# THE REACTION OF PERITONEAL CELLS AGAINST ASCARIS SUUM LARVAE

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## ABSTRACT

Sixty mice were used for the study on the reaction of peritoneal cells against Ascaris suum larvae. The mice were divided into 3 groups; gronp A–all were kept as Ascaris-free (non sensitized with Ascaris); group B–all mice were given inoculation with primary infection of Ascaris suum eggs; group C–all mice were given inoculation with secondary infection of Ascaris eggs. All of the mice in various groups were then inoculated peritoneally with Ascaris suum larvae. At interval of 2, 4, 6, 8 and 12 hours after inoculation, the peritoneal exudate was drawn for the study of the exudate cell reaction. It was shown that the adhesion of the cell on the cuticle of the larvae was not observed in parasitefree mice. By contrast, the adhesion of the cells on the cuticle of the parasite was shown in the sensitizedmice particularly on the group of mice which previously were twice sensitized. The type of the cells adhering to the larvae seem to be the result of immunologically mediated with cell reaction and the cell involving in this phenomena is probably the mononucleocyte of lymphomacrophage system.

## INTRODUCTION

During the study on the site of penetration of Ascaris suum larvea in experimentally infected mice (Keittivuti, 1974), the author accidentally found some of the larvae of Ascaris were partially or completely encased in the coagulated exudate in previously mice. These encased larvae seem to be inactive and non motile. The way by which the larvae become encased challenged the authors to study this phenomena, so that this experiment was carried on.

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## MATERIALS AND METHODS

**Preparation of Embryonated A. suum eggs**

Adult female Ascaris suum were obtained from the small intestines of slaughterd pigs at Bangkok Metropolis Slaughtered House ( Prakanong ). The worms were cleaned by washing in running tap water. The terminal one inch of the uteri of the worm was isolated in sterile physiological saline solution. The eggs in the uteri were dispersed by homogenizing at medium speed, in a small quantity of physiological saline solution in a grinder. The emulsion was filtered through two layers of gauze. The egg suspension was then poured into strile petri-dishes to a layer of 3 mm. In order to prevent bacterial contamination, formalin was added to make 2 percent solution. These cultures were incubated at room temperature, and were aerated by shaking at least once a day. The eggs were periodically examined for rate and stage of development. The majority of the eggs were embryonated after 3 weeks. The eggs were incubated for at least 4 weeks in order to allow adequate time for the first larval molt to take place and so become infective and then was kept at 4°C until used. Prior to use in experimental animals, the infectivity of the eggs was tested in mice. Recovery of larvae following artificial peptic digestion of the livers and lungs of mice 5 days after oral inoculation with embryonated eggs was the criterion of infectivity. The number and severity of lung hemorrhages were also considered as measures of viability and infectivity of the eggs.

**Preparation of Second Stage Larvae**

Infective stage A. suum larvae were obtained by hatching fully embryonated eggs. The embryonated eggs were decoated for about 2 hours at room temperature in a mixture of equal parts of 1 N sodium hydroxide and Clorox ( 5.25 percent sodium hypochlorite ). The decoated eggs were washed four times and then suspended in sterile saline and the concentration of the eggs was standardized to about 50,000 eggs per ml. The apparatus used for hatching decoated eggs (illustrated in Fig. 1 ) consisted of an appropriate size siliconized glass tube

into which was poured 20 ml. egg suspension in saline. Through the rubber a thin tube was connected to a compressed air cylinder. Another thin tube was used for exhaust. Only a slight air pressure was needed to give sufficient bubbling. When approximately 90 percent of the larvae were hatched, they were separated from the egg shells and debris aud collected by Baermann's technique. The hatched larvae were then standardized and used for intraperitoneal inoculation into experimental mice.

**Preparation of Third Stage Larvae**

The third stage larvae were obtained from the lungs of mice 6 days following oral inoculation with infective A. suum eggs. The lungs were minced with scissors and the larvae extracted by Baerman's technique.

