

**TOXOPLASMA INFECTION AT DIFFERENT AGES,  
STUDIED BY THE SKIN-TEST METHOD**

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Bearing in mind that human toxoplasmosis was quite unrecognized 14 years ago, it is surprising to find how widespread this infection has been shown to be in man. This knowledge is the outcome of numerous observations which have been made possible by improved diagnostic methods which have been placed at our disposal by American scientists. Of these methods, three have proved to be most serviceable in practical clinical work, namely, the dye test, the complement-fixation test, and the skin test. A fourth method, the technically troublesome neutralization test, has been abandoned in many places for routine examinations. Of the first three methods, the dye test has been shown to have a high degree of sensitivity and specificity, and seems to be the most suitable for diagnostic use. In large group examinations, however, even this technically simple test needs laboratory resources, which only few places in Sweden can offer. Also difficulties may well be experienced in obtaining voluntary blood samples from healthy adults and still more so from their children.

Even the more critical authors, who have retested the skin reaction introduced by Frenkel (1949*a, b*) seem to be unanimous about the practicability of the method for epidemiological group examinations. The reports about its reliability are, however, variable. Sabin carried out skin tests on a small number of patients who reacted positively to the dye test and obtained a positive skin reaction in only 61 % of them. Under identical conditions, Adams and co-workers (Adams, Kabler, Cooney & Adams, 1949) obtained a positive skin reaction in 81 % whilst, in 1950, Gard (1951) reported positive skin reactions in 88.5 % of 120 gravidae who reacted positively to the dye test. Further, among 180 gravidae with negative skin tests he found that fourteen, or about 8 %, gave positive serum reactions. Out of ninety-seven children with negative skin reactions, examined by Fischer in 1951, only one gave a positive dye test.

In Gard's investigations negative dye tests were obtained from 17 to 18 % of the cases that reacted positively to the skin test. This, however, only applied if the dye test titration had been done by the standard technique, the highest serum concentration used being 1/10. If the titration was made with more concentrated serum dilutions, as was done in a small number of these cases, even these sera gave a positive dye test; which is in accordance with Sabin's experience.

Granted that a positive dye test is a reliable proof of toxoplasma infection, past or present, the figures given by Gard show that a group examination of adults with the skin test will reveal at least as many infected subjects as an examination of the same group with a routine dye test. Only a small percentage, about 8 % of the negative reactors, will be erroneously excluded, whereas the routine dye test would

fail to reveal about 15 %. Fischer's results show that for children the skin test and dye test are practically of equal accuracy. Therefore the skin test seems to be a convenient, cheap and sufficiently exact method of examination for following the spread of toxoplasmosis among larger groups of the population. There is little or no risk of a false positive reaction, and the number of faulty exclusions is too small to be of any practical significance in the epidemiological evaluation of a mass examination.

The intention in the present investigation has been to study, by means of skin tests, the frequency of toxoplasma infection within different age groups. The subjects tested were children admitted to the Children's Department, pupils from Eskilstuna High School, and pregnant women and newly delivered women treated in the lying-in wards. No special selection was made, and only a few patients with widespread eczema, and a small number of premature babies, were not tested. All the persons examined were residents in the town of Eskilstuna and its neighbourhood, comprising a mixed industrial-agricultural district in Central Sweden. It should be noted that most of the test-subjects above the age of 15 were women. According to hitherto unpublished Swedish investigations there tends to be a higher frequency of positive reactions amongst women than amongst men. Such a finding is, however, contrary to many results previously published. Thus, Gard found the same frequency of positives amongst 1894 men and women who were tested.

The test extract which was used, toxoplasmin, was supplied by the State Bacteriological Laboratories in Stockholm, and was manufactured from toxoplasma cultures grown on embryonated hen's eggs. 0.1 c.c of the toxoplasmin was injected intracutaneously whilst the same amount of a control reagent was injected into another area of the skin. The control reagent consisted of an extract of non-infected embryonated hen's eggs. The reaction was read after 48-72 hr. and was deemed to be positive if redness and infiltration measuring at least  $10 \times 10$  mm. were manifest. In no case did the control reagent give rise to any skin reaction. Serological examinations were made at the State Bacteriological Laboratories on the sera from about sixty persons with positive skin reactions. The results have confirmed that a good 15 % of such sera give negative serum reactions in a routine titration (the highest serum concentration in the dye test was 1/10 whilst in the complement-fixation test it was 1/7.5). Otherwise no serological control tests were made, as it was not the idea of the investigation to evaluate the reliability of skin reactions which, as previously pointed out, we considered satisfactory for our purposes.

The results of the investigation are shown in Table 1. The frequency of positive reactions was the same for town and country dwellers. Figures from previously published investigations of a similar nature have been included in the table for comparison.

As the figures show a somewhat inadequate agreement, especially as regards the youngest age groups, it is necessary to discuss the composition of the populations tested in the different investigations. In the Cincinnati investigation this seems to satisfy all demands for an investigation without qualification except in regard to its size. With regard to the Stockholm investigations, it must be pointed out that

the figures are not representative in the case of the younger age groups, i.e. those under the age of 15 years. These subjects were derived from a hospital where most of the cases of manifest toxoplasmosis amongst Stockholm children were under treatment at the time of the investigation. Thus, all children under the age of 10 years with a positive reaction showed clinical signs of toxoplasma infection. All the investigations carried out in London were on inmates of institutions for the mentally defective; it is not improbable that the frequency of skin reactions amongst mentally deficient children is higher than it is in unselected children,

Table 1. *The frequency of positive toxoplasmin tests*

(Figures in brackets indicate number of test-subjects.)

