Perinatal Changes in the Population of Colonic Goblet Cells in the Rat

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During the late fetal period, the digestive tract undergoes changes in both morphology and physiology in preparation for adaptation to the neonatal life. The digestive tract of neonates must be more active in absorbing nutrients than that of fetuses receiving them through the placental circulation. It is well known that one of the principal functions of the large intestines is to eliminate and excrete undigested materials as feces [11]. In the large intestines, columnar cells reabsorb water and goblet cells secrete a large amount of mucus to facilitate elimination of the dehydrated materials and lubrication of the mucosal surface [2, 11]. However, a limited number of reports are available on the perinatal development of the colon with most emphasis on changes in the absorptive cells [1, 4, 5, 6, 8] and with only a little attention to goblet cells [8]. The present study was to examine changes in the population of goblet cells of the rat colon during the perinatal period.

Wistar rats were given water and commercial diet (Oriental pellets, NMF) both *ad libitum*. Rats were mated overnight and examined the following morning for the presence of sperm in the vaginal smear. The day on which sperm were found was regarded as day 1 of gestation. Fetuses on days 20, 21 and 22 of gestation and neonates on days 1, 2 and 3 of neonatal life were obtained. On day 20 of gestation, some fetuses were orally given 0.3g powdered milk (Weyth, SMA S-26, Japan) dissolved in 0.1ml distilled water *in utero* by the method of Morikawa *et al.* [9]. Two days later (on day 22 of gestation), the fetuses were autopsied. Non-treated littermate fetuses were used as controls.

For histometrical study, small portions of the descending colon were quickly removed, fixed in a mixture of 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.2 M phosphate buffer solution (pH7.4) for 1.5 hrs, and postfixed in 2.0% osmium tetroxide in phosphate buffer solution (pH7.4) for 1.5 hrs. The tissues were

dehydrated in a graded series of ethanol, soaked in propylene oxide and embedded in Epon-Araldite. Serial cross sections of the colon were cut at 1 μ m with an ultramicrotome (Sorvall, MT-5000). Several sections at least 20 μ m apart each other were mounted on a slide to avoid counting the same goblet cells twice. The sections were stained with 0.5% toluidine blue in 1/15 M phosphate buffer solution (pH7.4) by heating (80-85°C) for 5 min and were covered with immersion oil (Merck). Mature goblet cells were characterized by the presence of substances positive to toluidine blue in the cytoplasm. To facilitate counting goblet cells and absorptive cells in the surface epithelium except in the crypt, those areas in which the columnar cells were cut perpendicularly to the basement membrane were selected. The population of goblet cells was expressed as a percentage against the total number of epithelial cells counted (250 to 1,000 per sample). For scanning electron microscopy, some samples were prefixed in a mixture of 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.2M phosphate buffer solution (pH7.4) and postfixed in 2.0% osmium tetroxide in 0.2M phosphate buffer solution (pH7.4). After fixation, they were dehydrated in a graded series of acetone and subjected to critical point drying. The dried samples were mounted on aluminium stubs, coated with gold and observed by a scanning electron microscope (SEM) (Hitachi, S-800).

The population of colonic goblet cells was increased significantly day by day from fetal day 20 to neonatal day 2 (Table 1). A remarkable increase was noticed between fetal day 22 and neonatal day 1. SEM observation also revealed such a day-by-day increase of goblet cells (Figs. 1-3). Furthermore, oral administration of milk to fetuses *in utero* caused a significant increase in the population of goblet cells (Table 2). SEM observation confirmed this increment of goblet cells as well (Figs. 4 and 5).

