



OBSERVATIONS ON THE DIAGNOSIS OF CHRONIC TOXOPLASMA INFECTION IN MICE

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SUMMARY

The Authors present the results of their work on dye-test versus examination of brain sections, for detection of chronic experimental toxoplasmosis in mice.

The mice were survivors from lots used in experiments on the transmission of *Toxoplasma gondii* through blood-sucking arthropods. Out of 69 such animals on which 240 dye-tests were performed, only 4 gave positive results, whereas the examination of brain sections of 58 of them led to the finding of pseudocysts in 35 and glial nodes with parasites in 5 more.

On the other hand, in a miscellaneous group of mice infected by other means and in which the chronic stage was apparently reached only after a massive or prolonged contact of the host with the parasite, the percentages of positives by the dye-test and by brain examination were approximately identical.

In discussing the failure of the dye-test in the first group of mice, the Authors advance the hypothesis of toxoplasma having, among its so-called "proliferative forms" some organisms that are not truly proliferative — thus causing no disease and no antibody response — but specially destined to produce pseudocysts. Those organisms might be the "dormant" ones mentioned by COOK & JACOBS, and they also could be the resistant ones — that survive for a long period in the intestinal tract of blood-sucking arthropods and, probably, in other environments which are fatal to the true proliferative forms.

INTRODUCTION

While working on the transmission of *Toxoplasma gondii* through blood-sucking arthropods ^{2, 12}, we were confronted with the problem of detecting the so-called "unapparent" infection among experimental animals. Since it is mostly under the unapparent phase that naturally acquired toxoplasmosis occurs in man and animals, we feel that the natural mechanism — or mechanisms — of transmission must be such as to convey more often this type of infection rather than the acute fatal disease.

In the present paper we describe and discuss the results obtained in our attempts to diagnose experimentally induced toxoplasmosis in mice.

MATERIAL AND METHODS

Strain of Toxoplasma. — This we call the "N" strain, as it was supplied by NOBREGA, who, with his co-workers, isolated it during an outbreak of spontaneous toxoplasmosis among rabbits of the Instituto Biológico, São Paulo ¹⁰.

The strain has been maintained for several years in our laboratory, in white mice, through intraperitoneal passages made on every third day of infection. Its virulence

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for mice is high since almost 100 percent of those inoculated in routine passages die of acute toxoplasmosis quite regularly in 4-5 days. It must be mentioned, however, that on two occasions we noticed what appeared to be a lessening of virulence: the parasites became rarer and the mice longer lived, a few of these surviving to a chronic stage. The previous levels of parasitism and survival were re-established after a few more passages.

A detailed study of the pathology produced by the strain is yet to be made, but we are able to state that since the 2nd-3rd day following intraperitoneal inoculation the mice develop marked ascites, the exudate being rich in cells, fibrin and toxoplasmas. Both liver and spleen become enlarged but the former is always much richer in parasites. These are to be found also in the lungs, myocardium, brain and other organs and in thick blood smears. After several hundred passages through mice the "N" strain keeps its virulence for rabbits. As for white rats, we were never able to produce fatal infection among them, even with very heavy inocula, although pseudocysts have been found in brain sections of one rat killed several weeks after inoculation.

Animals. — Although a few white rats and rabbits were inoculated for special purposes, all our work on transmission has been done with white mice. Our experience confirms that of many other workers about the high susceptibility of mice to toxoplasma and the absence of spontaneous infection among them.

Mice were obtained as needed, usually in groups of twenty, from the colony maintained to supply all the laboratories of the Faculty. Great care was taken in handling, labelling, cleaning, sterilizing and disposing of animals, metal and glassware and we never had any evidence of stray infections among our mice.

