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SERUM IMMUNOGLOBULIN LEVELS IN FIVE VILLAGES OF THE REPUBLIC OF CHAD AND IN ONCHOCERCIASIS PATIENTS WITH AND WITHOUT MICROFILARURIA[1]

by

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1. INTRODUCTION

Buck et al. (1969, 1971) reported the recovery of microfilariae of Onchocerca volvulus in the urine of between 11 to 34% of the residents of Ouli Bangala, a village in southern Chad where onchocerciasis was hyperendemic. The following differences were found between patients with onchocercal microfilaruria and residents with onchocerciasis but without the urinary manifestations. In the microfilaruria group infection intensity was heavier; onchocercomata were more frequent; significantly more persons had inguinal lymphadenopathy; their weight/height ratios and antibody titres in haemagglutination tests with O. volvulus antigen were lower; levels of serum glutamic-oxalacetic transaminase were increased; the degree of lymphocytosis in differential counts of leucocytes was less pronounced; and their permanent residences were located at greater proximity to the breeding sites of Simulium damnosum, the only vector of onchocerciasis in the area. Among the many endemic diseases and infections were malaria, schistosomiasis mansoni and haematobium, loiasis, infections with Dipetalonema perstans and Wuchereria bancrofti. Of these, two were statistically associated with onchocercal microfilaruria, malaria parasitaemia in adults and frequency of infections with Loa loa. In exploratory examinations of immunoglobulin levels in serum samples kept frozen at -80°C, it was noted that the residents with the urinary manifestations had excessively high levels of IgG and IgA but a relative deficiency of IgD when compared with control subjects who had onchocerciasis but no microfilaruria. The present report describes the results of comparative studies of immunoglobulins between geographically different villages in the Republic of Chad.

2. MATERIALS AND METHODS

The population samples examined were drawn from five villages in the Republic of Chad, namely, Djimtilo at 12° 50′ N latitude and 14° 30′ E longitude near the mouth of the Chari river at Lake Chad; Ouli Bangala at 7° 50′ N and 15° 50′ E in the southernmost part of eastern Chad; Ouarai at 8° 40′ N and 17° 45′ E in the south-central parts of the country; Boum Khebir at 10° 10′ N and 19° 25′ E at the northern shore of Lake Iro; and Faya-Largeau at 17° 45′ N and 19° 10′ E, an oasis in the Sahara, and the administrative seat for the huge prefecture of Tibesti-Borkou-Ennedi. The predominant disease patterns in these places had many similarities, but also differences. Malaria (falciparum and malariae) was hyperendemic in all communities but the Saharan town; schistosomiasis was found in Djimtilo, Ouli Bangala and Ouarai; infections with Entamoeba histolytica and Giardia lamblia and with some of the non-pathogenic protozoa were detected in all places. Infections with Ascaris lumbricoides and Trichuris trichiura were infrequent in all communities while occasional cases of infections with Taenia saginata were found throughout. Among the observed differences between the places were the frequency of antibodies to tick-borne typhus (maximum in Djimtilo), the occurrence of onchocerciasis (Ouli Bangala), differences in the prevalence of microfilarial infections other than O. volvulus (maximum in Ouarai), hyperendemic yaws in Boum Khebir, and tuberculosis and amoebiasis (maximum in Faya-Largeau). All communities derived their livelihood from subsistence farming with the exception of the Saharan population where many families were engaged in seasonal caravan trade as well as in work on date plantations.

The population samples included in the study are composed of two major parts. The first is based on matching each of the originally found microfilaruria patients in Ouli Bangala (N = 32) with a control partner of the same sex and age in each village. The control set in the village of Ouli Bangala was doubled by matching two subjects for each case. Matching was achieved by the following technique. From the total populations of each village as determined in a house to house census (Buck et al. 1970) control persons for each case of microfilaruria were selected at random from the corresponding sex and 10-year age-group. After selection of the controls, their serum samples (stored at -80°C) were identified and subsamples withdrawn for determination of immunoglobulins.

