The distribution of certain diseases in Brazil as indicated by data obtained through viscerotomy

II) The incidence of malaria lesions in material collected from 1937-1946

by

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INTRODUCTION

The histopathological diagnosis of malaria by liver examination, in the acute as well as the chronic form of this disease, is based on finding the malarial pigment phagocytosed by cells of the histiocytic type. Before the discovery of the malaria parasite, the presence of the pigment in the peripheral blood was the most dependable diagnostic means for this infection. Lancisi (1717) was one of the first to draw attention to the dark or black hue of various internal organs in fatal cases of malaria. Meckel (1847), Virchow (1847) and Forsyth Meigs (1868) demonstrated that the black color of the spleen, brain, liver and other organs of individuals dying from malaria was due to the presence in the blood of pigment-bearing leucocytes, and to granular black pigment depositions in the viscera. Celli and Marchiafava (1884) were the first to identify the pigment as a hemoglobin derivative obtained through the gradual transformation of hemoglobin within the red cells. The malarial pigment, incorrectly named melanin by its discoverers, later was called hemozoin by Sambon (1909), under which name it is generally known up to the present. The more recent studies of Sinton and Ghosh (1934), and those of Morrison and Anderson (1942), have shown definitely that the pigment of the Plasmodium of Malaria which is derived from hemoglobin and constitutes an end product of the parasitic intracellular metabolism is, in fact, free hematin or, more accurately, ferrihemic acid.

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Secondary changes which may occur in the microscopic appearance of the liver in acute malaria are, in the order of frequency: a) more or less intense hyperplasia of the histiocitic elements of the parenchyma, particularly of the Kupffer cells; b) sinusoidal stasis of the motile cells, represented by mononuclear leucocytes, mainly of the macrophage type; c) hemosiderin deposits in the reticulo-endothelial and polygonal cells, especially in the central zone; d) active congestion; e) focal or diffuse cloudy swelling; f) focal necrosis injuring, generally, the central zone of lobules; g) scattered cellular necrosis, affecting irregularly contiguous lobules; h) presence of parasites, generally few in number, in phagocytosed or free red blood cells.

The above lesions are faint or absent in chronic malaria. This form is characterized by a relative abundance of pigment in the endothelial elements of the portal spaces and practically limited to them. Intense hypertrophy with hyperplasia of the perivascular connective tissue, mainly in the portal spaces is also marked. The neoformation of perilobular connective tissue is so intense at times, that it may be considered a true process of parasitic cirrhosis.

On microscopic examination of tissue section, malaria pigment is seen as small black or dark brown granules lying within reticulo-endothelial cells. The pigment is gathered at times in clumps of variable magnitude which always present a granular aspect. Two other chemically similar pigment may be confused with it: a) The so called "formaldehyde pigment" which may be differentiated by its microcrystalline structure and by the large size of its extracellular accumulations in the vascular spaces, and its general resemblance to a precipitate; b) The schistosomiase pigment. This pigment, the product of hemoglobin digestion regurgitated by adult tramatodes into the blood stream, also is deposited in the liver in the stellate cells of Kupffer and in the endothelium particularly that lining the Kiernan spaces. On microscopic examination it not rarely may be mistaken for malaria pigment, although it is generally a powdery or dust-like material of dark brown color, with a tendency to form clumps of variable size but with a roughly homogeneous appearence. A close chemical relationship must exist between malaria and schistosomiase pigments, as may be concluded from the careful study by Potenza (1941) who was unable to separate one from the other by histochemical means. However, Davis (1934) declares that it is possible to differentiate the pigments on microscopical examination alone and states that "... malaria frequently may be diagnosed in the presence of Schistosomiase". We do not share this view. Although, as a general rule, the microscopic aspect of the malaria pigment insures its identification, it seems to us that in the presence of characteristic lesions of schistosomiase, it is hazardous to diagnose malaria on the basis of the mere presence of a granular pigment of a darker hue. For, as is well known in experimental schistosomiase, the pigment may at times have the same appearence. Cases of this nature have therefore been omitted from the statistical analysis which follows.
MATERIAL AND METHODS

Data for the malarial incidence reported in this study were obtained by examination of liver specimens collected by viscerotomy throughout Brazil during the period of 1937 to 1946.

