March and 10th of April, 1979. On 24th of April, about two weeks after the second treatment, a flushing out of all the insects were tried by application of Dursban-methyl at a dose of 10 ppm per 10 minutes flow volume into the same spot. List of invertebrates (by Order and family) collected at this survey is shown in Table 1.

Table 1 List of invertebrates collected from the test stream (Tributary No. La. 4–1 of Los Lavaderos, Guatemala)

Ephemeroptera	– Caenidae
Plecoptera	– Perlidae
Trichoptera	– *Arctopsyche* sp.
	Dolophilodes sp.
	Micrasema sp.
	Trichoptera
Diptera	– Chironomidae
	Dixidae
	Psychodidae
	Simuliidae
	Tipulidae
Coleoptera	– Elmidae
Hemiptera	– Naucoridae
Odonata	– Anisoptera
	Zygoptera
Turbellaria	– Planariidae
Malacostraca	– Decapoda
Oligochaeta	
Anura	

The numbers of invertebrates collected from four Surbar-net samples (each from 1,000 m^2 area) set in the bottom of the stream 7 days before the insecticide application as well as those collected at weekly intervals from the insecticide application are shown in Table 2. The total number of blackfly larvae found before the insecticide treatment was 42 (including 16 *S. ochraceum* larvae), and constituted 11% of a total of 387 invertebrates found in the four Surber-net samples. On the next day of the treatment, still 25 blackfly larvae (including 22 *S. ochraceum* larvae) were found in the samples, but none thereafter (Table 2).

Table 2 Numbers of invertebrates found in four Surber-net samples before and after application of temephos (Stream No. La 4-1)

	DAYS AFTER THE APPLICATION						
	−7	1	7	14	21	28	Total
Caenidae	10	22	17	17	3	1	70
Perlidae			1	2		1	4
Arctopsyche sp.	24	11	17	15	5	2	74
Micrasema sp.		1			8	5	14
Chironomidae	70	17	7	7	15	4	120
Dixidae	2				1		3
Psychodidae					2		2
Simuliidae	42	25					67
Tipulidae	6	9	8	2	21	7	53
Elmidae	205	257	180	174	78	51	945
Naucoridae			1		2		3
Zygoptera	8	6	3		3	4	24
Planariidae	4	35	95	80	47	30	291
Decapoda		1			2	1	4
Oligochaeta	16	21	12	13	5	6	73
Anura		1				5	6
Total	387	406	341	310	192	117	1,753

The numbers of invertebrates collected by the drift net at half an hour or hourly intervals after application of temephos into the stream are shown in Table 3. Of a total of 358 invertebrates collected during the 5 hours period, 301 or 84.0% constituted blackfly larvae, among which 55% were those of *S. ochraceum* (Table 3).

Table 3 Numbers of invertebrates collected with a drift net at hourly intervals after application of temephos into a stream (No. La 4-1)

	Hours after the application						
	0.5	1	2	3	4	5	Total
Caenidae		1	1	1	2	1	6
Arctopsyche sp.	1	2	2			3	8
Chironomidae		3	1	1	3	3	11
Dixidae		2				1	3
Simuliidae	1		11	37	77	175	301
Tipulidae						2	2
Elmidae	4		4	5	4	6	23
Naucoridae			1				1
Zygoptera						2	2
Planariidae				1			1
Total	6	8	20	45	86	193	358

In the all-flushing experiment using Dursban (Chlorpyriphos) methyl carried out after two weeks of the second temephos treatment, a total of 3,849 invertebrates were collected with drift nets set 25 m, 50 m and 75 m down from the insecticide application spot, among which Simuliidae larvae were only 15 or 0.39% of the total invertebrates (Table 4).

Table 4 Numbers of invertebrates collected with drift nets during 2 hour period after application of Dursban methyl at a dose of 10 ppm per 10 minutes flow volume (two weeks after the second application of temephos, Stream No. La 4-1)

	Distrance from the application point			
	25m	50m	75m	Total
Caenidae	48	124	110	282
Perlidae			3	3
Arctopsyche sp.	185	307	869	1,361
Dolophilodes sp.	35	28	5	68
Micrasema sp.	7	9	2	18
Trichoptera	6			6
Chironomidae	155	93	152	400
Dixidae	8	13	14	35
Psychodidae	15	9	6	30
Simuliidae	4	3	8	15
Tipulidae	42	31	46	119
Elmidae	133	599	472	1,204
Naucoridae	1		1	2
Anisoptera	4	10	3	17
Zygoptera	3	3	17	23
Planariidae	24	2		26
Decapoda	94	1		95
Oligochaeta	27	78	40	145
Total	791	1,310	1,748	3,849

On two polyvynyl sheets (10 x 30 cm) set 7 days before the temephos application on rocks in the bottom of the stream, 31 and 50 blackfly larvae were found attached before exposure to the larvicide, but they all disappeared after 3 or 4 hours from exposure to the larvicide (Table 5).

Table 5 Numbers of blackfly larvae found attached on two polyvynyl sheets (10 x 30 cm) placed on the bottom of the stream before and after temephos application (Stream No. La 4-1)

Trap No.	Before application	Hours after the application					
		0.5	1	2	3	4	5
No. 1	31	31	26	9	3	0	0
No. 2	50	50	23	1	0	0	0

In another experiment conducted by Y. Tabaru and others on 8 August 1978, temephos was introduced into another stream (No. La 6-3) at a dose of 1 ppm per 10 minutes flow volume (1.7 times higher than in the previous experiment); out of a total of 1,837 invertebrates collected with drift nets after application of the larvicide, 1,317 or 72% were blackfly larvae and 55% among them were those of *S. ochraceum*: the most abundant among the other groups were those of Chironomidae, which constituted 21% of the total invertebrates thus collected (Table 6).

Table 6 Numbers of invertebrates collected with drift nets after application of temephos at a dose of 10 ppm per 10 minutes of flow volume (8 August 1978, Stream No. La 6-3)

	Hours after the application										
	0.5	1	1.5	2	3	4	5	10	15	20	Total
Caenidae					6						6
Arctopsyche sp.	4	3	1	15	20	4	5	11	1	4	68
Chironomidae	1	35	38	119	161	2	19	2	1	5	383
Psychodidae		1	5	5		1				1	13
Simuliidae	1	217	410	348	170	12	60	21	10	68	1,317
Tipulidae	1	1		1			3			3	9
Elmidae	1	2	2	5	4		3	3	3	6	29
Nucoridae			1								1
Anisoptera		2									2
Zygopterz		1			1						2
Decapoda							1				1
Oligochaeta				3	2						5
Anura	1										1
Total	9	262	457	496	364	19	11	37	15	87	1,837

It has been shown from these experiments that the applications of temephos into streams at doses of 0.6 or 1.0 ppm per 10 minutes flow volume were highly effective as the blackfly larvicide, but caused almost no or only a little toxic effects on other non-target invertebrates found coexisting in the same streams.

LABORATORY ANIMALS FOR STUDIES ON *ONCHOCERCA VOLVULUS* MICROFILARIAE

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Research on *Onchocerca volvulus* has been hampered by the lack of a small laboratory animal host. The chimpanzee has been the only acceptable experimental host for *O. volvulus* (Duke, 1962) but it is not possible to use large number of chimpanzees in the laboratory. In an attempt to maintain microfilariae in the laboratory, Nelson et al. (1966) and Rabalais (1974) introduced rodents as proxy hosts for the study of *Onchocerca gutturosa* and *O. cervicalis* microfilariae. Their success suggested that rodents might serve as an alternative to the chimpanzee for the study of *O. volvulus* microfilariae in proxy hosts.

Materials and Method

O. volvulus microfilariae obtained from onchocercomas were inoculated into mice subcutaneously in the inguinal region, in the scalp, or intraperitoneally. To recover microfilariae from animals, all tissues and organs were minced in saline in petri dishes. The fluids were centrifuged and the sediment was examined for microfilariae.

Results and Discussion

Microfilariae inoculated in the inguinal region disseminated rapidly, invading the tail, lungs and kidney within 1 h and the ears within 3 h postinoculation (PI). The number of microfilariae increased steadily with time in various organs and tissues with majority invading the tail. Live microfilariae of *O. volvulus* were recovered from mice over a period of 12 weeks. Approximately 14% of the injected microfilariae were recovered at 36 hr, 4.4% at 2 wk, and 0.6% at 4 wk PI (Table I). These results are lower in terms of longevity and recovery rates than the observations of Nelson et al. (1966) and Rabalais (1974). The distribution pattern of microfilariae in mice changed over the duration of infection. During the early period of infection microfilariae appeared in the ears, viscera, pelt, carcass and tail; later, the microfilariae tended to accumulate in the tail (Table I). In contrast to Nelson et al. (1966), our experiments demonstrated a concentration of microfilariae in the tail when inguinal and peritoneal inoculation were used, whereas microfilariae concentrated in the ears following inoculation into the scalp (Table II). These differences might be due to the fact that different species of microfilariae were used in each study. It is, however, interesting that the ears as well as the tail are the preferred site for *O. volvulus* microfilariae. Buck (1974) reviewed the dissemination of *O. volvulus* microfilariae in various organs other than the skin and eyes and the possible role of *O. volvulus* as the cause of systematic onchocerciasis. The rodents model may open the way for investigations into the dissemination of microfilariae and the pathologic changes induced by microfilariae.

