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THE IMMUNIZING POTENTIAL OF SPORULATED OOCYSTS OF *EIMERIA NIESCHULZI* EXPOSED TO HEAT AND CO-60 GAMMA-RADIATION*

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ABSTRACT: Sporulated oocysts of *Eimeria nieschulzi* Dieben 1924, a rat coccidium, were exposed to radiation, heat, or both in an effort to attenuate the parasite. Moderate levels of each treatment or combination thereof attenuated the parasite, reduced pathogenesis (as judged by oocyst discharge during primary infection), and produced immunity to challenge when the oocysts were subsequently inoculated into rats. Thus, heat- and/or radiation-treated *E. nieschulzi* oocysts fed to rats could reduce pathogenesis during a primary infection and yet give good homologous protection.

Many researchers (Albanese and Smetana, 1937; Honess, 1939; Waxler, 1941; Uricchio, 1953; Fitzgerald, 1965, 1968; Baldelli et al., 1966; Grosjean, 1969; Rose and Long, 1970; Abu Ali et al., 1972; Klimes et al., 1972; Singh and Gill, 1975; Wright, 1976) have examined the effects of radiation on coccidian species. There remain, however, a number of questions to be answered. One obvious point of uncertainty is the possibility of using a radiation-attenuated coccidian as an immunizing agent. Fitzgerald (1968) felt that a certain portion of γ-irradiated oocysts were rendered nonviable, and that the immunity produced could be effectively duplicated using smaller numbers of nonirradiated oocysts in the inoculum. Some workers (Albanese and Smetana, 1937; Waxler, 1941; Hein, 1963; Klimes et al., 1972) concluded that a simple reduction in the number of viable oocysts could not explain their results, and that the oocysts, in fact, had been attenuated by radiation. Baldelli and his co-workers (1966) stated that γ-irradiated oocysts could be of prophylactic value for coccidiosis of fowl. Little attention, however, was given to dose rate or other variables in those studies. The objective of the present study was to test a constant dose rate in conjunction with a second variable (heat) in an attempt to determine the feasibility of attenuating *Eimeria nieschulzi*, an intestinal coccidian of the common laboratory rat.

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MATERIALS AND METHODS

The *E. nieschulzi* isolate and inbred albino rats (SPF, Fischer 344 strain, female) were obtained and maintained for this study as outlined by Duszynski (1972). Sporulated oocysts were treated in identical, narrow screw top tubes containing a constant volume of 2.5% potassium dichromate ($K_2Cr_2O_7$). Treatment was of three types: radiation (15, 30, or 60 k-rads), temperature (35, 40, or 45 C), and temperature and radiation concurrently (15, 30, and 60 k-rads at 35 C, and 15 k-rads at 40 C). Irradiation was done by exposing the sporulated oocysts to CO-60 γ-radiation for 1 hr using the Gamma Irradiation Facility at Sandia Corporation, Albuquerque, New Mexico. All dosimetry was done by Dr. J. Pat Brannon of the Biosystems Research Unit at Sandia. Exposure of the sporulated oocysts to the desired temperatures (±0.5 C) was accomplished using a water bath monitored by thermograph for 1 hr.

Rats were infected *per os* with sporulated oocysts following light ether anesthetization. Each rat was given 5,000 treated, sporulated oocysts for 5 consecutive days (25,000 total) as a primary inoculation. Challenge inoculations were given on day 27 postinoculation (PI) and consisted of 5,000 untreated, sporulated oocysts.

Each rat was housed individually, and fecal samples were collected and processed at 24-hr intervals as described by Duszynski (1972). Fecal collections were made from days 7 to 16 PI, after challenge inoculations on days 34 to 39 PI (of original inoculation), and at least once between day 16 PI and challenge inoculation. The number of occysts discharged by each rat in a 24-hr interval was determined as follows. An appropriate aliquot, determined by preliminary examination of one sample from each treatment group, was taken from the total volume of $K_2Cr_2O_7$-fecal material sample. Each aliquot was put into a 15-ml centrifuge tube and then concentrated by flota-

TABLE I. *Total mean numbers of oocysts discharged after primary and challenge inoculations with heat-, radiation-, or both heat- and radiation-treated and untreated* Eimeria nieschulzi *oocysts.*

Groups	(*N*)	Oocyst treatments (k-rads/ temp C)	Oocyst discharged (in millions)	
			Primary inoculations*	Challenge inoculations†
Control	(18)	–/RT‡	111.6	0
I	(6)	–/35	89.6‖	0
II	(6)	–/40	74.6	0
III	(6)	–/45	21.1	0
IV	(6)	15/RRT§	2.5	0.7
V	(6)	30/RRT	0.4	22.0
VI	(12)	60/RRT	0.1	27.7
VII	(6)	15/35	2.7	9.4
VIII	(6)	30/35	0	46.8‖
IX	(6)	60/35	0	48.3‖
X	(6)	15/40	0	29.7
Challenge control	(12)	–/RT	–	67.8

* 5,000 sporulated, treated oocysts *per os* for 5 consecutive days; discharged oocysts collected days 7 to 15 PI.

† 5,000 sporulated, normal oocysts *per os* on day 27 after first primary inoculation; discharged oocysts collected days 7 to 12 postchallenge.

‡ RT = room temperature (19 to 21 C).

§ RRT = reactor room temperature (~ 28 C).