The methods of examination and the manner of interpretation of the viscerotomy data which were given in a previous paper (1949) are applicable to the present investigation. Specimens were considered to be malaria positive only in the presence of characteristic pigmentation.

The prevalence of malaria in Brazil as determined through viscerotomy is based on the presence of pigment in the liver constituting, thus, a pigmentation index of infection. This method of malarioniometry was first employed by Davis (1934) in his analysis of the fragments collected by viscerotome during the period of 1930 to 1933.

ANALYSIS OF THE STATISTICAL DATA

The number of liver specimens collected by viscerotomy in Brazil from 1937 to 1946 was 267, 107. A total of 16,559 malaria positive samples were found giving an incidence of $6.20 \pm 0.03$ for the country as a whole.

Liver samples were secured from 1,174 counties, 991 of which gave positive specimens. Thus approximately 85 cent of the countries are shown to be foci infection thereby confirming anew the extent and gravity of the problem in this country. This fortunately has already been recognized as such and is being vigorously combated by our public health authorities.

Geographical distribution — The incidence of malarial livers was determined for each locality or viscerotomy posts grouped by counties. Rates for counties alone are presented due to their greater precision.

a) Appraisal of the general situation — Cases of malaria were diagnosed in all States and Territories of the Republic (Tables 1 and 2).

The distribution of malaria in Brazil throughout the decade under study is given in the map of Figure 1. A pigmentation index above 25 per cent, which probably corresponds to an acute epidemic situation or a state of hyperendemicity, was arbitrarily designated as very high; indices varying from 15 to 25 per cent were given as high; those falling between 5 to 15 per cent as medium and under 5 per cent as low.

Examination of Figure 1 reveals some noteworthy characteristics. Two zones of high incidence are clearly delineated. The first, of vast extent, shaped like a large inverted “U”, extends over all the Amazon region and the States of Maranhão and Piauí and runs down the east and northeast of Goiás and west of Bahia to bend over the northwest of Minas Gerais. Covering the south of Goiás, as well as a large part of the “Triângulo Mineiro”, it reaches the south of Mato Grosso,
western São Paulo and northern and west Paraná. The second and much smaller zone, is limited to the long narrow strip of coastal lowlands extending from the State of Bahia to the State São Paulo, with additional "pockets" in the east of the State of Santa Catarina corresponding to the area of "bromeliad malaria". Between these two zones of high incidence there lies a third, clearly discernible extensive region of low incidence, which takes in the northeast of Brazil, the central region of Bahia, eastern and southern Minas, northeastern and southern São Paulo, eastern Paraná and the southern part of the Republic.

Medium or high malarial rates were obtained for some counties in the third zone. For some of these the index is based on a relatively small number of samples and the figures are of dubious value. However, for others in which sampling error is not a factor, favorable conditions for the prevalence of malaria must have existed or still be present. Thus, various counties of the States of Ceará and Rio Grande do Norte have high rates. These countries are in the region invaded by Anopheles gambiae between 1930 and 1938. This infestation was characterized by a tremendous epidemic outbreak of malaria in 1938 and 1939.

The percentile indices of incidence in the Brazilian States reach their highest value in the north. The maximum rates were secured in the State of Amazonas and the Territory of Rio Branco.

The incidence of malaria was lowest in the States of Pernambuco, Alagoas and Sergipe (Table 1).

A comparison of the malarial rates based on viscerotomy material collected in 14 States by Davis (1930-1933) and by us (1937-1946) is presented in Table 4. In 9 of the 14 States the values obtained by Davis are significantly higher than those secured by us. In 4 States alone (Amazonas, Maranhão, Piauí and Rio Grande do Norte) are the indices the same. In only one State, that of Ceará, is the incidence for the period of 1937 to 1946 significantly higher. This rise may be attributed to the great epidemic of gambiae-transmitted malaria to which reference has already been made.