The second population sample included all persons that were part of the follow-up study in Ouli Bangala conducted exactly three years after the original comprehensive epidemiological investigations in the same village (Buck et al. 1970). Rather than the total village, the second sample included only village residents who lived in the vicinity of the Lim river and whose infections were known to be heavy and clinical manifestations of onchocerciasis severe. Comparisons for differences in immunoglobulin levels were made between the cases of onchocercal microfilaruria and all others with a confirmed diagnosis of onchocerciasis but with negative urine specimens. The methods used for census taking, as well as for the protocols of the physical examinations, laboratory tests and methods of analysis of the data were described in detail in a previous publication (Buck et al. 1970).

IgG, IgA, IgM, and IgD levels were determined by a quantitative immunodiffusion method. The plates used were purchased from a commercial company (Meloy Laboratories, Inc., Springfield, Virginia 22151). Wells in the plates were filled with capillary pipettes. Unknowns and a standard reference serum (in serial twofold dilution) were tested simultaneously. Using the results obtained with the diluted reference serum, a curve was plotted on semi-log graph paper. Ig levels in the unknown sera were calculated from this curve. The incubation times and temperatures are listed below:

Immunoglobulin	Hours	Temperature
IgG	16-18	4°C
IgA	16-18	Room temperature
IgM	24-48	Room temperature
IgD	18-24	Room temperature

The normal ranges for human immunoglobulins for the method are shown below:

Immunoglobulin	mg/ml[a]	mg%	I.U/ml[b]
IgG	7.7-11.3	770-1 130	98-144
IgA	0.8-2.0	80-200	89-169
IgM	0.9-1.7	90-170	109-201
IgD	0-0.3	0-30	0-200

[a] mg/ml based on primary purified protein standards prepared by the commercial company. Range reflects one standard deviation from the mean.

[b] International units based on World Health Organization Research Standards for Human Immunoglobulins. (Rowe et al. 1970).

Tests for IgE were performed in The O'Neill Memorial Research Laboratories, The Good Samaritan Hospital, Baltimore, Maryland by Dr Kimishige Ishizaka. The methods used have been previously described (Johansson, 1967). Normal serum levels are thought to be <1000 I.U./ml.

3. RESULTS

3.1 Comparison of immunoglobulin levels between villages

Levels of immunoglobulins G, M and E in African populations are known to be significantly higher than those found in caucasians of corresponding age and sex (Johansson et al. 1968; Turner & Voller, 1966; McFarlane & Voller, 1966; Rowe et al. 1968; Crane et al. 1971; Cappuccinelli et al. 1971). In contrast, excessive levels of IgA and IgD have been observed only sporadically (Rowe et al. 1968). It has been mentioned that there is a certain danger in making comparisons between these entirely different populations, especially for defining ranges of "normal" immunoglobulin levels using the values from industrialized countries as standards of health (Turner & Voller, 1966). In the present study, comparisons between different villages and segments of the population of Chad were made by the use of a standardized mean index for each individual immunoglobulin class. This index determines the relative deviations from the country mean for individual villages or for patients with certain diseases and infectious states. By combining the standardized indices of different immunoglobulin classes in a single graph they can then be viewed together and evaluated in a fashion similar to the appraisal of differential counts of leukocytes or of serum protein fractions. Standardized mean indices were determined as follows. First, overall means were calculated for each individual immunoglobulin as shown in Table 1. Each of these means was given the relative value of 1.0 as the standard, regardless of the large differences in concentrations that exist between the individual Ig classes. By combining the four immunoglobulins (G, A, M, D) it is possible to draw a symmetrical figure, i.e. a square as shown in Fig. 1. Computation of the ratio: Ig mean of an individual community ÷ overall mean of Ig class, yields weighted and standardized mean indices that can then be plotted on the corresponding axis of the square designated for a particular immunoglobulin.