**Experimental Animals**

Six – week old mice from animal house of AFRIMS were used during the course of this study. A total of 60 mice were used in this experiment. They were divided into 3 groups as follows.

Group A. This group consisting of 20 mice was used as Ascaris – free control and no infective eggs were administered.

Group B. Mice in this group were given only a primary infection This group consisting of 20 mice was orally inoculated with 1,000 eggs permouse. After 10 days, the peritoneal exudate containing various infiltrating reactive cells was aspirated and observed.

Group G. The 20 mice in this group were given two oral inoculations of infective eggs of A. suum at a 14 day interval. The first inoculation consisted of 1,000 eggs per mouse while the second was 2,000 eggs per mouse. Ten days following the secondary inoculation, cells of the peritoneal exudate were aspirated and examined.

**Experimental Procedure**

Each experimental mouse was inoculated intraperitoneally with 1,000 second or third stage larvae in 0.5 ml. saline. At interval of 2, 4, 6, 8, and 12 hours after inoculation, two mice from each group were anaesthesized and inoculated with 1 ml. of sterile saline solution intraperitoneally and theis abdomens massaged gently for 1 – 2 minutes. They were then killed, their abdomens opened and the peritoneal fluid aspirated. For each time interval, the peritoneal

fluids of the two mice were combined and a drop of this fluid was placed on a slide and observed microscopically for larval activity among the peritoneal exudate cells. Turbidity of the peritoneal fluids was compared visually. A smear was air dried and stained with methyl green pyronin.

## RESULT

Fresh smears of peritoneal fluid obtained from mice 2, 4, 9, 8 and 12 hours following intraperitoneal larval inoculation were observed. When previously non – sensitized mice were used the larvae in the smears were actively motile. There were few leukocytes in these smears to interfere with their motility. Adherance of the leukocyte cells to the cuticle of these larvae was not observed (Fig. 2). By contrast, larvae obtained from the peritonaal cavity of mice previously sensitized by oral inoculation with A. suum embryonated eggs were covered with many adhering leukucytes and coagulated serous fluid (Fig. 3). When the smears were fixed and stained with pyronin methyl green, the red staining characteristic of many of thses cells indicated that they were mononuclear pyroninophilic leukocytes (Fig. 4,5,6). Some of them were of the large varieties while other were medium in size. The serous fluid around the larvae was also pyroninophilic. The exudate also contained small lymphocytes as well as polymorphonucleated cells (Fig. 5). In those mice orally inoculated twice with empryonated Ascaris eggs before intraperitoneal inoculation, the cellular exudate contained many more leukocytes. These cells also appeard to adhere to the larvae much earlier than in the once orally inoculated mice. Furthermore, the exudate in the twice inoculated mice seem to have coagulated faster around the larvae than in the once inoculated mice. This was particularly evident in mice twice previously inoculated 8 and I2 hours post intraperitoneal inoculation (Fig. 6).

The accumulation of the leukocytes and exudate the larvae in the peritoneal cavity of previously sensitized as well as non-sensitized mice appeared not to be affected by the stage of development of the intraperitoneally inoculated larvae. This exudate and leukocytic cells accumulated and adhered at the same intensity to the cuticles of the second stage larvae obtained by hatching embryonated eggs as they did to the thir stage larvae obtained from the lungs of other mice.

Gross observations of the peritoneal fluids from these groups of mice substantiated the effects of previous sensitization on intraperitoneally inoculated larvae. The turbidity and viscosity of the peritoneal fluid seem to be increased in the twice inoculated mice as compared to that from those once inoculated and those non-inoculated. It was also more prominent at 12 hours than at earlier periods (Fig. 7).

