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# IMMUNOLOGICAL RESPONSE TO TOXOPLASMOSIS IN POPULATION GROUPS OF THE STATE OF SÃO PAULO, BRAZIL, AS EVALUATED BY THE DISTRIBUTION OF SERUM TITERS IN THE DYE-TEST

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## SUMMARY

The present investigation analyses the pattern of frequencies of serum titers in positive reactants to the SABIN-FELDMAN dye-test from a total of 4,440 individuals resident in three different areas of the State of São Paulo, Brazil, most of which were suspected of having some clinical manifestation of toxoplasmosis. Over three quarters of the observations conformed to a theoretical chance distribution, disclosing one more indication about the specificity of the dye test. Observations with titers over 1:1,024 assembled into a separate distribution, being likely to correspond to recent cases of toxoplasmosis, independently of clinical manifestations.

## INTRODUCTION

SABIN & FELDMAN's [38] dye-test has been extensively used as a valuable diagnostic tool in clinical medicine, as well as in numerous epidemiological surveys investigating the degree to which population groups harbor infection by *Toxoplasma gondii*.

The intensity of a positive reaction is measured by the highest dilution at which blood serum can induce discoloration of the test organism, under specified conditions. Frequently the intensity of reaction, expressed as serum titer, is used to characterize severity of infection or acuteness of the disease. When the results of a series of tests are considered, the numbers of sera to which ordered titer values are assigned form a frequency distribution. Distributions of such kind have been used in several investigations to correlate results of the dye-test and other immunological means of diagnosis. In other instances, titer distributions have been used to compare groups of individuals of different sex, age, socio-economic condition or environment, in what regards the intensity of toxoplasma infection.

Less frequently, other possibilities of the study the distribution of positive titers to the dye-test have been explored. Notwithstanding, the pattern of such distribution of frequencies in fairly extensive surveys could in itself provide clues to the knowledge, on one hand, of the homogeneity of population groups concerning the dissemination of toxoplasma infection and their immune response; on the other hand, of specificity characteristics of the test under the conditions how it was performed in those groups.

With a view to the forementioned possibilities, the present investigation analyses the distribution of serum titers in positive reactants from a total of 4,440 individuals, resident in three different areas of the State of São Paulo, Brazil, most of which were suspected of having some clinical manifestation of toxoplasmosis. Since the indivi-

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duals by no means constitute representative samples of the population groups involved, the observed figures cannot be taken as expressing the incidence of toxoplasma infection in those groups. The figures nevertheless provide subsidiary information concerning the order of magnitude which the prevalence of toxoplasmosis can be expected to assume in this part of the country.

## MATERIAL AND METHODS

A) *Origin of the material studied* — All of the 4,440 serum samples were collected in private clinics from subjects suspected of having some form of toxoplasmosis, resident in three different areas of the State of São Paulo, Brazil. This State is situated in the southern area of the country, i.e, within the south-temperate climatic zone (Fig. 1).

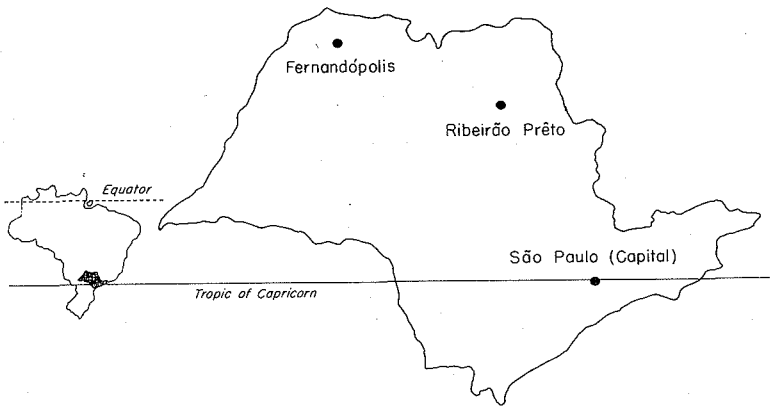


Fig. 1 — Immunological response to toxoplasmosis. Geographical situation of the areas studied. 1 — São Paulo (Capital); 2 — Ribeirão Prêto; 3 — Fernandópolis

3,731 Specimens were from São Paulo City, the capital of the State, a 1,509 square kilometer municipality, with a 3,109.9/km$^2$ population density (5.849,529 urban and 52,004 rural; 1970). Its altitude varies from 740 to 820 meters above sea level, and its average annual rainfall amounts to 1,640.1 mm.

389 Specimens were from Ribeirão Preto, an agricultural center 289 kilometers westwards from São Paulo, 1,048 square kilometers in area, with a 199.8/km$^2$ population density (194,800 urban and 14,627 rural; 1970). The local altitude averages 518 meters, and the annual rainfall 1,109 mm.

The remaining 320 sera were from Fernandópolis, also an agricultural center, 552 kilometers westward from São Paulo, with a 71.5/km$^2$ population density (28,480 urban, 10,510 rural; 1970). Its altitude averages 529 meters, and its annual rainfall 1,101 mm.

B) *Laboratory methods*

*Test sera* — The blood samples were collected asseptically and maintained at room temperature for about two hours, centrifuged at low speed (1,000 r.p.m.) for fifteen minutes, and the sera stored at + 4°C. Sera from residents of São Paulo were tested within two to three days upon collection, but those from Ribeirão Preto and Fernandópolis withstood periods of storage at + 4°C up to fifteen days, plus six do eight hour-periods at room temperature during surface delivery to São Paulo.

All of the sera were non-inactivated.

*Toxoplasma suspension* — The "N" strain of *Toxoplasma gondii* originally isolated by

TABLE I

Per cent distributions of sera positive to the dye-test (over the total numbers of positive sera)

