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Changes in the bacterial cecal flora of mice infected with Trichuris muris (Schrank, 1788)*

by

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Abstract: Cecal microorganisms of mice were categorized and enumerated weekly during the developmental cycle of infection with the whipworm, *Trichuris muris*. The cecal bacterial population consisted of *Escherichia coli, Proteus* spp, *Acinetobacter lwoffi (Mima polymorpha),* aerobic lactobacilli, staphylococci, enterococci, and anaerobes (bacteroides, streptococci, and lactobacilli) in control and *T. muris*-infected mice. The aerobic lactobacilli and the anaerobes constituted the greatest number of organisms in both groups. In week three there was a decrease in the number of these organisms, and in week four fewer of these and of all other organisms in the worm-infected mice when compared to controls. The most significantly reduced bacterial counts were observed during the period of *T. muris* self-cure.

Intestinal nematodes, except under conditions of crowding, inhabit a specific microcosm of the host's gastrointestinal tract. Whipworms of the genus *Trichuris* live in the cecum with their capillary anterior three-fifths interlaced through the mucosa.

The functions of the cecum in the maintenance of the homeostatic balance of the host are several. These include some reabsorption of fluid, the processing of secretions of vitamins and other bacterial metabolites, the production of secretory immunoglobulins, mucus production, and the movement of undigested or unabsorbed residues.

Studies of the bionomics of trichurids have revealed that although they may ingest blood they are not obligate blood-sucking nematodes (**Pike,** 1971; **Lotero** *et al.*, 1974). Their nutrition is considered to be derived from the cecal mucosa but the nature of the nutrients is ill-defined. *T. muris* has been shown to undergo self-cure which is related to the age, number, and length of time spent by the

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worms in the cecum, to the response of the mouse with and without immunosuppressive (cortisone acetate) treatment, and to a thymus-dependent immune response by the host **(Wakelin** and **Selby,** 1974a, 1974b).

The interactions of the bacterial cecal flora and *Trichuris* as they may relate to the nutrition of the worm and to the immune response of the host have not been examined.

The purpose of the present study was to observe the changes in the bacterial cecal flora of *T. muris*-infected (TMI) mice from the time of infection through patency.

MATERIAL AND METHODS

Strain and inoculation: The strain of *T. muris,* preparation of egg cultures and method of inoculation were as previously described **(Pike,** 1969). Inoculated mice each received 300 eggs.

Mice and housing: One hundred and forty male weanling DBA/2J mice (Jackson Laboratory, Bar Harbor, Maine) were assigned to cages using a table of random sampling numbers. The stainless steel cages containing wood shavings and five mice each were randomized as to treatment, 8 cages of control and 20 of inoculated mice. Purina laboratory chow and tap water were provided *ad lib.*

Cecal cultures: Cultures of the cecal contents were obtained weekly for seven weeks. Each week 5 control and 15 inoculated mice were used except at week zero when 10 were tested. The abdominal wall was opened under ether anesthesia. The cecum was tied off from the remainder of the large intestine and from the ileum and injected with 0.2 ml of sterile 0.85% saline. It was gently massaged for 30 seconds, 0.1 ml of the contents were then withdrawn and placed in 9.9 ml of brain-heart infusion broth (BHIB) (Difco).

Bacteriological techniques: The suspensions of the initial specimens were agitated on a mechanical shaker and serial 10-fold dilutions prepared in BHIB. Calibrated loopfuls of each dilution were spread on the surface of various selective media as described by **Schaedler** and **Dubos** (1962); **Schaedler** *et al.* (1965). In addition to the selective media outlined by the above authors, brain-heart infusion agar (Difco) and Mycosel (BBL) were used. Tubes of the latter medium were incubated both at 37 C and 22 C and retained for six weeks. All of the other cultures were incubated at 37 C, either aerobically for 24-48 hours or anaerobically (GasPak, BBL) for one week. Following incubation the number of colonies obtained per 1.0 ml of cecal contents were calculated from those obtained per loopful of the appropriate dilution.

