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COMPARATIVE EVALUATION OF TOXOPLASMOSIS INDIRECT FLUORESCENT AND SABIN-FELDMAN DYE TESTS IN A THOUSAND HUMAN SERA. A FEW UNEXPECTED RESULTS

Mário E. CAMARGO (1)

SUMMARY

Dye-test and indirect antiglobulin fluorescent test were compared in 1,000 human sera. A close agreement between results was found, with almost total coincidence as to reactivity and non-reactivity of sera in both tests. As for titers, in 97.7% of reagent sera results were the same or differed by only one dilution, the remaining cases differing by no more than two dilutions. A small number of cases was seen with unexpected temporary divergences, as they reacted only in the dye test when first examined. However, when tested again after being kept frozen for a few hours or days, they furnished totally negative results. A cytoplasm-modifying factor different from that responsible for immune-reactions is suggested to occur in such sera.

INTRODUCTION

Close agreement between results of dye and indirect fluorescent tests in toxoplasmosis serology have been reported (CAMARGO [1]; FULTON & VOLLER [3]; GARIN & AMBROISE-THOMAS [4]; STADTSBAEDER et al.[12]), although divergent results have also been observed (KELEN et al.[7]). As only limited number of sera were included in such studies, a more extensive investigation was thought necessary before routine use of the fluorescent technique.

A comparative evaluation of both serological methods in a large sample is now presented, with analysis of titer reproductibility and discussion of a few unexpected results.

MATERIAL AND METHODS

One thousand sera received in our Laboratory for toxoplasmosis serology during several months of 1964 were submitted to both tests. Blood collection in post-prandial periods was avoided. Sera were kept frozen (—20°C) from one to six days before testing, except occasionally when worked the same day.

Sabin-Feldman and indirect immunofluorescent antibody tests were carried out as previously described (CAMARGO [1]) in inactivated sera (30 minutes, 56°C) diluted fourfold in saline from 1/16 to 1/4,096 and, when necessary, diluted twofold from 1/4,000 on. As much as ten fluorescent reactions were run on each microscope slide (CAMARGO [2]) and Evan's blue at 1,0 mg% in the conjugate dilution was routinely employed as a counterstain, as modified from NICHOLS & MCCOMB [10].

RESULTS

Reproductibility of titers in the Sabin-Feldman dye-test: Duplicate titrations in 106 sera ranging from 1/16 to 1/8,000, done in

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(1) Laboratório de Sorologia, Instituto de Medicina Tropical de São Paulo, São Paulo, Brasil

different days, sometimes weeks or months apart, furnished same titers in 70 sera, or 66% (56.5% to 74.4%) *. In all remaining sera differences were of only one dilution. Several successive titrations of same sera have never shown larger differences.

Reproductibility of the indirect fluorescent test: Eighty four sera ranging from 1/16 to 1/32,000 were submitted to duplicate and 30 of them to triplicate titrations, done in different days. Same titers were found respectively in 53.6% (43.0 to 63.8%) and 50% (33.1% to 66.9%). Differences, when occuring, were never larger than one dilution. These results are show in Table I.

Three different conjugates were employed in fluorescent titrations of ten sera and no significative variations were observed (Table II).

Comparative results of dye and fluorescent tests: Agreement between results as to reactivity and non-reactivity (Table III) was observed in 993 from the one thousand tested sera, as 768 were reactive and 225 non-reactive in both tests. The 7 remaining sera reacted only in the fluorescent test, with titers not larger than 1/16.

* Estimated intervals are referred to 95% confidence limits

TABLE I

Titers of immunofluorescent test found in duplicate titrations of 84 sera and in triplicate titrations of 30 sera. Results indicated as $\log_2$