According to Helander [8], the volume density of goblet cells in colonic epithelium of rats did

Table 1. Percentage of goblet cells in the surface epithelium of the colon in perinatal rats

Day	No. of animals	% mean $\pm$ SEM	P
F ^{a)} -20	5 (4) ^{c)}	37.74 $\pm$ 1.27	p<0.05
F-21	5 (4)	41.27 $\pm$ 0.90	p<0.002
F-22	5 (4)	47.77 $\pm$ 1.10	p<0.001
N ^{b)} -1	5 (4)	65.56 $\pm$ 0.91	p<0.001
N-2	5 (4)	73.74 $\pm$ 0.79	NS ^{d)}
N-3	5 (4)	71.46 $\pm$ 1.07	

a) Fetal day.

b) Neonatal day.

c) Number of litters in parentheses.

d) Not significant.

Fig. 2. Scanning electron micrograph of the inner surface of the colon of a 22-day-old fetus. The population and size of goblet cells are increased as compared with those of the 20-day-old fetus in Fig. 1.

not alter from fetal day 21 to postnatal day 10 on the whole, with a transient increase just before sucking. In the present study, however, the populations of goblet cells in neonates on days 1, 2 and 3 were clearly and significantly greater than those in fetuses on day 22 of gestation. This discrepancy between our results and Helander's seems to be largely due to the difference of the method used, cell population versus volume density which means the volume ratio against the

Fig. 1. Scanning electron micrograph of the inner surface of the colon of a 20-day-old fetus. Small goblet cells (arrows) are scattered in the epithelium.

Fig. 3. Scanning electron micrograph of the inner surface of the colon of a 1-day-old neonate. The population of goblet cells is increased as compared with that of the 22-day-old fetus in Fig. 2.

total volume of epithelial region.

The stomachs of the neonates examined were filled up with maternal milk at autopsy. Thus, our

Table 2. Comparison of percentage of goblet cells in the epithelium of the colon between milk-given fetal rats and their littermate controls on day 22 of gestation

Group	No. of fetuses	% mean $\pm$ SEM	P
Milk-given	5 (4) ^a	62.48 $\pm$ 1.39	p < 0.001
Control	6 (4)	48.73 $\pm$ 0.62	

a) Number of litters in parentheses.

Fig. 5. Scanning electron micrograph of the inner surface of the colon of a milk-given fetus on day 22 of gestation. Milk was given 2 days earlier. The population of goblet cells is increased as compared with that of the littermate control fetus in Fig. 4.

present results indicate some positive relationship between the population of colonic goblet cells and the ingestion of maternal milk. Even before birth, the population of goblet cells was significantly increased from fetal day 20 to day 22, and this increase was further accelerated by giving milk to fetuses *in utero*. Incidentally, pancreatic acinar cells and gastric parietal cells can respond to milk given before birth as well [9, 10]. Also, it has been shown that postnatal growth and development of the small and large intestines can be accelerated by well-feeding [7, 12].

It has been reported that goblet cells appear

Fig. 4. Scanning electron micrograph of the inner surface of the colon of a non-treated littermate control fetus on day 22 of gestation. Many goblet cells are seen.

first on day 19 [8] or 20 of gestation [3, 6] in a well differentiated status in the rat [6]. These findings imply that the goblet cells are ready to respond to exogenous stimuli on late fetal days. In conclusion, the present results strongly suggest that differentiation of colonic goblet cells before birth can be further accelerated when some exogenous food materials such as milk are introduced into the digestive tract, and that the remarkably increased population of goblet cells just after birth is due to the intake of maternal milk.

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要 約

周生期ラットにおける結腸杯細胞の数的変化(短報): 森川嘉夫・井上 知・岡田利也(大阪府立大学農学部家畜解剖学教室)——ラットの結腸杯細胞は、胎生20日から生後2日にかけて日をおって有意に増加したが、胎生22日から生後1日にかけての増加が特に顕著であった。子宮内胎仔にミルクを経口投与すると、杯細胞は有意に増加した。このことから、杯細胞の発育分化が投与されたミルクによって促進されることが示唆され、生後の急激な杯細胞の増加は、仔の母乳取り込みによるものと推測された。