Microphallus pygmaeus: Effect of Long-acting ACTH Preparation on Establishment and Retention in Alimentary Canal of the Mouse

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The metacercariae of Microphallus pygmaeus (Levinsen 1881) form A (sensu James 1968a) develop in sporocysts within the hemocoel of the digestive gland and gonad of spent adults of the littoral prosobranch, Littorina saxatilis tenebrosa (Mont.) sensu James 1968b. In nature, the metacercariae develop into adults in the intestine of the Herring gull, Larus argentatus but James (1971) has shown that the laboratory mouse is an almost equally suitable host. In experimental infections in the Herring gull and in mice (James 1971), the sporocysts burst open in the host's stomach and although most metacercariae pass to the duodenum and develop into adults, some are digested. Those which survive become lodged between the villi of the duodenum and are very lightly attached by the oral and ventral suckers. Most mature into adults within 4 hr of infection in the duodenum and ileum and later migrate down the gut. Eight hours after infection 90–95% of the adult worms have already been voided with the feces, but some (2–10%) remain within the intestine for up to 9 days, the number of eggs produced by the retained worms progressively increasing. Previous studies (Hameed 1971) have shown that identical infections may result in a considerable variation in the number of worms initially retained and which develop in the intestine. Factors such as season, age of host, and the number of previous infections have been shown to be partly responsible for this variation by Hameed (1971).

The aim of the present study was to investigate the effect of an additional, not easily controlled, factor namely "stress" which might influence initial survival and subsequent intensity of infection, by Microphallus pygmaeus, of the laboratory mouse.
It is well known that variation in housing has many pronounced endocrinological and behavioral effects on rodents (Brain 1971). For example, an increase in group size increases the "stress" generated, as indicated by the elevation of adrenocortical activity. It is believed that the resulting increased titers of circulating glucocorticoids may have a pronounced immunosuppressive activity, greatly increasing the animal’s susceptibility to disease (Ader 1967). These compounds have been shown to affect hypersensitive responses to cestode proteins (Ahmed et al. 1970) and to have pronounced effects on nematode infections (Collette 1962; Campbell 1963; Wakelin 1967, 1970a and b; Harley and Gallichio 1970). The significance of cortisone treatment on immunity to digenean parasites has been clearly demonstrated (Halawani et al. 1969; Sinclair 1968a and b, 1970). It was decided to see whether the injection of adrenocorticotrophic hormone (ACTH), which is the pituitary factor responsible for the "stress" response, could influence the establishment and retention of a well-documented parasite, whose relations to the host tissues are less intimate than in the case of cestode and nematode parasites.

MATERIALS AND METHODS

Parasites

The collections of parasitized host winkles were made at Twr Gwlanod which is to the south of Aberystwyth, at the site of a gull colony. On returning to the laboratory the winkles were washed in filtered seawater and maintained for a short time at 4 C until required for use.

Animals

The mice used were outbred male, Swiss albino laboratory animals, aged 6 wk at the start of the experiment. They were kept under conditions of regular light cycling and at a temperature of 18–21 C. They were housed initially in groups of six in opaque plastic cages measuring 30 x 12 x 11 cm with wire tops, food and water being freely available, and the sterile bedding being replaced every 4 days.

Injection Schedule

Thirty-two animals were injected intramuscularly (im) on alternate days over the 22-day experimental period with 0.1 ml of a long-acting ACTH preparation (Cortrophin/Zn, Organon Laboratories, Ltd.), this volume being equivalent to 4 International Units (IU) of ACTH. Another 32 mice were injected at the same time with a placebo solution made up to the manufacturer’s specifications containing 2 mg/ml colloidal zinc hydroxide at a pH of 8.

Infection Procedure

Fully formed sporocysts, each containing 80 ± 5 metacercariae were dissected from the molluscan host, washed in a dish of artificial seawater, and 10 sporocysts were randomly selected and fed by stomach tube to each of the 64 recipient mice.

Parasitological Investigations

Eight mice (four experimental and four placebo injected) were killed at hourly intervals after infection and dissected. A period of 1–8 hr after introduction of the parasite was chosen because previous experiments have shown that most worms had matured during this time. The alimentary canal was removed in toto and cut into portions, namely, stomach, duodenum, ileum, cecum, colon, and rectum, and placed in physiological saline at 37 C.

The number of worms in each portion of gut was then counted. Fecal pellets voided during the killing of the mouse and pellets remaining in the rectum were also examined for worms.