| Area | Year | Number tested | Total positive | Titer (*) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $2^4$ | $2^6$ | $2^8$ | $2^{10}$ | $2^{12}$ | $2^{13}$ | $2^{14}$ | $2^{15}$ | $2^{16}$ | $2^{17}$ | $2^{18}$ |
| São Paulo ..... | 1961 | 168 | 76.8 | 10.9 | 22.5 | 34.1 | 13.9 | 9.3 | 3.1 | 1.5 | 3.9 | 0.8 | — | — |
| | 1962 | 461 | 76.1 | 4.8 | 17.9 | 35.6 | 21.9 | 7.4 | 2.6 | 2.3 | 2.9 | 2.9 | 1.7 | — |
| | 1963 | 686 | 70.3 | 7.5 | 15.8 | 29.9 | 23.8 | 9.1 | 0.2 | 2.1 | 2.7 | 2.5 | 2.1 | 4.3 |
| | 1964 | 554 | 75.0 | 6.7 | 15.1 | 31.5 | 27.7 | 8.9 | 1.7 | 1.9 | 2.4 | 1.4 | 1.7 | 1.0 |
| | 1965 | 843 | 68.0 | 4.5 | 13.3 | 34.4 | 25.7 | 8.2 | 2.4 | 2.6 | 2.4 | 1.7 | 1.6 | 3.1 |
| | 1966 | 1,019 | 69.6 | 3.5 | 15.4 | 24.1 | 24.4 | 10.6 | 4.1 | 3.9 | 2.7 | 3.1 | 3.7 | 4.5 |
| | 1961/66 | 3,731 | 71.3 | 5.5 | 15.6 | 30.5 | 24.2 | 9.1 | 2.4 | 2.7 | 2.7 | 2.3 | 2.2 | 2.8 |
| Ribeirão Prêto ... | 1961 | 60 | 83.3 | 14.0 | 22.0 | 40.0 | 12.0 | 10.0 | — | 2.0 | — | — | — | — |
| | 1962 | 60 | 78.3 | 4.3 | 17.0 | 42.6 | 25.5 | 8.5 | 2.1 | — | — | — | — | — |
| | 1963 | 74 | 79.7 | 5.1 | 13.5 | 37.3 | 22.0 | 15.3 | — | 3.4 | 1.7 | — | 1.7 | — |
| | 1964 | 114 | 84.2 | 5.2 | 18.8 | 28.1 | 30.2 | 12.5 | — | — | 1.0 | 3.1 | — | 1.0 |
| | 1965 | 81 | 86.4 | 4.3 | 11.4 | 50.0 | 21.4 | 8.6 | 4.3 | — | — | — | — | — |
| | 1961/65 | 389 | 82.8 | 6.2 | 16.5 | 38.5 | 23.3 | 11.2 | 1.2 | 0.9 | 0.6 | 0.9 | 0.3 | 0.3 |
| Fernandópolis ..... | 1962 | 62 | 82.3 | 11.8 | 27.4 | 41.2 | 11.8 | 5.9 | — | — | — | — | 1.9 | — |
| | 1963 | 71 | 85.9 | 4.9 | 19.7 | 37.7 | 21.3 | 8.2 | — | — | 3.3 | 3.3 | — | 1.6 |
| | 1964 | 28 | 92.9 | 7.7 | 23.1 | 23.1 | 26.9 | 11.5 | — | 7.7 | — | — | — | — |
| | 1965 | 96 | 84.4 | 4.9 | 9.9 | 32.1 | 34.6 | 11.1 | 3.7 | 2.5 | — | 1.2 | — | — |
| | 1966 | 63 | 65.1 | — | 22.0 | 26.8 | 19.5 | 17.1 | 4.9 | — | 2.4 | — | 2.4 | 4.9 |
| | 1962/66 | 320 | 81.3 | 5.8 | 18.8 | 33.5 | 23.8 | 10.4 | 2.0 | 1.5 | 1.2 | 1.2 | 0.8 | 1.2 |
| All areas ..... | 1961 | 228 | 78.5 | 11.7 | 22.4 | 35.7 | 13.4 | 9.5 | 2.2 | 1.7 | 2.8 | 0.6 | — | — |
| | 1962 | 583 | 77.0 | 5.6 | 18.9 | 37.0 | 21.2 | 7.3 | 2.2 | 1.8 | 2.2 | 2.2 | 1.6 | — |
| | 1963 | 831 | 72.4 | 7.0 | 16.0 | 31.4 | 23.4 | 9.6 | 0.2 | 2.0 | 2.7 | 2.3 | 1.8 | 3.6 |
| | 1964 | 696 | 77.3 | 6.5 | 16.2 | 30.5 | 28.1 | 9.7 | 1.3 | 1.8 | 2.0 | 1.7 | 1.3 | 0.9 |
| | 1965 | 1,020 | 71.0 | 4.6 | 12.7 | 35.6 | 26.2 | 8.6 | 2.8 | 2.4 | 1.9 | 1.5 | 1.2 | 2.5 |
| | 1966 | 1,082 | 69.3 | 3.3 | 15.7 | 24.3 | 24.1 | 10.9 | 4.1 | 3.7 | 2.7 | 2.9 | 3.6 | 4.5 |
| | 1961/66 | 4,440 | 73.0 | 5.6 | 16.0 | 31.6 | 24.1 | 9.4 | 2.2 | 2.4 | 2.3 | 2.1 | 1.9 | 2.4 |

(*) Reciprocal of serum dilution

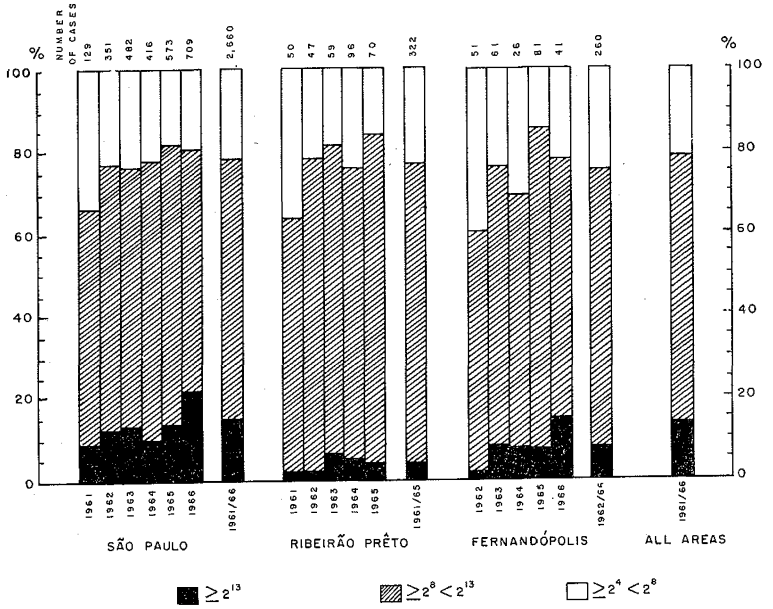


Fig. 2 — Graphical representation of the percent distribution of sera positive to the dye-test

NOBREGA [32] during an epizootics of rabbits, and laboratory-maintained by serial mouse-passage, was utilized as antigen. From mice inoculated two days earlier, a peritoneal exudate with an extracellular toxoplasma population ranging 20.000,000/ml was harvested. This exudate was diluted at 1:5 in cooled physiological salt solution added with 3.8 percent sodium citrate as anticoagulant, and kept in a refrigerator. Strict care was taken to have the test set up within one hour after the exudate harvesting.

*Accessory factor* — Obtained from volunteer donors, and routinely tested previously to storage in 1 ml aliquots, at — 20°C.

*Positive control serum* — The same precautions were observed concerning the positive control serum.

*Methylene blue solution* — This solution was prepared anew every 2-3 days, from a saturated alcoholic solution of methylene blue and an alkaline soda-borax solution of pH 11.

