

# The intradermal reaction in amebiasis

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*Summary: Studies on the amebiasis skin test were carried out in Amerindians living on reserves of Northern Saskatchewan. Results indicate the skin test to be highly sensitive in patients with acute amebic dysentery and in individuals with a history of amebic disease. A high percentage of asymptomatic school children living on a reserve where amebic disease is of common occurrence were also skin reactors. In a similar group of school children living on a reserve where amebic disease had never been reported but where *E. histolytica* infection rates are high there were very few reactors. A control group of white adults living in a non-endemic area were uniformly negative to the skin test. A comparison with the indirect hemagglutination test showed a good general correlation, but the skin test proved to be more accurate in cases of acute amebic dysentery in children 5 years of age or under. The skin test appears to have potential as a diagnostic technique and may be of considerable value in defining endemic areas of amebic disease.*

Knowledge of the intradermal reaction in amebiasis is limited. Early studies by Scalas<sup>1</sup> and Secret<sup>2</sup> with crude antigens prepared by extraction of mucus exudates and bits of tissue rich in amebae trophozoites gave contradictory results, the former claiming for the test specificity in amebic infections which the latter was unable to confirm. Spector,<sup>3</sup> using a heat-treated extract of *Entamoeba histolytica* trophozoites growing in culture with mixed flora, was unable to demonstrate specificity. But Leal<sup>4</sup> with a Seitz-filtered extract of amebae cultured with mixed flora reported a positive correlation in subjects with confirmed *E.*

*histolytica* infection. More recently Maddison, Kagan and Elsdon-Dew<sup>5</sup> studied the intradermal reaction in amebiasis using as an antigen a sonicated extract of *E. histolytica* trophozoites growing in a monobacterial culture. Their results showed that 81% of 42 patients with confirmed invasive amebiasis, either amebic dysentery or liver abscess, gave a positive reaction; in a control group of 78 persons in whom no symptoms attributable to clinical amebiasis were found, only 14% showed a positive reaction. The infection rate for *E. histolytica* in the control group was not determined, but in a stool survey of a similar population 13% were found infected with this parasite.

Further information on the nature and specificity of the skin reaction in amebiasis would be desirable because of its potential as a diagnostic aid and for the light it may shed on the nature of pathogenesis in this disease. Endemic

amebic disease occurs in several Indian reserves in Northern Saskatchewan,<sup>6,7</sup> and the Indian Health Service of the Federal Government initiated and is supporting a series of investigations aimed at its control.\* The occurrence of this circumscribed area of endemic invasive amebiasis in the reserves of Northern Saskatchewan provides a unique opportunity for studying various aspects of the host-parasite relationships in *E. histolytica* in man, a knowledge of which is essential to a control program. The present report deals with an investigation into the intradermal reactions as they occur in the different phases of infection and compares the results with those of the indirect hemagglutination test (HA).

## Materials and methods

### Antigen

The strain of *E. histolytica* which provided material for antigen was isolated from an Indian subject residing in the area of endemic amebic disease and is designated LLB strain. The amebae are grown in an all-liquid medium containing penicillin-inactivated *Bacteroides symbiosis*. By the time the amebae are harvested (between 48 and 72 hours) they have ingested most of the bacteria, and the remaining particulate matter consists almost entirely of amebae trophozoites.

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These are washed three times in cold physiological saline at a relative centrifugal force (RCF) of 250 G. We find that 1 ml. of packed trophozoites contains about 200 million amebae. The packed volume of amebae is measured and diluted in 49 parts of cold alkalinized double glass-distilled water (0.05 ml. NaOH per 100 ml. H<sub>2</sub>O). This is allowed to stand in the refrigerator for two to four hours, and rupture of the amebae which is induced by the hypotonicity of the solution is further encouraged by forcibly drawing in and ejecting the amebae suspension several times through a syringe with a No. 20 needle. This material is now clarified by centrifuging under refrigeration with RCF at 9000 G. for 20 minutes. The supernatant material, which is clear and faintly opalescent, is removed and constitutes the antigen in a dilution of 1:50. For the hemagglutination test it is frozen in amounts of 0.2, 0.5 or 1.0 ml. and stored at -20° C. When prepared for the skin test it is further diluted by adding equal parts of 1.7% cold NaCl to provide a 1:100 dilution of antigen in physiological saline, and passed through a membrane filter with apertures of 0.22 microns to ensure sterility. Sterility is further checked by culturing in meat broth. The antigen is stored frozen at -20° C. until use. We also have lyophilized the skin test antigen as a 1:50 dilution in distilled water prepared as above. Before use it is reconstituted with distilled water and diluted with equal amounts of 1.7% NaCl to give a 1:100 dilution in physiological saline.