Infection of the animals. — The methods employed in our experiments on transmission of toxoplasma through blood-sucking arthropods have been detailed elsewhere^{2, 12}. It is enough to mention here that mice have been infected by: 1) intraperitoneal inoculation of triturated triatomids or ticks, previously fed on sick animals, various time-

intervals having elapsed between feeding and inoculation; 2) mechanical transfer of parasites, by feeding triatomid bugs alternately on sick and clean mice; 3) ingestion of infected triatomid bugs, and, 4) the bite of ticks fed several days previously on sick animals.

Observations here described also include mice inoculated for the detection of unapparent infections among those used in the above mentioned experiments. In this case injection was always intraperitoneal, the inoculum being peritoneal exudate and/or triturated tissues (liver, spleen and brain). Penicillin was added to the inoculum when this was made up of triturated bugs or tissues.

Detection of acute infection. — Mice were daily watched and those found dead were examined whenever possible. Dying animals were killed and examined at once, so that examination could be made in good conditions, and also to prevent the surviving mice of feeding on the carcasses (a fact that occasionally occurred, despite the care to avoid it).

Marked ascites with a fibrinous milky exudate rich in cells and parasites was taken as proof that the animal had died (or was dying) of acute toxoplasmosis. This was usually the case when acute infection had been produced by intraperitoneal inoculation. Yet, sometimes mice died within a few days after inoculation, showing enlarged liver and spleen, and ascites, but no parasites could be detected or were so rare as to shed doubt on the diagnosis. In such cases, toxoplasma was considered as cause of the infection only if a sub-inoculation was positive. The diagnosis of acute toxoplasmosis was sometimes made prior to the animal's death, through the examination of peritoneal exudate obtained by paracentesis.

Among mice infected through other routes (i.e. not by intraperitoneal inoculation), some died of acute toxoplasmosis within various periods, but did not develop ascites. Spleen and liver were usually enlarged and richly parasitized.

Detection of chronic infection. — This has been quite a problem for us at the be-

ginning, and the way we solved it makes the main subject of this paper.

At first, relying on other workers' experience⁵, we tried "blind passages", that is, serial sub-inoculations of tissues suspensions into groups of clean mice. Some infections were thus detected but it soon became evident that the demand in technical help, animals, cages and so on, was more than we could expect to get. It was then decided to adopt the following scheme, based chiefly on DESMONT & LE TAN VINH's³ suggestions:

1) Between the 5th and 10th days of inoculation a search for parasites was made in peritoneal exudate aspirated by syringe, or — exudate being absent — in peritoneal washings obtained by injection and subsequent aspiration of (about 1 ml.) sterile saline. If no parasites were found, some of the animals were killed for sub-inoculations. The other negatives plus the surviving positives were kept under observation.

2) Beginning between the 20th and 30th days, dye-tests were performed at intervals of 5-10 days, on all surviving animals, blood being taken from the orbital sinus according to HALPERN & PACAUD's technique⁶.

3) After at least 45 days of observation and several dye-tests, surviving mice were anesthetized and bled from the heart for another dye-test.

If the results were negative all along, the animal was considered as not infected.

Dye-tests — were performed in accordance with the original technique proposed by SABIN & FELDMAN¹³, slightly modified in details as published in a former paper¹¹.

During the animals' life, blood was taken from the orbital sinus, mixed with saline, allowed to clot and then centrifuged. The amounts of blood and saline were calculated so as to give a final serum dilution of about 1:10, from which two more dilutions (1:50 and 1:100) were obtained. When the animal was sacrificed and a good amount of blood could be had from the heart, a first serum dilution of 1:5 was made. In almost all tests considered as negative the reading was compared with that of the accessory factor test tube. Only occasionally did a serum give a somewhat higher pro-

portion of unstained organisms, suggesting that perhaps a lower dilution would give a 50:50 or higher reading.

RESULTS

In vivo examination of peritoneal exudate or washings gave definitely positive results only in mice showing clinically evident infection. All these died of acute toxoplasmosis shortly after, except one that survived to a chronic stage. Among apparently healthy animals such examination only permitted the occasional finding (in fresh preparations) of a few parasites — the infection being sometimes confirmed by animal passages.

