MEMORANDUM

TO: Members of the Tropical Medicine Study Section

FROM: Irving Gerring, Executive Secretary

SUBJECT: 1) Conference on Toxoplasmosis
          2) Project Site Visit, E-173(R)

Enclosed is copy of a memorandum from Dr. Albert B. Sabin submitting a summary of the proceedings, the pertinent data presented, and the recommendations of the August 3, 1951, Conference on Toxoplasmosis, sponsored by the Tropical Medicine Study Section. Dr. Wright will report on this conference at the September 27 meeting, after which the Section members will discuss the recommendations for possible further action.

In addition, there is enclosed a copy of a letter dated August 24, 1951, from Dr. Johnstone relative to his project site visit on E-173(R).
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Memorandum and Recommendations on Toxoplasmosis

To: Members of Tropical Medicine Study Section

From: Albert B. Sabin, Chairman of Conference

SUBJECT: Summary and Recommendations of Conference dealing with "Problems and Responsibilities in the Serologic Diagnosis of Toxoplasmosis" held on August 3, 1951 at the National Institutes of Health under the sponsorship of the Tropical Medicine Study Section

1. The purpose of this conference was to determine from the most recent experience of a number of research laboratories:

   a) the clinical manifestations of human toxoplasmosis which can be diagnosed with reasonable certainty by the available serologic methods

   b) whether or not the serologic methods now in use, i.e., the dye test and complement fixation tests, lend themselves to performance in routine diagnostic laboratories

   c) whether or not the time has come to transfer some of the responsibilities for the diagnosis of human toxoplasmosis from the research laboratories to routine diagnostic laboratories.

2. A summary of the data and discussions presented at the conference is contained in the appendix to this memorandum. This provides the basis for the decisions and recommendations which follow.

3. It was the consensus of the participants in this conference that the time had come when the serologic diagnosis of certain conditions clinically suspect of toxoplasmic etiology should become a function of routine diagnostic laboratories, while the investigation of other conditions should continue in various research laboratories. The following clinical conditions were put in the category which called for routine diagnostic tests:

   a) The cerebral, ocular and visceral manifestations suggesting the possibility of congenital toxoplasmosis, e.g., encephalitis, jaundice, or hepatosplenomegaly in the neonatal period, and chorioretinitis, convulsions, mental retardation, etc., occurring in clinical patterns previously observed in the congenital disease.

   b) Exanthematous febrile illnesses, simulating certain rickettsial infections, which fail to respond to the antirickettsial antibiotics or which occur out of the tick season.
4. The relatively low incidence of clinically recognized disease due to toxoplasmic infection as well as the character of one of the diagnostic tests which needs to be used precludes any recommendation that this diagnostic service become the responsibility of individual state public health laboratories. It appeared, however, that this was a function that fell in the domain of the Communicable Disease Center of the Public Health Service. Accordingly, it was unanimously voted that, if it agreed with the conclusions of this conference, the Tropical Medicine Study Section request the Surgeon General of the U. S. Public Health Service to transmit the deliberations of this conference to the Communicable Disease Center for the practical implementation of these recommendations. Although the opinion was expressed that to begin with, it would be desirable to provide facilities and trained personnel in four regional laboratories to cover the needs of various parts of the U. S., it was realized that this was an arbitrary number. Administratively, it might be feasible to start with a smaller number of laboratories and increase it if necessary.

5. It was, furthermore, agreed that if, and when, such routine diagnostic service becomes available, the proceedings of this conference be publicized in various professional journals. This report would also include the following:

   a) A description of the clinical conditions which call for routine serologic tests for toxoplasmosis

   b) A brief description of the serologic tests in current use, the manner of obtaining and shipping specimens, and the interpretation of the results

   c) The laboratories which are prepared to perform such routine tests in various regions of the U. S.

Respectfully submitted,

/s/Albert B. Sabin, M. D.