Worm burdens: Following procurement of the cecal specimen used for the microbiological studies, the cecum and large intestine were opened and placed in 0.85% saline. During the early larval stages (weeks one through three) the entire intestine was cut into 1 cm lengths. Squash preparations of each length were made and scanned under the 3.5X objective of the compound microscope. The number of larvae present in each length and in the saline wash were enumerated. During weeks four, five, and six longer lengths of the intestine and the dissecting microscope were used and worms counted. A 1 cm length of the ileum was also examined but at no time were larvae or adults of *T. muris* found.

Statistical analysis: The Wilcoxon rank sum test **(Wilcoxon,** 1945) was used to test the statistical significance of differences between control and infected mice with respect to bacterial counts. The Wilcoxon test is a nonparametric technique which makes no distributional assumptions about the bacterial counts.

RESULTS

Larvae or adult worms were found at autopsy in each of the mice given *T. muris* eggs (Table 1). Three mice with heavy infections (over 200 larvae) died during week three. Evidence of self-cure occurred at weeks four and five when 7 out of 15 mice in week four, and 13 out of 15 in week five harbored 10 or less worms. Such low worm counts were not present in any of the mice during weeks one, two, and three and in only 3 out of 15 in week six.

The median and range of the colony counts per 1 ml of cecal suspension and the types of bacteria obtained each week are shown in Table 1. The aerobic lactobacilli and the anaerobic organisms, (bacteroides, streptococci, and lactobacilli) contributed the most colonies in both the control and TMI mice. During weeks three and four there were fewer of these colonies obtained from the TMI mice. During week three the difference was significantly lower ($P < 0.05$) than that obtained from control mice. A decrease in colony number obtained from the TMI mice was also observed with *Acinetobacter lwoffi (Mima polymorpha)* where the reduction was significant during weeks two, three, four and six. (In week five several cultures exhibited little or no growth; the figures were therefore less meaningful). Some significantly reduced counts of *Escherichia coli, Proteus* spp and staphylococci were also obtained from the TMI mice during weeks two, three or four.

All of the other bacterial counts regardless of levels showed considerable interweek variation and although there was an occasional significant rise (e.g. enterococci during weeks two, three, and six) or fall (e.g. aerobic lactobacilli week six) the trend was not consistent.

A species of *Candida* was isolated from two control mice during week six; fungal growth was absent from all other mycosel cultures.

DISCUSSION

This study revealed there was a reduction in the number of cecal bacteria of all types by week four of *T. muris* prepatency when compared to controls. Four of the seven groups of observations of that week were statistically significant ($P < 0.05$). In week three *E. coli, A. lwoffi,* aerobic lactobacilli, and anaerobic organisms also showed statistically significant reduced counts ($P < 0.05$). It was noted during procurement of the cecal suspensions that those from the TMI mice were visibly less turbid than were those from controls. This suggested a reduced amount of mucus or bacteria present. Specimens were taken and dilutions and platings were done under aerobic conditions. Subsequent studies have shown that adherence to strict anaerobiasis during procurement and handling of such specimens and media was essential for the survival and growth of the various fastidious anaerobic fusiform-tapered rods which predominate in the mucin layer of the cecum **(Gordon** and **Dubos,** 1971; **Lee,** *et al.,* 1971; **Savage** *et al.,* 1971).

In primary infections of *T. muris* self-cure occurs in 70-75% of mice during week three **(Wakelin,** 1967). The immunogens elaborated by *T. muris* may reach

TABLE 1

Worm[1] and bacterial[1] counts from the cecum of T. muris *infected and control mice*