#### Subjects studied

The following groups of subjects were studied; all but one were from Northern Saskatchewan Indian reserves.

A. Patients suffering from acute amebic dysentery. All cases were confirmed by finding erythrophagocytic trophozoites of *E. histolytica* in stools.

B. Patients who had a well-documented history of amebic dysentery or liver abscess and who had been treated during the previous 30 months. At the time of the test they were asymptomatic.

C. A group of school children on

an Indian reserve (Loon Lake) where confirmed cases of amebic disease are of common occurrence.

D. A group of school children on an Indian reserve (Mosquito) where *E. histolytica* infections occur, but where amebic disease is either absent or so rare that it has never been reported.

E. A random group of adults and children on the same Indian reserve as Group D (Mosquito) who had been studied the previous year.

F. A control group of adult non-Indian patients in a veterans hospital who suffered from a variety of chronic ailments, none of which were characteristic for amebic disease.

#### The skin test

The skin test was carried out in the usual manner with a tuberculin syringe and No. 27 needle. Approximately 0.1 ml. of antigen was injected intradermally on the flexor surface of the forearm and a similar injection of physiological saline was used as a control. Various strengths of antigen were tested and it was found that best results were realized with dilutions of 1:100. Several batches of antigen of the same strain of amebae (LLB) had been prepared and tested and with minor differences they all gave good results. Protein determinations using the method of Lowry *et al.*<sup>8</sup> gave results ranging from 15 to 35 mg. per 100 ml.

#### The hemagglutination test

This test was carried out with minor changes according to the

method described by Kessel *et al.*<sup>9</sup> We used sheep erythrocytes collected from blood defibrinated by the use of glass beads. The red cells were tanned with tannic-acid solutions in a dilution of 1:150,000 and our antigens gave best results when used in dilutions of 1:1500 to 1:4000, depending on the batch. The test was controlled by the use of positive sera of known strengths.

#### Stool examinations

Single stool specimens from individuals in the Loon Lake and Mosquito Reserves were collected, fixed and examined without concentration in a merthiolate-formalin solution and examined by one of us (M.J.M.).

## Results

#### Skin test

The criteria for a positive skin test must perforce be subjective to some extent and were arrived at after reviewing all the data. Firstly, the antigen reaction had to be at least twice the size of the saline control, and in general a wheal under 12 mm. in the long axis was not considered as positive unless it was notably turgid, showed pseudopod formation and was surrounded by a well-marked zone of erythema. In about 75% of positives the wheal was at least 14 mm. in the long axis. Areas of erythema ranged from 15 to 60 mm. across. The delayed reaction was considered positive when the area of erythema was at least 20 mm. in diameter without induration or 15 mm. with induration.