Sera no.	Tests 1st	2nd	3rd	Sera no.	Tests 1st	2nd	3rd	Sera no.	Tests 1st	2nd	3rd
66	8	8	—	94	10	8	—	125	8	8	—
67	8	10	8	95	8	8	—	126	10	10	—
68	8	8	—	96	8	8	—	127	8	10	—
69	8	8	—	97	10	12	—	128	8	6	—
70	10	8	—	98	8	10	10	129	12	12	—
71	8	8	8	99	8	8	8	130	14	15	15
72	8	8	8	100	8	8	—	131	8	10	8
73	8	8	—	101	8	6	8	132	10	10	—
74	8	10	—	103	8	10	—	133	4	4	—
75	10	10	10	104	10	10	10	134	6	6	—
76	8	8	8	107	13	14	—	135	8	10	—
77	10	12	—	108	8	10	—	136	8	10	—
78	10	12	—	109	10	10	10	137	12	12	—
79	8	10	—	110	8	8	—	138	8	8	—
80	8	10	10	111	12	12	12	139	8	8	—
81	10	12	—	112	10	10	10	140	8	8	—
82	8	8	8	113	15	15	—	141	8	8	8
83	8	10	—	114	12	12	—	142	6	6	—
84	8	8	—	115	10	12	12	143	10	8	8
85	8	8	—	116	10	8	8	144	10	10	—
86	8	10	—	117	8	10	—	145	8	10	—
87	8	8	—	118	10	8	10	146	10	8	10
88	8	10	—	119	10	10	—	147	8	8	—
89	8	10	—	120	10	8	10	148	12	10	—
90	8	8	8	121	12	10	10	149	10	8	—
91	10	10	10	122	10	10	—	150	10	8	8
92	10	8	10	123	8	8	8	151	12	12	—
93	10	10	—	124	8	8	8	152	10	8	—

TABLE II

Titers of 10 sera in dye and fluorescent tests with different conjugates. Titers indicated as $\log_2$

Sera	Dye-test	Fluorescent antibody test with conjugates		
		A	B	C
a	12	12	12	12
b	8	8	8	10
c	14	14	14	14
d	13	13	13	14
e	10	10	10	10
f	6	6	6	6
g	10	10	10	10
h	12	12	12	12
i	13	13	13	13
j	10	12	10	12

Conjugate A: F/P ratio = 5.6 × 10^{-3}; dilution for use = 1/60

Conjugate B: F/P ratio = 8.8 × 10^{-3}; dilution for use = 1/150

Conjugate C: commercial preparation; dilution for use = 1/80

TABLE III

Sera distributed as to reactivity in dye and fluorescent tests

Dye-test	Immunofluorescent test		Total
	Reactive	Non-reactive	
Reactive	768	0	768
Non-reactive ...	7	225	232
Total	775	225	1,000

As for the titers obtained in both reactions, a narrow agreement was also demonstrated. In Table IV we can see that identical titers were obtained in 407 or 52.5% (49.0% to 56.0%) of all reagent sera, titers differing by one dilution in 350 and by two dilutions in 18 sera. Larger differences did not occur. So, from 775 reactive sera, titers were the same or differed by one dilution in 775 or 97.7% (96.1% to 98.8%). In most cases of different titers, higher values were observed in the fluorescent test (308 sera or 83.7% of the 368 divergent cases).

This indicates the fluorescent as a somewhat more sensitive test than the Sabin-Feldman technique, as the differences observed are statistically significant to 0.05. *A few unexpected results:* During this study totally divergent results were eventually found, as a few sera were reactive in the dye and non-reactive in the fluorescent test. When submitted again to both reactions a few days later, results were also negative in the dye test. The previous divergence was attributed to some technical failure, inasmuch as dye test titers have always shown to be very stable in frozen sera even when duplicate titrations were done more than one year apart. However, the successive occurrence of such a phenomenon, its demonstration in two samples of blood from the same patient and the observation of decreasing titers in such cases, have led to the assumption of a labile toxoplasma — modifying factor not demonstrated through the fluorescent technique.

Only 12 sera from 11 patients have shown such characteristics throughout this study. Data on Table V indicate that most sera became non-reactive within six days of blood collecting. Such sera have always been tested after inactivation for 30 minutes at 56°C and kept frozen between successive reactions.

It is noteworthy that from the eleven patients, ten were children under twelve years of age. As the sera were obtained from adults as well as from children and respective percentages were approximately the same in the whole group of non-reactive sera included in this work (47% for adults and 53% for children), a chance distribution is very unlikely for such an occurrence.

A thorough study of the unexpected characteristics of sera here outlined was not possible to the moment. In a later series of cases, not included in this study, testing sera at the same day of collection revealed a few more similar cases, as summarized in Table VI.