Dye-tests were consistently negative in all the first lots of mice surviving inoculation with macerate of blood-sucking arthropods previously fed on sick animals.

So, up to a certain period in our experiments, the results could be classified as follows:

a) mice with clinical and parasitological evidence of acute toxoplasmosis, almost all dying in about 5-10 days;

b) mice with no clinical signs but showing a few parasites when examined between the 5th and 10th days and with the infection confirmed by passages in other mice, and,

c) mice with no clinical signs, parasitological examination negative or not confirmed through animal passages, and which, having survived more than 20 days, had several negative dye-tests.

The last group was considered as non infected.

The results being hitherto in accordance with the time period elapsed between the blood meal of the arthropods and their inoculation into mice, they seemed to indicate that: a) for only about 3 days enough toxoplasms survived (in the arthropods' digestive tract) to cause acute disease; b) from then up to about 10 days, infection was irregular, only a few parasites surviving in some of the bugs, causing mostly sub-acute disease, and, c) after the 10th day no parasite survived as no infection could be detected among the mice in which this was being tested.

These conclusions seemed perfectly satisfactory but, anyway, we later decided to save the brain of all mice dead or killed more than 20 days after inoculation. Sections of the organ were made and examined, and, to our surprise, we soon began to find typical pseudocysts in brain sections of mice which had had several negative dye-tests.

Dye-test versus brain examination. — Although a total of 448 dye-tests have been performed on a total of 133 mice surviving 20 days or more the experimental inoculations, Table I only shows the results obtained since we started to use both methods to detect the infection. As seen by the data on the Table I, an average of more than 3

tests were made per mouse. Actually, the number per mouse varied according, of course, to survival: on a few only one test was possible, but on the others two to six were made.

Out of 69 mice on which 240 tests were performed, only 4 gave positive results. Pseudocysts have been found in the brain of 3 of the positive mice. The fourth died of acute toxoplasmosis after a second inoculation had been made to test the presence of protective antibodies, and its brain was not examined.

Examination of brain-sections of 58 mice of the same group yielded 40 positive results. This means that, among the same group of mice of which only 5.8% had

TABLE I

Results of dye-tests and examination of brain sections of mice used in experiments on the transmission of *Toxoplasma gondii*

Lot No	Mice surviving 20 days or more									Acute toxo- plasmosis in other mice of same lot
	Total	Dye-tested				Brain examined				
		No of tests	No of mice	Positive mice		No of mice	Positives			
				No	%		With pseudocysts	With "nodes & parasites"	%	
27-1	1	4	1	—	—	1	1	—	—	?
29-1	4	12	4	—	—	3	2	—	—	—
29-2	4	14	4	1	—	4	4	—	—	—
30	1	4	1	1	—	—	—	—	—	+
31	2	10	2	—	—	2	2	—	—	—
32	1	5	1	—	—	1	1	—	—	?
34	2	11	2	—	—	2	1	—	—	+
34-1	3	15	3	—	—	3	2	—	—	—
35-1	3	9	3	—	—	1	1	—	—	+
36	4	15	4	1	—	3	3	—	—	—
36-A	4	15	4	1	—	4	2	—	—	—
37	4	14	4	—	—	3	3	—	—	—
37-A	3	12	3	—	—	3	2	—	—	—
39	2	6	2	—	—	2	1	—	—	—
40	2	8	2	—	—	2	1	—	—	—
41	10	38	10	—	—	10	4	2	—	—
44	4	16	4	—	—	4	1	1	—	—
45	3	5	3	—	—	1	1	—	—	—
46	3	7	3	—	—	2	—	1	—	—
47	4	12	4	—	—	4	2	—	—	—
48	2	3	2	—	—	1	—	1	—	—
49	3	5	3	—	—	2	1	—	—	—
Totals	69	240	69	4*	5.8	58	35	5	68.9	...