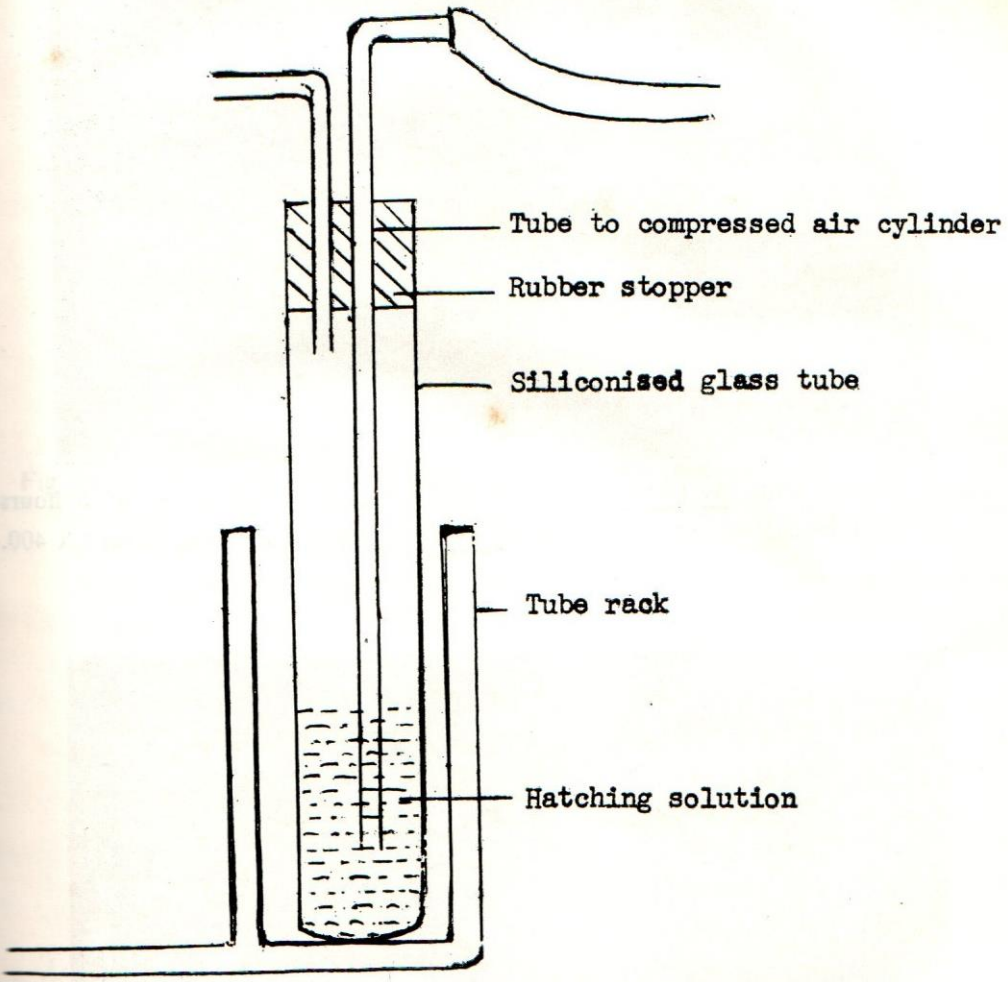


Fig. 1 Hatching Apparatus

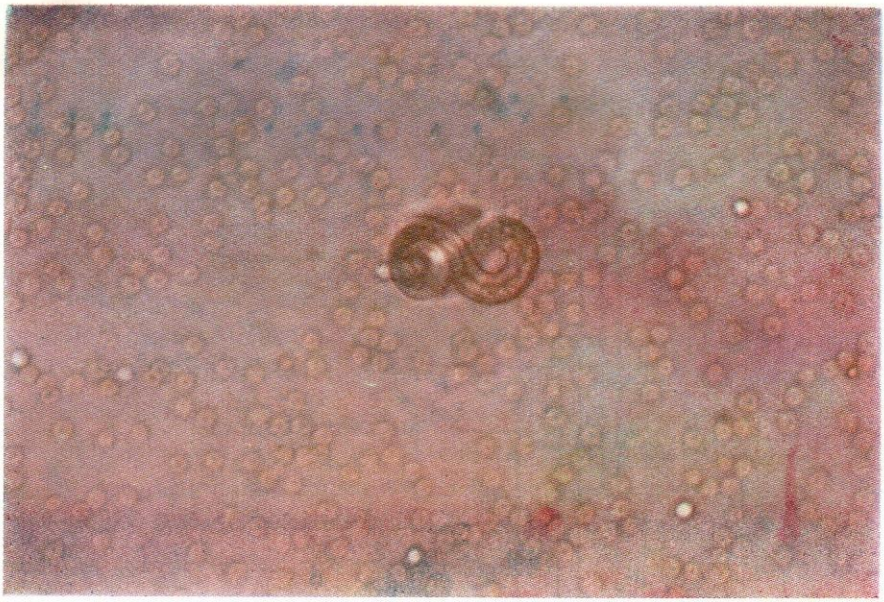

Fig. 2 Second stage A. suum larvae removed from peritoneal cavity of 6 hours after inoculation of larvae into an Ascaris - free mouse. Fresh smear : X 400.