Table 1 Distribution of *O. volvulus* microfilariae in mice at various times after subcutaneous inoculation into the inguinal region

Time of necropsy	No. of animal	% Recovery	No. of microfilariae recovered				
			Ears	Pelt & Carcass	Viscera	Tail	Others
36 hr	2	12.5 –15.1	79	8471	221	4160	407
1 w	2	2.2 –18.3	402	1682	479	4118	453
2 w	3	3.9 – 5.2	307	556	133	2325	79
4 w	3	0.4 – 0.8	17	43	9	783	3
6 w	3	0.01– 1.0	0	21	0	104	5
8 w	4	0.02– 1.1	6	43	2	331	3
12 w	4	0 – 0.04	1	8	0	1	0
16–18 w	8	0					

Table 2 Microfilarial distribution with site of inoculation

Mouse No.	No. inoculated (10^3)	Site of inoculation	Time of necropsy (wk)	No. of microfilariae recovered				
				Ears	Lungs	Spleen	Kidney	Tail
1	46	Groin	1	54	4	0	0	1,030
2	27		2	128	2	3	1	1,701
3	32		2	11	1	0	0	235
4	32	Peritoneal	1	187	11	4	11	1,550
5	55	Cavity	2	50	0	0	0	680
6	33		2	4	1	0	0	145
7	23	Scalp	1	315	30	4	2	138
8	23		1	117	7	2	6	88
9	23		2	336	19	2	13	186

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CIRCULATING IMMUNE COMPLEXES IN ONCHOCERCIASIS: ASSOCIATION WITH OCULAR AND SYSTEMIC COMPLICATIONS OF DIETHYLCARBAMAZINE THERAPY

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Diethylcarbamazine (DEC) continues to be utilized to treat human onchocerciasis despite significant complications associated with its use. The complications of DEC therapy of onchocerciasis appear to fall into two categories: (1) acute self-limited symptoms (pruritis, fever, malaise, dizziness) which resolve without apparent sequelae after several days of therapy; and (2) more long-lasting complications such as optic neuritis and chorioretinitis. These latter complications cannot be explained as simply a part of the natural progression of the disease process. Because the mechanisms of both of these types of complications are not understood, a rational approach to prevention cannot be formulated at present. This study was aimed at elucidating the etiology of the sequelae of DEC therapy of onchocerciasis.

Twenty men with *O. volvulus* infection in Liberia, West Africa were studied over a 6-month period during which DEC was administered daily for the first week, then weekly for the remainder of the treatment course. Ten men received DEC orally, and the remaining 10 transepidermally, in comparable dosages. These subjects were carefully evaluated by physical examination, ocular examination, and laboratory studies including sera for levels of circulating immune complexes (CIC) using a polyethyleneglycol precipitation I125-C1q binding assay.

Ocular examination before therapy showed normal visual fields by confrontation in all 20 men. Punctate keratitis was present in 2, early sclerosing keratitis in 5, and chorioretinitis in 11 persons prior to start of therapy. Limbitis, anterior uveitis, and optic disc abnormalities were not seen pre-treatment.

During the first week of therapy, all 20 men developed new lymphadenopathy, 18 of the 20 a rash, and 1 arthralgias. These findings resolved within two weeks of treatment and did not recur during the remainder of the 6-month course. Two men developed splenomegaly within the first two weeks which resolved by two months. There were no changes in visual acuity, color vision or pupillary reactions. Constriction of visual fields occurred in three persons. In two, the constriction was first noted at two weeks of treatment and progressively worsened over the 6-month period. In the third person, visual fields were normal up to two months, but were constricted by six months. Three individuals showed pallor of the optic nerve head at six months. New areas of chorioretinitis developed in five persons, and mild or moderate uveitis was seen in 16 of the 20 at some time during treatment. Punctate keratitis, present in two persons pre-treatment, appeared in a further 13 people during treatment, largely during the first two weeks of therapy. No progression in peripheral corneal haze (early sclerosing keratitis) was seen, though one person demonstrated transient acute limbitis in the first two weeks of therapy.

Proteinuria developed in seven persons during treatment. In four, the proteinuria persisted through the end of therapy, while in three, it was transient and was noted only between two and four months. The protein electrophoretic patterns of the urine was determined at six months from the persons with persistent proteinuria. A pattern suggestive of glomerular leakage was seen in one person, and in the other three it suggested tubular leakage. In the two people with

pre-existing proteinuria, the pattern suggested a glomerular source.

Pre-treatment levels of circulating immune complexes (CIC) were elevated. As can be seen in Figure 1, levels of CIC declined during treatment; however, there was a trend upwards at the end of the 6-month treatment period. If the study group is divided into those with initially abnormal and those with initially normal CIC levels, the slight increase in the mean CIC levels at the end of treatment for the initially abnormal group was not significant (Fig. 2). However, the increase in CIC levels in the initially normal group, from pre-treatment to six months, was significant, and the final mean level of this latter group fell into an elevated range (mean CIC level pre-treatment 7.8 ± 4.0; at six months 19.8 ± 12.8; $p < .05$, paired t test).

Pre-treatment levels of CIC were examined as potential predictors of subsequent complications. CIC levels greater than 30 percent binding were associated with subsequent constriction of visual fields ($p < .05$, Fisher exact test), and with development of proteinuria during therapy ($p < .04$). Levels of CIC consistently greater than 15 percent were strongly associated with development of visual field constriction during treatment ($p < .009$). Linear regression analysis of number of complications vs. pre-treatment levels of CIC showed a highly significant positive correlation ($p < .001$) (Fig. 3). Skin snip microfilarial counts and numbers of intraocular microfilariae did not show an association with complications. The mean numbers of intraocular Mf in the three individuals with visual field changes was 3.8 ± 3.6 per examination, compared to 3.2 ± 6.9 in those without visual field changes.

This study shows that increased levels of circulating immune complexes occur in onchocerciasis and persist despite treatment which suppresses the microfilarial burden. In fact, DEC treatment may increase the levels of CIC. Further, there is a strong association between levels of CIC and the development of ocular and systemic complications. Combined with the clinical nature of the complications, these findings provide compelling indirect evidence for the participation of immune complexes in the pathogenesis of the sequelae of DEC treatment of onchocerciasis.

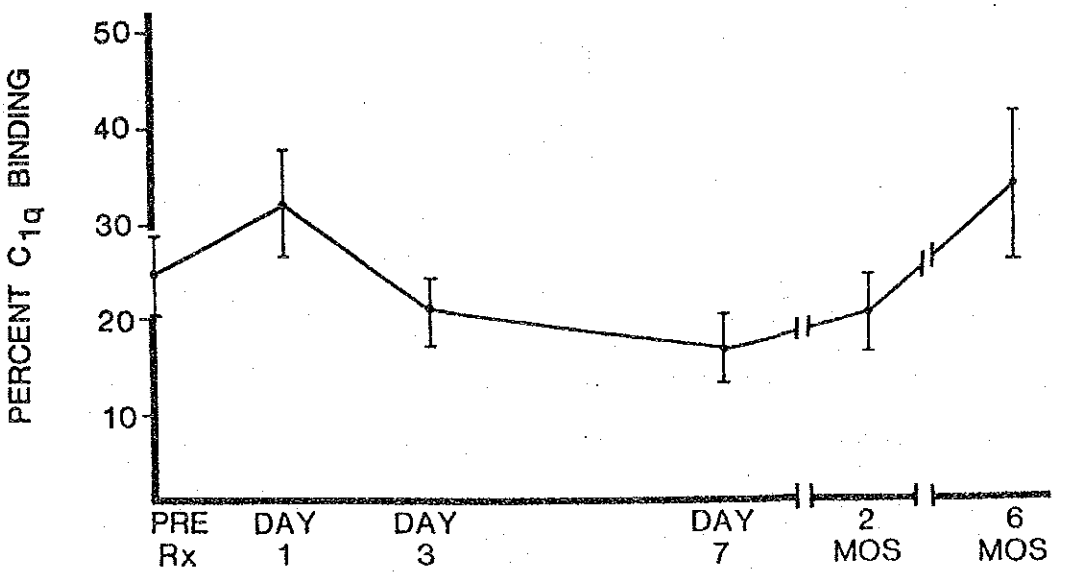

Figure 1 Mean levels of circulating immune complexes (by PEG precipitation $^{125}C1_q$ binding assay) in 20 men over 6 months while receiving diethylcarbamazine therapy. Upper limits of normal for uninfected controls was 15% $C1_q$ binding

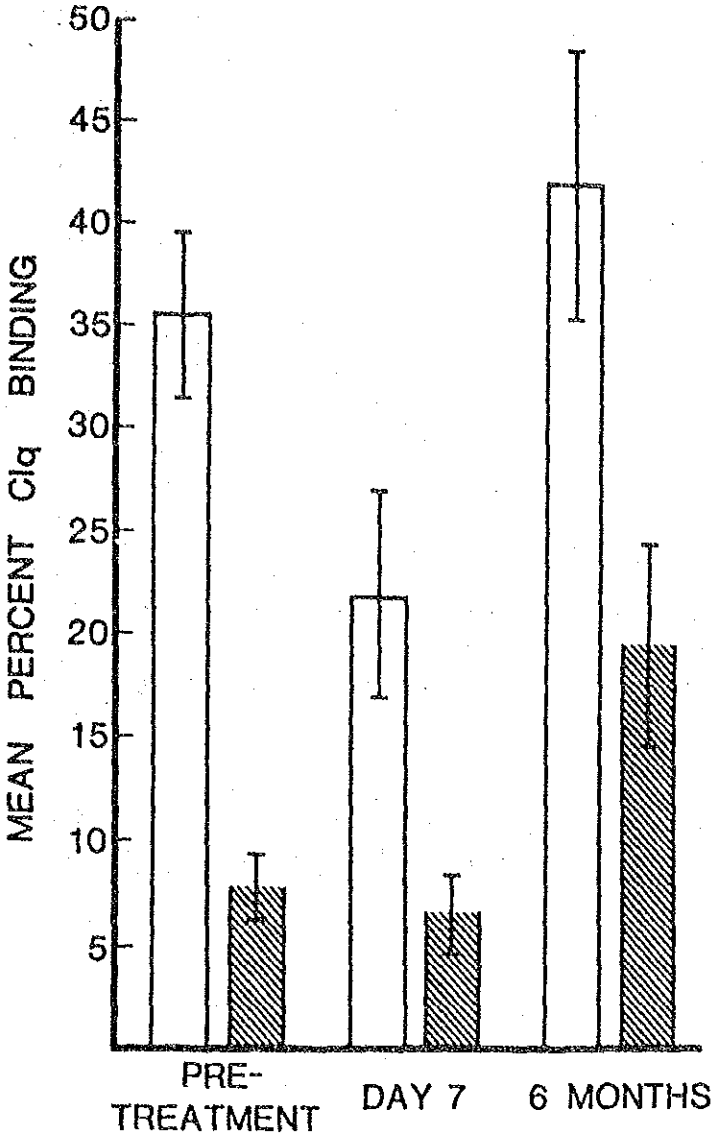

Figure 2 Mean percent C1q binding in individuals with elevated levels pretrement (open bars) compared to those with normal levels pretreatment (hatched bars) over 6 months while receiving DEC therapy