* In only one of these the brain was not examined; in the other 3 the brain had pseudocysts.

been positive by the dye-test, 68.9% had parasites in the brain.

Histological examination may look as too laborious a method but, actually, it is not so if one has the adequate facilities. As the sections may be kept almost indefinitely, examination may be done whenever possible and one may always go back to the original paraffin blocks for new sections. We usually immersed the whole brain in 10% commercial formalin. This is not the best way of obtaining a good fixation, but we found it good enough for the purpose in view. After inclusion in paraffin, 3-5 slides were made, each with 3-5 sections, stained with haematoxylin-eosin and examined under low power (oc. 5x, obj. 10x). After some training we were able to detect even the smallest cysts with such magnification. Anything unusual was, of course, checked under high power.

Data in Table I include mice in which no pseudocysts were found, but only "nodes with parasites". By this is meant the presence of glial reaction that we found to be quite frequent in positive animals. The reaction was sometimes discrete, more often compact, forming what we came to call "typical nodes". In most of these no identifiable toxoplasmas could be spotted, but some do contain a few or, occasionally, numerous bodies which were definitely recognized as parasites.

The absence of such reaction around intact pseudocysts was the rule. They were usually to be found elsewhere, in the same section or in another section of the same brain. Nevertheless we found a few pseudocysts surrounded by cell reaction. Sometimes it looked as if the cyst had ruptured and been invaded by cells. Occasionally an opposite interpretation seemed more likely, i.e. that small pseudocysts were forming in spite of tissue reaction and destruction of parasites in their immediate vicinity.

We tried the examination of fresh preparations of brain triturated in saline, as suggested by some³, but abandoned the method as unpractical and possibly dangerous.

Summarizing our results, we may say that the dye-test was an almost complete failure for the detection of chronic toxoplasmosis in this group of mice: on 35 mice with

pseudocysts, a total of 128 dye-tests revealed only 3 infections.

DISCUSSION

In an effort to clarify the reasons for this apparent failure of the dye-test, we tried to produce chronic toxoplasmosis through different means, and thus have other groups of mice tested for comparison. This proved to be quite a difficult task. Although we kept other less virulent strains of toxoplasma, we wanted all the work done with the "N" strain so as to have comparable results all along. Besides, the "N" strain was fairly well "stabilized" in mice and for years has been used in dye-tests for the diagnosis of human toxoplasmosis* and in a survey among blood-donors¹¹.

Trials made with the injection of very small amounts of diluted infected exudate, even if through the intradermal or subcutaneous route, resulted but in a delay of death due to acute infection. Successes were few and obtained as follows:

1) Nine mice were fed on peritoneal exudate or liver tissue of acutely infected animals: 7 of them died of acute toxoplasmosis. The 2 survivals were repeatedly dye-tested with negative results, but both had their brains examined and pseudocysts were found in one.

2) Thirty-eight mice were intraperitoneally inoculated with a small quantity of diluted peritoneal exudate and started immediately on a diet containing sulfadiazine in quantities calculated to yield about 4 mg. of the drug per mouse per day. After 12 days sulfa was discontinued, only 2 mice having died, both with positive exudate. The others looked healthy but some days later they began to die rapidly and all those that could be examined showed toxoplasmas; one of them, found dying on the 17th day of inoculation, was bled from the heart and had a positive dye-test at 1:50. Administration of sulfa was resumed but only 6 mice survived to the 30th day, when the drug was again discontinued and a dye-test was per-

* With very satisfactory results, including fatal congenital human cases from which the parasite was later isolated.

formed giving 100% positive results. Four mice were still alive 5 months later, when they were again dye-tested and again positive. Their brains were not examined.