		Week						
		0	1	2	3	4	5	6
Worms	I	...	43	70	126	11	2	82
		...	(19-93)	(35-150)	(23-201)	(0-140)	(0-184)	(1-245)
E. coli	C	0	18.2	0	2.3	20.0	10.6	1.0
		(0-5.1)	(0.2-29.1)	(0-2.2)	(1.4-5.1)	(0-120.0)	(0-15.6)	(0-2.7)
	I	...	5.3	1.5	0	0	0.2	9.2
		...	(0.4-44.0)	(0-17.5)	(0-64.0)**	(0-46.0)*	(0-80.0)	(0-80.0)
Proteus spp	C	0.4	0	0	0.3	3.0	0	0.2
		(0-8.9)	(0-0)	(0-11.5)	(0.1-0.4)	(0-186.0)	(0-0)	(0-1.2)
	I	...	0	0	1.9	0.1	0	0
		...	(0-0)	(0-3.6)*	(0-93.1)	(0-19.8)*	(0-1.0)	(0-42.0)
A. lwoffi (M. polymorpha)	C	0	18.2	8.2	18.7	1.7	0	0.4
		(0-0.5)	(0.2-29.1)	(3.8-26.6)	(3.9-43.1)	(0-3.6)	(0-0)	(0.2-1.4)
	I	...	2.0	5.9	1.2	0	0	0
		...	(0.4-9.0)	(0.1-45.7)*	(0-80.0)**	(0-0.2)**	(0-2.0)	(0-100.0)**

Aerobic Lactobacilli	C	192.0	119.0	170.0	370.0	270.0	330.0	300.0
		(8.0-558.0)	(20.0-344.0)	(60.0-280.0)	(170.0-970.0)	(113.0-457.0)	(280.0-370.0)	(260.0-430.0)
	I	...	205.0	310.0	189.0	210.0	337.0	196.0
		...	(80.0-507.0)	(66.0-670.0)	(2.0-830.0)*	(27.0-384.0)	(100.0-900.0)	(28.6-980.0)*
Anaerobic bacteria	C	119.0	59.0	100.0	210.0	90.0	20.0	50.0
		(5.0-472.0)	(3.0-110.0)	(40.0-279.0)	(160.0-372.0)	(4.5-190.0)	(10.0-60.0)	(5.0-160.0)
	I	...	100.0	110.0	101.5	66.0	100.0	27.8
		...	(0.3-408.0)	(29.0-950.0)	(25.0-310.0)**	(0-320.0)	(8.0-460.0)*	(0-390.0)
Staphylococci	C	0.2	3.4	2.5	0.2	10.0	0.8	0.8
		(0-8.1)	(2.7-13.3)	(0.7-2.7)	(0.1-7.9)	(0.6-33.2)	(0.4-2.4)	(0.1-1.3)
	I	...	3.9	1.7	0.9	0.2	0.4	0
		...	(0.3-78.0)	(0-10.3)	(0-40.1)	(0-10.0)**	(0-28.8)	(0-0.8)**
Enterococci	C	3.2	0	3.2	3.6	4.2	0.6	1.0
		(0-25.6)	(0-10.7)	(0.8-4.3)	(0-17.0)	(0.2-47.0)	(0-14.3)	(0-1.5)
	I	...	0	5.0	8.3	1.5	2.8	27.8
		...	(0-80.0)	(2.0-56.9)*	(0-97.0)*	(0-180.0)	(0.4-26.0)	(0-390.0)**

1 Worm counts =actual number, bacterial counts =number/of colonies (1 =$1x10^4$), median followed by range.
C Control mice N =10 week 0, N =5 weeks 1 through 6.
I Infected mice N =15 (week 3, deaths 3 N =12).
* $P < 0.05$
** $P < 0.01$

the tissues and blood stream through the lesion created by the mouth stylet of the worm as it feeds and moves within the epithelium of the cecal mucosa. The epithelial cells become damaged, there is increased cellularity and a rise in globule leucocytes (**Drum,** 1966). Blood leakage occurs in heavy infections (**Pike,** 1971). The immunogens elaborated by *E. coli, Proteus* spp, lactobacilli and enterococci (**Foo** and **Lee,** 1971) may also enter the tissues at this time via the worm lesions.

The observed reduction in bacterial counts during weeks three and four may thus reflect a response to individual bacterial immunogens or to possible cross-reacting bacterial and worm immunogens or to a state of synergy. Mice lightly infected with schistosome worms demonstrate a synergistic increase in resistance towards unrelated species of superinfecting microorganisms (**Collins,** *et al.*, 1972).