Perusal of Table 1 shows that the mean levels for IgG and IgM are very high by American standards but those for IgA and IgD fall within the range accepted as normal. It is a truism to make the general statement that the observed elevations are probably due to the abundance of infections by different parasites (Cappuccinelli et al. 1971a, b). The differences between the matched samples from the Chadian villages, as well as those between onchocerciasis patients with and without microfilaruria, are summarized in Fig. 1. On one extreme there is Ouli Bangala with excessively high IgG and IgA and to a lesser degree IgM levels and on the other extreme is Faya Largeau with the lowest levels of these immunoglobulins but with the highest concentration of IgD. Indeed, the immunoglobulin constellation in the latter community where malaria, schistosomiasis and filarial infections are rare resembles the one usually observed in caucasians.

3.2 Comparisons between onchocerciasis patients with and without microfilaruria

The results of the follow-up study for onchocerciasis in Ouli Bangala are summarized in Table 2. There is a tendency for IgG, IgA and IgM levels to increase with age. This trend is not apparent for the other two immunoglobulin classes (IgD and IgE). Statistically significant sex differences with male preponderance were observed for IgG and IgA; IgM levels were higher in females (Rowe et al. 1968). The observed dissimilarities of IgD and IgE were without statistical significance in the sample size examined.

The findings for residents with and without onchocercal microfilaruria are summarized in Table 3 and Fig. 2. As indicated by the graph, the shapes of the quadrangles are similar to those observed in the comparison of the matched samples. In addition to the already discussed regional differences, there is also a striking discrepancy in immunoglobulin concentrations between the residents of Ouli Bangala who had or did not have microfilaruria. Again, the findings are similar in the two studies. They indicate that the occurrence of onchocercal microfilaruria is associated with excessive IgG and IgA levels but also with relatively low concentrations of IgD.

3.3 Infection intensity and immunoglobulin levels

An analysis for association between the levels of the various immunoglobulins revealed a negative correlation ($r = 0.2$) between IgG and IgD for both sexes and in each age-group (Table 4). This is different from the findings of Rowe et al. (1968) who reported that there was no mutual relationship between immunoglobulin levels in Gambian adults. There is evidence that both the mean and the variance of immunoglobulins G and M increase with age (Rowe et al. 1968). High variance might indicate the existence of two subpopulations whose immunoglobulin levels tend to segregate in opposite directions. To obtain clues in support of this assumption, the following analysis was made independently for the males and females in the follow-up study in Ouli Bangala. In this village onchocerciasis has been the dominating endemic disease. Infection intensity was determined by skin snips and found to increase with age (Buck et al. 1969). Individuals in each of three age-groups, i.e. <10, 10-29 and 30+ were divided into two classes according to their microfilaria counts in skin snips. All persons with microfilaria counts exceeding the age-specific mean were classified as "severe" infections and those with lower counts as "light" infections. In both groups of persons the means increased with age but at a different scale. Fig. 3 shows the age-specific mean values for individual immunoglobulin classes separately for the "severe" and "light" infections. The increases of IgA and IgM levels with age were similar in the two groups but were different for IgG and IgD. The IgG means of persons with "light" infections show the typical age pattern while those of the "severe" group started with higher values in children but remained at approximately the same level in older age. The opposite tendency was observed for the IgD concentration. There was again a clear separation of the severe from the light infections with reversed age patterns. Results similar to those shown in Fig. 3 for the males were found also in the females of the population sample.

3.4 Immunoglobulin E and combined infection with *Schistosoma mansoni* and *Onchocerca volvulus*

Extremely high IgE levels are frequently found in rural African populations (Johansson et al. 1968; Cappuccinelli, 1971b). Our follow-up study in the village of Ouli Bangala included analyses for association between elevated IgE concentrations and infections with *S. mansoni* (45% prevalence) and *O. volvulus* (holoendemic), the two most important endemic diseases in the village. Table 5 shows the results in persons with single and combined infections. Because all individuals with eggs of *S. mansoni* also had onchocerciasis, it was not possible to determine IgE levels for single infections with *S. mansoni*. The highest IgE concentrations were observed for combined infections. The findings suggest that of the two endemic infections, schistosomiasis had the greater influence on the IgE levels, but the observed differences are not statistically significant.