TABLE IV

Sera distributed as to titers in dye and immunofluorescent tests

Dye-test titers	Immunofluorescent test titers										
	Non-reactive	1/16	1/64	1/256	1/1,024	1/4,096	1/8,000	1/16,000	1/32,000	1/64,000	Total
Non-reactive	225	7									232
1/16		4	8	7							19
1/64		1	32	42	8						83
1/256		1	5	123	92	1					222
1/1,024				33	175	110					318
1/4,096					4	34	14				52
1/8,000						4	19	8			31
1/16,000						1	3	12	6		22
1/32,000								8	8	5	21
1/64,000											0
Total	225	13	45	205	279	150	36	28	14	5	1,000

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TABLE V

Results of dye test successive titrations in a few sera, non-reactive, in the fluorescent test

Sera	Observed results					
	1st reaction	Days *	2nd reaction	Days	3rd reaction	Days
A	1/256	4	non-reactive	10	—	—
B	1/1,024	0	non-reactive	2	non-reactive	7
C	1/1,024	6	non-reactive	8	—	—
D	1/64	2	non-reactive	4	—	—
E	1/256	1	non-reactive	3	non-reactive	8
F	1/256	3	non-reactive	8	—	—
G	1/16	4	non-reactive	11	—	—
H	1/256	0	non-reactive **	2	—	—
I ***	1/1,024	1	non-reactive	12	—	—
J ***	1/64	6	non-reactive	8	—	—
K	1/64	5	1/16	12	non-reactive	14
L	1/64	0	1/16	2	non-reactive	7 **

* Days between blood collection and reaction
** Sera tested from 1/1 (undiluted) on. Other sera tested from 1/26 on
*** Sera from the same patient

TABLE VI

Divergent results of tests for toxoplasmosis in the sera of five children

Sera	Results of tests			
	On the same day of blood collection		Two days after blood collection	
	Dye test	Fluorescent test	Dye test	Fluorescent test
ML	1/64	non-reactive *	non-reactive	non-reactive
MS	1/1,024	non-reactive	non-reactive	non-reactive
EV	1/1,024	non-reactive	non-reactive	non-reactive
MSA	1/1,024	non-reactive	non-reactive	non-reactive
FV	1/256	non-reactive	non-reactive	non-reactive

* Non-reactive at 1/16

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DISCUSSION

Results here presented indicate the indirect fluorescent test as reliable a serological method for demonstrating toxoplasma antibodies as the dye-test, and much easier to perform. Coincidence of results of both tests was found for high-titred as for low-titred sera. Although some doubts have been held on the specificity of dye-test results, not only direct but also indirect evidences of such a specificity are today available. Examples of such evidences are the poor results to induce dye-test reactivity in animals through immunization with substances other than toxoplasma antigens (MAS BAKAL [8], PIEKARSKI et al.[11], etc.), the absence of high titers associated with infections other than toxoplasmosis (MEIRA et al.[9], KABELITZ [6], etc.), the demonstration of specificity of low-titred reactions in sera of patients harboring other infections (FULTON & VOLLER [3]) and statistical evidences of homogeneity of cytoplasm-modifying antibodies (MAS BAKAL [8]) in a population, since they occur in a normal frequency distribution curve. However, thermolabile cytoplasm-modifying factors not related to specific antibodies have been demonstrated by WESTPHAL & MÜHLPFORDT [13], and by JETTMAR [5], in non-inactivated sera. A different kind of cytoplasm-modifying factor is suggested by our findings of a short-lived lytic activity, rapidly disappearing from frozen sera although resistent to inactivation for 30 minutes at 56°C, non-reactive in the fluorescent test and seeming to occur mainly in sera from children.

STADTSBAEDER et al.[12], have also observed close agreement between dye and fluorescent test results when testing a few sera. However, two sera were found reactive in the dye-test and non-reactive both in fluorescent and complement fixation tests. Against an antibody nature for this labile cytoplasm-modifying factor, besides its non-reactivity with antiglobulin, we have observed in one case tested that it did not fix complement, as the serum was non-reactive also in the anticomplement fluorescent test. This is in contrast to the close agreement we have been finding between antiglobulin and anticomplement indirect fluorescent tests (unpublished results).

