Experimental ancylostomiasis in chickens: Effect of various dose of infective *Ancylostoma caninum* larvae on their migration and distribution

R.K. Agarwal and S.M. Agarwal[1]

Department of Bioscience, Ravishankar University, Raipur-492010 (M.P., India), July 29, 1981

Summary. White leghorn chickens, when infected with repeated doses of *Ancylostoma caninum* larvae, expel the larvae at a faster rate than when infected with a single dose. This suggests that the initial dose induces some resistance in the host. An initial dose of 1000 and 2000 larvae, followed by a 2nd dose of the same order, induces resistance in the alimentary tract causing the entire larval burden either to migrate to other tissues (organs) or to be expelled in 24 h.

Agarwal et al.[2] and Agarwal and Johri[3] have studied the migration and distribution of *A. caninum* larvae in chickens infected with single and repeated low doses. The earlier observation shows that the chickens develop immune response at a faster rate thus it serves an ideal experimental animal for the study of immune response due to hook worm infection. Earlier authors, Foster and Cross[4], Nicholas[5], Kono and Sawada[6], and Bhopale and Johri[7] have studied migration and distribution of *A. caninum* larvae in mice and Sasada[8] in chickens when infected with single or repeated doses.

Material and methods. Infective *A. caninum* larvae were cultured and counted according to the methods of Sen et al.[9] and Scott[10] respectively. 1-day-old male white leghorn chickens were divided into 4 groups, A, B, C and D of 50 chickens each, and were infected with single dose of 2000 and 4000 larvae to A and B groups and repeated doses of 1000+1000 and 2000+2000 to C and D groups, respectively. The repeated dose was given 12 h after the initial dose. Chickens from each group were necropsied for larval recovery at 4-h intervals upto 12 h, at 6-h intervals upto 48 h and 72 h. Larvae were collected and counted after the digestion of organs and musculature in artificial gastric juice by Baermann technique.

Results and discussion. Larval recovery from chickens of different groups necropsied at different intervals are shown in the table. Chickens of group D which were inoculated in 2 doses of 2000 larvae each, when compared with those of group B which were inoculated with 4000 larvae in 1 dose show much less larval recovery (hence much greater expulsion) at different intervals and this difference continues to increase in the necropsies 18 h after the 2nd dose. Similarly, larval recovery in chickens of group C, which were given larvae in 2 doses of 1000 each, when compared with chickens of group A, which were given a single dose of 2000 larvae, show much less larval recovery in necropsies at different intervals. However, the degree of difference although very noticeable in this set, was not pronounced as in the set discussed above. Maximum larval expulsion took place in group C (12.05%) and in group D (15.95%) in between 4 and 8 h, in contrast to the earlier observation of Agarwal et al.[2] where the initial repeated low doses of 250 and 500 led to maximum expulsion of larvae between 12 and 18 h and 18 and 24 h, respectively. Vardhani and

Showing percentage of *A. caninum* larvae recovered from singly and repeatedly infected male white-leghorn chickens. Readings are based on mean of recoveries made from 5 chickens

Hours of recovery	Control		Experimental	
	A 2000	B 4000	C 1000+1000	D 2000+2000
4	82.00 (± 10.46)	58.40 (± 6.26)	62.00 (± 3.80) t= 4.015*	55.40 (± 5.59) t= .79834
8	79.00 (± 9.79)	53.80 (± 9.23)	48.20 (± 5.30) t= 8.181*	43.40 (± 6.06) t= 2.105
12	76.20 (± 6.83)	53.20 (± 10.10)	38.20 (± 4.54) t= 10.347*	34.20 (± 3.76) t= 4.666*
18	66.60 (± 6.06)	42.00 (± 5.60)	32.20 (± 4.96) t= 3.031*	26.20 (± 5.40) t= 4.534*
24	50.00 (± 3.80)	51.20 (± 6.53)	29.00 (± 2.60) t= 10.126*	20.60 (± 3.36) t= 9.311*
30	47.60 (± 6.80)	50.40 (± 4.21)	25.20 (± 4.80) t= 5.90*	11.80 (± 2.86) t= 16.926*
36	34.20 (± 2.16)	35.60 (± 3.59)	16.20 (± 5.44) t= 6.869*	7.60 (± 2.31) t= 15.329*
42	22.20 (± 3.06)	25.40 (± 3.64)	11.80 (± 2.86) t= 5.574*	4.60 (± 1.14) t= 12.171*
48	19.00 (± 3.80)	15.40 (± 2.34)	6.00 (± 2.90) t= 6.061*	3.80 (± 0.83) t= 10.589*
72	6.90 (± 0.45)	4.60 (± 1.14)	3.80 (± 0.46) t= 0.7849	2.40 (± 0.54) t= 3.889*

Tabulated value of 't' = 2.306 at 8 degree of freedom and at 5% level of significance. *Significant.

Johri[11,12] stressed that the expulsion of larvae were probably due to increased histamine levels in the intestine. Complete larval migration from alimentary tract occurred at 30 h in group C and within 24 h in group D. Larval migration to heart, lung and muscles commenced within 4 h after the 2nd dose in experimental groups C and D whereas, but not in control groups A and B.

The complete elimination of larval burden from alimentary tract in groups C and D indicates that the initial high dose of 1000 and 2000 larvae have imparted severe resistance due to rapid sensitization which become intolerable for the larvae, resulting in their immobilization, death and ultimately expulsion. Murray et al.[13,14] reported vasoactive amines causing the expulsion of *N. brasiliensis* from infected mice. Other pathophysiological changes in intestine due to infection may also act as an effective barrier.