The marked diminution in malaria positive livers obtained in 9 States by viscerotomy throughout 1937 to 1946 may indicate a natural downward trend of the endemic curve for this period. This, in our opinion is more probable. Or, it may be an artifact in the sense that it reflects ponderable differences in the nature of the material examined or in the diagnostic criteria adopted. Undoubtedly, neither of these conditions occurred.

b) States and counties — All counties which were malaria negative or positive on viscerotomy during the decade of 1937 to 1946 are tabulated by States in Table 3. Detailed analysis of this table reveals the incidence of malaria in each Federal unit and its counties.
1. ACRE — In this Territory a high incidence, 16.18 per cent, was obtained. All of the seven counties provided with viscerotomy stations are positive. The rates are high in four of the counties and medium in the other three.

2. RIO BRANCO — Represented by a single country, Boa Vista, the Territory shows the very high incidence of 30.68 per cent.

3. GUAPORÉ — The rate in this Territory is also high (21.48 percent) as evidenced by the two counties with viscerotomy posts.

4. AMAPÁ — The prevalence of malaria here is also high (18.49 per cent). Uniform rates of infection are given by its three counties.

5. AMAZONAS — This States attains the highest incidence, 25.81 per cent. All of the 25 counties with viscerotomy stations were positive. The following are noteworthy for showing infection rates above 25 per cent: Manáus (25.38 per cent); Borba (49.52 per cent); Itacoatiara (37.68 per cent); Lábrea (41.38 per cent); Maués (29.19 per cent); Parintins (32.31 per cent); Uapés (30.36 per cent); and Urucurituba (34.04 per cent). Such rates necessarily signify the existence of local hyperendemicity with high prevalence of malaria.

6. PARÁ — All of the 52 counties in which viscerotomy posts are maintained are malaria positive. The incidence for the State as a whole is high, 20.83 per cent. Massive infection (rates above 25 per cent) is present in the following thirteen counties: Alenquer (26.57 per cent); Altamira (28.30 per cent); Cametá (25.77 per cent); Curralinho (30.46 per cent); Gurupá (26.00 per cent); Igaraçú-Miri (25.17 per cent); Itaituba (25.90 per cent); Juriti (31.58 per cent); Muaná (34.92 per cent); Ourém (27.32 per cent); Ponta de Pedras (34.80 per cent); São Sebastião da Boa Vista (28.16 per cent); and Viseu (26.72 per cent).

7. MARANHÃO — This State which is made up of 49 counties, in all of which malaria is present has a medium incidence of 12.55 per cent. However, high rates are given by 14 counties in two of which massive infection is to be found: Baixo Mearim (28.89 per cent) and Mirador (25.93 per cent).

8. PIAUÍ — A medium incidence of 13.64 per cent is obtained for the 34 counties of this State, all of which are malaria positive. High rates are found in 14 counties, in four of which massive infection was uncovered: Bom Jesus (27.50 per cent); José de Freitas (25.58 per cent); Oeiras (25.58 per cent) and Porto (29.63 per cent).

9. CEARÁ and RIO GRANDE DO NORTE — Both States have low malarial incidence. The values obtained are 4.93 per cent and 3.23 per cent, respectively. In Ceará, 71 of 79 counties samples are positive for malaria. In two counties alone are high indices obtained (Russas — 19.67 per cent and Jaguariúna — 20.31 per cent). In Rio Grande do Norte, 20 out of a total of 24 counties show infection with malaria. None give a high incidence.
A detailed examination will be made of the viscerotomy data of these two States covering the region of epidemic gambiae-transmitted malaria. Six counties in Ceará and four in Rio Grande do Norte, which lie within the infested area, and in which more adequate sampling was done, were chosen for study. The comparative prevalence of malaria in the counties of Aracati, Jaguariuna (formerly União), Icó, Jaguaribe, Morada Nova and Senador Pompeu in the State of Ceará and Areia Branca, Açú, Macau and Ceará Mirim in the State of Rio Grande do Norte for three 4 year periods: 1934 to 1937, the first or pre-epidemic phase; 1938 to 1941, the second or epidemic interval; and 1943 to 1946, the third or post epidemic period is presented in numbers in Table 5. A glance at the data reveals that the incidence of malaria in eight of the ten counties rose markedly in the epidemic period of 1938 to 1941 as compared with 1934 to 1937. Rates in the epidemic phase were double to fifteen times higher than those obtained in the first interval where it was low to nil. Apparent exception to this rise are the counties of Jaguaruana in Ceará and Ceará Mirim in Rio Grande do Norte. In the former, the index for 1934 to 1937 was moderately high. Even so the 1938 to 1941 rates attained more than twice this value. In Ceará Mirim the index for 1934 to 1937, in spite of being of dubious value due to the small number of samples on which it is based, is approximately double that for the epidemic period. The contradiction is explicable by the fact that the infestation of this county by A. gambiae occurred in 1931 and the epidemic of malaria took place in this same year (Soper and Wilson, 1945) prior, therefore to the period herein called epidemic. This clarifies the finding of only 1 liver with malarial pigmentation before 1938, perhaps as a residual finding, and which is responsible for the inversion of the indices obtained.

