



*Malaria - Immunity - Tunisia*

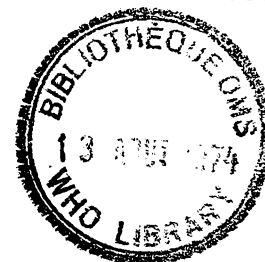
LONGITUDINAL SERO-EPIDEMIOLOGICAL  
STUDIES ON MALARIA IN TUNISIA

INDEXED

a 66.216

by

P. Ambroise-Thomas,<sup>1</sup> W. H. Wernsdorfer<sup>2</sup>  
B. Grab,<sup>3</sup> J. R. Cullen<sup>4</sup> and P. Bertagna<sup>5</sup>



CONTENTS

|   | <u>Page</u> |
|---|-------------|
| 1. Introduction . . . . .   | 3           |
| 1.1 The development of sero-epidemiology . . . . .                                | 3           |
| 1.2 Objectives . . . . .  | 4           |
| 1.3 Malaria in Tunisia . . . . .  | 4           |
| 1.3.1 General aspects . . . . .   | 4           |
| 1.3.2 Climate . . . . .   | 4           |
| 1.3.3 Development of the antimalaria programme . . . . .                          | 5           |
| 2. Material and methods . . . . .   | 5           |
| 2.1 Selection of stations - geographical and epidemiological considerations . . . | 5           |
| 2.2 Practical considerations. Surveys . . . . .                                   | 7           |
| 2.2.1 Schedule . . . . .  | 7           |
| 2.2.2 Personnel . . . . .   | 8           |
| 2.2.3 Recording . . . . .   | 8           |
| 2.2.4 Sampling . . . . .  | 8           |
| 2.2.5 Cooperation of population . . . . .   | 8           |
| 2.2.6 Blood collection . . . . .  | 9           |

<sup>1</sup> Professor of Parasitology, University of Grenoble, France.

<sup>2</sup> WHO, Geneva, formerly Senior WHO Adviser, Malaria Eradication Programme, Tunisia.

<sup>3</sup> Statistician, WHO, Geneva.

<sup>4</sup> WHO Technical Officer, Malaria Eradication Programme, Afghanistan, formerly Malaria Eradication Programme, Tunisia.

<sup>5</sup> Scientist, WHO, Geneva.

The issue of this document does not constitute formal publication. It should not be reviewed, abstracted or quoted without the agreement of the World Health Organization. Authors alone are responsible for views expressed in signed articles.

Ce document ne constitue pas une publication. Il ne doit faire l'objet d'aucun compte rendu ou résumé ni d'aucune citation sans l'autorisation de l'Organisation Mondiale de la Santé. Les opinions exprimées dans les articles signés n'engagent que leurs auteurs.

|  | <u>Page</u> |
|--|-------------|
| 2.3 Parasitological examinations . . . . .                             | 9           |
| 2.4 Immunofluorescence tests . . . . .                                 | 9           |
| 2.4.1 Reagents . . . . .   | 9           |
| 2.4.2 Practical execution . . . . .                                    | 10          |
| 2.4.3 Interpretation of results, checking of reproducibility . . . . . | 10          |
| 2.5 Processing of data . . . . .                                       | 11          |
| 3. Results . . . . .   | 11          |
| 3.1 Sampling performance . . . . .                                     | 11          |
| 3.2 History records . . . . .  | 11          |
| 3.2.1 Individual malaria history . . . . .                             | 11          |
| 3.2.2 Individual malaria treatment . . . . .                           | 11          |
| 3.2.3 Family malaria history . . . . .                                 | 11          |
| 3.3 Follow-up of sero-positives . . . . .                              | 12          |
| 3.4 Parasitological findings . . . . .                                 | 12          |
| 3.5 Serological findings . . . . .                                     | 13          |
| 3.5.1 General . . . . .  | 13          |
| 3.5.2 Geographical analysis . . . . .                                  | 13          |
| 3.5.3 Analysis by sex and age . . . . .                                | 16          |
| 3.5.4 Analysis according to individual malaria history . . . . .       | 17          |
| 3.5.5 Analysis according to previous treatment . . . . .               | 17          |
| 3.5.6 Analysis of follow-up results . . . . .                          | 18          |
| 3.5.7 Analysis in relation to the epidemiological situation . . . . .  | 19          |
| 4. Discussion . . . . .  | 20          |
| 5. Conclusions . . . . .   | 22          |
| Summary . . . . .  | 23          |
| Acknowledgements . . . . .   | 24          |
| References . . . . .   | 25          |

Between April 1970 and October 1972 sero-epidemiological studies were conducted in Tunisia during the course of which 18 localities were regularly visited and blood samples of 13 044 persons examined for the presence of malaria parasites and of fluorescent antibodies.

## 1. INTRODUCTION

### 1.1 The development of sero-epidemiology

Classical, well-proven methods such as measurement of spleen rate and parasite rate still constitute the basic means used in malariometric assessment. In recent years, however, they have been supplemented by serological techniques such as the passive haemagglutination test (IHAT) or the indirect immunofluorescence test (IFAT).

The IHAT is particularly suited for a quick study of a great number of serum samples. Although this technique has been used for epidemiological purposes by various workers, it is still under study.

The IFAT has gained widespread use, especially since Sulzer & Wilson (1967) introduced thick films as antigen. With this technique, various authors have studied in total more than 20 000 serum samples in numerous countries: for instance, in the Gambia (McGregor et al., 1965), in Surinam (Meuwissen, 1966), in Nigeria (Collins et al., 1967; Voller & Bruce-Chwatt, 1968), in Romania (Dranga et al., 1969), in Ethiopia (Collins et al., 1971; Collins & Skinner, 1972), in Tanzania (Lelijveld, 1971), in Malaysia (Collins & Skinner, 1972) in Amazonia and in different foci in Eastern Africa (Draper et al., 1972).

While the observations referred to above were made in countries endemic for malaria or in old foci where transmission had been interrupted for a long time, in Corsica the method was used for checking the extension of reintroduced malaria in a region which had been free of the disease for more than 20 years (Ambroise-Thomas et al., 1972).

It is recognized that some correlation exists between fluorescent antibodies and parasitaemia and that the serological results reflect previous experience with malaria among the persons examined and the amount of reinfection to which they were exposed.

The IFAT may, therefore, be considered as giving indications similar to those of splenometry, but with a higher precision and especially with a considerably superior specificity in areas of low endemicity or disappearing malaria. In this connexion, it should be remembered that Voller & Bruce-Chwatt in Nigeria (1968) have observed important serological fluctuations in relation to seasonal variations of malaria transmission, at least in certain age-groups.

Observations on the performance of the IFAT and on its application are at present being conducted in several laboratories, nearly all of which are situated in Europe and in the United States of America. Contrary to direct parasitological examination of blood slides and in spite of scientific fashion, the fluorescent antibody test has not yet been introduced to any significant degree to the developing countries, many of which are highly endemic for malaria. This is explained by the cost, the technical and operational demands and the indications of the technique.

Although IFAT is proving to be a suitable tool for sero-epidemiological studies in malaria, we do not yet know all its potentialities and limitations.

Apart from the studies of Collins et al. in Ethiopia (1971, 1972) and those of Lelijveld in Tanzania (1971), most observations were from cross-sectional surveys. We felt that longitudinal observations could yield valuable epidemiological information in conditions which generally are described as "disappearing malaria". This is why we have attempted longitudinal sero-epidemiological studies in Tunisia, for which the epidemiological background, objectives, material, methods and results are described in this paper.

## 1.2 Objectives

- (i) Qualitative and quantitative assessment of the degree of malaria sero-positivity in various parts of Tunisia, relating the results to parasitological findings.
- (ii) Evaluation of the changes of malaria sero-positivity in relation to the epidemiological development, especially under the impact of malaria eradication operations.
- (iii) Evaluation of the changes of malaria sero-positivity in relation to age, previous malaria experience and chemotherapy.
- (iv) Demonstration of the existence of any especially exposed population groups or communities.
- (v) Detection of any continued presence of residual malaria reservoir.
- (vi) Measuring the progress of malaria eradication operations and confirmation of continued interruption of malaria transmission, if applicable.

## 1.3 Malaria in Tunisia

### 1.3.1 General aspects

Situated on the northern tip of the African continent in the central part of the southern Mediterranean coast, Tunisia was affected by malaria since ancient times. The disease was hyperendemic in the northern and eastern maritime areas while it was hypoendemic in the west where the foothills of the Atlas range reach an altitude of 1500 m - but never constituting a geographical barrier to malaria transmission. The majority of oases around the Chott El Djerid and on the edge of the Sahara in the south were also malarious at various levels of endemicity. Only the extreme south was non-malarious. With the exception of some hyperendemic areas in the north, malaria was generally unstable.

The anopheline fauna is represented by 11 species with Anopheles labranchiae as the main vector in northern Tunisia and A. sergenti as the main vector in southern Tunisia. The role of A. multicolor as a potential vector has not yet been clarified. This species is found throughout central and southern Tunisia as far south as Fort Saint, some 400 km inside the Sahara.

### 1.3.2 Climate

The climate of Tunisia is dominated by subtropical maritime conditions whose influence extends far inland towards the west and south. In all areas, there are marked seasonal changes with two major seasons. The cold season extends from the end of October to the beginning of April in the north and from the end of November to the beginning of March in the south. The change from one season to another is usually quite abrupt. Rainfall is highest in the northern coastal hills where it exceeds 1000 mm per annum. The quantity decreases towards the south. In the southern areas, rainfall is very irregular and sporadic with marked fluctuations from year to year. In the coastal areas, relative humidity is at a high level with very little fluctuation. The relative proximity of the sea and the influence of irrigation keep the relative humidity in most of the southern oases rather high, even in the hot season.

Conditions become suitable for malaria transmission shortly after the onset of the warm season and remain so until the beginning of the cold season.

### 1.3.3 Development of the antimalaria programme

Starting from the hyperendemic areas and from foci in the hypoendemic zones, Tunisia was often subject to serious malaria epidemics which have shown a certain periodicity. After the ravaging epidemics of 1931 and 1932, malaria control measures were introduced on a systematic scale using drug prophylaxis and antilarval measures.

The malaria control measures suffered an interruption during the Second World War, but were resumed soon after. In 1965, malaria incidence again began to rise. This tendency came to an end when the malaria eradication programme was implemented in 1967. This programme was planned to cover the whole country by stages, following the classical phasing. While all northern areas were covered according to the original plan, the full inclusion of the southern Governorates was delayed until the second half of 1972. The attack measures consisted of residual house spraying with DDT. In the northern Governorates, full surveillance was established soon after the start of operations. In the south only passive case detection, epidemiological investigations and focal measures were applied. Radical treatment of all confirmed malaria cases was practised from mid-1968 onwards. The blood examination rate rose soon after the implementation of the programme, while slide positivity rate and malaria incidence have dropped to very low levels (see Table 1).

Figs 1-3 show the geographical aspects of the regression by comparing pre-operational conditions with those of 1970 and 1972.

The majority of the country, i.e. the 10 northern Governorates, passed into the consolidation phase in 1971 and 1972, while the southern Governorates are under preconsolidation, awaiting full inclusion in the programme. In 1972, four malaria cases were detected in the consolidation area while 15 occurred in the southern zone, 10 of them in one focus near Kébili in the Governorate of Gabès.

Malaria eradication had made steady, satisfactory progress from its implementation in 1967. The slide positivity rates, between April 1968 and December 1972 are shown in Fig. 4 in form of the three-monthly moving averages. The slide positivity rate in the northern Governorates has dropped consistently from the beginning of 1968, while a definite improvement in the south was only achieved in 1972.

## 2. MATERIAL AND METHODS

### 2.1 Selection of stations - geographical and epidemiological considerations

In total, 18 localities were selected. These localities are situated in eight different Governorates in areas representing the various major ecological and epidemiological conditions of the country. The intention was to have localities which were different as far as their size, their operational history and their epidemiological development were concerned. Furthermore, consideration was given to accessibility and cooperation of community leaders and population.

Table 2 identifies these individual localities and gives records of protection, surveillance and case incidence between 1967 and 1972.

The geographical distribution of the localities can be seen in the map of Fig. 5 which also shows the grouping of the localities into four major regions, reflecting a certain homogeneity as far as topographic, epidemiological, biological, climatic and demographic parameters are concerned.

About 2000 persons were covered at each and every of the six surveys which were conducted at six-monthly intervals starting in April 1970.

Region A: Eastern maritime

This area stretches along the Mediterranean coast from Cap Bon to Sfax and has a high relative humidity throughout the year. Summer comes early, rainfall is abundant in the north. The main vector is A. labranchiae, in the southern part A. multicolor may also play a role. Malaria transmission was in the past rather intense in the northern and central parts of the region, with hyperendemic foci in Cap Bon and Sousse Governorates; the part belonging to Sfax was less malarious.

Most recent malaria transmission in locality O1 took place in 1969, i.e. in the second year of the attack phase, while transmission in locality O2 probably ended in 1968. The case observed in 1970 has contracted the infection probably in 1968. Localities O3 and O5 are suburbs of towns which apparently had no malaria transmission already prior to the implementation of the malaria eradication programme. In the process of urbanization, these localities have had a considerable influx of population from the rural areas. This also explains the five malaria cases observed in locality O3 in 1968 which were probably not the result of local transmission.

Region B: Southern

This region consists of the three southern Governorates and the southern part of Sfax Governorate. The main features are semi-desert and desert with oases. Summer comes early and is long, rainfall scanty and irregular, but the maritime neighbourhood maintains the relative humidity at rather high levels even during the hot season. The main vector is A. sergenti; epidemiologically malaria in this area corresponds to oasis malaria. Following massive aerial spraying with insecticides for the control of desert locusts in the early sixties, A. sergenti became nearly extinct and malaria transmission was largely interrupted, leading to an important reduction of the malaria reservoir in this area which formerly had a very heterogeneous endemicity. Later A. sergenti propagated again, resuming its vectorial role.

Whereas the southern part of Sfax Governorate was included in the programme since 1969 with full surveillance, the Governorates of Gafsa, Gabès and Médénine were covered by passive case detection only and by focal measures which consisted of larviciding, DDT spraying and mass chemotherapy. Since the reappearance of A. sergenti, localized epidemics with a high transmission potential have occurred in several localities until 1971, especially in Gafsa and Médénine Governorates. These areas became the main reservoir of malaria in Tunisia but the indications are that the focal measures applied since 1969 have helped to reduce malaria prevalence and incidence to a low level.

Locality O4, near springs at the foot of a hill range, experienced a malaria epidemic with 97 cases in 1969; in locality O7, an oasis, 71 cases occurred in the same year. In both places, A. sergenti can breed throughout the year. Mass radical treatment was given in both localities. In locality O4, two cases were detected in each of 1970 and 1971, but these infections were apparently connected with an adjacent focus in Gafsa Governorate. While there was no recent indication of malaria transmission in locality O8, sporadic cases were observed in and near localities O6 and O9, both oases, in 1968. In July-September 1972 a small focus with 10 cases of falciparum malaria developed some 5 km from locality O6.

Region C: Western inland

This area consists of the Atlas foothills and various valleys of which the upper Medjerda valley is the most important. Summer in this region comes later than in the southern and eastern maritime regions, rainfall increases with altitude and towards the north. A. labranchiae is the main vector. In the past, malaria was mainly hypo- and mesoendemic in this region but cyclical epidemics did occur in this area of unstable malaria. Malaria eradication operations started in 1968 and were quite successful, but the area was for some time exposed to the importation of cases from adjacent Algeria. In 1972, the region was passed into the consolidation phase. For border protection, spraying operations are maintained along the Algerian frontier.

Locality 10 is part of Kasserine Town, the cases recorded in 1968 and 1969 were not autochthonous for this locality. Localities 11 and 12 are both rural. In the former malaria transmission had apparently ended in 1968, while in locality 12, it ended only in 1969. Near this locality, a focus with 20 cases was observed in 1968. Locality 13, also rural, and near the Algerian border, had eight cases in 1968; it is situated in an originally mesoendemic area which was notorious for malaria epidemics. Locality 14, a suburb of Jendouba Town, had experienced malaria transmission until 1969.

#### Region D: Northern maritime

This is a generally hilly area with various valleys and streams running to the northern coast. Summer is shorter than in the eastern maritime and southern regions, rainfall is abundant and humidity high throughout the year. This region with A. labranchiae as the main vector contained some of the most notorious hyperendemic foci of Tunisia, but the drainage scheme in the Tabarka basin had helped to eliminate the most important focus. Attack operations were conducted from 1968 to 1970/71. By 1972, malaria incidence in the whole area had come down to zero.

Locality 15, situated in the old Tabarka focus, was malarious until 1968, but the eight cases recorded during that year were probably all relapses. Locality 16 is on the outskirts of Menzel Bourguiba Town. The single cases recorded there in 1968 and 1969 are apparently not of autochthonous origin. In locality 17, situated near a small barrage lake, fairly intensive malaria transmission was observed in 1968. Among the seven cases detected in 1969, the majority were relapses. In locality 18 malaria incidence in 1967 was high with 23 cases, but the last malaria case was recorded in 1968.

## 2.2 Practical considerations. Surveys

When the plan for this comparative study was developed, it was realized that a sampling frame and a technical methodology had to be adopted which would permit comparability between the various surveys. Six months was considered to be the most suitable interval between these surveys which were conducted in April and September/October 1970, 1971, 1972, i.e. with a seasonal timing coinciding with a potential peak incidence of relapses or new cases respectively. As the malaria eradication programme had by 1970 reached an advanced stage - in 1969 the annual parasite incidence rate was already below 0.1 per thousand population - splenometric examinations could not be expected to yield any meaningful results. Therefore, the studies were limited to parasitological and serological observations in conjunction with epidemiological evaluation.

### 2.2.1 Schedule

After primary selection of the localities, a timetable was developed and due information was given to the health service administrations of the Governorates (regional administrations) concerned. Based on the experiences of the first round, the timetables of the subsequent surveys were prepared and included in the annual plans of action of the malaria eradication programme. These timetables were drawn up duly considering distances between the stations, suitability of timing in respect of the locality concerned in order to avoid undue absenteeism, and the feasible working capacity of the survey team. In general, one locality was surveyed per day when distances were considerable, while two localities were surveyed on days with lesser displacements. The surveys continued without interruption through weekends.

In view of the necessity of advising regional authorities and population of a precise timing, the surveys had to follow strictly the programme which did not permit revisiting of the individual localities during the same round.

### 2.2.2. Personnel

The survey team was composed of national and international personnel assigned to the central office of the malaria eradication programme, assisted by technical and laboratory personnel of the Governorate involved. For practical purposes, the team was divided into three units: one for registration and two for blood sampling. Registration was handled by the senior national technician, assisted by sector chiefs and, with the exception of localities 06, 07, 08 and 09, also by the local surveillance agent. Valuable help was rendered by cheikhs, village elders and other community leaders.

### 2.2.3 Recording

Recording presented one of the major difficulties in these studies as the various personal data had to be noted with adequate precision. The recording of the names of persons included in the survey required particular care. Certain names tended to occur quite frequently. While individual identification routinely covered three generations, it was essential in some cases to include a fourth name. Also age classification encountered some obstacles, especially in the remote rural areas. Again, the efforts of local personnel and community leaders helped with clarification.

On the basis of the experience of the first survey, a special survey form was developed in which data on name, sex, age, malaria history, treatment and malaria in the family were noted. A special follow-up record form was filled for each of the sero-positives detected in the first survey, and updated regularly.

In recording, labelling of blood slides and serological sampling, no major faults have occurred. Efficient liaison was maintained between the Malaria Eradication Programme in Tunisia, the Laboratory of Parasitology in Grenoble and WHO headquarters, Geneva.

### 2.2.4 Sampling

The sample size was fixed at 120 persons with about equal numbers from either sex, to be examined in each of the 18 selected localities (a total of 2160 individuals) during the first survey. The registration was made on a household basis in clusters liable to provide samples of required size. Because of the particular importance of young children in the present sero-epidemiological evaluation, their participation rate was purposely fixed at a higher level: efforts were made to include in the sample all children of the age-groups 6-23 months and 2-4 years from the registered households, while no attempt was made to cover more than two-thirds of the older children and juveniles (5-14 years) and one-third of the adults (15+ years). Infants of less than six months were not included in this study in order to reduce potential error caused by maternal antibodies in the IFAT.

As it was intended to follow all sero-positives detected at the first survey in all subsequent surveys the above described selection was only applied to complement the sample to the required size, from the second survey onwards.

In order to achieve the highest possible re-examination rate of previous sero-positives and satisfactory sampling among complementary persons, all regional administrations were advised well in advance and given the list of persons to be re-examined in order to notify them. In general, this system was efficient and even during the sixth survey 71% of the previous sero-positives were contacted again.

### 2.2.5 Cooperation of population

The cooperation of the population was satisfactory during the first and generally also in the second survey. In the third survey, the reexamination rate of previous sero-positives showed a marked drop. Moreover, it became very difficult to achieve qualitatively adequate sampling of complementary persons.



Measures were therefore taken to ensure a favourable disposition of the population, during the fourth to sixth surveys, by distribution of food rations kindly provided by the Comité National de Solidarité and the World Food Programme. The distribution of food rations could not offset genuine shortages in certain age-groups, e.g. male adults, who were mostly in the fields or at their place of employment, often even outside the region.

#### 2.2.6 Blood collection

At the moment of registration the persons to be examined were each given a paper slip or a stamped piece of cardboard with the registration number and data on sex and age, the latter as to avoid confusion if there was the possibility of exchange of paper slips, e.g. parents with small children. The paper slip or the piece of cardboard was presented to the blood sampling unit concerned.

