HISTOCHEMICAL STUDIES ON *Onchocercoa volvulus* MICROFILARIAE AND THEIR PREVALENCE IN NORTHERN NIGERIA

BY

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DEDICATION

This thesis is hereby dedicated to my grandfather, GALADIMA PYATA and my late grandmother KOKO GALADIMA for their role in my Primary School Education. It is often said that grandparents spoil children, but you put me in the right way that I should go. I may forget myself but I cannot forget you.

CERTIFICATION

This thesis entitled "Histochemical studies on *Onchocerca volvulus* microfilariae and their prevalence in Northern Nigeria" by MUSA GALADIMA meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DECLARATION

This is to certify that I carried out the work reported in this thesis at the laboratory of the Department of Microbiology, Ahmadu Bello University, Zaria, under the supervision of Dr. C.G. Vajime and Professor B.J. Harris of the Department of Biological Sciences, Ahmadu Bello University, Zaria.

This thesis is an original work carried out by myself and no part of it has been presented elsewhere for a degree or diploma. The work of other investigators is acknowledged and referred to in this presentation.

MUSA GALADIMA

ABSTRACT

This study reveals foci of Onchocerciasis, and prevalence rates, as well as acid phosphatase staining patterns of 0. volvulus microfilariae. A survey of Onchocerciasis was carried out in 7 villages in Kachia local government area of Kaduna State, 2 villages in Chenchaga local government area of Niger State and 2 villages of Mariga local government areaof Niger State. Diagnosis was based on the presence of micro-filariae of Onchocerca volvulus in skin biopsies. The population sampled ranged from children of less than one year old to adults aged above 55 years. This disease is reported for the first time from Iburu, Makyali, Kurmin Idon, Kurmin Biri, and Akupal villages of Kachia local government and from Bari village of Chanchaga local government and Kaliko village of Mariga local government. The prevalence rates of 0. volvulus infection were as follows: 24.1% for Kwakuti; and 20.4% for Bari villages of Chanchaga local government area, 4.7% for Kaliko village and 4.8% for Zungeru Primary School children of Mariga local government area. Villages in Kachia local government area had the following prevalence rates: Iri (41%)$_f$ Kurmin Idon (35.3%), Doka (42.0%), Makyali (33.3%), Iburu (42.1%) Akupal (14.9$) and Kurmin Biri (33.9%). Sex did not have any significant effect on the prevalence in

villages in Kachia local government area. The males in Chanchaga local government area were however about twice as infected as the females; results show that infection starts very early in life in the areas investigated. A child of less than one year of age was found to he infected. Projected figures show that there are over 200,000 infected people in Kachia local government area with over 16,000 people "blind, and in Chanchaga local government area there are aboiit 115,000 infected people with over 4,300 people blind. Other clinical, aspects of the disease in the areas include dermatitis, depigmentation of the legs and Onchocercomata.

Histochemical enzyme study, based on the demonstration of Acid-phosphatase activity in the microfilariae obtained from 7 of the savanna villages shows that the parasite Onchocerca volvulus is a complex. Two new types (Type XIV, Type XV) of the species complex as revealed by acid-phosphatase activity and seven previously described staining patterns (Types I, II, III, IV, V, VI, and IX) are reported and summarised below: Type I (Anal pore-Excretory pore) 5 Type II (Anal pore-Excretory pore-Innenkoper) 5 Type III (Anal pore Innenkoper); Type IV (Anal pore only); Type V (Negative). Other staining patterns found were Type VI (Amphids only); Type IX (Excretory pore only); Type XIV(Innenkoper only) and Type XV (Anal pore Excretory pore and between excretory pore and Amphids). In 6 out of the

7 villages where microfilariae were stained for acid phosphatase activity, type IV was found to be the most common staining type. An exception was found in one of the savanna villages in which the forest type (V) was the commonest. The significance of the staining patterns in relation to the Epidemiology of Onchocerciasis is discussed.

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TABLE OF CONTENTS

	TITLE	PAGE
	Title Page	i
	Dedication	ii
	Certification	iii
	Declaration	iv
	Abstract	v
	Acknowledgement	viii
	Table of Contents	xi
	List of Tables	xiv
	List of Plates	xvi
	CHAPTER ONE:	
1.	Introduction	1
	CHAPTER TWO:	
2.	Literature Review	6
2.1	Simuliidae: Taxonomy and Bionomics	6
2.2	Simuliidae as Vectors of Onchocerciasis	11
2.3	Sampling Methods for Simuliidae	18
2.4	Laboratory Rearing of Simuliidae	20
2.5	Morphology, life cycle and transmission of *Onchocerca volvulus*	25
2.5.1	Transmission of *O. volvulus*	32
2.6	Pathology of Onchocerciasis	38
2.6.1	Reaction to adult worms	38
2.6.2	Reaction to the Microfilariae	41
2.6.2.i	Onchocercal dermatitis	41
2.6.2.ii	Ocular Onchocerciasis	47

		PAGE
2.7	Epidemiology of Onchocerciasis	51
2.7.1	Onchocerciasis in Africa	51
2.7.2	Onchocerciasis in the American sub-region	56
2.7.3	Onchocerciasis in Nigeria	60
2.7.4	Factors affecting distribution of Simulium damnosum s.l.	64
2.8	Diagnosis of Onchocerciasis	69
2.8.1	Parasitological Methods	69
2.8.2	Mazzotti Reaction	73
2.8.3	Immunology and Immunodiagnosis of Onchocerciasis	74
2.9	Chemotherapy of Onchocerciasis	80
2.10	Control of Onchocerciasis	90
2.10.1	Noclulectomy	90
2.10.2	Chemical Control	92
2.10.3	The Onchocerciasis control Programme	97

CHAPTER THREE

3.	Materials and Methods	104
3.1	Study area	104
3.1.1	Kachia local Government (Kaduna State)...	104
3.1.2	Chanchaga Local Government (Niger State)	107
3.1.3	Mariga Local Government (Niger State) ...	108
3.2	Preliminary investigations on Onchocerciasis	108
3.2.1	Parasitological survey by skin snipping	108

3.2.2	Clinical Examination	110
3.3	Histochemical enzyme staining	111
3.3.1	Preparation of slides	111
3.3.2	Acid phosphatase staining	111
3.3.2.i	Reagents	111
3.3.2.ii	Staining Procedure	113

CHAPTER FOUR

4.1	Results of Preliminary Investigations on Onchocerciasis	115
4.1.1	Parasitological Results	115
4.2	Histochemistry	123
4.3	Field Observations	136

CHAPTER FIVE

5.	Discussion	138
	References	152
	Appendix	183

LIST OF TABLES PAGE

1. Prevalence rates of Onchocerca volvulus infections in seven villages of Kaduna: female population. 116

2. Prevalence rates of Onchocerca volvulus infection in seven villages of Kaduna State: male population. 117

3. Prevalence rates of Onchocerca volvulus infection in two villages in Niger State ... 119

4. Comparision of clinical patterns of Onchocerciasis in the affected villages 120

5. Total number of people sampled, percentage prevalence and projected prevalence of Onchocerca volvulus infection. 121

6. Prevalence of blindness in the affected population and in the projected population 122

7. Frequency of acid phosphatase staining patterns among people examined. 132

8. Types of combinations and frequency of acid phosphatase staining patterns 135

9. Total number of persons and microfilariae examined and the percentage distribution of various staining patterns of microfilariae in 8 areas. 135

APPENDIX

PAGE

1. Number of people sampled and number found infected with O. volvulus in villages in Kaduna and Niger States.... 183

2a. Histogram of % prevalence of Onchocerciasis in Kachia local government area of Kaduna State. 188

2b. Histogram of % prevalence of Onchocerciasis in Chanchaga local government area of Niger State. 189

2c. Histogram of % prevalence of Onchocerciasis in Kachia and Chanchaga local government areas of Kaduna and Niger States. 190

2d. Histogram of % prevalence of acid phosphatase staining types in the savanna 191

LIST OF PLATES

		PAGE
Plate 1 - 4	Acid phosphatase staining patterns of Onchocerca volvulus microfilariae	
Plate 1a	Enzyme activity at Anal pore and excretory pore.	125
Plate 1b	Enzyme activity at Anal pore, innenkoper and excretory pore	125
Plate 2a	Enzyme activity at Anal pore and innenkoper.	127
Plate 2b	Enzyme activity at Anal pore only	127
Plate 3a	No enzyme activity	129
Plate 3b	Enzyme activity at innenkoper only	129
Plate 4	Enzyme activity at Anal pore, excretory pore and between the amphids and excretory pore.	131

INTRODUCTION

Onchocerciasis or River blindness is caused by the filarial worm Onchocerca volvulus. The parasite was first isolated from a patient in Ghana by Leukart 1893 (cited by Sasa 1976). Onchocerciasis ranks as one of the world's most formidable infectious diseases. It causes an unsightly irritating skin disease which results in social ostracism due to the prematurely aged appearance of the atrophic skin and also because of the high prevalence in adults of ocular impairment especially in many communities in the savanna regions of Africa. Onchocerciasis affects about 40 million people in the endemic regions of tropical Africa. Mexico, Guatemala, Venezuela, Colombia and South Yemen. In patients with heavy infections, millions of microfilariae migrate into the skin, where they cause a prutitic and debilitating dermatitis. Microfilariae also migrate into the eye where they cause a variety of inflammatory changes that may progress to blindness. In some hyper endemic regions 80% of those aged 25 years and above are blind (Gibson et al, 1980). The prevalence and severity of the dermatitis, the eye disease and other complications vary from one endemic region to another. Onchocerciasis is an economically important disease. It incapacitates large sections of the community and makes them an economic burden to the

country. A whole village in a hyperendemic area may desert the area and the area becomes agriculturally useless. It follows that while one piece of fertile land may be deserted, another piece of land will become overcultivated with consequent decrease in the crop yield. The disease is widespread in Nigeria and was reported for the first time in 1908 in northern Nigeria. The vectors of the parasite which are the blackflies of the genus Simulium (Diptera: Simuliade) breed in fast flowing rivers where they deposit their eggs on rocks and submerged vegetation.

Simulium damnosum s.l. is the vector for Onchocerciasis in West Africa while Simulium woodi. Simulium naevei and Simulium ochracoum are the vectors of the parasite in East Africa, Guatemala and Mexico respectively. The forest and the savanna types of the Simulium damnosum are now known to have varying behavioural patterns which are associated with their different transmission pot entials for Onchocea volvulus and also with the differences in the clinical manifestations of the disease in the forest and savanna regions of West Africa (Duke, 1968; W.H.O., 1987). Cytogenetic studies by Vajime and Dunbar (1975) have shown that S. damnosum which used to be regarded as a single species is actually a species complex with 8 cytospecies so far elucidated; all are possible vectors of Onchocerca volvulus.

Diagnosis of the disease can be made by observation of some clinical symptoms, and by parasitological demonstration of the microfilariae in skin snips. Serological diagnosis which employs the indirect fluorescent antibody test (IFAT), indirect hemagglutination test (IHA), enzyme linked immunosorbent assay (ELISA), gel diffusion and several other techniques have been tried. At present the serological techniques do not give specific results. Cross reactions are obtained with other filariae worms and non filaria Nematodes (Kagan, 1963; Ambrose-Thomas, 1974). Improved techniques for parasite antibodies and circulating antigen detection will obviate the shortcomings of parasitological diagnosis, such as inability to detect low parasite load, and unisex infection (Ambrose-Thomas, 1974; Ottensen, 1984). At present, however, parasitological diagnosis based on the demonstration of microfilariae in skin biopsies and microfilaruria remains the only reliable means of diagnosis.

Several compounds have been tried for the treatment of onchocerciasis but suramin and diethyl carbamazine citrate stand out as acceptable curative chemotherapeutic agents. Both drugs have serious side effects which are often fatal (Budden, 1959; Anderson and Fuglsang 1976; Thylefors and Rolland, 1979, Taylor, 1984).

Diethyl carbamazine citrate is more tolerated and remains the drug of choice for treating onchocerciasis and other human filarial worm infections. Ivermectin which has been used successfully in animals (Goodwin, 1984) is now being tried for the treatment of onchocerciasis (Taylor, 1984). Field trial results show that it is less toxic than Diethyl carbamazine citrate (Awadzi et al., 1985).

Chemotherapy, vector control and Nodulectomy are means of controlling Onchocerciasis and of these, Vector control is so far the most important.

The cost of insecticides, resistance to such insecticides (W.H.O, 1987), shortage of qualified personnel, as well as inaccessibility of Simulium breeding sites are some of the many factors that militate against vector control.

Onchocerciasis has now assumed national importance in Nigeria and it has been incorporated into the national development plan (Crosskey, 1981). The disease which was thought to be confined to the savanna region of the country in the late fifties (Budden, 1956) has now been shown to be present in many other parts of the country, where it has been sought for (Crosskey, 1979). Information on the true situation of Onchocerciasis in Nigeria is scanty and this may be due to the fact that there are not many scientists working in the

field to provide the much needed information for the National Onchocerciasis Control Programme (NOCP).

The aims and objectives of this work have been directed towards achieving the national goal for the control and eradication of onchocerciasis, viz: (i) to find out areas of endemic Onchocerciasis in some communities in the savanna region of northern Nigeria, (2) to document the clinical manifestations of the disease in the study areas and (3) to find out the acid phosphatase staining patterns of O. voluvus microfilariae and its significance to epidemiology of Onchocerciasis.

CHAPTER 2

LETERATURE REVIEW

2.1 SIMULIIDAE: TAXONOMY AND BIONOMICS

Simuliidae (Blackflies) are widespread, and occur in all major land masses where there is a running water of sufficient permanance to provide the biological requirements of the development stages.

Blackflies are however absent in polar regions, barren deserts and coral island (Islands of volcanic lavas devoid of streams). Members of the genus Simulium are so widespread that its range is almost fully coincident with that of the simuliidae. There are about 1270 recognised species of the genus simulium s.l. Medically proved important members of this genus are: Simulium callidum. Simulium damnosum s.l., Simulium exiguum, Simulium guianense, Simulium metallicum and Simulium neavei group. Others are Simulium ochraceum. Simulium sanguineum and Simulium woodi (Crosskey, 1981 Raybould and White, 1979).

Even though the family is of small size, its classification is more difficult because of the remarkable morphological homogeneity that it possesses.

Naming a specimen from its morphological appearance becuase no other criteria are available remains a legitimate part of everyday taxonomy. The taxonomy of simuliids requires the incorporation data derived from other sources (i.e. cytotaxonomy, and zygotaxonomy) into the taxononic system. The homogeneity of blackflies makes suprraspecific classification difficult. The difficulty arises largely from the

almost unbroken evolutionary continum characterising the morphological attributes of simuliids.

Before Dunbar (1966) first performed cytogenetic studies on simulium damnosum s.l., the species was considered to be a single but morphologically and biologically variable species. Study of the giant polytene larval salivary gland chromosones is uniquely able to reveal the existence of sibling species for the following reasons:

(1) The banding patterns of the polytene chromosone possess enormous morphological details with sequential specificity unaffected by convergence phenomena.

(2) Sibling species generally differ by inversions, interchanges or other rearrangements in the banding sequence of one or more of their chromosones. (3) The principal ways in which sibling species differ chromosonally are (a) fixed sequence differences, commonly inversions or interchanges (b) Sex chromosone differences, for in many species the X and Y chromosones are differentiated in their banding sequence and these differentiated sex chromosones systems are species specific. (c) inversion polymorphism, that is, the occurence of alternative banding sequences that may occur heterozygously. By 1978, 26 cytospecies of S. damnosum s.l. had been identified (Dunbar and Vajime, 1972, Vajime and Dunbar 1975, Dunbar 1976). Eight of these cytospecies have been described and named by Vajime and Dunbar (1975) while the remaining 18 cytospecies have been identified by the locality from which it was first recognised.

cytospecies have been identified by the locality from which it was first recognised. 18 cytospecies have been found in East Africa while 10 sibling species have been found in West Africa with only 3 of these sibling species (dannosum. s.s., Sirbanum and Menge) found on both sides of the continent. The eight cytospecies which have been described and named by Vajime and Dunbar (1975) are S. dannosum, s.s., S. sirbanum. S. sudanense, S. dieguerense, S. soubrense, S. sanctipauli, S. yahense and S. squamosum. The criteria used in separating siblings are (1) a minimum of a single and interspecific inversion, (2) a difference in X and or Y chromosomes (3) differences in the sets of intraspecific inversion. Presence of one or more of the above criteria is differential. S. (E) dannosum, s.s. S. (E) Sirbanum and S. (E) sudanense S. (E) soubrense, S. (E) Squamosum, S.(E) sanctipauli are the cytospecies found in Nigeria (Vajime and Dunbar 1975). The splitting of the familiar Simulium dannosum into numerous species goes far towards explaining the bewildering diversity of behaviour recorded for this species. It is therefore submitted that, instead of just one species, many occur in this complex, each with its own distinct biological and ecological requirements. Western species prefer long rivers running through gentle rolling country while those of East Africa usually prefer mountain streams. Hydrochemical composition of the stream

concerned is apparently more important than the size of rivers. In East Africa several species of the sanga group have been observed breeding in the same river but at different locations. pH is the most important hydrochemical factor e.g. it has been found that the sebwe form occurs in the higher reaches at pH 6.4 and with increasing H^+ content, it is replaced by the turiani, sanga and finally the Nkusi forms (Dunbar and Gruno wald, 1974).

Morphological features which have been used in attempt to show variations in the species complex of S. damnosum are variations in dorsal thoracic pattern of the male and variations in the biting habits. Other useful observations are seasonal and regional changes in size of the adults, variations in colour of the antennae of females, differences in the setal tuft of the fore-tarsus, colour variation and wing size (Lewis and Duke, 1966). After studying variations in colour, thickness of the setal tuft of the foretarsus and wing length, Lewis and Duke (1966) concluded that S. damnosum from Northern savanna areas differ from forest areas and that these differences may have been partly of a clinical nature and due partly to factors affecting individual blackfly during development. By studying differences in the antennae, the number of teeth on the blade of the maxilla, number of spinules on the radial vein of the wing, Soponios and Peterson (1976), and Quillevere and Sechen (1978) were

able to separate the females of all six West African species of the complex. Garms (1978) confirmed that the colour of the basal wing tuft was useful in separating the savanna species from the forest species, that the length, shape and colour of the antennae separated the former from two of the latter and that different populations of the damnosum complex are distinguishable by morphometric means. According to Peterson and Deng (1981) the siblings from the savanna areas (S. damnosum s.s., and S. sirbanum) are collectively separable from those of the forest areas (Squamosum. sanctipauli. Soubrense and Yahense) by the following respective combination of characters; pale setae (versus dark setae) on the stem vein and basicosta of the wing and all pale setae (versus at least some dark setae mixed with pale) on the vertex and Postcranium. They further stated that it was possible to correlate the morphological variation of these adults with the cytotypes as distinguished by Vajime and Dunbar (1975).

Another possible method for separating sibling species is that of enzyme electrophoresis (Garms and Zillmann, 1984).

Members of the Simulium neavei group which are found in East Africa include S. atyophilum, S. ethiopiense, S. goinyi, S. hightoni, S. Kivuense, S. neavei, s.s., S. nyasalandicum, S. ovasmas, S. woodi and Ovazzae form (Raybould and White, 1979). Not all of them however bite man and may not therefore be involved in the transmission of Onchocerciasis. The S. neavei group

(subgenus lewisellum) includes all species with larvae and pupae that attach themselves to riverine crabs (Nelson, 1970, Raybould and White 1979).

2.2 Simullidae As Vectors of Onchocerciasis:

Simuliidae are small strongly built flies, the females of which may bite preferred species of animals or birds in order to obtain a blood meal providing nutrition for Oogenesis. The immature stages of Simulium species inhabit water courses, usually fast flowing streams or rapids in rivers. Juice of plants is the first food for the females and the only food for the male. However after coupling which takes place only once in the 24 hours after emergence, the female requires a blood meal for ovarian development (Nelson, 1970, Wenk, 1981). In one meal the female takes about one milligram of blood. Three to five days later laying of eggs takes place. Blackfly eggs are either scattered over water or laid in masses usually deposited on vegetation over hanging the water surface (Raybould and White, 1979, W.H.O., 1974). The larvae obtain food by filteration of organic matter or other substances carried by the water current. Ten days after eggs have been laid, the larvae are fully developed and begin to change to pupae in coccoon which is attached to a submerged supporting material. Three days later the adults emerge in a bubble of air and take flight as soon as they reach the surface (W.H.O., 1974).

Simulium bites during the day particularly in the overcast weather and in the shade. The West African

forms bite near the ground while the Central American forms bite in the upper part of the body.
The bite itself is not painful but itching starts rapidly causing discomfort which becomes intolerable when bites are numerous, (Nelson, 1970). In some places where fly population is very high, farmers and cattle rearers in West Africa often tie rags round their legs to prevent fly bites. The flies can bite very far away from the breeding place when conditions are favourable (Wenk, 1981, Garms et al., 1979). In wet weather the insects disperse rapidly at a distance of up to 40 kilometers and even 180 kilometers or more in gallery forests with favourable air current. They may then, if conditions are favourable establish new breeding places. The female Simulium is anthropophilic. In endemic areas there is thus a likelihood of its being infected with microfilariae in the skin. The highest bitting rates are in the morning and afternoon (W.H.O., 1974). It has been shown that many of the microfilariae fail to leave the midgut of the fly and that they are apparently trapped by the peritrophic membrane which forms around the blood meal (Duke, 1968, Omar and Garms 1977). The membrane provides an impenetrable barrier, and the microfilariae that fail to migrate die and desintegrate in the midgut. The mean intake of microfilariae per fly is 17 but less than 80% of those microfilariae that do escape eventually develop to the infective stage. If all the microfilariae that are ingested develop to maturity this would

undoubtedly cause heavy mortality in the flies (Omar and Garms, 1977). In nature, only about 2-5% of the fly population is infected. The distribution of flies is therefore more than the occurence of Onchocerca volvulus infection. According to Omar and Garms (1977) the mean microfilariae intake of more than 400 microfilariae per fly proved fatal to all the flies within the first 3 hours of infection. They proposed five reasons why flies with high intake of microfilariae die: (1) reverse migration of numerous microfilariae from the expanded to the tubular part of midgut where they cause serious injury and disintegration of gut epithelium; (ii) abrasive damage to the stomach epithelial cells by the invading microfilariae with occassional release of the gut content into the homocoele, (iii) interruption of the formation of the peritrophic membrane, particularly at its anterior and posterior ends with subsequent failure of the flies to digest the blood in the stomach, (iv) passage of large amount of parasitized blood from the stomach backwards into the hind gut leading to its mechanical obstruction, and (v) invasion and injury of various organs of the fly among them, the ventral nerve cord, brain, optic nerve, eye halters, fat body and flight musculature by excessive numbers of microfilariae. This study was carried out using O. volvulus microfilariae and Simulium metallicum. In a similar study involving O. volvulus microfilariae and S. ochraceum (Omar and Garms, 1975) it was demonstrated that high intake of microfilariae by the vector was not necessarily lethal to the fly

because most of the parasites were mechanically damaged by the buccopharyngeal armature during ingestion. It was shown that S. metallicum lacks buccopharyngeal armature and as such most ingested microfilariae reached the thoracic muscles undamaged. Duke (1968) and Blacklock (1926) showed that it is the parous flies that are involved in transmission. Forest Simulium damnosum s.l. has the highest biting density between 2-3 p.m. and the highest biting rate occurs in the months of June and July.