A combined test for association between IgE levels, reactivity in immediate-type skin tests with *S. mansoni* antigen and presence or absence of schistosome eggs in stool specimens, was made by Cochran's method (1954). The results are shown in Table 6. The IgE concentration was highest in persons who passed schistosome eggs and had a positive skin test. Interestingly, skin reactivity to schistosome antigen *per se* was not found to be associated with excessive elevation of IgE levels. Significant differences were observed only for the within group comparison of persons with positive skin tests who did or did not excrete schistosome eggs at the time of their examination.

4. DISCUSSIONS

Levels of individual immunoglobulin classes are affected differently and independently by disease processes and infections (McGregor et al. 1970). Therefore, comparisons of immunoglobulin patterns in populations must be concerned with the evaluation of the entire constellation of immunoglobulins. This can be facilitated by computing standardized mean indices for which the standard value of 1.0 can be computed separately for large areas or peoples with basically similar disease patterns and ethnic composition. Plotting of the indices derived

from specific study populations on a diagram outlining the relative "norms" for an entire area will easily depict specific differences in immunoglobulin patterns that may exist.

Among the Chadian villages the residents of Ouli Bangala had the most pronounced deviations of immunoglobulin levels from the country norm. In this village of Laka tribesmen, a variety of abnormal results from immunological tests have been observed. They include widespread anergy to tuberculins (Buck et al. 1969, 1970), lack of detectable HA antibodies to O. volvulus antigen in persons with severe onchocerciasis (Buck et al. 1969, 1971), deficient sensitivity of the complement fixation test for schistosomiasis (Buck & Anderson, 1972) and a low degree of serological responses to yellow fever vaccine (Buck et al. 1970). There is no satisfactory explanation for the different age patterns of IgG and IgD in persons with severe and mild onchocerciasis (Fig. 3). The severe group had lower weight/height ratios, a higher proportion of persons with elevated SGOT levels, higher microfilaria counts in skin snips but lower antibody titres in HA tests with O. volvulus and Dirofilaria immitis antigens. Their nutritional state was inferior, onchocercal microfilaruria more frequent and inguinal lymphadenopathy a common complication.

In contrast, IgE levels were not affected by either infection intensity or clinical severity of onchocerciasis. A combined analysis for association between IgE concentration and presence of mansonian schistosomiasis and onchocerciasis revealed that the presence of eggs of S. mansoni combined with a reactive skin test to the antigen of this fluke gave the highest degree of correlation.

5. SUMMARY

(1) A graphical method for evaluating immunoglobulin patterns is described.

(2) Excessively high levels of IgG, IgA and IgM accompanied by relatively low IgD concentrations were found to be associated with severe onchocerciasis complicated by microfilaruria.

(3) Comparative studies of IgG, IgA, IgM and IgD levels between persons with severe and mild onchocerciasis classified on the basis of microfilaria counts in skin snips revealed different age patterns. The natural increase of IgG levels with age was not found in the group with severe infections; while IgD concentrations continued to rise from the youngest to the oldest age-group.

(4) An analysis for association between IgE levels, presence of onchocerciasis (skin snips) and schistosomiasis (eggs in stool) and reactivity in the skin test for schistosomiasis showed the highest degree of correlation between IgE and presence of schistosome eggs accompanied by reactivity in the skin test with adult antigen of S. mansoni.

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TABLE 1. IMMUNOGLOBULIN G, A, M AND D MEAN LEVELS AMONG 32 RESIDENTS WITH ONCHOCERCAL MICROFILARURIA AND CONTROL SUBJECTS OF THE SAME AND OTHER CHADIAN VILLAGES MATCHED FOR AGE AND SEX[1]

Village	No.	Immunoglobulin mean levels in mg%			
		G	A	M	D
Djimtjilo (Lake Chad)	32	1 852	153.6	255.0	9.9
Ouli Bangala (SW Chad)					
Microfilaruria pts	32	3 026	198.4	273.9	7.4
Control subjects	64	2 732	176.0	303.5	10.6
Ouarai (S. Central Chad)	32	2 235	150.4	275.4	8.2
Boum Khebir (Lake Iro)	32	2 348	147.2	277.0	11.1
Faye-Largeau (Sahara)	32	1 468	147.8	186.2	12.3
Total	224	2 341	164.2	266.8	10.0

[1] For each case with microfilaruria control partners of the same age and sex were selected in each village from the list of the census data. After selection, the corresponding sera were identified and analysed. In Ouli Bangala two matched controls were drawn at random for each microfilaruria case.