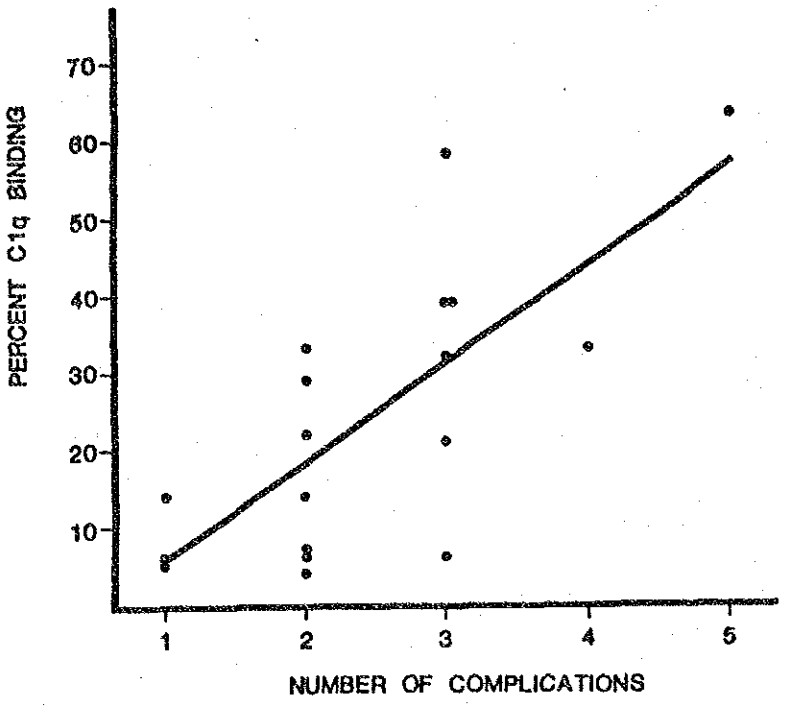

Figure 3 Pretreatment levels of CIC by percent Clq binding vs. number of complications over 6 months. The complications included in this analysis were proteinuria, uveitis, new chorioretinitis, changes in optic disc (pallor), visual field changes, and severe pruritis following treatment at 6 months

UNA BREVE REVISION Y PREREVISION DEL PROYECTO COOPERATIVO GUATEMALA-JAPON SOBRE INVESTIGACION Y CONTROL DE LA ONCOCERCIASIS

A BRIEF REVIEW AND PREVIEW OF THE GUATEMALA–JAPAN COOPERATIVE PROJECT ON ONCHOCERCIASIS RESEARCH AND CONTROL

Takeshi Suzuki

Jefe del Proyecto/Project leader

Guatemala-Japón Proyecto Cooperativo sobre Investigacion y Control de la Oncocerciasis

Guatemala–Japan Cooperative Project on Onchocerciasis Research and Control

1. *Introducción*

En 1915 el Dr. Rodolfo Robles Valverde reportó la existencia de la oncocerciasis americana en Guatemala. Este fue el primer informe sobre la enfermedad en el Hemisferio Occidental. Desde entónces, la Enfermedad de Robles (llamada así en la América Latina) ha sido descubierta en diversos países de Centro y Sur América: México (1923), Venezuela (1949), Colombia (1965) y Brasil (1967). Esta enfermedad también ya fue descubierta en el Ecuador.

1. *Introduction*

In 1915, Dr. Rodolfo Robles Valverde reported the existence of American onchocerciasis in Guatemala. This was the first report of the disease in the Western Hemisphere. Since then, Robles Disease (so-called in Latin America) has been found in various countries of the Central and South America: Mexico (1923), Venezuela (1949), Colombia (1965) and Brazil (1967). This disease has also been discovered in Ecuador.

Desde el descubrimiento de la Enfermedad de Robles, se han llevado a cabo extensos estudios epidemiológicos, parasitológicos y entomológicos en Guatemala. Sin embargo, nunca se ha lanzado un control sistemático en el país debido a las graves dificultades que se encuentran por las condiciones locales. La campaña de nodulectomía es una excepción, habiendose iniciado en *1915 y continuando* hasta la fecha. Desafortunadamente, se ha reportado que la nodulectomía es ineficaz para reducir la transmisión de la enfermedad, aunque sí alivia la frecuencia de severos síntomas oculares.

En 1975 se suscribió un Convenio entre el Gobierno de la República de Guatemala y la Agencia de Cooperación Internacional del Japón (JICA) para establecer un Proyecto de Cooperación para la Investigación y el Control de la Oncocerciasis en Guatemala. En el Acta de Discusiones sobre el Convenio, se expresaron los principios generales del Proyecto como sigue: (1) trabajos básicos de investigación para el control de la oncocerciasis, *incluyendo el control del vector* y quimioterapia; (2) práctica de control de la oncocerciasis, realzando el control del vector en el Area Piloto de San Vicente Pacaya, y (3) establecimiento de métodos eficaces para el control del vector, que puedan ser aplicados ampliamente en la República de Guatemala.

La duración de la cooperación japonesa para el Proyecto bajo este Convenio era de cinco años, comenzando desde octubre de 1975.

Durante este quinquenio, de octubre de 1975 a septiembre de 1980, un total de 43 Expertos Japoneses en epidemiología, parasitología, inmunología, oftalmología, *dermatología y entomología se unió al* Proyecto para llevar a cabo los trabajos de investigación y los ensayos de control de la enfermedad. Los resultados obtenidos durante este período serán presentados en la Conferencia Conjunta Guatemala-Japón sobre la Investigación y el Control de la Oncocerciasis, que se llevará a cabo en Guatemala durante el período comprendido entre el 12 al 16 de enero de 1981. El Acta de Sesiones de la

Since the discovery of Robles Disease, extensive studies on epidemiology, parasitology and entomology have been carried out in Guatemala. However a systematic control was never launched in the country, due to the severe difficulties encountered considering local conditions. The nodulectomy campaign is the exception, which was initiated in 1915, and continues to the present. Unfortunately, nodulectomy has been reported ineffective on reducing disease transmission, even though it alleviates the incidence of severe ocular symptoms.

In 1975, the Agreement was made between the Government of the Rebuplic of Guatemala and the Japan International Cooperation Agency (JICA) to establish the Cooperative Project on Onchocerciasis Research and Control in Guatemala. In the Record of Discussion (R/D), the outline of the Project was stated as (1) basic research works for onchocerciasis control including vector control and chemotherapy, (2) practice of onchocerciasis control, emphasizing vector control in San *Vicente* Pacaya Pilot Area, and (3) establishment of effective methods for vector control widely applicable in the Republic of Guatemala.

The duration of the Japanese cooperation for the Project under the R/D was five years commencing from October 1975.

During the five year period, from October 1975 to September 1980, a total of 43 Japanese Experts on epidemiology, parasitology, immunology, ophthalmology, dermatology and entomology joined the Project to carry out research work and control trials of the disease. The results obtained during this period will be presented in the Guatemala-Japan Joint Conference on Onchocerciasis Research and Control to be held in Guatemala, during the period 12 to 16 January 1981. The Proceedings for the Conference will be published by JICA after the Conference is over.

In May 1980, a new R/D was made between Guatemala and Japan, in which the Project was extended for additional three years, ending in September 1983.

In this small document, I would like to present the future activities of the Project

Conferencia será publicada por JICA después de su realización.

En mayo de 1980, se celebró un nuevo Convenio entre Guatemala y el Japón, por medio del cual el Proyecto se prorrogó por tres años más, que finalizarán en septiembre de 1983.

En este breve documento quisiera presentar las actividades futuras del Proyecto durante el período adicional de 1980–1983, juntamente con un breve resumen de las actividades pasadas del mismo.

2. *Actividades Pasadas del Proyecto*

Debido a una limitación de tiempo y de recursos, la mayor parte de las actividades se concentraron en el Area Piloto de Control de San Vicente Pacaya (SVP). San Vicente Pacaya es un área dentro del Departamento de Escuintla y se encuentra en la periferia sur-oeste de la principal zona endémica de la oncocerciasis en Guatemala. El área tiene aproximadamente 236 km^2, con una población de alrededor de 5,700 habitantes, de los cuales el 55%viven en el municipio de San Vicente Pacaya. El área está situada en la vertiente del Pacífico de la cadena volcánica 'Sierra Madre', estando constituida la mitad norte de terreno escarpado, donde varias especies de la mosca negra, incluyendo la especie *Simulium ochraceum*, el principal vector de la oncocerciasis, se reproducen en los numerosos riachuelos.

Durante la primera etapa del proyecto, se concentraron los esfuerzos en la determinación de los vectores, en un estudio de sus criaderos, estudios sobre el comportamiento de picadura de los vectores, estudios de la infección natural de las moscas negras antropofílicas y estudios de infección experimental, juntamente con los estudios epidemiológicos del Area Piloto y el trabajo básico de investigación para hacer posibles dichos estudios. Durante la segunda etapa, se iniciaron estudios sobre insecticidas, y finalmente, se inició un control a pequeña escala en SVP, usando temephos (ABATE) como larvicida. Al mismo tiempo, se inició una evaluación previa al control en SVP para obtener datos bases antes de iniciar un control general del área.

during the three year period, 1980–1983, together with a brief review of the past activities of the Project.