The young adult female of S. damnosum s.l. after emergence from the pupa has a well developed abdominal fat body. As the age of the fly increases the fat body disappears so that in the majority of flies which have taken a blood meal and laid eggs the fat body is either very reduced or almost entirely absent (Crosskey, (1958). S. damnosum s.l. with abundant fat body have normally not taken a blood meal are therefore nulliparous, whereas parous flies generally have little or no fat. Hence an assessment of the amount of fat body is useful in distinguishing a young nulliparous fly population from an older, largely parous population in which many flies will have already fed before capture and in which O. volvulus infections are more likely to occur. By weighing flies caught at different times of the day, Crosskey (1958) found out that the weight is heaviest in flies coming to bite in the early morning

and late afternoon, and lightest in flies biting in the middle part of the day. Examination of the fat body of the flies weighed showed that the weight distribution was closely correlated with the amount of fat body in the flies biting. As the mean weight of flies decreases towards the middle of the day, the proportion of flies with no fat body rises. Lewis (1958) used the structure of the ovarioles in distinguishing parous from Nulliparous flies. The absence of corpora Lutea was used as a criterion of Nulliparous fly. According to Omar and Garms (1975) the ovaries of nulliparous flies are clear and fall apart when teased with the dissecting needles. In parous flies most ovaries are yellowish in colour and could be stretched out and teased without breaking up. According to him the state of the fat body could not be relied upon for distinguishing parous from nulliparous flies. Duke (1968), Duke, Anderson and Fuglsang (1975) however considered the state of the fat body as adequate evidence for separating the two groups of the fly population. In savanna grassland permanent breeding sites are restricted to a few continously flowing rivers margined by gallery forest. Such lines of scattered bushes and small trees may extend along the banks of rivers flowing only seasonally also. During the dry season, S. damnosum s.l. may be active within these gallery forests but with the first rains and recommencement of flow in seasonal rivers, they

disperse linearly along the water surface under protection of the overhanging vegetation, thereby extending the breeding zone. Radial dispersal is observed in bush savanna during the wet season and throughout the year in forest (Wenk, 1981). Early in the day when humidity is high blackflies traverse the grassland by flying from one dump of bushes to another. They may then bite man in open fields or even in villages, many kilometers distant from their emergence sites. In the forest zone fly dispersal is not limited by dryness due to solar radiation but rather by the availability of sufficient light for flight orientation. The number of fly bites per man per year in the forest zones will therefore be in multiples of that in the savanna. This however is not necessarily the same for transmission potential in all cases since it depends to a great extent on the availability of Onchocerca volvulus microfilariae in the human population. In the savanna region nulliparous females of S. damnosum s.l. usually disperse inland away from their riverine emergence sites. Parous flies characteristically remain close to the banks of the rivers where they developed. The risk of infection should therefore be expected to be highest close to the breeding rivers. Duke (1975) however found proportions of parous and infective black flies higher inland and concurrently higher cases of onchocerciasis

than along the river banks. Many factors may contribute to fly dispersal to long distances from their breeding sites. It has to be assumed that endogenous diurnal rhythms of appetite behaviour and exogenous stimuli such as light, temperature, odour and humidity activate the blood seeking flight. Air movement may influence its direction. Odour, CO_2 output, colour, body shape, and movement of the host affect the attraction and landing of the fly (Wenk, 1981). In the Cameroon rainforest, electric fan traps baited with trousers which had been worn by man for several days captured more females of S. damnosum s.l. than did traps baited with worn shirts of similar material. A number of human body fluids were used as bait but only sweat appeared to contain the olfactory attractant(s). The fluid portion of filtered arm sweat attracted more flies than did the residue (Thompson, 1976). Captured flies fed on sugar solution could live up to 63 days (Davies, 1953). After coming out of the pupa until just after the first blood meal, all the ovarioles remain at the same stage of development. Three sections of the ovariole are evident: the germarium, partially developed second Oocyte and a large further developed first oocyte. After commencement of oviposition a small follicular relic or "rest body" becomes evident below the largest oocyte. A single blood meal is necessary and sufficient to ensure maturation of an

egg batch. The first gonotrophic cycle takes about 3-4 days while subsequent ones require 4-5 days (Duke, 1968). parous and nulliparous flies differ in the following ways (Wenk, 1981).

	Nulliparous	Parous
Follicular relics	Never present	Present
Ovaries	tough, disrupt as soon as stretched	Loose and easily stretched
Fat body	Large and numerous cells present	Reduced or completely absent
Malpighian tubules	Rather dark	Progressively clear with age.

The first oviposition comprises 400-600 eggs, the number however depends on the size of the female. The average size of eggs is larger in forest than in savanna taxa of S. damnosum s.l. Simulium damnosum s.l. females have maximum longevity of one month. Simulium damnosum s.l. longevity is much greater in the savanna than in the forest; and in the savanna in the dry season as compared with rainy season.

2.3 SAMPLING METHODS FOR SIMULIIDAE

Various sampling methods for adult flies have been well documented (Service, 1981). Both bait

collections undertaken during day light get more representative samples of anthropophilic blackflies than other methods. The human bait method is more usuful in sampling S. damnosum s.l. than S. callidum and S. metallicum which are termed shy feeders which are best collected by descending nets. Dark skinned people are generally more attractive to blackflies than lightskinned people (Crosskey, 1955). Animal bait collections has been used in sampling black flies which are of Medical and veterinary importance. In this method the collector could either remain with the bait animal or inspect it from time to time at 15 minute intervals to collect black flies attracted to it. This procedure may make it difficult to differentiate flies attracted to the baited animals from those attracted to the collector. Bed nets have been used to enclose a variety of bait animals and large descending nets have been used to catch Simuliids attracted to large Mammals such as horses and reindeer(Bennett, 1960; Disney 1972) commonly the bird is enclosed in a Mesh cage which is placed on a base-board either at ground level or raised to various heights. At intervals a cage is lowered over it so as to enclosed it and all blackflies attracted to it. In Ghana, Odotcyinbo (1969) using bird baited traps caught 931 blackflies over 11 days. 88.9% of which were of the S. damnosum complex. In East Africa, Raybould (1965) placed on

inter nittently-activated fan over a chicken and caught several hundreds of S. adersi while in Cameroon, Thompson (1977) used fan traps to catch the S. damnosum complex attracted to chicken and sheep. CO_2 traps which employs release of CO_2 from cylinders or by dry ice sublimation has also been used. Other attractant method of sampling Simulium include the use of silhouette (Bradburry and Bennett, 1974, Fallis and Raybould, 1975), light traps (service, 1979) and attractive sticky traps (Walsh, 1972). Non attractant methods of sampling simuliidae which provide useful informations on dispersal, oviposition, swarming and vector feeding have also been used (Service, 1981). These include non-attractant sticky traps, sunction traps and vehicle mounted traps.

2.4 LABORATORY REARING OF SIMULIIDAE

Laboratory rearing of Simulium damnosum s.l. will provide research tool for cytotaxonomic studies, insecticidal test and experimental transmission of Onchocerciasis. Adults of S. damnosum s.l. appear to survive better when kept singly in small tubes than when grouped together in larger containers (Raybould and Mhiddin, 1974). The use of plastic tubes is preferred over glass tubes because in the latter water condenses and traps the flies causing their death. The tubes are usually placed in trays floating on water in drum. Adult S. damnosum s.l. require

sugar, which is usually provided in solution absorbed in cotton-wool or foam rubber. However sucrose crystals and water can be supplied separately. They can also be fed on fruits such as sultanas and sliced apple, or flowers on which simuliids naturally feed (Raybould and Grunewald 1975). Few cases of S. damnosum s.l. mating in captivity have been observed (Wenk and Raybould, 1972, Raybould and Grunewald, 1975). Specially designed emergence cages have been used for mating experiments. These cages (40cm x 40cm x 60cm) are made of black cloth with a triangular opening about 40cm high on one side. This opening is covered with netting through which light enters and on the surface of which (light enters and on the surface of which)the blackflies move and copulate.

Another obstacle encountered in the laboratory rearing of S. damnosum s.l. is the difficulty with which S. damnosum s.l. are induced to take blood meal Some of the few instances where S, damnosum s.l have been induced under laboratory condition to take blood meal have been reported by Muirhead - Thompson (1977), and Mcmahon (1968). Mcmahon (1968) successfully fed several species of African simuliids including those of S. damnosum complex using an appratus incorporating a feeding tube containing warmed human or ox-blood covered by a membrane. Both Wuild and laboratory reared adults engorged through the membrane. The work of Grune wald and Wirtz (cited by Raybould, 1981).

showed that the type of animal used as well as the temperature affect the willingness of the flies to take blood meal. Using a rabbit, the feeding rates obtained with kibwesi and Kisiwani females were respectively 41 and 51% on a brown rabbit but only 14 and 21% on a white one. When the same authors offered a mixture of heated blood and saturated sucrose solution (absorbed on filter paper) to four African Simulium species (including two in the S. damnosum complex) nearly all individuals took blood but subsequently produced very few eggs. S. damnosum s.l. females only rarely lay eggs in captivity unless induced to do so by immersion in water or decaptitation (Lewis et al. 1961). Frequently only a minority of laboratory laid eggs give rise to larvae. Those of the West African Members of the complex hatch after two days at the temperature of about 30°C while those of Kibwezi take four days at 20°C. The eggs of S. damnosum s.l. can be kept up to a week at an optimum temperature during storage, and at 15°C and below hatching is delayed.

Larval development requires suitable water flow and turbulence, correct chemical conditions and right kind of food. The West African members of the S.damnosum complex can all be reared in the same culture water obtained from aquatic site of any of them. Methods which have been used for rearing blackflies are based on agitation and oxygenation of water in a closed system, and those based on a continuous flow of pumped water.

The most successful rearing techniques for the S.damnosum complex have been based on the continuous circulation of pumped water over an inclined trough through pipes or tubes. Most blackfly larvae are very sensitive to a high ammonia content and water pollution. There are two main approaches to overcoming this problem. One is to either use a large volume of water or to regularly change the water. The other is to introduce self purification system (Raybould and Grune Wald, 1975). The main feature of the later method is that the ionic balance and pH of the culture water (obtained from a blackfly stream) are adjusted and maintained at similar levels to those in the natural habitat (Grune Wald 1976). The water is purified by its slow circulation through a gravel filter, an activated charcoal filter and a container with the submerged roots of Monstera sp (Araceae) which remove excess phosphates and nitrogen compounds. Development is rapid if the larvae are adequately fed. Suitable food includes Tetra (R) conditioning food and cultures of algae. The technique developed by Raybould (1979) in which lesser volume of water is used than in the purification system and which also the larvae are fed on living algae (chlorophyceae) has given better results for the rearing of S. damnosum complex. At a temperature of about 25°C larval development is very rapid, pupation normally commences between about 10 and 14 days after hatching and full size adults

sometimes develop in under two weeks. The non anthropophilic kibwezi form and S. goubronse are the only laboratory mating species of the S. damnosum complex.

2.5 MORPHOLOGY LIFE CYCLE AND TRANSMISSION OF O.VOLVULUS

Onchocerca volvulus was first described by Leuckart in 1893 and was known by the name Filaria volvulus. It belongs to the class Nematoda. The living worms are white opalescent and transparent with distinct transverse striations of the cuticula. The males measure 19 to 42mm in length by 130 to 210 microns in diameter (Faust et al, 1970 Neafie, 1972). The females measure 33.5 to 50cm in length by 220 to 400 microns in diameter. The worms are wire like, Filiform and blunt at both ends. At the anterior extremity there are 8 small submedian sessile papillae, arranged in 2 rings and a pair of large oval lateral pipillae. In the male the posterior end of the worm curves ventrally (Faust, et al, 1970). The cuticle varies in thickness from 3 to 5 microns. The hypodermis is a layer lying beneath the cuticle which is inconspicuous except in four areas where it buldges into the body cavity to form dorsal and ventral chords. While the dorsal and ventral chords are prominent only in a minute segment of the worm anterior to the nerve ring lateral chords are prominent throughout the length of the worm. Martinez-palona (1978) observed that there is no plasma membrane covering the cuticle in the microfilaria while Jane et al (1974) reportedly observed membrane in the female Onchocerca volvulus which is projected into numerous folds substantially increasing the surface area. According to Martinez-Palona (1978) this lack of external membrane in the

living microfilaria cellular elements are hidden from the exterior by the acellular cuticle. This may explain the lack of cellular reaction usually found around living O. volvulus microfilariae in the dermis of Onchocerciasis patients. A layer of muscle lies beneath the hypodermis and the muscle cells are divided into four quarters by the lateral chords (Neafie 1972). The body cavity is internal to the muscle layer and is filled with fluid and connective tissue cells in which the digestive and reproductive organs are suspended. In the male worm the diggestive tube is comprised of a mouth, Oesophagus, intestine, cloaca and anus. In the female the rectum replaces the cloaca (Neafie, 1972). The mouth is simple and terminal. The Oesophagus is cylindrical and begins at the anterior end of the worm. It is 630 microns long and has a maximum diameter of 25 microns (Martinez-palomo, 1978). Its anterior portion is muscular and its posterior portion is glandular. The nerve ring located 140 microns from the anterior end of the worm, surrounds the muscular Oesophagus. The intestine which is straight tube has a uniform diameter of 25 microns and lies between the Oesophagus and the cloaca. The cloaca is a short narrow duct 140 microns from the posterior end of the worm. It is 75 microns long and serves as passage way for spermatozoa and feaces. It terminates at the anus which is located 65 microns from the posterior tip of the worm. The male reproductive

system is a straight tube running almost the entire length of the worm and consists of a testis, vas deferens and ejaculatory duct. This testis has two regions, a proximal germinal region 1.7mm long, followed by a growth region 10mm long. The germinal region of the testis is concerned with germ cell production and spermatogonia undergoing mitosis are seen there (Neafie, 1972). The growth region of the testis has three distinct divisions based upon spermatic maturation. There is a proximal portion 5mm long, a short middle zone 1.7mm long and a distal portion 3.3mm long. The proximal portion contains spermatogonia that are maturing into primary spermatocyte. The short middle zone contains primary and secondary spaermatocytes undergoing the first and second meiotic division respectively. The secondary spermatocyte divides to form spermatids. The distal portion of the growth region of the testis is filled with spermatids. The vas deferens is 7.5mm long and is filled with spermatids or spermatozoa. The ejaculatory duct is 500 microns in length. It is a narrow thick walled duct that contains few spermatozoa. The ejaculatory duct narrows abruptly before uniting with the intestine to form the cloaca. The male worm has two copulatory spicules. One is 135 microns in length and the other 60 microns in length.

The female worms are much longer than the males. The hypodermis, cuticle and musculature are basically the same as that of the male. The intestine is a

straight tube that varies greatly in diameter from 20 to 150 microns and extends from the oesophagus posteriorly to the rectum. The rectum is 100 microns in length and terminates at the worm (Neafie, 1972). The reproductive system consists of paired ovaries, oviducts, seminal receptacles and uteri. The two uteri unite to form the vagina which terminates at the vulva. The ovaries, oviducts and seminal receptacles are located only in the posterior 35mm of the worm. Each of the reproductive tubes begins as an ovary in the posterior portion of the worm. Each ovary has two regions of about equal length, a proximal germinal region and a distal growth region. Production of germ cells are limited to the germinal ovary and dividing Oogonia are seen there. The growth region of the ovary is larger in diameter than the germinal region and contains primary (maturing) Oocytes. The ovaries lead anteriorly into the oviduct, each about 3mm long. The diameter and histologic appearance of the oviduct varies considerably throughout its length. The oviduct is usually small in diameter, thick walled with prominent epithelium and has a narrow lumen that contains mature primary Oocytes. The seminal receptacles are 4.5 and 2-5mm long respectively. Fertilisation usually occurs in the seminal receptacles and meiotic division of the mature

primary Oocyte occur only after fertilisation. The seminal receptacles lead but ocassionally loop back and forth many times. The uterus is devided into three regions. There is a short proxinal region, an extremely long middle, and a short distal region. The short proxinal region contains numerous spermatozoa, zygotes and many embryos in an early stage of development characterized by a spherical mass of dividing cells enclosed with a thin Membranous sheath (Neafie, 1972). The middle zone at least 200mm long accounts for most of the length of the worm. The embryos in this portion of the uterus have differentiated into elongated microfilariae with recognisable anterior and posterior ends. The microfilariae are coiled and remain enclosed within the thin membranous sheath. The short distal region contains mature microfilariae in which the membranous sheath is not identifiable.

The Microfilariae of Onchocerca volvulus measure 220 to 360µm in length by 5 to 9µm in diameter. They are unsheathed. The body bears transverse striations. There is a cephalic space of 7 to 13µm long between the anterior end and the first nucleus.

The caudal space measures 9 to 15µm between the terminal nucleus and the posterior end (Gibson et al 1976, Gibson et al., 1980). The nerve ring, the excretory and the R_1(R_1=first Rectal cell) are situated 65um, 100um,

120µm, and 225µm respectively from the apex. R_2 and R_3 cells are situated 30µm from R_1 R_4 is 40µm from R_1 and 10um from the anal pore. The tail is 52um long (Bain, 1968). The cuticle of O. volvulus microfilariae has several layers. The outermost dense surface layer is about 11um. There is a trilaminate membrane which is about 7um in diameter (Gibson, et al 1976). Between the surface layer and the trilaminate membrane there is an electron-translucent sublayer (electrolucent zone) which is abotu 1.5µm in diameter. Beneath the trilaminate membrane is a dense outer cortical layer about 15µm thick. Next is the less dense inner fibrous layer 40-50µm thick. Treatment with DEC has no noticeable effect on the cortical and fibrous layers of the cuticle. Beneath the cuticle is a hypodermis that contains filamentous mitochondria. Below the hypodermis are bands of muscle cells.

In man the adult worms, males and females, live together in tangled masses each comprising many worm bound together in a fibrous reaction of the hosts tissue to form an onchocercal nodule. Nodules vary in size from that of a split pea to a wallnut. Some lie superficially and can be palpated just under the skin over knees, ribs and scapula or over the scalp.

Others are impalpable, lying deep in relation to the joint capsules and bones especially around the hip joints. The females are vivi parous, bringing forth large numbers of motile embryos known as microfilariae (Duke, 1971). The formation of the microfilariae is the only stage in the life history at which any multiplication takes place. The microfilariae invade the skin, mainly in the same anatomical quarter as the nodule, and lying in the subepidermal layer, that await ingestion by the feeding Simulium. Blackflies biting an infected person may ingest microfilariae along with the meal. The microfilariae penetrate the fly's gut enter the flight muscles and grow through several molts to become infective larvae. The first stage larvae when fully matured (96 hour larvae) measure 260µm long by 40µm wide. The nerve ring and the excretory cells are 50um and 70µm respectively from the apex. The excretory pore is not vissible. The Oesophagus is 135µm long. The second stage larvae (5 days) measure 435µm long by 39µm wide. The nerve ring and excretory cell are 50µm and 75um respectively from the apex. The infective larvae measure 650µm long by 22µm wide. The nerve ring and excretory cell are 65µm and 110µm respectively from the apex (Bain, 1968). The tip of the tail is long (25µm) in the first stage larva and short in the second stage larva (10-12um).

The third stage larva has. blunt tail. A period of about 6 days is required from the time that microfilariae are ingested to the time that infective stage larvae are produced (Duke, 1968). The infective larvae move to the head of the fly ready to be injected into the human host when the fly takes its next blood meal. From the time that the infective larvae are injected into human host to the time that they mature into adult worms little is known about their development (Duke, 1971). During this period there is no multiplication and the build up of a heavy worm load in man depends on the repeated inoculation of infective larvae from the bites of many infected flies. The adult worms have a life span of about 15 years while the microfilariae live for about 2 years (Bain, 1968, Duke, 1971, Nelson, 1970).

TRANSMISSION

Blacklock (1926) was the first person to prove that Simulium damnosum s.l. was the vector responsible for the transmission of Onchocerca volvulus to man. Blacklock observed that the flies bit between 6.00 a.m. - 6.00 p.m. They did not bite during heavy rainfal. Flies died within 20-60 seconds when exposed directly to hot sun shine (for dully fed flies) and for 1-2 minutes for partially fed flies. Blacklock also observed that the feeding time varied from $1\frac{1}{2}$ - 5 minutes. The flies were easily disturbed in the early stages of their feed but after they had reached blood and commen ed

to engorge, they were not disturbed by placing a test tube over them. The engorgement is remarkable and the abdomen distends visibly owing to the large accumulation of blood in the midgut. In an experimental infection of wild S.damnosum s.l. with microfilariae from infected individual at Bolo, West Cameroon, Duke (1968) found out that if a nulliparous fly takes a blood meal on day 0, it does not go back for a second blood meal until about day 3 when ovarian development and egg deposition would have taken place. Similarly he found that the third blood meal occurs on day 6. The experimental flies at this stage contained infective larvae in their head. The development of the parasite from microfilaria in the blood meal to the infective stage larva requires a period of six days (Blacklock, 1926; Duke 1968). Duke and More (1968) estimated that an infected fly habours a mean of 11 microfilariae and a mean of 4.2 infective larvae.

One of the most important indices in connection with the transmission of onchocerciasis is the infective biting density of the simulium vector which may be defined in general terms as the number of infective flies biting man in unit time (Duke 1968). Another index of importance is transmission potential which is simply the product of the infective biting density and the arithmetic mean number of infective larvae

per infective fly. These two indices which indirectly can give idea of the severity of Onchocerciasis in an area are themselves dependent on a number of factors such as number of flies in relation to man, their longevity, their ability to support the development of Onchocerca volvulus, their degree of Zoophily and the size and availability of the reservoir of Microfilariae in the human population on which they are feeding (Duke, 1968). In a forest village of Bolo, Cameroon, Duke (1968) found that there was a high infective biting density of 14000 infective bites/man/year and a transmission potential of 92,000 infective larvae/man/year. There were however very few serious eye lesion encountered. The infective biting density and the transmission potential vary from season to season. Duke (1968) showed that the proportion of parous flies infected and the geometric mean number of infective parasites per infective fly fell during the period of July to September, This is the season of the year when there is most rain and least shining of sun. During this period also there was a drop in the ratio of infective flies to flies with developing infections. This indicates that there was a greater fly mortality between the second and the third blood meals and this can reduce both the infective biting density and the transmission potential Duke (1968) determined the infective biting potential of S. damnosum s.l. in different bioclimatic zones and

its influence on the transmission potential and found out that the infective biting density and the transmission potential were higher for a forest area than some savanna (Guinea and Sudan) areas. There was however no corresponding severity in terms of eye lesions. This observation therefore raises doubt as to the validity of the hypothesis that the greater longevity and more limited dispersal of S. damnosum s.l. in the northern savanna zones lead to an exceptionally high degree of transmission and thus to the development of human infections of maximal density which frequently progresses to blindness. Duke, et al,(1975) determined transmission potentials and associated pattern of onchocerciasis at four Cameroon Sudan Savanna villages. They found out that transmission potential perse could not be held responsible for severity of onchocercias. Areas with perenial transmission had greater severity of onchocercias, than areas with seasonal transmission. While high transmission potentials in the savanna paralleled onchocercal eye lesions, the same was not true for the feres area. Duke et al., (1975) therefore concluded that perential transmission, parasite strain and transmission potential together determine the severity of the disease as a cause of blindness. Thylefors, Philipon and Prost (1978) determined the transmission potentials of Onchocerca volvulus and associated intensity of onchocerciasis in 4 Sudan

Savanna villages (3 in Upper Volta and 1 in Ivory Coas) and found that the transmission potentials for the four villages were 220, 1200, 1600 and 2000 infective larvae/man/year. Villages with Annual transmission potential between 1,200 and 2,000 infective larvae/man/year had prevalence of onchocerciasis of about 70%. It was therefore concluded that in this study, prevalence did not correlate well with level of transmission. They however found out that there was an increasing intensity of infection in villages with higher transmission potential and this was also associated with an increasing proportion of blindness and severe ocular lesions due to onchocerciasis. There was no case of sclerosing Keratitis or complicated anterior Uveitis in the villages that had transmission potential of 200 infective larvae/man/year. On the other hand there were several cases of posterior segment lesion which per se does not lead to blindness.

Onchocerca volvulus is not a uniform species but comprises biologically or physiologically distinct race each with its own inherent properties which determine the pattern of transmission and clinical picture. De leon and Duke (1966) conducted experimental studies on the transmission of Guatemalan and West African strains of Onchocerca volvulus by three species of Guatemalan blackflies. Carriers of the Guatemalan strains were exposed to bites of local blackflies. S. ochraceum was found to be the most efficient vector of O. volvulus.

It ingested the highest number of Microfilariae, and the lowest mortality rate after being infected and allowed the development of the highest number of infective larvae. When the same 3 species of the Guatemalan blackflies (S. ochraceum, S. callidum, S. metallicum) were exposed to 2 patients habouring the African strain of Onchocerca volvulus it was found out that the Guatemalan flies ingested 20 to 25 times the number of microfilariae from persons habouring the Guatemalan strain than from those habouring the African strain. Ingested microfilariae of the African forest strain developed to the infective larval stages while those of the African Savanna strain did not develop to the infective larval stage in the Guatemalan blackflies. These results show that the Guatemalan strain of O.volvulus is physiologically distinct from either the forest or Sudan Savanna strain of O. volvulus. Duke (1967) conducted studies on the intake and subsequent fate of microfilariae of a Central American strain of O.volvulus in West African Simulium damnosum s.l. A group of 1000 Simulium ochraceum in Guatemala was infected with microfilariae from 2 Guatemalan O. volvulus carriers, transported by air across the Atlantic to Cameroom and the infective larvae collected and then inoculated to seven chimpanzees. Feeding experiments were carried out with forest and savanna form of S. damnosum. For a known concentration of microfilariae in the skin of the Chimpanzee, forest form of S. damnosum s.l.

ingested 2 to 4-5 times as many microfilariae of the forest strain of O. volvulus as the Guatemalan strain. In both forms of S. damnosum, ingested parasites of the Guatemalan strain were rapidly eliminated as microfilariae and very few developed to infective larvae. These results again indicated that the Guatemalan strain and the West African strain of O. volvulus are physiologically different with respect to their compatibility with the West African Similium vector.

2.6 PATHOLOGY

The pathological changes produced as a result of O.volvulus infection in man may roughly be classified into two categories: those caused mainly by the adult worms and those caused by the microfilariae. The skin nodules or onchocercomata are the main features of clinical manifestation caused by the adult worms. On the other hand the microfilariae are apparently responsible for a variety of other often very serious pathological changes especially in the skin and eyes.