3) Another group of 25 mice was inoculated through the peritoneum with mixtures of peritoneal exudate (rich in toxoplasmas), plus blood, or broth, and penicillin, kept for various periods of time. Mice injected with the oldest mixtures presented no evidence of infection, but in the 5 lots inoculated with mixtures 4 to 10 days old, 7 mice died of acute toxoplasmosis and 9 survived. Dye-tests were performed on all 9, being positive in 7; 2 of these had the brain examined and both had pseudocysts.

Results obtained in this miscellaneous group of mice are presented on Table II,

together with those seen in 4 other mice that, at different times and for unknown reasons, survived the routine inoculations made for the preservation of the strain. The percentage of dye-test positives here — 81.8 — is obviously quite different from that — 5.8 — seen among the mice used in our transmission experiments (Table I). Besides, while in the miscellaneous group only 1 out of 5 found with a positive brain had negative dye-tests, in the other group this ratio was of 37 to 40.

On Table III are given the maximum titers obtained in all positive dye-tests among mice of both the first group (listed on Table I) and the miscellaneous group (listed on Table II).

TABLE II

Results of dye-tests and examination of brain sections on a miscellaneous group of mice surviving inoculation with *Toxoplasma gondii*

Mice of group	Type of inoculum, route of inoculation, etc.	Mice surviving 15 days or more							Mice dead of acute toxoplasmosis
		Total	Dye-tested			Brain examined			
			Nº	Positives		Nº	Positives		
				Nº	%		Nº	%	
M-1	Tissues acutely infected mice; orally.	2	2	—	—	2	1	50.0	7
M-2	Peritoneal exudate of acutely infected mice; intra-peritoneally. Treated with sulfa.	7	7	7	100.0	—	—	—	31
M-3	Peritoneal exudate of acutely infected mice, kept several days; intra-peritoneally.	9	9	7	77.8	2	2	100.0	7
M-4	Peritoneal exudate of acutely infected mice; intra-peritoneally. Routine passages.	4	4	4	100.0	2	2	100.0	many
Totals		22	22	18	81.8	6	5	83.3	...

TABLE III

Maximum titers obtained in dye-tests performed on various groups of mice experimentally infected with *Toxoplasma gondii*

No	Mice of group	Type of inoculum, route of inoculation, etc.	Time intervals after inoculation					5 months
			in days					
			15-19	20-29	30-39	40-49	50 +	
1	29-2	Experiments on transmission through arthropods	1:50	...	1:50	—
2	30		...	neg.	1:10	1:10	—	—
3	36		1:10	neg.	(neg.)	—
4	36-A		neg.	1:10	(neg.)	—
5	M-2	Peritoneal exudate of acutely infected mice; intra-peritoneally; treated with sulfa.	1:50	—	—	—	—	—
6			1:5000	...	1:1000	—
7			1: 100	...	1: 100	1:250
8			1: 100	...	1: 100	1:250
9			1: 100	—	—	—
10			1:5000	...	1: 500	1:500
11	1: 100	...	1: 100	1:250
12	M-3	Peritoneal exudate of acutely infected mice, kept several days; intra-peritoneally.	...	1:10	(neg.)	—	—	—
13			...	1:10	—	—	—	—
14			...	1:10	—	—	—	—
15			...	1:10	neg.	—	—	—
16			...	neg.	1:10	(neg.)	—	—
17			...	1:10	neg.	—	—	—
18	...	1:50	1:10	...	(neg.)	—		
19	M-4	Peritoneal exudate of acutely infected mice; intra-peritoneally; routine passages.	1:10	...	neg.	(neg.)	—	—
20			1:10	—	—	—
21			1:10	—	—	—
22			1:10	—	—	—

neg. = negative from 1:10 dilution
 (neg.) = negative from 1:5 dilution
 ... = test not performed
 — = animal not alive or control suspended

