Some Factors Which Influence Intrinsic Factor Content and Its mRNA Level in the Rat Stomach

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Factors which regulate Intrinsic Factor (IF) content and IF mRNA level were examined with an abc-ELISA and Northern blot analysis in growing rats, and compared with pepsinogen (Pg) and in terms of the increased need for vitamin B_{12} ($V.B_{12}$). Increases in IF content and IF mRNA level gradually occurred from day 13 after birth, whereas those of Pg and Pg mRNA started from day 16. The effects of a few related hormones on the expression of IF mRNA were examined. The injection of hydrocortisone induced IF and Pg mRNA expression in 5-d-old postnatal rats. Furthermore, adrenalectomy-induced decreases in IF content and IF mRNA level in adult male rats were recovered with hydrocortisone administration.

IF content and IF mRNA level were measured in the artificially and physiologically created needs for $V.B_{12}$, the first being regeneration of the liver, the $V.B_{12}$ storing tissue, following partial hepatectomy, and the second pregnancy or lactation. During regeneration of the liver, increases in IF content and IF mRNA level were marked, followed by reduction toward the original level after accomplishment of regeneration. Increases in IF content and IF mRNA level were also seen in lactating rats, but no increases were obtained in pregnant rats. These results suggest that the IF content and IF mRNA level are regulated not only by corticosteroids but also by the increased need for $V.B_{12}$.

Key words intrinsic factor; vitamin B_{12}; mRNA; hydrocortisone; partial hepatectomy; lactation

Intrinsic Factor (IF) is the vitamin B_{12} ($V.B_{12}$) binding protein that carries $V.B_{12}$ from the stomach to the ileum mucosa, and is secreted from the stomach.[1] Since $V.B_{12}$ is a coenzyme of methionine synthase (methylcobalamin) and methylmaronyl-Co A mutase (deoxyadenosyl cobalamin), it is an important factor in metabolism, and its deficiency leads to hematological and neurological disorders. We have already reported that IF is synthesized in the chief cells of the rat stomach and secreted with the stimulation of carbachol, cholecystokinin and secretin in the same manner as pepsinogen (Pg).[2] However, regulation of the gene expression of IF has not yet been clarified.

In general, the gene expression of a transporter for an essential nutrient would be regulated not only by neural and humoral factors but also by the need of a nutrient, as was shown in the glucose transporter.[3] Dieckgraefe, *et al.* reported that IF appeared in the stomach during the suckling period of postnatal rats, and a remarkable increase in IF occurred with hydrocortisone administration.[4] The phenomenon of enhanced corticosteroid secretion occurring during the suckling period has been known,[5] so the question which is crucial in the expression of IF mRNA whether a circulating corticosteroid concentration still remains.

In this paper, we confirmed the exact time of IF and IF mRNA appearance in comparison with that of Pg and Pg mRNA. Also, If the mechanism of effective $V.B_{12}$ intake is prepared so as to avoid a lack of $V.B_{12}$, IF biosynthesis should be stimulated by an increased need for $V.B_{12}$ in the body. Therefore, we examined IF content and IF mRNA level in two cases of increased $V.B_{12}$ need. The first was the a surgically created increased need, such as a partial hepatectomy, and the other involves a physiologically created increased need, such as pregnancy or lactation.

MATERIALS AND METHODS

Materials IF and Pg were isolated from the stomach of Wistar rats, and IF was immunized to Japanese white rabbits as described previously.[2,6] Half of the rabbit anti-rat IF IgG fraction was biotinylated before use. Horseradish peroxidase-avidine D, L-thyroxine and hydrocortisone were purchased from Sigma Chemical Co.(St Louis, U.S.A.). Tetragastrin was from MECT (Tokyo, Japan). Antisense oligodeoxynucleotides for IF and Pg mRNA were customized by Greiner Japan (Tokyo, Japan), as follows: for IF, 5′-GGGA-GAATCTGGGCAATGGACATGGGGTTCTGGAA-3′, which was estimated by Dieckgraefe, *et al.*,[7] and for Pg, 5′-TGTGTCTACAATGCCTTGGCAGCCCTG-3′, which was estimated by Ichihara, *et al.*,[8] then labeled by ^{32}P using an Oligonucleotide 5′-End Labeling System (NEN Life Science Products, Boston, U.S.A.). Only RNA from the stomach was markedly hybridized with IF and Pg antisense oligodeoxynucleotides, so that antisense oligodeoxynucleotides of IF and Pg were specific for their mRNAs. β-Actin cDNA (Wako Pure Chemical Industries, Tokyo, Japan) was used for the detection of β-actin mRNA after treatment with a Random Primer Plus Extension Labeling System (NEN Life Science Products). All other reagents were of the best commercial quality available.