2. *Past activities of the project*

Due to limited time and resources, most project activities were concentrated in San Vicente Pacaya (SVP), the Pilot Control Area. San Vicente Pacaya is a county within the Department of Escuintla and is located in the south-western periphery of the major Guatemalan endemic zone of onchocerciasis. The area is approximately 236 km^2 and has a population of about 5,700, among whom 55% are living in the municipal town of San Vicente pacaya. The area is situated on the Pacific slope of the Sierra Madre volcanic range, with the northern half composed of rugged terrain, where several blackfly species, including *Simulium ochraceum*, the principal vector of onchocercisasis, breed in the numerous small streams.

In the first stage of the Project, efforts were concentrated on the determination of vectors, survey of their breeding sites, studies on vector biting behaviour, natural infection studies of anthropophilic blackflies, and experimental infection studies, together with the epidemiological surveys of the Pilot Area and basic research work necessary to enable such surveys. In the second stage, insecticide studies were commenced, and eventually a small scale control was launched in SVP, using temephos (ABATE) as a larvicide. At the same time, pre-control evaluation was initiated in SVP to obtain baseline data prior to overall control of the area.

Up to the present, considerable information on various aspects has been gathered, which is briefly summarized below.

2.1 *Entomology and vector control*

(1) *Simulium horacioi* Okazawa and Onishi, a new anthropophilic species closely related to *S. metallicum*, was described, which commonly breeds in small streams of San Vicente Pacaya.

(2) *S. ochraceum* was incriminated as the principal vector of onchocerciasis in the pilot area, through natural and experimental

Hasta la actualidad, se ha recopilado bastante información respecto a diversos aspectos, la cual se resume a continuación.

2.1 *Entomología y control del vector*

(1) El *Simulium horacioi* Okazawa y Onishi, una nueva especie antropofílica relacionada con el *S. metallicum*, fue descrito. Esta especie se reproduce comunmente en pequeños riachuelos de San Vicente Pacaya.

(2) El *S. ochraceum* resultó ser el principal vector de la oncocerciasis en el área piloto, según estudios de infección natural y experimental. El papel del *S. metallicum* y del *S. horacioi* en la transmisión de la enfermedad todavía es incierto; sin embargo, se confirmó que las Mf del *O. volvulus* podían llegar a una tercera etapa en cualquiera de estas especies, pero en un alcance menor que en el *S. ochraceum.*

(3) La mayoría de los criaderos de *S. ochraceum* se limitan a riachuelos de correinte rápida en las áreas montañosas, con un volumen de flujo que fluctúa entre 0.1–10 litros/seg., en tanto que el *S. metallicum* prefiere riachuelos más grandes.

(4) En mayo, al inicio de la estación lluviosa, la reproducción del *S. ochraceum* disminuye, manteniendo este bajo nivel hasta octubre o diciembre, cuando comienza la estación seca. De acorde a lo anterior, la densidad de hembras tiene su cúspide en el período de diciembre/enero.

(5) En cuanto a la ingesta de sangre, el *S. ochraceum* prefiere a los humanos sobre los caballos, el ganado u otros animales. Ingieren sangre más a menudo de la parte superior del cuerpo humano, donde generalmente se encuentran las densidades más altas de microfilarias. Sin embargo, el *S. metallicum* se alimenta con más frecuencia en la parte inferior del cuerpo humano, y prefiere otros animales sobre los humanos.

(6) La duración de un ciclo gonotrófico del *S. ochraceum* fue observada en el laboratorio, siendo de 3 a 5 días, dependiendo de la temperatura.

(7) Existe la tendencia a que, en un lugar de mayor altura, la mortalidad del *S. ochraceum* es baja, pero el período de incubación de

infection studies. The role of *S. metallicum* and *S. horacioi* in the transmission of the disease is still uncertain, yet it was confirmed that *O. volvulus* Mf were able to reach third stage in either species, but in lesser extent than in *S. ochraceum.*

(3) Most *S. ochraceum* breeding sites are limited to small rapid-flowing rivulets in the mountainous areas, with the flow volume range of 0.1–10 litre/sec.; whereas *S. metallicum* prefers larger streams.

(4) In May, at the onset of the rainy season, breeding of *S. ochraceum* declines. Maintaining this low level until October or December, with the initiation of the dry season. Reflecting this, the female density shows its highest peak in the December/January period.

(5) For blood meals, *S. ochraceum* prefers humans to horses, cattle or other animals. They take blood more often from the upper region of human bodies, where high Mf density is usually found. *S. met.*, however, will generally feed on the lower part of man, although prefers other animals to humans.

(6) The duration of one gonotrophic cycle of *S. ochraceum* was observed in the laboratory as 3 to 5 days, depending on temperature.

(7) There exists a tendency that, in a locality with a higher altitude, mortality of *S. ochraceum* is low but incubation period of *O. volvulus* in the flies is long; whereas in a lower altitude locality, fly mortality is high, but the incubation period is short. This may be one of the reasons limiting endemic foci of the disease in a certain altitude range in Guatemala.

(8) Although chlorphoxim and chlorpyrifos-methyl demonstrated even higher effectiveness than temephos in laboratory tests, it was decided, for the time being, to adopt temephos as a larvicidal agent in the field control, because of its low toxicity to humans and non-target organisms.

(9) At the first stage of the studies, a solid form of temephos was adopted as a standard formulation for field control trial. The solid briquettes, placed in small wire-cages, were set in the streams, with expected dissolution time of 10 minutes or more. This formulation was used in the control operation of the Barre-

O. volvulus en las moscas es largo; en tanto que en un lugar de menor altura, la mortalidad de las moscas es alta, pero el período de incubación es corto. Esta podría ser una de las razones que limita los focos endémicos de la enfermedad a ciertos límites de altitud en Guatemala.

(8) Aunque el clorofoxima y el cloropirifos-metil demostraron una más alta eficacia aún que el temephos en pruebas de laboratorio, se decidió, por el momento, adoptar el temephos como agente larvicida en el control de campo, debido a su baja toxicidad para los humanos y otros organismos que no son la meta del control.

(9) En la primera etapa de los estudios se utilizó el temephos en forma sólida como formulación estándard para el ensayo de control de campo. Las briquetas sólidas, colocadas en jaulas de alambre, fueron puestas en los riachuelos. Estas tenían un tiempo estimado de disolución de 10 minutos o más. Esta formulación se usó en la operación de control de la Cuenca de Río Barretal, habiéndose logrado un gran éxito. Estudios posteriores revelaron, sin embargo, que los polvos dispersibles en agua eran tan eficaces como el larvicida en forma sólida y mucho más convenientes en términos de operación.

(10) Se concluyó tentativamente que las aplicaciones de insecticida se deberían repetir cada dos semanas, como resultado de un estudio sobre la reaparición en pequeña escala de los *S. ochraceum* aún no plenamente desarrollados, después de inundarlos con una aplicación de insecticida.

(11) Estudios recientes han revelado que una dosis mayor de temephos no necesariamente aumenta la distancia de efectividad, que depende más en la condición de los riachuelos.

(12) En la Cuenca del Río Barretal, en el Area Piloto, se lanzó un ensayo de control en marzo (en junio, en algunos riachuelos) de 1979, con aplicaciones cada dos semanas de 3.3 ppm de temephos por 10 minutos de flujo de corriente. Se notó una marcada reducción en la densidad de las hembras de *S. ochraceum* uno o dos meses después de la primera aplicación. La baja densidad se ha mantenido así hasta la actualidad.

tal River Basin with great success.

Later studies revealed, however, that wettable powder was as effective as the solid form and operationally more convenient.

(10) It was concluded tentatively, that insecticide applications should be repeated fortnightly, as a result of a small-scale reappearance study of *S. ochraceum* immatures, after flusing-out with an insecticide application.

(11) Recent studies revealed that the increased dose of temephos does not necessarily increase the effective distance, which depends more on the condition of the streams.

(12) In the Barretal River Basin of the Pilot Area, a control trial was launched in March (in some streams in June) of 1979, with fortnight application of 3.3ppm temephos per 10 minutes flow volume. Marked reduction of female *S. ochraceum* density was observed one or two months after the first application. The low density has been maintained to the present.

2.2 *Epidemiology and parasitology*

(1) For skin biopsy, the Holth-type punch proved most appropriate from comparative studies with three types of instruments, i.e. Holth-type and Walser-type sclerocorneal punches and disposable scalpel. In routine surveys, biopsy samples were taken with a Holth-type punch from both iliac crest and the scalpular region in males, and one sample from both sides of scapular region in females.

(2) Studies on microfilariae of *O. volvulus* revealed that (i) more Mf are distributed in the skin of upper torso, than in head or in the lower part of a human body, (ii) high Mf density is not always found in the skin near nodules, (iii) sometimes Mf appear in human urine, (iv) Mf appearance in the skin is subperiodic; the density is slightly higher during 7–9 hours and slightly lower during 17–19 hours, and (v) about half of the persons with more than 500 MfD in the skin harbour Mf also in the anterior chambers of eyes.

(3) Through palpation and nodulectomy studies, some information was obtained on the relationship between size of nodules and existence of adults and Mf in the nodules, in addition to the reappearance of nodules after

(1) Para las biopsias cutáneas, las punzadoras tipo Holth demostraron ser my apropiadas, tal como se desprende de estudios comparativos con tres tipos de instrumentos, v.g., las punzadoras esclerocorneales tipo Holth y tipo Walser, y un escalpelo desechable. En los estudios de rutina, las muestras de biopsias fueron tomadas con una punzadora tipo Holth, tanto de la cresta ilíaca como de la región escapular en los y una muestra de ambos lados de la región escapular en las mujeres.

(2) Estudios sobre las microfilarias de *O. volvulus* revelaron que (i) hay más Mf distribuidas en la piel del torso superior, que en la cabeza o en la parte inferior del cuerpo humano; (ii) no siempre se encuentra una alta densidad de Mf en la piel cerca de los nódulos; (iii) algunas veces las Mf aparecen en la orina humana; (iv) la aparición de las Mf en la piel es subperiódica; la densidad es un poco más alta durante 17–19 horas, y (v) alrededor de la mitad de las personas con más de 500 de densidad microfilárica en la piel tienen Mf en las cámaras anteriores de los ojos.