Examination of the indices secured in all counties during the post-epidemic period reveals that the pigmentation index behaves similarly to the spleen index. Thus, following an acute outbreak of malaria in a previously uninfected region, the pigmentation index falls considerably — sometimes returning to zero.

A noteworthy finding, moreover, is the surprising agreement, as shown in Table 5, between the values obtained for the indices of malarial incidence during the epidemic period of 1939-1941, as determined through viscerotomy and by parasite indices. In five of the seven counties in which it was possible to compare our results with those obtained by Soper and Wilson (1945) a marked degree of agreement was evidenced. In our opinion, this fact confers upon the pigmentation index the same importance which is assigned in malarioriometry to the spleen and parasite indices. The value obtained for the pigmentation index permits recognition of the prevalence of malaria in a given zone. Its fluctuations provide a basis for interpretation of the epidemiology.

10. PARAÍBA — Twenty of the 25 counties which sent in viscerotomy specimens were found to be malaria positive. The percentual
incidence is low, equal to 2.39. Only one county, that of Ingá, shows a moderate incidence. However, this falls in the lower range.

11. PERNAMBUCO — Forty-four of a total of 57 counties show evidences of infection. The total incidence is low, giving an index of 1.93 per cent. Moderate values were obtained for 10 of the counties. In one of these, Surubim, the rate attains the higher range.

12. ALAGOAS — The incidence of malaria in this State is low, with an index of 1.87 per cent. Fourteen of 16 counties sampled are positive. A low incidence prevails in all, with the exception of Cururipe and São Luís do Quitunde which have indices of medium values.

13. SERGIPE — Malarial livers were secured from 23 of 31 counties covered by viscerotomy. A low index of 1.80 per cent is obtained for the State. Only 2 of its 23 malarial counties, Japaratuba and Darcilena, give medium rates.

14. BAHIA — This State also has a low incidence — 4.96 per cent. Eighty-six of 110 counties which sent in viscerotomy specimens are malaria positive. The prevalence of infection is high in 4 counties. In one of these Geremobobo, the incidence is very high. However, this may be a chance occurrence, as the number of samples is small. The other three counties with high rates are: Cachoeira, Taperoá and Cai-rú.

15. ESPÍRITO SANTO — There is a moderate incidence of malaria in this State, with an index of 7.25 per cent. Liver specimens were obtained from 32 counties, 31 of which are found to be malarial. Four counties — Anchieta, Aracruz, Linhares and São Mateus present a high incidence.

16. RIO DE JANEIRO — The pigmentation index for the entire State is moderate, attaining 5.79 per cent. All but one of the 50 counties covered by viscerotomy are malaria positive. Only five of these show a high incidence: Araruama, Itaguai, Mangaratiba, São Pedro da Aldeia and Silva Jardim.

17. MINAS GERAIS — Two hundred and seventeen of the 263 counties which were sampled are malaria positive. A low incidence, with an index of 2.52 per cent is given by this State. Only 4 counties show high rates: Carlos Chagas, Divinópolis, Raul Soares and São Romão. In the first two, the incidence reaches a very high level.