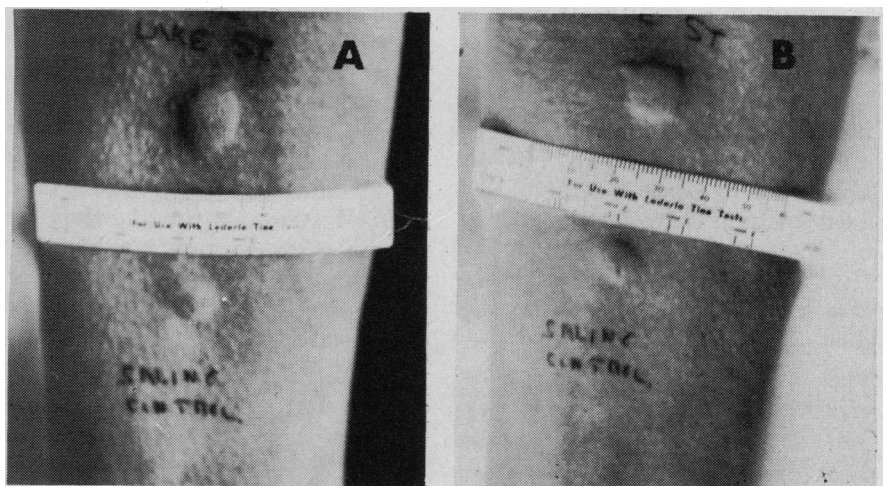


FIG. 1.—Forearm of amebic dysentery subject showing typical intradermal reaction. The characteristic wheal with pseudopods is seen at the site of injection above the ruler and the saline control below. (A) shows the reaction at five minutes and (B) at 15 minutes after injection.

The large majority of positives showed an immediate reaction which appeared within two to five minutes of injection. Usually it showed pseudopod formation and continued to enlarge, reaching a maximum size within five to 15 minutes. Between 15 and 20 minutes the wheal started to subside and it disappeared completely in one to three hours (Figs. 1a and 1b). There was a surrounding zone of erythema which appeared quickly and often followed the course of the wheal. In a percentage of cases, between 10 and 12 hours after injection a delayed reaction appeared as an erythematous patch with or without induration. In some cases it persisted in a gradually diminishing form for from three to four days. It never showed necrosis or sloughing. More detailed results for each of the groups studied are presented below and in Table I.

**Group A**—This group included 23 patients with acute amebic dysentery. They were of both sexes and ranged in age from less than 1 year (11 months) to 67 years. All subjects gave a positive skin test with the immediate and/or the delayed reaction. Twenty-one (91%) showed an immediate reaction, 16 (70%) a delayed reaction, and 13 (about 57%) were positive for both. The two patients showing a delayed reaction only were children aged 18 months and 2 years respectively.

**Group B**—In this group there were 21 persons with a confirmed history of amebic disease, the majority with amebic dysentery but several with amebic liver abscess. All had received specific therapy, and none showed evidence of amebic disease at the time of skin-testing. They were of both sexes and ranged in age from 3 to 60

**TABLE II**  
**Results for the indirect hemagglutination test for amebiasis in six groups of subjects being investigated for the amebiasis skin test reaction (for description of groups see text)**

| Group | Numbers studied | Total positive |    | Strongly positive* |    | Moderately positive† |    | Weakly positive‡ |    | Negative§ |     |
|-------|-----------------|----------------|----|--------------------|----|----------------------|----|------------------|----|-----------|-----|
|       |                 | No.            | %  | No.                | %  | No.                  | %  | No.              | %  | No.       | %   |
| A     | 23              | 16             | 70 | 4                  | 17 | 8                    | 36 | 4                | 17 | 7         | 30  |
| B     | 21              | 17             | 81 | 5                  | 24 | 7                    | 34 | 5                | 24 | 4         | 19  |
| C     | 24              | 14             | 58 | 0                  | 0  | 10                   | 42 | 4                | 17 | 10        | 42  |
| D     | 24              | 0              | 0  | 0                  | 0  | 0                    | 0  | 0                | 0  | 24        | 100 |
| E     | 19              | 0              | 0  | 0                  | 0  | 0                    | 0  | 0                | 0  | 19        | 100 |
| F     | 26              | 0              | 0  | 0                  | 0  | 0                    | 0  | 0                | 0  | 26        | 100 |

\*Strongly positive: positive in sera dilutions of 1:7290 or greater.  
 †Moderately positive: positive in sera dilutions of 1:810 or greater but less than 1:7290.  
 ‡Weakly positive: positive in sera dilutions of 1:270 but less than 1:810.  
 §Negative: sera showing no reaction or positive in dilutions of less than 1:270.

years. Twenty (95% of the group) were positive, showing an immediate and/or delayed reaction; one was negative for both. There were 17 (80%) with an immediate reaction and 13 (about 60%) showing a delayed reaction; 10 (roughly 50%) were positive for both.

**Group C**—This group included 24 school children of both sexes from 8 to 16 years, chosen at random from the classrooms. At the time of the testing none complained of illness. Nineteen (about 79%) showed a positive skin test. All positives gave an immediate reaction, and of these, six or roughly one-third showed a delayed reaction as well.