For parasitological sampling, two slides were taken from each person during the first survey, placing thick and thin films on the same slide, while one such slide each was taken in the five subsequent surveys. Every slide carried the village code and the individual serial number. The slides were stored in horizontal storage boxes and 24 hours later packed for despatch, and with the least possible delay forwarded for further processing to the Central Laboratory of the Malaria Eradication Programme, Tunis, in order to keep the interval between preparation and staining of the blood films below seven days.

For serological sampling, two blood samples of 70 microlitres each were taken from every person included in the survey, using heparinized microcapillaries and following the technique described by Ambroise-Thomas & Kien Truong (1968). This method permits particularly precise sampling. The contents of each capillary were absorbed onto squares of special filter paper on which locality code and individual serial number were inscribed using a lead pencil, prior to impregnation. After completion of the survey in the locality, the impregnated papers were put on a string and duly protected against flies. Particular care was taken to avoid contact between the samples and fixing agents such as spirit, formalin, etc. The samples were dried either in the open air or in a drier at temperatures not exceeding 40°C. Prior to packing, the samples were sorted and rearranged in numerical order. As after drying, the samples may be preserved at temperatures below 40°C for up to 20 days, it was possible to send the samples by airmail from Tunisia to the laboratory in Grenoble, where they were preserved at -20°C until examination. The two series of samples were despatched separately so as to reduce the risk of loss.

#### 2.3 Parasitological examinations

The number of persons examined in the individual rounds varied between 2170 and 2182, the total of the six surveys was 13 044. At the Central Laboratory of the Malaria Eradication Programme, the slides were stained with Giemsa stain using the mass block staining method for thick films outlined in the "Manual for the Processing and Examination of Blood Slides in Malaria Eradication Programmes".<sup>1</sup> The two slide series of the first survey were read by different microscopists. Every slide of the other five rounds was examined twice, by different well experienced microscopists, normally charged with the activities of a reference laboratory.

#### 2.4 Immunofluorescence tests

##### 2.4.1 Reagents

(i) Antigens were prepared as thick films following the technique of Sulzer & Wilson (1967), utilizing blood of Macaca mulatta which was splenectomized eight days before infecting it intravenously with a strain of Plasmodium cynomolgi bastianellii. The

---

<sup>1</sup> Document MHO/PA, 262.61, World Health Organization, 1961, 2nd ed., Geneva.

blood, taken seven to eight days after infection when infected cells reached 5 to 10%, was heparinized and washed, three or four times, with phosphate buffer pH 7.2. The thick films were placed on slides which were entirely silicone-coated with the exception of six circles of 7 to 8 mm diameter. One thousand to 1500 such slides can be prepared in one session. After drying, the antigen preparations were placed in a deep freeze at  $-70^{\circ}\text{C}$  for up to 15 months.

- (ii) Serum samples. For the immunofluorescence tests, the dissolved plasma was utilized. It was obtained by placing each filter paper square in 0.7 ml of buffer solution at pH 7.2 and leaving it overnight at a temperature of  $+4^{\circ}\text{C}$ . This corresponds to a final dilution of 1/20.

Repeated controls have shown that this micromethod produces results which are practically identical with those obtained directly from the sera. This is in agreement with the findings of Vaisman et al. (1963).

It should be stated, however, that the haemoglobin contained in the plasma solution inhibits fluorescence to a certain extent. It is, therefore, not exceptional that a reaction appears less intensively positive at a dilution of 1/20 than at higher dilutions. Hence, every serum sample must be checked in at least two dilutions.

Each sample was tested at dilutions of 1/20 and 1/40 and in the case of a positive result, further dilutions were tested until negativity, using the plasma diluate within a maximum of 24 hours. If this limit was passed, e.g. on weekends, replicates were run from the duplicate samples, starting again from the dilutions 1/20 and 1/40.

- (iii) The fluorescent conjugate is a commercial product (fluorescent antiglobulin human sera) available from the Institut Pasteur, Paris. It is generally employed at a dilution between 1/50 and 1/100, a dilution which must be precisely determined for every new batch of conjugate, e.g. 1/75 for batch No. 70.176.

#### 2.4.2 Practical execution

The tests were carried out following the classical technique described by Ambroise-Thomas et al. (1969). They were read using a fluorescent microscope fitted with a bright field dry condensor, an emission filter BG 12 of 6 mm thickness and an objective 25 x. Generally, one technician was able to examine 30-50 sera per working day, depending on the range of positivity.

#### 2.4.3 Interpretation of results, checking of reproducibility

For the interpretation of serological findings certain aspects of test sensitivity and reproducibility of results have to be considered. The fact that parasites themselves are used as the antigen in the test is of course an element of stability, particularly when the same antigen is employed throughout as it has been the case in our investigations. The antigen should show good reproducibility. Variation may be caused by other components of the reaction, e.g. fluorescent conjugates and microscopic equipment. Consequently, the reproducibility was checked systematically throughout these studies. For this purpose, 150 samples from the preceding survey were retitrated whenever a new series of survey material came under processing. The results were compared with those of the first aliquots and they were generally identical. In the rare cases of disparity, the variation did never exceed one dilution. The reproducibility of this method therefore permits its use in longitudinal studies.

As far as the specificity of the test is concerned, the first significant dilution hitherto recognized by all authors is 1/20. This level has been experimentally determined and is backed by the examination of several thousands of serum samples. Obviously, tests which are positive at this initial dilution only have a very limited practical value and may simply indicate past, even rather remote contact with the plasmodium.

In these studies we have retained the dilution of 1/20 as the critical dilution, being aware that the corresponding quantum of fluorescent antibodies may very well be subject to discussion. In our opinion, it is not permissible to draw conclusions at the individual level. Our interest was centred on a global evaluation based on the population of localities, locality groups and the whole country, especially in the context of longitudinal observations.

## 2.5 Processing of data

After every survey round, a brief compilation of data pertaining to the sample was prepared in Tunisia. After reception of parasitological and serological results at Geneva, the data were processed on a computer of the International Computing Center, Geneva.

## 3. RESULTS

### 3.1 Sampling performance

Table 3 gives the data on the composition of the sample of the first survey, on locality basis, by sex and age, previous malaria history, malaria treatment and malaria of family members. In Table 4, the same data are summarized for the four groups of localities, while Table 5 gives a review of global data from the first to the sixth survey. In Table 6, the observed age percentage distributions of the total sample examined at each survey are compared with the corresponding expected distribution calculated on the basis of the 1966 Population Census of Tunisia, under the assumption that only two-thirds of the children of 5-14 years and one-third of the adults (15 years and over) were represented in the sample. These tables show that, as far as the total number of individuals examined and their age distribution are concerned, the actual performance was quite close to the requirements specified in section 2.2.4. The global sex-ratio was also satisfactory at each survey.

### 3.2 History records

#### 3.2.1 Individual malaria history

The data in Tables 3 and 4 pertain to confirmed cases only and show considerable differences in the various localities and groups of localities. Group B with the foci of El Bouaa (4) and Talalet (7), active in 1969, shows the highest number of old malaria cases. Also Group D has a rather high number which was due to the foci of El Bridj (18) and El Guaria (17), active in 1967 and 1968 respectively. The two localities without old malaria cases are Merkez Ben Halima (5) in Group A and Ragouba (8) in Group B. From Table 5 it is apparent that the number of old malaria cases contained in the samples was sharply reduced after the first survey. When referring to Table 2, it is evident that in most localities the majority of old confirmed cases was examined in the first survey. Most of them were sero-negative and therefore not convened for the subsequent surveys in which the composition of complementary persons was subject to considerable change.

#### 3.2.2 Individual malaria treatment

As seen in Table 3, for the first survey, the number of persons having received treatment for malaria was identical with that of malaria cases in all localities except Talalet (7), where mass radical treatment was administered in 1969 and received by all persons examined except one. In the subsequent surveys (Table 5), the difference between the numbers of old malaria cases and persons having received treatment is more pronounced as in mid-1970 mass radical treatment was also administered in El Bouaa (4).

#### 3.2.3 Family malaria history

This item was tentatively included, but in view of the lack of precision it has not been considered in the subsequent analysis.

### 3.3 Follow-up of sero-positives

In the first survey a total of 406 sero-positives were found. It was intended to follow up as many as possible through the subsequent surveys. Table 7 shows the number and the relative percentages which could be reexamined in the respective groups of localities and in total.

In the global figures, a sharp reduction is seen until the third survey, but subsequently the percentage rose again due to the special measures taken and it stayed above 70% in the fifth and sixth surveys as well. Deaths and migration were found to account for considerable loss; in Table 8 the reasons for absenteeism in the sixth round are quoted and the corrected reexamination rates given.

About half the absenteeism of old sero-positives observed in the sixth round was due to death or emigration from the region. In some of the localities, persons were originally included which apparently did not belong to the area.

When applying correction for death and emigration, the global reexamination rate of the sixth survey comes to 85.7%, the rates of 15 localities exceed 75%.

In total, 166 of the sero-positives of the first round presented themselves at each of the five subsequent surveys, i.e. 40.9%, or 49.4% if corrected for death and permanent absence.

### 3.4 Parasitological findings

In the blood examinations, only one person among 2171 examined was found positive in the first survey while all 10 873 slides of the subsequent surveys were negative. The positive of the first survey was an infection due to P. vivax in an adolescent of 14 years found in locality 2, Beni Hamed, Cap Bon. This locality still had malaria transmission in 1968. All confirmed cases were then radically treated and no signs of transmission were apparent in 1969. The infection in question must have been contracted before 1970, but the patient declared that he did not suffer from any fever attacks during or before 1969. The patient was sero-positive at a titre of 1/80.

The parasitological results correspond to expectation, even considering that most localities had malaria transmission shortly before or at the beginning of the attack phase. The first survey was conducted when the northern operational area had just moved into the third year of the attack phase. Intensive malaria control measures prior to the eradication programme had reduced the parasite reservoir to such an extent that full surveillance could be implemented after the first spraying cycle. The quality of active and passive case detection was adequate so that the probability of acute symptomatic cases having escaped detection was limited. This does not fully apply to the southern Governorates, localities 6-9, where case detection activities relied exclusively on passive case detection. Nevertheless, a fair degree of coverage was reached there as well.

In 1968, a total of 131 cases had been diagnosed in the localities under study. In 1969, the number was 191, due not to a general rise of malaria incidence but to the inclusion of the two major foci of malaria transmission of 1969, amounting to 168 cases. The fact that no positives were subsequently found among the survey slides of these localities may be explained by mass radical treatment. In 1970, the year of survey rounds 1 and 2, the malaria incidence had receded to three cases, and in 1971 to two cases. In 1972 no cases were observed. The annual average number of slides collected by active and passive case detection in the 18 localities amounts to approximately 4000.

### 3.5 Serological findings

#### 3.5.1 General

The serological studies were conducted under two major aspects: to confirm the presence or absence of malaria transmission and to measure a regression of sero-positivity after full or partial interruption of malaria transmission.

For the first objective, individual cases have to be considered after selecting an appropriate critical serum titre, while trend studies may suffice for the second.

The results of the serological examinations are summarized in Table 9. The data indicate a continuous reduction of the proportion of sero-positives from the first until the fifth survey. There was also a gradual disappearance of high titres (1/160+). In the sixth survey, the number of sero-positives increased again, but with the exception of one person these were low titres. Considering that the mean titre of sero-positives was further reduced, this increase has apparently a limited significance.

#### 3.5.2 Geographical analysis

(i) By locality. The results of the serological examinations of the six surveys are presented in Table 10 by locality. The development of the percentage of sero-positives is graphically illustrated in Fig. 6. Localities 1, 2, 3 and 5 started with the highest degrees of sero-positivity. In the case of localities 1 and 2, both rural communities in Cap Bon region, this is consistent with the epidemiological history. In localities 4 and 7, the original percentage of sero-positives was also higher than in the average of the other localities. In both, malaria epidemics had occurred in 1969, but radical treatment was given to all confirmed cases. In the majority of the other localities, the percentage of sero-positives was already below 10% in the first survey. Surprisingly, the percentage of sero-positives was low in localities 15, 17 and 18, all in formerly hyperendemic areas and in the case of No. 17 and 18 also with a recent history of malaria incidence.

The sero-positivity rate generally showed a marked tendency to diminish which was unbroken in localities 2 and 12. In localities 9, 11, 13, 14, 15, 17 and 18, the rate rose slightly in the second survey. This phenomenon may in part be ascribed to the sampling procedure in which the old sero-positives were reexamined and the complementary persons exchanged, among whom one would expect a certain number of sero-positives to be detected for the first time.

Several localities showed an increased number of sero-positives in the sixth round, most pronounced in localities 4 and 6 where epidemiological features and population movements could possibly explain a maintenance of levels observed in the fourth survey, but not an increase. In other localities with the exception of No. 13 the increases are slight, produced by positivity at the lowest titre and apparently without epidemiological significance.

Considering the trends of sero-positivity in the individual localities, it is not surprising that at the outset El Arima (1) and Beni Hamed (2) showed the highest levels as these were formerly hyperendemic areas. In El Arima, high titres persisted until the third survey. In this locality, malaria transmission was apparently more intensive than would be apparent from the case detection data. The serological findings suggest that malaria transmission continued to be interrupted in 1970-1972.

The comparatively high sero-positivity in Beni Khiair (3) is surprising as this semi-urban community has apparently not had local malaria transmission for more than 10 years. However, the dispensary has records of confirmed malaria cases treated there

(five in 1968). These cases were from among the surrounding rural areas, migrants and newcomers. The rather high sero-positivity at the outset can be interpreted as largely representing the conditions of the surrounding region, and influenced by migrants from further distant areas. The migrant population appears to constitute an important part - five of the sero-positives found in the first survey belong to this group, underlining the potential importance of mobile population groups in the introduction of malaria. This may be illustrated by the fact that in winter 1969/70 a migrant was found positive in Cap Bon. By the time of the laboratory diagnosis, he had already quit Cap Bon, and he and his group were traced some 400 km away in the Governorate of Sfax, when three more persons of the group suffered from malaria.

El Bouaa (4), situated in an originally hypoendemic area, was subject to an epidemic outbreak of vivax malaria in 1969. Comparatively low percentage of sero-positives and titre levels are surprising but can be explained by rapid detection and radical treatment of the cases in 1969, followed by mass radical treatment in summer 1970, between the first and second surveys, when there were indications that malaria transmission resumed after one new case had turned up in June 1970. The serological results showed the presence of four persons with titres of 1/160+, three of them with 1/1280, and thus confirmed that the administration of mass radical treatment was well founded. The two cases found in 1971 were contracted outside the locality and under focal measures malaria stayed interrupted in 1972. In the sixth survey, 13 persons were found sero-positive, all at a titre of 1/20. Complementary information was collected from 12 of these. Four were young children (2-4 years), born before the outbreak of 1969, but who had not participated in the mass radical treatment in 1970. Two more persons had probably suffered from malaria before. One infant had suffered from an apparently non-malarious febrile disease immediately before the survey. One adult, previously sero-negative, had just before the survey returned from Libya where he had passed all summer 1972. The remaining four persons gave no indication of any recent febrile disease.

Merkez Ben Halima (5), a suburban part of Sfax, was apparently not subject to malaria transmission within the last 20 years. Relatively high sero-positivity is therefore considered to be due to population influx from inland areas. Sero-positivity and titres receded and gave no indication of malaria transmission.

In Rabta (6) sero-positivity at the outset was low and there were no signs of malaria transmission throughout. Passive case detection in this locality is of a good standard. In the sixth survey, 16 persons were sero-positive, two at titre 1/40, the others at 1/20. A special investigation has shown that both persons with 1/40 titres were nomads who had left the locality soon after the survey. Most of the other persons have participated in several surveys and were then sero-negative. The majority of them had been ill with fever and diarrhoea shortly before, or at the time of the sixth round. This outbreak of febrile diarrhoea showed a certain geographical focalization. During the outbreak, slides were taken regularly by the dispensary and examined at the regional laboratory, Gabès. They were all negative. The phenomenon is therefore to be ascribed to nonspecific antigenic stimulation, probably due to a gastrointestinal infection.

Talalet (7) had an epidemic outbreak of vivax malaria, with 71 cases, in 1969. Mass radical treatment was administered, mosquito control and surveillance were introduced. The sero-positivity rate was rather high in the first survey and receded until the third. After that, low-titre sero-positivity was maintained at less than 10%, without there being an indication of local malaria transmission after 1969. This area is subject to fairly intensive migration between Libya and Algeria in which the local population participates. Most of the sero-positives there were adults or adolescents.

In Ragouba (8) and El Hamma (9), initial sero-positivity was low and receded almost completely as it did in Cité Nour (10) and Sidi Harrath (11).

Haidra (12), where some malaria transmission had occurred in 1968 and 1969, had moderate initial levels of sero-positivity which have receded completely.

In Sidi Mbarek (13), positivity did not recede as fast as seen in other localities. As from the second round, most of this positivity was among complementary persons and not among previous sero-positives. Only one of the latter kept on producing positive reactions, in the sixth round with a titre of 1/160. This was a young man of 17 years who had suffered from fever attacks between 1966 and 1969. There were no fever attacks after 1969. Slides were taken again in 1973 which were negative. As seen in Table 10, another sero-positive with a titre of 1/40 was found in the same locality during the sixth round. This was a young woman of 20 years who fell ill in 1969 with fever, chills and vomiting. In March 1973 she developed the same symptoms. Slides were taken, but were negative. This locality, situated near the Algerian border, is subject to a considerable degree of population movement.

Zehoua (14), El Houamdia (15), El Guaria (17) and El Bridj (18), similar to Sidi Mbarek (13), had a low degree of initial sero-positivity, but a slight rise was uniformly observed in the second survey, a rise which was almost exclusively limited to 1/20 titres. The phenomenon resembles that observed in localities 3, 4 and 5 in the sixth round and may have a similar cause as there was no indication of malaria transmission in 1970. It is interesting to note that Tindja (16) did not follow the trend of the other localities of the same geographical group in the second round, but had a rise - exclusively titres of 1/20 - in the sixth round.

When considering sero-positivity at a titre of 1/80 as an indication of an existing or a recently passed malaria infection, 61 persons examined in the first survey do so qualify, with particular concentration in known foci. The number decreased to 32 and six in the second and third rounds and was zero in surveys four and five. In the sixth survey, there was one positive at the titre of 1/160, the young man already mentioned from Sidi Mbarek (13), who had a titre of 1/640 in the first survey and was still positive in the fifth round. In this case, the history points to a malaria infection contracted in or prior to 1969, but there were no symptoms after 1969 and an epidemiological investigation in the locality produced no indication of local malaria transmission after 1969.

(ii) By group of localities. The development of sero-positivity rates is graphically shown in Fig. 7, while the geometrical mean reciprocal titres (GMRT) of sero-positives of the total samples and of groups A and B are given in Fig. 8. The low number of sero-positives in groups C and D after the second survey did not permit the expression of the GMRT in graphical form. The initial sero-positivity rate was highest in group A, while the rates were lowest and almost identical in groups C and D, the western inland and northern maritime. This last zone was previously one of rather high endemicity and one could have expected conditions similar to those of group A. The slope following the first survey is most pronounced in group A, but evident in the other groups as well. The rise after the fifth survey is slight in groups A, C and D and more marked in the southern group B. Much of this rise is due to locality 6 where nonspecific factors were apparently responsible in the majority of low sero-titres.

The GMRT was highest in group A where it showed a clear tendency to drop faster than the sero-positivity rate. In the regression lines of the total sample (Figs 7 and 8), this feature is lost due to the relative weight of groups B, C and D, which started with lower sero-positivity rates and lower GMRTs.

### 3.5.3 Analysis by sex and age

(i) Sex. In the total sample and in the larger groups, i.e. those of 5-14 years and 15+ years, the differences are very limited. Somewhat more marked differences are occasionally found in the two younger age groups; but they were in no case significant and apparently related to the limited size of these groups. An analysis by locality groups yielded similar results and it can therefore be concluded that sero-positivity did not show differences connected with the sex (Table 11).

(ii) Age. The results of the serological examinations according to age are given in Table 12, separately for the groups of localities and the six surveys. The development of the sero-positivity rates in the various age groups are shown in Fig. 9 for the four groups of localities and the total sample, while Fig. 10 shows the development of the GMRT of sero-positives, by age group, in the total sample.

As was to be expected, the initial levels were generally lowest in the youngest age group, though that of the 2-4 years' old was usually quite close to it. The apparent reversal in the total sample is due to a distortion caused by the differing weight of the age group samples in the various groups of localities. This becomes clear from the graphs of the locality groups. In group A, surprisingly the highest percentage of sero-positives and incidentally also the highest mean titre were observed among the 5-14 years' old, while in the other locality groups the adults showed the highest levels.

In group C and more so in group D, a rise in the percentage of sero-positives was observed between the first and second survey, a rise in which the adults did not participate and which was most pronounced in the infants. This may be a phenomenon similar to that observed in locality 6, Rabta, during the sixth survey. As it was not related to any rise of malaria incidence in an area with fairly adequate surveillance, it may be indicative of factors related to other infections.

When analysing the rise of sero-positivity according to age, it is seen that the increase is highest in the youngest age group, followed by the 2-4 years' old, while the least response is observed in adults. While the rise is most pronounced in locality group B, it is practically absent in group C. As high titres were quite rare, more than 92% of all sero-positives having a titre of 1/20 only, it seems that an antigenic stimulation other than by plasmodia was responsible and from the observations in locality 6, Rabta, gastrointestinal infections should be taken into consideration. These infections find more suitable conditions to spread in warm and humid conditions, which would explain the absence of the phenomenon in the dry western inland zone. This would also be compatible with the peculiar age pattern of the rise which would be hard to interpret in the light of specific antigenic stimulation, the more so as the epidemiological malaria situation does not offer any explanation.

The recession of sero-positivity is most clearly seen in locality group A. The fastest drop is observed in the youngest age groups, while the drop is slowest in adults as is also evident in the other zones.