*The test*(*) — The SABIN & FELDMAN [38] dye-test technique modified by SABIN et al. [37] in 1952, was followed. Every serum was submitted to a screen test covering the range of $2^{-4}$ to $2^{-12}$ (in four-fold) dilutions, the sera reacting at the last dilution being subsequently tested in two-fold dilutions from $2^{-12}$ up to $2^{-18}$. Control reactions with known positive sera were carried out simultaneously to each series of tests, the negative control being the accessory factor itself.

C) *Criteria for dye-test positiveness* — The highest serum dilution preventing the stain-intake in 50 per cent of the toxoplasma cells, was considered as the titer of the given serum in the dye-test, these titers being here expressed as the logarithm to base 2 of the corresponding dilution.

## RESULTS

*Occurrence of positive sera* — Sabin & Feldman's dye test was performed in the sera of 4,440 individuals from the three forementioned areas during a period of six

(*) Carried out at the "Laboratório de Análises Brooklin", São Paulo, Brazil

years, from 1961 to 1966. The numbers of positive and negative sera from each area on each year, as well as the distribution of positive reactants according to serum titer, are presented in Table I.

Altogether 73.0 per cent of the sera were found to be positive, with titers varying from 1:16, which was the lowest dilution tested, to 1:262,144 ($2^{-18}$). As shown by the figures in Table I and the graphical representation in Figure 2, only small fluctuations were observed from year to year in the percentages that positive tests represented of the total in each area; an exception was the decrease of about 20 per cent observed at Fernandópolis in 1966. The average percentages of positive sera in the 6-year period were 71.3 per cent in São Paulo, 82.8 per cent in Ribeirão Preto and 81.3 per cent in Fernandópolis, the total numbers of tested sera being 3,731, 389 and 320 for each area, respectively.

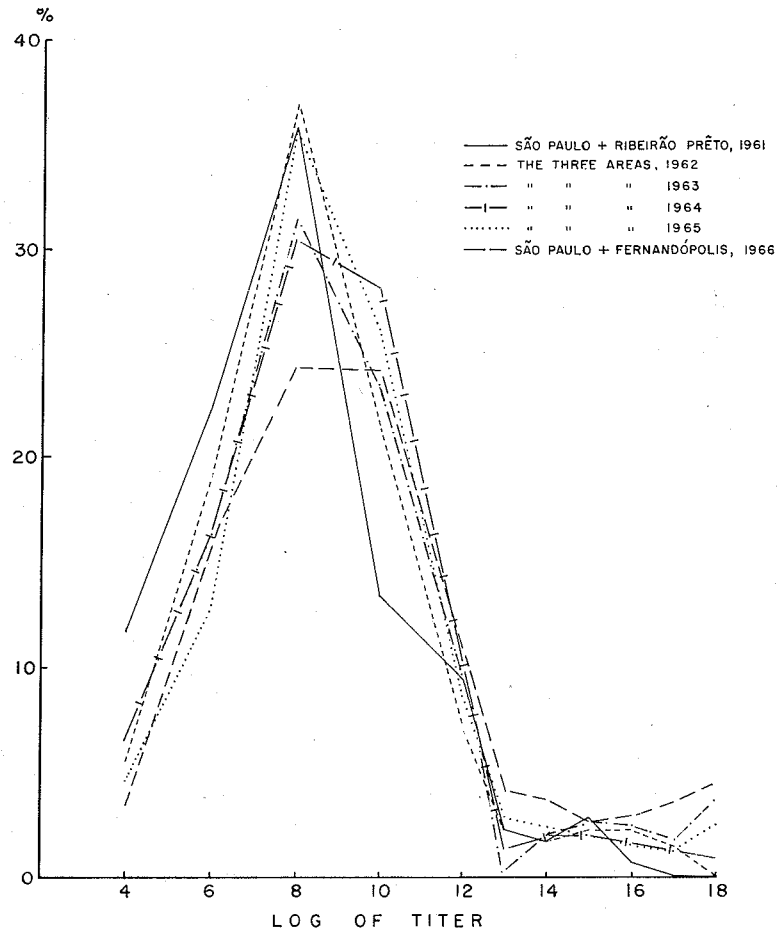


Fig. 3 — Comparison of the frequency distribution of the total sera positive to the dye-test in the three areas studied, corresponding to different years.

In Fig. 2 differently shaded areas represent the numbers of sera with high (higher than 1:4,096), intermediate (from 1:256 to 1:4,096) and low titers (lower than 1:256), expressed as percentages of the total number of sera tested on each year and area.

Considering the whole period of observation, sera with high titers were more frequent in São Paulo (10.7 per cent of the total) than in Ribeirão Preto (3.7 per cent), with intermediary frequency in Fernandópolis (6.3 per cent). A similar relationship holds true for the separate results corresponding to each year. Furthermore, a slight trend toward an increase of high titers is apparent on successive years in each area of investigation.

Sera with intermediate and low titers were in general less frequent in São Paulo than in Ribeirão Preto for Fernandópolis during the whole period of observation.

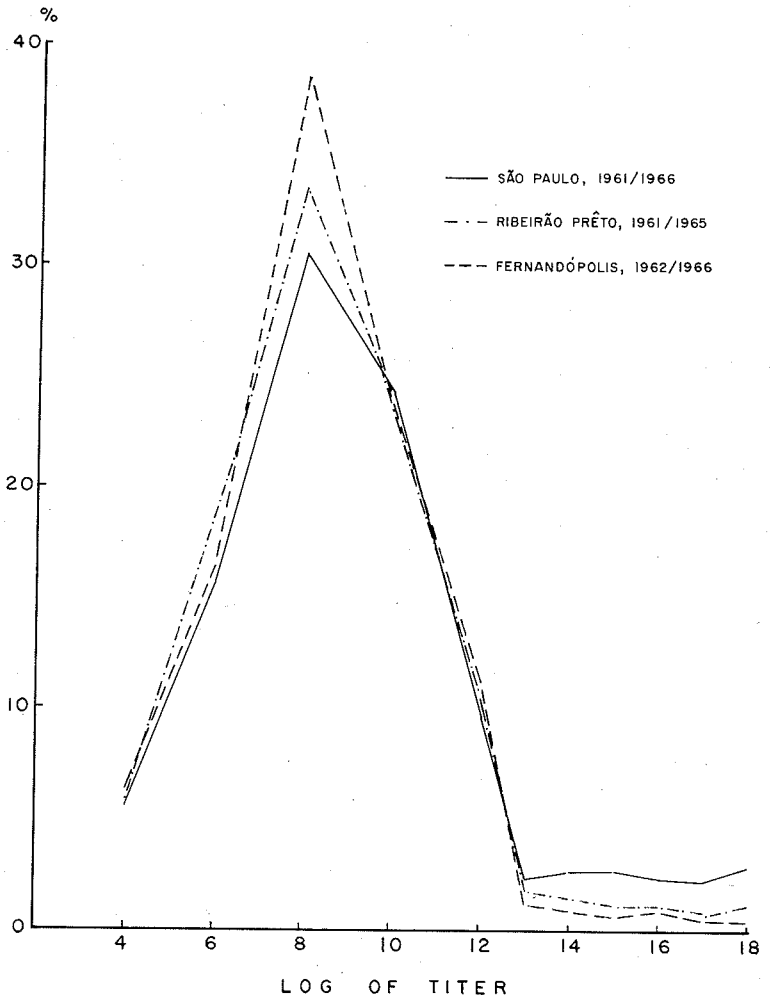


Fig. 4 — Totals of sera positive to the dye-test corresponding to the 6-year period separately for each area, expressed as percent of the total number of positive sera in the respective area

The occurrence of positive sera at different titers can be better analysed when, for each year and area, percentages are taken over the total numbers of positive sera instead of the total numbers of sera tested. Those percentages are also given in Table I, and their distributions are considered in the following section.