In view of the results here described we came to consider the dye-test as very specific, but not quite so sensitive as to justify the assumptions that negatively dye-tested mice should be considered uninfected. We realize the importance of the dye-test in minimizing "the possibility of confusion of *Toxoplasma* with a morphologically similar organism" — as said by JONES *et al.*⁸. We thought, indeed, about the possibility that we might be dealing with another parasite that could have originally infected some of the mice, or the bugs. This idea was, however, laid aside by the following reasons:

- 1) Among mice infected through blood-sucking arthropods, pseudocysts had the same appearance both in the negatively and in the positively dye-tested mice;
- 2) They had again the same appearance as those pseudocysts found in mice (and one rat) infected with material directly taken from mice with acute toxoplasmosis;
- 3) They were also identical with pseudocysts found in fatal human cases of the congenital disease;
- 4) Nothing like toxoplasma pseudocysts was found in 16 carefully examined mice

taken at various times and from different lots of the stock-colony;

5) The triatomid bugs, used in most of the transmission experiments, were taken from a colony long maintained and extensively used in studies on *Trypanosoma cruzi*, by several people in the Laboratory:— no parasite similar to toxoplasma was ever found either in the bugs or in animals infected through them.

From all the results presented in this paper the obvious inference is that the inoculum may be insufficient to stimulate the building up of "cytoplasm-modifying" antibodies to detectable levels and yet be still enough to result in pseudocyst production. A positive test may probably be expected if the organism of the host has had the opportunity of a sufficient contact with the antigen furnished by the proliferative phase of the parasite, through either a heavy inoculum of dead parasites, or a prolonged infection, or both.

Our positively dye-tested mice were mostly survivals from lots in which the same inoculum caused acute fatal disease to some or, even, to most of the animals. The group that furnished the highest titers was precisely the one in which the infection was so severe as to cause death to the great majority, despite prolonged sulfa treatment. For another group of positives the inoculum was probably poor in living parasites (although enough to cause fatal disease to many), but certainly rich in antigen furnished by dead toxoplasmas. On the other hand, no acute disease was detected in many lots of mice of which some showed pseudocysts — and all of which had consistently negative dye-tests.

It is obvious that for only a few days the material conveyed in the intestinal tract of triatomid bugs, contains parasites capable of causing acute or sub-acute disease. From then on the parasites are only enough to cause chronic toxoplasmosis.

And now, we ask ourselves if this "enough" means simply "number of parasites", i.e. if we are actually dealing here "with the late part of a survival curve which is becoming asymptotic", as explained by JACOBS⁷ when referring to infectivity of stored infected tissues. As it has been noticed with other

strains^{4,9}, the "N" strain is so virulent that it seems theoretically probable that just one parasite taken from an acutely infected mouse may cause fatal infection to the recipient mouse. On the other hand, the proliferative phase of the "N" strain, just as others', has a limited ability for surviving in different media. Why should some individual parasites survive for a much longer period in some environments — such as the intestinal tract of triatomid bugs — where most of them are quickly destroyed? And why should the few forms that manage to survive longer, be not capable anymore of rapid multiplication in the host's cells (causing no acute or sub-acute disease), but still be able to resist the host's defenses and produce pseudocysts?

We think of a hypothesis that could perhaps answer these and many another questions:— that among the so-called "proliferative forms" of toxoplasma there might be individuals with distinct physiological properties and of which the destiny would be to produce pseudocysts, just as, for instance, of each schizont of plasmodia some merozoites develop to gametocytes while the others continue the schizogonic cycle. Such forms, if more resistant to conditions that adversely affect the true proliferative forms, could assure the transmission from host to host by several different means, even before they have a chance to form pseudocysts. On the other hand, if less invasive than the true "proliferative forms", they would, thereby, cause no disease and no antibody response.

We have not known of such a hypothesis being expressed by others, but it seems to us that there is a suggestion of it in the following period by COOK & JACOBS¹: "The possibility may be considered, therefore, that under some circumstances, dormant forms of the parasites may persist in cultures cleared of proliferating toxoplasmas by pyrimethamine. This hypothesis suggests that it may be profitable to study *in vitro*, by the use of drugs and immune serum, the mechanism by which pseudocysts are formed."