2.6.1 REACTION TO ADULT WORMS:

Adult worms become lodged in subcutaneous tissues and gradual fibrous encapsulations develop around them. In early stage of infections, however, the worms lie freely in the subcutaneous tissues and do not produce any noticable symptoms (Nnochiri 1964). In later

stages, chronic inflammations without acute symptoms develop around the adult worms which eventually become nodules of various sizes. In Guatemala and Mexico the great majority of skin nodules are found on the scalp or near the eye but in Africa majority of nodules are found on the chest, lower trunck or near joints. The nodules contain varying numbers of adult worms and also numerous microfilariae. They are from a few millimeters to several centimeters in diameter. The nodules tend to lie over bony prominences, especially skull, scapula, ribs, elbows, tronchanters, iliac-crests, sacrum and knees (Gibson et al. 1980). Characteristically the nodules are firm, tend to be flattened or bean shaped, easily moveable and nontender. Nodules may be discrete or bound together as conglomerate masses. By themselves nodules, and the worms they contain are innocuous but the millions of microfilariae release migrate through the nodule into the skin, eye, lymph nodes and sometimes deep organs. At these various sites the microfilariae degenerate and provoke inflammation and if unchecked, gradually increasing amounts of scar tissue. The nodules may be deep seated or superficial. At the Enugu Central laboratory (Onuigbo, 1975) some of the onchocercal nodules were misdiagnosed as fibroma, cancer, lipoma, lymph nodes, dermoid cycts and tumors. Microscopically Onchocercal nodules have an outer shell of hyalinised and vasculari-

sed scar tissue that surrounds one or more coiled and inter wined adult worms (Connor et al 1970). Some nodules are comprised entirely of hyalinised scar tissue with tunnels to accommodate the worms. Other nodules show portions of the worms lying in a soft center containing variable inflammatory components including neutrophilis, firbin, eosinophilis, giant cells, lymphocytes, plasma cells and Russel bodies. From histological studies Isreal (1959) reported that each nodule contains a large proportion of disintegrating worms. Half of the total number of nodules he examined contained only dead worms. According to Burchard et al (1979) Electron Microscopical study of ochocercal nodule showed that the bulk of the cells in the center of the nodules consists of lymphocytes and macrophages. The lymphocytes can be observed in the various stages of differentiation. A majority of the macrophages show degenerate changes with increasing lysosome and increase of fatty vacuoles. Ngu et al (1981) showed that nodules contain live adult worms and that microfilariae are produced by the worms in these nodules which are subsequently released into the skin. In the African form of onchocerciasis nodules are more common in patients in the forest zone than in the savanna zone (Nelson, 1970).

2.6.2 Reactions to the Microfilariae

Irrespective of the strain of parasite, the most severe clinical manifestation of onchocerciasis are due to reactions to the presence of the microfilariae.

Onchocercal Dermatitis

Onchodermatitis usually begins with a puritis which may be accompanies by a papular rash. This may affect any part of the skin but tends to be most severe on the lower trunk, the pelvic girdle, and thighs (Nelson, 1970, Gibson, et al 1980). There is often intense itching and scratching which may result in secondary infection. In many patients the purities persists throughout the whole course of the disease, especially in Africa where there is no difficulty in recognising an affected community becuase the whole population seems to be busy scratching (Nelson, 1970). Not all infected person however experience itching although the microfilariae are readily seen in skin snips. If these patients are given low dose (80mg or less) of diethyl carbamazine citrate they will usually develop an acute erythematous and prutitic reaction due to the killing of Microfilariae; this is the basis of the diagnostic test known as Mazzotti test (Mazzotti, 1951). It is generally believed that the main clinical features of onchocerciasis are the result of an immunopathological response and the lesions are probably proportional in their severity to the number of microfilariae that die in the skin or eyes (Ottesen, 1984).

The second stage of onchodermatitis is characterised by thickening of the skin due to intradermal oedema. In Central America the patients may develop "Erisipela de la costa" affecting usually the face or upper trunk and there may be attacks of "Mal morado" with a purplish eruption resembling lichen planue affecting the upper part of the body (Gibson, et al 1980, Sasa, 1975).

Eventually most people develop some degree of lichenification or thickening of the skin especially over the buttocks and thighs (W.H.O., 1966, Browne, 1976, Connor et al 1969). Later acanthosis, hyperkeratosis and parakeratosis appear associated with an infiltrate of histiocytes, eosinophilis and lymphocytes in the dermis.

Reticulum, elastic and collagen fibers proliferate and the small dermal vessels become tortuous. This is followed by dermal atrophy which becomes thickened with scar tissue. In some patients there is defibrinoid degeneration of dermal collagen and focal granulomas in the upper corium. The clinical varieties of onchocerciasis known as "craw-craw" skin", "elephant skin" and "lizard skin" are some of the observed dermal changes (Nelson, 1970). Often it is impossible to distinguish the skin changes from these of avitaminosis A and Rodgers (1962) has suggested that either the parasite may compete for vitamin A in the skin or that vitamin A may predispose to heavier infections. The

last stage is characterised by a trophy of the skin with loss of elasticity which gives the patient a prematurely aged appearance (Zubaidy, 1976, Nelson, 1970) and according to Laigret, 1929 (cited by Sasa Manabu 1976) "Onchocerciasis makes young people look like old and old people look like lizards". This is one of the most distressing features of onchocerciais. Even relatively young members of the community appearing to be old and decrepit.

In the Yemen Arab Republic, onchocerciasis frequently produce a characteristic combination of changes that are often sharply limited to one limb. These changes are difuse Oedema, hyper pigmentation, papules and regional lymphadenopathy. This syndrome is known as Sowda - derived from the Arabic word meaning dark or black (Buttner et al 1982, Gibson et al 1980). The skin in the affected regions becomes blue-black. It occurs most often on the legs, especially the skin and the inner side of the thigh (Anderson and Fuglsang, 1973). The affected skin is very itchy and is slightly scaly, rough and thickened. I is frequently asymmetrically bilateral. An outstanding feature is the enlargement of the groin glands.

Depigmentation is a feature of chronic onchodermatitis and it is usually most pronounced on the legs. This condition may be confused with leprosy and Nelson (1970) has reported seeing many patients with onchocercal depigmentation in leprosaria. Edungbola et al (1983) found depigmentation of skin (leopard skin) to be a common feature of Onchocerciasis in the Babana

district of Kwara State. The loss of elasticity is responsible for the development of "hanging groin". These pendulous sacs are most commonly seen in Adult males. They often contain inguinal or femoral lymph glands and may hang down as far as the knee (Nelson, 1970), Zubaidy 1976). Cherry (1959) described a similar complication in women which resembles the "hottentot apron". a genital distortion which was at one time fashionable in females in South West Africa. In Africa the loss of elasticity of the skin of the groin reulsts in exceptionally high prevalence of hernias (Nelson 1958, Williams and Williams 1966, Duke, 1972). Onchocerciasis is undoubtedly one of the most important causes of hernia and especially femoral hernia in man. Another clinical picture of the disease, though not very common is elephantiasis of the scrotum (Gibson et al, 1980, Duke, 1972, Omen, 1967). The difference between Onchocercal elephantiasis and that of Bancroft filariasis is that there is no accompanied hydrocoele of the legs in the case of Onchocercal elephantiasis. Lympha deno pathy is a common and typical sign of Onchocerciasis. The glands are enlarged and firm but not painful. This symptom is found most commonly in the inguinal region in African cases, but in Central America, it may also be present in the axillae and the neck. In such cases the microfilariae are often found in the glands, and hyperplasia of the lymphatics and later diffuse fibrosis are recognised (Sasa, 1976

Anderson and Fuglsang 1973). Gibson and Connor (1978) showed that the inguinal, femoral, occipital, postauricular and axillary lymphnodes could be involved in Onchocerciasis patients. Lymphnodes from Yemenites were found to be soft and slightly tender. In many African patients Onchocercal lymphadenities was found to be accompanied by hanging groin and or elephantiasis that is restricted to the genitalia.

Microfilariae of O. volvulus were found in the vessels of liver, kidney, spleen, lung by Rodhain and Gavrilov (1935). Microfilariae have also been found in body fluids and secretions including blood, urine, cerebrospinal fluid, tears, sputum, vaginal secretion and ascitic fluid (Buck et al, 1971, Fuglsang and Anderson, 1974), Meyer et al (1977) found O. volvulus microfilariae in the renal glomeruli, pancrease, thoracic aorta, the pulmonary alveoli and the choroid plexus. Transplacental infection of newborn infants of heavily infected mothers have also been reported (W.H.O. 1977). The presence of microfilariae in the blood, dermal lymphatics, lymphnodes and deept organs indicates that microfilariae of O. volvulus are disseminated by way of the hemolymphatic system.

Histopathological changes in early onchocereal dermatitis are minimal and may be overlooked. Gradually however inflammatory cells, mainly lymphocytes, histiocytes, plasma cells and eosinophils collect

around vessels and appendages. Dermal fibrolasts appear and increase in number as the infection becomes more and more severe. Most cells are often present in increased numbers but neutrophils are usually absent from the dermis. Later in the infection, there may be hyperkeratosis, focal parakeratosis, acanthosis, Melanophores in the upper dermis, dilated lymphatics, tortuous dermal vessels and increased mucin in the upper dermis (Gibson et al 1980).

Fibrosis is probably the most serious change. It begins early and continues throughout the course of infection eventually replacing the dermal papillae and normal strands of dermal collagen by hyalinised scar. Speciemens from areas of spotty depigmentation reveal an absence of Melanin in the epidermis, mild to severe inflammation of the underlying dermis and very few microfilariae beneath the depigmented areas in contrast to adjacent pigmented areas which may contain many microfilariae (Buck, 1974). Specimens through atrophic skin (in very late stages of Onchocercal dermatitis) reveals a thin Keratin layer that usually separates from the epidemis (during processing); a very thin epidermis and loss of rete ridges which gives the epidermis a smooth under surface (Buck, 1974). In case of sowda which is characterised by diffuse oedema, hyperpigmentation, papules and regional lymphadenopathy, the most stricking histo pathologic feature is an extensive inflammatory cell

infiltrate of the upper dermis composed of many plasma cells and fewer lymphocytes, eosinophils and histiocytes. The infilatrate forms broad cuffs around dermal vessels and appendages, greatly thickens the dermis and extend into the subcutaneous adipose tissue. Sclerosis and oedema of the dermis are prominent. Changes in the epidermis include hyperkeratosis, parakeratosis, follicular plugging, acanthosis with elongation of rete ridges and scarttered foci of intra epidermal oedema (Gibson et al. 1980). Inguinal and femoral lymph nodes excised from Africans with onchocerciasis show varying degrees of scarring of capsule, peri capsular tissue and the interior of the nodes. This commonly involves 20 to 80 percent of the cross sectional area of the node but sometimes 90 percent or more of the lymphoid tissue is replaced by scar (Connor et al, 1969). Other histopathologic changes in the lymph nodes include sinus histiocytosis and infiltrate of plasma cells, eosinophils and neutrophils. Germinal centers shrink and disappear as fibrosis and other changes advance. Within the medulla of the lymph nodes there are often large numbers of eosinophils, plasma cells, mast cells, histiocytes and occassionally giant cells (Gibson et al; 1980, Connor et al, 1969).

2.6.2 OCULAR ONCHOLERCIASIS

The single most important aspect of onchocerciasis is that of blindness (Duke 1972). The damage to the

eye is caused by microfilariae of O.volvulus in the eye tissue. Examination by slit lamp has revealed living or dead microfilariae in the cornea, anterior chamber, retrolental space and the vitreous. It has been estimated that 20 microfilariae or more in the anterior chamber constitute a risk factor of blindness (W.H.O. 1976). The presence of living microfilariae in the cornea is an indicator of severity of infection (Anderson and Fuglsang, 1973). The associated ocular lesions are punctate Keratitis, sclerosing Keratitis, iridocyclitis, chorioretinitis and optic atrophy (Gibson et al, 1980, Nelson, 1970, Buck, 1974). Punctate Keratitis is characterised by snow flake opacities that can develop in all parts of the cornea but are most common at its periphery. Each opacity is inflammatory reaction around degenerating microfilariae, and they are about 0.5mm across and mostly circular. Sclerosing keratitis is a stromal inflammation that results from invasion of the cornea by large numbers of microfilariae, producing a white hazziness of the strain. Sclerosing Keratitis is a common cause of blindness in the African Savanna and in Central America (W.H.O. 1982 , 1884). Iridocyclitis results from the presence of microfilariae in the anterior chamber where they appear as small wriggling silver threads through the slit lamp microscope. Severe iridocyclitis is associated with large numbers of microfilariae in the skin (Gibson et al 1980). In

chorioretinitis there are varying degrees of atrophy of the retinal pigment epithelium and the choriocapillaries. Severe chrioretinitis involves such atrophy over a wide area of the posterior fundus. Chorioretinitis usually affects both eyes but often to different degrees. Optic atrophy in onchocerciasis appears to be caused by inflammation of the optic nerve and it often accompanies onchocercal chorioretinitis but may also be an isolated finding. Onchocercal disease (both ocular and dermal) appears to result not from mechanical damage caused by the presence of adult worms or microfilariae but by inflammatory reactions around certain microfilariae. Histophatological studies indicate that living microfilariae generally evoke no inflammatory reaction in the eye or in the dermis but that damaged microfilariae are usually surrounded by cells, particularly eosinophils and macrophages (Gibson et al, 1976; Henson, et al 1979; Mackenzie, 1980).

The presence of microfilariae has been demonstrated in all ocular tissue but the route of entry of microfilariae into the eye is still not known for certain and remains a subject of much controversy. Microfilariae have been found in the juxta-vascular position and it has been suggested that perhaps they pass along the sheaths of the short ciliary vessels and nerves into the eye (Neuman and Gunders, 1973).

Duke (1975) suggested that the eyes might be invaded by microfilariae spreading locally from impalpable adult worms located in or near the orbit. The possibility of blood borne microfilariae reaching the eye has been suggested (Fuglsang and Anderson 1974). It is also possible that microfilariae may pass via the cerebrospinal fluid to reach the optic nerve sheath (Duke et al., 1976). Another hypothesis is that the microfilariae may produce enzymes digesting mucopoly saccharides and Collagen fibrils, which would enable them to migrate in the sclera (Thylefors and Tonjum, 1978).

Blindness due to onchocerciasis is a common feature of the disease in the savanna zone of West Africa (Duke 1972; W.H.O. 1976, 1982). There are many areas in the Savanna region of Africa where 30% of the population may have impaired vision as a result of Onchocerciasis and the blindness rate in the adult population may be over 10%. The pathetic picture of a string of blind men being led to the fields by a child so that they may do their limited work to help with growing the village crops is in some places too obvious (Duke 1972). The eye lesions usually take many years to develop and the worst affected section of the community is the adult male population, over the age of 40 years but with the Central American form of the disease there may be an acute onset in young adults especially where

the adult worms are located around the head (W.H.O. 1976; Anderson et al. 1975; Fuglsang et al. 1976; Fuglsang and Anderson, 1977).

2.7 EPIDEMIOLOGY OF ONCHOCERCIASIS

2.7.1 Onchocerciasis in Africa

Onchocerciasis was first described in Africa in 1893 when Leuckart isolated the parasite from nodules under the skin of a native of Ghana. An estimated 17,120,500 people are infected in the African Sub Region with Onchocerca volvulus with 326,600 people totally blind from onchocerciasis. The disease is found in 27 African countries (W.H.O. 1987) namely Angola, Benin, Burkina Faso, Burindi, Cameroon, Central African Republic, Chad, Congo, Cote d'ivore and Equatorial Guinea. Others are Ethiopia, Gabon, Ghana, Guinea, Guinea Bisau, Liberia and Malawi. Also found to be plagued by the disease are Mali, Niger, Nigeria, Senegal, Sierra Leone, Togo, Uganda, United Republic of Tanzania and Zaire (Nelson, 1970; Sasa, 1976, Gibson et al. 1980; W.H.O. 1987). Beaver et al. 1983) reported finding Onchocerca volvulus infection in a child in Zambia. This was the first such documented case in an indegenous person and if confirmed will perhaps represent the Southermost limit of the known occurence of human onchocerciasis in Africa.

There are two forms of onchocerciasis in West Africa (Nelson, 1970; W.H.O., 1976; 1984). In the

forest area, gross skin and gland lesions are seen, nodules are abundant and blindness rate seldom reach 3% in onchocerciasis patients. In the Sudan Savanna zone, on the other hand, nodules are less numerous, gross skin and gland lesions are less frequent but microfilarial loads are often very high producing a high incidence of sclerosing Keratitis, this often associated with severe iridocyclitis, and these two regions are largely responsible for high blindness rate of up to 10%. In Central and Eastern Africa, another clinical variant has been noted in areas where the parasite is transmitted by members of the S. neavei complex. For example it has been noted in Zaire that the highest blindness rates and many head nodules are found in the Southerly forest of the Kasai, where S.neavei is the vector. Further north on the forest border in Uele, where S. damnosum transmits the parasite there are few eye lesions but gross skin lesions, hanging groins and scrotal elephantiasis are common (W.H.O. 1984). In the Yemen Republic a form of O. volvulus is found which produces maximal concentration of microfilariae in the lower legs, and ankles, and the hypertrophic, blackened skin lesions called "Sowda" are common (Anderson and Fuglsang, 1973 ; Gibson et al. 1980; Butner et al, 1982).

The differences in clinical manifestation of onchocerciasis in different bioclimatic zones are associated with a number of factors such as different

Simulium vectors (Duke, 1968; Duke et al, 1975; Garms, 1978; Butner et al, 1982), differences in level of annual transmission potentials (Duke, 1968; Duke et al, 1975) and different types of the parasite (Bryceson et al., 1976). In West Africa this difference in strain/pathogenicity is particularly obvious.

Considering the geographical differences in the relationship between annual transmission potential (ATP) figures and the clinical patterns of onchocerciasis (e.g. relatively moderate ATP figures associated with very severe ocular onchocerciasis in the Savanna (S. damnosum/S. sirbanum areas) versus much higher ATP figures in the forest (S. yahense, S. soubrense, S. saneti pauli area) without the occurence of severe ocular lesions) in-depth research is urgently needed on the intra-specific identity of the infective O. volvulus larvae carried by the various vector species in different bioclimatic zones (W.H.O., 1984). Bryceson et al (1976) using immunogical techniques found that there were antigenic differences in the Savanna and forest O. volvulus. Even though he found that even worms from the same individual could differ in their antigen composition there was a distinct Savanna/forest difference which could be detected serologically. Enzyme histochemistry based on the detection of Acid phosphatase has also been used for possible identification of Savanna from forest O. volvulus. Braun-Muzinger and Southgate (1977) carried out histochemical differentiation of Onchocerca

volvulus microfilariae from Togo and found that there were four distinct enzyme staining patterns. They deduced from their study that a number of biological strains or variants of Onchocerca volvulus coexist in West Africa. Omar (1978) carried out similar investigation using O.volvulus microfilariae from West African rain forest (Liberia) Sudan Savanna (Upper Volta), Guatemala and the Yemen. Although the method for classifying staining type differed from that of Braun-munzinger and Southgate (1977), Omar identified 5 staining patterns in the microfilariae population. The staining types were as follows: type I, with enzyme activity and Anal pore and excretory pore; type II, enzyme activity at Anal pore and inner body; type III, with enzyme activity at Anal pore, inner body and Excretory pore; type IV with enzyme activity at Anal pore only, and type V with no enzyme activity at all. He also found that type IV was more common in the Savanna specimen and also type V was more common in the forest area. Ufomadu et al. (1986) were able to identify 13 staining patterns in microfilariae of O. volvulus from the Guinea Savanna (Jos) of Northern Nigeria. Omar and Schulz-key (1978) determined Acid Phosphatase activity in the larval stages of O. volvulus developing in the vector Simulium damnosum s.l. and reported that there were no detectable differences in the staining patterns between the forest and Sudan Savanna strains. Omar and Garms (1981) however were able to differentiate developing O. volvulus

larvae in S. damnosum from the larvae of other filarial worms and nematodes that also develop in S. damnosum s.l. Even though different staining patterns of O. volvulus microfilariae have been found in the different Bioclimatic zones of West Africa, it is not yet possible to associate a particular staining pattern with a given clinical picture of onchocerciasis. Yarzabal et al. (1983 found four staining types in the microfilarial population in Upper Orinoco Basin of Venezuela. Type I (17.2%) Type II (0%) Type III (0.5%) and Type IV (75.6%). Omar et al. (1982) carried out enzyme histochemical studies on Onchocerca volvulus microfilariae from rain-forest and Sudan savanna areas of the onchocerciasis control programme in West Africa and recorded the five staining types earlier mentioned. In the rain forest areas where onchocerciasis is transmitted by Simulium yahense, S. sanctipauli, S. soubrense, S. squamosum there were no significant differences of microfilarial staining patterns in patients, by age and sex, between the three Simulium - Onchocerca complexes studied. There was a close relationship between the "Strain differences" as revealed morphoenzymatically, and the clinical picture of the disease in both the forest and the Sudan Savanna zones.

In the forest zone of Liberia where Simulium yahense and the S. sanctipauli subcomplex are the vectors, there is little blindness due to onchocerciasis, despite annual transmission potentials (ATP) above 5000 (W.H.O. 1987). Similarly in Cote d'ivore there is almost no

blindness in the forest although transmission by S. yahense is severe. The onchocerciasis in the Savanna of Cote d'ivore transmitted by S. damnosum s.s. and S. sirbanum results in a higher prevalence of severe ocular lesions in both the anterior and posterior segments of the eye, with more ocular microfilariae, greater number of nodules, and higher microfilarial concentration, as well as more dermatitis vitiligo, lymphadenopathy and hanging groin.

2.7.2 Onchocerciasis in the American Sub-Region

Guatemala, Onchocerciasis was first discovered in America in 1915 by the Guatemalan Scientist Rodolfo Roble. S. ochraceum, S. metallicum, S. callidum, and S. oxiguum (S. gonza-lezi) are the known vectors in Guatemala.

Tada et al. (1979) carried out another Epidemiological study in San Vicente Palaya. Using a single skin snip biopsy, they examined 2,153 persons of whom 664 (30.8%) proved positive for O. volvulus. They also found that altitude played an important role in the parasitosis with transmission occuring mainly at altitude between 600 and 1,300m. Out of 1,217 persons examined by simultaneous biopsy and nodule palpation 587 proved positive by one or both methods. 101 nodules were detected mostly in children or persons who had lived in areas of low endemicity. Ocular onchocerciasis is distributed in two areas - that occupied by the Sierra Madre mountain

range along the South coast on the Pacific (at an altitude of 600-1200m), and that of the cuchumatanes areas of Huehuetenango department. About 30,000 out of 343,000 inhabitants in the area are thought to be infected (Perez, 1986).

Mexico

In 1923 Fullebor reported seeing Onchocerciasis nodules on the head of a five year old Mexican child who attended his clinic in Hamburg, Germany. In 1931 Torcella first observed microfilariae in the anterior chamber of the eye of an onchocerciasis patient. S. metallicum, S. callidum, S. ochraceum and S. gonzalezi are the vectors of onchocerciasis in Mexico. (Prez, 1986). S. haematopotum and S. veracruzanum are other two potential vectors. Transmission occurs at altitude of 500-1,600 meters. No transmission occurs at sea level. Salazar-Mallen (1975) reported that prevalence close to 70% existed in some endemic areas. Overall it is roughly estimated that some 40,000 people are infected in Mexico. The focus with the highest number of infection is in Soconusco in Southern Chiapas followed by a focus in the state of Chiapas and the third focus is in the state of Oaxaco (Salazar-Mallen, 1975; Perez, 1986). Coast Erysipelas of the kind in Guatemalan has not been found in Mexico, however notable skin discoloration has been seen sometimes producing a greenish tingle, and this appears to be the source of the so called "Mal morado" (Purple

Sickness). By 1983 Onchocerciasis in Mexico was not thought to be of any public health significance and blindness was very small.

Venezuela: The first known onchocerciasis focus in Venezuela was discovered in the Eastern part of the country in late 1940s, S. metallicum, S. exiguum, S. pintoi, S. yarzabali, S. incrus-tatum and S. cuasisanguineum are the blackflies known to transmit onchocerciasis in Venezuela (Perez, 1986). In 1984 Rivas et al (cited by Perez 1986) found the overall prevalence of onchocerciasis in nine states to be 39.94 per thousand of inhabitants examined. The clinical part of the study detected nodules in 23% of the onchocerciasis patients studied. The commonest ocular lesion found, accounting for 47.59% of the total was Keratitis. In 1974 a total of 1,628,370 persons living in the onchocerciasis areas were tested for onchocerciasis and 40,091 proved positive by the mazzortti test. In 1983 there appeared to be three foci in Venezuella. The first involved the states of Arogua, Carabobo, Cojedes, Guarico, Miranda and Yaracuy. The second focus included the states of Anzoategui, Monagas and sucre. The third focus is located in the Upper Caura, in the Cedeno District of Bolivar State, in the parima mountains, and from the upper Ventuari to the upper Orinoco to the East of the Federal Territory of Amazonas (Perez, 1986).

Brazil: Onchocerciasis was first diagnosed in Brazil in 1967 by Bearzoti et al (cited by Perez 1986). The foci of the disease are found near the Toototobi river in the North Central part of the state of Amazonas, the Surucucu mountains where the Yanomama Indians live in the West of the Federal Territory of Roraima and in Auris in the extreme North-Western part of the Roraima Territory. Prevalence rates range between 30% and 91.7% in these foci. The vectors of the disease includes Simulium pintoi, S. incrus-tatum, S.amazonicum and S. cuasisanguineum, (W.H.O. 1979; 1982).

Colombia: The first case of onchocerciasis discovered in colombia was reported in 1965 (Assis-Mari and Little, 1965) among a group of patients suffering from ophthalmic complications. The endemic area lies between the settlements of lopez and San Antonis (Latitude 3°N and longitude 77° 23W). Little and D'Alessandro (1970) examined 292 persons from the Micay River area and found infection in 44 people representing 15% of the population studied. Non palpable nodules were found in 20 people. The principal vector of Onchocerciasis in Colombia is S. exiguum (Tidwell et al. 1980).