Infants and young children with sero-titres of 1/80+ were specially investigated:

(a) In the first survey, five infants and young children were found to have titres of 1/80+, three positive at 1/80, one at 1/160 and one at 1/640. All were from locality group A: two from El Arima (1), one from Beni Hamed (2) and two from Beni Khiair (3). Both children of El Arima were between two and four years of age at the first survey, and positive at 1/640 and 1/160 respectively; both had a history of fever and could have contracted malaria in 1968 or 1969, when there was



malaria transmission in El Arima. One of these two children also had a suppurative otitis media at the time of the first survey. Both children were completely followed up and became sero-negative in the fifth survey. The infant from Beni Hamed, born in August 1969 and positive at the titre 1/80 was febrile on the day of the survey. Brother and mother were also sero-positive. This infant died shortly afterwards from measles. Of the two infants from Beni Khlar, both positive at 1/80, one did not belong to the locality; the other has often suffered from febrile gastroenteritis and was ill at the time of the survey. Blood slides were always negative. Brother and sister died at the age of one year due to gastrointestinal toxicosis.

(b) In the second survey, three children of 2-4 years were found to be positive at a titre of 1/80. One of them, from El Arima (1), was previously positive at the titre of 1/160 and probably a malaria case having received an infection in 1968 or 1969. One was a child from Zehoua (14), who was born in France and lives in an institution at Gammarth (near Tunis). This child suffers from a mental disorder and was only for a very brief visit at Zehoua, coinciding with the second survey. There is no indication that this child had ever contracted malaria. The third child, from Tindja (16) suffers from thalassaemia, a condition which could produce nonspecific sero-positivity. There was no indication of a malaria infection. This child was often ill and admitted to Menzel Bourguiba Regional Hospital where the diagnosis of thalassaemia was confirmed.

(c) In the third survey, a child of four years at El Arima was found to be sero-positive at a titre of 1/160+. This child was found positive, though at a lower level, in the first survey, and there is the probability that it had contracted malaria in 1969. Later, the child became sero-negative.

#### 3.5.4 Analysis according to individual malaria history

In the first survey 136 former confirmed malaria cases were examined while the other 2035 persons never had malaria or had not known about it. Among the former malaria cases 31.5% were sero-positive while the percentage among the other persons was 19.7% (rates standardized for age). The reciprocal mean titres of the sero-positives in the two groups, however, were very similar with 33.63 and 34.93 respectively.

Table 13 provides details on the results during the six surveys. The data may give the impression that the proportion of sero-positives has stayed unduly high among the former malaria cases. This is explained by the fact that only the sero-positives of the first survey were followed up, not so sero-negative former malaria cases, what also accounts for the drop in the number of former malaria cases observed after the first survey. Hence the majority of former malaria cases examined after the first survey were sero-positives under follow up and the regression of sero-positivity in this group seems to be similar to that observed in the other sero-positives. A simple comparison between the two groups is given in Table 14.

Percentage of sero-positives and mean reciprocal titres do not show any appreciable differences as one would expect when the antigenic stimulus leading to sero-positivity is the same. Still, the former malaria cases had all received radical treatment, a point to be considered in para. 3.5.5. An analysis by group of localities has yielded no new aspects.

#### 3.5.5 Analysis according to previous treatment

Apart from the population of two localities, El Bouaa (4) and Talalet (7), all previously treated persons were confirmed malaria cases. In Talalet mass radical treatment was carried out in summer 1969, in El Bouaa in summer 1970, i.e. between the first and second surveys. This accounts for an increase of the number of treated persons in the second round in spite of a reduced number of former malaria cases.

Table 15 shows the serological results according to individual treatment history, suggesting a higher degree of sero-positivity among the previously treated persons, which becomes evident in Fig. 11. Such a difference is to be expected as individual treatment was confined to confirmed cases and mass radical treatment, given according to epidemiological indications in foci of then active transmission. Much of the higher sero-positivity among treated persons is concentrated in localities 4 and 7 where one observes a generally higher percentage of sero-positives and initially also higher mean titres than in the other localities (Table 16). In both localities malaria transmission had occurred more recently than in the others. As for the rise in the sixth survey an explanation has to be sought in nonspecific causes as indicated in chapter 3.5.3.

Considering the time elapsed since the treatment, it is obvious that the proportion of sero-negatives is higher in those persons who had received radical treatment one year or longer ago. In the first survey 110 persons out of the total of 236 had received treatment 12 or more months ago. Of these 22 were sero-positive (20%) while 41 of the 126 more recently treated were sero-positive (32.5%). In the third survey the same phenomenon was encountered with 4.5% of the earlier treated and 16.6% of the more recently treated persons being sero-positive.

### 3.5.6 Analysis of follow-up results

Of the 406 sero-positives found in the first survey, between 278 and 306 were reexamined in the five subsequent rounds, reexamination rates thus were ranging between 68.5% and 75.4%. Only 166 of the originally sero-positives (40.9%) presented themselves at all the subsequent surveys. In Table 17 the serological results obtained in the originally sero-positives as available throughout the follow-up are compared with those in the 166 which constitute a proper cohort.

The comparison of percentage of sero-positives and mean reciprocal titres in these two groups (see Fig. 12) demonstrates a close resemblance. Thus it may seem to be permissible to utilize the data obtained from all originally sero-positives available. An analysis by locality group indicates, however, that the rate of complete follow-up was subject to wide variation (see Table 18 and Fig. 13). While the rate of complete follow-up was lowest in group A, well above average in group B, and highest in group C, it was found that the age groups of 2-4 and 5-14 years were followed best. Analysing according to the type of locality, a lesser follow-up rate was recorded in the semi-urban communities (Nos. 3, 5, 10, 14, 16), while the rural localities produced higher rates, as was to be expected. In the semi-urban places the 2-4 years' old were followed up the best, while it was the age group of 5-14 years in the rural localities.

In view of the differences of rates of partial and complete follow-up it was decided to limit further analysis to the cohort of 166 persons which were followed throughout.

In the cohort the percentage of sero-positives shows a fast regression between spring 1970 and spring 1971, i.e. from first to third survey (Fig. 12), while a slight rise is seen in the fourth survey, a phenomenon which is repeated to a lesser extent in the sixth round. These rises were due to positivity at low titres, almost exclusively 1/20. An analysis by group of localities (Fig. 14) indicates that the rise in the fourth survey was most marked in group A, while both groups A and B participated in the rise during the sixth round. The analysis by age (Fig. 15) shows that the rise in the fourth round involves particularly the 2-4 years' old, followed by the youngest age group, while in the higher age groups there was little or no reaction. This suggests that a nonspecific antigenic stimulation was responsible for this phenomenon.

The general serological development is summarized in Table 19, comparing the results at discriminative titres of 1/20+ and 1/40+.

Conversion to sero-negativity between first and second round was relatively slower than between second and third round. A high number of reversions to sero-positivity was observed between the third and fourth rounds at titre level 1/20+ only. The phenomenon is transient and not seen at titre level 1/40+, thus indicating its nonspecific nature. The same applies to the apparent rise between fifth and sixth survey which is practically limited to 1/20 titres.

Normally most of the titre changes, increases and decreases, extended over one titre step only. Occasionally, only in the earlier period, changes over a wider range of concentrations were observed; these were exclusively decreases.

The regression of the sero-positivity rate at a discriminative titre level of 1/40+ corresponds, in this cohort, to an overall rate of approximately 83% per annum.

### 3.5.7 Analysis in relation to the epidemiological situation

In analysing the regional trends it appears appropriate to relate percentage of sero-positives (1/20+) to the slide positivity rate rather than the malaria incidence as the epidemiological data prior to the beginning of 1968 are not based on surveillance activities. In Fig. 16 these indices are given for the areas with adequate data, i.e. for the Governorates of Cap Bon, Kasserine, and Bizerte, while the data for the three southern Governorates are combined.

The curves bear close resemblance, in particular those with relatively high starting points of both slide positivity rate and sero-positivity rate, e.g. for Cap Bon and the southern Governorates, especially if the nonspecific rises of the sixth survey are disregarded. The serological trend confirms the epidemiological picture as obtained by surveillance.

For a global comparison, malaria incidence and sero-positivity rates of the whole northern operational area under malaria eradication are illustrated in Fig. 17, using for the sero-positivity rate the titre levels of 1/20+ and 1/40+. It is seen that the sero-positivity rate at 1/40+ moves with due delay practically parallel to the incidence line. This has also permitted the elimination of the apparently nonspecific rise observed in the 1/20+ titre line in the sixth round.

Analysing the regression in the various age groups using 1/40+ as the discriminating titre as done in Fig. 18 is more illustrative than at titres 1/20+ (Fig. 9). The adults started off with the highest sero-positivity rate which was also the slowest to recede. Next, as to be expected, followed the 5-14 years' old. The youngest age group surprisingly had a higher initial sero-positivity rate than the 2-4 years' old. This could have been to some extent due to the sample of the 6-23 months' old being smallest and therefore least homogenous. This group has shown the fastest regression.

Among persons born and exposed to malaria transmission pre-operationally, only the mechanism of expiring contact with the antigen after interruption of transmission is apt to produce a regression of sero-positivity. At the time of the first survey most of the 2-4 years' old were born in the pre-operational era but time-wise had less opportunity of acquaintance with malaria than did older persons. The 6-23 months' age group, initially mainly composed of infants born in the first year of the attack phase, was in course of the study period completely replenished by infants born in the third or fourth year of the attack phase or even in the consolidation phase. Maintained negativity in this group also indicates absence of malaria transmission.

On the whole, the serological results seem to confirm the conclusions reached by classical epidemiological evaluation. The sero-positivity recedes at about the same rate as malaria incidence did before. Apart from some focal activity in El Bouaa (4) in 1970 there were no signs of malaria transmission in the localities studied between 1970 and 1972.

#### 4. DISCUSSION

The studies described in this paper have aimed at developing the serological picture in relation to the epidemiological features of malaria in Tunisia. In view of yet undefined limits and interpretation of serological results it was necessary to conduct these exploratory observations in association with a programme having qualitatively adequate surveillance and having achieved operational success to such an extent that the range of epidemiological heterogeneity was not too wide.

In this respect Tunisia offered suitable opportunities: since its inception in 1966/67 the malaria eradication programme had made steady progress and interruption of malaria transmission was achieved in the majority of areas within the first year of the attack phase. The fact that the programme started at fairly low parasite reservoir levels and that surveillance was introduced at a very early stage had produced rapid success and it is felt that the sero-epidemiological studies would have yielded even more information, had they started one or two years earlier.

Besides indications of residual malaria transmission in a focus with recent proven transmission and of the prolonged existence of potential reservoir in another locality, the serological studies provided valuable information on trends which conform with the picture of disappearing malaria in Tunisia.

For the IFAT it would certainly have been preferable to use the specific homologous antigens, i.e. P. vivax and P. falciparum antigens. Lacking these antigens and in view of the prevalence of vivax infections in Tunisia and particularly in the areas under study, the use of P. cynomolgi bastianellii was the next best solution. Although heterologous, this antigen provides quite reliable detection of vivax fluorescent antibodies and a fair proportion of falciparum infections is picked up as well. It would have been interesting to run tests with P. malariae antigen too since this species was not recorded in Tunisia for several years before the start of the eradication programme.

Sero-titres of 1/80+ indicate with high probability recent or continuing individual malaria experience and could therefore be used as monitors which are more sensitive than parasitological blood examination. For trend studies, however, it seems to be justified to set the critical sero-titres lower. While under the local conditions of Tunisia the limit of 1/20+ is obviously subject to some nonspecific interference, the limit of 1/40+ seems to be nearly free of such interference. For these trend studies the sero-positivity rate has generally provided more valuable information than did the mean reciprocal titres of sero-positives.

The studies have met the objectives mentioned in para. 1.2. It was possible to assess the degree of malaria sero-positivity and to relate it to the past and current epidemiological situation in Tunisia. They have also permitted the evaluation of the impact of malaria eradication operations and made evident an age-gradient of regression, as one would expect according to the different degrees of malaria experience in the various age groups, especially after transmission had been interrupted. Former confirmed malaria cases were more frequently sero-positive than persons without or with doubtful malaria history. But the rate of regression was the same in both groups - probably the result of radical treatment which had been given in every confirmed case. Among the treated, whether former confirmed malaria cases or mass-treated persons, sero-positivity was more frequent among those who had received the treatment more recently.

In the absence of marked post-seasonal rises of sero-positivity (1/40+) in the second, fourth and sixth surveys there was nothing to suggest maintained malaria transmission except in El Bouaa (4) in the summer of 1970. Considering the limited information provided by the third and fifth surveys one may question, under the conditions of Tunisia, the necessity of such intermediate surveys when the objective of the serological observations is to clarify the epidemiological trend and to measure the progress of a programme.

Under these circumstances one serological survey per year may very well suffice in areas with seasonal malaria transmission and may suitably be conducted some six to eight weeks after the end of the transmission season, so as to give the latest infected persons the possibility to produce detectable antibodies. In areas with vivax infections showing a high frequency of delayed incubation this conclusion may not apply and an intermediate serological survey might be indicated at the beginning of the transmission season. In areas with perennial malaria transmission, mostly areas with high malariogenic potential, it may be necessary to assess the profiles at shorter intervals.

Sampling in these studies experienced some difficulties, especially regarding the regular follow-up of old sero-positives. It was shown that the age groups of 2-4 and 5-14 years are the most accessible. Depending on the progress of a programme, most of the essential information can usually be gathered from these two age groups, except that which can only be provided by observations on infants. For a more detailed analysis, especially in advanced programmes, it may be appropriate to choose narrower divisions in the younger age groups, e.g. 6-23 months, 2-3, 4-5, 6-7, 8-9, 10-14 years, but it is realized that it may be difficult to obtain sufficient group sizes.

The problem of following the sero-positives of the first survey was technically solved by proper recording and from the third survey onwards by stimulating cooperation. Migration and death were the main cause of absenteeism. Only 40.9% of the original group have participated in all six surveys. Retrospectively it would have been interesting to follow also a cohort of initially sero-negatives in view of potential conversion to sero-positivity. In the case of Tunisia the virtual absence of high titres after the third round indicates post hoc that such follow-up was not necessary under these particular circumstances.

In these studies serological technique has proved to be an epidemiological tool which may be suitable to fill the methodological gap in the assessment of malaria eradication operations in the advanced attack and the consolidation phases. For the early attack phase - leaving aside the particular case of Tunisia - direct parasitological evaluation as laid out in the Tenth Report of the WHO Expert Committee on Malaria (1964) and described by Black (1968) is adequate. It becomes insufficiently sensitive and reliable once the parasite rate is about to descend below the level of 1-2%. This is the time when active and passive case detection are to take over as an instrument of surveillance, monitoring and evaluation. As many examples have shown, case detection alone may not give a realistic picture of the epidemiological situation and therefore it would be desirable to use a tool which takes over from and links with parasitological surveys, when the latter are about to become obsolete.

When starting from high malaria endemicity levels not much is to be gained from serological evaluation since a uniformly high contact with malaria is apt to keep sero-positivity at high levels. In our opinion it would be appropriate to introduce serological assessment post-seasonally before the parasite rate is expected to drop below the level of 1%, in the case of areas of seasonal malaria transmission, or about six months before this critical level is going to be passed in areas with perennial transmission. At this stage a fairly high sero-positivity rate and high mean reciprocal titres are to be expected, but judging from the experience in Tunisia, a rapid regression will set in once the annual parasite incidence drops below 0.1 per thousand population. From then onwards the slope of the sero-positivity rate, in our studies with a critical titre level fixed at 1/40+ and an annual regression to approximately 17% of the previous year's level, closely resembles the ideal slope of parasite rates if malaria transmission is interrupted. For the practical execution of this type of trend study it would be important to maintain continuity with early attack phase assessment, i.e. to carry on with serological evaluation in the selected indicator areas and to proceed with the estimates for sample size according to recognized statistical procedure, replacing the expected parasite rate by the expected sero-positivity rate based on the estimated ideal rate of regression.

For this kind of routine evaluation, it would be indicated to choose a follow-up system, i.e. to conduct it on a cohort, whether initially sero-positive or not. Significant titre rises, especially conversion to sero-positivity of formerly sero-negatives, will point to persistence of malaria transmission, while a general regression conforming with the ideal rate will confirm the status of interrupted malaria transmission.

Apart from routine epidemiological assessment of the progress of malaria eradication it appears that serological methods are particularly suited to be applied in epidemiological investigations around recently detected malaria cases and in the follow-up of foci. The former to detect sources of transmission which may have ceased to show overt parasitaemia at the time of the case investigation and are largely responsible for the classification "cryptic", and to detect asymptomatic collateral cases. The latter to demonstrate potential focal activities which may escape attention if the classical methods of focal follow-up are applied.

It would also be useful to use serological methods in parallel with the classical technique in the follow-up of confirmed, treated malaria cases. This would provide valuable information on the efficacy of treatment as sero-titre rises over two dilutions will with near certainty be indicative of a persistence of the old infection unless a new one was acquired.

## 5. CONCLUSIONS

(a) The sero-epidemiological studies in Tunisia have permitted the assessment of the contribution that this serological technique may provide in the evaluation of a malaria eradication programme in an advanced stage, which has the characteristic features of disappearing malaria.

(b) The degree of sero-positivity observed in the various geographical regions of Tunisia generally corresponded to the past and recent epidemiological history, with higher levels in areas which had maintained higher degrees of malaria endemicity and experienced malaria transmission until the start of malaria eradication or even in the beginning of the attack phase.

(c) The age differential of sero-positivity shows the evidence of cumulative malaria experience in the higher age groups, while the sero-positivity was less in the younger groups where it also disappeared faster than in adults.

(d) Sero-positivity was more frequently found among former confirmed malaria cases than among persons without or with only doubtful malaria history. The rate of regression was the same in both categories, probably under the influence of treatment which was given to each confirmed case.

(e) Sero-positivity was higher among the recently treated than among those having received treatment earlier. Since treatment was only given in cases of confirmed existence of parasitaemia or particular exposure to infection, this difference expresses mainly an expected time-related regression of sero-positivity following the cessation of contact with the antigen.

(f) Serological results have shown that signs of active transmission were evident in one locality in summer 1970, and have indicated the potential persistence of an infection in another locality.

(g) In the case of Tunisia it was found that serological trend studies based on a critical titre of 1/20+, though generally providing useful information, seasonally tend to be subject to nonspecific interference in the lowest titre range.

(h) The annual regression of sero-positivity at titres 1/40+ corresponds to a drop to approximately 17% of the previous year's level and thus resembles the natural regression of parasite reservoir following the interruption of malaria transmission.

(i) The regression of sero-positivity confirms the epidemiological trends in the various parts of Tunisia covered by the study and has, with the exception mentioned under (f), indicated the continued absence of malaria transmission.

(j) A cohort study on initially sero-positives, completely successful in 40.9% of this group, has shown that follow-up in the age groups of 2-4 and 5-14 years was more satisfactory than in the youngest group and in adults.

(k) The sero-epidemiological observations have met the objectives of the study. One more survey may be indicated in autumn 1974 to assess the later development of the sero-epidemiological situation.

(l) The study has shown that this serological technique can be used as a valuable tool in epidemiological assessment which may advantageously be used for routine evaluation in antimalaria programmes in advanced stages, for epidemiological investigations around confirmed malaria cases and for the follow-up of individual cases and foci.

#### SUMMARY

In a longitudinal study between April 1970 and October 1972 a total of 13 044 persons in 18 selected localities of nine Governorates in Tunisia were examined parasitologically for the presence of malaria parasites in the peripheral blood and serologically for the presence of fluorescent malaria antibodies using the heterologous P. cynomolgi bastianellii antigen. Six surveys were conducted at six-monthly intervals.

While parasitological examinations yielded negative results with the exception of one vivax infection detected in the first round, the initial sero-positivity rate at a titre level of 1/20+ was 18.7% and 8.8% at a titre level of 1/40+, with generally higher sero-positivity in the eastern maritime and southern regions. There was a clear age gradient, showing higher sero-positivity, qualitatively and quantitatively, in the older groups.

The sero-positives detected in the first survey were followed up, reexamination rates varying between 68.5% and 75.4% per survey and 166 persons (40.9%) participated in all six surveys.

The serological results indicated residual malaria transmission in one locality in summer 1970. In the other localities sero-positivity (1/40+) receded from the first survey onwards, showing a regression rate which is very similar to that of the natural regression of malaria infections after interruption of transmission. When using a discriminative titre level of 1/20+ nonspecific titre rises were seen, usually in the summer seasons and normally confined to the lowest titre (1/20).

The serological results were evaluated and correlated to the past and present epidemiological situation in Tunisia. The serological picture corresponds to that of disappearing malaria in the absence of new infections. It has been shown that this serological technique is a suitable tool for complementing epidemiological assessment and would be particularly valuable in antimalarial operations once the parasite rate has dropped below the level at which classical evaluation through mass surveys in indicator areas is meaningful.

#### ACKNOWLEDGEMENTS

The writers are grateful for the assistance received from the late Dr T. Hachicha, Director Preventive Medicine, Ministry of Health of Tunisia, and for the most valuable technical cooperation of Dr R. Vande Voorde and Mr T. Redissi, of the personnel of the Malaria Eradication Programme and all who have facilitated these studies. Miss A. Decuyper, Technician at the Laboratory of Parasitology, Faculty of Medicine, University of Grenoble, be thanked for her untiring assistance in serological testing. Thanks also are due to the Comité national de Solidarité, Tunis, and the World Food Programme, whose assistance has to a large measure secured the continuity of observations.

#### RESUME

Au cours d'une étude longitudinale qui s'est déroulée d'avril 1970 à octobre 1972, un total de 13 044 personnes habitant 18 localités situées dans 9 gouvernorats de Tunisie ont été soumises à des examens parasitologiques visant à établir la présence d'hématozoaires dans le sang périphérique, ainsi qu'à des examens sérologiques pour la recherche des anticorps fluorescents du paludisme au moyen de l'antigène hétérologue de Plasmodium cynomolgi bastinaellii. L'étude a comporté six enquêtes, faites à intervalles de six mois.