After permitting ourselves this little theorizing, we only wish to emphasize that the dye-test, useful as it undoubtedly is, should not be relied upon too much when it comes to detect unapparent toxoplasma infections among laboratory animals.

RESUMO

As Autoras relatam sua experiência no diagnóstico da toxoplasmose latente em camundongos.

Quando estudavam a possibilidade de transmissão do *Toxoplasma gondii* por artrópodes hematófagos verificaram que, dos camundongos utilizados nas experiências, uma parte morria de toxoplasmose aguda, sendo a infecção facilmente evidenciada pelo encontro de parasitas no exsudato peritoneal, no fígado e no baço. Em outros camundongos que não apresentavam o quadro típico da infecção aguda, os parasitas puderam ser demonstrados por sub-inoculações. Muitos animais, porém, sobreviveram mais de 20 dias, sem sinais de doença. Para comprovar a possível existência de infecções latentes nestes animais, as Autoras lançaram mão da prova do corante (reação de SABIN & FELDMAN). As provas eram iniciadas entre o 20º e 30º dia após a inoculação e repetidas a intervalos de 5-10 dias, sendo o sangue colhido do plexo retro-orbital. Depois de 45 dias ou mais e de várias reações de SABIN-FELDMAN negativas, os animais eram sacrificados e sangrados do coração para nova reação.

Baseadas nas afirmativas de vários pesquisadores as Autoras, a princípio, consideravam negativos e desprezavam todos os animais que tinham tido várias provas de SABIN-FELDMAN negativas.

Posteriormente, entretanto, resolveram examinar o cérebro (cortês histológicos) de todos os animais utilizados nas experiências e que houvessem sobrevivido mais de 20 dias à inoculação, tendo então — com surpresa — verificado a presença de pseudocistos em muitos camundongos nos quais diversas provas de SABIN-FELDMAN tinham sido negativas. Assim é que, de 69 camundongos nos quais fôra feito um total de 240 reações, só 4 tinham sido positivos, enquanto que o exame do cérebro de 58 deles revelou pseudocistos em 35 e nódulos inflamatórios com parasitas em 5 outros.

Por outro lado, num grupo miscelâneo de camundongos infectados por outros modos, as percentagens de positividade foram quase idênticas tanto pela prova do corante como pelo exame do cérebro. Neste grupo de camundongos, aparentemente a fase crônica foi

atingida só depois de um contacto duradouro (através de uma infecção prolongada), ou maciço (devido à grande quantidade de parasitas mortos presentes no inoculum) do parasita com o organismo do hospedeiro. Aqui teria havido, portanto, suficiente estímulo para a produção dos anticorpos “modificadores do citoplasma” evidenciados pela positividade da prova do corante.

Continuando a discutir os resultados de suas observações, as Autoras apresentam a hipótese de que, na chamada “fase proliferativa”, tenha o toxoplasma, ao lado das formas realmente proliferativas, outras destinadas a evoluir para pseudocistos — da mesma maneira que, por exemplo, em cada esquizonte de plasmódio alguns merozoítas evoluem para gametócitos enquanto que os demais continuam o ciclo de multiplicação esquizogônica. As formas não proliferativas seriam provavelmente pouco virulentas, incapazes de causar doença e resposta imunitária do hospedeiro, mas, por outro lado, mais resistentes, sobrevivendo por longo tempo em ambientes — tais como o tubo digestivo de certos artrópodes — nos quais as demais formas são rapidamente destruídas.

Concluindo seu trabalho, dizem as Autoras que, baseadas em sua própria experiência, consideram a prova do corante muito útil e específica, porém não tão sensível como a julgam vários pesquisadores, segundo os quais devem ser tomados como não infectados os animais em que a prova houver sido negativa.

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