Ecuador: Arzube (1982) described onchocerciasis in the first endemic focus of Ecuador. Out of 87 persons examined O. volvulus microfilariae were found in 72 (83%), of these 62 (86%) were blacks and 10 (14%) were Indians belonging to the Lapaya group. 42 (58%)

were males and 30 (42%) were females. The following clinical manifestations were found in the positive cases: nodules in 45 subjects (63%) dermatitis, pigment changes, and or skin atrophy in 31 (43%); lymphadenopathy and/or lympacedema of the external genitalia in 19 (26%), corneal opacity in 32 (44%) and dermatitis and/or pruritis in 49 (68%). Three species of Simulium, viz S. quadrivitatum S. exiguum and S. amtillarum have been found in the oncherciasis area with S.quadrivitatum and S. exiguum confirmed to be capable of transmitting onchocerciasis (Shelley, and Arsubo, 1985).

A total of 97,200 people are said to be infected in the American subregion. 1400 of the infected population are blind due to onchocerciasis (W.H.O., 1987).

ONCHOCERCIASIS IN NIGERIA

Onchocerciasis was first reported in Northern Nigeria in 1908 by Parsons (cited by Budden 1956). In 1926 Dyce sharp found microfilariae of Onchocerca volvulus in the skin of 35 out of 100 prisoners at Kaduna. The first report that ocular onchocerciasis occured in Northern Nigeria was made by Dr. J.L. Mcletchie, (cited by Budden 1956) who "stated that in Adamawa province he saw a group of elderly people all of whom had Onchocercal nodules and varying degrees of loss of vision up to total blindness". Later ocular

onchocerciasis was found to be an important cause of blindness in many parts of Northern Nigeria (Budden, 1956, Crosskey 1955). The first comprehensive report on onchocerciasis in Northern Nigeria is that given by Budden (1956). His report shows that onchocerciasis is present in all the provinces of the then Northern Nigeria. Prevalence rate as well as severity of the disease varied from one community to the other. Prevalence in some places was 1-250 per 1000 persons (light infection) while it was 251-500 per 1000 persons in moderate infection and 501-750 per 1000 persons in heavily infected communities. In heavily infected area blindness rates reached 570 per 1000 persons. Budden estimated that 16,836,000 were exposed to the disease, 339,000 infected and 19,400 persons were blind due to onchocerciasis. Nwokolo (1950) described 100 cases of onchocerciasis in Enugu district of Eastern Nigeria. Out of the 100 cases, 59 had onchodermatitis, 38 had demestrable onchocercomata. 7 had onchocercomata of the scalp, 2 had swelling of the face and 1 person complained of redness of the eye but there were no cases of blindness. One patient had synovitis and microfilariae were demonstrated in the centrifuged deposit of the synovial fluid. Onuigbo (1975) reported the occurence of Onchocercal nodules in the Ibos of Nigeria. He recorded 90 cases out of 5000 biopsies. Prevalence of infection was $1\frac{1}{2}$ times higher in males than females. He found that the forehead

was the favourite location of nodules. In another report, Onuigbo (1975) stated that some of the onchocercal nodules were misdiagnosed as fibroma, cancer, lipoma, lymph nodes, dermoid cysts calcified hematoma, periostoma, porokeratosis and unspecified cysts and tumors.

Based on serological evidence Nnochiri (1964) reported that onchocerciasis was prevalent in the Ibadan, Iwo, Shagamu, Oshogbo and Oyo areas of Western Nigeria. Wyatt (1971) carried out survey of Onchocerciasis in the Ibarapa area of Western Nigeria. Out of 1,119 persons examined by skin snip 442 (39.5%) were positive. 239 out of 1135 persons had nodules (21%). Although only one person was reported blind, lichenification, depigmentation, skin atrophy and velvet skin were noticed.

At Bonu village of Suleja Emirate of Niger State, Galadima (1982) found 42% prevalence rate of Onchocerca volvulus infection in the population aged 10 years and above. Bradley (1976) found a prevalence of 81.3% O. volvulus infection in the lowland villages of the Hawal valley of Nigeria, with a mean blindness rate of 5.8% and 5.8% prevalence with 0.7% blindness rate in the upland villages. Edungbola (1982) reported that Onchocerciasis was prevalent in the Ile-Ire district of Kwara State. In a related survey Edungbola et al (1983) found that "Leopard skin" due to Onchocerciasis was a common feature in the Babana district of Nigeria.

Out of 1,310 persons examined for the characteristic Onchocercal depigmentation 26.4% were found to be affected. Nock (1985) reported 9.4% of O. vulvulus infection in the Dalle village of Kaduna State. Gemade and Dipeolu (1983) studied the prevalence of Onchocerciasis in the Benue State of Nigeria and found infection rates of 61% (Kucho), 71% (Gube) and 72% (Manor). They found the peak of infection to occur in the 31-40 year age group. Blindness due to Onchocerciasis was 11.2%.

It is now known that onchocerciasis occurs in almost all the states of Nigeria except perhaps Lagos and Rivers States (W.H.O. 1987, Crosskey 1979). "Nigeria is the country with the largest number of infected persons in the whole of Africa" (W.H.O., 1987). By 1986 (W.H.O., 1987), it was estimated that 38,919,500 people were at risk in onchocerciasis endemic areas; 6,962,000 were infected with Onchocerca volvulus and 113,600 persons were blind as a result of onchocerciasis. Onchocerciasis is more prevalent in the Northern part of the country (Budden 1956, Crosskey 1979). This disparity is associated with some physical features of Northern Nigeria which make the area more suitable for the breeding of the Simulium flies. The distribution of Simulium damnosum and onchocerciasis has been given by Crosskey (1956, 1979).

2.7.4 FACTORS AFFECTING DISTRIBUTION OF SIMULIUM DAMNOSUM S.L.

The physical features that favour the distribution of S. damnosum s.l. in Northern Nigeria were listed by Crosskey (1956) and they include Geology, River System, Altitude, Climate and Vegetation. River systems that have rocky beds favour the breeding of S. damnosum s.l. Much of the land surface of Northern Nigeria is formed of hard ancient precambriam rocks. Where the precambrain forms the surface the rivers are often rocky bedded with rapids suitable for S. damnosum breeding but in the sedimentary areas suitable rivers scarcely ever occur. An example of this is seen along the River Niger which between Yelwa and Jebba flows over precambrian rocks and at this stretch S. damnosum s.l. occurs at intervals but is not found on the Niger further down stream where it flows over sedimentary deposits. Granites and volcanic laval eruptions in Jos Plateau and the Biu Plateau have also resulted in rocky bedded rivers and S. damnosum s.l. occurs in both the Jos Plateau and the Biu-Tangale-Waja areas (Crosskey 1956). "The Hawal River on the Southern edge of the Biu Plateau has a much-fissured laval bed that provided a fly breeding ground for several miles (Crosskey, 1956).

River Systems:

Northern Nigeria is extremely well drained (Crosskey 1956). The bigger rivers which flow

perenially maintain breeding of S. damnosum s.l. throughout the year and serve as sources of reinvasion of smaller rivers.

Altitude:

The greater part of Northern Nigeria lies between 3.00 and 800m above sea level with some areas like the Mambila Plateau reaching 1,700-2000ft above sea level. Although altitude seems to play no part in the distribution of Simulium flies in Northern Nigeria (Crosskey 1956), altitude seems to be a factor in the distribution of Simulium vectors and the disease itself in the Yemen and South America (Perez 1986, W.H.O. 1987). In the Yemen infection appears to be limited to a few permanent major Wadis at altitude of 300 - 1200m.

Rainfall and Dry Months:

In the North, rainfall varies between 75 inches a year in the South east to as little as 15 inches in the extreme North. S. damnosum s.l. foci appear always to be centered on a perenially flowing river of moderate or large size (Crosskey 1956). Dry season survival appears to be assured by continuous breeding on a small scale in those rivers that just manage to maintain flow, however reduced, throughout the drought period.

Vegetation:

S. damnosum s.l. foci exist principally in the Guinea Savanna. In this zone the breeding rivers

generally have a highly or moderately developed forest. Only a few fly foci are known from the Sudan Savanna area, for it is in this zone that the rivers largely dry up in the dry season and are sandy bedded, rainfall is low and the dry season very protracted and dessicating. In the Southern part of the North, South of the Niger and Benue, many fly foci are in the area of derived savanna (Crosskey 1956).

Several cytospecies of the S. damnosum complex have been identified in Nigeria, namely: S. damnosum s.s., S. sirbanum, S. soubrense, S. sanctipauli, and S. squamosum (Vajime and Dunbar 1975). S. sirbanum and S. damnosum s.s. occur in the Northern savanna; S. squamosum is widespread in the forest - savanna mosaic/forest area especially South and West of the Niger river on the Jos Plateau fringes, and also East of the lower Niger. The S. sanctipauli subcomplex occurs mainly South and East of the Niger river.

Socio-Economic Aspects of Onchocerciasis

Onchocerciasis is one of the world formidable diseases. Although it does not kill, it incapacitates the sufferer making him an economic burden. The itching which is one of the early manifestation can lead to loss of several man hours per day (W.H.O. 1974). In areas of high endemicity a whole village may move their settlement to a less fertile land with the result that large Agricultural areas are deserted (W.H.O. 1966). In the onchocerciasis zone of Upper Volta only 40 - 45 percent of the population compared

with the national average of 50 - 52 percent work (W.H.O. 1974). Blindness is the major complication of onchocerciasis that makes the disease a public health problem. Blindness may affect a great many persons in communities where Onchocerciasis occurs and produces permanent and irreversible disability, particularly in West African Savanna areas. Blindness strikes mainly at economically active adults in the prime of life, it is the direct cause of substantial excess mortality and the indirect cause of stagnation. or decline in the population of communities. In Burkina Faso the annual blindness incidence rate ranges from 4 to 7 cases per 1000 persons in hyperendemic onchocerciasis areas, 1 to 2 cases in Mesoendemic areas and 0.3 - 0.6 cases per 1000 persons in hypoendemic areas (W.H.O. 1987). According to Prost and Prescott (1984) blindness rate in some communities in the Onchocerciasis control area could go up to 100 - 130 per 1000 persons observed. Gemade and Dipeolu (1983) reported 11.2% blindness rate among the Tiv people living in the Kwande local Government area. Bradley (1976) found blindness rate due to onchocerciasis to be about 5.8% in the Hawal valley of Nigeria. Budden (1956) found that in some hyper endemic areas of Northern Nigeria blindness rate due to oncherciasis was 57 per 1000 persons. In the Onchocerciasis control are the onset of blindness starts at about the age of 39 years and life span is lowered by about 13 years (W.H.O. 1987). Similarly, mortality is higher among

blind people (3 - 4 times) than for non blind people. Onchocerciasis is a major cause of depopulation in the Onchocerciasis control areas (Philippon, 1977). Bradley (1976) showed that Onchocerciasis was the chief factor influencing settlement patterns in the Hawal valley of Nigeria. According to Budden (1956) Blindness was one of the causes of migration of people from endemic areas and this was well known to the native chiefs. According to Budden, "the population density tends to fall as the intensity of infection in the community rises. Moreover, the average population density in heavily and moderately infected areas (25 and 43 per square mile) is lower than the average for Northern Nigeria as a whole (60 per square mile". Gabathular and Gabathular 1947 (cited by Budden 1956) suggested that habitual abortion might sometimes be due to onchocerciasis. Ikejiani (cited by Budden 1956) reported two cases of habitual abortion in females suffering from Onchocerciasis who were treated with hetrazan and afterwards produced healthy children. According to Vajime (1982) the economy can be affected by Onchocerciasis in the following ways:

- "In hyper endemic areas blindness in 20% or more adult males often lowers farming capacity below survival level".

- "Partly because of farming failure, partly becuase of the intolerable nuisance of Simulium swarms, river valleys eventually become deserted. Uplands,

which usually have less fertile soil, become over-crowded. This process has direct economic effects especially in subsaharan West Africa".

- "Fishing in rivers infected with Simulium may be impossible or may lead to blindness. Thus available source of protein is lost."

- "Labour forces engaged in activities such as building dams in Africa have had to be protected against the attention of Simulium at considerable cost."

2.8 DIAGNOSIS OF ONCHOCERCIASIS

2.8.1 Parasitological Methods:

Parasitological methods are still the most frequently used techniques to assess the prevalence and severity of onchocerciasis. The pitfall in parasitological diagnosis however is that it may not detect light or early infections and it is completely unable to detect prepatent infection.

Adult Worms:

The detection of nodules is of limited use for establishing the diagnosis of onchocerciasis since some people, especially those who are lightly infected, do not have detectable nodules. This method may therefore have a low sensitivity but, because of its specificity and ease of application, may be of great use for the rapid preliminary assessment at a community level.

The presence of nodules in patients living in areas where transmission has been interrupted for several years is less conclusive since onchocercal nodules may contain only dead worms (Isreal 1959). The presence of onchocercal nodules can be used as an adjunct to parasitological diagnosis based on demonstration of Onchocerca volvulus microfilariae and as a danger sign to the eye when they are present on the head near the eye (Onuigbo, 1975; Fuglsang et al 1976).

Microfilariae

Evidence of the presence of O. volvulus microfilariae in the skin is the most frequently used method to determine prevalence and intensity of infection (W.H.O, 1974, 1987). Although microfilariae have been found in the sputum, cerebro spinal fluid urine and blood (Buck 1973; Duke et al. 1976; Roux and Picq, 1973; Fuglsang and Anderson, 1974; Botto et al, 1984), demonstration of microfilariae in the skin remains the practical method of establishing onchocerciasis (Nelson, 1970; Buck, 1974; Kirwood et al 1983). The oldest method of skin snipping has been that of using needle and razor blade (Budden, 1956; Buck 1974). In this method a needle is used to raise a cone of skin which is then cut with a sharp razor blade. The snip which is about 3mm in diameter is then put in drops of physiological saline (Sowa and Sowa 1975) and incubated at room temperature for 30 minutes or more. Apart from Normal Saline as incubating medium other workers have used other incubating media such as Hanks

Balanced Salt Solution (Ngu et al 1981), Tyrode solution (Nock 1985) and phosphate Buffered Saline (Ngu et al. 1981). For quantitative assessment, standard methods which employ calibrated instruments such as the corneoscleral punch (Fuglsand and Anderson, 1977, Kale 1983) and the weighing procedure using torsion balance (Kale, 1983; Awadzi and Roulet, 1980) have been used. The use of the puch makes it possible to express the level of microfilariae in the skin as sumber of microfilariae per a given size of skin snip. Where torsion balance is used the concentration of microfilariae in the skin can be expressed as number of microfilariae/mg of skin. This procedure of quantifying the level of microfilariae in the skin is particularly useful in assessing the efficacy of chemotherapeutic agent (Awadzi and Gilles 1980, Rivas-alcala et al 1984); determination of endemicity of Onchocerciasis and the level of microfilariae in the skin near the eyes signifying ocular involvement (W.H.O, 1987, Fuglsang and Anderson, 1977). Incubation of skin snips for longer periods, for example 24 hours has lead to the increase in the sensitivity of the technique as well as increasing the number of microfilariae that emerge from the snips (Awadzi and Roulet, 1980). Nelson et al (1966) observed that unless the snips are teased not many of the microfilariae will emerge. Kale et.al. (1977) advised against teasing the skin snip which is harmful and diminishes the number of microfilariae which emerge.

The use of collagenase to digest skin snips (Schulz-key, 1978) makes it possible to count the total number of microfilariae contained in that snip. The use of collagenase increase the sensitivity of this test significantly only in patients with very low parasite loads. However the collagenase technique can be useful for the examination of a limited number of snips such as when monitoring drug efficency or for other research purposes. Collagenase digestion can be performed on ethanol - fixed material stores at ambient temperature (Schulz-key and Karam, 1984). The skin snip technique can be improved in terms of specificity by taking multiple snips and from different body regions (Kale, 1982; Alfons and Wenk 1983; Kale, 1983). The prefered site for obtaining skin snips in Afria is the pelvic girdle usually below the iliac crest (Buck, 1974, Alfons and Wenk, 1983) whereas in the Americans the prefered site is the torso (Brandling Bennett and Darsie 1983). Other body sites from which snips could be taken are the calf, the knee, shoulder and the outer canthus of the eye (W.H.O., 1982; Ufomadu et al 1986). People who have more than 15 microfilariae in a skin snip taken near the eye are at risk of developing ocular onchocerciasis (Fuglsang and Anderson 1977). Microfilariae of Onchocerca volvulus do not exhibit periodicity, so that skin snips can be taken at any time of the day (Nelson, 1970).

Edungbola et al (1983) used the "Leopard skin"

for the purpose of diagnosis of onchocerciasis in the Babana District of Nigeria. Although a sign of onchocerciasis "Leopard skin" is not sensitive enough for general diagnosis of onchocerciasis due to the fact that not all onchocercal patients develop this manifestation and its appearance is usually in the chronic stage of the disease (W.H.O, 1966). Microfilariae of O. volvulus have been found in the urine of heavily infected people and this test for microfilaruria has been used to measure the level of endemicity in an area (Sasa, 1976). The number of microfilariae in urine increases with intake of Diethyl Carbamazine citrate (Fuglsand and Anderson, 1973, 1974; Roux and Picq, 1973; Anderson et al 1973).

Diagnosis of ocular onchocerciasis has been made by either observing the microfilariae in the eye or by opthalmoscopic examination of the eye for the typical onchocercal lesions (W.H.O. 1987). It has been estimated that 20 microfilariae or more in the anterior chamber and 50 microfilariae or more in the cornea constitutes a risk factor of blindness (W.H.O, 1982).

2.8.2 Mazzotti Reaction:

This is an indirect method for diagnosis of onchocerciasis. The mazzotti reaction is probably the most sensitive technique for diagnosis (Duke 1974; Buck, 1974; W.H.O. 1966). The patient is given a

single oral dose of diethylcarbamazine (DEC) usually 50mg (W.H.O. 1987). Intense itching begins about 20 to 90 minutes after taking the drug in infected individuals. The itching may involve the whole body but begins first usually over the lower trunk, buttocks, and thighs. The intense pruritus may be accompanied by erythema, oedema, urticaria and the development of vesicles and papules. Lymph nodes often become swollen, tender and painful. Oedema of the head and neck and especially the eye lids may follow especially in patients with onchocercal eye disease (Gibson et al 1980). The reactions reach a maximum amount of 24 hours after DEC and subside over the following 48 to 72 hours. Patients with light infections that microfilariae are not found will have a positive mazzotti reaction which confirms the diagnosis. The mazzotti reaction is specific and sensitive yielding only a very small false - negative and virtually no false positive (Sasa, 1976).

2.8.3 IMMUNOLOGY AND IMMUNODIAGNOSIS OF ONCHOCERCIASIS

Onchocercal disease (both ocular and dermal) appears to result not from mechanical damage caused by the presence of adult worms or microfilariae but by inflammatory reactions around certain microfilariae. Histopathological studies indicate that living microfilariae generally evoke no inflammatory reaction in the eye or in the dermis but that damaged microfilariae are usually surrounded by cells, particularly eosinophils and macrophages (Gibson et al, 1976; Henson et al 1979;

Mackenzie, 1980). What Immunological Mechanisms (Immediate hypersensitivity (Donnelly et al, 1983), delayed hypersensitivity (Bartlett et al 1978), immune complexes (Steward et al 1982), etc initiate these inflammatory response and result in the expression of clinical pathology is not clear. Immunity to parasite antigen is associated with localised dermal pathology ("Sowda") and low microfilariae count. While lack of responsiveness to parasite antigen is associated with generalised infection and high microfilarial count in the skin (Bartlett et al. 1978; Buttner et al., 1982). The antigens of O. volvulus have not been fully characterised. Crude extracts of adults worms and the microfilariae have been used in immunlogical studies (Kagan 1974; Marcoullis and Grasbeck, 1973). In view of the fact that the important pathological signs of onchocerciasis are due to the microfilariae much study is being centered around the antigenic composition of the microfilariae. The microfilariae antigens that have received greater attention are the surface antigens and the Excretory and Secretory antigens (E-S) (Ottesen, 1984) as well as the antigen of the Larval stage (L3) (W.H.O, 1987). Antigenic studies have shown that Onchocerca volvulus possesses a complex set of antigens. Even an individual could habour Onchocerca parasites of different antigenic composition (Marcoullis and Grassbeck 1973, Bryceson et al, 1976). Infection in man leads to the production of antibodies against many parasite antigens.

Immunoglobulin levels of various isotypes, especially IgG, IgM and IgE are elevated non specifically. In many people who are infected with O. volvulus, IgE levels are raised to extreme high levels and a greater proportion of IgE antibody is directed against parasite antigens (W.H.O, 1987). In a study on the serum immunoglobulin levels in 5 villages at the Republic of Chad, Buch et al (1973) found excessively high levels of IgG, IgA and IgM accompanied by relatively low IgD, associated with severe onchocerciasis complicated with microfilaruria. Comparative studies of IgG, IgA, and IgD levels between persons with severe and mild onchocerciasis, classified on the basis of microfilaria count in the skin snips revealed different age patterns. The natural increase of IgG levels with age was not found in the group with severe infection, while IgD concentration continued to rise from the youngest to the oldest age group. Ngu and Blackett (1975) found specific IgG and IgM antibodies against surface antigens of microfilariae in the sera of Onchocerciasis patients in Cameroon. Ngu and Blackett (1976) found significantly higher levels of immunoconglutinin, IgG and IgM and alteration in C_3 levels in the population infected with O. volvulus than in the non infected population. Merino and Brand (1977) reported that during the course of the disease the immunological apparatus suffers only slight modification since no great variations were found in immunoglobulin levels, the C_3, the reactivity to

2-4 dinitrochlorobezene or the response of peripheral blood lymphocytes to mitogens. They also found that under treatment the levels of serum IgM are increased and there is a decrease in C_3 levels.

Circulating antigen - antibody complexes are found in the sera of a high percentage of persons infected with O. volvulus (Steward et al 1982) but the significance of this observation is not clear. It is possible that immune complex deposition leads to acute inflammation and tissue damage or that the complexes contribute to a down-regulation of the host immune response.

Persons infected with O. volvulus are known to have impaired cell mediated immune response(Ottesen, 1984; W.H.O, 1987). This impairment of cell mediated immunity appears to be more pronounced in persons with little cutaneous reaction (and microfilariae distributed through the skin) than in those with highly reactive skin disease (and few demonstrable microfilariae). The precise mechanism by which dampening of the cell mediated immune response occurs are as yet unknown (W.H.O, 1987).

Although some people remain free from infection in endemic onchocerciasis areas despite high transmission potential, immunity to onchocerciasis does not occur in infected individuals. People who have been free from infection by chemotherapy are susceptible to reinfection (Ottessen, 1984, W.H.O. 1987).

The main stay of diagnosis is the demonstration of microfilariae in skin biopsies. However because

microfilariae can be present in small numbers and because the means to detect them parasitologically are relatively insensitive, immunodiagnostic techniques have been routinely employed for diagnosis (Ambroise-Thomas 1974). Traditionally these have been serological or skin test determination of antibodies generated by the host. Since all these assays however, share the major draw back of being unable to discriminate between past exposure and current infection and because the magnitude of the response bears no relation to the parasite burden, recent attention has focused instead on the direct detection of parasite antigens in patients' blood or urine (Ottesen, 1984). Ambroise-Thomas (1974) and Kagan Kagan (1963) have given extensive review of the literature on immunodiagnosis of filariasis including Onchocerciasis. The immunodiagnostic tests that have been used for the diagnosis of onchocerciasis include skin test (Kagan 1963), complement fixation test (Ambroise-Thomas 1974), passive heamagglutination test (Ikeda et al, 1978, 1979; Bryceson et al 1976) and double gel diffusion and immuno-electrophoresis (Ngu et al 1981, Bryceson et al, 1976). Others are indirect fluorescent antibody test (Ten-Eyeck, 1973; Ngu and Blackett, 1976), radioallergo - sorbent test (Somorin and Heiner, 1976) and Enzyme linked immunosorbent assay (Bartlett et al, 1975, Voller et al, 1975). The antigens that have been used in these studies are either heterologous antigens derived from animal sources (Kagan, 1963, 1974; Ambroise-

Thomas 1974) or sections and crude extracts of homologous species and excretory-secretory products of the microfilariae. A major set back in all these techniques for the detection of antibodies is that there is not only a limit in the sensitivity of the techniques but there is also cross reaction among the filariae and also in other nematode and non parasitic diseases (Ngu et al 1981), Galadima 1982). Ten-Eyeck (1973) was able to detect antibody presence in 102 out of 105 (97%) onchocerciasis positive patients using frozen section of O. volvulus. When using antigen from Wuchereria bancrofti, he was able to detect antibody in 86% of onchocerciasis patients. False positive reactions were observed by Mueller et al (1973) when they found that there were cross reactions between O. gutturosa antigen and sera obtained from people infected with other intestinal nematodes apart from filariasis.

