Reinitiation of merogonous development by sporozoites released from autoinfective oocysts and recycling of type I meronts (*10, 13, 14*) are features of the life cycle of *Cryptosporidium* that may explain why a small number of oocysts are capable of producing overwhelming infections in succeptible animals and man (*8*). These features may also explain why immunodeficient individuals may develop persistent, life-threatening intestinal infections in the absence of repeated oral exposure to oocysts (*3*), and why very heavy *Cryptosporidium* infestations can develop in the respiratory (*6*) and biliary (*7*) tracts of such patients.

In HFL cells, it appears that sporozoites develop into mature type I meronts within 16 hours (Table 1). The presence of type I meronts through 96 hours after inoculation is most likely due to a recycling of one or both asexual stages since sporozoites were not seen beyond 8 hours and no apparent autoinfective cycle was observed. Recycling of type I meronts has been reported in experimentally infected mice (*3, 13, 14*) and chicken embryos (*10*).

Since human and calf isolates of *Cryptosporidium* exhibit little or no host specificity (*1, 3*), it was not surprising that PK-10 and PCK cells also supported complete development (from sporozoite to sporulated oocysts) of the human isolate. However, the number of parasites developing in these two cell types was smaller than those in HFL cell cultures.

We are aware of only one other coccidian species, *Eimeria tenella*, which has been reported to complete its entire developmental cycle (from sporozoites to unsporulated oocyst) in cell culture [see (*15*) for review]. Unlike *E. tenella*, however, oocysts of *Cryptosporidium* complete sporogonous development within the host cells grown in vitro. Not only were the in vitro derived oocysts of *Cryptosporidium* sporulated by morphologic criteria, but they were also infective to mice.

Cryptosporidiosis is most ominous in its effects on morbidity and its contributions to mortality in patients with AIDS (*16*). No effective therapy is available to eradicate this agent once it becomes established in an immunodeficient individual (*5*). The growth of *Cryptosporidium* in cell culture not only provides a means of studying its behavior, development, and metabolism but it also provides a mechanism for rapid evaluation of potentially useful therapeutic agents.

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17. Some of these findings were presented at the Fourth International Symposium on Neonatal Diarrhea (*14*). Supported in part by cooperative agreement No. ARS-587B30-3-482 between the U.S. Department of Agriculture and the Alabama Agricultural Experiment Station.
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Suppression of Prolactin in Pigs by *Escherichia coli* Endotoxin

Abstract. *An endotoxin produced by Escherichia coli caused a decrease in prolactin concentrations in the plasma of sows when given at low dosages 2 days postpartum. Five to tenfold increases occurred in the plasma cortisol concentrations. Piglet growth, used as an indicator of milk secretion by the sows, was significantly depressed after the endotoxin administration. Some cases of lactation failure in the periparturient sow may thus be due to endotoxins suppressing prolactin concentrations. This appears to be the first report of a bacterial endotoxin having an effect on prolactin in any species.*

Insufficient milk production by sows and the resultant malnourishment of piglets may be directly responsible for between 6 and 17 percent of all preweaning deaths in commercial pig production facilities and represents a multimillion dollar annual loss to the American pork industry (*1, 2*). Malnourishment also contributes to the neonatal susceptibility of piglets to transmissible gastroenteritis, *Escherichia coli* enteritis, and crushing by the sow, the primary causes of death in the neonatal pig (*2*). Sows with insufficient milk frequently show one or more of the following clinical signs: pyrexia, anorexia, leukopenia, lethargy, mastitis, metritis (infrequently), and blanching of the mammary glands (*3*).

Sows with agalactia frequently have clinical signs of mastitis, which in many instances is due to Gram-negative bacteria, including *E. coli* (*4*). When placed in the mammary gland or uterus of postpartum sows, *E. coli* endotoxin is readily absorbed and can be assayed in the blood (*5*). Prolactin concentrations in the plasma of agalactic animals is reduced (*6*), and treatment of sows with *E. coli* endotoxin produces signs typical of natural cases of hypogalactia (*7*).

For the experiments described here we used normal sows of Yorkshire/Lan

Table 1. Changes in mean piglet weight (grams per hour per piglet) for days 1 to 3 postpartum divided into three intervals per day (2400 to 0800 hours, 0800 to 1600 hours, and 1600 to 2400 hours). Analysis of variance for each interval on day 2 revealed no statistically significant difference between groups ($P = 0.05$). Data are expressed as means (standard error) ($N = 3$ for each group at each dose of endotoxin).