Si les épreuves parasitologiques ont donné des résultats négatifs, mis à part une infection à P. vivax détectée au cours de la première enquête, le taux initial de séro-positivité atteignait 18,7 % pour le titre de 1/20+ et 8,8 % pour le titre de 1/40+, les valeurs les plus hautes étant généralement enregistrées sur la côte est et dans le sud. Une nette gradation en fonction de l'âge a été constatée : qualitativement aussi bien que quantitativement, la séroposivité était plus marquée chez les sujets âgés.

Les cas séro-positifs détectés au cours de la première enquête ont été suivis, avec des taux de réexamen variant entre 68,5 % et 75,4 % par enquête; au total, 166 personnes (40,9 %) ont participé à chacune des six enquêtes.

Les résultats des épreuves sérologiques ont montré que dans l'une des localités, il y avait eu, au cours de l'été 1970, une transmission résiduelle du paludisme. Ailleurs, la séro-positivité (1/40+) s'est constamment abaissée après la première enquête et le taux de régression a été très semblable à celui qui s'établit naturellement pour le paludisme après interruption de la transmission. En prenant comme titre critique celui de 1/20+, on a observé, généralement au cours de l'été, des accroissements non spécifiques des titres, le plus souvent limités au bas de l'échelle (1/20).

Les résultats des enquêtes sérologiques ont été évalués et analysés en fonction de la situation épidémiologique passée et présente en Tunisie. Le tableau qui en résulte correspond à celui du paludisme en voie de disparition en l'absence de nouvelles infections. On a pu ainsi montrer que la technique sérologique employée est un bon moyen de compléter les évaluations épidémiologiques et serait particulièrement utile dans les opérations antipaludiques, lorsque l'indice plasmodique est descendu à un niveau où les évaluations classiques par enquête systématique dans les secteurs témoins ne sont plus significatives.



REFERENCES

- Ambroise-Thomas, P. & Kien Truong, T. (1968) Nouvelle technique de micro-prélèvements sanguins par la réaction d'immune-fluorescence, Bull. Ass. Dipl. Microbiol., Nancy, 112, 29-33
- Ambroise-Thomas, P. et al. (1969) L'immune-fluorescence indirecte dans le séro-diagnostic du paludisme. Modalités techniques et principaux résultats, Rev. Inst. Pasteur, Lyon, 2, 275-287
- Ambroise-Thomas, P. et al. (1972) Réapparition du paludisme en Corse. Intérêt du dépistage séro-épidémiologique, Bull. Soc. Path. Exot., 65, 533-542
- Black, R. H. (1968) Manual of epidemiology and epidemiological services in malaria programmes. WHO, Geneva, Offset publ. ME/68.5
- Collins, W. E. et al. (1967) Fluorescent antibody studies in human malaria. V. Response of sera from Nigerians to five Plasmodium antigens, Amer. J. trop. Med. Hyg., 16, 568-571
- Collins, W. E. et al. (1968) Studies on the relationship between fluorescent antibody response and ecology of malaria in Malaysia, Bull. Wld Hlth Org., 39, 451-463
- Collins, W. E. et al. (1971) Serological malaria survey in the Ethiopian Highlands, Amer. J. trop. Med. Hyg., 20, 199-205
- Collius, W. E. & Skinner, J. C. (1972) Amer. J. trop. Med. Hyg., 21, 690-695
- Dranga, A. et al. (1969) Contribution à l'étude de l'immunité résiduelle en Roumanie, Bull. Wld Hlth Org., 40, 753-761
- Draper, C. C. et al. (1972) The epidemiologic interpretation of serologic data in malaria, Amer. J. trop. Med. Hyg., 21, 696-703
- Lelijveld, J. L. M. (1971) Sero-epidemiological studies of malaria in Tanzania, Doctors Dissertation, Catholic University, Nijmegen, Netherlands, 137
- Meuwissen, J. H. E. Th. (1966) Fluorescent antibodies in human malaria, especially in Plasmodium ovale, Trop. Geogr. Med., 18, 250-259
- McGregor, I. A. et al. (1965) Immunofluorescence and the measurement of immune response to hyperendemic malaria, Trans. Roy. Soc. Trop. Med. Hyg., 59, 395-414
- Sulzer, A. J. & Wilson, M. (1967) The use of thick-smear antigen slides in the malaria fluorescent antibody test, J. Parasit., 53, 1110-1111
- Vaisman, A., Hamelin, A. & Guthe, T. (1963) Bull. Wld Hlth Org., 29, 1-6
- Voller, A. & Bruce-Chwatt, L. J. (1968) Serological malaria surveys in Nigeria, Bull. Wld Hlth Org., 39, 883-897
- World Health Organization (1964) WHO Expert Committee on Malaria, Tenth Report, Techn. Rep. Ser., No. 272, WHO, Geneva

TABLE 1. EPIDEMIOLOGICAL DATA ON MALARIA IN TUNISIA, 1966-1972

| Year | Number of slides |          | $\frac{a}{\text{ABER}}$ | $\frac{b}{\text{SPR}}$ | $\text{API}^c$<br>(per thousand population) |   |
|------|------------------|----------|-------------------------|------------------------|---|---|
|      | Examined         | Positive |                         |                        | Northern<br>Zone<br>(MEP)                   | Southern Zone<br>(intensified<br>control) |
| 1966 | 93 494           | 4 790    | 2.06                    | 5.12                   | 1.259                                       | 0.108                                     |
| 1967 | 107 847          | 2 360    | 2.32                    | 2.19                   | 0.593                                       | 0.100                                     |
| 1968 | 193 779          | 2 200    | 3.92                    | 1.14                   | 0.541                                       | 0.075                                     |
| 1969 | 536 198          | 457      | 10.58                   | 0.085                  | 0.091                                       | 0.099                                     |
| 1970 | 508 804          | 94       | 9.81                    | 0.018                  | 0.0073                                      | 0.073                                     |
| 1971 | 469 933          | 100      | 8.68                    | 0.021                  | 0.0025                                      | 0.101                                     |
| 1972 | 449 552          | 19       | 8.11                    | 0.004                  | 0.0009                                      | 0.016                                     |

$\frac{a}{\text{ABER}}$  = Annual Blood Examination Rate (in % of total population).

$\frac{b}{\text{SPR}}$  = Slide Positivity Rate (in %).

$\frac{c}{\text{API}}$  = Annual Parasite Incidence (number of confirmed malaria cases per thousand population).

TABLE 2. GENERAL DATA OF LOCALITIES SELECTED FOR SERO-EPIDEMIOLOGICAL STUDIES

| Locality |             | Governorate       | Group | Size of population | Characteristics               | Spraying operations (DDT) | Mass radical treatment | ACD <sup>a</sup> | PCD <sup>b</sup> | Number of confirmed malaria cases |      |      |      |           |
|----------|-------------|-------------------|-------|--------------------|-------------------------------|---------------------------|------------------------|------------------|------------------|-----------------------------------|------|------|------|-----------|
| Code No. | Code letter |                   |       |                    |                               |                           |                        |                  |                  | 1967                              | 1968 | 1969 | 1970 | 1971 1972 |
| 01       | A           | El Arima          | A     | 251                | Rural, agricult.              | 1968-1971                 | -                      | 1968-            | 1968-            | ?                                 | 0    | 4    | 0    | 0         |
| 02       | B           | Beni Hamed        | A     | 300                | Rural, agricult.              | 1968-1971                 | -                      | 1968-            | 1968-            | ?                                 | 19   | 0    | 1    | 0         |
| 03       | C           | Beni Khia         | A     | 8 711              | Semirural suburb (Nabeul)     | 1968-1970                 | -                      | 1968-            | 1968-            | ?                                 | 5    | 0    | 0    | 0         |
| 04       | D           | El Bouaa          | B     | 1 000              | Rural, past.-agricult.        | 1971, 1972                | 1970                   | 1968-            | 1968-            | ?                                 | 3    | 97   | 2    | 2         |
| 05       | E           | Merkez Ben Halima | A     | 1 370              | Suburb of Sfax                | 1968                      | -                      | 1968-            | 1968-            | 1                                 | 0    | 0    | 0    | 0         |
| 06       | F           | Rabta             | B     | 1 408              | Oasis                         | -                         | -                      | 1972-            | 1968-            | ?                                 | 0    | 2    | 0    | 0         |
| 07       | H           | Talalet           | B     | 528                | Oasis                         | -                         | 1969                   | -                | 1968-            | ?                                 | 0    | 71   | 0    | 0         |
| 08       | K           | Ragouba           | B     | 1 191              | Rural, past.-agricult.        | -                         | -                      | -                | 1968-            | ?                                 | 0    | 0    | 0    | 0         |
| 09       | L           | El Hama           | B     | 891                | Oasis                         | -                         | -                      | -                | 1968-            | ?                                 | 0    | 2    | 0    | 0         |
| 10       | M           | Cité Nour         | C     | 11 502             | Quarter of Kasserine          | -                         | -                      | 1968-1971        | 1968-            | ?                                 | 12   | 2    | 0    | 0         |
| 11       | N           | Sidi Harrath      | C     | 1 101              | Rural, past.-agricult.        | 1968-1970                 | -                      | 1968-            | 1968-            | ?                                 | 5    | 0    | 0    | 0         |
| 12       | O           | Haidra            | C     | 1 271              | Rural, past.-agricult.        | 1968-1972                 | -                      | 1968-            | 1968-            | ?                                 | 2    | 4    | 0    | 0         |
| 13       | P           | Sidi Mbarek       | C     | 487                | Rural, agricult.              | 1968-1971                 | -                      | 1968-            | 1968-            | ?                                 | 8    | 0    | 0    | 0         |
| 14       | R           | Zehoua            | C     | 1 836              | Suburb of Jendouba            | 1968-1971                 | -                      | 1968-            | 1968-            | ?                                 | 1    | 1    | 0    | 0         |
| 15       | S           | El Houamdia       | D     | 1 673              | Rural, agricult.-forestry     | 1968-1971                 | -                      | 1968-            | 1968-            | ?                                 | 8    | 0    | 0    | 0         |
| 16       | T           | Tindja            | D     | 3 305              | Semirural suburb (Ml. Bourg.) | 1968-1971                 | -                      | 1968-            | 1968-            | ?                                 | 1    | 1    | 0    | 0         |
| 17       | X           | El Guaria         | D     | 574                | Rural, agricult.              | 1968-1971                 | -                      | 1968-            | 1968-            | ?                                 | 66   | 7    | 0    | 0         |
| 18       | Y           | El Bridj          | D     | 359                | Rural, agricult.              | 1968-1970                 | -                      | 1968-            | 1968-            | 23                                | 1    | 0    | 0    | 0         |

<sup>a</sup> ACD = Active case detection.

<sup>b</sup> PCD = Passive case detection.

TABLE 3. AGE AND SEX COMPOSITION OF SAMPLE, HISTORY RECORDS  
FIRST SURVEY, BY LOCALITY

| Locality |   | Number examined |             |             |       |     |       |        |       |       |     |       |     | Having suffered from malaria |        | Having received malaria treatment |     | With family member having suffered from malaria |      |
|----------|---|-----------------|-------------|-------------|-------|-----|-------|--------|-------|-------|-----|-------|-----|------------------------------|--------|-----------------------------------|-----|---|------|
|          |   | Male            |             |             |       |     |       | Female |       |       |     |       |     |                              |        |                                   |     |   |      |
|          |   | Serial No.      | Code letter | Grand total | 6-23M | 2-4 | 5-14  | 15+    | Total | 6-23M | 2-4 | 5-14  | 15+ | Total                        | Number |                                   |     |   |      |
| 1        | A |                 |             |             | 120   | 5   | 11    | 19     | 27    | 62    | 4   | 6     | 24  | 24                           |        | 58                                | 2   | 1.7   | 2    |
| 2        | B | 115             | 6           | 6           | 31    | 15  | 58    | 1      | 5     | 15    | 36  | 57    | 6   | 5.2                          | 6      | 5.2                               | 3   | 2.6   |      |
| 3        | C | 114             | 18          | 8           | 20    | 15  | 61    | 10     | 7     | 16    | 20  | 53    | 4   | 3.5                          | 4      | 3.5                               | 8   | 7.0   |      |
| 4        | D | 120             | 4           | 9           | 21    | 15  | 49    | 1      | 12    | 22    | 36  | 71    | 34  | 28.3                         | 34     | 28.3                              | 14  | 11.7  |      |
| 5        | E | 120             | 3           | 13          | 18    | 35  | 69    | 5      | 12    | 15    | 19  | 51    | 0   | 0                            | 0      | 0                                 | 0   | 0   | 0    |
| 6        | F | 124             | 10          | 16          | 12    | 14  | 52    | 12     | 12    | 15    | 33  | 72    | 2   | 1.6                          | 2      | 1.6                               | 2   | 1.6   | 1.6  |
| 7        | H | 120             | 6           | 11          | 16    | 14  | 47    | 2      | 13    | 22    | 36  | 73    | 19  | 15.8                         | 119    | 99.2                              | 15  | 12.5  | 12.5 |
| 8        | K | 120             | 7           | 9           | 24    | 13  | 53    | 5      | 8     | 24    | 30  | 67    | 0   | 0                            | 0      | 0                                 | 0   | 0   | 0    |
| 9        | L | 117             | 1           | 14          | 13    | 26  | 54    | 3      | 7     | 18    | 35  | 63    | 1   | 0.9                          | 1      | 0.9                               | 0   | 0   | 0    |
| 10       | M | 120             | 5           | 14          | 23    | 16  | 58    | 5      | 13    | 20    | 24  | 62    | 7   | 5.8                          | 7      | 5.8                               | 26  | 21.7  | 21.7 |
| 11       | N | 120             | 11          | 17          | 19    | 17  | 64    | 6      | 11    | 24    | 15  | 56    | 3   | 2.5                          | 3      | 2.5                               | 24  | 20.0  | 20.0 |
| 12       | O | 120             | 2           | 20          | 27    | 15  | 64    | 4      | 12    | 20    | 20  | 56    | 3   | 2.5                          | 3      | 2.5                               | 6   | 5.0   | 5.0  |
| 13       | P | 120             | 3           | 10          | 26    | 19  | 58    | 4      | 7     | 22    | 29  | 62    | 2   | 1.7                          | 2      | 1.7                               | 8   | 6.7   | 6.7  |
| 14       | R | 120             | 4           | 18          | 19    | 17  | 58    | 7      | 11    | 28    | 16  | 62    | 4   | 3.3                          | 4      | 3.3                               | 17  | 14.2  | 14.2 |
| 15       | S | 120             | 6           | 15          | 18    | 18  | 57    | 9      | 18    | 20    | 16  | 63    | 6   | 5.0                          | 6      | 5.0                               | 7   | 5.8   | 5.8  |
| 16       | T | 132             | 10          | 15          | 21    | 17  | 63    | 9      | 21    | 24    | 15  | 69    | 3   | 2.3                          | 3      | 2.3                               | 0   | 0   | 0    |
| 17       | X | 122             | 11          | 13          | 20    | 14  | 58    | 4      | 19    | 19    | 22  | 64    | 10  | 8.2                          | 10     | 8.2                               | 36  | 29.5  | 29.5 |
| 18       | Y | 127             | 8           | 12          | 23    | 22  | 65    | 2      | 13    | 22    | 25  | 62    | 30  | 23.6                         | 30     | 23.6                              | 13  | 10.2  | 10.2 |
| TOTAL    |   | 2 171           | 120         | 231         | 370   | 331 | 1 050 | 93     | 207   | 370   | 449 | 1 121 | 136 | 6.3                          | 236    | 10.9                              | 179 | 8.2   | 8.2  |

TABLE 4. COMPOSITION OF SAMPLE, FIRST SURVEY, BY GROUP OF LOCALITIES

| Group of localities | Number examined |       |     |      |     |       |        |     |      |     | Having suffered from malaria |        | Having received malaria treatment |        | With family member having suffered from malaria |        |      |
|---------------------|-----------------|-------|-----|------|-----|-------|--------|-----|------|-----|------------------------------|--------|-----------------------------------|--------|---|--------|------|
|                     | Grand total     | Male  |     |      |     |       | Female |     |      |     |                              |        |                                   |        |   |        |      |
|                     |                 | 6-23M | 2-4 | 5-14 | 15+ | Total | 6-23M  | 2-4 | 5-14 | 15+ | Total                        | Number | %                                 | Number | %   | Number | %    |
| A                   | 469             | 32    | 38  | 87   | 92  | 249   | 20     | 30  | 71   | 99  | 220                          | 12     | 2.6                               | 12     | 2.6   | 11     | 2.3  |
| B                   | 601             | 29    | 58  | 87   | 82  | 256   | 22     | 53  | 100  | 170 | 345                          | 56     | 9.3                               | 156    | 26.0  | 31     | 5.2  |
| C                   | 600             | 25    | 79  | 114  | 84  | 302   | 26     | 54  | 114  | 104 | 298                          | 19     | 3.2                               | 19     | 3.2   | 81     | 13.5 |
| D                   | 501             | 34    | 56  | 82   | 71  | 243   | 25     | 70  | 85   | 78  | 258                          | 49     | 9.8                               | 49     | 9.8   | 56     | 11.2 |
| Total               | 2 171           | 120   | 231 | 370  | 329 | 1 050 | 93     | 207 | 370  | 451 | 1 121                        | 136    | 6.3                               | 236    | 10.9  | 179    | 8.2  |

TABLE 5. COMPOSITION OF SAMPLES IN FIRST TO SIXTH SURVEY, GLOBAL DATA

| Serial<br>number<br>of survey | Number examined |       |     |      |     |       |        |     |      |     |       |     | Having<br>suffered<br>from malaria |     | Having received<br>malaria treatment |     | With family member<br>having suffered<br>from malaria |  |
|-------------------------------|-----------------|-------|-----|------|-----|-------|--------|-----|------|-----|-------|-----|------------------------------------|-----|--------------------------------------|-----|---|--|
|                               | Grand<br>total  | Male  |     |      |     |       | Female |     |      |     |       |     |                                    |     |                                      |     |   |  |
|                               |                 | 6-23M | 2-4 | 5-14 | 15+ | Total | 6-23M  | 2-4 | 5-14 | 15+ | Total |     |                                    |     |                                      |     |   |  |
|                               |                 |       |     |      |     |       |        |     |      |     |       |     |                                    |     |                                      |     |   |  |
| 1                             | 2 171           | 120   | 231 | 370  | 329 | 1 050 | 93     | 207 | 370  | 451 | 1 121 | 136 | 6.3                                | 236 | 10.9                                 | 179 | 8.2   |  |
| 2                             | 2 182           | 120   | 203 | 375  | 351 | 1 049 | 96     | 206 | 371  | 460 | 1 133 | 35  | 1.6                                | 261 | 12.0                                 | 19  | 0.9   |  |
| 3                             | 2 173           | 88    | 177 | 474  | 321 | 1 060 | 71     | 161 | 433  | 448 | 1 113 | 51  | 2.3                                | 280 | 12.9                                 | 150 | 6.9   |  |
| 4                             | 2 170           | 86    | 218 | 375  | 358 | 1 037 | 90     | 199 | 359  | 485 | 1 133 | 39  | 1.8                                | 245 | 11.3                                 | 122 | 5.6   |  |
| 5                             | 2 175           | 84    | 216 | 384  | 375 | 1 059 | 94     | 206 | 383  | 433 | 1 116 | 31  | 1.4                                | 203 | 9.3                                  | 69  | 3.2   |  |
| 6                             | 2 173           | 83    | 192 | 421  | 391 | 1 087 | 80     | 188 | 374  | 444 | 1 086 | 28  | 1.3                                | 225 | 10.4                                 | 48  | 2.2   |  |

TABLE 6. COMPARISON BETWEEN EXPECTED AND OBSERVED AGE  
PERCENTAGE DISTRIBUTION OF THE SAMPLES

| Age groups | Age percentage distribution |                       |      |      |      |      |      |
|------------|-----------------------------|-----------------------|------|------|------|------|------|
|            | Expected                    | At successive surveys |      |      |      |      |      |
|            |                             | 1st                   | 2nd  | 3rd  | 4th  | 5th  | 6th  |
| 6-23M      | 10.9                        | 9.8                   | 9.9  | 7.3  | 8.1  | 8.2  | 7.5  |
| 2-4 years  | 19.8                        | 20.2                  | 18.7 | 15.6 | 19.2 | 19.4 | 17.5 |
| 5-14 years | 35.3                        | 34.1                  | 34.2 | 41.7 | 33.8 | 35.3 | 36.6 |
| 15+ years  | 34.1                        | 35.9                  | 37.2 | 35.4 | 38.8 | 37.1 | 38.4 |
| Total      | 100                         | 100                   | 100  | 100  | 100  | 100  | 100  |

TABLE 7. FOLLOW-UP OF ORIGINALLY SERO-POSITIVES THROUGH FIVE  
RESURVEYS, BY GROUP OF LOCALITIES

| Group of localities | No. in 1st survey | Resurveys |      |     |      |     |      |     |      |     |      |
|---------------------|-------------------|-----------|------|-----|------|-----|------|-----|------|-----|------|
|                     |                   | 2nd       |      | 3rd |      | 4th |      | 5th |      | 6th |      |
|                     |                   | No.       | %    | No. | %    | No. | %    | No. | %    | No. | %    |
| A                   | 207               | 142       | 68.6 | 126 | 60.9 | 136 | 65.7 | 132 | 63.8 | 136 | 65.7 |
| B                   | 127               | 107       | 84.3 | 95  | 74.8 | 108 | 85.0 | 98  | 77.2 | 9   | 73.2 |
| C                   | 42                | 33        | 78.6 | 35  | 83.3 | 37  | 88.1 | 37  | 88.1 | 36  | 85.7 |
| D                   | 30                | 24        | 80.0 | 22  | 73.3 | 23  | 76.7 | 22  | 73.3 | 22  | 73.3 |
| Total               | 406               | 306       | 75.4 | 278 | 68.5 | 304 | 75.0 | 289 | 71.2 | 288 | 70.9 |

TABLE 8. ANALYSIS OF REASONS FOR ABSENCE OF ORIGINALLY  
SERO-POSITIVES, SIXTH SURVEY, BY GROUP OF LOCALITIES

| Group of<br>localities | Total no. of<br>sero-positives<br>in basic survey | No. reexamined<br>during<br>sixth round | Reasons for absence (6th round) |                        |                         |                                   | Reexamination rate (%) |                         |  |
|------------------------|---|---|---------------------------------|------------------------|-------------------------|-----------------------------------|------------------------|-------------------------|--|
|                        |   |   | Death                           | Unknown in<br>locality | Left region<br>for good | Other reasons<br>(incl. refusals) | Gross                  | Corrected<br>for deaths | Corrected for<br>deaths and<br>permanent absence |
| A                      | 207   | 136                                     | 6                               | 8                      | 28                      | 29                                | 65.7                   | 67.7                    | 82.4   |
| B                      | 127   | 94                                      | 2                               | 1                      | 15                      | 15                                | 74.0                   | 75.2                    | 86.2   |
| C                      | 42  | 36                                      | -                               | 3                      | 1                       | 2                                 | 85.7                   | 85.7                    | 94.7   |
| D                      | 30  | 22                                      | 1                               | -                      | 5                       | 2                                 | 73.3                   | 75.8                    | 91.7   |
| Total                  | 406   | 288                                     | 9                               | 12                     | 49                      | 48                                | 70.9                   | 72.5                    | 85.7   |



TABLE 9. GLOBAL RESULTS OF SEROLOGICAL EXAMINATIONS, FIRST TO SIXTH SURVEY

| Survey No. | Number of persons |          |                      |     |    |     |     |     |       | % sero-positive* | Geometr. mean recipr. titres (sero-pos. only)* |
|------------|-------------------|----------|----------------------|-----|----|-----|-----|-----|-------|------------------|--|
|            | Examined          | Negative | Positive at titre 1/ |     |    |     |     |     |       |                  |  |
|            |                   |          | 20                   | 40  | 80 | 160 | 320 | 640 | 1280+ |                  |  |
| 1          | 2 171             | 1 765    | 216                  | 129 | 39 | 11  | 1   | 6   | 4     | 20.1             | 34.93  |
| 2          | 2 182             | 1 880    | 194                  | 76  | 17 | 9   | 2   | 1   | 3     | 14.9             | 30.16  |
| 3          | 2 173             | 2 017    | 124                  | 26  | 2  | 1   | 1   | 1   | 1     | 7.7              | 25.33  |
| 4          | 2 170             | 2 077    | 85                   | 8   | 0  | 0   | 0   | 0   | 0     | 4.9              | 21.49  |
| 5          | 2 175             | 2 156    | 14                   | 5   | 0  | 0   | 0   | 0   | 0     | 1.3              | 24.11  |
| 6          | 2 173             | 2 096    | 71                   | 5   | 0  | 1   | 0   | 0   | 0     | 3.5              | 21.85  |

\* Standardized for age.