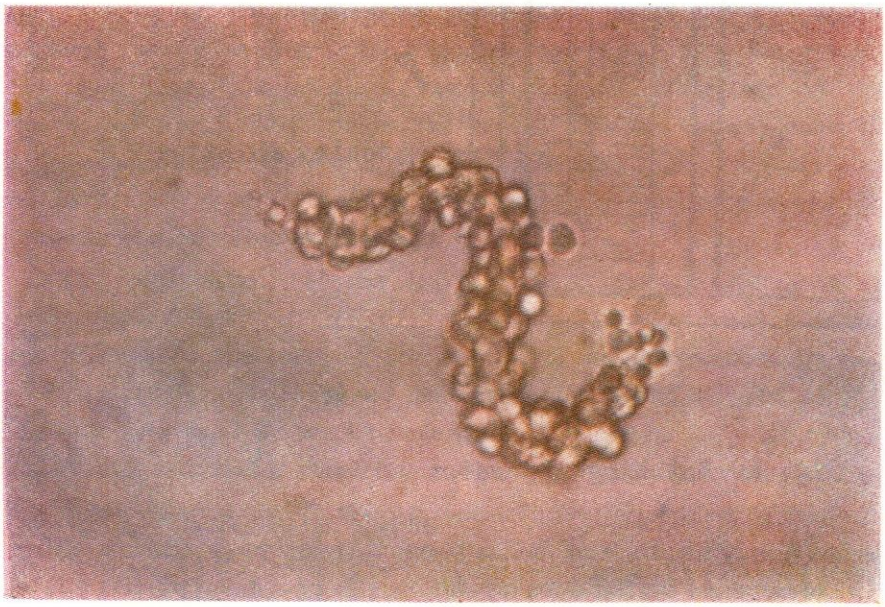

Fig. 3 Second stage A. suum larvae showing cell adhesione around the cuticle of the worms removed from the peritoneal cavity 6 hours after inoculation of larvae into a mouse twice infected with A. suum. Fresh smear : X 400.