*Distributions of positive sera according to titer* — The frequency distributions of sera positive to the dye-test on each year and area present as a general characteristic an accumulation of cases in correspondence to titers 1:256 and 1:1,024, with gradually decreasing numbers for titers progressively lower or higher than those values.

Since the number of observations is not large for each year and area separately, especially in Ribeirão Preto and Fernandópolis, the totals of the three areas are first considered to compare the distributions corresponding to different years. The data, expressed as percentages of the total number of positive sera observed on each year, are plotted in Fig. 3 in relation to the logarithm of the serum titer. Apart a few irregularities, the general appearance of the curves is not suggestive of essential differences corresponding to any particular year.

On the other hand, when the totals corresponding to the 6-year period are considered separately for each area and expressed as percentages of the total number of positive sera in the respective area, the curves plotted on Fig. 4 result. Here a striking similarity is apparent, indicating for the different areas a very good agreement in

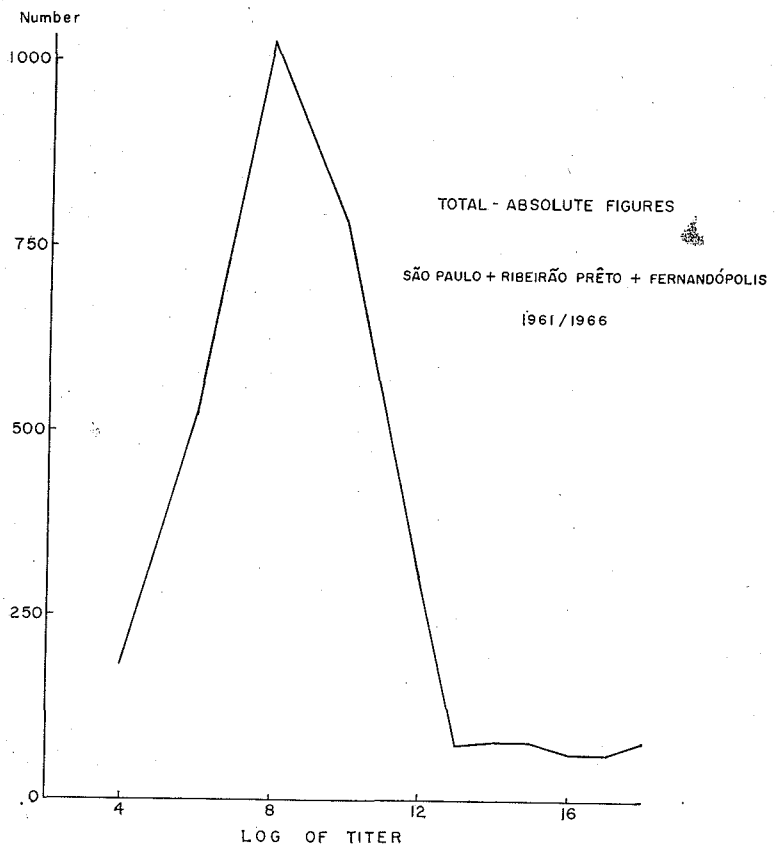


Fig. 5 — Pooled distribution of the 3,242 sera positive to the dye-test

the shape of the distribution of positive sera in relation to the logarithm of the titer.

Inasmuch as the distributions corresponding either to different areas or to different years do not show definite peculiarities characteristic of a certain year or area, but rather seem to exhibit a common pattern, the pooled distribution of 3,242 positive sera according to log titer can be considered as typical of the whole set of data and is plotted in Fig. 5.

The main portion of the resulting curve reminds the bell-shaped configuration of the normal probability curve. However, truncation is apparent in correspondence to the lowest dilution tested, whereas an extended tail corresponds to the highest serum dilutions, rendering the curve definitely assymetrical. Nevertheless, a major portion of the data could still possibly follow the normal distribution, partially masked by superposition of the distribution or distributions corresponding to the remaining data. In order to check this possibility, an expedient resource is the graphic test of normality, which in this case consists of plotting as ordinates the probits corresponding to the cumulative percentages of positive sera observed up to each titer, and as abcissae the logarithms of the respective titers: when the graph approximates a straight line, the original data can be considered as normally distributed.

The graphic test of normality was performed with the data of Table II and is shown in Fig. 6. It is seen that the first four points in the graph are disposed on an approximately straight line, whereas the six points corresponding to the highest serum dilutions form a slightly curved, concave up line, at an angle with the straight

TABLE II

Overall distribution of sera positive to the dye-test

| Log titer (*) | Observed frequency | Cumulative frequency | Cumulative per cent of total | Probit |
|---|---|---|---|---|
| 4 | 181 | 181 | 5.61 | 3.41 |
| 6 | 518 | 699 | 21.58 | 4.21 |
| 8 | 1,023 | 1,722 | 53.13 | 5.08 |
| 10 | 782 | 2,504 | 77.25 | 5.75 |
| 12 | 304 | 2,808 | 86.62 | 6.11 |
| 13 | 73 | 2,881 | 88.87 | 6.22 |
| 14 | 78 | 2,959 | 91.28 | 6.36 |
| 15 | 76 | 3,035 | 93.62 | 6.52 |
| 16 | 67 | 3,102 | 95.68 | 6.72 |
| 17 | 61 | 3,163 | 97.56 | 6.98 |
| 18 | 79 | 3,242 | 100.00 | — |

(*) Logarithm to base 2 of the reciprocal of serum dilution

line. As shown by that graph, two different distributions appear to have been superimposed: the observations corresponding to sera which were positive at dilutions varying from 1:16 to 1:1,024 follow an approximately normal distribution; those corresponding to sera which were positive at higher dilutions diverge from the former.