Aug. 21, 1951
A. Conference on Problems and Responsibilities in the Serologic Diagnosis of Toxoplasmosis

Sponsored by
The Tropical Medical Study Section
Division of Research Grants
National Institutes of Health, Public Health Service

Dr. Albert B. Sabin, Chairman

Agenda

1. Statement of problem and definition of objectives of conference

2. Reports by individual participants regarding experience of their laboratories with serologic tests for toxoplasmosis during the past 2 years

3. Consideration of precautions to be observed in laboratories engaged in work with Toxoplasma

4. Recommendations for future performance of tests and reporting to physicians

5. Responsibility of Public Health Service in providing facilities for performance of diagnostic tests

6. Consideration of advisability of drawing up a statement for publication in various journals regarding:
   a) Clinical conditions which call for routine serologic tests for toxoplasmosis
   b) The serologic tests at present available and the interpretation of the results
   c) The laboratories prepared to perform such routine tests in various regions of the United States

August 3, 1951 - 10:00 A.M.
Building T-6, Conference Room 1057
National Institutes of Health
Bethesda, Maryland
B. Participants

Conference on Toxoplasmosis

August 3, 1951

Chairman: Dr. Albert B. Sabin, Children's Hospital, Cincinnati, Ohio

Dr. Heinz Eichenwald
New York Hospital

Dr. Harry A. Feldman
New York State Medical Center

Dr. Joel Warren
Walter Reed Army Hospital

From National Institutes of Health:
Dr. Victor H. Haas
Dr. Willard H. Wright
Dr. Leon Jacobs
Dr. John Bozicevich

Dr. Don E. Eyles
Laboratory of Tropical Diseases
Public Health Service

Dr. Jacob Frenkel
Rocky Mountain Laboratory
Public Health Service
Present Status of Congenital Toxoplasmosis

The accumulated published reports have established beyond doubt that the most commonly encountered clinical manifestations of human toxoplasmosis are the result of congenital infection. Neither the manifestations relative to damage of the nervous system, which are most frequent, nor those relative to involvement of the viscera (e.g. neonatal jaundice, hepatosplenomegaly) are sufficiently characteristic to permit a diagnosis on clinical grounds alone. The specific diagnosis can be established by serologic methods. The chief practical benefit to be derived from a specific diagnosis of congenital toxoplasmosis is concerned with the good prognosis for subsequent children. Other conditions responsible for congenital types of ocular and cerebral damage, simulating those of congenital toxoplasmosis, carry an uncertain prognosis, since there are instances of multiple abnormal children in the same family.

It is difficult to estimate the actual incidence of congenital toxoplasmosis in the U. S., since its recognition depends both on clinical awareness and diagnostic facilities. The experience of two of the participants in this conference, Dr. Feldman in Syracuse, N. Y., and Dr. Eichenwald in New York City, who without any publicity during the past 2 to 3 years accepted serum specimens from various physicians, was of special interest. Dr. Feldman established the serologic diagnosis of congenital toxoplasmosis in 44 children, and Dr. Eichenwald in 79; the clinical manifestations which were found in these patients are described in their statements contained in this appendix. The tests on hundreds of specimens performed in the Tropical Diseases Laboratory of the N. I. H. by Drs. Bozicevich and Jacobs could not be evaluated in this respect, because the specimens were only rarely accompanied by adequate clinical histories. However, I know from personal correspondence with many physicians, who wrote to me for interpretation of the serological results reported to them from the N. I. H., that a certain number of cases were also diagnosed by the N. I. H.