(3) Por medio de palpaciones y estudios de nodulectomía, se ha obtenido alguna información sobre la relación entre el tamaño de los nódulos y la existencia adultos y Mf en los nódulos, adicionalmente a la reaparición de los nódulos después de una nodulectomía. Sin embargo, aún no existe certeza respecto a si los vermes oncocercóticos se mueven fuera de los nódulos y si existen nódulos profundos en la piel, que no se puedan palpar.

(4) Se confirmó que tanto *O. gutturosa* y *O. cervicalis* están ampliamente distribuidos en el ganado y los equinos de Guatemala, lo cual realza la importancia de identificaciones discriminatorias de las larvas de las especies de *Onchocerca* en las moscas negras.

(5) Pruebas comparativas de actividad usando tres clases de antígenos en las pruebas cutáneas, revelaron que el antígeno de adultos de *O. volvulus* producía tasas positivas más altas y tasas falso-negativas más bajas que el antígeno FST-3 de *D. immitis* o que el antígeno de las microfilarias de *O. volvulus*.

(6) Se realizó un ensayo para inocular microfilarias oncocértuicas en ratones. Durante el

nodulectomy. It is not yet certain, however, whether onchocercal worms move outside nodules and whether there exist any nodules deep beneath the skin, which can not be palpated.

(4) It was confirmed that both *O. gutturosa* and *O. cervicalis* are widely distributed among cattle and equines of Guatemala, which emphasizes the importance of discriminating identification of *Onchocerca* spp. larvae in blackflies.

(5) Comparative activity tests, using three kinds of antigens for the skin tests, revealed that antigen from *O. volvulus* adults produced higher positive rate and lower false negative rates than the FST-3 antigen from *D. immitis* or antigen from *O. volvulus* microfilariae.

(6) A trial was made to inoculate onchocercal microfilariae to mice. During the early period of experimental infection, Mf appeared in eyes, ears, viscera, pelt, tail and carcass, but later Mf accumulated in the tail. Live Mf were recorded up to 12 weeks post-inoculation.

(7) It was confirmed that both ophthalmological and dermatological symptoms in Guatemalan onchocerciasis are not as severe as those in the African endemic foci. The prevalence rate of blindness caused by the disease was as low as 0.4%, even in the high endemic foci.

(8) The main ophthalmological manifestations of onchocerciasis in Guatemala are corneal changes, with a high incidence of iritis. Punctate keratitis is most frequently observed. Typical cases of exudative fibrous iridocyclitis are rare, although the incidence of the so-called inactive iritis is noticeably high. Statistical analysis revealed a significant correlation between head nodules and ocular symptoms, especially in the anterior segment of the eye.

(9) A total of 252 patients from onchocerciasis endemic foci were given diethylcarbamazine (DEC) treatment. The doses of DEC varied between 3 to 10 mg/kg of body weight during 10 days. Only 7 patients were withdrawn from treatment due to detrimentral side reactions. Of 242 treated patients, with whom follow-up was possible, 139 were negative in skin biopsy, while 103 continued

primer período de infección experimental, aparecieron Mf en los ojos, oídos, vísceras, piel, cola y esqueleto, pero después las Mf se acumulaban en la cola. Se observaron Mf vivas hasta 12 semanas después de la inoculación.

(7) Se confirmó que tanto los síntomas oftalmológicos y dermatológicos en la oncocerciacias guatemalteca no son tan severos como los de los focos endémicos africanos. La tasa de incidencia de ceguera causada por la enfermedad resultó tan baja como 0.4%, aún en los focos de alta endemicidad.

(8) Las principales manifestaciones oftalmológicas de la oncocerciacis en Guatemala son cambios en la cornea con una alta incidencia de iritis. Los casos típicos de iridociclitis exudativa fibrosa son raros, pero la incidencia de la llamada iritis inactiva es marcadamente alta. Los análisis estadísticos revelaron una correlación significativa entre los nódulos en la cabeza y los síntomas oculares, especialmente en el segmento anterior del ojo.

(9) Un total de 252 pacientes de focos endémicos de oncocerciacis recibieron tratamiento con dietilcarbamazina (DEC).Las dosis de DEC que fueron administradas fluctuaron entre 3 a 10 mg/kg de peso corporal durante 10 días. Sólamente a 7 pacientes se les retiró de este tratamiento debido a reacciones secundrias perjudiciales. De 242 pacientes tratados, a los que sí fue posible hacerles un seguimiento, 139 resultaron negativos en las biopsias cutáneas, en tanto que 103 continuaron siendo positivos, aunque con una densidad de filarias reducida.

(10) Los estudios realizados en San Vicente Pacaya revelaron que aproximadamente 30% de los habitantes resultaron ser positivos a las Mf por el método de corte cutáneo. Tanto la tasa de Mf y la densidad de Mf resultaron ser más altas en los hombres que en las mujeres en cualquier grupo etario. En los estudios oftalmológicos, alrededor de un 6% tenían Mf en las cámaras anteriores.

(11) Para poder obtener datos epidemiológicos básicos antes de la operación de control, se seleccionaron 6 aldeas y fincas en SVP en 1978. De 1978 a la fecha, se han repetido estudios epidemiológicos con biopsias cutáneas, palpación de nódulos, pruebas cutáneas, juntato be positive, although with reduced Mf density.

(10) Surveys in San Vicente Pacaya Pilot Area revealed that approximately 30% of the inhabitants were Mf positive by skin-snip method. Both Mf rate and Mf density were higher in males, than in females in any age group. In the ophthalmological surveys, about 6% had Mf in the anterior chambers.

(11) In order to obtain base-line epidemiological data prior to the control operation, 6 villages and plantations were selected in SVP in 1978. From 1978 to the present, epidemiological surveys with skin biopsy, nodule palpation, skin tests, together with ophthalmological surveys have been repeated in the localities once a year. The results will later be compared with those obtained after the overall control operation was launched.

(12) Data recording and analysis system was established, in order to provide more effective and informative data. Original data sheets of the surveys in SVP, containing information for individual identification, life history, parasitological and immunological diagnosis, and ophthalmological and dermatological findings, were coded according to the coding manual prepared in advance.

3. *Future activities of the project*

3.1 *Expansion of vector control operation*

At the present, a small area of approximately 7.6 km^2 is under vector control, which is about one-eleventh of the entire *S. ochraceum*-breeding area of San Vicente Pacaya. The experience in this small control area raises the question, whether a residual adult population in the area originated from those actually bred in the streams under larvicidal treatment, or invaded from the neighbouring areas, in which no control measures have ever been taken.

In the second term of the Project, the first priority is given to the expansion of the vector control activities. In Guachipilín, a large river basin of approximately 22 km^2 of rugged terrain, comprehensive surveys of all streams were made in 1979, while large-scale stream tests of larvicides were conducted in 1980. The overall control opera-

mente con estudios oftalmológicos en esos lugares anualmente. Estos resultados serán comparados posteriormente con los que se obtuvieron después del lanzamiento de la operación general de control.

(12) Se estableció un sistema de registro de datos y de análisis para proporcionar datos más eficaces e informativos. Las hojas de datos originales de los estudios realizados en SVP, que contienen información para identificación individual, historiales, diagnósticos parasitológicos e inmunológicos, y los hallazgos oftalmológicos y dermatológicos, fueron puestos en clave de conformidad con el manual de claves que fuera preparado con anticipación.

3. *Actividades Futuras del Proyecto*

3.1 *Amplicación de la operación de control de vectores*

En la actualidad, una pequeña área de aproximadamente 7.6 km^2 está bajo control de vectores. Esta área representa alrededor de una onceava parte de toda el área de reproducción de *S. ochraceum* de San Vicente Pacaya. Esta pequeña área de control hace surgir la pregunta de si una población residual en el área se originó de aquellas reproducidas verdaderamente en los riachuelos que han estado bajo tratamiento larvicida, o si invadieron estos lugares de áreas circundantes en las cuales nunca se han tomado medidas de control.

En el segundo período del Proyecto, la primera prioridad se da a la ampliación de las actividades de control de vectores. En Guachipilín, una cuenca fluvial grande de aproximadamente 22 km^2 de terreno escarpado, se realizaron estudios completos de todos los riachuelos en 1979, en tanto que en 1980 se llevaron a cabo pruebas a gran escala con larvicidas en los riachuelos. La operación general de control en Guachipilín será lanzada a principios de 1981. Como siguiente paso, la Cuenca del Río Chilar será estudiada, seguido por la operación de control. Finalmente, un área de 63 km^2 en total habrá sido cubierta por aplicaciones de larvicida para finales de 1982.

Tal como fuera mencionado anteriortion in Guachipilín will be launched in the early part of 1981. As the next step, the Chilar River Basin will be surveyed, followed by control operations. Finally, an area of 63 km^2 in total will be covered by larvicide application by the end of 1982.

As previously mentioned, the pre-control epidemiological evaluation was initiated in 1978. Sicne then, the evaluation has been conducted every year during the June-August period. Similar surveys will be continued once a year until 1983. It is expected that these surveys clarify the effect of the vector control operation on human inhabitants of the area.

3.2 *Extensive multidisciplinary surveys*

Extensive multidisciplinary surveys will be carried out to cover major areas belonging to the so-called "second endemic focus" of Guatemala; and, if possible, certain sampled areas belonging to other endemic foci. The surveys will also be extended to the neighbouring areas, which have not been designed as endemic foci.

The objectives of the surveys are (i) to obtain up-to-date epidemiological information on onchocerciasis in each area; (ii) to clarify the correlation between epidemiological and entomological findings, (iii) to compare both epidemiological and entomological findings between inside the endemic focus and outside although nearby the focus, and (iv) to obtain information on possible modification of vector control procedures according to each local condition.

A survey team consisting of an epidemiologist, a parasitologist, an ophthalmologist, an entomologist and their assistants, will visit each area to carry out surveys by standard methods, filling prefixed formats. Thirty to fifty areas will be surveyed during the period mid-1981 to mid-1983.