‖ $P < 0.05$; all other values at least $p < 0.05$ when compared to controls for primary inoculations and challenge controls for challenge infections.

tion in Sheather's sugar solution. The number of oocysts/aliquot was determined by microscopic examination. The assessment of the effects of radiation and/or heat was determined by comparing the results from rats infected with treated oocysts to those obtained from rats infected with untreated oocysts. Oocyst discharge in the treated and untreated groups was examined for significant differences at the α 0.05 level using single classification analysis of variance tests for both equal and unequal sample sizes (Sokal and Rohlf, 1969).

RESULTS

All treatment groups (Table I) except Group I showed a statistically significant decrease in oocysts discharged during primary infection when compared to the control group. Radiation lowered oocyst production more markedly than did heat, while combination treatments had an additive effect in lowering oocyst production at all but the smallest radiation and heat level (Group VII). Neither radiation nor heat treatments (Group I–VI) used were sufficient to halt oocyst production entirely, although Groups VIII and IX combining both radiation and heat treatments did halt oocyst production completely (Group X produced oocysts in small numbers during primary infection). Only Group VII (mixed treatment) did not show a significant decrease in oocyst production when compared to its single treatment counterpart (Group IV). This is not surprising since 35 C (Group I) does not adversely affect oocyst production. It should also be noted that although the room temperature and reactor room temperature differed, both were still well below the 35 C temperature of Group I which was not significantly different from the control.

Oocyst production during challenge infections correlated inversely with oocyst production during primary infections (Table I). If oocysts were produced during primary infection, then few or no oocysts were produced on challenge. This is obvious in the control and temperature-treated Groups (I–III) which produced high numbers of oocysts during primary infection and no oocysts on challenge. It is also apparent in the radiation treatment Groups (IV–VI) and 15 k-rad Groups VII and X which produced some oocysts during primary infection but had a significant decrease in oocyst production on challenge when compared to challenge controls. Conversely, Groups VIII and IX which produced no oocysts during primary infection were not significantly different in oocyst production during challenge infection from the challenge controls.

DISCUSSION

A number of reports suggest that sporozoites can be attenuated by radiation, i.e., they are not killed and are capable of invading tissue following exposure to mild levels of irradiation. Klimes et al. (1972) exposed sporulated oocysts of *E. tenella* to γ-radiation and found that the sporozoites were not killed and were able to excyst and invade chicken kidney cells in culture, though further development was impaired. Similar results were observed for *E. tenella* by Sokolič et al. (1973). Studies done in our laboratory showed that most *E. nieschulzi* sporozoites are not killed by radiation and/or heat (up to 60 k-rads at 35 C, i.e., treatments corresponding to Groups I–IX in Table I) and that ~ 20% of treated sporozoites are capable of excystation (Conder and Duszynski, 1976).

Other studies, using *E. tenella*, have shown that the parasite is capable of completing its life cycle when exposed to moderate γ-radiation levels (Hein, 1963; Baldelli et al., 1966).

Several researchers (Hein, 1963; Baldelli et al., 1966; Uricchio, 1953) feel that sporozoites attenuated by radiation or heat can be used to produce immunity against further coccidial infection without the normal level of pathogenesis taking place during primary infection. Baldelli et al. (1966) found that a patent infection was necessary to produce immunity to *E. tenella*, but that radiation levels of 20 to 30 k-rads would annul the pathogenicity, i.e., reduce the number of oocysts discharged by the host, while not significantly altering the parasite's developmental cycle. Hein (1963) was able to produce immunity using irradiated *E. tenella* sporozoites even though the parasite did not successfully complete second generation schizogony. Uricchio (1953) showed that high (45 C) and low (−5 C) temperatures diminished pathogenicity in *E. tenella* infections even though patency occurred and immunity developed. In contrast, Fitzgerald (1968) using γ-irradiated oocysts was only able to obtain immunity to *Eimeria bovis* in cattle when animals infected with treated oocysts produced oocysts at a level similar to controls infected with untreated oocysts. Further, the groups which demonstrated immunity displayed no noticeable reduction in pathogenesis during primary infection. Thus, he concluded that the use of a large number of irradiated oocysts had no advantage over a smaller number of non-irradiated oocysts in causing immunizing infections.

In rats, successful endogenous development following primary infection as evidenced by oocyst discharge during patency produced good immunity (Table I). However, significantly fewer oocysts were discharged after primary infection in treatment Groups II and III and complete immunity was still effected as determined by the lack of oocyst production on challenge infection (Table I). Whenever oocysts were discharged following a primary inoculation, regardless of the number of oocysts produced, at least some level of immunity to challenge could be seen. Thus successful endogenous development is necessary to develop immunity to *E. nieschulzi* in rats. However, without further information on what stage actually elicits the immune response, we must not dismiss the possibility that those individual sporozoites, which did not result in oocyst production, may also have contributed to producing the immune response. As shown in Table I, Groups I–IX, where the number of viable sporozoites is relatively constant (~ 20% based on ability to excyst), oocyst production following primary inoculation decreases greatly with increasing treatment levels. This would imply that attenuation of the parasite has indeed occurred, i.e., a given number of viable sporozoites produce oocysts in numbers inversely proportional to the level of treatment with radiation and/or heat. The endogenous stages prior to oocyst production, primarily schizonts, have been shown to produce immunity (Horton-Smith, 1947, 1949; Kendall and McCullough, 1952), and although irradiated sporozoites are not fully functional, they do excyst (Conder and Duszynski, 1976), maintain invasive properties (Klimes et al., 1972; Sokolič et al., 1973), and undergo at least some development. Therefore, we think that radiation- and/or heat-attenuated oocysts can be of immunizing value. Further, the stage of the coccidian life cycle which actually induces immunity and the level of infection necessary for effective immunity to develop should be considered in any future studies of this nature.

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