Additional important information relative to the prognosis for subsequent children following the birth of a proved case of congenital toxoplasmosis was reported at the conference. Data were presented on 38 mothers (21 by Eichenwald, 16 by Feldman and one by Frenkel) who had given birth to infected children and subsequently became pregnant 57 times. Among these 57 subsequent pregnancies, there were 6 miscarriages and 3 premature births with 2 deaths. Two of the miscarriages and one of the prematures were examined for evidence of toxoplasmosis but none was found. It is the opinion of obstetricians that this incidence of miscarriages and prematurity can be encountered in the population at large. All the live children proved to be normal with no evidence of toxoplasmic infection. Some of the normal children followed a previous miscarriage or premature birth. This experience added to previous observations forms a firm foundation on which a favorable prognosis can be given for subsequent children.
and constitutes a most compelling reason for providing a toxoplasma diagnostic service for the physicians of this country.

Present Status of Other Conditions in which the Role of Toxoplasmosis is under Investigation

a) Chorioretinitis of unknown etiology in adults and children acquired after birth. - No evidence for toxoplasmic etiology has been found thus far. Among 22 adults studied by Feldman and 13 over the age of 6 years by Frenkel, the incidence and titers of toxoplasma antibodies was similar to that encountered in the "normal" population of similar age. The same was true of the results obtained in the Tropical Diseases Laboratory of the N. I. H. on serum specimens submitted to them from 66 patients with "eye disease".

b) Infectious encephalitis of unknown etiology. - Regarded as still a research problem in isolated instances. Routine serologic tests for toxoplasmosis in all cases of encephalitis of unknown etiology is regarded as unwarranted and impractical at this time.

c) Spontaneous abortions of unknown etiology. - Dr. Feldman's studies are still incomplete, and the question is regarded as being in the realm of research.

d) Fevers of unknown etiology. - Feldman has studied 100 or more of such cases without obtaining any significant results. Recent published reports by Westphal and his associates from Hamburg, Germany did not inspire confidence regarding the reliability of the results obtained or the validity of the conclusions.

e) Detection of inapparent acute toxoplasmosis among women in prenatal clinics. - Preliminary experience of Drs. Sven Gard in Sweden and Feldman in this country suggests that thousands might have to be tested before one is detected. The serologic investigation of unexplained febrile illnesses during pregnancy may be a worthwhile research problem, but cannot as yet be included among conditions which would call for routine diagnostic tests.

f) "Glandular fever" or lymphadenitis of unknown etiology. - Recent studies in Denmark and Sweden have indicated that certain ill-defined illnesses with lymphadenopathy are associated with very high titers of toxoplasma antibody. This also was regarded as a worthy research project, rather than as an indication for routine diagnostic tests.

g) Cerebral palsy and retrolental fibroplasia. - Preliminary studies by Dr. Feldman have yielded negative results. This suggests that the congenital cerebral and ocular damage for which routine diagnostic tests for toxoplasmosis may be indicated can be defined in accord with the
clinical manifestations most commonly encountered in congenital toxoplasmosis.

h) Exanthematous fevers of unknown etiology, simulating rickettsial infections. - The fatal cases in adults associated with skin rash reported by Pinkerton and Henderson in 1941, the recent fatal case in a 60-year old woman in Boston, studied serologically by Feldman, the fatal case, which recently occurred in a laboratory technician in the Laboratory of Tropical Diseases at Memphis, and several, severe non-fatal laboratory infections in recent years in Sweden and Czechoslovakia, almost all associated with rash, suggested that a clinical syndrome might be defined for which routine toxoplasma diagnostic tests could be performed. There was some discussion in this connection with regard to the effect of aureomycin and chloramphenicol on experimental toxoplasmosis, and Drs. Frenkel, Eichenwald and Feldman indicated that tests which they had performed yielded negative results. Negative results were also reported in publications by Forrest Adams and his associates, but Dr. Summers in this country and Drs. Steen and Kass in Norway reported positive results. Dr. Feldman suggested the possibility that some other substance associated with certain lots of the early preparations of aureomycin, rather than the aureomycin itself, might have been responsible. The point of this discussion was that exanthematous fevers, suggestive of rickettsial infection, which either occurred out of season or failed to respond to the rickettsiostatic antibiotics, might be suitable candidates for routine serological tests for toxoplasmosis.