Isolation and characterisation of specic antigens from either the adult worms or microfilariae of O. volvulus together with improved serological technique will be of paramount importance in the general diagnosis of onchocerciasis as well as evaluating the effectiveness of control measures. Klenk et al (1984) carried out serodiagnosis of human onchocerciasis using purified Litomosoides carinii antigen and found the ELISA techniques to be more sensitive (92.9%) than latex agglutination (89.1%) and Double diffusion (82.1%). The ELISA technique however had 10.3% false positive as against 6.8% false

positive for the latex agglutination and 0% for the double diffusion test. IgE levels are raised to extrmely high levels probably higher than in any other helminthic infection) and a greater portion of IgE antibody (compared to IgG antibody) is directed against parasite antigen (Buttner et al, 1982). Semorin and Heiner (1976) however could only detect IgE in 31 out of 57 onchocerciasis patients. Detection of circulating antigen instead of antibody detection is being investigated for possible use in the diagnosis of onchocerciasis but not much information has accumulated yet to warrant judgement of this technique (Ottesen, 1984).

In onchocerciasis, the immune response to certain immunogens and other infections has been reported to be depressed. In a study to examine the effect of onchocerciasis on tetanus immunisation, Prost et al (1983) found that only 7.1% of Onchocerciasis patients became immunised after two months of vaccination with a single 0.5ml dose of concentrated tetanus toxoid. In a control group that had no O. volvulus infection, 44.5% developed immunity after two months vaccination with the same amount of tetanus toxoid. Similarly non reaction to BCG, and pulmonary tuberculosis has been found in hyper endemic onchocerciasis are (W.H.O. 1987).

2.9 CHEMOTHERAPY OF ONCHOCERCIASIS

Numerous compounds have been produced which will either kill the microfilariae or the adult worm or

sterilise the female worms and prevent them from producing microfilariae. Out of these numerous compounds, the most tested ones include suramin (Duke et al 1981) Diethylcarbamazine (Domingue et al 1983), Ivermectin (Awadzi et al 1985), Melarsonyl potassium (Duke, 1970) melarsen W. (Goodwin, 1984), Metronidazole, oxaminiquine, tinidazole, mebendaxole, Tricholorophone, Pyrantel Pamoate and Levamisole. (Kale, 1978, 1982; Fuglsang and Anderson, 1978, Awadzi et al 1982). Others are Flubendazole (Kale, 1982) and Nifurtimox (Fuglsang and Anderson, 1978). Out of these drugs only suramin and diethylocarbamazine citrate have been registered for treatment of onchcerciasis. Ivermectin which is now the most promising drug for onchocerciasis treatment is under field trial and is yet to be registered for clinical use. Suramin, which kills both microfilariae and adults worms, is an inherently dangerous drug because of its high protein binding affinity with alteration of enzyme function (Hawking, 1978; 1978). The risk of optic neuropathy, nephrotoxicity, and occasional death associated with suramin treatment severely limits its usefulness, especially for mass treatment (Anderson et al. 1976). Budden (1959) used Suramin for the treatment of Onchocerciasis and obtained the following results: Microfilariae rapidly disappeared from the anterior chamber and cornea, ocular inflammation ceased, the number of microfilariae in the skin fell dramatically, pruritus stopped after an initial exercabation and skin texture improved. Two of the

patients died in hospital with what appeared to be a hypersensitivity reaction, prolonged high fever, prostration, arthritis, exfoliation of the skin, severe ulceration of the buccal muscosa and descending infection of the bronchial tract. The death which occured in heavily infected patients was thought to have resulted from massive liberation of filarial "toxin". Thylefors and Rolland (1979) observed optic atrophy following treatment of Ocular Onchocerciasis with Suramin.

Suramin is a complex water soluble derivative of urea that is under brand names including Bayer 205, Germanin and Moranyl (Gibson et al 1980). It is normally given intravenously at weekly intervals for 6 weeks. The toxicity associated with Suramin can be divided into three categories: (1) Toxic effects of Suramin itself. This includes tenderness of the palms, soles, thickening of keratin layer, polyurea and increased thirst plus tiredness, anorexia and Malaise. Other toxic effects include ulceration of mouth and tongue, chronic diarrhoea, severe prostration lasting weeks or months (Duke, 1974).

(2) Reactions attributed to death of adult O. volvulus worms. This includes urticaria and swelling of affected parts of the body, tenderness and swelling around nodules, painful immobilization of hip joint and pain above the knee from reaction around worm bundles.

(3) Reactions to death of microfilariae of O. volvulus. This includes itching of microfilariae infected skin,

proceeding to indurated dermatitis or to popular or vesicular eruptions with subsequent desquamation, pyrexia and sometimes associated with bronchitis. Other effects include iritis, with red eye, flare, posterior synechiae, swelling, pain and limitation of movement of the joints especially of fingers, writs, elbows, toes and knees (W.H.O. 1978; 1966).

Ocassionally the treatment may result in death (Budden, 1959; W.H.O. 1987). If treatment is discontinued at the first appearance of any adverse signs, they will usually disappear. These adverse effects can also be alleviated by the systemic administration of corticosteroide (Sasa, 1976; W.H.O. 1982). Diethyl carbamazine citrate is microfilaricidal when given to an infected person but has virtually no effect on adult Onchocerca (Goodwin, 1984). Diethyl carbamazine citrate is available under several brand names including Banocide, Hetrazan and Notezine (Gibson et al 1980). Diethyl carbamazine citrate has been the mainstay of treatment for onchocerciasis for many years (Taylor, 1984). There are however two major disadvantages with the use of Diethyl carbamazine citrate (DEC). One is the host response induced by the in-vivo killing of microfilariae (Bryceson et al 1977) and the other is the lack of effect on the adult worm (Taylor et al. 1980), so that the therapeutic effect is only temporary. After DEC treatment the skin is rapidly repopulated by new microfilariae over a period of 6-12 months and

the skin snip counts usually return to pre-treatment level within a year (Duke 1968). Minutes to hours after the administration of dietyl carbamazine to most patients with onchocerciasis, a series of events occur that include dermal, ocular and systemic components known collectively as the mazzotti reaction (Mazzotti, 1951). Systemic reactions include increased itching, rash, hoadache, aching muscles, joint pain, pain, swelling, and tenderness of the lymph nodes; fever, tachy cardia and hypotension and vertigo (W.H.O. 1987). Fuglsand and Anderson (1974) reported that some people collapsed after treatment with Banocide. The toxic effects following DEC therapy are more pronounced in heavily infected individuals and in people who already have ocular involvement (Anderson and Fuglsang; 1977; Anderson et al 1976; W.H.O. 1977). Bird et al (1980) reported that optic neuritis also occurs following DEC treatment, which leads to visual field loss. Banocide therapy has also been reported to lead to the mobilization of microfilariae and they are found in increased numbers in blood urine, lymph nodes as well as in the cornea (Fazen, et al. 1976; Anderson and Fuglsang, 1976, Roux and Picq, 1973; Anderson et al. 1975). They may also appear in the epidermis, synovial fluid, cerebrospinal fluid, hydrocoele, tears and in the sputum (Sasa, 1976). Bryceson et al (1977) reported a case involving nine Nigerians with severe onchocerciasis who were treated with diethyl carbamazine

The patients developed clinical changes ranging in severity from mild itching to distress, cough and syncope. Physiological changes such as fever, tachy pnoea, tachycardia or hypertension were seen in 8 patients. In five patients the systolic blood pressure fell by more than 25mm Hg and one patient collapsed on attempting to sit up. Circulating eosinophils decreased profoundly in all cases reaching their lowest levels just before or during the clinical and physiological changes. A fall in serum complement (C_3) was also found associated with the reaction. Some studies have used eye drops of aqueous 3 to 5 percent DEC solutions (Anderson, 1977; Ben-Sira et al; 1970; Anderson and Fuglsang, 1973, 1977). Anderson and Fuglsang (1973) found that in heavily infected patients with severe ocular involvement topical DEC (3 percent) produced local complications of sufficient importance to necessitate stopping treatment.

A cream containing DEC, designed for the treatment of Onchocerciasis by inuction (Langham et al, 1978) was tested on patients in Ibadan, Nigeria and at Wau, Southern Sudan and was shown to produce allergic response. at least as severe as those following the oral administration of the drug. The use of the cream was therefore not recommended. The use of small doses of Banocide do not seem to be enough to take care of the possible serious side effects (Goodwin, 1984, Taylor, 1984). In an attempt to block the mazzotti

reaction, many anti-inflammatory drugs have been studied. Studies at Tamale showed that prednisolone abolished the cardiovascular and lymphatic components of the mazzotti reaction provoked by DEC but did not prevent pruritus or skin rash (Awadzi et al, 1982).

Cyprohepatadine, triprolidine and cimetidine and Anti-inflammatory agents such as indomethacin and Kebuzone have failed to alter either the nature or severity of the reaction (Awadzi et al 1982). Other anti-inflammatory agents which have been used but with little success in suppressing the exercabation of the disease following DEC are dexamethasome, betamethasone, triamcinolone, methysergide, aspirin and diazepan (W.H.O. 1987).

It should be noted however that not all patients develop these severe side effects and it seems that these reactions have some genetic linkage (Nelson, 1970, Henson et al, 1979). In view of the possible severe side effects of DEC, indiscriminate use of Banocide has been advised against. DEC should not be used indiscriminately in patients with onchocerciasis. Its use should be restricted to carefully selected patients who are kept under observation and given steroid "cover" against inflammatory reactions. The major indication for treatment with currently available drugs is the presence of onchocerciasis that is severe enough to threaten sight. Suggested indications for chemotherapy of onchocerciasis using diethyl carbamazine and Suramin are as follows (W.H.O. 1987).

Major Indications: Sight - threatening disease as indicated by:

1. 20 or more microfilariae in either anterior chamber.
2. 50 or more microfilariae in either cornea
3. Severe uveitis in patients with onchocerciasis
4. Early sclerosing keratitis
5. Visual field constriction in patients with active disease.

Other indications: Severe onchodermatitis, high microfilarial count, 100 microfilariae or more per mg of skin and 15 microfilariae or more per mg of outer canthus skin snip. Because of the possible serious side effects with either Suramin of Banocide, it has been recommended that the course of treatment should be as follows (W.H.O. 1987): "Patients being treated should be hospitalised or treated under close medical supervision". Treatment should begin with diethyl carbamazine given under corticosteroid coverage and later followed by Suramin treatment; patients should be monitored carefully for side effects and the treatment modified or interrupted if major adverse reactions occur; treatment should not be given indiscriminately or without due consideration for the patient's present and future status and the likelihood of reinfection.

The mechanism by which DEC kills microfilariae remains to be proven. Basic to the problem is the finding that DEC does not kill microfilariae in vitro

(Rivas-alcala et al., 1984). According to Hensam et al (1979), "DEC does something to microfilariae which greatly increase their ability to provoke an inflammatory reaction in the host". They listed several possible mechanisms for this exacerbation of the inflammatory response to microfilariae that is caused by DEC:

1. That DEC kills microfilariae which then become highly irritant.

2. DEC treatment removes the hypothetical coat of human antigen.

3. That DEC activates complements on the microfilarial surface.

4. DEC stops production of complement inhibitor.

5. DEC breakds tolerance to microfilarial antigens.

6. DEC alters the status of cyclic nucleotides in microfilariae.

Despite the serious side effect commonly associated with DEC treatment, it is presently the drug of choice for treating onchocerciasis (Taylor, 1984; W.H.O. 1987).

Chemotherapeutic agent which may most likely get priority for onchocerciasis treatment is an Aver-mectin called iver-mectin (Taylor, 1984). Ivermectin is a macrocyclic lactone, a synthetic derivative of avermectin B, which is a fermentation product of Streptomyces avermitilis. Ivermectin is highly active against O. gibsoni

microfilariae in cattle. It has a broad spectrum of antiparasitic activity and has for several years been used in veterinary medicine as an intestinal anthelmintic and for the control of arthropod ectoparasites (Goodwin, 1984). Aziz et al. (1982) tested ivermectin on patients with O. volvulus infections with a single dose of 50 to 200ug and observed a 90 percent reduction in the skin microfilarial load in one week. There was no mazzotti reaction except in few cases in which the reaction was very mild. Adwadzi et al, (1985) treated nineteen patients who had moderate to heavy infections with O. volvulus with single doses of 50, 100, 150 or 200ug per kilogram of invermectin slowly eliminated microfilariae from the skin and eye without serious adverse clinical or ocular reactions in all treated groups; very low levels of skin microfilariae were maintained for nine months. Dose dependent stimulation of embryogenesis was noticed in the first one month after initiating therapy and then suppression of embryogenesis 3 months after therapy. The mechanism of action of ivermectin against O.volvulus has not yet been clearly established. It's effect is thought to result from ivermectin's activity in enhancing both the presynaptic release of gamma-amino butyric acid (GABA) and the post-synaptic enhancement of GABA-binding to a chloride channel linked receptor (W.H.O., 1987). Although ivermectin has been shown to have macrofilariciadal activity in some animal filarial species, there is no evidence suggesting that such an effect occurs against O. volvulus.

The Benzimidazoles, Metrifonate and the arsenicals, although they have been tried and some of them found to have possible beneficial effects for the treatment of onchocerciasis, they are not in clinical use due to either their serious mazzotti effects, teratogenic effects, encephalopathy, or the intolerance of high doses of such drug associated with prolonged use in treatment (Taylor, 1984; Goodwin, 1984; W.H.O. 1987). Nodulectomy is one of the earlier methods of treating onchocerciasis and has been widely used for more than 40 years in Mexico, and Guatemala where head nodules are relatively common (Nelson, 1970). Although this practice is very common in Guatemala and Mexico it has been difficult to assess its benefit as a treatment for onchocerciasis. Some of the nodules are impalpable and some worms may not have formed nodules yet (Kale, 1982; Albiez, 1983; Taylor 1984). Removal of head nodules may only delay the onset of ocular lesion but in areas where there is no vector control it can only be a temporary measure. In Africa nodulectomy is not a common practice and the little information on this available showed that nodulectomy did not significantly alter skin snip counts when the patients were examined later (Kale, 1982b; Albiez 1983; Meyers et al. 1977).

2.10 CONTROL OF ONCHOCERCIAIS

2.10.1 NODULECTOMY: Control measures can either be carried out against the parasite, the vector or both. The parasite may be controlled by excision of nodules which

by destroying the adult worms, also eliminates the microfilariae after a few months, thus interrupting the transmission (Nelson 1970, Kale, 1982). Control of onchocerciasis by excision of nodules has been a prominent feature in Guatemala and Mexico where medical auxillaries go about removing head nodules. While this practice may reduce the risk of ocular involvement it can only serve as a temporary measure in lowering the microfilaria load of the skin as some worms might not have formed nodules yet. It is virtually impossible to remove all nodules as some of them are impalpable, moreover, from the medical point of view it is not feasible to cut out large number of nodules from many people who may in any case be reinfected at any time (W.H.O. 1974). Nodulectomy has not been an important method of controlling onchocerciasis in African (Albiez, 1983; Meyers et al., 1977; W.H.O. 1987). Destruction of worms by Suramin is not possible except under medical supervision. It is a dangerous drug which cannot be used in mass campaign (Budden, 1959, Nelson, 1955). Destruction of microfilariae by diethyl carbamazine also produces considerable adverse reaction and as such cannot at present be used in mass campaign. This leaves us with destruction of vector. The vector control can be done under certain conditions. The first is that campain should last for quite a long time. It should interrupt transmission throughout the cumulative life of the adult worms and the last microfilariae produced

by the adult, that is 16-18 years. The second condition is that the ecological area should be quite large.

2.10.2 CHEMICAL CONTROL

Until the appearance of DDT, the methods proposed and tested for blackfly control were notably ineffective. They were uneconomical (sweeping larvae from stones or removing vegetation in rivers), ineffective, and or involved the application of materials highly injurious to non-target fauna (oil emulsion, xylol, kerosine, arsenicals (Jamback, 1981). The first reprot on the effectiveness of DDT on Simulium is that of Bishop in 1943 (cited by Jamback, 1981). He dripped an emulsion consisting of kerosine solution of DDT, soap, and water into a small stream and noticed that within a few hours the well developed larvae of Simulium venustum on rocks in the stream disappeared for a distance of at least several hundred feet downstream. Fairchild and Barreda 1945 (Cited by Jamback 1981) showed that DDt when applied at very low dosage rates directly into water courses was extremely effective in killing Simulium larvae. Following this demonstration, numerous Simulium control schome have been executed in Africa in the 1940s and 1950s. Vector control or eradication has led to onchocerciasis eradication from Kenya and some foci in Uganda (Raybould and White, 1979). In Kenya the eradication of S. neavei s.s. was completed in 1956 (McMahon et al. 1958; McMahon, 1967). The control was carried out using 18% DDT in emulsion oil applied to

rivers and streams at dosages of 0.5 to 1mg/liter. The control covered an area of 40,000km^2 and lasted for 10 years. There has been no report of resistance to DDT by S. damnosum s.l. in East Africa whereas resistance has been reported in West Africa (Walsh, 1970). The environmental advantages of employing biodegradable compounds such as temephos (Abate), against vectors belonging to the S. naevei group are offset by the fact that these species tend to breed in cool streams where the potency of temephos would be reduced by the low temperature (Raybould and White, 1979). Factors which lead to the effective control of the disease in Kenya has been attributed to thorough pre-control surveys for both early stages and adult forms of S. neavei. The breeding sites were plotted on maps and the flight range of the adult fly along the water courses was recorded. Another factor which facilitated the scheme is the geographical isolation of the affected area reducing the chances of reinfection from outside. The accessibility of many dosing points by road also facilitated the programme (Raybould and White, 1979).

The effective control of vectors of onchocerciasis has led to the improvement of life and economy of the affected areas, for example, in Uganda, biting rates of over 300 S. damnosum per man hour prior to control discouraged cultivation of the exellent agricultural land along the Victoria Nile. Onchocer-

ciasis infection rates in those days were very high, being 80-100 percent in people aged 15 years and above, and about 65 percent in the overall population. As a result of the control and intermittent eradication of the vectors, infection rates declined to 17% in 1967 and to less than 0.2% in 1974. The area concerned has now become one of the major food producing parts of Uganda (Raybould and White, 1979).

Very limited vector control has been carried out in Guatemala. In March 1979 larviciding operation was launched in the Lavaderes river of the pilot control area using 10 percent temephos briquettes at two weekly intervals. Results showed that larval density decreased rapidly soon after the temephos application, subsequent searching revealing neither old larvae nor pupae in most tributaries. Collections from a bait-boy showed that the number of adult Simuliids attempting to bite at first fluctuated between 26 and 524 during three hours observation periods before pesticide treatments but then decreased remarkably, so that from June to December 1979, adult density remained between 0 and 21 (Ogata, 1981). In the Bametal and Zapote river basin adjacent to the Lavaderos river, two-weekly larviciding was commenced in June 1979 at 83 sites on 44 tributaries. Although results showed absence of larvae in the post treatment assessments, the decrease in adult fly population proved much less marked than in the lavaderos river basin (Ogata, 1981). The reason for this was suspected

to be invasion from the Southern area not under blackfly control.

In West Africa, where onchocerciasis is widespread and frequently a serious problem, the first attempt of vector was made at Mayo Kebbi, Chad but did not succeed (Taufflieb 1955 cited by Walsh et al, 1979). In Nigeria the then Northern Region Ministry of Health decided in 1955, that a pilot project should utilize the newly available insecticides and see whether S. damnosum s.l. control was practicable at a relatively local level. The primary objective was to see if "it would be possible to obtain significant vector control by annual larviciding of the breeding site" (Crosskey 1979). After one year of preliminary investigation, control work was started in the Abuja Emirate in 1956. The aims of the project were" (i) to determine the possibility of controlling S. damnosum s.l. in an area not isolated from other fly areas, (ii) to evolve a practical and economic technique for routine annual control and (iii) to assess the effect of vector control on the prevalence of onchocerciasis within the project area" (Davies 1963). The control which was carried out between 1956 and 1960 employed DDT. The major rivers were treated with DDT at a concentration of 1.0 parts per million of river water over a period of 30 minutes at weekly intervals for a period of 12 weeks. Results evaluation carried out by Davies (1963, 1968) showed that the Simulium population was reduced by 90 percent but there was no such corresponding

decrease in the prevalence of onchocerciasis. The Gurara river was too large to be included in the scheme and formed a constant source of reinfection along the Western boundary. This contributed to the failure of the scheme. Other factors that contributed to the failure of the scheme were the short duration of the scheme and the small size of the area which was bounded by other endemic zones. The construction of the Kainji dam also necessitated Simulium control to project the workers at the project site (Hitchem and Goiny, 1966; Walsh, 1970). Control operations were concentrated on the Oji river and its major tributaries, the Kontagora river complex and the Niger. Wet season larviciding continued from 1961 to 1968 using 25% DDT emulsifiable concentrate (Arkotine) at dosage rates ranging from 0.03 to 1.0PPM. During the construction of the Oji river power station a short stretch of the river was dosed with DDT to protect the work force. The Kaduna river and the river Hawal are other places that have received limited control (Crosskey, 1981). The main lesson learnt from the control work in Nigeria mentioned above is that the very widespread areas of closely connected breeeding sites made it very difficult to control on a small scale (Hitchen and Goiny, 1966; Garms et al 1978). This together with the discovery of the disease in more parts of Nigeria and the likely success of the large scale OCP was quickly noticed in Nigeria and considerations were given to the development of a National control programme. Various scientists visited the OCP to see the methods

in use and the problems to be faced. Senior scientists from OCP and WHO consultants visited Nigeria in the late 1970's. They used the information available to produce studies in relation to the development of an onchocerciasis control campaign (W.H.O. 1978, Crosskey, 1981). A National Onchocerciasis control programme (NOCP) has now been set up in Nigeria and the country has been divided into zones. Actual control on National scale is yet to start. It is suggested that at least two aviation centers will be needed when the whole country is under control. The precise length of the rivers that will have to be treated by air in the National onchocerciasis control programme is yet undetermined. However the number and complexity of river systems supporting S. damnosum s.l. in Nigeria is much greater than in the Onchocerciasis control programme. "The logistics of aerial application are going to be much more complicated and expensive. Many of the fuel and insecticide dumps in the OCP are in remote areas and are left unattended. Nigeria has a much greater and often a more densely dispossed population. Many more dumps will have to be sited near population centers and, at the very least, cooperation with the village heads will have to be sought to prevent pilferage" (Gregory, 1982).

2.10.3 THE ONCHOCERCIASIS CONTROL PROGRAMME

This programme which started in February 1975 initally covered 7 West African countries (Walsh et al.

1981). The programme now has involved 11 West African countries (W.H.O. 1987) namely, Benin, Burkina Faso, Cote d'Ivoire, Ghana, Guinea Bissau and Mali, Other countries are Niger, Senegal, Sierra Leone, Guinea and Togo. The OCP had its genesis from a meeting in 1968 at Tunis sponsored by USAID to discuss the feasibility of onchocerciasis control (W.H.O. 1969). The meeting concluded that control was technically feasible, and that the chances of obtaining successful and lasting effects would be greater if the control was carried out in ecological zones that were sufficiently large to obviate the need for continuous protection of the whole area against reinvasion by the blackfly vector. The meeting further suggested that the area centered on the densely populated headwaters of the volta Basin would offer the greatest prospect of success. The Tunis meeting led to the establishment by W.H.O. of a mission (the PAG Mission) charged with the task of preparing a comprehensive plan for Onchocerciasis control. The mission submitted its report in 1973 to a conference of participating countries, potential donors, and sponsoring agencies (FAO, UNDP, the World Bank and W.H.O.). The operational plans and budget were approved and an onchocerciasis fund managed by the World bank was established. The programme is a corporate undertaking of 11 participating countries in West Africa and the control programmedonor communities (countries, foundations and development banks) and four United Nations Agencies (UNDP, FAO, the World Bank and W.H.O.) which together

make up its governing body, the joint programme committee (JPC). W.H.O. is the executing agency with the World Bank as the financial trustee (Walsh et al 1979; Walsh et al 1981; W.H.O. 1987). The objective of the programme is to eliminate onchocerciasis as a disease of public health and socio-economic importance throughout the area covered by the programme and to ensure that there is no recrudescence of the disease in the future. The programme covers an area of more than 750,000km^2 with a river system of 23,000km. Temephos (Abate) is the insecticide that has been used in more than 50 percent of the programme area (Walsh et al. 1979; Walsh et al. 1981; W.H.O., 1987). For smaller, more heavily wooded streams, helicopters are used for delivering the insecticide. For control of larger, more open rivers, fixed-wing aeroplanes are used. The rivers are treated with insecticide emulsion at rates of between 0.05 and 0.1PPM measure over a 10 minute period of flow. The lower dosage rates are used for fairly large fast flowing rivers during the wet season and the high dosage rates are used in small stream and during dry season conditions of intermittent flow in the larger rivers. On large rivers during the wet season treatments are made at 40km intervals, whereas in extreme dry season, each separate breeding site is given individual treatment (Walsh et al 1979). Treatments are carried out at weekly intervals. Control activities have continued since February 1975 and the programme is expected to last for 20 years, a time based on the

postulated life-span of the parsite Onchocerca volvulus s.l. At the initiation of the programme it was estimated that one million people within the control area were infected of whom at least 70,000 were blind or had serious impairment of sight. Entomological, epidemiological and socioeconomic evaluations have been used to assess the effectiveness of the control (Walsh et al. 1979; W.H.O., 1987). Prior to the initiation of the control programme, the Annual biting rate (ABR) in some areas such as in the White Volta basin, along the Bougouriba, the Upper Comoe, the Leraba and the Upper Bandama rivers reached levels of 50,000-250,000 (W.H.O. 1987). In the central well controlled area, S. damnosum s.l. has been virtually eliminated. Annual biting rates are between zero and 100 throughout the area (Walsh et al 1979; Anonymous, 1987). In the less well controlled areas even though the flies have not been elimiated, they have been reduced to low densities with annual biting rates normally below 500 and rarely exceeding 1000. Infective flies have also been reduced to very negligible level. In some of the reinvaded area, however, annual transmission potentials and annual biting rates remain close to precontrol levels (Walsh et al, 1979; Garms et al 1979; W.H.O, 1987).