TABLE 10. RESULTS OF SEROLOGICAL EXAMINATIONS, 1ST-6TH SURVEY, BY LOCALITY

| Locality | First survey |            |     |     | Second survey |            |     |     | Third survey |            |       |     | Fourth survey |            |     |       | Fifth survey |            |    |    | Sixth survey |            |     |    |    |       |      |      |   |      |
|----------|--------------|------------|-----|-----|---------------|------------|-----|-----|--------------|------------|-------|-----|---------------|------------|-----|-------|--------------|------------|----|----|--------------|------------|-----|----|----|-------|------|------|---|------|
|          | Neg.         | Rec. titre |     |     | Neg.          | Rec. titre |     |     | Neg.         | Rec. titre |       |     | Neg.          | Rec. titre |     |       | Neg.         | Rec. titre |    |    | Neg.         | Rec. titre |     |    |    |       |      |      |   |      |
|          |              | 20         | 40  | 80  |               | 160+       | 20  | 40  |              | 80         | 160+  | 20  |               | 40         | 80  | 160+  |              | 20         | 40 | 80 |              | 160+       | 20  | 40 | 80 | 160+  |      |      |   |      |
| 1        | 63           | 19         | 22  | 6   | 10            | 82         | 15  | 13  | 6            | 4          | 104   | 9   | 3             | 0          | 4   | 101   | 17           | 3          | 0  | 0  | 116          | 2          | 2   | 0  | 0  | 122   | 2    | 0    | 0 | 0    |
| 2        | 55           | 21         | 29  | 8   | 2             | 80         | 26  | 10  | 3            | 1          | 102   | 13  | 5             | 0          | 0   | 116   | 4            | 0          | 0  | 0  | 121          | 0          | 0   | 0  | 0  | 121   | 0    | 0    | 0 | 0    |
| 3        | 68           | 20         | 18  | 7   | 1             | 97         | 12  | 6   | 1            | 4          | 97    | 17  | 6             | 0          | 0   | 115   | 8            | 0          | 0  | 0  | 126          | 0          | 0   | 0  | 0  | 115   | 6    | 0    | 0 | 0    |
| 4        | 88           | 19         | 9   | 2   | 2             | 91         | 10  | 15  | 0            | 4          | 101   | 17  | 2             | 0          | 0   | 113   | 6            | 1          | 0  | 0  | 119          | 1          | 0   | 0  | 0  | 107   | 13   | 0    | 0 | 0    |
| 5        | 76           | 25         | 10  | 7   | 2             | 95         | 20  | 3   | 3            | 0          | 108   | 13  | 0             | 0          | 0   | 108   | 12           | 0          | 0  | 0  | 121          | 0          | 0   | 0  | 0  | 115   | 5    | 1    | 0 | 0    |
| 6        | 105          | 13         | 6   | 0   | 0             | 114        | 5   | 1   | 0            | 0          | 117   | 3   | 0             | 0          | 0   | 115   | 4            | 1          | 0  | 0  | 120          | 0          | 0   | 0  | 0  | 104   | 14   | 2    | 0 | 0    |
| 7        | 84           | 22         | 12  | 2   | 0             | 102        | 16  | 6   | 0            | 0          | 119   | 5   | 0             | 1          | 0   | 110   | 10           | 1          | 0  | 0  | 112          | 5          | 3   | 0  | 0  | 111   | 9    | 0    | 0 | 0    |
| 8        | 91           | 24         | 5   | 0   | 0             | 115        | 5   | 2   | 0            | 0          | 113   | 6   | 1             | 0          | 0   | 117   | 3            | 0          | 0  | 0  | 120          | 0          | 0   | 0  | 0  | 118   | 3    | 0    | 0 | 0    |
| 9        | 106          | 7          | 3   | 1   | 0             | 108        | 11  | 1   | 0            | 0          | 113   | 4   | 2             | 0          | 0   | 117   | 3            | 0          | 0  | 0  | 120          | 0          | 0   | 0  | 0  | 118   | 2    | 0    | 0 | 0    |
| 10       | 117          | 3          | 0   | 0   | 0             | 119        | 4   | 0   | 0            | 0          | 115   | 5   | 0             | 0          | 0   | 121   | 0            | 0          | 0  | 0  | 120          | 0          | 0   | 0  | 0  | 120   | 0    | 0    | 0 | 0    |
| 11       | 113          | 7          | 0   | 0   | 0             | 109        | 9   | 6   | 1            | 0          | 112   | 6   | 3             | 0          | 0   | 119   | 4            | 0          | 0  | 0  | 123          | 1          | 0   | 0  | 0  | 120   | 3    | 0    | 0 | 0    |
| 12       | 98           | 14         | 4   | 3   | 1             | 115        | 3   | 2   | 0            | 1          | 117   | 7   | 0             | 0          | 0   | 115   | 5            | 0          | 0  | 0  | 119          | 3          | 0   | 0  | 0  | 120   | 0    | 0    | 0 | 0    |
| 13       | 113          | 5          | 0   | 1   | 1             | 105        | 17  | 0   | 0            | 1          | 115   | 5   | 0             | 0          | 0   | 114   | 4            | 2          | 0  | 0  | 119          | 1          | 0   | 0  | 0  | 115   | 3    | 1    | 0 | 1    |
| 14       | 117          | 1          | 1   | 0   | 1             | 112        | 4   | 3   | 1            | 0          | 116   | 4   | 0             | 0          | 0   | 118   | 2            | 0          | 0  | 0  | 120          | 0          | 0   | 0  | 0  | 121   | 0    | 0    | 0 | 0    |
| 15       | 118          | 0          | 1   | 0   | 1             | 109        | 9   | 2   | 0            | 0          | 119   | 2   | 0             | 1          | 0   | 119   | 1            | 0          | 0  | 0  | 120          | 0          | 0   | 0  | 0  | 120   | 0    | 0    | 0 | 0    |
| 16       | 124          | 7          | 1   | 0   | 0             | 113        | 6   | 0   | 1            | 0          | 115   | 5   | 0             | 0          | 0   | 120   | 1            | 0          | 0  | 0  | 120          | 0          | 0   | 0  | 0  | 112   | 8    | 0    | 0 | 0    |
| 17       | 112          | 4          | 5   | 1   | 0             | 105        | 13  | 2   | 1            | 0          | 115   | 3   | 3             | 0          | 0   | 120   | 0            | 0          | 0  | 0  | 120          | 1          | 0   | 0  | 0  | 120   | 1    | 0    | 0 | 0    |
| 18       | 117          | 5          | 3   | 1   | 1             | 109        | 9   | 4   | 0            | 0          | 119   | 0   | 1             | 0          | 0   | 119   | 1            | 0          | 0  | 0  | 120          | 0          | 0   | 0  | 0  | 117   | 2    | 1    | 0 | 0    |
| Total    | 1 765        | 216        | 129 | 39  | 22            | 1 880      | 194 | 76  | 17           | 15         | 2 017 | 124 | 26            | 2          | 4   | 2 077 | 85           | 8          | 0  | 0  | 2 156        | 14         | 5   | 0  | 0  | 2 096 | 71   | 5    | 0 | 1    |
| In %     | 81.3         | 9.9        | 5.9 | 1.8 | 1.0           | 86.2       | 8.9 | 3.5 | 0.8          | 0.7        | 92.8  | 5.7 | 1.2           | 0.1        | 0.2 | 95.7  | 3.9          | 0.4        | 0  | 0  | 99.1         | 0.6        | 0.2 | 0  | 0  | 96.46 | 3.27 | 0.23 | 0 | 0.05 |

TABLE 11. PERCENTAGE OF SERO-POSITIVES (1/20+) ACCORDING TO  
SEX AND AGE, FIRST TO SIXTH SURVEY

| Survey<br>No. | % sero-positive |      |           |      |            |      |           |      |       |      |
|---------------|-----------------|------|-----------|------|------------|------|-----------|------|-------|------|
|               | 6-23 months     |      | 2-4 years |      | 5-14 years |      | 15+ years |      | Total |      |
|               | Male            | Fem. | Male      | Fem. | Male       | Fem. | Male      | Fem. | Male  | Fem. |
| 1             | 11.5            | 17.2 | 15.6      | 10.1 | 19.5       | 19.5 | 22.8      | 22.2 | 20.4  | 19.9 |
| 2             | 10.0            | 12.5 | 9.9       | 11.2 | 11.5       | 14.3 | 16.8      | 17.4 | 14.2  | 15.6 |
| 3             | 3.4             | 7.0  | 5.1       | 6.8  | 6.3        | 6.9  | 8.1       | 9.4  | 7.0   | 8.3  |
| 4             | 0               | 2.2  | 2.8       | 2.0  | 4.8        | 2.8  | 6.7       | 6.0  | 5.4   | 4.4  |
| 5             | 0               | 0    | 0         | 0    | 0          | 0.3  | 2.9       | 1.6  | 1.6   | 1.0  |
| 6             | 6.0             | 3.8  | 3.1       | 5.3  | 3.3        | 2.4  | 2.8       | 4.3  | 3.2   | 3.8  |

TABLE 12. RESULTS OF SEROLOGICAL EXAMINATIONS, 1ST-6TH SURVEY,  
BY AGE GROUP AND GROUP OF LOCALITIES

| Group of localities | Age group | First survey |            |     |         | Second survey |            |      |         | Third survey |            |      |         | Fourth survey |            |     |         | Fifth survey |            |    |         | Sixth survey |            |     |         |     |
|---------------------|-----------|--------------|------------|-----|---------|---------------|------------|------|---------|--------------|------------|------|---------|---------------|------------|-----|---------|--------------|------------|----|---------|--------------|------------|-----|---------|-----|
|                     |           | Neg.         | Rec. titre |     |         | Neg.          | Rec. titre |      |         | Neg.         | Rec. titre |      |         | Neg.          | Rec. titre |     |         | Neg.         | Rec. titre |    |         | Neg.         | Rec. titre |     |         |     |
|                     |           |              | 20         | 40  | 80 160+ |               | 20         | 40   | 80 160+ |              | 20         | 40   | 80 160+ |               | 20         | 40  | 80 160+ |              | 20         | 40 | 80 160+ |              | 20         | 40  | 80 160+ |     |
| A                   | 6-23M     | 31           | 7          | 11  | 3       | 0             | 40         | 5    | 3       | 0            | 0          | 20   | 3       | 0             | 0          | 0   | 34      | 0            | 0          | 0  | 0       | 36           | 2          | 0   | 0       | 0   |
|                     | 2-4       | 40           | 15         | 11  | 0       | 2             | 78         | 10   | 4       | 1            | 0          | 55   | 6       | 1             | 0          | 1   | 72      | 6            | 0          | 0  | 0       | 81           | 3          | 0   | 0       | 0   |
|                     | 5-14      | 77           | 30         | 29  | 21      | 1             | 127        | 28   | 11      | 4            | 4          | 209  | 23      | 6             | 0          | 0   | 155     | 17           | 0          | 0  | 0       | 171          | 3          | 0   | 0       | 0   |
|                     | 15+       | 114          | 33         | 28  | 4       | 12            | 109        | 30   | 14      | 8            | 5          | 127  | 20      | 7             | 0          | 3   | 179     | 18           | 3          | 0  | 0       | 196          | 2          | 2   | 0       | 0   |
| B                   | 6-23M     | 44           | 5          | 2   | 0       | 0             | 56         | 6    | 2       | 0            | 0          | 47   | 3       | 0             | 0          | 0   | 51      | 2            | 0          | 0  | 0       | 44           | 0          | 0   | 0       | 0   |
|                     | 2-4       | 94           | 13         | 4   | 0       | 0             | 111        | 6    | 4       | 0            | 0          | 87   | 7       | 1             | 0          | 0   | 114     | 3            | 0          | 0  | 0       | 113          | 0          | 0   | 0       | 0   |
|                     | 5-14      | 145          | 32         | 9   | 1       | 0             | 156        | 11   | 10      | 0            | 1          | 220  | 13      | 1             | 1          | 0   | 185     | 5            | 0          | 0  | 0       | 222          | 1          | 0   | 0       | 0   |
|                     | 15+       | 191          | 35         | 20  | 4       | 2             | 207        | 24   | 9       | 0            | 3          | 209  | 12      | 3             | 0          | 0   | 222     | 16           | 3          | 0  | 0       | 212          | 5          | 3   | 0       | 0   |
| C                   | 6-23M     | 50           | 1          | 0   | 0       | 0             | 60         | 4    | 0       | 0            | 0          | 52   | 2       | 0             | 0          | 0   | 61      | 0            | 0          | 0  | 0       | 65           | 0          | 0   | 0       | 0   |
|                     | 2-4       | 126          | 6          | 1   | 0       | 0             | 89         | 6    | 0       | 1            | 0          | 103  | 2       | 0             | 0          | 0   | 125     | 1            | 0          | 0  | 0       | 123          | 0          | 0   | 0       | 0   |
|                     | 5-14      | 215          | 9          | 3   | 1       | 0             | 219        | 8    | 5       | 1            | 0          | 212  | 9       | 2             | 0          | 0   | 196     | 5            | 1          | 0  | 0       | 211          | 0          | 0   | 0       | 0   |
|                     | 15+       | 167          | 14         | 1   | 3       | 3             | 192        | 19   | 6       | 0            | 2          | 208  | 14      | 1             | 0          | 0   | 205     | 9            | 1          | 0  | 0       | 202          | 5          | 0   | 0       | 1   |
| D                   | 6-23M     | 58           | 1          | 0   | 0       | 0             | 36         | 4    | 0       | 0            | 0          | 32   | 0       | 0             | 0          | 0   | 28      | 0            | 0          | 0  | 0       | 33           | 0          | 0   | 0       | 0   |
|                     | 2-4       | 121          | 3          | 2   | 0       | 0             | 88         | 8    | 2       | 1            | 0          | 73   | 1       | 1             | 0          | 0   | 96      | 0            | 0          | 0  | 0       | 105          | 0          | 0   | 0       | 0   |
|                     | 5-14      | 159          | 7          | 1   | 0       | 0             | 148        | 10   | 2       | 1            | 0          | 206  | 5       | 0             | 0          | 0   | 170     | 0            | 0          | 0  | 0       | 162          | 0          | 0   | 0       | 0   |
|                     | 15+       | 133          | 5          | 7   | 2       | 2             | 164        | 15   | 4       | 0            | 0          | 157  | 4       | 3             | 1          | 0   | 184     | 3            | 0          | 0  | 0       | 180          | 1          | 0   | 0       | 0   |
| TOTAL               | 6-23M     | 183          | 14         | 13  | 3       | 0             | 192        | 19   | 5       | 0            | 0          | 151  | 8       | 0             | 0          | 0   | 174     | 2            | 0          | 0  | 0       | 178          | 0          | 0   | 0       | 0   |
|                     | 2-4       | 381          | 37         | 18  | 0       | 2             | 366        | 30   | 10      | 3            | 0          | 318  | 16      | 3             | 0          | 1   | 407     | 10           | 0          | 0  | 0       | 422          | 0          | 0   | 0       | 0   |
|                     | 5-14      | 596          | 78         | 42  | 23      | 1             | 650        | 57   | 28      | 6            | 5          | 847  | 50      | 9             | 1          | 0   | 706     | 27           | 1          | 0  | 0       | 766          | 1          | 0   | 0       | 0   |
|                     | 15+       | 605          | 87         | 56  | 13      | 19            | 672        | 88   | 33      | 8            | 10         | 701  | 50      | 14            | 1          | 3   | 790     | 46           | 7          | 0  | 0       | 790          | 13         | 5   | 0       | 1   |
| TOTAL in %          | 6-23M     | 85.9         | 6.6        | 6.1 | 1.4     | 0             | 88.9       | 8.8  | 2.3     | 0            | 0          | 95.0 | 5.0     | 0             | 0          | 0   | 98.9    | 1.1          | 0          | 0  | 0       | 100.0        | 0          | 0   | 0       | 0   |
|                     | 2-4       | 87.0         | 8.4        | 4.1 | 0       | 0.5           | 89.5       | 7.3  | 2.4     | 0.7          | 0          | 94.0 | 4.7     | 0.9           | 0          | 0.3 | 97.6    | 2.4          | 0          | 0  | 0       | 100.0        | 0          | 0   | 0       | 0   |
|                     | 5-14      | 80.5         | 10.5       | 5.7 | 3.1     | 0.1           | 87.1       | 7.6  | 3.8     | 0.8          | 0.7        | 93.4 | 5.5     | 1.0           | 0.1        | 0   | 96.2    | 3.7          | 0.1        | 0  | 0       | 99.9         | 0.1        | 0   | 0       | 0   |
|                     | 15+       | 77.6         | 11.2       | 7.2 | 1.7     | 2.4           | 82.9       | 10.9 | 4.1     | 1.0          | 1.2        | 91.1 | 6.5     | 1.8           | 0.1        | 0.4 | 93.7    | 5.5          | 0.8        | 0  | 0       | 97.8         | 1.6        | 0.6 | 0       | 0.1 |

TABLE 13. SEROLOGICAL FINDINGS ACCORDING TO PREVIOUS MALARIA HISTORY  
FIRST TO SIXTH SURVEY

| Survey<br>No. | Former malaria cases (Paras. confirmed) |      |      |                     |    |    |     |      | Persons with negative or unknown<br>malaria history |       |      |                     |     |    |     |      |
|---------------|---|------|------|---------------------|----|----|-----|------|---|-------|------|---------------------|-----|----|-----|------|
|               | Total                                   | Neg. | Pos. | Rec. titres of pos. |    |    |     |      | Total   | Neg.  | Pos. | Rec. titres of pos. |     |    |     |      |
|               |   |      |      | 20                  | 40 | 80 | 160 | 320+ |   |       |      | 20                  | 40  | 80 | 160 | 320+ |
| 1             | 136                                     | 102  | 34   | 20                  | 9  | 4  | -   | 1    | 2 035   | 1 663 | 372  | 196                 | 120 | 35 | 11  | 10   |
| 2             | 35                                      | 24   | 11   | 4                   | 4  | 1  | 2   | -    | 2 147   | 1 856 | 291  | 190                 | 72  | 16 | 7   | 6    |
| 3             | 51                                      | 47   | 4    | 3                   | -  | -  | 1   | -    | 2 122   | 1 970 | 162  | 121                 | 26  | 2  | -   | 3    |
| 4             | 39                                      | 35   | 4    | 3                   | 1  | -  | -   | -    | 2 131   | 2 042 | 89   | 82                  | 7   | -  | -   | -    |
| 5             | 31                                      | 31   | 0    | -                   | -  | -  | -   | -    | 2 144   | 2 125 | 19   | 14                  | 5   | -  | -   | -    |
| 6             | 28                                      | 26   | 2    | 2                   | -  | -  | -   | -    | 2 145   | 2 070 | 75   | 69                  | 5   | -  | 1   | -    |