Diagnostic Criteria for Congenital and Acquired Toxoplasmosis

The high incidence of toxoplasma antibodies, mostly of low titer, increasing with age in the normal population as a result of inapparent or unrecognized infections, presents special problems in the serologic diagnosis of toxoplasmosis. It is clear that the mere presence of toxoplasma antibody does not establish toxoplastic etiology for a given clinical condition. The relative rarity of toxoplasma antibodies among children under 6 years of age, and the constant occurrence of such antibodies and their persistence in high titer for many years in both the mothers and affected children renders the diagnosis of congenital toxoplasmosis relatively easy up to about the age of 6 years - when dye test titers of 1:256 or greater are almost the rule. Over the age of 6 years, the association of certain clinical manifestations known to occur in congenital toxoplasmosis, (e.g., chorioretinitis, convulsions, mental retardation, cerebral damage with calcification, etc.) with dye test antibodies of similar or lower titer in both mother and child, still renders the diagnosis highly probable. The diagnosis cannot be made in the absence of "dye-test" antibodies in the child, regardless of how high the titer may be in the mother's serum. The placentally transmitted antibodies in an uninfected child disappear within 3 to 6 months, and the occurrence of high titers in a child after the age of 6 months is indicative of congenital infection if the mother has similar high titers.
The mere presence of complement-fixing antibody is not an indication of either recent or active infection since it has been found to persist for many years, often in fairly high titer, in "healthy" women who had given birth to one child with congenital toxoplasmosis, and subsequently to normal children. The complement-fixing antibody, however, disappears more rapidly than the dye-test antibody. A negative complement fixation test, therefore, does not rule out toxoplasmosis. There is an indication from observations on experimental animals and human beings that the complement-fixing antibody is not demonstrable during the first few weeks after infection, while the dye test antibody reaches high peaks more rapidly. Thus, by the use of both the complement fixation and dye tests it is often possible to obtain serologic evidence of a very recent or acute toxoplasmic infection, when the dye test alone may fail to indicate a rising titer. For example, if a newborn child exhibits a high dye test titer and a negative complement-fixation test, while the mother's serum contains both dye test and complement-fixing antibodies in high titer, the probability is that the infection is quite recent and still very acute; chemotherapy with sulfonamides would be indicated in such a situation.

It is also evident from this that the serologic diagnosis of acquired toxoplasmic infection in the individual case is possible only during the first weeks of the infection by a rising titer of dye test or complement-fixing antibodies (at least 8-fold and preferably greater) - the dye test reaching a level of at least 1:256 in an individual who survives several weeks after onset. In suspect cases of acquired toxoplasmosis, the association of a high dye test titer with a negative complement fixation test should be regarded as presumptive evidence for acute, active infection calling for intensive chemotherapy; the appearance of complement-fixing antibodies in such patients in subsequent weeks would constitute conclusive serologic evidence for the diagnosis of recent active toxoplasmic infection.

Both Dr. Eyles and Dr. Eichenwald presented data having a bearing on this question. In the fatal laboratory infection studied by Dr. Eyles, the patient's serum had no toxoplasma antibodies at all before infection, dye test titers of 1:16, 1:64 and 1:256 respectively on the 4th, 6th and 8th days of the disease but no complement-fixing antibodies. The laboratory infection, experienced by Dr. Eichenwald, was subclinical: he had no antibodies before infection and tests at 4-day intervals revealed that the dye test antibodies in a titer of 1:256 first appeared 16 days after exposure (reaching a level of 1:16,000 fourteen days later), while the complement-fixing antibodies did not appear until 32 days after exposure.