Comparison of epidemiological data also shows that the control programme has effected interuption of transmission. Out of a total of 6,700 children examined, all of whom were born after the start of control, only one was found infected compared to the expected number

400, in the central well controlled area had there been no control (Prost and Prescott, 1984, W.H.O., 1984, 1987). The microfilarial load in the skin of adults, and children born 10 years before the start of control has also been reported to have fallen (W.H.O. 1987). The level of microfilariae in the cornea has also fallen.

Prost and Prescott (1984) reported that blindness rate for Burkina Faso used to be 7-9 per 1000, while in many hyper endemic districts could be up to 30-50 per 1000 and in some selected villages 100-130 per 1000 blindness rates could be observed prior to control. Blindness rate decreased to 2.5 per 1000 in hyperendemic areas during the period 1975 - 1978 and 3.2 per 1000 of overall blindness during the period 1975 - 1980.

The control has also had positive impact on the socioecnonomic aspect of the affected areas. Significant increase has been reported in the amount of cultivatable land which had hitherto been abandoned as a result of onchocerciasis (W.H.O. 1987).

Some of the problems encountered in the OCP area have been those of reinvasion of controlled areas and resistance to insecticides (Garms et al. 1979; W.H.O. 1987). The vast majority of invading flies belonged to the 2 cytospecies, S. damnosum s.s. and S. sirbanum.

Resistance against chlorphoxin and temephos has been reported in the OCP area (W.H.O. 1987). In either case, Bacillus thuringiensis H-14 substitute has resulted to reversion to normal susceptibility some months after

the withdrawal of the insecticide.

Apart from controlling black flies for the purpose of eliminating the disease, Onchocerciasis, black-fly control in many parts of the world have been carried cut purely for economic purposes. In the USSR blackfly control has been necessitated by major hylroelectric projects (Dubitskii, 1981) in regions of Siberia. The blackflies encountered in this area included Gnus cholodkovskii, G. jacuticum, Simulium morsitans, S. reptans, Schoenbaueria pusillum and Schoenbaueria brachy-arthra. In the construction area, working capacity was known to be seriously reduced through biting and the persistent and extremely annoyance caused by blackflies forming a cloud about the head and entering the mouth, nose, earns and eyes. During periods of massive blackfly attack, human productivity was known to decline by 20-30 percent. Also the number of ocupational injuries increased significantly (Dubitskii, 1981). In areas where biting fly control was not carried out, there were recorded instances of consequent economic losses ranging from tens of thousands to hundreds of thousands of roubles daily. Similarly, in the dairy industry, the average daily milk yield was shown to be reduced by up to 45% through blackfly attack and egg laying of domestic birds was severely reduced. Fattening of stock was also found to be substantially retarded.

Control of Simulium articum in Northern Alberta, Canada was necessitated by the great economic loss

inflicted to livestock farmers (Charnetski and Haufe, 1981). In 1971 severe hundreds thousand dollars were lost by livestock owners from blackfly menace.

In the USA blackflies have been known to constitute a serious menace at Parks, campgrounds, resort hotels and other recreational facilities where these are developed in thousands, with abundant running water sources suitable for the breeding blackflies (Lascala and Burger, 1981). The biting and annoyance of female blackflies in certain areas has been found to create a nuisance for residents and vacationers during the tourist season, resulting in suspected economic losses in resort areas of high blackfly population. Lascala and Burger (1981) reported that "a severe blackfly season in 1975 prompted the Balsams Hotel management to seek assistance from the department of entomology at the University of New Hamshire". The blackfly involved was identified to be Simulium decorum.

Blackfly control, especially vectors of onchocerciasis can be very expensive. Prost and Prescott (1984) reported that 10.2 million dollars was used by the OCP between the period 1975 - 1980 in Burkina Faso. The results of effective control however warrants such investment.

CHAPTER THREE

MATERIALS AND METHODS

MATERIALS AND METHODS

STUDY AREAS

The study areas consisted of 7 villages in Kachia local governement area of Kaduna state, 2 villages in Chanchaga local government (Niger State) and 2 villages in Mariga local government area (Niger State),

Kachia Local Government: (Kaduna State)

The villages that came under study in the Kachia local government area are: Iri, Kurmin Idon, Doka, Iburu, Makyali, Kurmin Biri and Akupal (see Fig1). These villages are found in the Guinea Savanna. The area falls between latitude 10° 7'N - 10° 21'N and longitude 7° 40'E - 8° 40'E. There are two major rivers in the area namely: River Kaduna to the West and River Iri to the East. These two rivers flow throughout the year. The population of the villages ranges from 1,300 - 7,400. The inhabitants of these villages speak Kadara language. They are mainly Christians and Animists. Each of these villages has a primary school but none of them has a post primary institution. There is a rural health center at Idon, after village Makyali. The nearest General Hospital is that of Kachia, the local government headquarter which is about 50km distant. Water for domestic purposes is obtained from rivers and streams. The major motorable road in the area is the Kaduna-Kachia highway. Iburu, Kurmin Idon, Doka, Makyali and Kurmin Biri are situated on the Kaduna - Kachia

MAP OF STUDY AREA WITH LOCALITIES

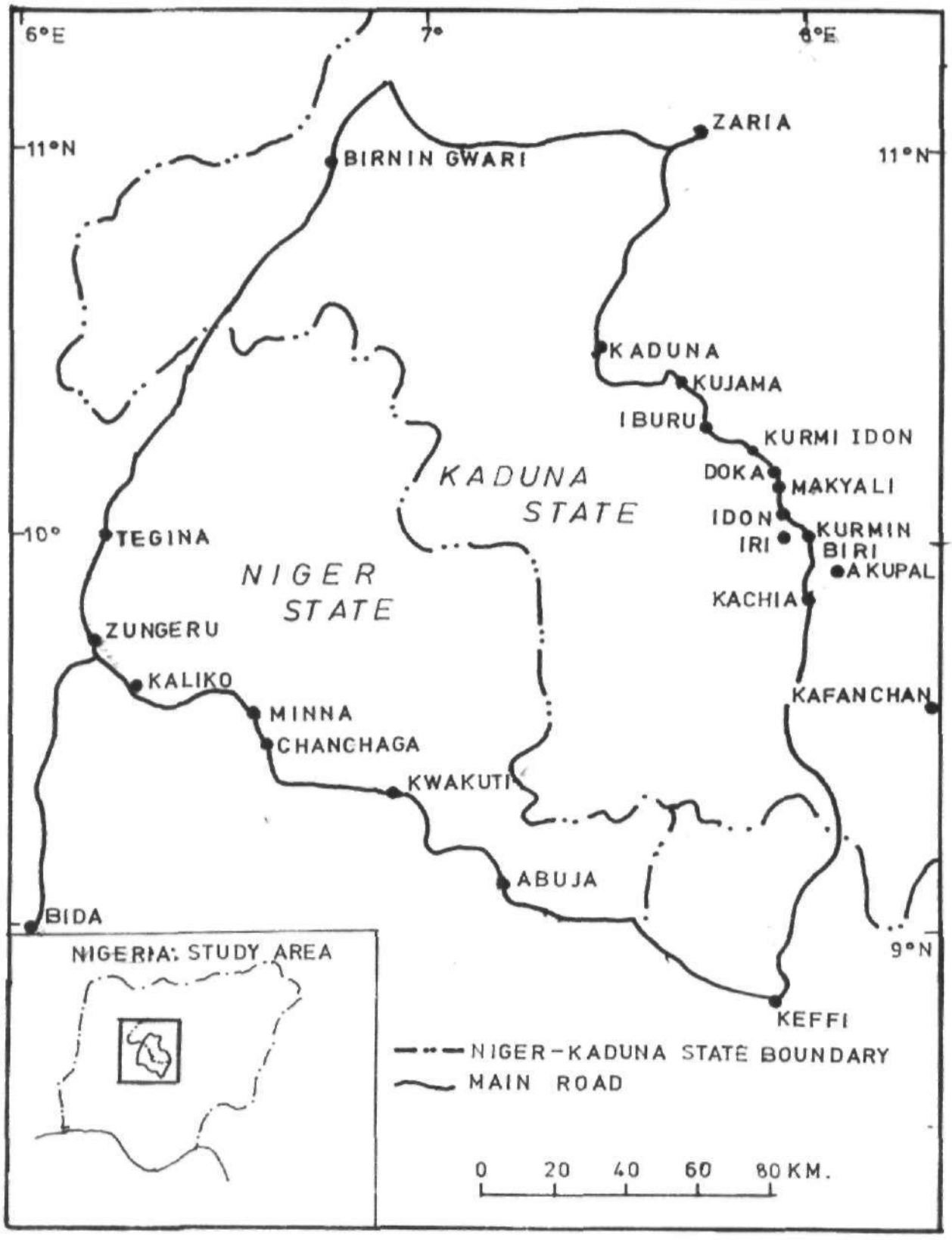


Fig.1

highway. The other villages do not have good access roads and the roads to these villages become nearly unmotorable during the rainy season.

The annual rainfall is about 1,500mm. Oral interviews revealed that most of the villages had moved from areas nearer to one of the rivers. Half of the people at Kurmin Idon had once lived near the River Kaduna while the other half were once near the River Iri. Doka village moved to the present site from somewhere near the River Kaduna. This was also the case with Makyali village. village. Iburu village moved from its former settlement near River Iri, to where it is now. Akupal and Iri villages seem to have remained in their old settlements. No one could say exactly when they moved to their present sites but the reasons most of them gave are that: (1) their former settlements were too forested and (2) that they wanted to be nearer to the major road (Kaduna-Kachia road).

Agriculture is the main occupation of the people. Both men and women are actively involved in farming. The main crops cultivated in the area are maize, sorghum, yams and rice. Other crops cultivated are millet, beniseed, tomatoes, pepper and cocoayam.

Although, the people are familiar with the vector, Simulium damnosum s.l., they are not aware that the flies transmit any disease. The fly is called 'Ovue' in their local language. They said that the flies are usually abundant during the first rains in May/June.

They believe that the flies bite mostly between 10.00 a.m. and 12.00 noon, and between 4.00 p.m. and 6.00 p.m.

Chanchaga Local Government (Niger State)

2 villages namely Bari, near Chanchaga, and Kwakuti were surveyed. These villages lie between latitude 9° 14'N - 9°35'N and longitude 6° 49'E - 7° 10'E. Bari village is very close to Chanchaga river in Minna while Kwakuti is near the Gurara fall. Both villages are inhabited by the Gwari people. The villages are situated in the Guinea Savanna. The annual rainfall is about 1316.6mm. Bari village has a primary school. Health Institutions close to the village are the Chanchaga leprosarium and the Minna General Hospital. The road from this village to Minna or Chanchaga is hardly motorable. Kwakuti is situated on the Minna-Suleija highway. There is a primary school and a Basic health clinic in the village. There is a bore hole which is the main source of water supply. The inhabitants of these two villages are both christians and moslems. Agriculture is the main occupation of the people. Only the males are actively involved in farming. The main crops cultivated in the area are yams, maise, sorghum, and rice. Other crops grown in the area are beans, tomatoes and pepper. Oral communication shows that Kwakuti villagers moved there from somewhere not too distant from their present settlement. Although the former settlement area is more forested than the present one, the people could not give any reason why they decided

to move. Bari village was reported also to have moved from somewhere else. Their explanation for movement was that where they were staying before was too forested.

Mariga Local Governement (Niver State)

Kaliko village and Zungeru town of Mariga local government fall between latitude 10° 7'N - 10° 14'N and longitude 6° 17'E - 6° 28'E. The two areas are situated in the Guinea Savanna with annual rainfall of about that of Minna (1316.6mm).

The main occupation of the people (Gwari) is agriculture. The main crops grown in the area are yams, sorghum and rice. There is a primary school in the village and the nearest health centre is the rural Health Center at Zungeru. Zungeru has become cosmopolitan with people from all parts of the country. Rice is the main crop grown in the area. Zungeru is an old town but Kaliko is a recent settlement on the Zungeru-Minna highway.

3.2 Preliminary Investigation on Onchocerciasis

Parasitological survey and clinical examination of onchocerciasis were carried out as preliminary investigations to the main histochemical studies.

3.2.1 Parasitological Survey by skin Snipping

Due to the unavailability of corneoscleral punch, hypodermic needles of 23G and razor blades were used for skin snipping (Buck, 1974, W.H.O. 1987). The name, age,

sex and clinical examination (skin itch, blindness, impaired vision, depigmentation of legs) were recorded for each person to be skin snipped. The pelvic region below the iliac crest was chosen as the site for snipping (Nelson; 1970, Sasa, 1976; Alfons and Wenk 1983). The area of the skin to be snipped was prepared by cleaning with Methanol soaked in cotton wool (W.H.O. 1987) and allowed to dry. Using a hypodermic needle of about 23G a cone of skin was raised and then cut with a sharp razor blade. Two snips were taken from each person. The snips were then incubated in phosphate buffered saline (PBS) has pH 7.2 or Normal Physiological saline in Microliter wells (Awadzi and Roulot, 1980; Ngu et al. 1981, W.H.O, 1987). The incubation time lasted for 6-24 hours before the snips were examined. At the end of the incubation period pasteur pipettes were used to transfer drops from each well onto separate microscope slides. The slides were examined with X10 objective without cover slips. Some slides were examined in the field using monocular microscope while others were examined in a nearby government clinic. Samples that were taken on the last day of field trip were kept in the refrigerator after returning to the University and examined during the following days. The presence of microfilariae in the drops of saline was recorded as a positive case of O. volvulus infection. Microfilariae were not counted since the snips were not of uniform size and were not weighed due to absence of a suitable balance.

3.2.2 Clinical Examination

Aspects of clinical examination were restricted to cases of body itch, depigmentation of the legs, total blindness, impaired vision and presence of palpable nodules. The subjects were asked (before taking a skin snip) if they experienced body itch. Visual impairment was recorded for those who said they had difficulty in seeing. Blindness was assessed by total inability to see. Nodules were looked for on the knees, lateral abdominal wall and in the pelvic region (Nwokolo, 1950; Kale, 1982). The legs were examined for depigmentation (Nelson, 1970; Buck, 1974; Edungbola *et al.*, 1983).

3.3 HISTOCHEMICAL ENZYME STAINING OF ACID PHOSPHATASE

3.3.1 Preparation of Slides: After the initial examination for the presence of microfilariae, skin snips that showed microfilariae were allowed to dry on the slides. The microfilariae were fixed using cold acetone. Acetone was kept cold in a bottle stored in a cool box containing ice blocks (Omar, 1978). The slides were again allowed to dry, after fixing in cold acetone for 2 minutes. Slides are wrapped singly in tissue paper and kept packed in polyethylene bags and transported to the laboratory on cold ice blocks. Slides were kept in the freezer until they could be stained. The slides were stained not later than three months after collection.

3.3.2 Acid Phosphatase Staining

Staining for acid phosphatase activity of the microfilariae was done according to the method of Chalifoux and Hunt (1971).

3.3.2.1 Reagents.

Solution I: Michaeli's veronal acetate buffer pH 10.

Sodium acetate ($NaC_2H_3O_2 3H_2O$)	9.714g
Sodium barbital	14.714g
Distilled water	500ml

This was stored at $5^{o}C$.

Solution II: Naphthol AS-TR phosphate

Naphthol AS-TR phosphate	0.05g
N,N-Dimethyl formamide	5.0ml

This was prepared fresh any time it was to be used.

Solution III: Pararosanilin

Pararosanilin hydrochloride	1g
Distilled water	20ml
Concentrated hydrochloric acid	5ml

Pararosanilin was added to the distilled water, heated to dissolve and the hydrochloric acid added. This was cooled and stored at $4^{\circ}C$.

Solution IV: Sodium Nitrite, 4%

Sodium Nitrite ($Na\ NO_2$)	4g
Distilled water	100ml

This was stored at room temperature or at $4^{\circ}C$.

Solution V: 1% Methyl green in phosphate buffer.

(A) 0.2M Sodium phosphate

$Na_2\ HPO_4$	28.396g

(B) 0.1M Citric acid

$CH_4\ (CH)\ (COOH)_2\ H_2O$	21.011g
Distilled water	1000ml

Working Solution:

Solution A	77.1ml
Solution B	122.9ml
Methyl green	2.0g

This was kept at room temperature

Preparing Enzyme Substrates:

20ml of solution I were added to 50ml of distilled water in a coplin jar. 4ml of solution II were added to the above mixture. 3.2ml of solution III and 3.2ml of solution IV were mixed in a test tube and the mixture

added to the contents of the coplin jar. The pH was adjusted to exactly 5 using a pH meter and NaOH solution.

3.3.2ii Staining Procedure:

Slides were placed horizontally on staining racks with the surface containing the microfilariae uppermost. By means of pasteur pipettes the slides were flooded with the substrate (Naphthol AS-TR phosphate) and allowed to stay for two hours at room temperature. Substrate was added from time to time to ensure that the slides did not dry up. After the two hours period of incubation the slides were rinsed in distilled water. The slides were then counter-stained with 1% methyl green for 2-3 minutes and then rinsed in distilled water. The smears were then dehydrated, first in 95% ethyl alcohol and then in absolute ethyl alcohol. After dehydration, slides were rinsed in xylene and then mounted using Canada Balsam. The smears were examined for the precipitated red azodye indicating acid phosphates activity.

Classification of staining types according to the sites of enzyme activity in the microfilariae was determined using the classification of Omar (1978). The microfilariae on each slide were examined for the type of enzyme activity.

Photomicrographs:

Photomicrographs of microfilariae were taken using Leitz microscope at 630 magnification.

Photographic tracing: The photographs were traced to show more clearly the acid phosphatase staining patterns.

Measurement of Microfilariae: Microfilariae were measured using Camera Lucida.

CHAPTER FOUR

RESULTS

R E S U L T S

4.1 RESULTS OF PRELIMINARY INVESTIGATIONS ON ONCHOCERCIASIS

4.1.1 PARASITOLOGY

A total of 1531 people consisting of 781 females and 750 males (Tables 1 and 2), were skin snipped in 7 villages of Kachia local government area (see Fig. 1). 271 females (34.7%) of the samples) were infected with the parasite Onchocerca volvulus but the infection varied from 0 to 100% (see Table 1) with infestation showing a tendency to increase with age. 292 males (38.9%) were infected (Table 2) with the parasite Onchocerca volvulus. Again the infestation rate varied from 0 to 100% with a tendency to increase with age. The prevalence rates for the female population area were as follows: Iri (36.10%), Kurmin Idon (35.0%) Doka (36%), Makyali (31.30%), Iburu (39.50%, Akupal (14.5%) and Kurmin Biri (42%). Table 1 shows that infection started early in life (1-5 age group) in most of the vilages.

The prevalence rates for the male population for the same villages were as follows: Iri (45.6%), Kurmin Idon (35.0%), Doka (48%), Makyali (35.4%), Iburu(43.2%), Akupal (15.4%) and Kurmin Biri (191%). As was noticed in the female population, infection started quite early in the male population. A case of a boy less than one year old, infected with the parasite, was detected (Table 2).

TABLE 1: **Prevalence rates of** Onchocerca volvulus **infections in seven villages** of Kaduna State: female population.

Age Group	Iri No.	%	Kurmin Idon No.	%	Doka No.	%	Makyali No.	%	Iburu No.	%	Akupal No.	%	Kurmin Biri No.	%	Total No.	%
<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-5	8	25%	8	0%	22	9.1%	20	25%	-	-	-	-	-	-	58	16.8%
6-15	37	19%	4	14.6%	34	11.8%	41	19.3%	87	34.5%	7	0%	1	0%	248	14.2%
16-25	8	37.5%	69	15.9%	31	22.6%	14	35.7%	19	47.4%	7	0%	14	35.7%	162	27.80%
26-35	16	43.8%	38	63.2%	28	50%	12	58.30	10	40%	3	0%	8	62.5%	115	45.40%
36-45	7	71.4%	21	95.2%	36	61.1%	13	46.20%	6	66.7%	8	12.5%	11	54.60%	102	58.20%
46-55	2	100%	2	0.0%	19	57.9%	3	0%	6	50%	5	20%	-	-	37	38.0%
755	5	80%	3	100%	2	100%	9	44.4%	1	100%	32	21.5%	7	57.1%	59	71.90%
Total	83	36.10%	183	35%	172	36%	112	31.30%	129	39.5%	62	42.0%	41	42.0%	781	34.7%

No. = Number of people skin snipped

% = Percentage of people infected

TABLE 2: Prevalence rates of Onchocerca volvulus infection in seven villages of Kaduna State: Male population

Age Group	Iri		Kurmin Idon		Doka		Makyali		Iburu		Akupal		Kurmin Biri		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
∠1	1	100%	-	-	-	-	-	-	-	-	-	-	-	-	1	100%
1 - 5	19	21%	17	11.8%	30	16.70%	11	36.4%	-	-	1	0%	1	0%	79	14.4%
6 -15	36	39%	58	20.7%	45	15.60%	71	29.6%	78	47.4%	8	12.5%	8	25%	304	27.10%
16-25	10	40%	18	72.2%	30	23.30%	6	66.7%	21	28.6%	4	0%	5	0%	94	33.0%
26-35	9	55.60%	15	66.7%	22	63.60%	10	40%	12	25%	5	0%	10	20%	83	38.70%
36-45	5	60.0%	18	72.2%	40	82.50%	5	40%	13	46.10%	4	0%	8	25%	93	46.60%
46-55	3	100%	7	11.80%	25	84.0%	5	60%	5	60%	4	25%	8	25%	57	52.20%
7 55	7	100%	1	100%	8	100%	5	60%	3	100%	13	30.8%	2	0%	39	70.10%
Total	90	45.6%	134	35%	200	48%	113	35.4%	132	43.20%	39	15.4%	42	19.1%	750	38.90%

No. = Number of people skin snipped

% = Percentage of people infected.

Of the 4 villages sampled in Niger State, only two (Kwakuti and Bari) had an appreciable number of cases of Onchocerciasis; both villages are in Chanchaga local government area (see Table 3). The prevalence varied from 12%-75% in males and 2.2%-100% in female samples, the overall prevalence in males (32.4%) being greater than in females (11.80%). There was tendency for infestation to increase with age.

The other two localities in Niger State, Kaliko village and Zungeru town gave different results. At Kaliko village 85 individuals were skin snipped (age 5 - 63 years) and only 4 (4.7%) of the sample) were infected (all were adults, over 25 years). At Zungeru, 145 persons were skin snipped at a Primary School. Of this sample 3 teachers and 4 pupils were infected.

Table 4 shows that skin manifestations were the commonest symptom of infection in all the villages. Blindness which is the most serious effect of Onchocerciasis was also found to occur in appreciable percentage of the infected population. No case of blindness was found in the population sampled at Akupal, Bari, Kaliko and in pupils at Zungeru Primary school. Depigmentation occured invariably on the legs. There were only 3 villages in which no cases of depigmentation was found (Bari, Kaliko, Zungeru) (Table 4). Although Onchocercomata is known to be a main feature of onchocerciasis in the forest area, cases of up to 10.5% in some of the villages were encountered. The nodules were found around the

TABLE 3: Prevalence rates of Onchocerca volvulus infection in two villages in Nigeri State

Age Group	MALES						FEMALES					
	Kwakuti		Bari		Total		Kwakuti		Bari		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
∠1	-	-	-	-	-	-	-	-	-	-	-	-
1- 5	7	28.60%	5	40%	12	34.30%	45	2.20%	-	-	45	2.20%
6 -15	155	23.90%	25	12%	180	17.9%	130	2.30%	21	9.5%	151	5.90%
16-25	19	63.20%	10	30%	29	46.6%	63	22.20%	9	0%	72	11.10%
26-35	40	35%	7	57.1%	47	46.1%	31	6.5%	13	7.7%	44	7.10%
36-45	12	75%	8	50%	20	62.5%	11	90.90%	8	12.5%	19	51.70%
46-55	12	41.7%	5	40%	17	40.8%	10	80%	1	100%	11	90%
755	19	73.7%	-	-	19	73.7%	3	100%	-	-	3	100%
Total	264	35.20%	60	30%	324	32.70%	293	14%	52	9.62%	345	11.80%

No. = Number of people skin snipped

% = Perceentage of people infected.