TABLE 14. SEROLOGICAL FINDINGS IN ORIGINALLY SERO-POSITIVES  
ACCORDING TO INDIVIDUAL MALARIA HISTORY  
FIRST TO SIXTH SURVEY

| Survey | Originally sero-positives |               |              |               |
|--------|---------------------------|---------------|--------------|---------------|
|        | Former malaria cases      |               | Others       |               |
|        | No. examined              | No. sero-pos. | No. examined | No. sero-pos. |
| 1      | 34                        | 34            | 372          | 372           |
| 2      | 28                        | 10            | 278          | 116           |
| 3      | 28                        | 4             | 250          | 31            |
| 4      | 27                        | 3             | 277          | 34            |
| 5      | 24                        | 0             | 265          | 6             |
| 6      | 28                        | 2             | 260          | 8             |

TABLE 15. SEROLOGICAL FINDINGS ACCORDING TO PREVIOUS ANTIMALARIA TREATMENT  
FIRST TO SIXTH SURVEY

| Survey No. | Treated persons |      |      |                     |    |    |     |      | Untreated persons |       |      |                     |     |    |     |      |
|------------|-----------------|------|------|---------------------|----|----|-----|------|-------------------|-------|------|---------------------|-----|----|-----|------|
|            | Total           | Neg. | Pos. | Rec. titres of pos. |    |    |     |      | Total             | Neg.  | Pos. | Rec. titres of pos. |     |    |     |      |
|            |                 |      |      | 20                  | 40 | 80 | 160 | 320+ |                   |       |      | 20                  | 40  | 80 | 160 | 320+ |
| 1          | 236             | 173  | 63   | 36                  | 21 | 5  | -   | 1    | 1 935             | 1 592 | 343  | 180                 | 108 | 34 | 11  | 10   |
| 2          | 261             | 201  | 60   | 29                  | 24 | 1  | 3   | 3    | 1 921             | 1 679 | 242  | 165                 | 52  | 16 | 6   | 3    |
| 3          | 280             | 254  | 26   | 23                  | 2  | -  | 1   | -    | 1 893             | 1 763 | 130  | 101                 | 24  | 2  | -   | 3    |
| 4          | 245             | 227  | 18   | 16                  | 2  | -  | -   | -    | 1 925             | 1 850 | 75   | 69                  | 6   | -  | -   | -    |
| 5          | 203             | 194  | 9    | 6                   | 3  | -  | -   | -    | 1 972             | 1 962 | 10   | 8                   | 2   | -  | -   | -    |
| 6          | 225             | 203  | 22   | 22                  | -  | -  | -   | -    | 1 948             | 1 893 | 55   | 49                  | 5   | -  | 1   | -    |

TABLE 16. SEROLOGICAL FINDINGS IN TREATED PERSONS FROM LOCALITIES 4 AND 7  
AND IN TREATED PERSONS FROM LOCALITIES 1-3, 5, 6, 8-18

| Survey | Treated persons, localities 4 & 7 |             |                  | Treated persons, other localities |             |                  |
|--------|-----------------------------------|-------------|------------------|-----------------------------------|-------------|------------------|
|        | No. exam.                         | % sero-pos. | GMRT (sero-pos.) | No. exam.                         | % sero-pos. | GMRT (sero-pos.) |
| 1      | 152                               | 34.3        | 31.30            | 84                                | 22.8        | 46.78            |
| 2      | 239                               | 22.0        | 36.54            | 22                                | 43.0        | 54.52            |
| 3      | 242                               | 10.7        | 22.04            | 39                                | 2.6         | 20.0             |
| 4      | 220                               | 7.9         | 21.65            | 24                                | 5.6         | 20.0             |
| 5      | 185                               | 5.1         | 25.43            | 16                                | 0           | -                |
| 6      | 207                               | 8.4         | 20.00            | 15                                | 0           | -                |

TABLE 17. SEROLOGICAL FINDINGS OF FOLLOW-UP IN ORIGINALLY SERO-POSITIVES

| Group  | Survey | Total | Neg. | Pos. | Rec. titres of pos. |     |    |     |      | Reexam.<br>Rate<br>% |
|--|--------|-------|------|------|---------------------|-----|----|-----|------|----------------------|
|  |        |       |      |      | 20                  | 40  | 80 | 160 | 320+ |                      |
| Sero-pos. of<br>first survey<br>as available                     | 1      | 406   | -    | 406  | 216                 | 129 | 39 | 11  | 11   | -                    |
|  | 2      | 306   | 180  | 126  | 67                  | 40  | 11 | 5   | 3    | 75.4                 |
|  | 3      | 278   | 243  | 35   | 25                  | 7   | 1  | 1   | 1    | 68.5                 |
|  | 4      | 304   | 267  | 37   | 34                  | 3   | -  | -   | -    | 74.9                 |
|  | 5      | 289   | 283  | 6    | 4                   | 2   | -  | -   | -    | 71.2                 |
|  | 6      | 288   | 278  | 10   | 9                   | -   | -  | 1   | -    | 70.9                 |
| Sero-pos. of<br>first survey<br>present at<br>all six<br>surveys | 1      | 166   | -    | -    | 84                  | 57  | 15 | 5   | 5    |                      |
|  | 2      | 166   | 101  | 65   | 32                  | 22  | 7  | 3   | 1    |                      |
|  | 3      | 166   | 146  | 20   | 16                  | 2   | 1  | 1   | -    |                      |
|  | 4      | 166   | 142  | 24   | 23                  | 1   | -  | -   | -    |                      |
|  | 5      | 166   | 163  | 3    | 3                   | -   | -  | -   | -    |                      |
|  | 6      | 166   | 159  | 7    | 6                   | -   | -  | 1   | -    |                      |

TABLE 18. ANALYSIS OF FOLLOW-UP OF ORIGINALLY SERO-POSITIVES

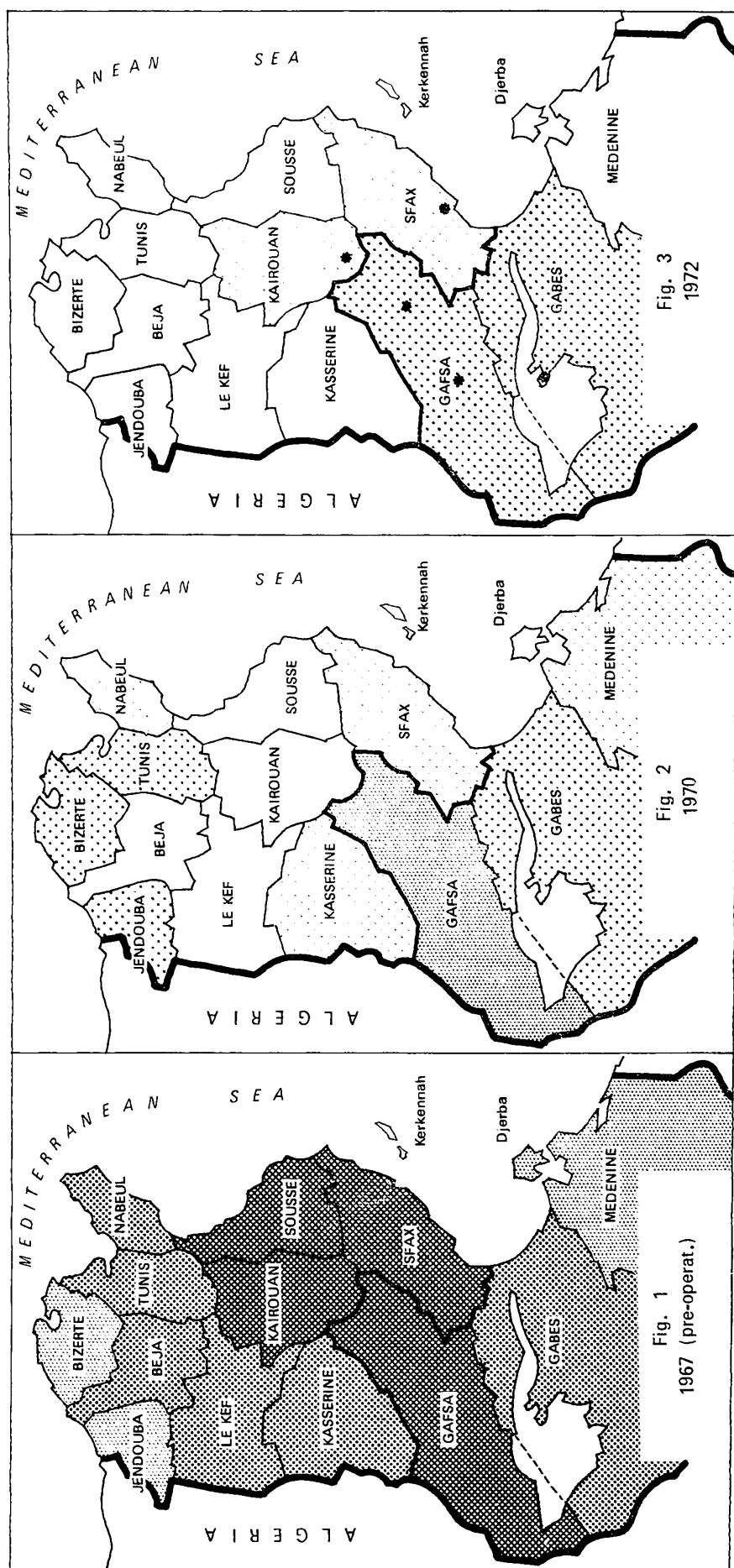
| Group of localities | Number of persons             |     |      |     |       |                        |     |      |     |       | % completely followed up (Total) |
|---------------------|-------------------------------|-----|------|-----|-------|------------------------|-----|------|-----|-------|----------------------------------|
|                     | Sero-positive in first survey |     |      |     |       | Completely followed up |     |      |     |       |                                  |
|                     | 6-23M                         | 2-4 | 5-14 | 15+ | Total | 6-23M                  | 2-4 | 5-14 | 15+ | Total |                                  |
| Group A             | 21                            | 28  | 81   | 77  | 207   | 6                      | 14  | 29   | 15  | 64    | 30.9                             |
| Group B             | 8                             | 16  | 42   | 61  | 127   | 5                      | 6   | 25   | 30  | 66    | 52.0                             |
| Group C             | 1                             | 7   | 13   | 21  | 42    | 0                      | 4   | 9    | 12  | 25    | 59.5                             |
| Group D             | 1                             | 5   | 8    | 16  | 30    | 0                      | 2   | 3    | 6   | 11    | 36.7                             |
| Total               | 31                            | 56  | 144  | 175 | 406   | 11                     | 26  | 66   | 63  | 166   | 40.9                             |

TABLE 19. SEROLOGICAL DEVELOPMENTS IN ORIGINALLY SERO-POSITIVE COHORT

| Survey | Titre level 1/20+        |                         |                          |                         |                          | Titre level 1/40+            |                          |                         |                         |                         |
|--------|--------------------------|-------------------------|--------------------------|-------------------------|--------------------------|------------------------------|--------------------------|-------------------------|-------------------------|-------------------------|
|        | No. of pos.<br>at outset | No. of<br>negativations | No. of neg.<br>at outset | No. of<br>positivations | No. of pos.<br>at outset | No. of neg.<br>negativations | No. of neg.<br>at outset | No. of<br>positivations | No. of<br>negativations | No. of<br>positivations |
| 1→2    | 166                      | 98                      | -                        | -                       | 82                       | 52                           | -                        | -                       | -                       | -                       |
| 2→3    | 68                       | 52                      | 98                       | 4                       | 30                       | 27                           | 52                       | 1                       | 1                       | 1                       |
| 3→4    | 20                       | 15                      | 146                      | 21                      | 4                        | 4                            | 78                       | 1                       | 1                       | 1                       |
| 4→5    | 26                       | 25                      | 140                      | 2                       | 1                        | 1                            | 81                       | -                       | -                       | -                       |
| 5→6    | 3                        | 2                       | 163                      | 6                       | 0                        | 0                            | 82                       | 1                       | 1                       | 1                       |



# SLIDE POSITIVITY RATES (%) IN TUNISIA, BY GOVERNORATE



Note to Fig. 1 : the pre-operational rates of Soussse and Kairouan refer to 1966 as attack operations in these Governorates have started in 1967

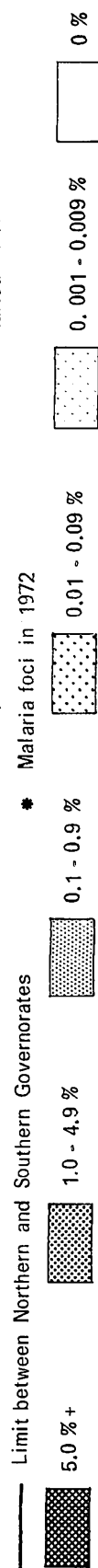


FIG 4 SLIDE POSITIVITY RATES, TUNISIA 1968 - 1972 (3-MONTHLY MOVING AVERAGES)

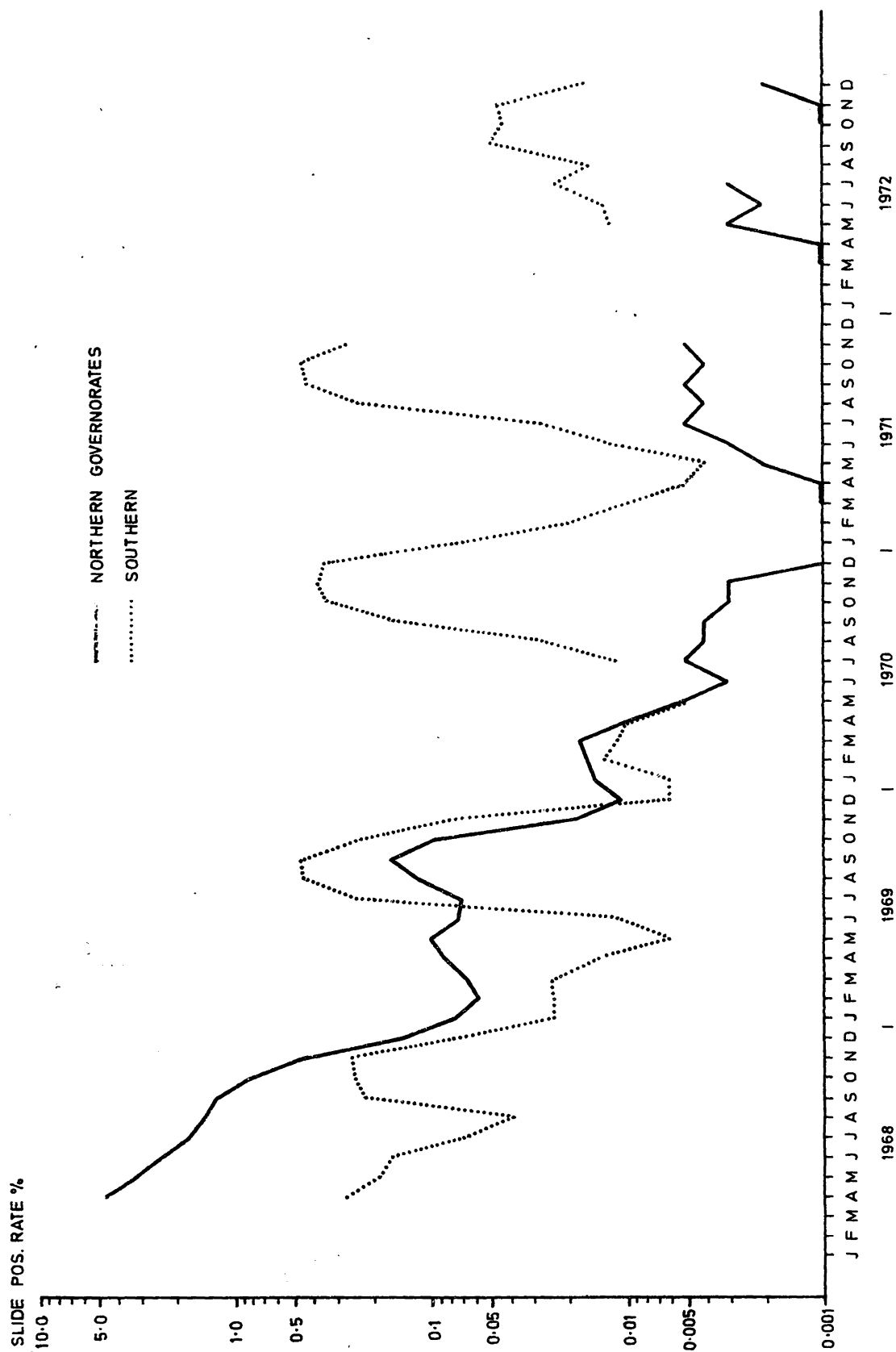
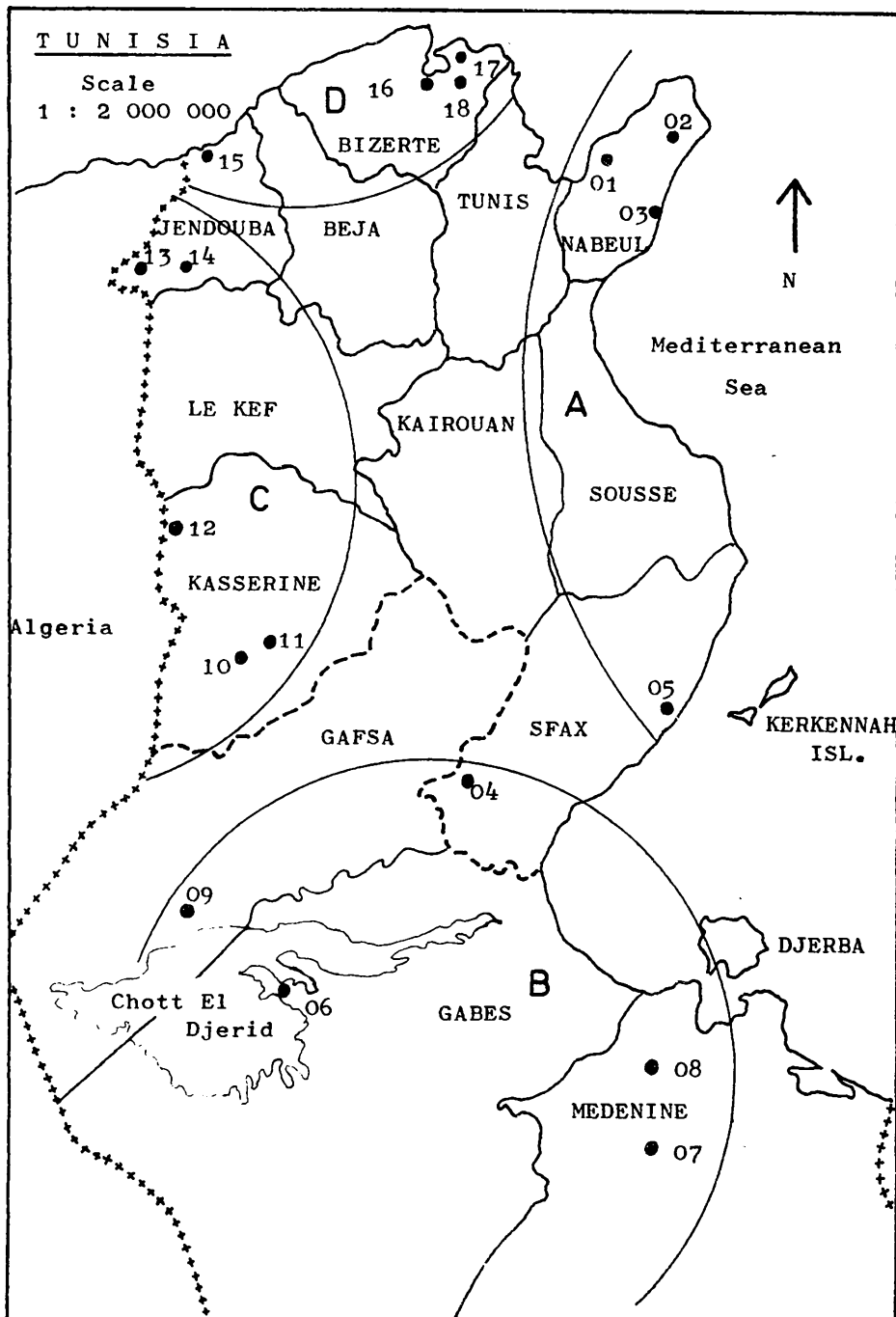


Fig.5 DISTRIBUTION OF LOCALITIES (01 - 18) UNDER STUDY  
AND GROUPING OF LOCALITIES (A - D)