Dose of endotoxin (mg)	Day 1 postpartum			Day 2 postpartum			Day 3 postpartum		
	1	2	3	1	2	3	1	2	3
4	2.0 (1.2)	2.5 (1.2)	5.2 (0.4)	5.5 (2.3)	−1.5* (1.6)	6.3 (0.4)	5.1 (0.6)	2.5 (0.7)	9.2 (1.6)
8	4.6 (0.6)	2.2 (1.0)	5.3 (0.3)	6.2 (0.7)	−3.1* (1.0)	3.8 (0.6)	5.9 (0.7)	2.9 (0.9)	10.2 (1.1)
16	6.4 (0.6)	1.1 (0.4)	8.7 (1.9)	5.0 (1.0)	−3.2* (1.1)	7.1 (1.7)	5.3 (1.4)	2.4 (1.6)	10.2 (0.5)

*Value significantly different from corresponding period for groups 1 and 3 postpartum ($P < 0.025$, $P < 0.002$, and $P < 0.03$ for the groups receiving 4, 8, and 16 mg of endotoxin, respectively).

drace crossbreeding after their first farrowing. All animals farrowed on either day 113 or 114 of gestation. Individual sows received endotoxin (4, 8, or 16 mg; groups 1, 2, and 3, respectively) in saline on day 2 and saline on days 1 and 3 postpartum, so that each animal served as its own control. Since there is substantial interanimal variation in basal prolactin concentrations [up to three times the difference in clinically healthy animals (*8*)] and potential lactational performance, this design permitted within animal comparisons of responses to endotoxin and saline.

Radioimmunoassays were used to measure plasma glucocorticoids (*9*) and prolactin (*10*) with the use of antibody ALM-5 (from P. V. Malven) and the NIH reference preparation SP-162C (16 IU/mg). Plasma prolactin declined after endotoxin administration (Fig. 1). Endotoxins blunted the suckling-induced surge in prolactin in groups 1 and 3 when the piglets were given access to the sow on day 2 postpartum at 1300 hours, but prolactin concentrations increased in group 2 after the piglets suckled. These results suggest that endotoxins can interfere with the normal mechanisms involved in prolactin release in the sow. It is not known whether the effects are mediated by way of the hypothalamus or directly at the anterior pituitary, or at both locations.

Table 2. Normalized prolactin values for days 1 to 3 postpartum for intervals 1 to 4 as depicted in Fig. 1.

Interval	Dose of endotoxin		
	4 mg	8 mg	16 mg
	Day 1 postpartum		
1	99 ± 2*	104 ± 2	103 ± 3
2	107 ± 7	97 ± 7	94 ± 3
3	97 ± 12	82 ± 10	103 ± 11
4	103 ± 7	90 ± 9	114 ± 6
	Day 2 postpartum		
1	101 ± 2	100 ± 3	106 ± 2
2	104 ± 5	102 ± 2	96 ± 2
3	74 ± 3	83 ± 8	86 ± 4
4	29 ± 2	73 ± 3	40 ± 3
	Day 3 postpartum		
1	105 ± 5	106 ± 2	100 ± 3
2	95 ± 14	92 ± 2	86 ± 2
3	78 ± 14	85 ± 9	95 ± 14
4	96 ± 10	123 ± 12	122 ± 9

*Mean ± standard error of the mean.

Changes in average piglet weight were used as an index of milk production by the sow. The presence of people in the farrowing building (sample collection, feeding, piglet weighing, and cleaning) is reflected by a relative decline in piglet growth during the second weighing interval (0800 to 1600 hours) (Table 1) on days 1 and 3 postpartum compared to the first and third intervals. The rate of piglet growth for interval 2 (after endotoxin administration) on day 2 was 4.1, 5.7 and 5.0 g less than the corresponding values for days 1 and 3 postpartum for the groups receiving 4, 8, and 16 mg of endotoxin, respectively. These decreases are significantly different (see Table 1). The results demonstrate that small amounts of endotoxin are capable of significantly suppressing lactation as measured by piglet growth.

Endotoxin administration to the sows also caused other changes typical of exposure to bacterial endotoxin. These included pyrexia, neutropenia, lymphopenia, lethargy, and blanching or mottling of the skin. A marked increase in plasma glucocorticoids from mean concentrations of 20 to 25 ng to over 100 ng per milliliter of plasma occurred after endotoxin injection. Glucocorticoids can suppress basal prolactin or prolactin release stimulated by thyrotrophin-releasing hormone in other species (*11*). However, treatment of other sows with adrenocorticotropic hormone (0.5 IU/kg) on day 4 postpartum caused a similar rise in plasma glucocorticioids with no concomitant decrease in prolactin.