RESULTS

The graph (Fig. 1) of total parasites recovered against time shows that although over the entire experimental period, the ACTH-treated mice have greater numbers
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of living parasites than placebo-injected mice the pattern in the placebo group is identical to that observed in untreated mice. The significant ($P < 0.001$) decline in worm burden over the 8-hr study period in both ACTH and placebo-treated mice was shown by regression analysis to follow virtually parallel slopes. These were $b = 0.0153$ and $b = 0.0151$, respectively. Thus the major effect of ACTH treatment appears to be to increase the number of worms surviving in the gut during the first 1-hr period of infection. This observation is confirmed by the fact (see Fig. 2) that the number of parasites voided in the feces of the ACTH category is initially lower than in the placebo-treated category. Fully formed adult parasites bearing 10–20 eggs were first found 2 hr after initial infection in the ACTH treated mice, but only after 4 hr in the placebo-treated controls.

In addition, the histogram (Fig. 3) indicates that in the first 4 hr of infection the effect of ACTH is to promote the retention of worms in the preferred anterior region of the gut. Thus parasites recovered from the duodenum were significantly higher in the ACTH-treated mice ($t = 11.59, P < 0.001$). Similarly, greater numbers were recovered from the ileum than in the controls ($t = 9.02, P < 0.001$). More posteriorly, however, the situation is reversed, the placebo-treated mice having more parasites than the ACTH-treated mice. The relevant
Fig. 3. Histogram showing the mean total number of parasites recovered from each region of the gut over the first 4 hr of the observation period in both ACTH and placebo-treated categories. Each mean is for 16 animals and standard deviations are indicated.

$t$ and $P$ values are, cecum ($2.51, P < 0.02$), $P < 0.001$). Examination of fecal pellets colon ($4.28, P < 0.001$), and rectum ($6.13$, showed higher numbers of worms voided.

Fig. 4. Histogram showing the mean total number of parasites recovered from each region of the gut over the last 4 hr of the observation period in both ACTH and placebo-treated categories. Each mean is for 16 animals and standard deviations are indicated.
from placebo-injected animals than from ACTH-treated mice ($t = 9.32, P < 0.001$).

Over the final 4-hr period the number of worms were consistently higher in all gut regions in the ACTH-treated mice (Fig. 4). The relevant $t$ and $P$ values are, duodenum (4.64, $P < 0.001$), ileum (4.59, $P < 0.001$), cecum (4.54, $P < 0.001$), colon (2.33, $P < 0.05$), and rectum (1.90 n.s.). The number of parasites in the fecal pellets were not significantly different in the two categories.

**DISCUSSION**

The most striking effect of ACTH is the marked increase in the number of parasites present in the preferred regions of the gut, namely the duodenum and the ileum, during the first hour of infection. This initial difference is maintained as the rate of decline in parasite numbers is the same in ACTH and placebo-treated animals during the subsequent 7-hr period. Thus it is possible that ACTH influences the viability of the metacercariae in the stomach of the host resulting in greater numbers reaching the duodenum. Alternatively, ACTH treatment may induce a more favorable environment in the host duodenum and ileum, thus enabling more parasites to be maintained in these clearly preferred regions of the gut. The more rapid development to sexual maturity of the parasites in ACTH-treated mice may also reflect the longer periods of time spent by the parasites in these preferred gut regions.

The exact mechanism of the action of ACTH on susceptibility to infection by these parasites is interesting in view of the fact that the parasite is only lightly attached to the gut villi, and careful histochemical examination reveals that there are no obvious differences between parasitized and healthy gut mucosa. It is difficult to see how the immunosuppressive action of the adrenals could have effect as no known immune response appears within 8 hr and corticosterone, the major glucocorticoid produced by the mouse, does not have a marked immunosuppressive action. Steroidal effects have been demonstrated, by the administration of cortisone, to alter the immune response of the intestinal wall (Murray et al. 1971) as have castration and ovariectomy (Waddel et al. 1971). The possible effect on the action of gut cell mediated antibody by the adrenal glucocorticoids is worth further investigation.

It has been previously demonstrated that many environmental effects can strongly influence the activity of the pituitary–adrenocortical axis in laboratory rodents. “Stress” hormones are stimulated by such factors as crowding, the incidence of fighting behavior (especially important in male rats and mice), noise and handling (see review by Brain 1972). It follows from the experiment described in this paper that such factors inducing endogenous changes in glucocorticoids could have a strong influence on the number of parasites retained in the gut of laboratory mice. Thus, these factors should be rigidly controlled in parasitological studies otherwise many differences in infestation may be due to some noncontrolled environmental variation, such as group size or handling—rather than a change in the infectiveness of the parasite.

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