The experiment suggests that the initial low dose of 250 and 500 larvae took some time to induce sufficient resistance in the host for maximum expulsion and the immune response is much greater in high repeated dose than in low repeated doses.

1 Acknowledgment. We are grateful to the Council of Scientific and Industrial Research, New Delhi for the award of Post-doctoral fellowship to RKA.
2 Agarwal, R.K., Vyas, M., Vardhani, V., and Johri, G.N., Bioresearch *3* (1979) 45.
3 Agarwal, R.K., and Johri, G.N., J. Helminth. *54* (1980) 109.
4 Foster, A.O., and Cross, S.K., Am. J. trop. Med. *14* (1934) 565.
5 Nicholas, R.L., J. Parasit. *42* (1958) 363.
6 Kono, M., and Sawada, T., Kitakanto Igaku *11* (1961) 431.
7 Bhopale, M.K., and Johri, G.N., J. Helminth. *49* (1975) 179.
8 Sasada, T., Keio-Igaku *17* (1937) 491.
9 Sen, H.G., Joshi, U.N., and Seth, D., Trans. R. Soc. trop. Med. Hyg. *59* (1965) 684.
10 Scott, J.A., Am. J. Hyg. *8* (1928) 158.
11 Vardhani, V., and Johri, G.N., Indian J. Parasit. *2* (1978) 55.
12 Vardhani, V., and Johri, G.N., Experientia *34* (1978) 122.
13 Murray, M., Miller, H.R.P., Stanford, J., and Jarrett, W.F.H., Int. Archs Allergy appl. Immun. *40* (1971) 236.
14 Murray, M., Jarrett, W.F.H., and Jennings, F.W., Immunology *21* (1971) 17.

0014-4754/83/080905-02$1.50 + 0.20/0

Transmembrane potential of J774.2 mouse macrophage cells measured by microelectrode and ion distribution methods[1]

D.J. McCaig[2] and R.D. Berlin

Department of Physiology, University of Connecticut Health Center, Farmington (Connecticut 06032, USA), December 30, 1982

Summary. The transmembrane potential (E_m) of J774.2 macrophage cells measured by microelectrodes was -24.1 ± 0.7 mV (mean ± SEM). E_m measured by lipophilic ion distribution was -35 ± 2 mV or -40 ± 2 mV, using a cation or anion, respectively. By any method, colchicine reduced E_m by ~ 3 mV.

Transmembrane potential (E_m) of macrophage cells has been measured by intracellular recording with microelectrodes[3-6], or calculated from lipophilic ion distribution[7], but comparison of these methods in a single cell line has not been made. Therefore, in this study, the E_m of cultured J774.2 mouse macrophages has been determined both by impalement with microelectrodes and by distribution of a lipophilic cation triphenyl methyl phosphonium ($TPMP^+$) or a lipophilic anion, thiocyanate. The effects of the microtubule disrupting agent colchicine have been examined, since this agent alters cell volume and electrolyte composition of J774.2 cells[8] and might therefore modify E_m.

Methods. Cells of the J774.2 mouse macrophage line[9] were grown in Dulbecco's modified Eagle's minimal essential medium, supplemented with 20% horse serum, as previously described[10]. Cells, cultured in monolayer and mounted in 2 ml culture medium (at room temperature or 33–37 °C, containing 10 mM HEPES buffer), were impaled with microelectrodes (3 M KCl-filled, resistance 50–70 MΩ) and voltage signals were recorded using standard techniques.

In order to estimate $TPMP^+$ uptake, cell suspensions ($1-3 \times 10^6$ cells ml^{-1}) were incubated at 37 °C in 5% CO_2 with tritiated $TPMP^+$ (0.1 μCi ml^{-1}, 2.8×10^{-8} moles l^{-1}). Cell volume was estimated in identically treated samples of the cell suspensions from the distribution of tritiated H_2O and ^{14}C inulin. Cpm were measured in cell pellets obtained by spinning samples through silicone fluid. Cellular $TPMP^+$ uptake was calculated and used to estimate E_m from the equation:

$$E_m = -61 \log \frac{(TPMP^+)\ \text{inside}}{(TPMP^+)\ \text{outside}}$$

Thiocyanate distribution was determined in cell suspensions containing 0.1 μCi ml^{-1} ^{14}C KSCN (1.6×10^{-6} moles l^{-1}), using the same protocol.

$(K^+)_{outside}$ was raised in some experiments by adding KCl to the commercially obtained culture medium. Since osmolarity was changed, control experiments were performed in which osmolarity was increased with sucrose, and E_m was found to be unchanged.

Results. The mean E_m, measured by microelectrode impalement, was -24.1 ± 0.7 mV (mean ± SEM, 558 cells from 26 cultures) and the distribution of E_m values is shown in figure 1. The range of E_m-values was large, but more than 75% of cells were in the range -10 to -35 mV. E_m was the same whether measured at 33–37 °C or at room temperature (~ 20 °C). A small number of cells (< 10%) exhibited hyperpolarization during recording (E_m -60 to -75 mV) but, with continuous observation by phase contrast microscopy, it was found that these cells invariably developed surface blebbing, suggesting irreversible cell damage.

E_m in cells incubated with $TPMP^+$ was -35 ± 2 mV (range -27 to -47 mV, 25 experiments). $TPMP^+$ uptake was slow