TABLE 4: COMPARISON OF CLINICAL PATTERNS OF CCHOCERCIASIS IN THE AFFECTED VILLAGES

Villages	No. of People Sample	No. of People Infected	Clinical Manifestations		
			Blindness	Skin Manifestation	Nodules
Iri	173	71	10(14.0%)	43(60.6%)	6(8.4%)
Kurmin Idon	316	117	6(5.1%)	72(61.5%)	8(6.8%)
Doka	372	158	19(12.0%)	105(66.5%)	10(6.0%)
Makyali	225	75	6(8.0%)	30(40.0%)	2(2.7%)
Iburu	261	110	8(7.3%)	72(65.0%)	3(2.7%)
Akupal	101	15	Nil	3(20%)	Nil
Kurmin Biri	83	28	3(10.7%)	10(35.7%)	1(3.6%)
Bari	113	23	Nil	8(34.7%)	Nil
Kwakuti	557	134	10(7.5%)	65(48.5%)	14(10%)
Kaliko	85	4	Nil	4(100%)	Nil
Zungeru	145	7	Nil	2(28.5%)	Nil

TABLE 5: Total number of people sampled, percentage prevalence and Projected Prevalence of Onchocerca volvulus infection

Village	No. of people Sampled	No. of people Infected	% Prevalence	Projected Population	Estimated people Infected in the Projected population
Iri	173	71	41.0%	2,949	1,209
Kurmin Idon	316	117	35.3%	2,595	917
Doka	372	158	42.0%	3,697	1,553
Makyali	225	75	33.3%	NA	NA
Iburu	261	110	42.1%	1,285	541
Akupal	101	15	14.9%	NA	NA
Kurmin Biri	83	28	33.9%	NA	NA
Total for Kachia LGA	1,531	574	37.4%	549,824	205,634
Bari	113	23	20.4%	NA	NA
Kwakuti	557	134	24.1%	NA	NA
Total for Chanchaga LGA	670	157	22.2%	517,119	114,800

NA = Not available. The projected populationsof villages in Kachia local government area are those of 1988 obtained from the Ministry of Economic Planning, Statistic Division, Kaduna. The projected population for Chanchaga local government area is that of 1984 obtained from Niger State Brochure: Niger State. The land of untapped resources. February, 14th-21st, 1987.

TABLE 6: BLINDNESS RATE IN THE INFECTED POPULATION AND IN THE PROJECTED POPULATION

Village	No. of Persons Infected	% Blind	Projected Number Infected	Projected Number Blind
KACHIA LGA:				
Iri	71	14%	1,209	169
Kurmin Idon	117	5.1%	917	47
Doka	158	12%	1,553	186
Makyali	75	8%	NA	NA
Iburu	110	7.3%	541	40
Akupal	15	Nil	NA	Nil
Kurmin Biri	28	10.7%	NA	NA
Total	574	8.2%	205,634	16,862
CHANCHAGA LGA:				
Bari	23	Nil	NA	NA
Kwakuti	134	7.5%	NA	NA
Total	157	3.80%	114,800	4,305

NA = Not available

waist region. An estimation based on total population of Kachia and Chanchaga local government areas shows that 205,634 people are probably infected in Kachia local government area and 114,800 people are probably infected in the Chanchaga local government area (Table 5). It is also estimated that 16,862 individuals in Kachia local government area and 4,305 individuals in Chanchaga local government area are probably blind due to onchocerciaisis.

4.2 HISTOCHEMISTRY

Acid phosphatase was used as a marker to identify possible physiological strains of Onchocerca volvulus microfilariae from skin snips from the following areas: Iri, Makyali, Ibura, Akupal, Kurmin Biri, (Kachia local government area of Kaduna State). A total of nine different staining patterns were observed which are designated types I - VI, types IX, and two new types: XIV and XV.

From Table 7 it can be seen that a higher number of individuals harbour the type IV microfilariae in the savanna villages. 102 individuals out of 119 were found to habour type IV microfilariae in their body. This was followed by type V which was found in 53 persons. Type I was found in 39 persons while types II and III were found in 9 and 11 individuals respectively. Very few individuals had types IV, IX, XIV and XV (Table 7). Bari village was the only savanna area in which type V was higher than any other type.

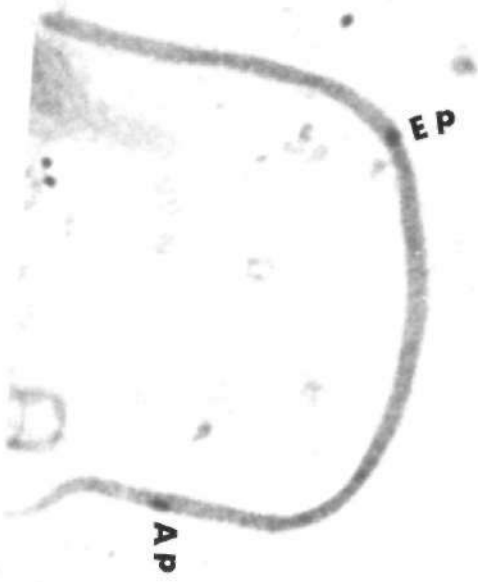
Plate 1a: Microfilaria of Onchocerca volvulus with acid phosphatase activity at Anal pore and Excretory pore (Type I).

Plate 1b: Microfilaria of O.volvulus with acid phosphatase activity at Anal pore, excretory pore and innenkôper (Type II)

AP = Anal Pore

EP = Excretory pore

IB = Innenkoper



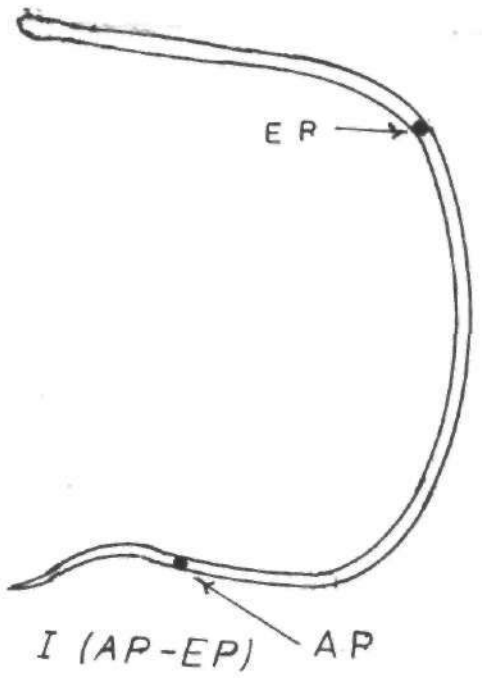


Plate 1a

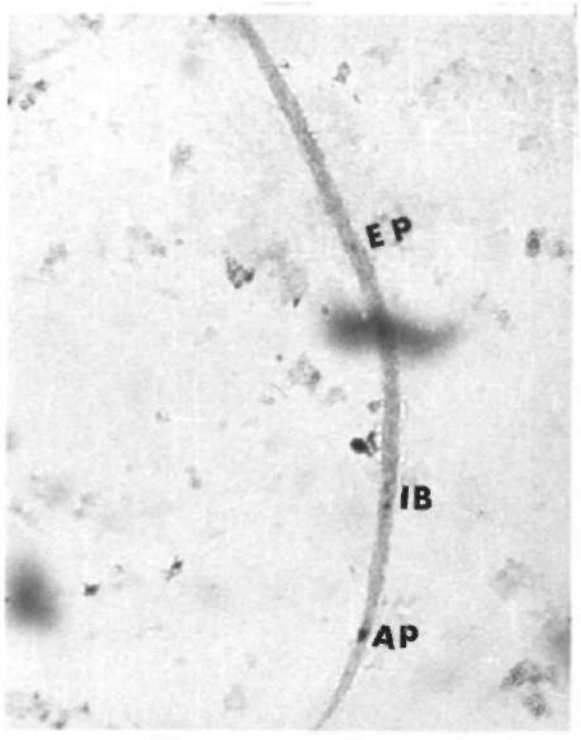


Plate 1b

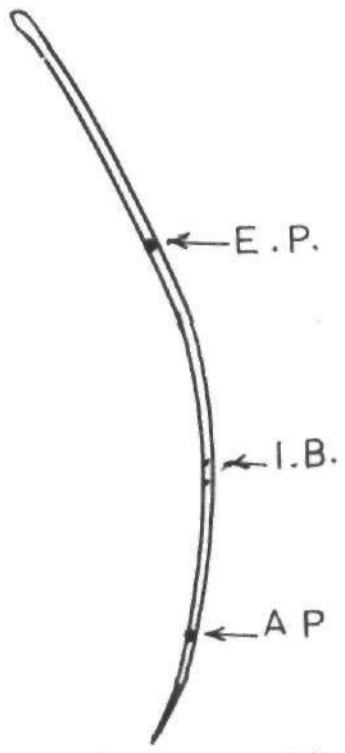


Plate 2a: Microfilaria of O.volvulus with acid phosphatase activity at Anal pore and innenkörper (Type III)

Plate 2b: Microfilaria of O.volvulus with acid phosphatase activity at Anal pore only (Type IV)

AP = Anal pore

IB = Innenkörper

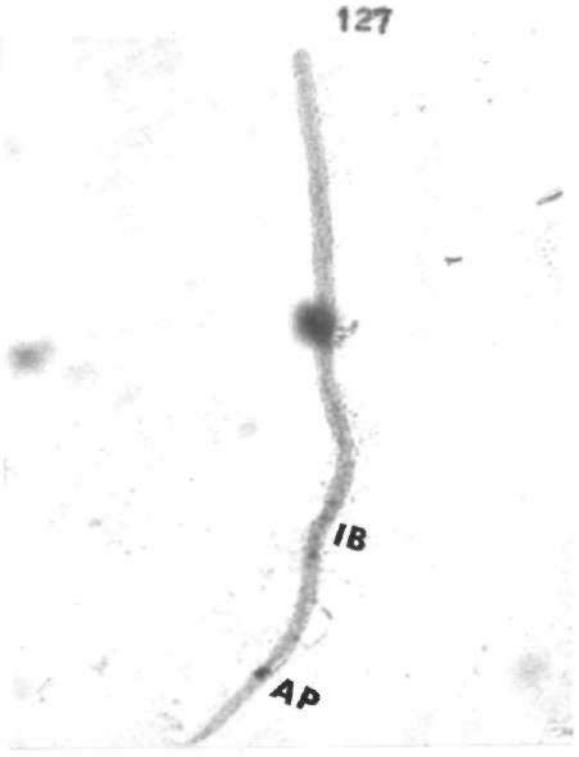
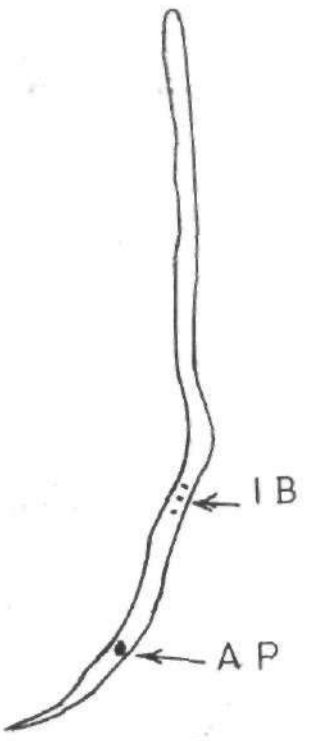


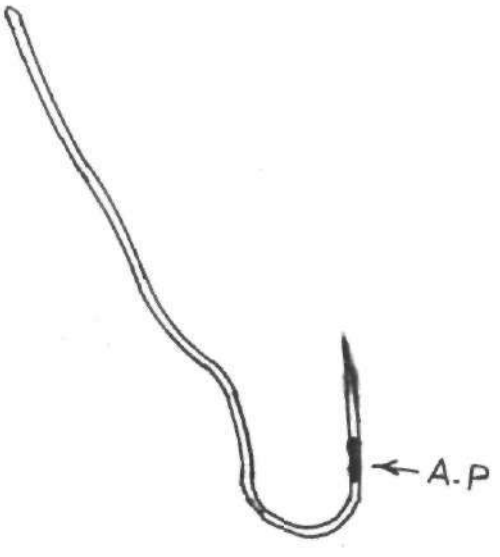
Plate 2a



III (AP-IB)



Plate 2b



IV (AP)

Plate 3a: Microfilaria of O.volvulus with no enzyme activity (Type V)

Plate 3b: Microfilaria of O.volvulus with acid phosphatase activity at the innenköper only (Type XIV)

IB = Innenköper

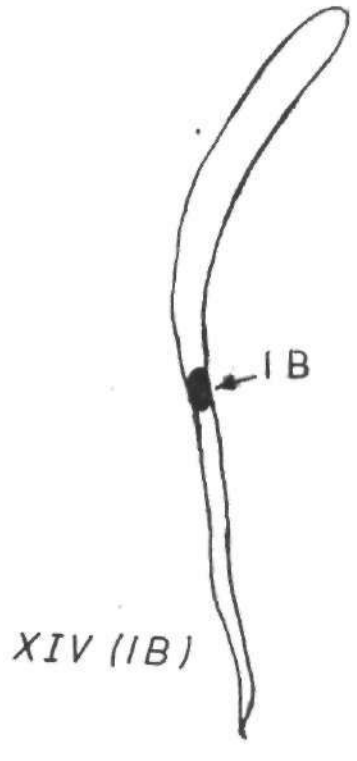




V (Negative)

Plate 3a





XIV (IB)

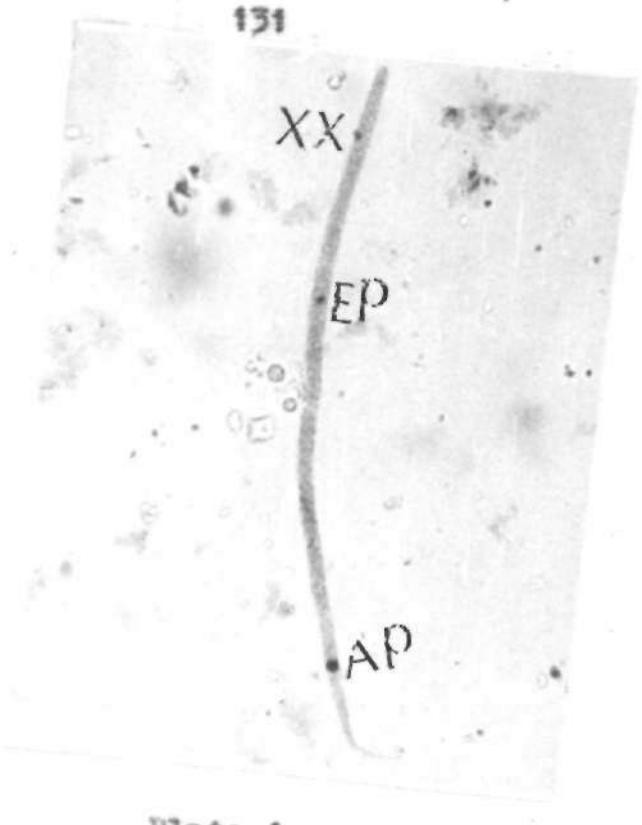
Plate 3b

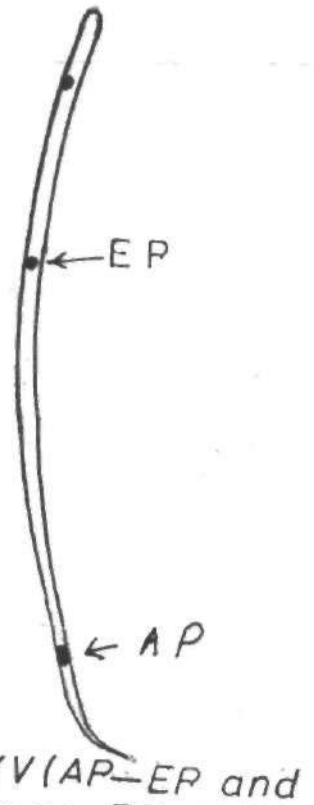
Microfilaria of O.volvulus showing acid phosphotase activity at the anal pore, excretory pore and in the region between the excretory pore and the amphids (Type XV).

AP = Anal Pore

EP = Excretory pore

XX = Between excretory pore and amphids.





XV (AP—EP and between EP-AMP)

Plate 4

TABLE 7: FREQUENCY OF ACID PHOSPHATASE STAINING PATTERNS AMONG PEOPLE EXAMINED

Village/Town	No. of People Examined	Frequency of Staining Patterns								
		I	II	III	IV	V	VI	IX	XIV	XV
Savanna Area										
Iri	10	3	3	4	10	2	0	0	1	0
Makyali (adults)	14	4	2	1	14	11	0	1	0	0
Iburu	8	2	0	0	7	2	0	0	0	0
Akupal	11	6	0	1	10	3	0	0	0	0
Kurmin Biri	18	10	2	2	18	1	0	0	0	0
Bari	17	2	0	1	8	17	0	1	1	0
Kwakuti	34	9	2	2	29	16	1	1	2	1
Total	112	36	9	9	96	96	52	1	3	4	1

TABLE 8: TYPES OF COMBINATION AND FREQUENCY OF ACID PHOSPHATASE STAINING PATTERNS

No. of Combinations	Types of Combinations	Frequency
1	I II III IV V	5
2	I II III V	2
3	II III IV	1
4	I II IV V XIV	1
5	III IV	2
6	IV III IV	3
7	II IV	1
8	II IV V	1
9	IV V	18
10	I IV V	3
11	V IX	1
12	II IV	18
13	I III IV V	2
14	I II IV V	1
15	I IX	1
16	I II IV	2
17	I V	1
18	III IV V XIV	1
19	I II III IV	1
20	IV V IX	1
21	I IV XV	1
22	III IV IX	1
23	I II IV IX	1
24	I III IV IX XIV	1
25	IV ALONE	27
26	V ALONE	10
27	I ALONE	4
28	IX ALONE	1
	Total	112

A comparison of acid-phosphatase staining patterns in microfilariae in adults and children of Makyali village of Kaduna State (Kachia local government area). Type IV accounted for 73.6% of microfilariae from adults and 75.8% of microfilariae from children. Type I accounted for 6.4% and 18.2% of microfilariae from adults and children respectively.

While type II was found in 3.6% of the microfilariae from adults, this type was not found in children. Type III accounted for 1.8% of microfilariae from adults but there was none in the case of microfilariae from children. Type V was found in 13.6% and 6.1% of microfilariae from adults and children respectively. Type IX was found in 0.9% microfilariae from adults but none in the case of children.

Table 9 gives the percentage occurence of the various staining patterns of microfilariae from 7 savanna villages. Six out of the seven savanna villages had type IV as the most common type. The average for the 7 villages shows that type IV accounts for 72.2% of total microfilariae followed by types V (11.4%), I(10.2%) III (2.5%) and II (2.3%).

TABLE 9: Total number of persons and microfilariae examined and the percentage distribution of various staining patterns of microfilariae in 8 areas

Town/ Village	No. of Persons Examined	No. of Microfilariae	STAINING PATTERNS								
			I	II	III	IV	V	VI	IX	XIV	XV
SAVANNA Area:											
Iri	10	550	40(7.3%)	40(7.3%)	50(9.1%)	360(65.5%)	50(9.1%)	0	0	10(1.8%)	0
Makyali	14	1100	70(6.4%)	40(3.6%)	20(1.8%)	810(73.6%)	150(13.6%		10(0.9%)		
Iburu	8	450	20(4.4%)	0	0	400(88.9%)	30(6.7%)				
Akupal	11	710	210(28.4%)	20(1.3%)	20(1.3%	450(63.4%)	10(0.7)				
Kurmin Biri	18	1520	200(13.2%)	20(1.3%)	20(1.3%	1270(83.6%)	10(0.7%)				
Bari	17	170	3(1.8%)	0	3(1.8%)	23(13.5%)	138(81.2%	1(0.6%	1(0.59%)	1(0.56%)	
Kwakuti	34	1720	90(5.2%)	20(1.2%)	40(2.3%)	1180(68.6%)	320(18.6%)		10(0.58%)	20(1.6%)	20(1.1%)
Total	112	6220	633(10.2%)	140(2.3%)	153(2.5%)	4493(72.2%)	708(11.4%	-	21(0.3%)	31(0.5%)	20(0.3%)

Twenty four combinations of acid phosphatase staining patterns were observed (Table 8) in 112 sampled individuals. The combinations IV and V, and I and IV had the highest frequencies (18 each). The new staining patterns (XIV and XV) only occured in combination where type IV also occured. Types IV, V, I, and IX also occured alone in the following respective frequencies: 27, 10, 4 and 1.

The length of the microfilariae ranged from 210.6um - 272.3 um. The excretory pore occured at a distance of 55.3um - 93.6 um from the apex. The position of the anal pore was 170.2um - 234um from the apex. One of the new types (type XIV) was found to be extremely smaller than the other microfilariae. It's total length was 85.3um with the innenkoper situated 44.1um from the apex.

4.3 FIELD OBSERVATIONS

a. Flies:

Numerous breeding sites of Simulium damnosum s.l. were noticed in the River Iri. The larvae were mostly found on leaves of weeds growing in the river. At a point in the river very close to the houses of the people of Iri village, was an embankment built by the Nigerian Railway Corporation, to trap water for locomotive engines. Larvae of S. damnosum s.l. were found growing abundantly on the embankment. Partly dressed children were seen very often at this point swimming and catching fish. Some man-

biting Simulium damnosum s.l. were caught between the hours of 8.00 a.m. and 2.00 p.m., besides the embankment. When dissected, some of the flies contained microfilariae in their blood meals and others had developing larave in the head.

During a visit to River Kaduna during one of the surveys, up to 25 Simulium flies were caught between 5.00 p.m. and 6.00 p.m. They were much bigger and more vociferous than those seen at River Iri.

b. Human activities:

Both the men and women were seen on their farms. Even children less than one year old were carried to the farms. In most cases the women had their legs and their shoulder regions exposed. The men and the younger children wore in most cases pants or short trousers without any from of clothing for the trunk. Most of the people in the villages of Kachia local government area were noticed to be taking their bath in the streams and rivers.

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

DISCUSSION

This study reveals foci of Onchocerciasis, and prevalence rates as well as acid-phosphatase staining patterns of O.volvulus microfilariae. The disease is reported for the first time from Iburu, Makyali, Kurmin Idon, Kurmin Biri, Akupal, Bari and Kaliko villages (see Fig. 1). In addition, although Budden (1956) found the disease to be present in Doka, Iri and Kwakuti villages (Fig. 1), this is the first time that prevalence rates have been given for these villages. In general, Onchocerciasis is found to be mesoendemic in Iri, Iburu and Doka villages of Kachia local government. The disease is hypoendemic in the remaining villages of Kachia local government (Tables 1 and 2) (Kurmin Idon, Makyali, Kurmin Biri and Akupal), and it is also hypoendemic for the four villages of Niger State (Table 3) (Bari, Kwakuti, Kaliko and Zungeru). The prevalence rates (Table 1, 2 and 3) of the infection are comparable with that of Wyatt (1971) who recorded 39.5% prevalence rate in the Ibarapa area of Western Nigeria. The prevalence rates of Onchocerciasis in the villages surveyed are low however, compared with those recorded by Gemade and Dipeolu (1983) in Benue State, where prevalence ranged from 61% - 71%.

The prevalence rates in the villages investigated, were also comparable with that of Galadima (1982) who reported 42% infection rate at Bonu village of Niger State, but they are higher than that of Dalle village of

Kaduna State (9.4%) reported by Nock (1985). It should be borne in mind that while most other investigators based their data on the adult population, who are more likely to be infected with the parasite Onchocerca volvulus, this study took into account both adults and children. As can be seen in Tables 1, 2, and 3, infection was found to start early in life. Children below primary school age had a high prevalence of infection in some areas such as Iri, Makyali and Doka villages of Kachia local government. This finding is of serious medical concern because microfilariae will have a better opportunity of reaching the eyes as the children grow older (Nelson, 1970). Although some of the children complained of visual impairment, it was not possible in this study to find out whether such apparent defects in vision were due to Onchocerciasis or to other factors. On one occasion at Iri village (Table 2) infection was found in a male child under one year old. Infection in children less than one year old has been reported by Brinkman et al. (1976). As can be seen in Tables 1 and 2, infection generally increased with age. This observation has been made by other investigators (Bradley, 1976; Galadima, 1982; Gemade and Dipeolu, 1983). Although it is difficult, from the results of this study to state exactly the peak of infection in terms of age group, it does seem that a stable peak is reached in the 36 - 45 age group. No decline in prevalence is noted in age groups beyond the 36 - 45 age group. The fewer people examined in the age groups of 46 years and above may be responsible

for the 100% prevalence noted in some areas (Tables 1, 2).

The social activities of the people in the areas investigated as noted under field observations, are contributing factors for the high prevalence rate of Onchocerciasis in the area. These activities which include farming, hunting, palm wine tapping, fishing and wood cutting, lead to better man-fly contact and the attendant higher risk of infection. The prevalence rate (38.9%) in the female population of Kachia local government area is close to that of the males (47.8%). It is generally agreed that the males are usually more infected than the females (Bradley, 1976; Budden, 1956, Galadima, 1982). The males by their activities usually stand a higher risk of infection. When compared with the results obtained from the 2 villages of Chanchaga local government area where infection was 11.8% for the females and 32.4% for the males, it would appear that there is a greater difference in prevalence rate between males and females of Chanchaga local government area than in Kachia (see Tables 1, 2, and 3). In Kachia local government area, the Kadara people who occupy the villages investigated have agriculture as their major occupation. Both men and women spend a major part of the day time in the farm and this probably explains the observation that the prevalence rates in the males and females are similar. The Gwari people who occupay the Chanchaga local government area have similar social activities to those of Kadara people, but the Gwari females do not actively participate in farming. The time spent by the Gwari

women in fetching water and firewood or taking food to men in the farm is short, compared to the farming hours of the Kadara women, this can explain the difference in the prevalence rate in females in the two areas.