The bulk of the data, however, corresponds to the normally distributed portion, which represents 77.3 per cent of the total. From the probit graph, estimates of the arithmetic

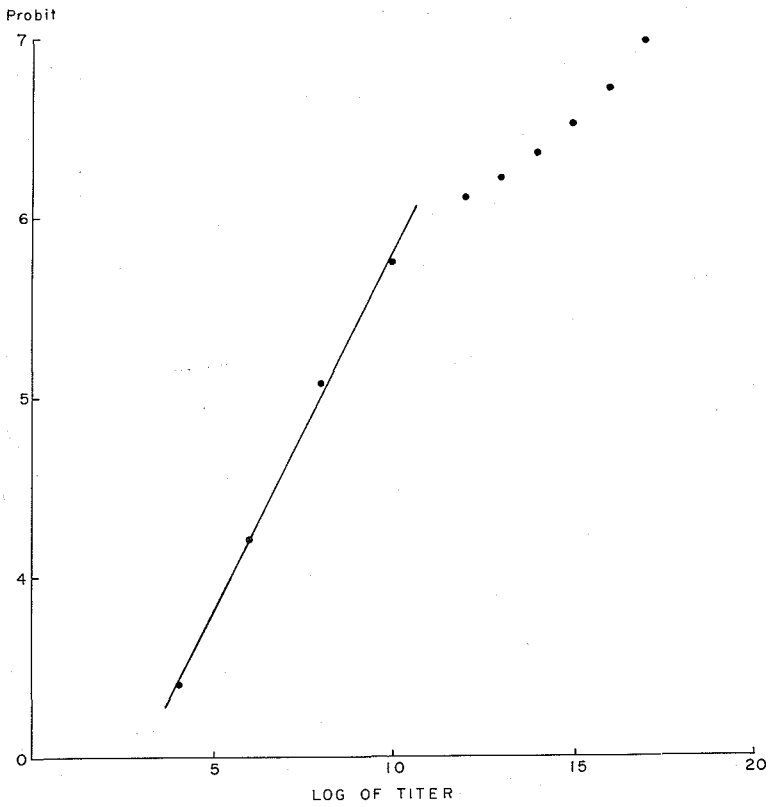


Fig. 6 — Overall distribution of sera positive to the dye-test. Graphic test of normality

mean and standard deviation of the log titers in that portion can be derived. The mean — which is also the logarithm to base 2 of the geometric mean of the titers — is the abcissa value corresponding to probit 5 as the ordinate, and was found to be 7.98. The standard deviation is the reciprocal of the slope of the probit line, and was found to be 2.54.

For the whole set of positive tests, the calculated mean of log titers was 9.31 and the standard deviation was 2.66.

## DISCUSSION

The large proportion of positive tests which was found consistently in the three areas investigated, no doubt points out that toxoplasmosis is widely prevalent in those areas. That finding in itself bears no direct relation to the extent to which cases with clinical symptoms of toxoplasmosis should be expected in the involved communities. Latent infection by *Toxoplasma gondii* is probably responsible for the large majority of positive serological tests which have been found in most surveys of population groups in different countries. In fact, high as well as low rates of positiveness to the dye test appear to have been found independently of the inclusion or not of suspected clinical cases of toxoplasmosis in the investigated group. Considering investigations which included suspected cases, such as the present one, the proportions of positive sera were similarly high in many instances, as shown in the upper section of Table III; however, in other instances, as shown in the lower section of the same table, much lower pro-

TABLE III

Frequency of positive dye test in groups including suspected cases of toxoplasmosis

| Authors | Locality | Number tested | Per cent positive | Observations |
|---|---|---|---|---|
| CAMARGO, M. E. [8] | São Paulo, Brazil | 1,000 | 76.8 | |
| REUSS, K. [36] | Frankfurt/Main, Germany | 4,240 | 62.3 | |
| KUDICKE, H. & POEHLIG, W. [25] (A) | Lower Saxony, Germany | 1,234 | 65.7 | |
| KUDICKE, H. & POEHLIG, W. [25] (B) | Lower Saxony, Germany | 2,194 | 71.4 | |
| HAHN, E. [21] | North Italy | 1,000 | 77.3 | |
| LEINZIGER, E. et al. [26] | Linz, Australia | 484 | 85.5 | Female patients; Landesfrauenklinik |
| SCHOLZ, R. [39] | Linz, Australia | 473 | 97.5 | Mothers; Landeskinderkrankenhaus |
| SCHOLZ, R. [39] | Linz, Australia | 138 | 71.0 | Children; same hospital |
| GAGLIERIS, A. N. [7] | Pavia, Italy | 93 | 40.8 | |
| VON ZEIPEL, G. & LINDER, L. A. [42] | Gottenborgh, Sweden | 328 | 38.4 | |
| MERCIER, P. et al. [28] | Greece | 170 | 30.6 | |

portions of positiveness have been found. On the other hand, in surveys of groups consisting of supposedly normal individuals, the proportions of positive sera were often below 30 per cent (Table IV, upper section), but other times well above 60 per cent (lower section, same table). Those discrepancies could be expected if one considers that the chances for the appearance of clinical symptoms of toxoplasmosis do not necessarily run parallel to the positiveness rates indicating spread of the organism in different population groups. But such discrepancies might also raise doubts about the specificity of the dye regarding infection by toxoplasma. Experimental data reported by MUEHLPFORD [31], HEIN [23], MICHALZIK [29], WILDFUEHR [14], AWAD [1], AWAD & LAINSON [2] and GROENROOS [20], appear to indicate that positive dye tests may result from the presence of other infectious agents or from the action of serum properdin. The evidence in that respect has been denied by FELDMAN & MILLER [13] as well as by CATHIE [9], BORGEN & BJOERNSTADT [8], and MAS BAKAL [27], who on the basis of other experimental findings uphold the reliability of the dye test in the diagnosis of toxoplasmosis.

Significant indications about specificity of the dye test are disclosed by analysis of the titer distribution of positive tests, such as performed in the present paper. As described above, over three quarters of our observations conformed to a theoretical chance distribution, the normal probability curve, when classified according to logarithms of the serum titers.