The clinical changes observed in this study after endotoxin administration closely mimic the changes observed in

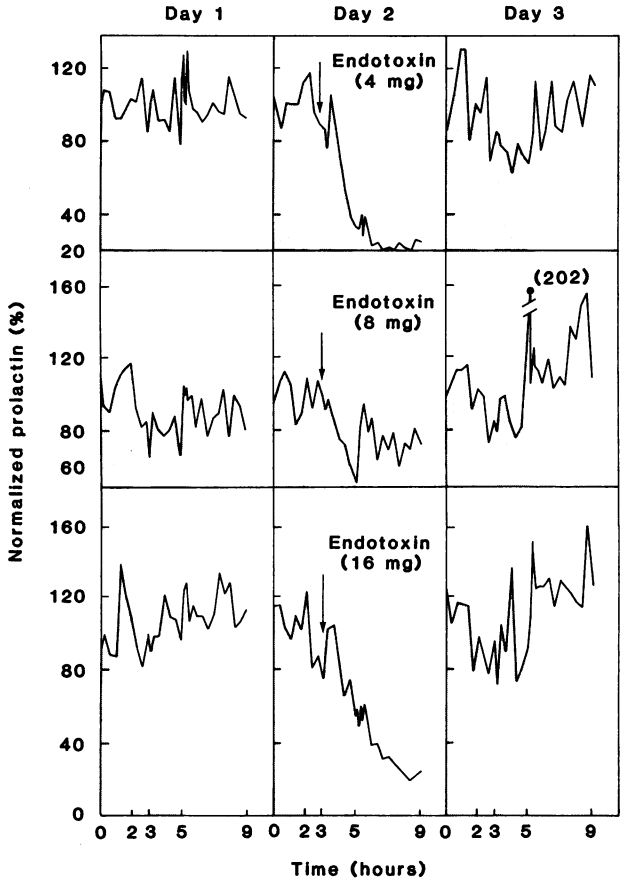

Fig. 1. Changes in prolactin concentrations after the administration of saline on days 1 and 3 postpartum or endotoxin in saline on day 2 postpartum. Blood sample collection started at 0800 hours on all days and was designated as 0 hours. Groups 1, 2, and 3 (three sows per group) were treated with 4, 8, or 16 mg of endotoxin (055:B5 strain, Difco), respectively, given as a single subcutaneous injection on day 2 postpartum at 3.0 hours. The time of endotoxin administration is marked with an arrow for each group. Blood samples were collected via indwelling jugular cannulas surgically implanted 3 to 5 days prepartum. Piglets were permitted to nurse at will between 0 and 2 hours, after which they were penned adjacent to the sow's head behind a wire mesh barrier and not permitted access to the sow until 5 hours when they were again permitted to nurse. This procedure facilitated separation of changes in prolactin concentrations associated with endotoxin administration and changes attributable to the suckling stimulus. To permit comparisons between days, the prolactin concentrations for each animal were normalized for each day with the mean concentrations for samples collected between 0800 and 1100 hours arbitrarily set equal to 100 and all other concentrations computed as a percentage of this value. For statistical analysis the prolactin concentrations were pooled into one of four intervals (0 to 2, 2 to 3, 3 to 5, and 5 to 9 hours) and the means compared by an analysis of variance. The standard errors of the means for groups 1, 2, and 3 on days 1, 2, and 3 postpartum were 10.6, 8.3, and 7.9 percent, respectively. Mean prolactin concentrations were either not different or higher when intervals 3 or 4 were contrasted with intervals 1 or 2 on day 1 and 3 postpartum. Prolactin was significantly lower in interval 4 when contrasted with either intervals 1 or 2 on day 2 postpartum ($P < 0.0001$, $P < 0.046$, and $P < 0.0001$ for groups 1, 2, and 3, respectively), by the least-squares mean procedure for multiple comparisons from the S.A.S. program (S.A.S. Institute, Cary, North Carolina).