**Group D**—This group included 24 school children of both sexes from 6 to 15 years, chosen at random from the classrooms. Two of the 24 children reacted positively to the skin test, one with an immediate and the other with a delayed reaction.

**Group E**—In this group there were 19 subjects of both sexes ranging in age from 11 to 72 years. Three (about 16%) had a positive skin test; all showed an immediate

reaction and two of the three a delayed reaction as well. The three positive subjects were aged 16, 42 and 72 years respectively. There were 10 subjects less than 16 years and all were negative; of the nine remaining subjects, 16 years or older, three were positive.

**Group F**—This is the control group and included 26 adult male patients in a veterans hospital; they ranged in age from 28 to 82 years and 14 were below 60 years. All were negative for signs and symptoms of amebic disease and were in hospital for a variety of illnesses including liver cirrhosis, malignant disease, bronchitis, cardiovascular problems, etc. All skin tests were negative.

#### Indirect hemagglutination test

The results for the hemagglutination tests on subjects in the different groups are summarized in Table II. Only subjects showing a reaction in dilutions of 1:270 or higher were considered to be positive. In Group A, where all subjects were suffering from acute amebic dysentery, 16 of 23 (about 70%) were positive. This group included nine adults and two teenagers; the remaining 12 patients were 5 years of age or less. There was a total of seven with a negative serology and all were in the age group 5 years or under.

In Group B, which included asymptomatic subjects with a history of previous amebic disease, 17 of 21, or 81%, showed a positive serology. There were four subjects 5 years of age or less, and two of these were negative to the indirect hemagglutination test; of the older

**TABLE I**  
**Results obtained with the amebiasis skin test in six groups of subjects (for description of groups see text)**

| Group | Numbers studied | Total positive |     | Immediate reaction |    | Delayed reaction |    | Immediate and delayed |    | Negative |     |
|-------|-----------------|----------------|-----|--------------------|----|------------------|----|-----------------------|----|----------|-----|
|       |                 | No.            | %   | No.                | %  | No.              | %  | No.                   | %  | No.      | %   |
| A     | 23              | 23             | 100 | 21                 | 91 | 16               | 70 | 13                    | 57 | 0        | 0   |
| B     | 21              | 20             | 95  | 17                 | 80 | 13               | 60 | 10                    | 50 | 1        | 5   |
| C     | 24              | 19             | 79  | 19                 | 79 | 6                | 25 | 6                     | 25 | 5        | 21  |
| D     | 24              | 2              | 8   | 1                  | 4  | 1                | 4  | 0                     | 0  | 22       | 92  |
| E     | 19              | 3              | 16  | 3                  | 16 | 2                | 10 | 2                     | 10 | 16       | 84  |
| F     | 26              | 0              | 0   | 0                  | 0  | 0                | 0  | 0                     | 0  | 26       | 100 |

children and adults who made up the remaining 17 in the group, only two were negative. Of the 24 asymptomatic school children on Loon Lake Reserve (Group C), where amebic disease is highly endemic, 14 (58%) were positive. All the subjects from Mosquito Reserve (Groups D and E), where amebic disease has not been reported, as well as the controls (Group F), were negative to the hemagglutination test.

In Groups A and B at least one-quarter of those with positive serology showed titres of 1:7290 or greater (strongly positive), while none of the positive HA reactions in the Loon Lake school children (Group C) reached titres of this level.

#### Stool surveys

Stool examinations for intestinal parasites were carried out on two Indian reserves, Loon Lake and Mosquito, the former an endemic area for amebic disease and the latter apparently free from this disease. The common intestinal protozoan infections were seen on both reserves, with higher rates in the Loon Lake population. *E. histolytica* infections were diagnosed on the basis of cyst morphology and size; cysts under 10 microns in diameter were considered to be *E. hartmanni*. *E. histolytica* cysts ranged from 10.5 to 14.5 microns in diameter.

The *E. histolytica* infection rates for the two reserves are shown in Table III. At Loon Lake 58 subjects were examined and 20 (34%) were found positive. These results are based on examination of a single unconcentrated stool examination. A comprehensive survey including repeated stool examinations and concentration techniques would have doubled at least the observed infection rate. On Mosquito Reserve, 36 subjects were examined and six (17%) were found positive for *E. histolytica*. Here again re-

peated stool examinations with concentration techniques would have revealed an infection rate probably twice as high. A breakdown by age group indicates that children 15 years and younger on both reserves show a higher infection rate than do older age groups.