In naturally unresponsive or tolerant mice *T. muris* larvae are not eliminated and grow to maturity (**Wakelin,** 1967). In the present study 10 of the 30 mice remaining at weeks five and six were unresponsive. Worm counts of 80 or more were observed in those weeks. In addition reduced bacterial counts were much less than in weeks three and four suggesting insufficient immunogenic stimulation either specific or cross-reacting. The lowered bacterial counts of the two previous weeks and the presence of adult worms known to be less immunogenic than larvae (**Wakelin,** 1967) may have been the reason.

Alternatively it could be postulated that the lower counts may have resulted from the secretions of a bactericidal or bacteriostatic metabolite or larvae and not of adult *T. muris.* This explanation is less plausible, because the ability of nematodes to carry out such functions has not been demonstrated.

Just how *T. muris* embeds itself into the cecal mucosa remains unclear. **Nimmo-Smith** and **Keeling** (1960) studying the hydrolytic enzymes of *T. muris* were unable to detect "enzymes which could definitely be implicated in penetration of the host's intestinal mucosa". The ability of enteric microorganisms to degrade intestinal mucins, particularly their carbohydrate moieties, has been established (**Hoskins** and **Zamcheck,** 1968). The resultant moieties may be a source of nutrition either to other microorganisms, the worms, or both. Additionally, this degradative action of the bacteria may facilitate penetration of the mucosa by the worms. A reduction in the number of enteric organisms could thus place the worm at a disadvantage.

The cecal cycle of *T. muris* influences and is influenced by the presence of other cecal-inhabiting nematodes or protozoa, and blood and tissue protozoa. Mice carrying natural or experimental infections of mouse oxyurids exhibited a low susceptibility to infection with *T. muris,* a critical factor being the chronological order of infection (**Keeling,** 1961). Mice infected with *T. muris* and then challenged with *Entamoeba histolytica* showed a higher rate of amebic infection and were more susceptible to amebic tissue invasion than were controls (**Knight** and **Chew,** 1974). Earlier, **Phillips** and **Gorstein** (1966) showed the participation of bacteria in the etiology of amebic enteritis in guinea pigs. **Ross** and **Knight** (1973) however did not find a relationship between amebic infection and bacterial flora or the rat but the role of anaerobes was not considered in their study. The effect of *Plasmodium berghei* and *Trypanosoma brucei* infections on the immune expulsion of *T. muris* depended upon the experience of the mice with *T. muris* immunogens, and to the chronological order of infection (**Phillips** *et al.*, 1974). Both acute malaria and trypanosome infections initiated at the same time as the *T. muris* infection suppressed worm expulsion.

Selective adherence by certain microorganisms to the cecal epithelium **(Savage,** 1972; **Fubara** and **Freter,** 1973) may be altered by *T. muris* thereby affecting the physiology of the mucosal surface. The synergism between the effects of local immunity and bacterial antagonism **(Shedlofsky** and **Freter,** 1974) may be changed. Lastly, a stressful environment for the mucosal microorganisms such as competition for available nutrients **(Tannock** and **Savage,** 1974) may exist. Bacterial survival could thus be influenced particularly during the developmental larval stages of the worm.

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RESUMEN

Los microorganismos cecales de ratones infectados con *Trichuris muris* fueron examinados y contados cada semana durante su ciclo de desarrollo. La población de bacterias cecales fue de *Escherichia coli, Proteus* spp., *Acinetobacter lwoffi (Mima polymorpha),* lactobacilos aeróbicos, stafilococos, enterobacterias y anerobios (bacteroides, stafilococos y lactobacilos) tanto en ratones infectados con *T. muris* como en los testigos. En ambos grupos los organismos más numerosos fueron los lactobacilos aeróbicos y los anerobios. Durante la tercera semana hubo una disminución en el número de estos organismos, y durante la cuarta semana hubo menos de éstos y de los demás organismos en los ratones infectados que en los testigos. Los recuentos bacterianos más reducidos se observaron durante el período de auto cura de *T. muris.*

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