TABLE 2. IMMUNOGLOBULINS G, A, M, D, AND E BY AGE AND SEX
OULI BANGALA, 1970

Age	No. examined		IgG			IgA			IgM			IgD			IgE		
			Mean (mg%)			Mean (mg%)			Mean (mg%)			Mean (mg%)			Mean (ng/ml)		
	♂	♀	♂	♀	both	♂	♀	both	♂	♀	both	♂	♀	both	♂	♀	both
0-9	10	12	2 540	2 268	2 392	91	114	104	216	418	326	11.0	12.7	11.9	8 190	4 519	6 188
10-19	24	11	2 900	2 583	2 800	197	114	174	383	427	397	10.5	8.0	9.7	6 204	7 920	6 743
20-29	7	11	2 400	2 583	2 512	190	153	167	268	413	357	7.6	7.2	7.4	6 917	4 910	5 691
30-39	13	18	3 535	2 882	3 156	230	183	203	456	339	388	10.5	4.5	7.0	11 083	7 050	8 741
40-49	12	9	3 409	2 776	3 138	268	162	223	324	344	333	12.3	11.3	11.9	10 492	5 075	8 170
50+	5	3	2 240	2 920	2 495	247	278	259	255	587	380	9.4	12.0	10.4	5 060	13 326	8 160
Total	71	64	2 956	2 583	2 779	203	155	180	343	427	383	10.5	7.6	9.1	8 065	6 373	7 263
Mean diff ♂ - ♀ ÷ SE diff			$\frac{+373}{131} = \underline{2.8}$			$\frac{+48}{15.3} = \underline{3.1}$			$\frac{-84}{43} = \underline{1.95}$			$\frac{2.9}{1.64} = \underline{1.76}$			$\frac{1\ 692}{994} = \underline{1.70}$		

TABLE 3. IMMUNOGLOBULIN MEAN LEVELS AMONG LOCAL RESIDENTS OF OULI BANGALA WITH AND WITHOUT MICROFILARURIA, FOLLOW-UP JULY 1970

	IgG (mean mg%)	IgA (mean mg%)	IgM (mean mg%)	IgD (mean mg%)
Without microfilaruria (94 subjects)	2 850	171.1[a]	332.9	10.3[a]
With microfilaruria (42 subjects)	2 980	212.8[a]	359.5	7.8[a]
Normal range (114 subjects)	600-1 200	50-240	50-150	0-30

[a] Statistically significant at P = 0.05.

TABLE 4. CORRELATION BETWEEN IMMUNOGLOBULIN G AND IgA, IgM, IgD AND IgE LEVELS AMONG MALES[a]

Age	No.	IgG versus A	Versus M	Versus D	Versus E
		r[b]	r[b]	r[b]	r[b]
0-9	10	0.19	0.44	-0.34	0.33
10-29	31	0.30	0.0	-0.03	0.19
30+	30	-0.08	0.03	-0.27	0.36
Total	71	0.13	0.08	-0.174	0.28

[a] Female sample: N = 65; IgG versus IgD r = -.196.
[b] Correlation coefficient r.

TABLE 5. IgE LEVELS FOR INDIVIDUALS WITH AND WITHOUT MICROFILARIAE OF ONCHOCERCA VOLVULUS IN SKIN SNIP AND EGGS OF SCHISTOSOMA MANSONI IN SINGLE STOOL SPECIMENS

	No. in group	IgE mean in ng/ml	SE
A. Neither eggs nor microfilariae	7	6 085	1 833
B. Microfilariae only	66	6 626	737
C. Microfilariae and eggs[a]	44	8 134	991

[a] There were no persons who had eggs but no microfilariae.