18. SAO PAULO — The incidence of malaria for the State as a whole is low, 4.39 per cent. A total of 156 counties were covered, of which 118 are positive. High indices prevail in 7 counties — Glicério, Miracatu, Pereira Barreto, Salto Grande, Xiririca, Itaporanga and Oswaldo Cruz. The incidence in Itaporanga is above 25 per cent, an indication of massive infection.

19. PARANÁ — A medium incidence with an index of 7.07 per cent, is given by Paraná. Thirty-four of the 47 counties sampled are
infected. High indices are presented by the following counties: Tibagi, Tomazina, Assai and Venceslau Braz.

20. SANTA CATARINA — The index for this State is low — 2.85 per cent. Viscerotomy material was obtained from 32 counties, 23 of which are malaria positive. Six counties give rates between 5 and 11 per cent, of medium value, therefore. The counties with the highest rates are Brusque and Nova Trento.

21. RIO GRANDE DO SUL — A low incidence, 2.64 per cent, was secured for this State. Eleven of its 15 counties are positive. But in only 3 — São Luis de Gonzaga, São Borja and Torres — do the indices attain medium values.

22. GOIÁS — Thirty-seven of 40 counties which were covered are malaria positive. The incidence for the entire State is moderate with an index of 6.65 per cent. The counties of Itumbiara, Natividade, Rio Verde, Mataúna and Morrinhos give high indices.

23. MATO GROSSO — The incidence for this State also, is moderate. An index of 6.84 per cent was obtained. Twenty of the 24 counties sampled are malaria positive. Of these only 3 have high rates: Aquidauana, Diamantino and Nioaque.

*Age Distribution* — Circumstances, which have already been given in our paper on schistosomiasis, forced us to restrict the analysis of age distribution to the material examined during 1938. During this year 1,268 cases of malaria bearing data on age were obtained from a total of 30,819 liver specimens secured through viscerotomy. This represents a total incidence for the entire country of 4.11 ± 0.074.

Distribution of the 1,268 cases by age groups is presented in Figure 2 and Table 6. As may be seen, malaria affects about equally each of the six age groups given. However, there is greater prevalence of infection in adolescents and adults in the 10 to 19, 20 to 29 and 30 to 39 age groups, among which groups no significant difference in incidence exists. There are significant differences shown, however, between the 0 to 9 and 10 to 19 groups (— 1.1 ± 0.32) as well as between the latter and the 50 years and over group (1.54 ± 0.34).

Thus, it may be state that the incidence of malaria is less for the first and last age groups, between which no significant difference is demonstrated. The decrease in prevalence noted for the 40 to 49 age group is not statistically significant in relation to that of the groups which precede it nor the one which follows it.

**CONCLUSIONS**

The examination of 267,107 liver specimens obtained by viscerotomy during the period of 1937 to 1946 established the diagnosis of malaria in 16,559 samples, which gives Brazil a percentile incidence of 6.20 ± 0.03.
A detailed analysis of the geographical distribution of the positive specimens was made by States and counties. It is noted that malarial livers were obtained from all Federal units of the country.

The present analysis is based on the diagnosis of malaria by the presence of the characteristic pigment in the liver. The difficulties inherent to this diagnostic method are pointed out in the text.

The percental index of pigmentation in viscerotomy livers obtained for any region by this manner as may be inferred from the comparative data given, partakes, in our estimation, of the same importance in the interpretation of epidemiological studies on malaria, as the well known and widely employed spleen and parasite indices.

A criterion for the classification of the values of the pigmentation index is established. Indices above 25 per cent are considered to denote a very high incidence, and may reflect an acute epidemic condition or, in general, a hyperendemic state. Indices between 15 and 25 per cent are high; those between 5 and 15 per cent medium or moderate; while those under 5 per cent are low.

The prevalence of malaria is high or moderate in the Northern and Northeast regions. The incidence is moderate in the Midwest and in the States of Espírito Santo and Rio de Janeiro in the Eastern Meridional region, and in the State of Paraná in the South. Incidence is low for the remaining regions of Brazil taken as a whole.

The age distribution of malaria, as evidenced by livers obtained through viscerotomy, is calculated for 1938 alone. Analysis showed the distribution to be quite uniform for all age groups although there was greater prevalence, significant albeit slight, of cases among adolescents and adults between 20 and 40 years of age.