In what concerns the observations with titers over 1:1,024, which appeared to make up a separate distribution, they are likely to correspond to recent cases of toxoplasmosis, independent of clinical manifestations. The notion that high titers of the dye test are associated with recent infection, rather than with the appearance of symptoms, seems to be well established (SABIN et al. [37];

TABLE IV

Frequency of positive dye-test in groups of supposedly normal individuals

| Authors | Locality | Number tested | Per cent positive | Observations |
|---|---|---|---|---|
| BERENGO, A. et al. [3] | Siena, Italy | 1,200 | 4.1 | Blood donors |
| FELDMAN, H. A. & MILLER, L.T. [13] | Arizona, USA | 236 | 4.2 | Navajo Indians |
| MIDTWEDT, T. [30] | Lapland, Norway | 100 | 5.0 | School children |
| HARBOE, A. [22] | Oslo, Norway | 1,599 | 8.5 | |
| FELDMAN, H. A. & MILLER, L.T. [13] | Iceland | 108 | 11.1 | |
| MIDTWEDT, T. [30] | Norway | 200 | 15.5 | School children |
| FELDMAN, H. A. & MILLER, L.T. [13] | Portland, Ore., USA | 293 | 17.4 | |
| FELDMAN, H. A. [11] | USA | 2,294 | 19.0 | Military recruits |
| BEVERLEY, J. K. A. et al. [5] | Sheffield, England | 581 | 19.5 | |
| REMINGTON, J. S. et al. [34] | USA | 359 | 19.5 | Students of Harvard University |
| GIBSON, C. L. [17] | Tennessee, USA | 627 | 20.4 | Urban Negro population |
| GIBSON, C. L. et al. [19] | Tennessee, USA | 987 | 21.8 | Rural Negro population |
| COOK, I. [10] | Austrália | 760 | 24.0 | |
| GROENROOS, P. [20] | Finland | 1,176 | 25.6 | |
| FELDMAN, H. A. & MILLER, L.T. [13] | St. Louis, Mo., USA | 184 | 25.6 | |
| MAS BAKAL, P. [27] | Holland | 200 | 66.0 | Blood donors |
| FELDMAN, H. A. & MILLER, L.T. [13] | La Lima, Honduras | 266 | 66.2 | |
| JAMRA, L. F. [24] | São Paulo, Brazil | 300 | 67.0 | |
| NUSSENZWEIG, R. S. [33] | São Paulo, Brazil | 334 | 71.3 | Blood donors |
| FERRARIS, G. & BERETTA, L. R. [14] | Bari, Italy | 676 | 74.1 | Pregnant women |
| FELDMAN, H. A. & MILLER, L.T. [13] | Tahiti | 121 | 82.0 | |
| GIBSON, C. L. & COLEMAN, N. [18] | Costa Rica | 156 | 88.5 | |
| GIBSON, C. L. & COLEMAN, N. [18] | Esciuntha, Guatemala | 100 | 94.0 | |

GROENROOS [20]; FELDMAN [12]; BEVERLEY [4]; FLECH & LUDLAN [15]; SCHOLZ [39]; TOLENTINO [40]). At any rate, the forementioned observations with high titers, as evidenced by the probit graph, do not fit into the distribution pattern exhibited by the bulk of our data. The latter form a homogeneous aggregate, suggesting that a stable system of causes was operative in the production of titer variability.

In other words, 77.3 per cent of the observed sera can be considered as samples of the same "universe" of variation, in which the titer values result from the interaction of multiple causes, effective to different degrees in each case, but constantly present. Conformity to one and the same probability distribution, indicating stability of the variation pattern, points out the underlying operation of a constant-cause system. Such an effect could hardly originate in a system where specificity of the involved test — one of the causes in operation — were not constantly present. Therefore, demonstration that the system of chance causes in operation to produce the observed pattern of titer variability is constant, represents strong evidence in favor of specificity of the dye test to diagnose one and the same infection.

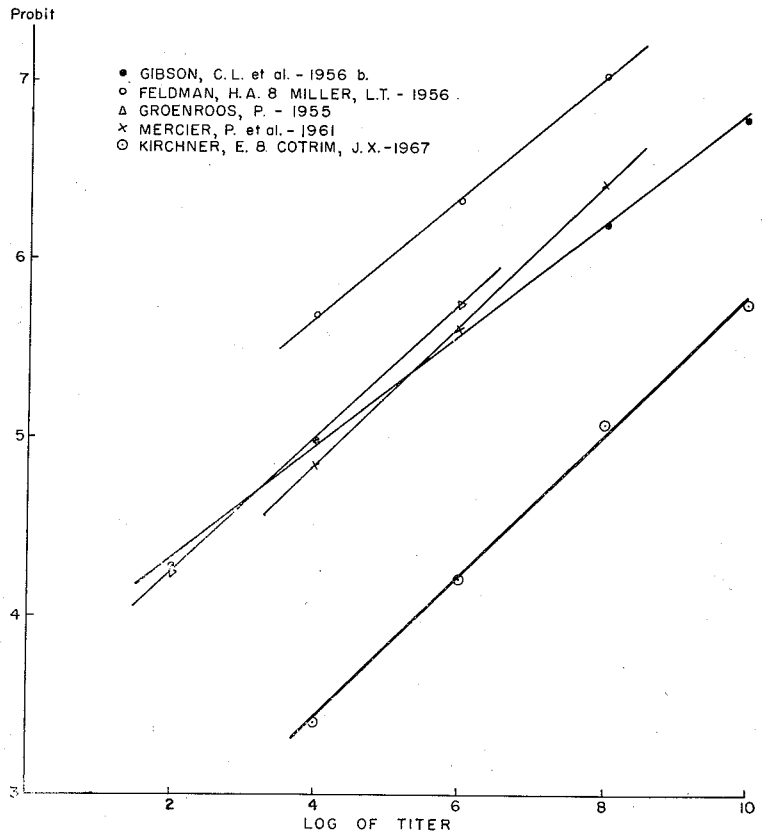


Fig. 7 — Graphic tests of normality. Data reported by:

— ● — GIBSON, C. L. et al.[19] — Rural Negro population, Tennessee, USA

— O — FELDMAN, H. A. & MILLER, L. T.[63] — Population; Portland, St. Louis and Pittsburgh, USA

— △ — GROENROOS, P.[20] — Normal; blood donors and patients, toxoplasmosis excluded; Finland

— × — MERCIER, P. et al.[28] — Suspected of toxoplasmosis; Greece

— Θ — KIRCHNER, E. & COTRIM, J. X. — Suspected; São Paulo, Brazil

If such is the case, the titer observed in other dye test surveys should also be expected to conform to the normal probability distribution, at least as far as the considered group is homogeneous in what concerns toxoplasma infection, the corresponding immune response, and the conditions under which the test was performed.

The data of 3 other surveys carried out in São Paulo, Brazil, as well as those corresponding to 14 population groups investigated in other countried were analysed and led to the probit graphs represented in Figures 7-10. As a matter of fact, as shown in Table V in 16 out of the 18 groups considered, over 70 per cent of the