TABLE 6. TEST FOR ASSOCIATION BETWEEN IMMUNOGLOBULIN E SERUM LEVELS, REACTIVITY IN SKIN TESTS WITH S. MANSONI ANTIGEN, AND PRESENCE OF EGGS OF SCHISTOSOMA MANSONI

VILLAGE OF OULI BANGALA

	Deviation of immunoglobulin E level from group mean[a]			
	+	-	Total	IgE mean
	(%)			ng/ml
Skin test reactive				
eggs: present	12 (75.0)	4	16	11 533
absent	8 (23.5)	26	34	5 841
All skin tests reactive	20 (40.0)	30	50	7 662
Skin test non-reactive				
eggs: present	5 (35.7)	9	14	8 053
absent	9 (36.0)	16	25	7 964
All skin tests non-reactive	14 (35.9)	25	39	7 998

[a] 7810 ng per ml.

Test criterion	Degree of freedom	Sum of squares	Chi square	P
Skin test reactive eggs + versus -	1	2.88	12.2	<0.001
Skin test non-reactive eggs + versus -	1	0.000073	0.0003	+0.5
Skin test reactive versus non-reactive	1	0.03687	0.156	+0.5
Total	3	2.919	12.4	0.01

Fig. 1 STANDARDIZED MEAN INDICES FOR IMMUNOGLOBULINS G, A, M AND D IN 32 CASES OF ONCHOCERCAL MICROFILARURIA AND CONTROL SUBJECTS IN FIVE VILLAGES MATCHED FOR AGE AND SEX