In Kachia local government area, Doka, Iri and Iburu villages stand out as villages where Onchocerciasis is more prevalent than in others (Tables 1 and 2). Inquiries among the inhabitants of all these villages (with probably Iri and Akupal as exceptions) revealed that the people moved from sites closer to the two rivers (River Iri and River Kaduna) at some time in the past. The degree of proximity of the villages and their farming areas to the rivers, probably plays an important role in the differences, in the prevalence of the disease, between one village and the other (Bradley, 1976). Akupal village has an exceptionally low prevalence of Onchocerciasis. Although this village is nearer to River Kaduna than Kurmin Biri village, O.volvulus infection is lower than that of Kurmin Biri. Due to the inavailability of suitable instruments, the load of microfilariae per skin snip was not determined but casual microscopic examinations showed that the people in Iri, Kurmin Iden, Doka, Iburu and Kurmin Biri are heavily infected. Akupal village however showed a very low level of microfilariae.

In Chanchaga local government area(Niger State) the prevalence of infection at Bari (near Chanchaga, Minna) and its clinical picture (Table 4) resemble that at Akupal village (Kaduna State). The River Chanchaga which

is less than 10km from Bari is therefore likely to be the source from which the vector population transmits the same strain of O.volvulus at Bari and at Akupal villages. The Chanchaga River was found to support the breeding of Simulum damnosum s.l. and cases of O.volvulus infection were reported by Budden (1956) at Chanchaga village. The proximity of Kwakuti village (Niger State) to the Gurara fall (Budden, 1956) which has been known to be a major breeding site for Simulium damnosum s.l., explains the high prevalence of Onchocerciasis in the area, especially in the male population (Table 3).

At Kaliko village (Niger State), Onchocerciasis was found to have very low prevalence (4.7%). This village is about 15km from Zungeru on the Minna - Zungeru road. It is not surprising that a low prevalence is found here. Zungeru has been reported to have only a moderate prevalence of Onchocerciasis (Budden, 1956), but blind people likely to be victims of onchocercal blindness can be seen by the Zungeru bridge across the River Kaduna. Inquiries showed that Kaliko village is a young settlement and the people might have come from a more distant area where transmission is low. At Zungeru town (Niger State), examination of school children and their teachers revealed a very low prevalence (4.8%). With the exception of Akupal and Bari villages, this prevalence of 4.8% is low compared to that found in primary school aged children in the other villages surveyed (see Tables 1,2 and 3). Zungeru which has been known to have a moderate number of cases of Onchocerciasis has witnessed some urbanisation compared to the other study areas which are typically rural. Activities of the school children in an urban area

like Zungeru are not likely to take them to places where they could be greatly exposed to infective bites of Simulium damnosum s.l.

Interesting clinical aspects of Onchocerciasis encountered include blindness, depigmentation, Onchocercomata (nodules) and itching i.e. Onchodermatitis (Table 4). Overall blindness rate ranged from 0 - 14% of the infected people. No cases of blindness were seen at Bari, Kaliko and Zungeru in the population samples. At Iri there were 10 blind people per 173 samples, 6 per 316 at Kurmin Idon, 19 per 372 at Doka, 6 per 225 at Makyali and 8 per 261 at Iburu. Others were as follows: 10 per 557 at Kwakuti and 3 per 83 at Kurmin Biri. These blindness rates are quite high when compared to 5.8% in the Hawal valley (Bradley, 1976) and 5.7% for some hyperendemic areas of Northern Nigeria (Budden, 1956) all in the Savannah Zone. When compared to reports from the forest zone, the blindness rates observed in this study are higher than those reported by Nwokolo (1950) in Enugu, Eastern Nigeria and by Wyatt (1971) at Ibarapa Western Nigeria; each of the above authors found only 1 case of blindness in over 1000 people. As reported by Duke et al (1975), the forest strain of O.volvulus is known to be associated mainly with skin manifestations of Onchocerciasis, while the Savannah strain causes eye problems ranging from visual impairment to total blindness. Duke et al's (1975) observation explains the sharp contrast in the blindness rates in the villages reported in this study and those of the forest areas mentioned above. Similarly the prevalence

of skin manifestations ranged from 0-9.5% and Onchocercomata which ranged from 0-10.5% are lower than those reported by Wyatt (1971) and Edungbola et. al. (1983). Itching was the most common, though not the most important aspect of Onchocerciasis in the villages surveyed. This finding is in agreement with the report by Nelson (1970) that "In many patients the pruritus persists throughout the whole course of the disease, especially in Africa where there is no difficulty in recognising an affected community because the whole population seems to be busy scratching".

Population estimates in Kachia and Chanchaga local governments, based on the 1963 census, suggest that the number of people infected with O.volvulus could be 205, 634 and 114,800 in Kachia and Chanchaga local government areas respectively. Similar deductions suggest that 16,862 and 4,305 persons are victims of Onchocercal blindness in Kachia and Chanchaga local government areas respectively (Tables 4 and 5). Projections based on sample populations are however likely to give underestimated figures because some areas with high prevalence rates (often exceeding 70%) are sometimes not sampled during field surveys (W.H.O., 1987). It is therefore likely that there are more infected people in the two areas than are suggested.

Acid phosphatase staining patterns of microfilariae from savanna areas (Tables 7 - 9) show that there is variation or polymorphism, in terms of staining, in the microfilaria population. This polymorphism seems to

exist among microfilariae progeny of a single female worm or of different female worms (Omar, 1978). In the present study a total of nine staining patterns were found in the microfilaria population (Tables 7 -9 and plates 1 - 4). These staining patterns include types I - V already reported (Omar, 1978, Omar et al. 1982) and types VI - XIII reported by Ufomodu et al. (1986) from the Jos Plateau of Nigeria.

Although the findings on prevalence rates are preliminary, they will nevertheless provide additional information in areas where onchocerciasis exist. Similarly, any new strain of O.vulvulus encountered can help in explaining any aberant manifestation of Onchocerciasis that may be encountered in the study area.

Reported for the first time in this work are microfilariae distinguished by two staining patterns namely type XIV with staining at the inner body alone, and type XV with staining at the anal pore, excretory pore and the region between the excretory pore and the amphids. While Omar (1978) and Omar et al. (1982) consistently reported type IV (Staining at the anal pore only) to be the more frequent type in the savanna, and type V as the more frequent type in the forest area, Ufomadu et al. (1986) contrastingly showed that type V was more frequent in the savanna villages of the Jos Plateau than the expected type IV. Type V had the highest frequency distribution of 28.1% followed by type IV with 18.8%.

In the present study findings in Kachia and Chanchaga local government areas gave staining patterns very similar to those of Omar (1978) and Omar et al. (1982) but differing considerably from the report by Ufomadu et al. (1986). Of the nine different staining patterns found in specimens obtained from the savanna villages of Kachia and Chanchaga, type IV was clearly found to be the more frequent, with a frequency ranging from 13.5% to 88.9%, the only exception being Bari village (Niger State) where type V was more frequent (81.2%) and type IV was much less common (13.5%). This Bari village result was similar to that of forest areas (Omar 1978), where type V staining was more frequent (72.1%) than type IV (14.0%).

In a comparison between children and adults, conducted at Makyali village and considering only a limited number of specimens, it was found that there was no striking contrast between the staining types in the two groups. However, type V was higher in adults (13.6%) than children (6.1%), but type I was higher in children (18.2%) than adults (6.4%). Types II, III and IX were not detected in children.

The similarity in the clinical pictures of Onchocerciasis between Bari village of Niger State and that of Akupal in Kaduna State (Table 4) is not supported by the acid phosphatase staining patterns of microfilariae from these two villages (Table 9). Type IV is shown to be more frequent at Akupal while type V is predominant at Bari village. The presence of the more frequent forest

strain (type V) in this savanna village is supported by the work of Ufomadu et. al. (1986) which showed type V to be more frequent in the Jos area, and by the work of Nock (1985) which showed that there are some areas of Onchocerciasis in the savanna zone with patterns like that of the forest area. Pockets of forest S.damnosum s.l. species within savanna zone suggest that forest type strains of O.volvulus can be found within savanna. This seems to be the case in the previous (Ufomadu et al. 1986) and present study.

Another interesting observation is the presence of the typical savanna strain at Akupal, where the clinical picture of Onchocerciasis is so different from those of other savanna villages (Table 4). Perhaps the low level of microfilariae in the skin of the infected people in this particular village is a contributing factor to this observation. The level of microfilariae in the skin has been used as an indication of severity of infection (Gibson et. al., 1980, Buttner et. al. 1982, W.H.O. 1987). It is suggested that with the presence of type IV in Akupal, if the transmission potential is increased, with subsequent increase in the level of skin microfilariae, the clinical manifestations at this village can become more typical of the savanna zone in which blindness and visual impairment are the most common features of Onchocerciasis (Duke, 1968, Nelson, 1970; W.H.O., 1984).

The presence of microfilariae with varying staining patterns in individuals is an indication of variation in the parasite; and the presence of differences in the staining patterns of microfilariae between individual hosts, between villages and in different ecological zones, suggests that O.volvulus is a complex (W.H.O.,1987) just as the vector S.damnosum s.l. is a complex (Vajime and Dunbar 1975). In some individuals up to five different staining types were found.

The presence of multiple staining patterns in the same individual, suggests (1) the possibility of multiple infections (2) variations in either the microfilariae population or in the adult worms. It may be noted that it's presence in most of the combinations (Table 8), type IV is of savanna O. volvulus microfilariae. Other staining patterns may either arise from type IV or give rise to it. Hardly any of the samples lacked type IV (19 out of 112).

One question which, when answered, will shed more light on this issue of variation is whether the adult worms have the inherent property of producing microfilariae having such enzyme staining variations or the property is acquired later as a result of ageing or due to some external environmental factors (host factors inclusive).

Omar (1978) stated that acid phosphatase activity first appears at the anal pore in intra uterine microfilariae, followed by activity at the excretory pore. He therefore suggested that type I (AP-EP) staining pattern in the skin represents the microfilariae that arrive early

in the skin, and that type II (AP-IB-EP) and type III (AP-IB) represent transisent populations. If this explanation is acceptable, it could be inferred that types IV and V represent older microfilariae with type V being characteristic of the forest area and type IV being characteristic of the savanna area. The other staining types should therefore be regarded as transient phases in the life span of the microfilariae. The reasons for this assumption are that none of these other staining types has been found to be exclusively in an individual; in addition where these other types occur together, they are in very low numbers. For example type VI was found in only one microfilaria and type XV in only 20 microfilariae. If the ageing theory of Omar is accepted it can be stated that the microfilariae produced by the forest strain of O.volvulus s.l. get older more rapidly than the savanna strain or, on the other hand, that the enzyme activity at the anal pore of the savanna strain is more stable than that of the forest area.

Microfilariae from children did not reveal large numbers with type I (AP-EP) staining pattern which according to Omar (1978) represents the type of microfilariae arriving early in the skin. Omar's explanation however seems plausible from the results obtained in this work in which type I was the third, in terms of frequency after types IV and V. It can therefore be postulated that the ageing process takes place very rapidly, perhaps as soon as the microfilariae are released by the worms. Perhaps in vitro culture of the adult

worms will shed more light on the process of variation, based on enzyme activity in the microfilaria population. If gravid female worms are raised in the laboratory, a continuous study can be made and this will provide more information. A further study by chromatography, after separating the various staining types, of their antigenic composition and immunological reactions would definitely provide a greater understanding of the relationship between strains and pathogenicity. In future, it might be possible to ascribe particular clinical entities to particular staining types. As of now, however, it is only possible to say that type IV is the most common type of microfilaria in the savanna area and it may be responsible for the typical picture of Onchocerciasis in the savanna, while type V which is the most common type in the forest area is responsible for the type of Onchocerciasis in the forest. The results from Bari village (a savanna village) in which type V characteristic of the forest strain (and the findings of Ufomadu et al. (1986) in which type V was the most common in the Jos area) should stimulate further study. This should include specimens from areas which are transitional between the savanna and the forest. Correlation between clinical picture, level of parasite in the skin, acid phosphatase staining patterns and the species of Simulium damnosum s.l. in any given area would greatly enhance our understanding of the relationship between parasite strain and epidemiology of Onchocerciasis. The staining pattern of the microfilariae in a given area may influence the transmission of Onchocerciasis by various species of the Simulium damnosum complex.

Apart from the relationship between staining type and Onchocerciasis, acid phosphatase staining pattern can also be of great use in tracing the source of reinfection or reinvasion in Onchocerciasis control areas.

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APPENDIX 1

Number of people sampled and number found infected with O. volvulus in villages in Kaduna and Niger States.

IRI VILLAGE	MALES		FEMALES	
Age group (Yrs)	No. Sampled	No. Infected	No. Sampled	No. Infected
1	1	1	-	-
1-5	19	4(12%)	8	2(25%)
6-16	36	14(39%)	36	7(19%)
16-25	10	4(40%)	8	3(37.5%)
26-35	9	5(55.6%)	16	7(43.8%)
36-45	5	3(60%)	7	5(71.4%)
46-55	3	3(100%)	2	2(100%)
55	7	7(100%)	5	4(80%)
Total	90	41(45.6%)	83	30(36.1%)
KURMIN IDON				
1	0		0	-
1-5	17	2(11.76%)	8	0
6-15	58	12(20.7%)	41	6(14.6%)
16-25	18	13(72.2%)	69	11(15.9%)
26-35	15	10(66.7%)	38	24(63.2%)
36-45	18	13(72.22%)	21	20(95.2%)
46-55	7	2(11.8%)	2	0(0%)
55	1	1(100%)	3	3(100%)
Total	133	53(39.9%)	183	64(35.0%)

Appendix 1 Contd:

IBURU VILLAGE	MALES		FEMALES	
Age group (Yrs)	No. Sampled	No. Infected	No. Sampled	No. Infected
1	-	-	-	-
1 - 5	-	-	-	-
6 - 15	78	37(47.4%)	87	30(34.5%)
16 - 25	21	6(28.6%)	19	9(47.4%)
26 - 35	12	3(25%)	10	4(40%)
36 - 45	13	6(46.2%)	6	4(66.7%)
46 - 55	5	3(60%)	6	3(50%)
55	3	3(100%)	1	1(100%)
Total	132	58(43.2%)	129	51(39.5%)
AKUPAL VILLAGE				
1	-	-	-	-
1 - 5	1	0	-	-
6 - 15	8	1(12.5%)	7	0(0%)
16 - 25	4	0(0%)	7	0(0%)
26 - 35	5	0(0%)	3	0(0%)
36 - 45	4	0(0%)	8	1(12.5%)
45 - 55	4	1(25%)	5	1(20%)
55	13	4(30.8%)	32	7(21.9%)
Total	39	6(15.4%)	62	9(14.5%)

Appendix 1 Contd:

DOKA VILLAGE	MALES		FEMALES	
Age group (Yrs)	No. Sampled	No. Infected	No. Sampled	No. Infected
1	0	5(0	0	0
1 - 5	30	5(16.7%)	22	(9.1%)
6 - 15	45	7(15.6%)	34	4(11.8%)
16 - 25	30	7(23.3%)	31	7(22.6%)
26 - 35	22	14(63.6%)	28	14(50%)
36 - 45	40	33(82.5%)	36	22(61.1%)
46 - 55	25	21(84%)	19	11(57.9%)
55	8	8(100%)	2	2(100%)
Total	200	96(48%)	172	62(36%)
MAKYALI VILLAGE				
1	0	0	0	0
1 - 5	11	4(36.6%)	20	5(25%)
6 - 15	71	21(29.8%)	41	8(19.5%)
16 - 25	6	4(66.7%)	14	5(35.7%)
26 - 35	10	4(40%)	12	7(58.3%)
36 - 45	5	2(40%)	13	6(46.2%)
46 - 55	5	2(40%)	3	0(0%)
55	5	3(60%)	9	4(44.44%)
Total	113	40(35.4%)	112	35(31.3%)

Appendix 1 Contd:

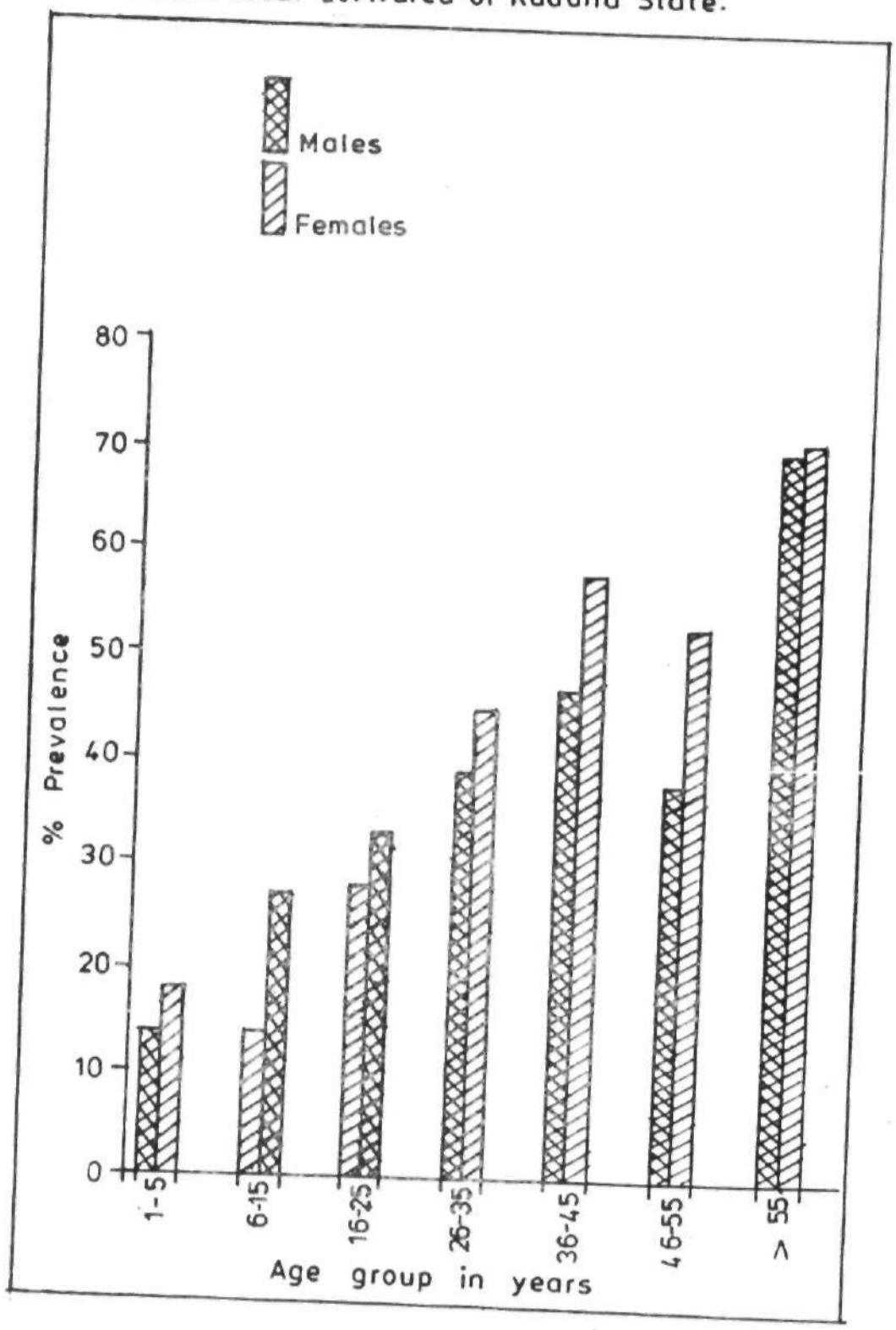
KURMIN BIRI	MALES		FEMALES	
Age group (Yrs)	No. Sampled	No. Infected	No. Sampled	No. Infected
1	-	-	-	-
1-5	1	0(0%)	-	
6-15	8	2(25%)	1	0(0%)
16-25	5	0(0%)	14	5(35.7%)
26-35	10	2(20%)	8	5(62.5%)
36-45	8	2(25%)	11	6(54.6%)
46-55	8	2(25%)	-	-
55	2	0(0%)	7	4(57.1%)
Total	42	8(19.1%	41	20(48.8%)
BARI VILLAGE				
1	-	-	-	-
1-5	5	2(40%)	-	-
6-15	25	3(12%)	21	2(9.5%)
16-25	10	3(30%)	9	0.(0%)
26-35	7	4(57.1%)	13	1(7.7%)
36-45	8	4(50%)	8	1(12.5%)
46-55	5	2(40%)	1	1(100%)
55	-	-	-	-
Total	61	18(29.5%)	52	5(9.6%)

Appendix 1 Contd:

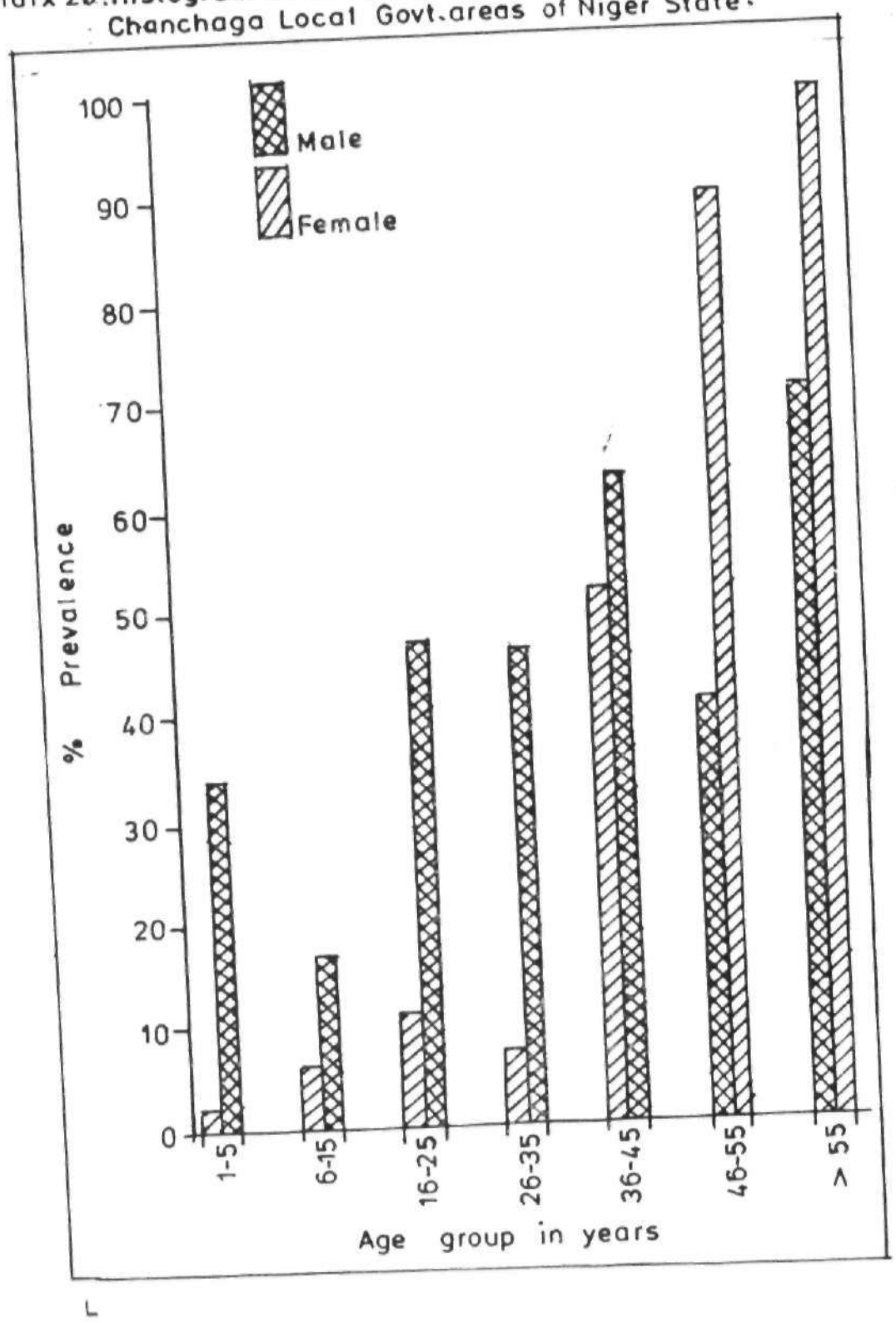
KWAKUTI VILLAGE:

Age group (Yrs)	MALES		FEMALES	
	No. Sampled	No. Infected	No. Sampled	No. Infected
1	0	0	0	0
1-5	7	2(28.6%)	45	1(2.2%)
6-15	155	37(23.9%)	130	3(2.3%)
16-25	19	12(63.2%)	63	14(22.2%)
26-35	40	14(35%)	31	2(6.5%)
36-45	12	9(75%)	11	10(90.9%)
46-55	12	5(41.7%)	10	8(80%)
55	19	14(73.7%)	3	3(100%)
Total	264	93(35.2%)	293	41(14%)

Appendix 2a : Histogram of % Prevalance of onchocerciasis in Kachia Local Govt. area of Kaduna State.



Appendix 2b: Histogram of % Prevalance of onchocerciasis in Chanchaga Local Govt. areas of Niger State.



Appendix 2c: Histogram of % Prevalance of onchocerciasis in Kachia and Chanchaga Local Govt areas of Kaduna State and Niger State.