Helminth infections found included the fish tapeworm *Diphyllobothrium latum*, the dwarf tapeworm *Hymenolepis nana* and eggs of the trematode superfamily Opisthorchioidea.

#### Discussion

##### *The significance of the intradermal reaction in amebiasis*

The results of our studies indicate that the intradermal test for amebiasis is both sensitive and specific. Admittedly the number of patients tested to date is small, but of 23 confirmed cases of acute amebic dysentery all showed a positive reaction while a control group of 26 subjects negative for signs or symptoms of amebic disease in a region where the disease is not endemic were uniformly negative. The immediate reaction occurs more consistently, but in a small percentage of cases the delayed reaction alone was seen. With one exception all subjects in the group with a history of amebic disease continued to show positive skin tests for periods of at least 30 months. In this group, as in the acute cases, the immediate reaction occurs more frequently, but again a small minority of cases show only the delayed reaction. Whether the persistence of the intradermal reaction is due to the initial disease or to reinfection cannot be stated. Because of the high *E. histolytica* infection rates in this community, reinfection must be common.

The results of skin testing a group of apparently disease-free children (Group C) living in an area where amebic disease is highly endemic (Loon Lake) are most interesting, as we find here

that over three-quarters of the children are reactors (Table I). A similar group (Group D) in an area in which amebic infection is endemic but who are apparently free from amebic disease (Mosquito Reserve) showed less than 10% reactors although one-third of the small group of adults (nine) tested here showed a positive skin test.

A positive amebiasis skin test, according to our results, may indicate acute disease, past disease, or prolonged residence in an area where amebic disease is highly endemic. The results of our stool surveys show that the *E. histolytica* infection rate on Loon Lake Reserve (Group C) where amebic disease is common is very high indeed and in certain age groups is probably close to 100%. Thus the large number of skin reactors found on this reserve could be interpreted as a measure of the *E. histolytica* infection rate rather than disease. That this is not the case is clear from results obtained on Mosquito Reserve where, with an infection rate which is probably close to 50% of that occurring in Loon Lake, less than 10% of the children show a positive skin test.

It would appear that infection with *E. histolytica* does not necessarily result in a positive skin test and, in fact, we have discovered several subjects on the Mosquito Reserve who had a negative skin test but who were passing cysts of *E. histolytica*. Conversely, it is of interest to note that in the small group of adults tested on this same reserve the skin reaction rates were 33% as compared to 8% for the children, yet infection rates for *E. histolytica* were found to be higher in children. This may be explained by the longer exposure period to infection in adults, who would have had a greater opportunity to be repeatedly infected and eventually sensitized.

We can only speculate on the course of events in subjects with amebic infection which result in the appearance of an intradermal reaction. It is reasonable to assume that tissue invasion by the parasite is a prerequisite for a positive skin test. This may explain the high incidence of positive skin reactions in the asymptomatic school

**TABLE III**  
The *Entameba histolytica* infection rates in two Indian reserves based on a single stool examination (unconcentrated) for each subject

| Reserve   | Subjects |          | 1-4 yrs. |         | 5-9 yrs. |         | 10-15 yrs. |         | 16 yrs. + |         |
|-----------|----------|----------|----------|---------|----------|---------|------------|---------|-----------|---------|
|           | No.      | Pos.     | No.      | Pos.    | No.      | Pos.    | No.        | Pos.    | No.       | Pos.    |
| Loon Lake | 58       | 20 (34%) | 15       | 5 (33%) | 23       | 8 (35%) | 13         | 6 (46%) | 7         | 1 (14%) |
| Mosquito  | 36       | 6 (17%)  | 2        | 1 (50%) | 4        | 0       | 15         | 3 (20%) | 15        | 2 (13%) |

children tested on the Loon Lake Reserve as compared to the low rates seen in a similar group on Mosquito Reserve. In the former, invasive amebiasis resulting in ulcerative colitis and liver abscess is relatively common, while on Mosquito Reserve invasive amebiasis leading to disease has never been reported. At the same time the fact that a large majority of an asymptomatic group of school children (Loon Lake) are skin-positive may also indicate that tissue invasion of the gut wall by amebae does not always result in successful tissue colonization and disease.