General Applicability of the Complement Fixation and Dye Tests for Serologic Diagnosis of Toxoplasmosis

It was generally agreed that for optimum results it was desirable to use both tests, although with certain exceptions mentioned above,
the dye test can be used by itself for the diagnosis of both congenital and acquired toxoplasmosis. The consensus of those who have worked with the dye test for several years is that it yields reproducible results in different laboratories and the technique is simple enough to be learned by technicians for routine use. The chief drawback in this test is that it requires living toxoplasma. This requirement makes it unsuitable for performance in routine laboratories, which may receive only occasional or irregular requests for this test. While the complement fixation test does not share this drawback, since the antigen is stable on storage for long periods of time, it cannot be used alone because a negative test does not rule out toxoplasmosis. It was pointed out that the routine use of both tests would greatly benefit from the availability of standard control antisera of known titer. The unfortunate laboratory infections with toxoplasma which have occurred during the last few years led to the suggestion that only technicians possessing fairly good titers of toxoplasma antibody be employed and that all possible precautions ordinarily used in working with infectious material be exercised.
D. Summary of Material Presented at the Toxoplasma Conference at the N.I.H.

Heinz Eichenwald, M. D.

In the past 3 years, over 700 sera were submitted to us from pediatric services from hospitals in and about New York to be studied for serologic evidence of toxoplasmosis. Only cases where there was a suspicion of this disease were submitted, and an adequate clinical history as well as careful physical and laboratory examinations were available in all. 79 patients with adequate serologic evidence of toxoplasmosis were found among this group, not including the mothers of congenital cases. Seventy-five of these were in children, and all of these probably represented the congenital form. Approximately 65% of patients were below the age of 1 year when tested. Slide neutralizing titers (dye test) in children below the age of 5 years ranged from 1:1024 to as high as 1:16,000; above that age, titers ranged from 1:256 up. The complement-fixing titers roughly paralleled this, although in much lower titer. However, in acute cases in the neonatal period, a dissociation between C-F and dye test titers was noted repeatedly, even though the mother had high titers by both tests. In addition, children past the age of 5 years had not infrequently negative C-F tests with positive dye tests, probably due to the fact that the latter antibodies persist longer in a detectable form. Skin sensitivity tests were not found particularly useful in the detection of clinical cases.

Clinically, the most constant (80%) finding was chorioretinitis, although it should be noted that in not a single infant was this lesion detected prior to hospitalization. Cerebral calcifications occurred in 60%; spinal fluid changes, hydrocephalus, pathologic jaundice each in 23%; microcephaly, hepatosplenomegaly each in 15%; rash, other ocular changes, etc., in less than 10%. Convulsions were the most common feature in the history, occurring in about 30% prior to hospitalization, and in about 50% within the age of 18 months. Almost 1/4 of the patients were admitted to hospitals because of non-specific complaints of vomiting, fever, and listlessness, and about 15% because of jaundice and/or hepatosplenomegaly.

Eight patients died; one at the age of 15 months following an operation, 3 at age 2-3 months, the remainder below 1 month. Autopsies were obtained in 6 cases, with toxoplasma being isolated in 4, and demonstrated microscopically in another. The 6th case in whom no organisms were demonstrated was the 15-month-old, who, however, had healed lesions suggestive of toxoplasmosis. In all autopsied cases, adequate serologic evidence of the disease had been obtained prior to death.
Follow-up studies in patients and their mothers are being conducted. The high incidence of mental retardation in congenital cases is appalling, occurring in about 80%. Twenty-one mothers who had given birth to infected children subsequently became pregnant 29 times. This resulted in 4 miscarriages, of which 2 were examined without evidence for Toxoplasmosis being found, 2 premature births, of which one died and was examined with negative results, and the remainder were normal healthy babies. The passive, transplacental transmission of antibodies previously described was noted again, and, therefore, all infants tested prior to the age of 4 months were rechecked at age 5 months or later. All infants with passively acquired immunity had negative serology by the age of 5 months.