Age	Cincinnati			
	(Sabin & Feldman, 1949)	London (Fischer, 1951)	Stockholm (Gard, 1951)	Eskilstuna 1951
0-4	0% (20)	0.5% (186)	3.5% (537)	0% (201)
5-9	5% (20)	4.3% (322)	6.5% (123)	1% (104)
10-14	14% (20)	7% (216)	21% (42)	0% (74)
15-19			45% (40)	22% (73)
20-29	20% (20)		45% (332)	34% (122)
30-50	57% (40)		50% (374)	47% (74)
(20-50)	45% (60)	33% (59)	48% (706)	40% (196)

owing to the connexion between toxoplasmosis and brain damage in children. Further, it is clear that the choice of mental defectives may give rise to misleading figures for the higher age groups too, since the living conditions of those under investigation and especially their opportunities for acquiring infections are abnormal.

If regard is given to these considerations, agreement between the different investigations is satisfactory. Some differences remain, however. It would seem that toxoplasma infections permeate the population in Stockholm more readily than in less densely populated places, such as Eskilstuna and its surroundings. This idea gains further support from an investigation made by Gard in Stockholm, which is not incorporated in the table, and which was carried out on unselected schoolchildren of 12-13 years of age. Amongst 128 children examined skin reactions were positive in 15%. In the corresponding age group in Eskilstuna no positive cases were found.

The figures from Central Sweden, however, do not seem to apply to the whole country. In an investigation in one of the most northerly towns of Sweden it was found that out of 173 adults between the ages of 20 and 50 years there were only 10% who manifested positive skin reactions. Moreover, about half of the positive persons had previously been living in more southerly parts of the country. A similar observation has been made in regard to the occurrence of toxoplasmosis in wild hares, found dead in the woods. In the north of Sweden toxoplasma could only very rarely be found in the dead animals, but farther south it has not been very rare. As the mortality among hares with toxoplasma infection is about 100%, this observation is of value as a proof that the spread of the disease is subject to significant geographical variations.

## DISCUSSION

There are two questions of interest from an epidemiological point of view, namely, where is the reservoir of infection and how is the disease spread. Neither of these questions can be answered as yet with any certainty. The only definitely known channel of infection is the transplacental, but this is too rare to be of any epidemiological interest.

Grouping of infected individuals into ages suggests that the inhabitants of Central Sweden are exposed to a fairly constant risk of acquiring toxoplasmosis and the high incidence of positive diagnostic reactions indicates that susceptibility amongst mankind is general. In Northern Sweden the risk of infection is obviously less, even in the relatively densely populated area where investigations have been carried out. These interesting facts give rise to a number of reflections. From the point of view of epidemiological experience the geographical variations speak against the idea that the infection is spread by direct human contact, even though this assumption finds support from the curve of the age groups. The observation that uninfected rats can live together with infected ones without contracting the disease, as long as they do not eat dead animals, is a further strong argument against the theory of contagion. The hypothesis has been put forward that biting insects might act as vectors. But as the blood-sucking insects of Sweden are not confined to any particular area they cannot be made to account for the uneven geographical distribution of the disease except by assuming that the reservoirs of infection are extrahuman and subject to geographical variation. Further, investigations on dogs have shown that toxoplasma can be recovered from the evacuations of infected animals, and that food contaminated thereby can carry the disease to healthy puppies.

It is worth considering the possibility that food contaminated by evacuations from infected subjects could carry the infection from man to man or from infected animals even to the populations of large cities. In Stockholm, where faecal contamination of drinking water is rigorously controlled, toxoplasma infection is nevertheless very common. That food should get soiled by human excreta to any great extent does not seem probable. On the other hand, vegetables, etc., may obviously become heavily infected by manure from domestic animals, whilst corn and other stored food is liable to get soiled by, above all, rats. These considerations seem to favour the opinion that if human beings are infected by contaminated food, the reservoir of infection is probably extrahuman.

With the reservation that this reasoning is based less on proved facts than on theoretical arguments, many of which are open to discussion, the following points may be summarized. The reservoir of infection may be human or extrahuman. The mode of transmission is probably not by direct contagion. On considering two other possibilities, transfer by biting insects and by contaminated food, the arguments seem to favour the conception that the reservoirs of infection are extrahuman. Whether this opinion is correct remains to be proved, but there is a warrant for it in the fact that a variety of animals are known to harbour toxoplasma.

## SUMMARY

On the basis of previously published work, the skin test for toxoplasmosis has been judged to be a convenient and sufficiently reliable method for carrying out epidemiological group investigations intended to elucidate the spread of the disease. With it one can probably detect as many persons with serologically demonstrable antibodies against toxoplasma as when the same persons are examined by means of the routine dye test. An investigation employing skin testing has been carried out on 648 persons between the age of 0 and 45 years, all living in or adjacent to a town in Central Sweden. The reagent used (toxoplasmin) was prepared from toxoplasma cultures grown on embryonated hen's eggs by the State Bacteriological Laboratories, Stockholm. Amongst 379 children under the age of 15 years, only one gave a positive reaction, whereas in the higher age groups the frequency of positives rose rather rapidly to 40% in the age group 30–45 years. The results show fair agreement with those previously reported from similar investigations, with one very noticeable exception: in North Sweden skin reactions have proved positive in a bare 10% of adults studied. The significance of this observation is discussed in regard to the epidemiological characters of the disease. Having due regard to our incomplete knowledge of the subject, attention is drawn to the possibility that reservoirs of infection are extrahuman and that transfer of the disease to human beings occurs through the medium of biting insects or contaminated food.

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