As a diagnostic technique for acute disease, the intradermal reaction may prove to be of considerable value in those patients who are not permanently resident in areas highly endemic for amebic disease, while a negative test would appear to be highly significant in all cases.

#### *A comparison of the intradermal and indirect hemagglutination tests*

In general there was good correlation between the intradermal and hemagglutination tests, but the former reaction was apparently the more sensitive. Amerindian subjects studied in the areas where *E. histolytica* infection is endemic totalled 111, and of these 67 were positive to the skin test as compared to 47 with a positive HA reaction. Nevertheless, there were three subjects negative to the skin test but positive in low titres to the HA test, one with 1:810 and the other two 1:270. The former was an asymptomatic 10-year-old with a history of treated amebic dysentery, and the latter two were asymptomatic school children on the Loon Lake Reserve where amebic disease is common.

All patients with amebic dysentery were positive to the skin test while 30% of this group were negative to the HA reaction. An analysis by age of the group of false negatives showed that all were children aged 5 years or under. Further, there were 12 children with amebic dysentery in this age group and seven of these were

negative to the HA test. These results suggest that for infants and toddlers with amebic dysentery the intradermal reaction is diagnostically more reliable than the HA test.

Correlation in the two groups of asymptomatic school children (Groups C and D) was also good, the intradermal reaction and the HA test respectively showing 79% and 58% positives for the Loon Lake group and 8% and zero for the Mosquito Reserve children. In view of the fact that *E. histolytica* infections were shown to be prevalent in both groups but amebic disease was endemic at Loon Lake only, the results of these tests would appear to be of much greater significance than parasite studies for determining the prevalence of amebic disease in a community. The value of the serological approach for studying the epidemiology of amebiasis has been noted elsewhere.<sup>10</sup> Our work suggests that the intradermal reaction, particularly in school children, could be equally useful in this regard and is much simpler to use.

Recent studies by Krupp<sup>11</sup> indicate that agglutinin antibody levels in patients treated for amebiasis start to drop in about one month and the fall in titre continues for at least the next six to 12 months. We found subjects treated 30 months earlier still showing a positive intradermal reaction. As already stated, because of the high risk of reinfection, we do not know whether the reaction had persisted since the initial infection or was due to reinfection.

#### *The immediate versus the delayed reaction*

On the basis of results obtained to date, we are unable to define the significance of the immediate versus the delayed skin reaction in amebiasis. There was no correlation of either one with the status of the host-parasite relationship, nor was there a consistent pattern to relate either with the HA test. We cannot confirm the findings of Maddison and his colleagues<sup>5</sup> that the so-called "carrier" infections show a higher incidence of delayed skin reactions. In our asymptomatic

group of school children where 79% were positive to the skin test, all showed the immediate reaction, but only one-third the delayed reaction. By contrast, of the amebic dysentery patients, all of whom were reactors, almost three-quarters showed the delayed reaction.

#### **Résumé**

##### *La cutiréaction dans l'amibiase*

La cutiréaction pour la recherche de l'amibiase a été appliquée à des Amerindiens vivant dans des réserves de la Saskatchewan septentrionale. Ces essais ont révélé que ce test est très sensible dans des cas aigus de dysenterie amibienne et chez des sujets ayant eu des antécédents de cette parasitose. Un fort pourcentage d'écoliers asymptomatiques, mais vivant dans une réserve connue pour être un foyer d'endémie, ont également présenté des réactions positives. Chez un groupe similaire d'écoliers vivant dans une autre réserve exempte d'amibiase mais où l'infection à *E. histolytica* était fréquente, le nombre de cutiréactions positives a été très minime. Un groupe de témoins, composé d'adultes de race blanche vivant dans une région non endémique, ont tous eu une cutiréaction négative. On a noté une bonne corrélation générale entre la cutiréaction et l'épreuve indirecte d'hémagglutination mais la cutiréaction s'est révélée plus précise dans les cas aigus de dysenterie amibienne chez des enfants ayant au plus 5 ans.

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