A case of acute, subclinical adult toxoplasmosis was reported due to a known laboratory infection. Dye test titers first appeared 16 days after the infection and reached levels of 1:16,000 by 30 days. C-F titers did not appear until 32 days had elapsed. These observations agree with the results obtained in experimental animals.

During these investigations it became apparent that the results obtained by serologic methods were readily reproducible by other workers in the same laboratory, and by other laboratories. This was particularly true for the slide neutralization (dye test) method.
E. Summary of Material Presented at the Conference on Toxoplasmosis Conducted on August 3, 1951 at the National Institutes of Health

Harry A. Feldman, M.D.

A. Dye Test Technique

1. Activator is produced from defibrinated human blood which permits rapid separation and distribution for freezing.

2. The methylene blue is added to the tubes immediately following incubation. 0.02 cubic centimeter is added to each tube containing 0.2 cubic centimeter of toxoplasma-serum mixture.

3. Serum dilutions may be made on any day, numbered tubes into which they are distributed are frozen in a mechanical deep freeze until used.

B. Dye Test Reproducibility

Among individuals whose serum was tested on three or more monthly occasions (pregnant women in prenatal clinics), the dye test results varied as follows:

- 74 were always negative
- 10 were always 1:4
- 7 varied between negative, undiluted, and 1:4
- 4 varied between undiluted and 1:4

C. Congenital Toxoplasmosis

1. The following was collected from among 44 instances of congenital toxoplasmosis in whom the diagnosis was based upon serological evidence. Among 44 offspring, 18 were male and 24 were female. Of 41 offspring 10 were premature and 31 were born at term. Two of the premature died as compared to 3 of those who were born at term. Of these 44 mothers, 16 had 27 pregnancies subsequent to the birth of the toxoplasma infected baby. Of the 27 pregnancies, 24 terminated in normal babies, 2 ended with miscarriages and one gave birth to a premature which died.

2. Dye test titers were plotted against age and it was found that whereas the titers up to 3 years of age are principally at the level of 1024 or higher, that even as late as age 17 all the patient titers are 256 or higher with the exception of one 7-year-old child which had a titer of 1:64. At 16 and 17 years there is one mother each with a titer 1:64.

3. Passive transfer antibody seems to fall in a straight line from birth to 6 months of age and generally reaches the 1:64 level
at age 3 months. Therefore, high titers encountered at 3 months or older should be significant.

4. Among 33 congenital cases, 14 had all components of the tetrad while three had chorioretinitis, hydrocephaly or microcephaly, and psychomotor retardation. One had chorioretinitis, cerebral calcification, and psychomotor retardation; 4 had chorioretinitis and cerebral calcification; 2 had chorioretinitis and psychomotor retardation and 5 had chorioretinitis only. Nine had microophthalmia in addition to other findings and 8 had strabismus as well as other signs.

D. Acquired Toxoplasmosis

Serological information obtained from the study of a fatal case of proven toxoplasmosis, which occurred in a 60-year-old white female, who became ill in Boston, was presented as follows:

<table>
<thead>
<tr>
<th>Day of Disease</th>
<th>Dye Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Negative</td>
</tr>
<tr>
<td>48</td>
<td>1:16,384</td>
</tr>
<tr>
<td>55</td>
<td>1:16,384</td>
</tr>
<tr>
<td>73 (Died)</td>
<td>1:32,768</td>
</tr>
</tbody>
</table>

E. Surveys

Surveys under way in a number of widely scattered American cities show that a great proportion of the adult population has antibodies for toxoplasma by either skin or dye tests. One incomplete study still in progress among the residents of New Orleans turned up one child 2 years old with a titer of 1024, another 3-1/2 years old with a titer of 1:64, and one 4 years old with a titer 1:4096. These children are being investigated further, but they are the first to be encountered in the surveys who are less than 5 years of age and have demonstrable antibodies for toxoplasma by dye test.
F. Summary of Data Presented - J. K. Frenkel, M. D.