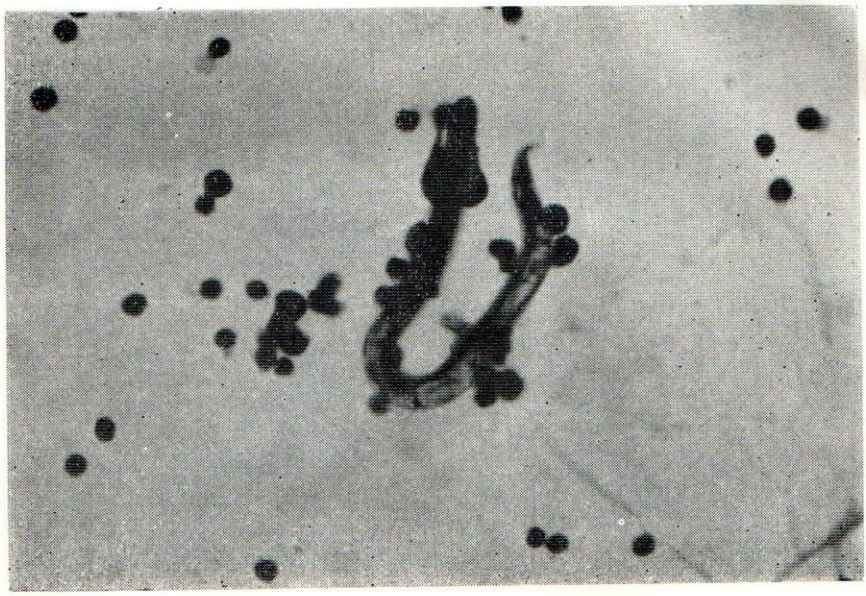

Fig. 4 An A. suum infective stage larvae showing few cells adhering on the cuticle of the worm 4 hours after intraperitoneal inoculation of larvae into a mouse previously twice infected with A. suum eggs. Notice amoeboidal appearance of the cells at the anterior end of the worm. Methyl green pyronin stain : X 400.

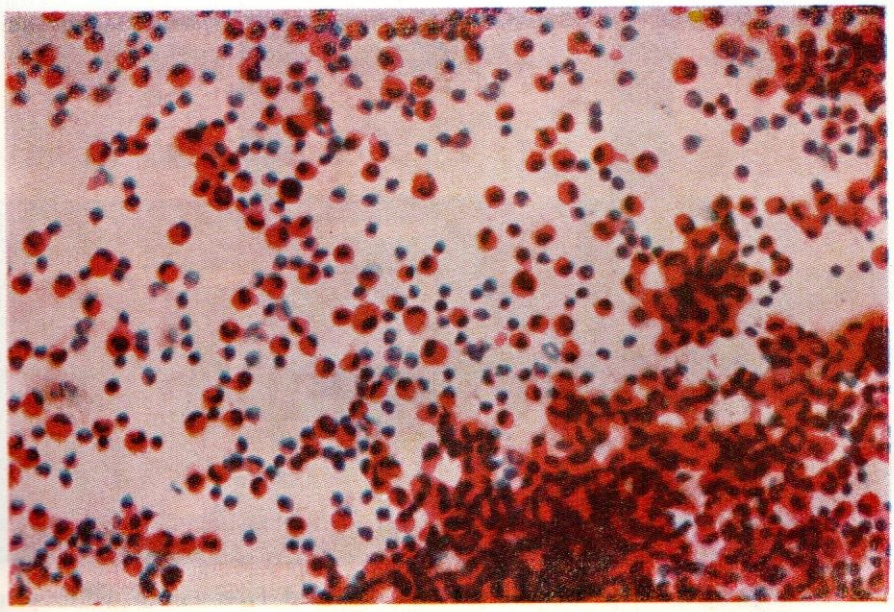

Fig. 5 Peritoneal exudate cells of mouse previously sensitized with A. suum eggs. These cells were removed from the peritoneal cavity after inoculation of larvae into mouse. Notice the different sizes of red cytoplasmic pyrininophilic cells. Methyl green pyronin stain : X 400.

Fig. 6 A. suum infective stage larvae removed from the peritoneal cavity 8 hours after inoculation of a mouse previously twice inoculated with A. suum eggs. Notice extensive cell adhesion to the worm surface. Methyl green pyronin stain : X 400.