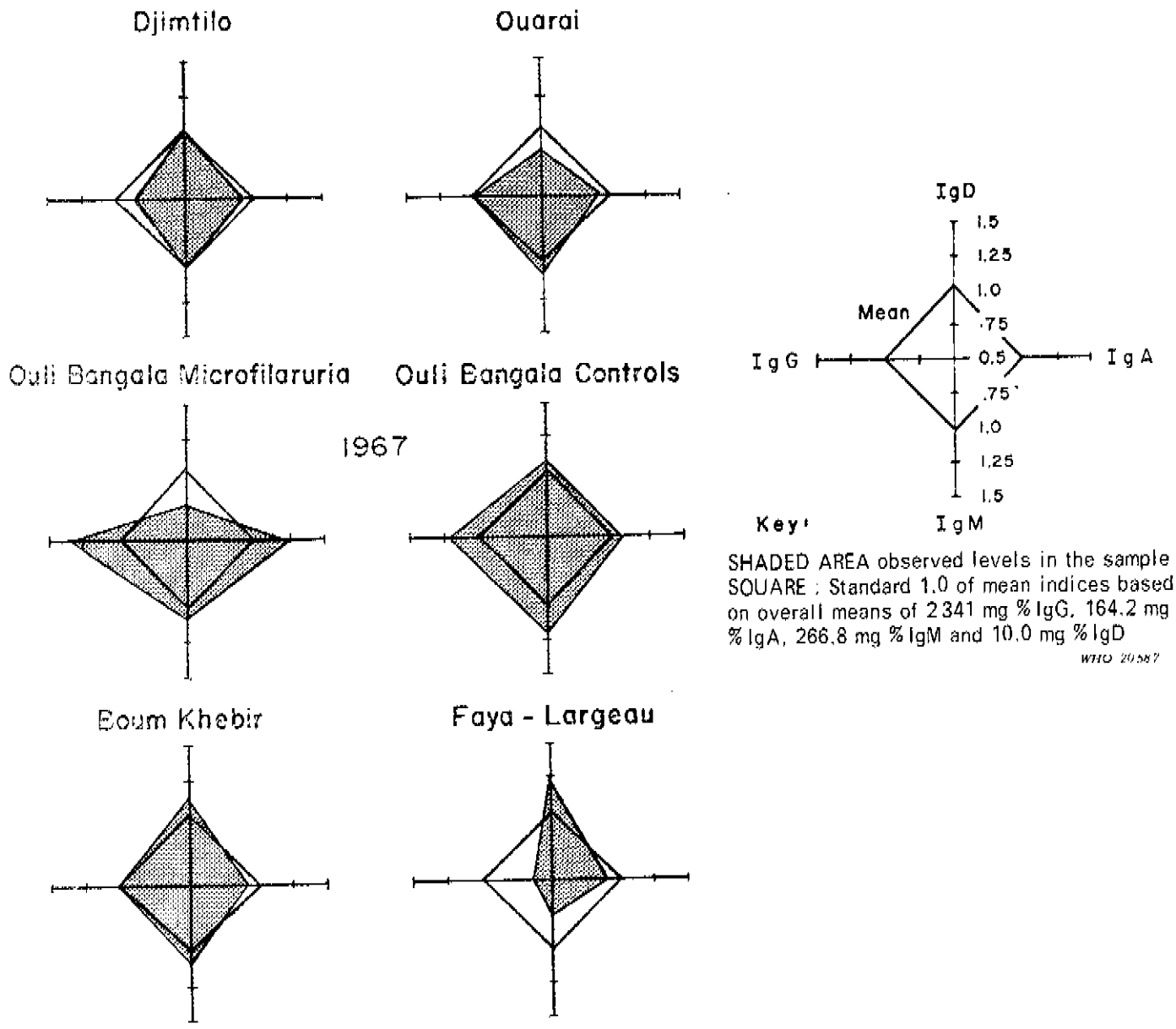
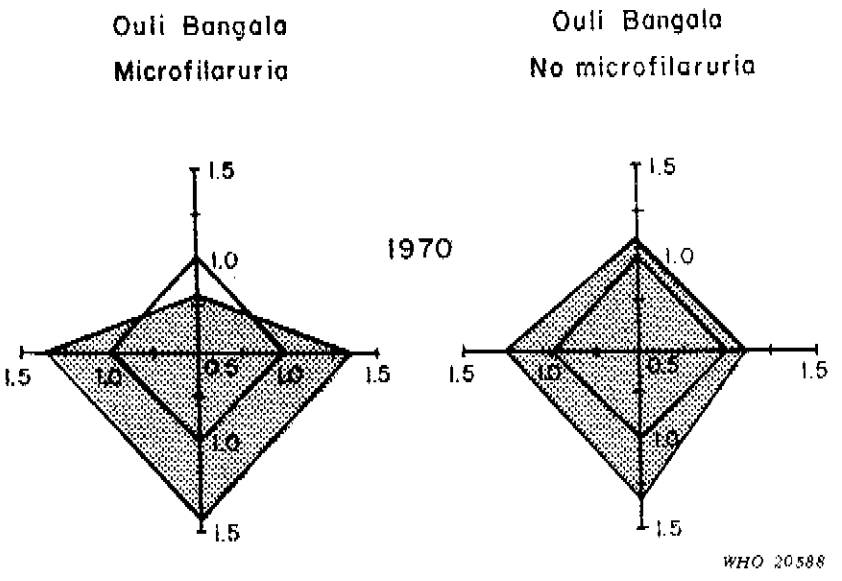


Fig. 2 **STANDARDIZED MEAN INDICES FOR IMMUNOGLOBULINS G, A, M AND D IN 42 RESIDENTS OF OULI BANGALA WITH ONCHOCERCAL MICROFILARURIA AND 94 RESIDENTS WITH ONCHOCERCIASIS WITHOUT URINARY COMPLICATIONS**



KEY: As in Figure 1

Fig. 3 AGE SPECIFIC MEAN VALUES OF IgG, A, M AND D IN PERSONS WITH ONCHOCERCIASIS WITH "SEVERE" INFECTIONS (SKIN SNIP COUNTS ABOVE GROUP MEAN) AND "LIGHT" INFECTIONS (SKIN SNIP COUNTS BELOW GROUP MEAN)