3.3 *Intensive longitudinal surveys/Advanced vector control trial*

Two or three areas will be selected in one of the endemic foci. Intensive longitudinal surveys will be carried out in the areas, mainly on entomology but also on epidemiology,

mente, la evaluación epidemiológica previa al control se inició en 1978. Desde entónces, la evaluación se ha llevado a cabo cada año durante el período de junio-agosto. Se continuará realizando estudios similares una vez por año hasta 1983. Se espera que estos estudios aclaren qué efecto ha tenido la operación del control de vectores sobre los habitantes de esta área.

3.2 *Amplios estudios multidisciplinarios*

Se llevarán a cabo amplios estudios multidisciplinarios para cubrir las áreas principales que pertenecen al llamado "segundo foco endémico" de Guatemala, y, de ser posible, en ciertas áreas escogidas que pertenecen a otros focos endémicos. Los estudios también se ampliarán a otras áreas circundantes que no han sido designadas como focos endémicos.

Los objetivos de los estudios son (i) obtener información actualizada sobre la oncocerciasis en cada área; (ii) aclarar la correlación que existe entre los hallazgos epidemiológicos y entomológicos; (iii) para comparar tanto los hallazgos epidemiológicos como entomológicos obtenidos dentro y fuera (aunque cerca) del foco endémico; y (iv) obtener información sobre posibles modificaciones a los procedimientos de control de vectores de conformidad con cada condición local.

Un grupo de estudio compuesto por un epidemiólogo, un parasitólogo, un oftalmólogo, un entomólogo y sus asistentes, visitará cada área para llevar a cabo estudios por métodos corrientes, y llenará formatos determinados con anticipación. Se estudiarán de treinta a cincuenta áreas durante el período de mediados de 1981 a mediados de 1983.

3.3 *Estudios longitudinales intensivos/Ensayo avanzado de control de vectores*

Dos o tres áreas serán seleccionadas en uno de los focos endémicos. Se llevarán a cabo estudios longitudinales intensivos en las áreas, principalmente sobre entomología, pero también sobre epidemiología, parasitología y oftalmología.

Los objetivos de los estudios son (i) obtener información sobre la prevalencia

parasitology and ophthalmology.

The objectives of the surveys are (i) to obtain information on seasonal prevalence of vector blackflies and infection rate of the vectors, as well as seasonal fluctuation of vector breeding and of water flow in streams, (ii) to elucidate population dynamics in the areas, together with man-vector contact throughout the year, and (iii) to obtain basic information on transmission dynamics of the disease.

A survey team consisting mainly of an entomologist and his assistants will visit each area once or twice a month during the whole year or more. The surveys will begin in the second quarter of 1981.

Thereafter, a control trial will be started in one or two of the areas, by larvicide application in a limited period of the year, and/or to the limited breeding streams. Detailed planning will be made later, based on the information obtained during the first year.

3.4 *Improvement of vector control measures*

Since onchocerciasis control activities, now being carried out, are based exclusively on vector control, improvement of the control measures should be an essential part of future activities of the Project. The following studies are now being planned:

(1) *Insecticide susceptibility tests*

There is a possibility of the vector blackflies developing resistance to insecticides under the situation of continued use. In order to monitor susceptibility level of the larvae, insecticide susceptibility tests will be conducted periodically.

(2) *Effectiveness tests of insecticides*

Comparative studies on various insecticides, including new chemicals, will be done both in the laboratory and in the field, in order to find out more effective control agents and also to prepare substitute chemicals in case the vectors develop resistance to temephos.

(3) *Improvement of larvicide formulations*

Both solid and wdp formulations of temephos are effective in the field, yet the effective distance is limited. Studies will be done to improve the formulations, based on the dispersion studies of the chemical released

estacional de las moscas negras vectores y la tasa de infección de estos, así como la fluctuación estacional de la reproducción de vectores y del volumen del flujo de agua en los riachuelos; (ii) poner en claro las dinámicas de población en las áreas, juntamente con el contacto hombre-vector durante todo el año; y (iii) obtener información básica sobre la dinámica de transmisión de la enfermedad.

Un grupo de estudio, compuesto principalmente de un entomólogo y sus asistentes visitará cada área una o dos veces por mes durante un año completo o más. Los estudios comenzarán durante el segundo trimestre de 1981.

Más adelante, se iniciará un ensayo de control en una o dos de las áreas, llevando a cabo la aplicación de larvicida en un período limitado del año, y/o en riachuelos limitados donde existen criaderos. Más adelante se harán planes detallados en base a la información obtenida durante el primer año.

3.4 *Mejoramiento de las medidas de control de vectores*

Puesto que las actividades de control de la oncocerciasis que se están llevando a cabo en la actualidad se basan exclusivamente en un control de vectores, el mejoramiento de las medidas de control deberán formar parte esencial de las actividades futuras del Proyecto. Se tiene proyectado realizar los siguientes estudios:

(1) *Pruebas de susceptibilidad de insecticidas*

Existe la posibilidad de que las moscas vectores desarrollen una resistencia a insecticidas bajo una situación de uso continuado. Para monitorear el nivel de susceptibilidad de las larvas, se llevaran a cabo pruebas de susceptibilidad de los insectos periódicamente.

(2) *Pruebas de eficacia de insecticidas*

Se llevarán a cabo estudios comparativos sobre diversos insecticidas, incluyendo nuevos productos químicos, tanto en el laboratorio como en el campo, para encontrar agentes de control más eficaces y también para preparar agentes químicos de sustitución en caso de que los vectores desarrollen una resistencia al temephos.

(3) *Mejoramiento de formulaciones larvicidas*

in the streams.

(4) *Further studies on interval of larvicide application*

Since vector breeding sites in Guatemala are scattered throughout rugged mountainous areas, periodical visits of the sites with a longer interval than two weeks as in the present operation could save man-power tremendously. Studies will be done in various types of streams on reappearance of immature stages of vectors, particularly of pupae, after flushing-out with an insecticide application.

(5) *Trials of adult control*

In Guatemala, larval control seems more practical and effective, than adult control. Nevertheless, it is worthwhile to have trials of adult control using methods such as ULV application. It is noted that in certain areas, blackflies are also an annoying nuisance.

(6) *Basic studies on biological control*

Although practical biological control of vector blackflies could not be expected in near future, basic studies on biological control agents will be carried out.

3.5 *Special studies*

The results obtained by the Project during the first five year period are certainly informative, but some of them are unfortunately inconclusive, mainly due to shortage of time assigned to each subject. The following special studies are more or less basic, but, in a long range point of view, they could be the essential components in solving the problems of onchocerciasis control.

Priority will be given later to each of these studies. Studies under low priority will be done only when sufficient budget, manpower or sometimes a new methodology is available.

(1) *Semi-field and laboratory rearing of vector blackflies*

A trial was made to rear vector blackflies in the laboratory with partial success. Mass rearing under semified or laboratory conditions, if successful, will benefit bionomics, transmission and insecticide studies.

(2) *Cytotaxonomic studies of blackflies*

The recent description of *S. horacioi* threw at least dim light on the species com-

Tanto la formulación sólida y la dispersible en agua del temephos son eficaces en el campo, pero, sin embargo, la distancia de eficacia es limitada. Se llevarán a cabo estudios para mejorar las formulaciones en base a estudios de dispersión del producto químico liberado en los riachuelos.

(4) *Estudios adicionales sobre intervalo de aplicaciones de larvicida*

Puesto que los criaderos de vectores en Guatemala están diseminados en todas las áreas montañosas abruptas, las visitas periódicas a estos lugares a intervalos mayores de dos semanas podría significar un gran ahorro en recursos humanos. Se llevarán a cabo estudios en diversos tipos de riachuelos en relación con la reaparición de vectores en sus etapas de inmadurez, especialmente en la etapa de pupas, después de sacarlos por medio de una aplicación de insecticida.

(5) *Ensayos de control del vector adulto*

En Guatemala el control de las larvas parece ser más práctico y eficaz que el control de los vectores adultos. Sin embargo, vale la pena llevar a cabo ensayos sobre el control de vectores adultos usando métodos tales como la aplicación de insecticida de UBV (ultra bajo volumen). Se ha notado que en ciertas áreas las moscas negras también son una gran molestia.

(6) *Estudios básicos sobre control biológico*

Aunque no se puede esperar un práctico control biológico de las moscas negras vectoras en el futuro cercano, se llevarán a cabo estudios básicos sobre agentes de control biológico.

3.5 *Estudios especiales*

Los resultados obtenidos por el Proyecto durante los primeros cinco años son ciertamente informativos, pero algunos de estos desafortunadamente no son concluyentes, principalmente debido al corto tiempo asignado para cada tema. Los siguientes estudios especiales son más o menos básicos pero, desde el punto de vista a largo plazo, podrían ser componentes esenciales para resolver los problemas del control de la oncocerciasis.

Más adelante se le asignarán prioridades a cada uno de estos estudios. Los estudios de baja prioridad se llevarán a cabo al disponer

plex problems of *S. metallicum*. For further progress, cytotaxonomic studies of main anthropophilic species are required.

(3) *Experimental infection studies*

Further experimental infection studies with anthropophilic blackflies species will be carried out to decide the role of each species in onchocerciasis transmission.

(4) *Geographical and geological studies of streams*

So far vector control relies on larvicide application, therefore studies of streams based on geographical and geological knowledge are essential. Preliminary studies along this line have given us useful information on area mapping. Further detailed studies are needed.

(5) *Development of blackfly traps*

Several types of carbon-dioxide traps were already designed and utilized with little success for *S. ochraceum* collection. Efficient traps, if successfully developed, will certainly benefit in bionomic studies, as well as evaluation of control operations.

(6) *Flight range of vector blackflies*

In the flight range studies, the difficulty lies in collecting a large number of blackflies during a limited time. The studies will be done, if enough man-power and time is available.

(7) *Transmission dynamics of vector blackflies*

Since eradication of vector flies seems infeasible, it is essential to determine the level of vector density below which transmission could not be maintained. A preliminary study along this line was previously performed, however, further studies are needed. Threshold level of annual biting rate (ABR) and annual transmission potential (ATP) should also be explored.