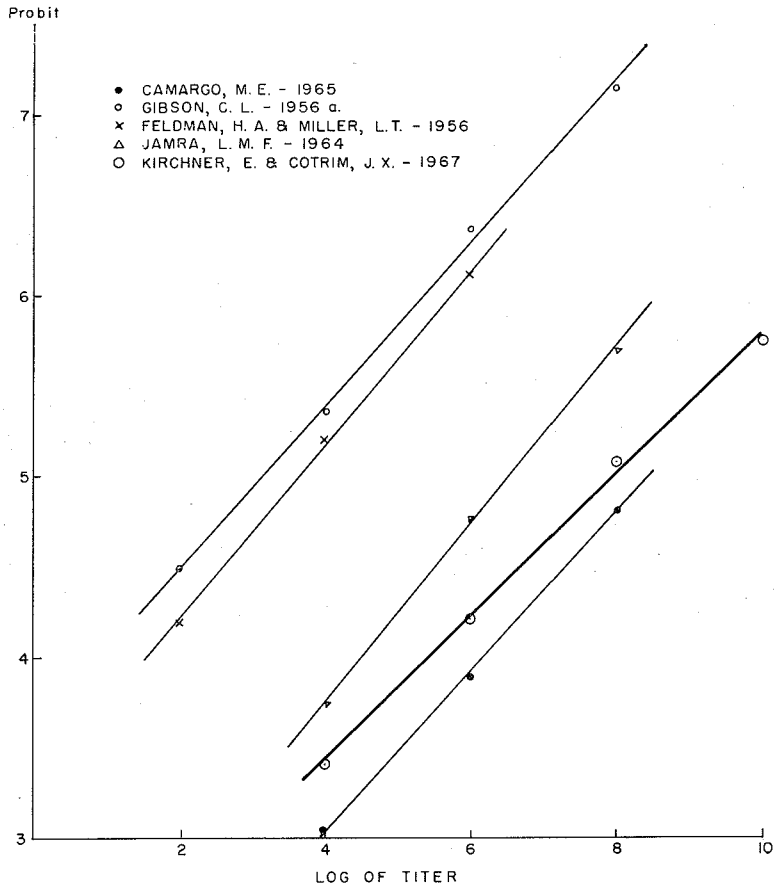


Fig. 8 — Graphic tests of normality. Data reported by:

— ● — CAMARGO, M. E.[8] — Suspected of toxoplasmosis; São Paulo, Brazil

— O — GIBSON, C. L.[17] — Urban Negro population; Tennessee, USA

— × — FELDMAN, H. A. & MILLER, L. T.[13] — Population; New Orleans, La, USA

— △ — JAMRA, L. M. F.[24] — Urban population; São Paulo, Brazil

— ⊖ — KIRCHNER, E. & COTRIM, J. X. — Suspected; São Paulo, Brazil

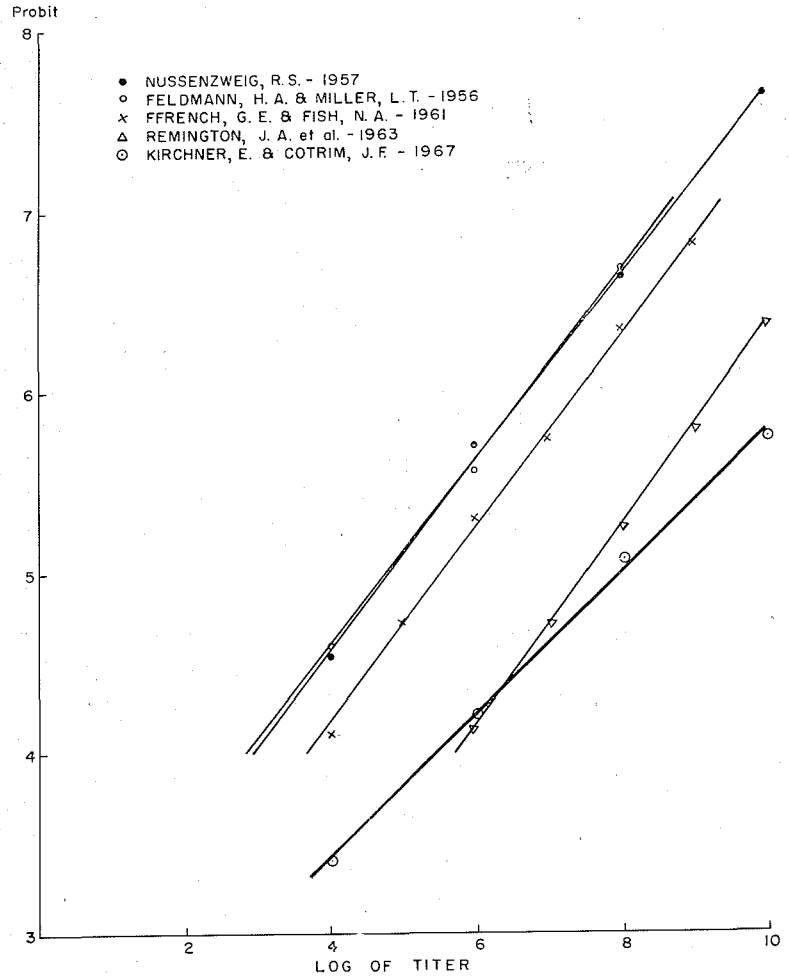


Fig. 9 — Graphic tests of normality. Data reported by:

— ● — NUSSENZWEIG, R. S.[33] — Blood donors; São Paulo, Brazil

— O — FELDMAN, H. A. & MILLER, L. T.[13] — Population; Honduras

— × — FFRENCH, G. E. & & FISH, N. A.[16] — Hospitalized patients; Ontario, Canada

— △ — REMINGTON, J. A. et al.[34] — Students at Harvard University; Boston, Mas., USA

— Θ — KIRCHNER, E. & COTRIM, J. X. — Suspected of toxoplasmosis; São Paulo, Brazil

sera exhibited titers which could be considered as normally distributed.

As to the possible characterization of these population samples in regard to their including or not actual cases of toxoplasmosis, neither the variability range of the titer distributions (*b* values in Table V or relative inclination of probit lines in Figs. 7-10) nor the remaining values investigated tentatively (s, $X_5$, $\overline{X}$ etc.), do allow such delimitation.