----- Limit between Northern and Southern Governorates

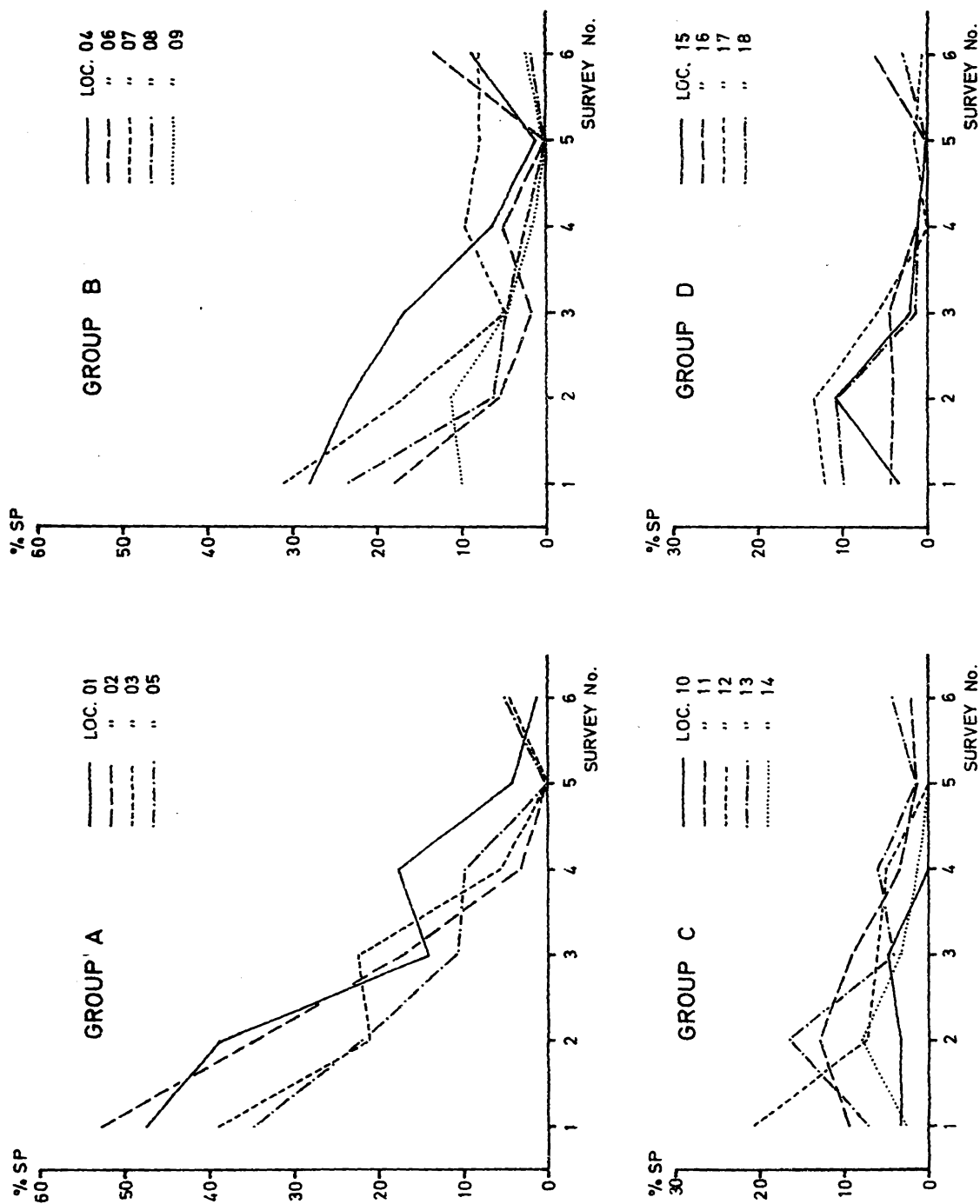


Fig.6 DEVELOPMENT OF THE PERCENTAGE OF SERO-POSITIVES (1/20+),  
BY LOCALITY, FIRST TO SIXTH SURVEY

% SP = % SERO-POSITIVE

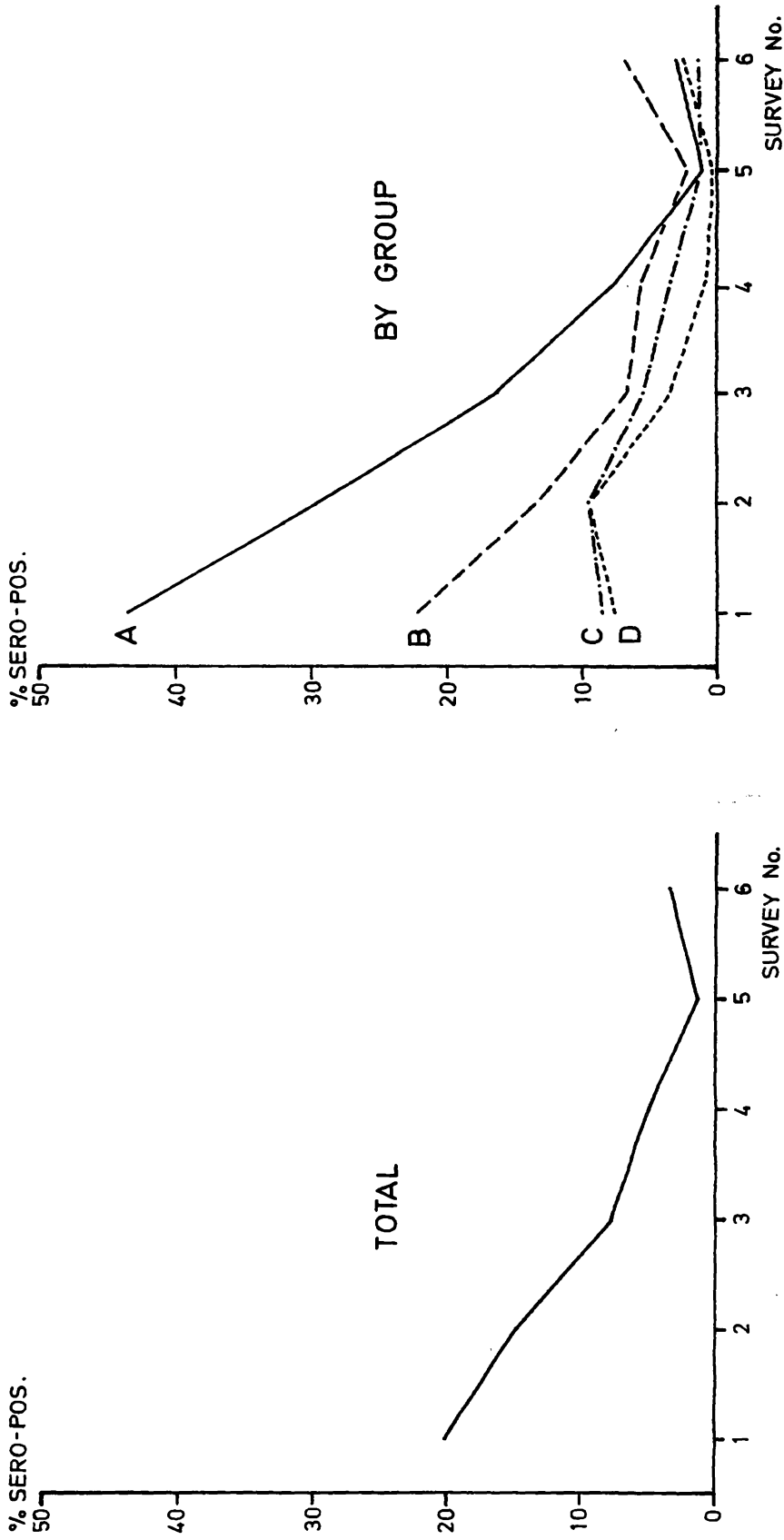


Fig. 7 SERO-POSITIVITY RATE (TITRES 1/20+) IN TOTAL SAMPLE  
AND BY GROUP OF LOCALITIES (STANDARDIZED FOR AGE),  
FIRST TO SIXTH SURVEY

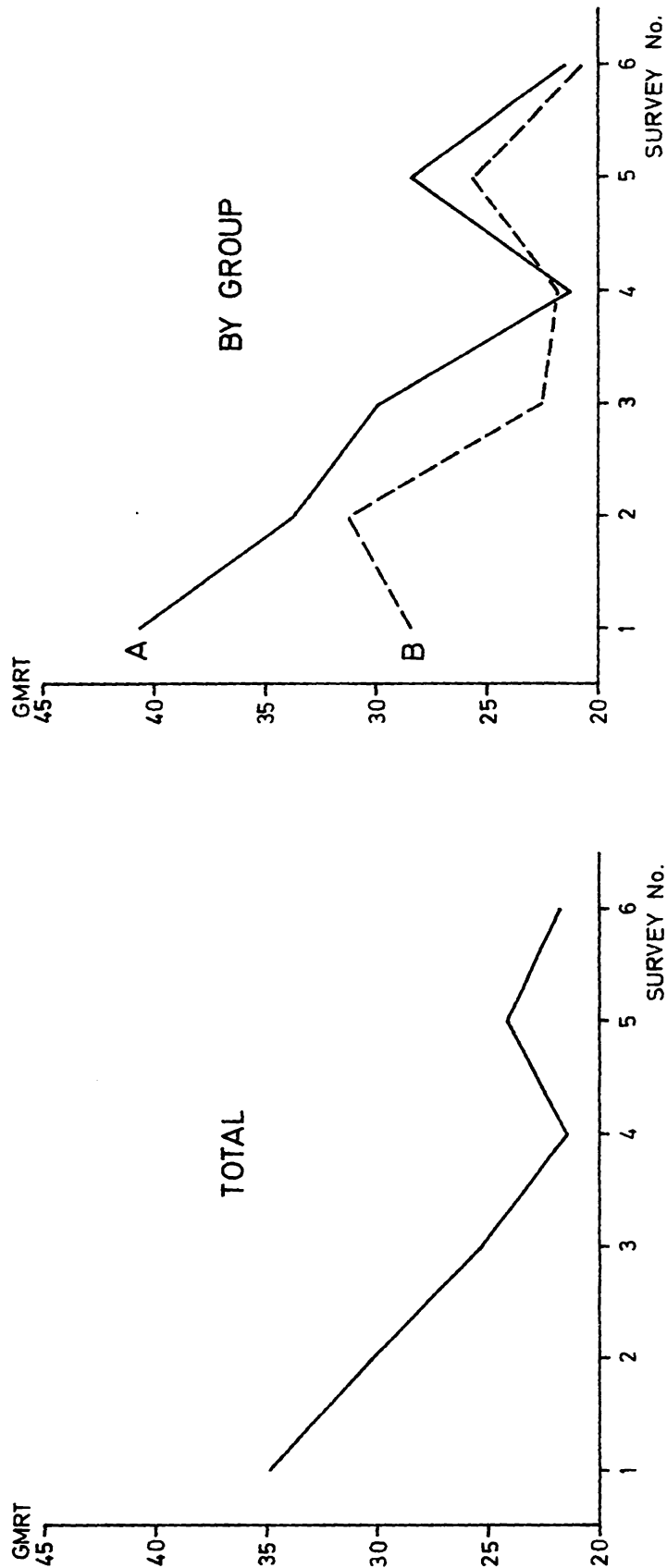


Fig. 8 GEOMETRICAL MEAN RECIPROCAL TITRES (GMRT) IN ALL SERO-POSITIVES AND THOSE OF GROUPS A & B (STANDARDIZED FOR AGE), FIRST TO SIXTH SURVEY

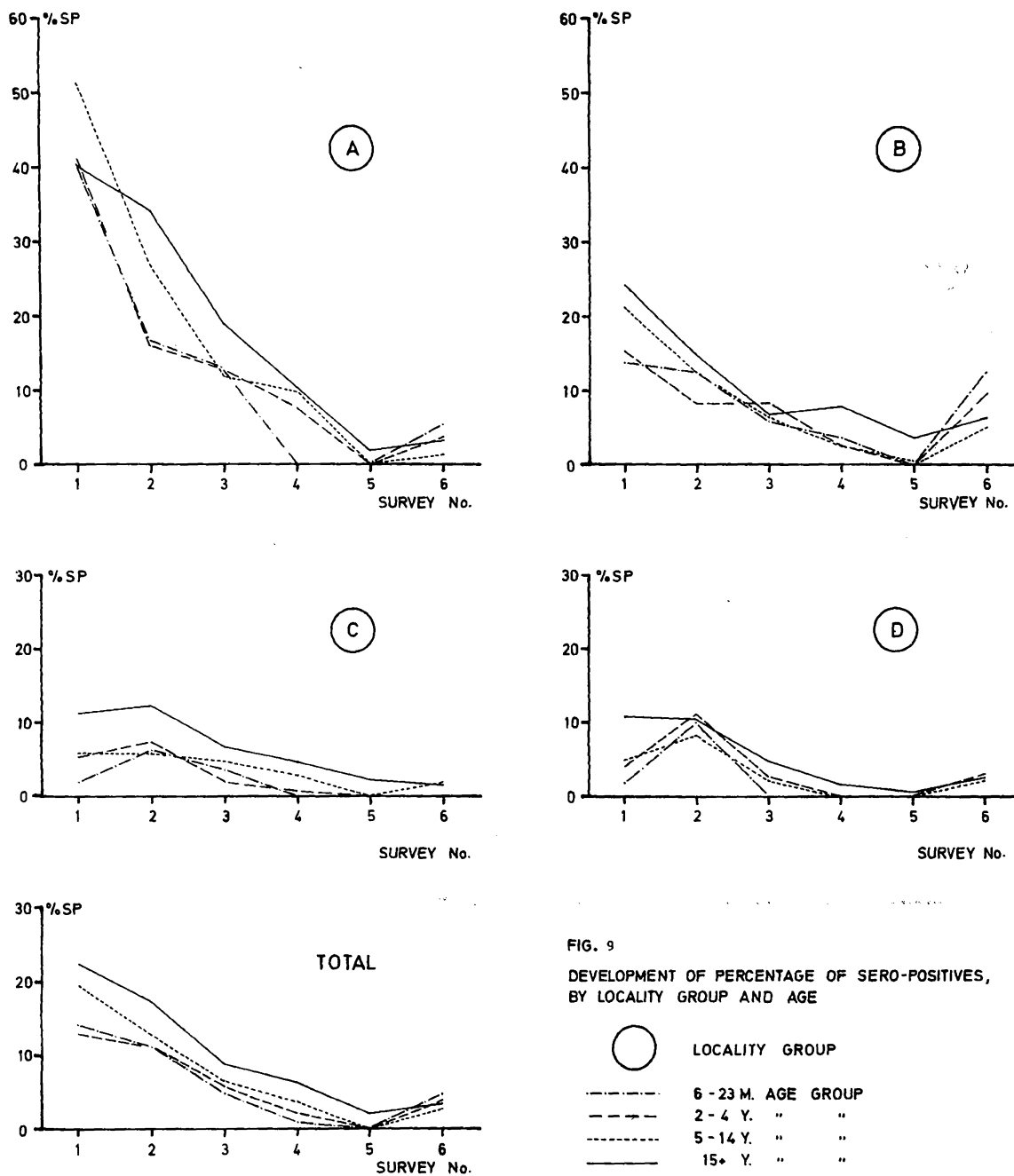


FIG. 9  
DEVELOPMENT OF PERCENTAGE OF SERO-POSITIVES,  
BY LOCALITY GROUP AND AGE

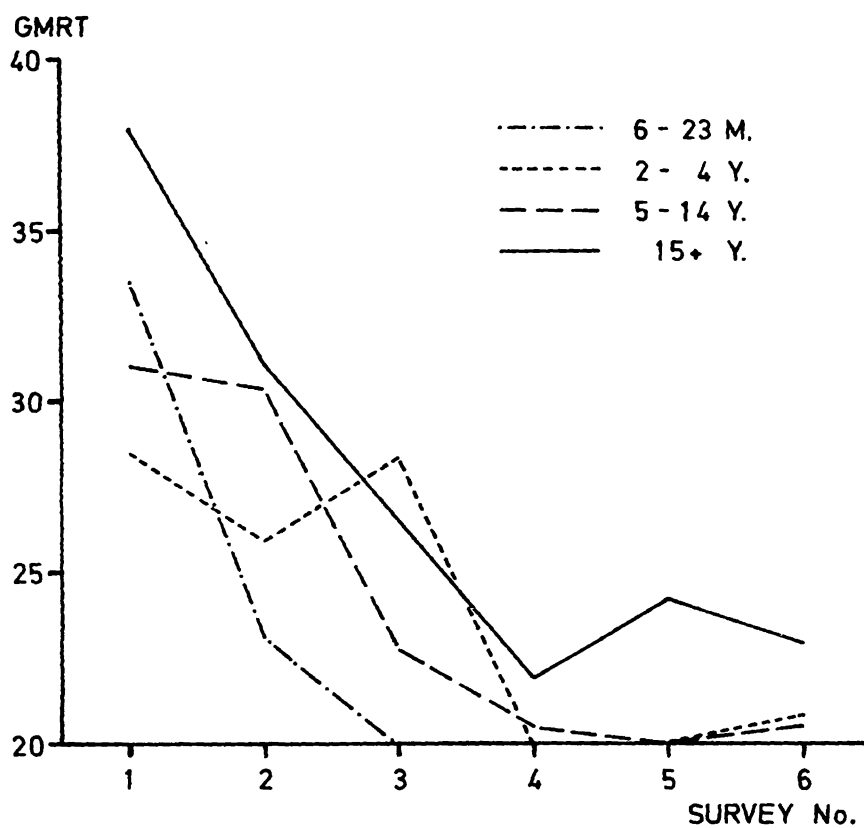
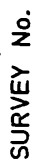


Fig.10 DEVELOPMENT OF GEOMETRICAL MEAN RECIPROCAL  
TITRES (GMRT) IN SERO-POSITIVES (1/20+)  
ACCORDING TO AGE, WHOLE SAMPLE, FIRST TO  
SIXTH SURVEY





**Fig. 11**

TREATED PERSONS

UNTREATED PERSONS

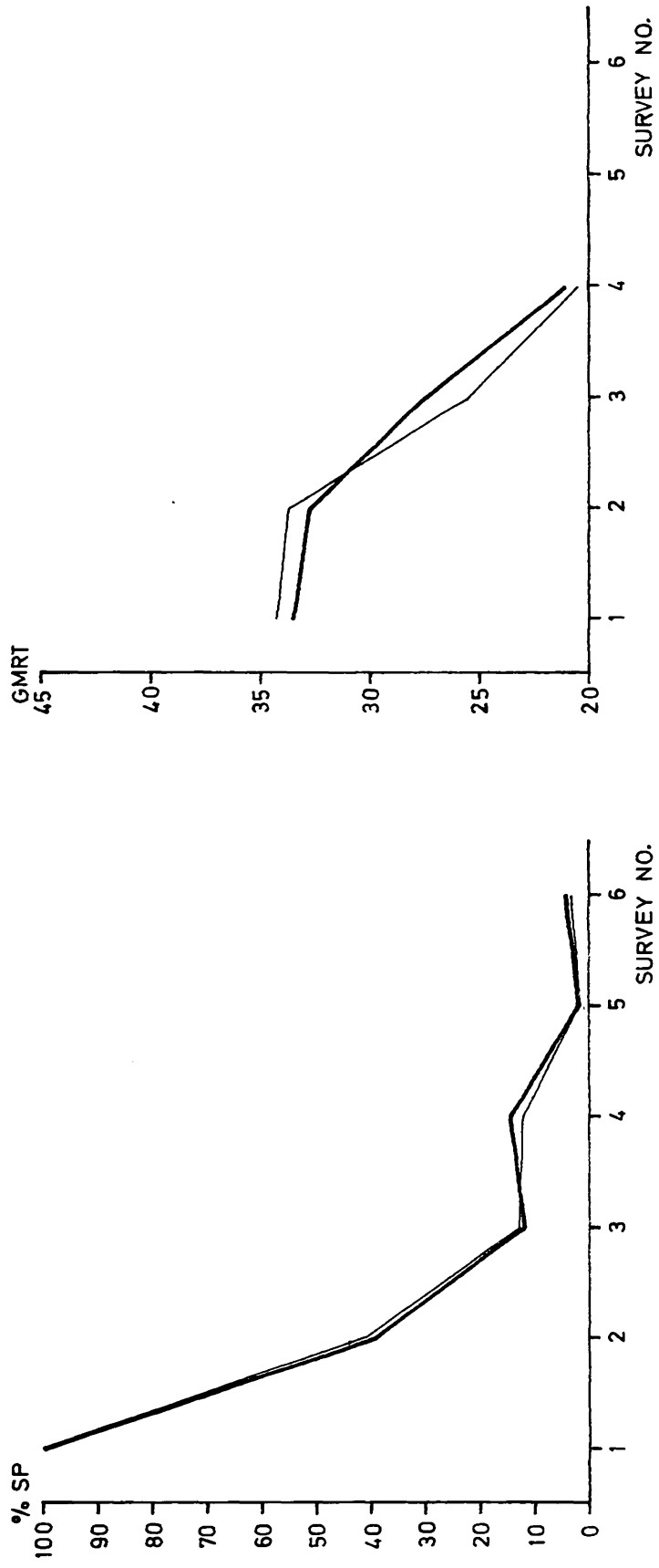


Fig.12 SERO-POSITIVITY RATE (1/20+) AND GMRT OF SERO-POSITIVES AMONG  
ORIGINALLY SERO-POSITIVES FOLLOWED COMPLETELY OR AS AVAILABLE

— FOLLOWED THROUGH ALL SIX SURVEYS      % SP = % SERO-POSITIVE  
- - - FOLLOWED AS AVAILABLE                      GMRT = GEOM. MEAN REC. TITRE