1. Comparison of dye and C-F data in 176 selected patients showed:

- 51 sera negative by both tests
- 0 " positive by C-F test alone
- 70 " by dye test alone (range 1:2-1:512; 8 were 1:96 or higher)
- 55 " positive by both C-F and dye test

  - lowest dye test associated with C-F test: D-1:32 - CF-1:6,1:24
  - lowest C-F test associated with dye test: CF-1:2 - Dye 1:128
  - highest dye test 1:32,000 associated with C-F tests 1:12 and 1:512

Not included (above) the following sera:

<table>
<thead>
<tr>
<th>C-F</th>
<th>DYE</th>
</tr>
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<tbody>
<tr>
<td>Child, aet. 3 mo. (born 1/2 mo. premat)</td>
<td>1,000,000</td>
</tr>
<tr>
<td>Mother</td>
<td>262,000</td>
</tr>
</tbody>
</table>

2. Serial serological titers in patients with neonatal toxoplasmosis and their mothers showed:

a. lack of parallelism in course of dye and C-F titers

b. titers of mothers and their children showed different trends of change

c. C-F titer of one mother had disappeared 4.3 years after giving birth

d. C-F titer of a child had disappeared 6.1 years of age

3. Comparison of dye and C-F titers of guinea pigs produced by 8 strains of toxoplasma, 1 month after infection, showed great variation of titers achieved. Differences between animals infected with the same strain were as great as the differences between animals infected with different strains.

4. Record of one mother giving birth to one normal child after a toxoplastic one.

5. All three fathers and 7 siblings tested had no evidence of recent infection, whereas the 5 mothers who lived in the same environment had given birth to toxoplastic children and had serologic evidence of recent infection.

6. Majority of patients with active chorioretinitis and positive skin tests had only moderately high dye test titers (range: 1:4-1:256, median 1:32). If chorioretinitis had followed acute infection with toxoplasma much higher titers would have been expected. The infrequency of C-F titers in the same 13 patients likewise indicates old infections (11/13 were negative). (In a control group the in-
Frenkel's Summary, concluded

cidence of positive skin tests was 10%, in the chorioretinitis group, active plus inactive, 70%). This indicates a significant correlation between the presence of chorioretinitis and positive tests for toxoplasmosis.

7. Spectroscopic and chemical comparison of a methylene blue sample sent by Dr. Westphal and said to be inferior for the dye test revealed no significant differences when compared to the dye sample used by myself (spectral peak, width, median; dye content, Azur B fraction).
G. Statement Submitted by Drs. Leon Jacobs and John Bozicevich

1. Of 424 serum specimens tested by both the dye test and the complement fixation test, 46 gave titers of 1:1024 on the former. Of these 46, only 1 was negative on the complement fixation test.

2. When the antibody titers detected by the dye test were 1:256, the complement fixation test was positive in 33 of 46 cases. The complement fixation test was positive in 13 of 49 cases with a dye test titer of 1:64. The complement fixation test was consistently negative when the dye test was positive with undiluted serum or at 1:16.

3. The complement fixation test was performed with an optimal dilution of antigen and serum dilution of 1:5. Of 23 sera with a dye test titer of 1:1024 or higher, which were complement fixation-tested with serum dilutions of 1:5 and higher, 14 gave positive complement fixation tests at 1:5, 9 at 1:10, and 1 at 1:80 serum dilutions.
H. Statement Submitted by Dr. Don E. Eyles

With regard to our laboratory infection of toxoplasmosis, I presented data on the dye test titer. Three sera taken before illness, the last two months before the patient became sick, all gave negative dye test reactions. The serum taken on the fourth day of the disease gave a positive titer of 1:16, the sixth day of the disease a titer of 1:64, and the eighth day of the disease a titer of 1:256. Complement fixation reaction, done by Dr. Bozicevich, was negative throughout. In our case report we made the point that since a large proportion of the population gives positive dye test reactions, it would be necessary to have two or more sera taken at intervals during the course of the disease in order to confirm a diagnosis by means of the dye test. Of course, our case was fulminating and a less severe case would probably eventually show a high titer which in itself might be of significance.