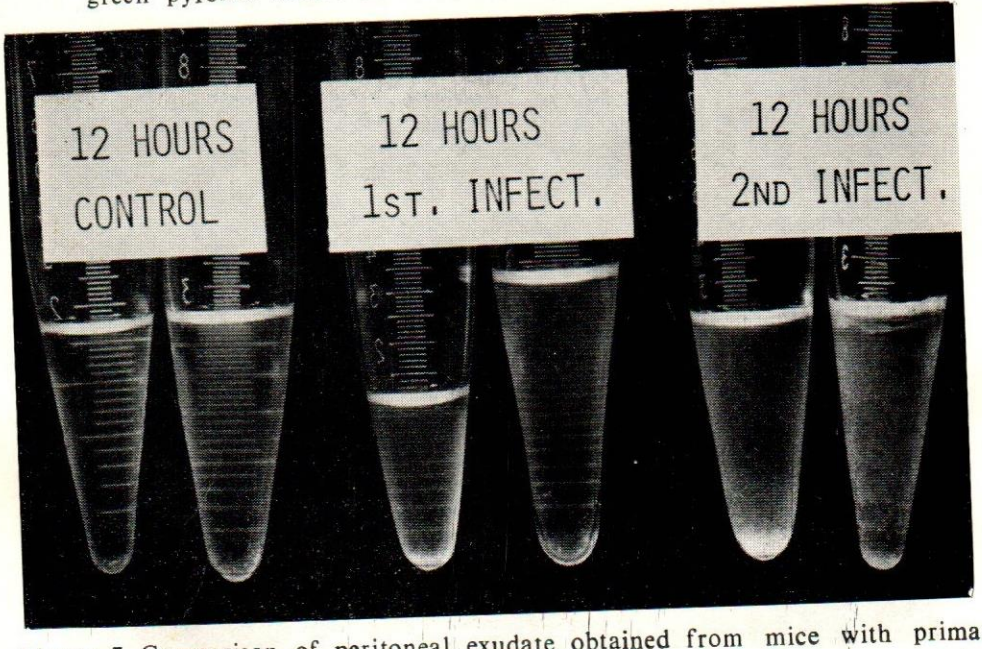


Figure 7 Comparison of peritoneal exudate obtained from mice with primary infection and secondary infection of A. suum eggs and exudate obtained from Ascaris-free mice. Note the fluid obtained from mice with secondary infection is more turbid than those obtained from Ascaris-free mice and from mice with single infection.

## DISCUSSION

The type of cellular response surrounding the larvae in the peritoneal cavity of sensitized mice in this study is probably similar to those described by Holst (1922) as a result of intraperitoneal inoculation of foreign materials. The presence of foreign materials apparently has an effect on free cells in the peritoneal cavity. Inoculation of tuberculin in the peritoneal cavity of tuberculous guinea pigs enhanced the coagulation of the peritoneal fluid presumably through the increase in the number of peritoneal cells and changes in the character of the exudate. There are few reports in the literatures describing the adhesion of peritoneal cells to larval stages of the parasitic nematodes. Soulsby (1961 a, b) reported that eosinophils are predominent cell type adhering to the lung stage larvae of A. suum when placed into the peritoneal cavity of a sensitized rabbit. This reaction was not evident when the lung stage was placed into non-sensitized animal or when the lung stage larvae were in the peritoneal cavity of a sensitized rabbit. The results obtained from the present study indicated the cell adhesion reaction did not occur in parasite-free mice whem these mice were inoculated intraperitoneally with artificiailly hatched larvae or larvae obtained from the lung of previously infected animals. By contrast adhesion of cells to the larvae occurred when these mice were previously sensitized by oral inoculation with embryonated A. suum eggs. Jeska (1969) indicated that cell adhesion to larvae also occurred in parasite-free animals. He postulated that this phenomenon may be mediated through a non-immunological mechanism characterixed by the presence. of a prodominantly polymorphonuclear cell exudate. The cell adhesion to the larval stage of A. suum in the present study apparently does not involve the polymorphonuclear leukocyte. These cells were not observed to participate in the cell adhesion reactions although they were seen trapped in large coagulated masses or coagulated masses or scattered in the peritoneal exudate. The adhesion of the cells on the cuticle of the worm seem to be selective rather than mechanical. At similar post inoculation times the cell adhering to the larvae were more numerous in the twice sensitized than in the once sensitized and non-sensitized