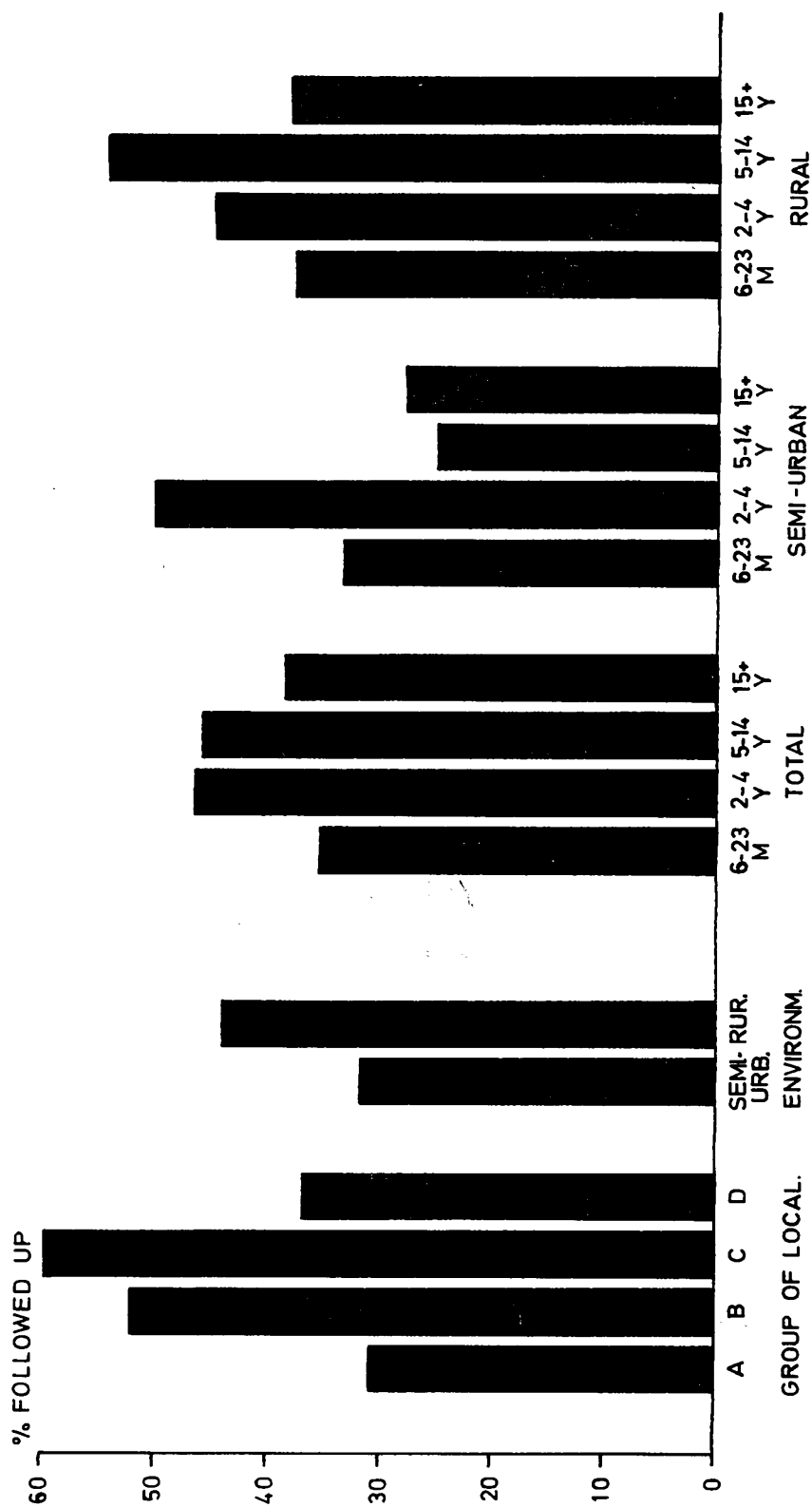


Fig.13 PERCENTAGE OF ORIGINALLY SERO-POSITIVES FOLLOWED THROUGH ALL SIX SURVEYS,  
ACCORDING TO GROUP OF LOCALITIES, ENVIRONMENT, AGE GROUP, AND ENVIRONMENT

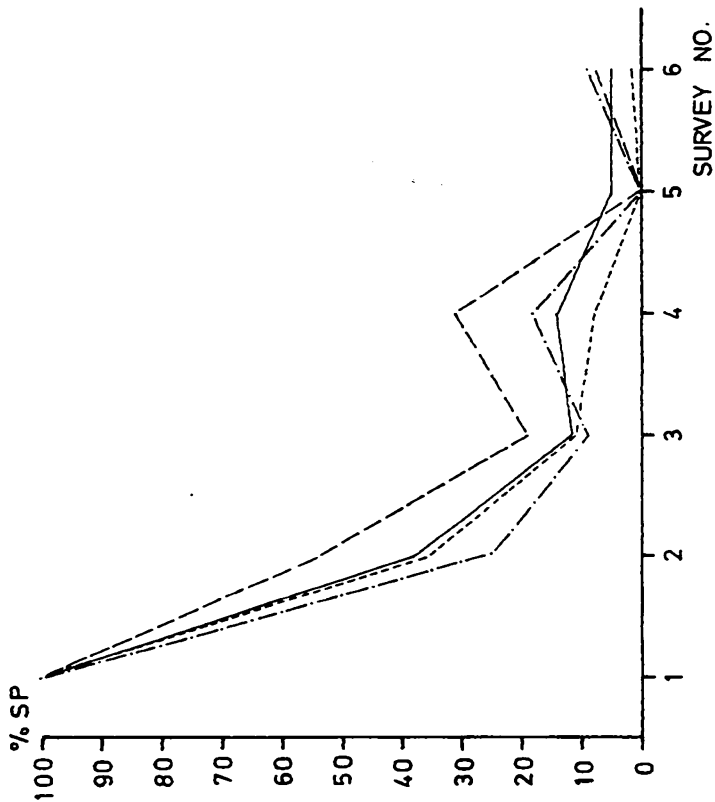


Fig.15 SERO-POSITIVITY RATE (1/20+) AMONG ORIGINALLY SERO-POSITIVES FOLLOWED COMPLETELY FROM FIRST TO SIXTH SURVEY, ACCORDING TO AGE AT FIRST SURVEY

..... 6 - 23 MONTHS    % SP = % SERO-POS.  
----- 2 - 4 YEARS  
- . - . 5 - 14 YEARS  
\_\_\_\_\_ 15+ YEARS

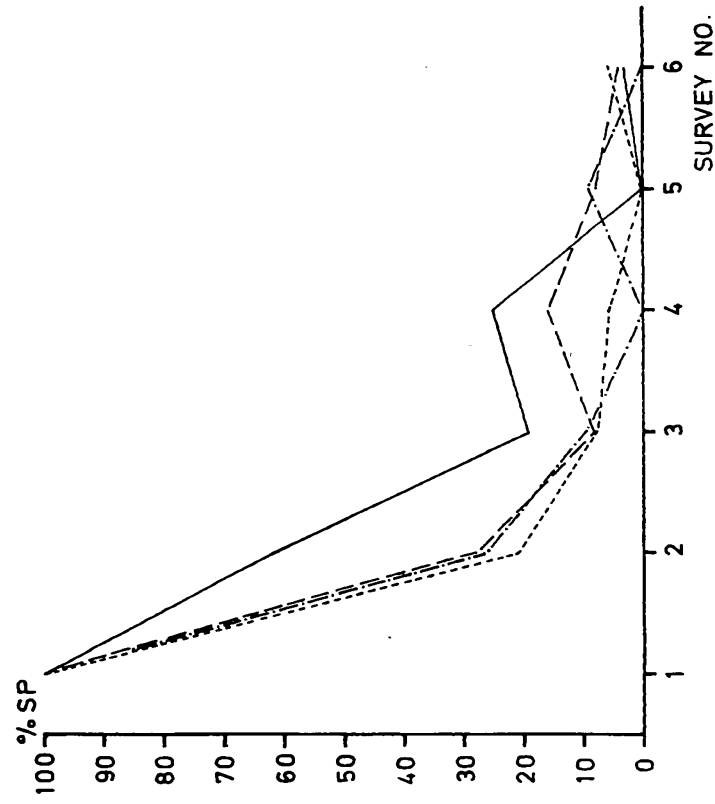


Fig.14 SERO-POSITIVITY RATE (1/20+) AMONG ORIGINALLY SERO-POSITIVES FOLLOWED COMPLETELY FROM FIRST TO SIXTH SURVEY, BY GROUP OF LOCALITIES

\_\_\_\_\_ GROUP A    % SP = % SERO-POS.  
----- GROUP B  
..... GROUP C  
- . - . GROUP D

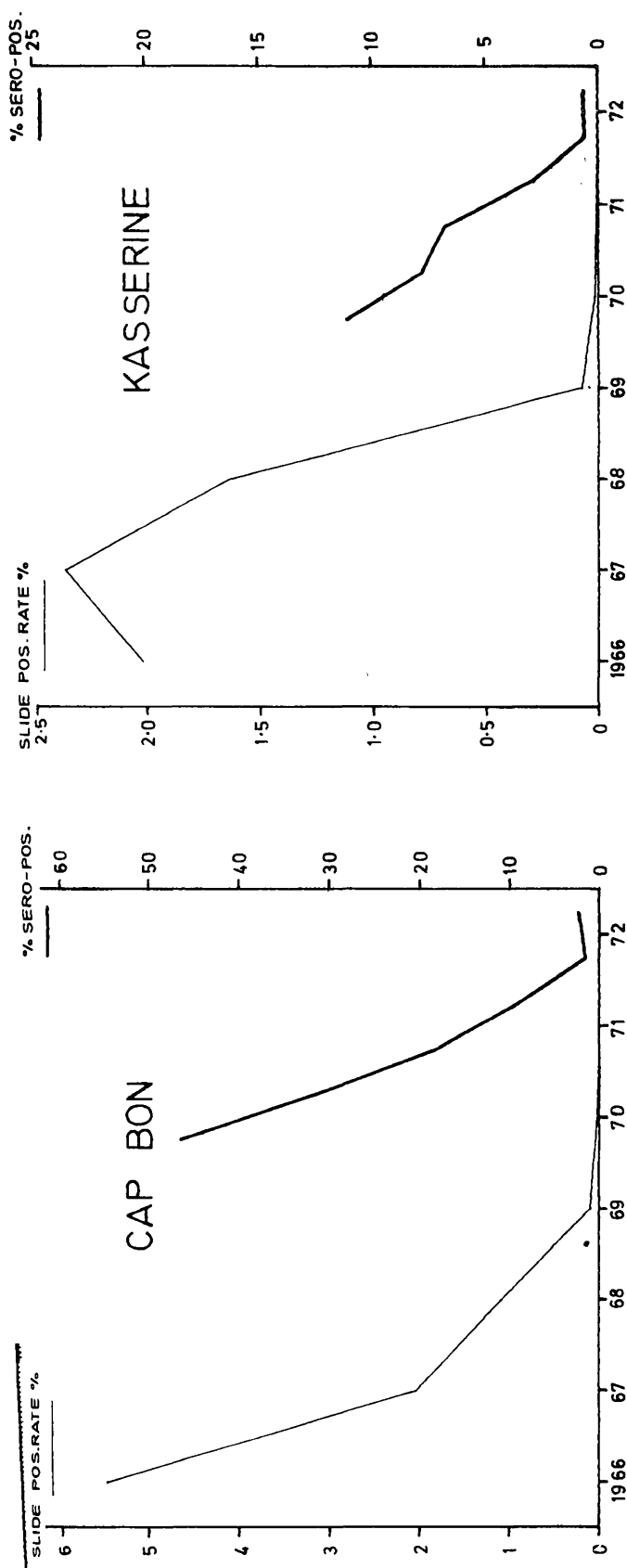
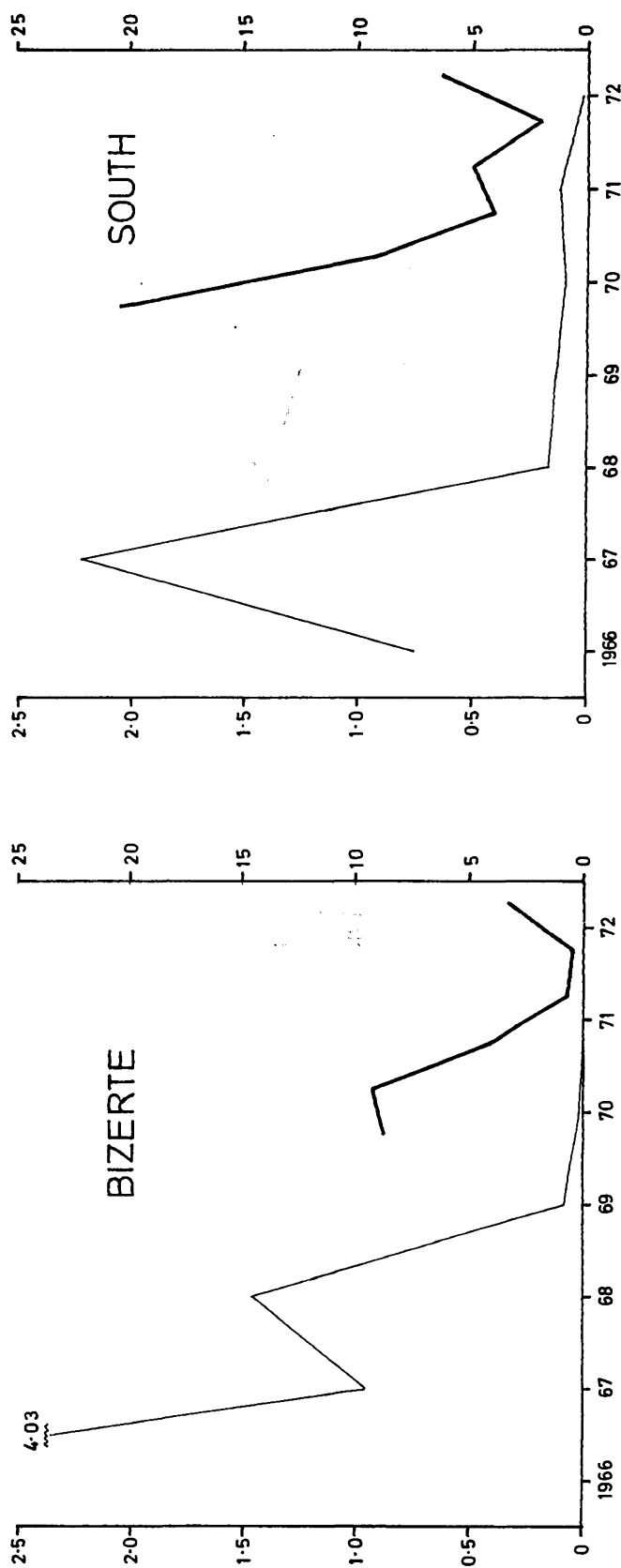


Fig. 16 SLIDE POSITIVITY RATE 1966 - 1972 AND SERO-POSITIVITY RATE 1970 - 1972  
(TITRE LEVEL 1/20+) IN CAP BON, KASSERINE, BIZERTE AND SOUTHERN GOVERNORATES



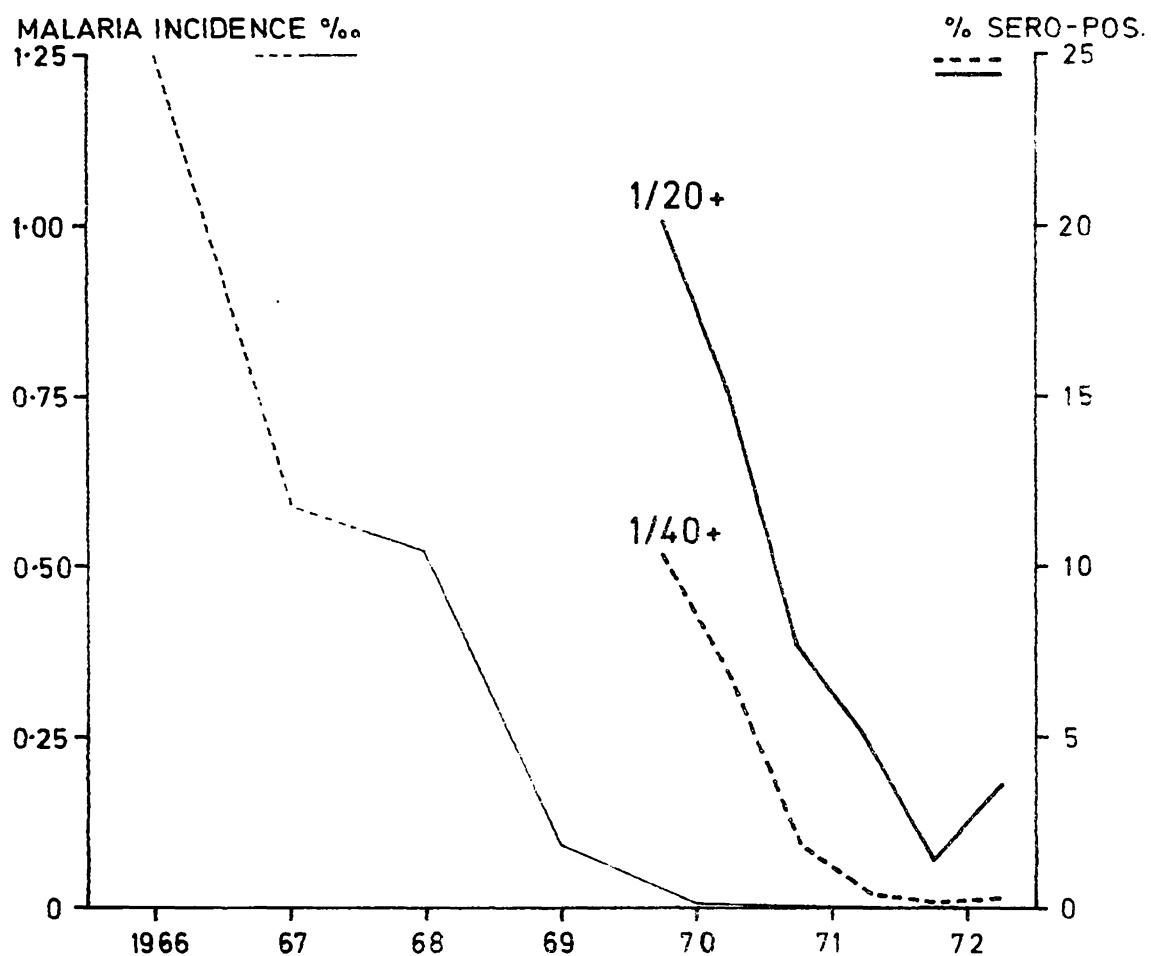


Fig.17 MALARIA INCIDENCE 1966 - 1972 AND SERO-POSITIVITY RATES 1970 - 1972  
(TITRE LEVELS 1/20+ AND 1/40+) IN ATTACK AND CONSOLIDATION PHASE AREAS OF TUNISIA

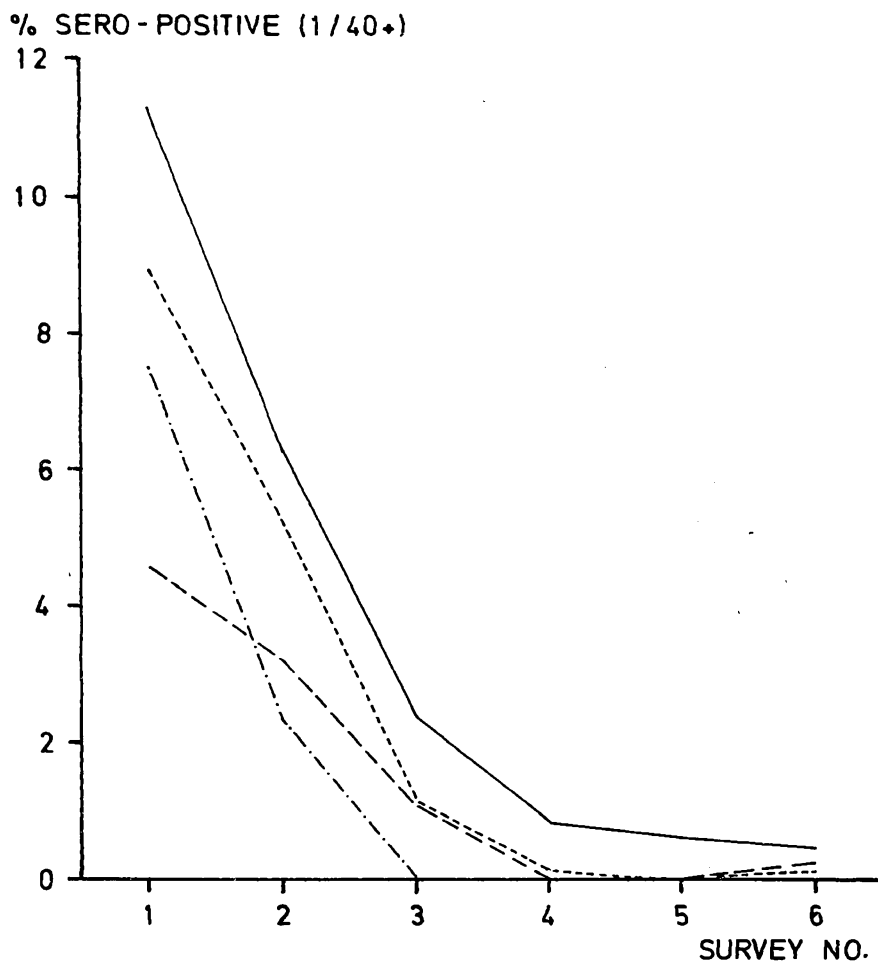


Fig.18 DEVELOPMENT OF SERO-POSITIVITY RATES AT TITRE LEVEL 1/40+ THROUGH SIX SURVEYS, 1970 - 1972, BY AGE GROUP

|       |               |                   |
|-------|---------------|-------------------|
| ..... | 6 - 23 MONTHS | } AT FIRST SURVEY |
| ----- | 2 - 4 YEARS   |                   |
| ----- | 5 - 14 YEARS  |                   |
| ————  | 15+ YEARS     |                   |

The purpose of the WHO/MAL series of documents is threefold:

- (a) to acquaint WHO staff, national institutes and individual research or public health workers with the changing trends of malaria research and the progress of malaria eradication by means of summaries of some relevant problems;
- (b) to distribute to the groups mentioned above those field reports and other communications which are of particular interest but which would not normally be printed in any WHO publications;
- (c) to make available to interested readers some papers which will eventually appear in print but which, on account of their immediate interest or importance, deserve to be known without undue delay.

It should be noted that the summaries of unpublished work often represent preliminary reports of investigations and therefore such findings are subject to possible revision at a later date.

The mention of manufacturing companies or of their proprietary products does not imply that they are recommended or endorsed by the World Health Organization.

\* \* \*