Animal inoculations were made from our case as follows: Whole blood was put into mice on the fourth and sixth day of the disease. Mice subsequently developed infections in both cases. Spinal fluid was put into mice on the fourth and ninth day of the disease and both lots of mice subsequently showed infections. Brain, spleen and liver were put into mice post-mortem and each lot of mice became infected. Lung tissue was put into mice post-mortem but the suspension was overheated in the Waring Blender and no infection resulted in the mice.

Our case report has been completed and has been submitted to the JAMA. We have not heard from them as to whether or not they are going to accept this paper. I will not attempt to summarize the clinical findings except to remind you that psychosis was one of the earliest symptoms and this prevented us from obtaining any history from the patient.

Although we believe that we used more than the customary precautions before our tragedy, we have since instituted rigidly enforced precautions for the handling of the disease. I am enclosing an outline of these precautions. I note that some elementary precautions which one might take for granted are not included in these lists but perhaps they will be helpful to you.

I had a letter from Dr. Gard yesterday and it would appear that they have had still another laboratory infection. Including Dr. Alm's there have been four in Sweden so far. Dr. Gard is now employing only technicians with positive titers.

I also presented at the meeting a few data on a sampling of the rural colored population of Fayette County, Tennessee. You showed particular interest in the four, five and six year group. We found 12 positives among 60 sera. Positives were as follows: 2,1:16; 4,1:64; 4,1:256; 1,1:1024 and 1,1:4096. In the entire group we found a fairly typical age incidence except that our incidence in the four to ten year group was 23 per cent which I believe is higher than usual. We also found that there appeared to be a tendency for the positive reactors to group themselves in families.
Safety Precautions for Toxoplasmosis Laboratory

A. Passage of strains, autopsy, and other animal dissection work --

1. All dissection, tapping, autopsy, etc., to be performed under glass hood using gowns, gloves and masks.

2. All infected animals to be handled with tongs unless anesthetized.

3. All hood wastes to be placed in formalin before removal. All waste tissues and mice to be kept in formalin for 48 hours before disposal.

4. Dissection instruments to be packaged sterilly and package broken under hood for dissection work. After use all instruments to be placed in cresol solution. Containers with cresol will be kept under hood at all time.

5. Only dead (killed) or anesthetized mice to be used for obtaining peritoneal exudate.

6. Only enough peritoneal exudate to insure maintenance of strains will be kept in refrigerator. No food or drinks will be kept in this refrigerator.

7. Hands will be washed in cresol solution before removing gloves and masks. After removal hands are to be washed with green soap.

8. Formalin will be placed in dissection pan after each dissection and surface of hood will be sponged with formalin at frequent intervals.

B. Dye test procedures --

1. Peritoneal exudate will be added to dilutions in animal room under hood.

2. Dilutions and mixtures will be prepared only with mechanical pipettes -- ABSOLUTELY NO MOUTH PIPETTING.

3. Slides will be prepared under hood.

4. Rubber gloves will be worn during all dye test procedures.

5. All tubes and containers will be placed in cresol solution immediately after use.

6. Slides will be placed in a dish of cresol solution immediately after examination. This dish will be kept by microscopes at all times.
7. Microscope will be sponged with antiseptic solution after work is concluded.

C. Care of animals --

1. Animal attendants will wear gown, mask and gloves for routine care.

2. Litter will not be changed on routine passage animals since they live only about four days.

3. When litter must be changed on drug test animals, etc., tongs will be used and animals will be transferred to clean jar.

4. Used jars will be sterilized or disinfected before reuse.

5. Litter will be treated with formalin before removal from jars.

6. Animal room will be treated with residual insecticide at frequent intervals.