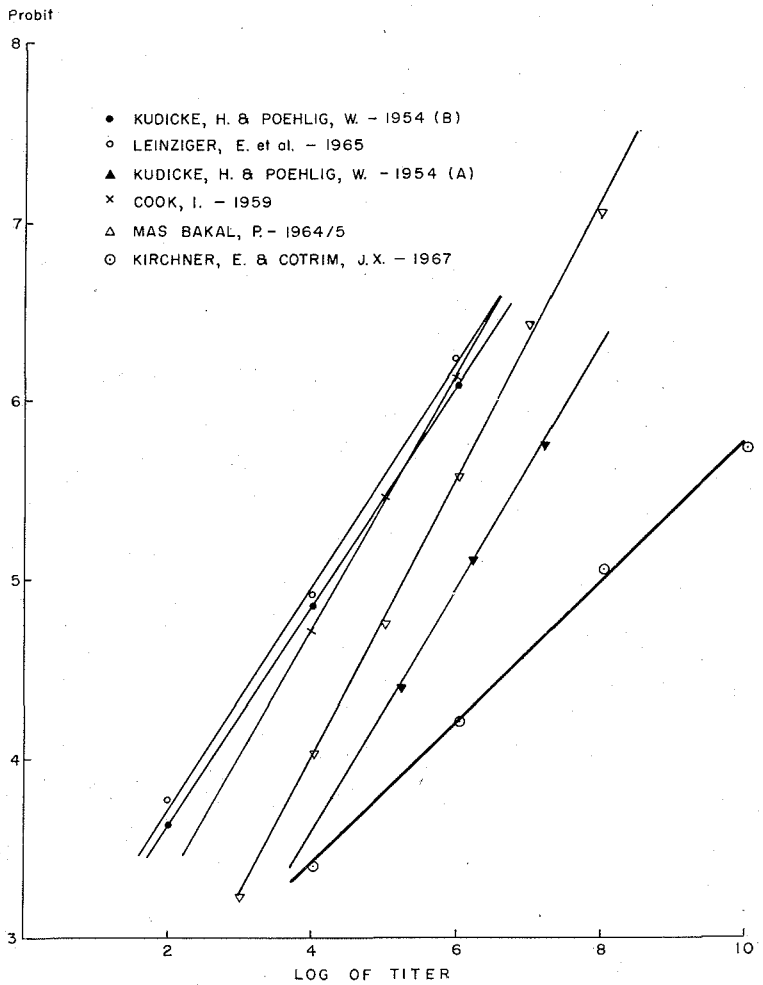


Fig. 10 — Graphic tests of normality. Data reported by:

— ● — KUDICKE, H. & POEHLIG, W.[25] (B) — Patients of various diseases, including toxoplasmosis; Saxony, Germany

— O — LEINZIGER, E. et al.[26] — Obstetric Clinic, Linz, Austria

— ◆ — KUDICKE, H. & POEHLIG, W.[25] (A) — Patients of various diseases, including toxoplasmosis; Saxony, Germany

— × — COOK, I.[30] — Population; Queensland, Toowoomba and Tableland, Australia

— △ — MAS BAKAL, P.[27] — Blood donors; Netherlands

— Θ — KIRCHNER, E. & COTRIM, J. X. — Suspected of toxoplasmosis; São Paulo, Brazil

TABLE V

Immunological response to toxoplasmosis as measured by the dye-test. Analysis of the data corresponding to eighteen population groups

| Authors | Sample studied | no. of sera | b | s | $X_5$ | Modal value X | Average X observed | Per cent positive over total | Per cent positive comprised in probit line |
|---|---|---|---|---|---|---|---|---|---|
| GIBSON, C. L. et al. [19] | Rural Negro population; Tennessee, USA | 987 | .3120 | 3.205 | 4.154 | 4 | 5.1 | 21.8 | 81.4 |
| FELDMAN, H. A. & MILLER, L. T. [13] | Population; Portland, St. Louis and Pittsburg, USA | 621 | .3375 | 2.963 | 2.010 | 4 | 4.4 | 31.9 | 73.2 |
| GROENROOS, P. [20] | Normal; blood donors and patients, toxoplamosis excluded; Finland | 1,176 | .3750 | 2.667 | 4.000 | 6 | 5.3 | 25.6 | 77.3 |
| MERCIER, P. et al. [28] | Suspected of toxoplasmosis; Greece | 170 | .3925 | 2.548 | 4.403 | 4 | 5.8 | 30.6 | 92.2 |
| KIRCHNER, E. & COTRIM, J. X. | Suspected; São Paulo, Brazil | 4,440 | .3945 | 2.535 | 7.982 | 8 | 9.3 | 73.0 | 77.3 |
| CAMARGO, M. E. [8] | Suspected; São Paulo, Brazil | 1,000 | .4400 | 2.273 | 8.477 | 10 | 9.3 | 76.8 | 42.2 |
| GIBSON, C. L. [17] | Urban Negro population; Tennessee, USA | 627 | .4480 | 2.232 | 3.125 | 4 | 3.8 | 20.4 | 75.0 |
| FELDMAN, H. A. & MILLER, L. T. [13] | Population; New Orleans, La., USA | 270 | .4825 | 2.073 | 3.648 | 5 | 5.0 | 39.3 | 86.8 |
| JAMRA, L. M. F. [24] | Urban population; São Paulo, Brazil | 300 | .4875 | 2.051 | 6.551 | 8 | 7.7 | 67.0 | 75.5 |
| NUSSENZWEIG, R. S. [33] | Blood donors; São Paulo, Brazil | 334 | .5130 | 1.949 | 4.788 | 6 | 5.9 | 71.3 | 99.6 |
| FELDMAN, H. A. & MILLER, L. T. [13] | Population; Honduras | 266 | .5225 | 1.914 | 4.828 | 6 | 6.0 | 66.2 | 92.0 |
| FFRENCH, G. E. & FISH, N. A. [66] | Hospitalized patients; Ontario, Canada | 650 | .5371 | 1.862 | 5.556 | 6 | 6.2 | 40.0 | 96.5 |
| REMINGTON, J. A. et al. [34] | Students at Harvard University; Boston, Mas., USA | 359 | .5500 | 1.818 | 7.535 | 8 | 8.1 | 19.5 | 75.8 |
| KUDICKE, H. & POEHLIG, W. [25](B) | Patients of various diseases, including toxoplasmosis: Saxony, Germany | 2,194 | .6150 | 1.626 | 4.228 | 6 | 5.3 | 71.4 | 86.3 |
| LEINZIGER, E. et al. [26] | Obstetric Clinic, Linz, Austria | 484 | .6200 | 1.613 | 4.065 | 6 | 5.1 | 85.5 | 89.4 |
| KUDICKE, H. & POEHLIG, W. [25](A) | Patients of various diseases, including toxoplasmosis: Saxony, Germany | 1,234 | .6800 | 1.471 | 6.068 | 6.2 | 6.4 | 65.7 | 67.7 |
| COOK, I. [10] | Population; Queensland, Toowoomba and Tableland, Australia | 760 | .7050 | 1.418 | 4.376 | 4 | 5.2 | 24.0 | 87.1 |
| MAS BAKAL, [27] | Blood donors; Netherlands | 200 | .7760 | 1.289 | 5.264 | 6 | 5.7 | 66.0 | 98.4 |

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RESUMO

*Resposta imunológica à toxoplasmose em grupos populacionais do Estado de São Paulo, avaliada pela distribuição de títulos da reação de Sabin-Feldman*

A presente investigação analisa o padrão de freqüências de títulos séricos em reagentes positivos ao teste de Sabin-Feldman, de um total de 4.440 indivíduos residentes em três áreas diversas do Estado de São Paulo, Brasil, a maioria dos quais constituída por casos suspeitos de apresentarem alguma manifestação clínica de toxoplasmose. Mais de três quartas partes das observações enquadram-se como distribuição teórica ao acaso, proporcionando mais uma indicação favorável à especificidade da prova do corante. Verificou-se ainda que os resultados com títulos superiores a 1:1.024 constituem uma distribuição separada, parecendo, assim, representar casos recentes da doença, independentemente de manifestações clínicas.

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