(8) *Studies on Onchocerca spp. in animals*

Studies on *Onchocerca* spp. originating from animals may be useful in solving the identification problem of onchocercal larvae found in blackflies.

(9) *Affect of onchocerciasis on socio-economic development*

This subject was rarely studied in the past. These studies are the primary requirements for onchocerciasis control activities.

de suficientes fondos, recursos humanos o, en al unos casos, cuando hayan nuevas metodologías disponibles.

(1) *Reproducción de moscas vectores*

Reproducción de moscas vectores en condiciones parciales de campo y de laboratorio, habiéndose logrado un éxito parcial. La reproducción en masa bajo condiciones parcialmente de campo o de laboratorio, de tener éxito, serán de beneficio para los estudios de bionómica, de transmisión y de insecticidas.

(2) *Estudios citotaxonómicos de las moscas negras*

La reciente descripción de *S. horacioi* nos io, por lo menos, una leve idea sobre los complejos problemas de especies de *S. metallicum.* Para lograr un avance adicional, se necesitan estudios citotaxonómicos de las principales especies antropofílicas.

(3) *Estudios sobre infección experimental*

Se llevarán a cabo estudios especiales sobre infección experimental con especies antropofílicas de la mosca negra para decidir el papel de cada especie en la transmisión de la oncocerciacis.

(4) *Estudios geográficos y geológicos de riachuelos*

Hasta la fecha, el control de vectores se basa en aplicaciones de larvicidas y, por lo tanto, es necesario llevar a cabo estudios de riachuelos en base a conocimientos geográficos y geológicos. Estudios preliminares sobre estas bases nos han dado información util sobre el levantamiento de mapas del área. Se necesitan estudios detallados adicionales, sin embargo.

(5) *Desarrollo de trampas para moscas*

Hay varios tipos de trampas de diόxido de carbono ya diseñadas y que fueron utilizadas con poco éxito para la captura de *S. ochraceum.* De ser desarrolladas trampas que sean eficientes, esto ciertamente beneficiará los estudios bionómicos, así como la evaluación de las operaciones de control.

(6) *Alcance de vuelo de las moscas vectores*

En los estudios sobre el alcance de vuelo, la dificultad yace en la captura de gran número de moscas negras durante un período limitado de tiempo. Estos estudios se llevarán a cabo de tener a la disposición un suficiente número de personal.

(10) *Analysis of effect of nodulectomy*

Considerable data on nodulectomy have been compiled in Guatemala, since its initiation in 1915. Epidemiological analysis of these data could throw light on the effect of nodulectomy, either on transmission or incidence of blindness.

(11) *Improvement of immunological tests*

Extensive studies have been carried out in the Project on this subject. Further studies, utilizing the latest methodology, could give conclusive information on the availability of the method in evaluation of control operations.

(12) *Establishment of animal models*

Lack of adequate animal models for onchocerciasis, with an exception of chimpanzee, hampers transmission studies and chemotherapy of the disease. This is the most important study for onchocerciasis control from a long range point of view.

(13) *Studies on chemotherapy*

Because of side effects of DEC and Suramin, chemotherapy has been done only in a limited scale. Integrated control trial, both by vector control and chemotherapy should be considered.

3.6 *Staff training*

During the first five year period, emphasis was not always laid on staff training. It was unfortunate, but unavoidable, because senior staff in the Project was entirely involved in the heavy work burden of basic and operational research. As the Project has now proceeded to the second stage, high priority should be given to such activities. The detailed programmes of the staff training will be mentioned elsewhere.

3.7 *Standardization of vector control measures*

The vector control measures now being adopted are based on fortnight application of an insecticide to vectror breeding streams throughout the year. Although the amount of insecticide needed to cover all breeding sites is extremely small, man-power for the operation is remarkable. In future nation-wide control campaign of onchocerciasis, a simplified, less time- and manpower-consuming method should be devised. In SVP or else-

(7) *Dinámica de transmisión de las moscas vectores*

Puesto que la erradicación de las moscas vectores parece no ser factible, es esencial determinar el nivel de la densidad de vectores debajo del cual la transmisión no se podría mantener. Un estudio preliminar respecto a esto se llevó a cabo anteriormente. Sin embargo, se requieren estudios adicionales.

El nivel umbral de la tasa anual de picaduras (TAP) y el potencial anual de transmisión (PAT) también se deberá explorar.

(8) *Estudios sobre las especies* Onchocerca *en animales*

Estudios sobre las especies de *Onchocerca* que se originan en animales podrían ser útiles para resolver el problema de identificación de las larvas oncocérquicas encontradas en las moscas negras.

(9) *Efecto de la oncocerciasis en el desarrollo socio-económico*

Este tema ha sido estudiado muy poco en el pasado. Estos estudios son requisito primordial para las actividades de control de la oncocerciasis.

(10) *Análisis del efecto de la nodulectomia*

En Guatemala se ha recopilado un número considerable de datos sobre la nodulectomía desde su iniciación en 1915. Los análisis epidemiológicos de estos datos nos podrían dar una idea sobre el efecto de la nodulectomía, ya sea sobre la transmisión o sobre la incidencia de la ceguera.

(11) *Mejoramiento de las pruebas inmunológicas*

Se han llevado a cabo estudios extensos sobre este tema en el Proyecto. Estudios adicionales utilizando la última methodología podrían darnos información concluyente sobre la disponibilidad del método en el evaluación de las operaciones de control.

(12) *Establecimiento de animales modelos*

La falta de animales modelos adecuados para la oncocerciásis, con la excepción del chimpancé, obstaculiza los estudios de transmisión y quimioterapia en relación a la enfermedad. Este es el estudio más importante para el control de la oncocerciasis, desde un punto de vista a largo plazo.

(13) *Estudios sobre quimioterapia*

where, control trials will be made, using simplified measures based on the results of studies mentioned before. Cost efficiency analysis will also the made, in order to estimate the cost for the nation-wide campaign.

Finally standard control measures should be established. Based on this, a manual entitled "OPERATION MANUAL OF ONCHOCERCIASIS CONTROL IN GUATEMALA" will be prepared. The manual will consist of the following chapters:

(1) Organization,
(2) Survey and mapping of area,
(3) Preparation of insecticide,
(4) Application of insecticide,
(5) Entomological evaluation and
(6) Epidemiological evaluation.

This is the final target of the Project. The three year period may be insufficient to accomplish such a big task, but it is hoped that devoted efforts from all staff members of the Project can attain these final goals, in order to benefit all affected people in Guatemala, and to provide information on control of onchocerciasis to other countries of Latin America.

Debido a los efectos secundarios del DEC y del Suramín, la quimioterapia solamente se ha llevado a cabo en una escala limitada. Se deberá considerar un ensayo de control integrado, tanto por medio del control de vectores como por quimioterapia.

3.6 *Capacitación del personal*

Durante el primer período de cinco años, no siempre se le dio suficiente énfasis a la capacitación del personal. Fue desafortunado, pero inevitable, ya que el personal de más antiguedad en el Proyecto estaba totalmente involucrado en la dura carga de trabajo de las investigaciones básicas y de operación. Puesto que el Proyecto ahora ha pasado a su segunda etapa, se le deberá dar una alta prioridad a estas actividades. Más adelante se mencionará un programa detallado para la capacitación del personal.

3.7 *Estandarización de las medidas de control de vectores*

Las medidas de control de vectores que ahora se están adoptando se basan en el aplicación bimensual durante todo el año de un insecticida en los riachuelos donde existen criaderos de vectores. Aunque la cantidad de insecticida que se necesita para cubrir todos los criaderos es bastante pequeña, el personal para llevar a cabo este trabajo es numeroso. En futuras campañas nacionales para el control de la oncocerciasis, se deberá pensar en un método simplificado que tome menos tiempo y utilice menos personal. En SVP o en cualquier otra parte, se llevarán a cabo ensayos de control usando medidas simplificadas en base a los resultados de los estudios mencionados anteriormente. También se hará un análisis costo-eficacia para poder estimar el costo de una campaña nacional.

Finalmente, se deberán establecer medidas estándar de control. En base a esto, se preparará un manual intitulado "OPERACIONES DE CONTROL DE LA ONCOCERCIASIS EN GUATEMALA". Este manual tendrá los siguientes capítulos:

(1) Organización
(2) Estudio y levantamiento de mapas del área

(3) Preparación de insecticidas
(4) Aplicación de insecticidas
(5) Evaluación *entomológica*, y
(6) Evalucaión epidemiológica

Esta es la meta final del Proyecto. Podría ser que el período de tres años fuese insuficiente para llevar a cabo tan grande tarea, pero se espera que los esfuerzos dedicados de todos los miembros del personal del Proyecto hagan posible el logro de estas metas finales, para así beneficiar a todas aquellas personas afectadas por la enfermedad en Guatemala, y proporcionar información sobre el control de la oncocerciasis a otros países de América Latina.