mice. This response was also evident through the higher turbidity and viscosity of the peritoneal fluid obtained from the sensitized animals (Fig. 7). The reaction of the peritoneal cells associated with the cell adhesion on the cuticle of the larval stages of A. suum in the present experiments appear to be the result of immunolgically mediated white cell reactions. This phenomenon of immunological cell adherence to parasite cuticles has been observed in other species of parasitic worms. White cells were observed adhering to the surface of Nippostrongylus larvae in the peritoneal cavities of appropriately sensitized rabbits (Soulsby, 1963).

The type of cell adhering to the cuticle of the larvae inoculated intraperitoneally in previously sensitized mice is pyroninophilic in its staining characteristics. The red cytoplasmic staining was obseaved in various size cells of the peritoneal fluid in mice previously sensitized with A. suum. These types of leukocytes are considered to be associated with an immune phenomen. Some investigators considered them active immunocytes or transformed lymphocytes on the basis of the basis of their pyroninophilic character. The participation of macrophages as well as transformed lymphocytes in the adherence phenomena to worm cuticle was suggested bo Jeska (1969). It was noted in the present study that these adhering cells seem to possess amoeboid movement characteristic particularly in the actively attached cells (Fig. 4). It is difficult sometimes to distinquish the cell types of transformed lymphocytes and macrophages. Some authors mentioned that transformed lymphocytes may become macrophage like upon combining with cytophilic antibody (Coulsom, Gurner and Coomb, 1967). The discrete adherence of pyroninophilic cells to the intraperitoneally inoculated A. suum larvae to be most active at 2 to 6 hours post inoculation. Thereafter, these cells seem to be extremely crowded in the coagulative mass around the trapped larvae. The complexity of the nature and mechanism of cell adhesion reaction to the parasite is not well elucidated. Further study is needed to establish the role played by the cells and the basic mechanism of the reactions.

## CONCLUION

This experimcnt was conducted to observe the peritoneal cell reaction against larval stage of Ascaris suum in experimental mice. The result obtained from this experiment indicated that the cell adhesion did not occur in parasite-

free mice that were inoculated intraperitoneally with artificially hatched larvae or larvae or larvae obtained from the lung of previously infected animals. By contrast, adhesion of the cells to larvae occurred when these mice were previously sensitized by oral inoculation of embryonated Ascaris suum eggs. The reaction of the peritoneal cells associated with the cell adhesion on the cuticle of the larval stages of Ascaris suum in the present study appear to be the result of immunologically mediated with cell reaction. The type of the cells adhering to the cuticle of larvae in previously sensitized mice is pyroninophillic in its staining. The cell probably will be mononucleocyte of the lymphoid macrophages system which is variable in size.

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## REFERENCES

1. Coulson, A. S., Gurner B. W. and Coombs, R. R. A., 1967, Macrophage like proper of some guinea pigs transformed cells. Int. Arch. Allergy, 32:264.
2. Holst, P.M., 1922. Studies on the effect of tuberculin IV. Experiments with phagocytosis. Tubercle. Lond. 3:337.
3. Jeska, E.L., 1967. Mouse peritoneal exudate cell reactions to parasitic worms. 1 Cell adhesion reactions. Immunology. 16:761 – 771.
4. Keittivuti, B., 1974. Sites of Penetration of Ascaris suum in experimentally infected mice and swine. Dissertation, Purdue Univ., Lafayette, Indiana.
5. Soulsby, E.J.L., 1961a. Immune mechanism in helminth infection. Vet. Rec. 73:1053.
6. Soulsby, E.J.L., 1961b, Immunity to helminths and its effect on heminth infections. Colston Pap. 13:165.
7. Soulsby, E.J.K., 1963. The nature and origin of the functional antigens in the imnune response to nematode infections. Ann. N.Y. Acad. Sci. 113:221 – 229.