field cases of lactation failure. These results suggest that endotoxins may play a pivotal role in the pathogenesis of some cases of lactation failure in the sow. Sources of endotoxin may well be foci of bacteria, for example, *E. coli*, in one or more mammary glands of affected sows. This hypothesis is supported by Morkoc [(*12*); see also (*13*)] who reported a significantly higher incidence of detectable endotoxin concentrations in hypogalactic sows than in paired control animals. Although the endotoxins exert several effects that are deleterious to normal lactation, for example, disruption of the microvasculature, immunologic stimulation, and alterations in the endocrine profile of the animals, the relative importance of each to the disruption of lactation is unknown. Since prolactin is an apparent prerequisite for normal lactation in the pig (*14*), the magnitude of the observed prolactin decline is sufficient to produce lactation failure in the absence of the other changes induced by endotoxins. We therefore propose that small amounts of *E. coli* endotoxins are capable of suppressing prolactin concentrations in the sow and are a significant factor in the pathogenesis of lactation failure in the periparturient animal.

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Antigens on HTLV-Infected Cells Recognized by Leukemia and AIDS Sera Are Related to HTLV Viral Glycoprotein

Abstract. *Cross-reactive antigens of molecular size of 61,000 to 68,000 daltons are found on the surface of human cells infected by human T-cell leukemia-lymphoma virus (HTLV). They are recognized by antibodies from patients with adult T-cell leukemias and lymphomas, from healthy carriers of HTLV, and from patients with the acquired immunodeficiency syndrome (AIDS). The latter finding has been one of the major reasons for suggesting an association of HTLV with AIDS. However, whether these antigens are of cellular or viral origin has not been clear. These antigens have now been shown to be associated with the presence of viral proteins in the cells, and a cross-reactive glycoprotein of molecular size of 46,000 daltons has been found to be a consistent structural part of viruses purified from several HTLV-producer cell lines. The findings thus suggest a viral (HTLV) origin of these antigens.*

Human T-cell leukemia-lymphoma viruses (HTLV) are a family of exogenous T-lymphotropic type C retroviruses strongly associated with adult T-cell leukemia (ATL) (*1*). Recently, additional attention has been given to these viruses as a result of the demonstration that a high proportion of patients with acquired immunodeficiency syndrome (AIDS) have antibodies that react with antigens on the surface of HTLV-producing transformed human T cells, such as the Hut 102 (*2*) and the MT 2 (*3*) cell lines (*4*). In some cases, HTLV was isolated from these patients or was shown to be integrated in the cellular genome (*5, 6*). In particular, one of the surface antigens, which is also widely recognized by antibodies from ATL patients (*7*), appears to be gp 61, a glycoprotein of molecular size 61,000 daltons (*4*). Whether this antigen is of viral or cellular origin is, however, still unknown. If it is cellular in origin, its expression might be induced by HTLV infection. However, another etiological agent yet to be identified might induce the same cellular protein. Therefore, it is unclear whether the antibodies indicate a direct involvement of HTLV in AIDS, and it has become imperative to define the origin of these antigens.

Using an HTLV-transformed virus-producing cell line we call G-25/MI (*6*), we found an antigen of 63,000 to 67,000 daltons (p65) which, like gp 61 in Hut 102 cells (*4, 7*), reacted strongly with sera from both ATL patients and healthy carriers of HTLV but did not react with sera from seronegative normals, as shown by a strip radioimmunoassay (RIA) based on the "Western blot" technique (*8*) (Fig. 1A). The G-25/MI cell line was established from the leukemic T cells of a black ATL patient from the Caribbean (*6*) and produces HTLV of type I. Although all of the sera from ATL patients or seropositive normals also detected the viral *gag*-related antigens p24 or p19 (or both) (*9, 10*), the recognition of p65 was much stronger in many sera. An antigen of 54,000 daltons was previously identi-

Table 1. Competition of p65 and *gag*-related antigens by various cellular and viral extracts. Competition RIA's were done on G-25/MI cell strips as described in Fig. 1. Symbols indicate that competition was +++, complete; ++, strong; +, marginal; or −, absent.

Competing agent	Competition with	
	p65	*gag* antigens
Cells (50 μg of protein)		
G-25/MI	+++	+++
JM (Jurkat)	−	−
Normal human T cells	−	−
MT 2	+	+++
Hut 102	+	+++
G-11/MJ	+	+++
Purified viruses (10 μg of protein)		
G-25/MI	−	+++
MT 2	++	+++
Hut 102	++	+++
G-11/MJ	++	+++
Virus-producer and nonproducer cells (500 μg)		
G-11/MJ	+++	+++
NIH82/C2 (HTLV-producer)	+++	+++
NIH82/15B (HTLV-infected but nonproducer)	−	−