ANNEXO

ANNEX

Annexo/Annex 1

LISTA DE LA PUBLICACIONES DEL PROYECTO
LIST OF THE PUBLICATIONS OF THE PROJECT
(enero/January 1981)

1. Tada, I.; Aoki, Y.; Rímola, C.E.; Ikeda, T.; Matsuo, K.; Ochoa A., J.O.; Recinos, M.M.; Sato, S.; Gody B.; H.A.; Castillo O.; J.J. & Takahashi, H.
   Onchocerciasis in San Vicente Pacaya, Guatemala.
   WHO/ONCHO/77. 140. 10pp. 1977.
2. *Onishi, O.*; Okazawa, T. & Ochoa A., J.O.
   Clave gráfica para la identificación de los simúlidos del área de San Vicente Pacaya, por los caracteres externos de larvas y pupas.
   GJCRCPO-MENSAP. Serie No. 2, Guatemala, 1977. 11 pp.
3. Matsuo, K.; Okazawa, T.; Onishi, O. & Ochoa A., J.O.
   Maintenance of the adults of Guatemalan blackfly, *Simulium ochraceum,* in the laboratory.
   Jap. J. Sanit. Zool., 29(3), 251–254, 1978.
4. Matsuo, K.; Okazawa, T.; Onishi, O. & Ochoa A., J.O.
   Experimental observation of developmental period of *Onchocerca volvulus* in black fly, *Simulium ochraceum.*
   Jap. J. Parasit., 29(1), 13–17, 1980.
5. Matsuo, K. & Ochoa A., J.O.
   Scanning electron microscopic studies on Guatemalan black flies.
   I. Abdominal dorsal hairs of larvae of 6 species.
   Jap. J. Sanit. Zool., 30(4), 329–333, 1979.
6. Matsuo, K. & Ochoa A., J.O.
   Scanning electron microscopic studies on Guatemalan black flies.
   II. The hairs of abdominal surfaces of larvae of 5 species.
   Jap. J. Sanit. Zool. (in preparation)
7. Ikeda, T.; Tada, I. & Aoki, Y.
   The indirect hemagglutination test for onchocerciasis performed with blood collected on filter paper.
   J. Parasitol., 64(5), 786–789, 1978.
8. Hashiguchi, Y.; Kawabata, M.; Zea F., G.; Recinos C., M.M. & Flores C., O.
   The use of an *Onchocerca volvulus* microfilarial antigen skin test in an epidemiological survey of onchocerciasis in Guatemala.
   Trans. Roy. Soc. Trop. Med. Hyg., 73(5), 543–548, 1979.
9. Tada,I.; Aoki, Y.; Rímola, C.E.; Ikeda, T.; Matsuo, K.; Ochoa A., J.O. Recinos C., M.M.; Sato, S.; Godoy B., H.A.; Castillo O., J.J. & Takahashi, H.
   Onchocerciasis in San Vicente Pacaya, Guatemala.
   Amer. J. Trop. Med. Hyg., 28(1), 67–71, 1979.
10. Ikeda, T.; Aoki, Y. & Tada, I.
    A sero-epidemiological study of onchocerciasis with the indirect hemagglutination test.
    J. Parasitol., 65(6), 855–861, 1979.
11. Kawabata, M.; Tada, I.; Hashiguchi, Y.; Yoshimura, T.; Zea F., G.; Flores C., O. & Recinos C., M.M.
    Diagnostic evaluation for skin biopsies in Guatemalan onchocerciasis patients. I. Skin snipping methods and microfilarial densities in a given minute area of the skin. (in preparation)

12. Zea F., G.; Hashiguchi, Y.; Kawabata, M.; Aoki, Y.; Tada, I.; Recinos C., M.M. & Flores C., O.
Diagnostic evaluation for skin biopsies in Guatemalan onchocerciasis patients. II. Distribution of microfilariae in the skin.
Helminthol., (in preparation)

13. Watanabe, M.
Observations on the age determination, follicular development and gonotrophic cycle of *Simulium ochraceum* in Guatemala.
Jap. J. Sanit. Zool. (in press)

14. Ogata, K.
Preliminary report of Japan-Guatemala onchocerciasis control pilot project in Guatemala.
"Blackflies" – The future for biological methods in integrated control. Edit. Dr. M. Laird., Academic Press. (in press)

15. Hashiguchi, Y.; Tada, I.; Ochoa A., J.O.; Recinos C., M.M. & Molina, P.A.
Bovine and equine onchocerciasis in Guatemala, especially in San Vicente Pacaya municipio.
J. Parasitol., 66(6), 1980. (in press)

16. Tada, I.; Mimori, T.; Sakaguchi, Y.; Kusano, M.; Hashiguchi, Y. & Recinos C., M.M.
The use of aceto-orcein stained squash preparations for enumeration of nuclei in microfilariae of various filarial parasites.
(in preparation)

17. Takaoka, H.
Pathogens of blackfly larvae in Guatemala and their influence on natural populations of three species of onchocerciasis vectors.
Am. J. Trop. Med. Hyg., 29(3), 467–472, 1980.

18. Poiner, G.O. & Takaoka, H.
Isomermis benevolus sp. n. (Mermithidae, Nematoda), a parasite of *Simulium metallicum* (Dieptera: Simuliidae) in Guatemala.
Jap. J. Sanit. Zool., 30(4), 305–307, 1979.

19. Aoki, Y.; Recinos C., M.M. & Hashiguchi, Y.
Life span and distribution of *Onchocerca volvulus* microfilariae in mice.
J. Parasitol., 66(4), 1980. (in press)

20. Ochoa A., J. O.
Biological studies of blackflies and their relative importance as vector of *Onchocerca volvulus* in Guatemala.
(in preparation)

21. Nonaka, S.; Hashiguchi, Y.; Kawabata, M.; Aoki, Y.; Tada, I.; Figueroa M., H. & Zea F., G.
Dermatological survey of onchocerciasis in Guatemala.
J. Dermatol., 7(1), 61–70, 1980.

22. Okazawa, T. & Onishi, O.
Description of a new species of *Simulium (Simulium)* Latreille and redescription of *Simulium (Simulium) metallicum* Bellardi from Guatemala (Diptera: Simuliidae).

23. Yamada, H.
Onchocerciasis (Robles disease, River-blindness) in Guatemala and Ghana. Clinical features and epidemiological research.
Folia Ophthalmol. Jpn., 29 (11) 1817–1837, 1978.

24. Yamada, H.
Fluorescein angiographic findings in ocular onchocerciasis in Guatemala, with reference to findings of ERG of Ghanaian patients.
Acta Soc. Ophthalm. Jap., 83(7), 874–886, 1979.

25. Yamada, H.; Rímola, E.C. & Zea F., G.
Oncocercosis

Ann. Rept. of Ohara General Hospital., 22(29), 21–30, 1979.
26. Undeen, A.H.; Takaoka, H. & Hansen, K.
The evaluation of *Bacillus thuringiensis* var. *israelensis* de Barjac as a larvicide for use against *Simulium ochraceum*, the Central American vector of onchocerciasis. (in preparation)
27. Nakamura, Y.; Yamagata, Y.; Takaoka, H.; Takahashi, M.; Ochoa A., J.O.; Molina, P.A. & Takahashi, H.
Control trial of the vector of onchocerciasis, *Simulium ochraceum* (Diptera: Simuliidae) in the Lavaderos River valley, Guatemala.
Jap. J. Sanit. Zool., 32(1), 1981. (in press)
28. Tanaka, I.; Hashigushi, Y.; Okazawa, T.; Ochoa A., J.O. & Tada, I.
Duration of blood feeding of *Simulium ochraceum* in relation to intake of *Onchocerca volvulus* microfilariae.
Jap. J. Sanit. Zool., 31(3), 209–214, 1980
29. Ito, S.; Tanaka, I. & Ochoa A., J.O.
Comparative studies on the affinities of two black flies, *Simulium metallicum* and *S. ochraceum* for the larvae of *Onchocerca volvulus* in Guatemala.
Jap. J. Sanit. Zool., 31(4), 1980. (in press)
30. Akiyama, T.; Anan, S.; Nonaka, S. & Yoshida, H.
Immunological studies on onchocerciasis in Guatemala.
I. Immunoglobulins levels in Guatemalan onchocerciasis.
(in preparation)
31. Poinar Jr., G.O. & Takaoka, H.
New mermithids (Nematoda) from Guatemalan blackflies (Diptera: Simuliidae).
(in preparation)
32. Takaoka, H.
Effects of temperature on infectivity of *Simulium ochraceum* with a Guatemalan strain of *O. volvulus*.
(in press)
33. Takaoka, H.
Further studies of pathogens of blackfly larvae in Guatemala and their influence on natural populations of three species of onchocerciasis vectors.
(in preparation)
34. Takaoka, H.
Seasonal breeding of *S. ochraceum* in temporal stream system in Guatemala.
(in preparation)
35. Takaoka, H.; Hansen, K.; Takahashi, H.; Ochoa A., J.O. & Juárez, E.L.
Development of *Onchocerca volvulus* larvae in *Simulium ochraceum* at various altitudes in Guatemala with special reference to the ambient temperature.
(in preparation)
36. Hashiguchi, Y.; Kawabata, M.; Tanaka, I.; Okazawa, T.; Flores C., O. & Recinos C., M.M.
An examination in a single endemic area of onchocerciasis in Guatemala, with special reference to the seasonal variation of the microfilarial skin concentration.
Trans. Roy. Soc. Trop. Med. Hyg. (in preparation)
37. Hashiguchi, Y.; Kawabata, M.; Ito, S. & Recinos C., M.M.
The intake and damage of *Onchocerca volvulus* microfilariae by *Simulium ochraceum* in Guatemala.
Brit. J. Helminthol. (in preparation)
38. Yoshimura, T.; Hashiguchi, Y.; Kawabata, M.; Flores C., O.; Gudiel P., O.O. & Chester, E.
Prevalence and incidence of Onchocerciasis in San Vicente Pacaya, Guatemala.
(in preparation)

39. Anan, S.; Akiyama, T.; Ushijima, N.; Nonaka, S.; Yoshida, H.; Sakamoto, M.; Aoki, Y. & Zea F., G.E.
Immunological studies on onchocerciasis in Guatemala. II. Detection of specific IgG and IgE antibodies to *Onchocerca volvulus* antigen using ELISA technique.
Am. J. Trop. Med. Hyg.
or J. Dermatol. (Tokyo) (in preparation)

40. Okazawa, T. & Takahashi, H.
Blackflies (Simuliidae; Dipteral) in highland streams in Guatemala with special reference to their life cycle.
(in preparation)

Annexo/Annex 2

LISTA DE LOS PARTICIPANTES JAPONESES EN EL PROYECTO
LIST OF THE JAPANESE PARTICIPANTS IN THE PROJECT
(Abril/April 1976 – Diciembre/December 1980)

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Jefe del Proyecto/Project Leader

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Takeshi SUZUKI Oct. '80–	1-4-5 Tamatsutsumi, Setagaya-ku, Tokyo 158, Japan

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Yoshiki AOKI May '76–Aug. '77	Department of Parasitology, Institute for Tropical Medicine, Nagasaki University, Nagasaki 852, Japan
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