RESUMO

Estudo comparativo das reações de imunofluorescência indireta e de SABIN-FELDMAN *para a toxoplasmose, em 1.000 soros humanos. Alguns resultados inesperados*

Compararam-se as reações de imunofluorescência indireta e de SABIN-FELDMAN para a toxoplasmose, em 1.000 soros humanos. Encontrou-se estreita concordância quanto a reatividade ou não reatividade dos soros. Quanto a títulos, em 97,7% dos 775 soros reagentes verificou-se concordância ou diferenças de sòmente uma diluição. Nos demais 2,3% de soros reagentes, estas diferenças não excederam de duas diluições. Em raros soros, entretanto, observaram-se divergências entre os resultados, negativos na reação de imunofluorescência e reagentes na de SABIN-FELDMAN. Porém tal reatividade apresentou-se instável, tendendo a desaparecer ràpidamente dêsses soros, embora mantidos congelados a —20°C, em contraste com a estabilidade de títulos observada habitualmente nos soros reagentes.

Sugere-se a presença de fator modificador de citoplasma, de natureza inespecífica, para os soros de reatividade instável, diverso do fator específico presente no comum dos soros reagentes.

REFERENCES

1. CAMARGO, M. E. — Improved technique of indirect immunofluorescence for serological diagnosis of toxoplasmosis. *Rev. Inst. Med. trop. São Paulo* 6:117-118, 1964.

2. CAMARGO, M. E. — Preparation of microscopical slides to simplify immunofluorescence serological titrations. *Rev. Inst. Med. trop. São Paulo* 7:39-40, 1965.

3. FULTON, J. D. & VOLLER, A. — Evaluation of immunofluorescent and direct agglutination methods for detection of specific *Toxoplasma* antibodies. *Brit. M. J.* 2:1173-1175, 1964.

4. GARIN, J. P. & AMBROISE-THOMAS, P. — Le diagnostic sérologique de la Toxoplasmose par la méthode des anticorps fluorescents (technique indirecte). *Presse Med.* 71:2485-2488, 1963.

5. JETTMAR, H. M. — Zum Nachweis toxoplasmafeindlicher Qualitäten in Menschenserum. *Wien klin. Wchnschr.* 66:276, 1954.

CAMARGO, M. E. — Comparative evaluation of toxoplasmosis indirect fluorescent and SABIN-FELDMAN dye tests in a thousand human sera. A few unexpected results. *Rev. Inst. Med. trop. São Paulo* **8**:62-68, 1966.

6. KABELITZ, H. J. — Untersuchungen über unspezifische Mitreaktionen und Titerschwankungen in den Toxoplasma-Seroreaktionen. *Klin. Wchnschr.* **38**:602-606, 1960.

7. KELEN, A. E.; AYLLON-LEINDL, L. & LABZOFFSKY, N. A. — Indirect fluorescent antibody method in serodiagnosis of toxoplasmosis. *Canad. J. Microbiol.* **8**:545-554, 1962.

8. MAS BAKAL, P. — The specificity of Sabin-Feldman's dye test in the diagnosis of toxoplasmosis. *Trop. geogr. Med.* **14**:56-66, 1962.

9. MEIRA, J. A.; AMATO Neto, V.; NÓBREGA, P. & TRAPP, E. E. — Resultados de reações sorológicas para o diagnóstico da Toxoplasmose efetuadas com o sôro de pacientes com protozooses. *Hospital* (Rio) **55**: 641-648, 1959.

10. NICHOLS, R. L. & McCOMB, D. E. — Immunofluorescent studies with trachoma and related antigens. *J. Immunol.* **89**:545-554, 1962.

11. PIEKARSKI, G.; SAATHOFF, M. & KORTE, W. — Zum Problem der Spezificität der Toxoplasma-Seroreaktionen. *Z. Tropenmed. Parasit.* **8**:356-367, 1957.

12. STADTSBAEDER, S.; TELLIER-VERHEYDEN, N. & WEBER, E. M. — Diagnostic sérologique de la toxoplasmose par l'immunofluorescence. *Acta Clin. Belg.* **19**:161-166, 1964.

13. WESTPHAL, A. & MÜHLPFORDT, H. — Untersuchungen über wesen und fehlerquellen des Toxoplasmose-Serofarbtestes nach Sabin und Feldman. *Z. Hyg. Infekt. Kr.* **131**: 423-439, 1950.

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