Measurement of IF and Pg Under ether anesthesia, stomachs from starved adult rats for 5 h and non-starved postnatal rats were excised and washed with ice-cold saline. The stomachs were homogenized with 9-fold 50 mM phosphate buffer (pH 7.5), and the homogenates were frozen for more than 2 h. After thawing, the homogenates were centrifuged at 12000×***g*** for 15 min. IF in an aliquot of supernatant was measured by the enzyme immunoassay with anti-rat IF IgG, biotinylated IF IgG and peroxidase conjugated avidine, which was established in our laboratory.[2] Pg was estimated according to the peptic activity of the homogenate as

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described previously.[6]

Measurement of IF and Pg mRNAs Total RNA was extracted using the acid–guanidine thiocyanate–phenol–chloroform method[9] from half of the same stomach used for IF and Pg measurement. An aliquot of extracted RNA was electrophoresed on 0.9% agarose–formaldehyde gel in a buffer (pH 7.0) containing 3-morpholinopropanesulfonic acid, sodium acetate, and EDTA. After blotting onto a sheet of nylon membrane from agarose gel, RNA was hybridized with antisense IF or Pg^{32}P-oligodeoxynucleotide. Hybridized nylon membrane was exposed to a sheet of X-ray film. After development of the X-ray film, quantification of the spots was carried out with NIH Image.

Preparation of Adrenalectomy and Hepatectomy Adrenalectomy was performed following the method of Grollman.[10] After adrenalectomy, male rats were maintained with free access to food and saline instead of tap water for one week. Hydrocortisone (50 mg/kg in olive oil) was subcutaneously injected into the rats.

Partial hepatectomy was performed as follows: about 2/3 of the liver was removed from the male rats of about 7 weeks old under pentobarbital anesthesia.[11] At the 2nd, 4th, 6th, 8th and 10th d after the operation, IF content and IF mRNA level in the stomach were measured.

Statistical Analyses The results are expressed as mean±S.E. Differences between the two groups were analyzed by Student's *t*-test.

RESULTS

Developmental Changes in IF, Pg and Their mRNAs in the Stomachs of Growing Rats, and the Effect of Hydrocortisone IF, Pg and their mRNAs in the stomachs were measured from birth for 5 weeks. Small amounts of IF mRNA and Pg were detected, even during the early postnatal period. Increases in IF content and IF mRNA level gradually occurred from about day 13, and after the maximal increase at around day 21 they converged to the adult level by about day 33 (Fig. 1). Similar increases in Pg content and Pg mRNA level occurred from day 16, and reached the adult level by day 21.

Effects of hydrocortisone, thyroxine and tetragastrin on IF and Pg content, and their mRNA levels, were examined in postnatal rats (Fig. 2), in which only hydrocortisone-induced increases were seen. When hydrocortisone (50 mg/kg) was subcutaneously injected into 5-d-old rats, increases in IF content and IF mRNA level in the stomach started within 2 d (7-d-old) and reached a maximum level by 3 d (8-d-old). Also, increases in Pg content and Pg mRNA level occurred within 1 d and reached a constant level by 3 d. Augmentation of IF and Pg content and their mRNA levels with hydrocortisone was maintained for at least 5 d (10-d-old) after the injection. However, the IF and Pg content and their mRNA levels remained at a low level with a single injection of thyroxine (100 μg/kg) or tetragastrin (100 μg/kg).

Effects of Hydrocortisone on IF Content and IF mRNA Level in Adrenalectomized Adult Rats Hydrocortisone (50 mg/kg) was subcutaneously injected into adrenalectomized adult male rats at one week after operation, and IF content and IF mRNA level in the stomach were measured, respectively (Fig. 3). After adrenalectomy, the IF content and IF mRNA level significantly dropped to 34% and 52% of the sham operation levels. However, they recovered to 85% and 94% one day after the hydrocortisone injection, respectively.

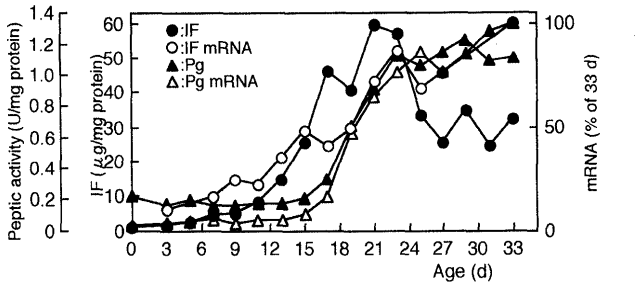

Fig. 1. Developmental Changes in IF and Pg Content and Their mRNA Levels in Postnatal Rat Stomachs

IF and Pg mRNA levels were expressed as a % of the ratio to β-actin mRNA at day 33 after birth. Each value is the mean of those from 6—11 rat stomachs.

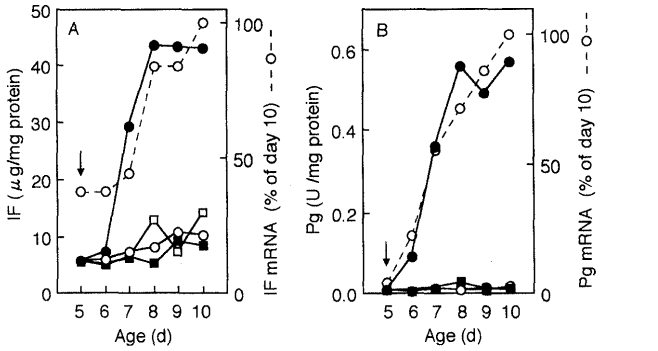

Fig. 2. Effects of Hydrocortisone, Thyroxine and Tetragastrin on IF and Pg Content and Their mRNA Levels in Postnatal Rat Stomachs

Each chemical was subcutaneously injected at the arrow, and IF(A) and Pg (B) contents were measured for 5 d. Symbols represent control (■), hydrocortisone (●), thyroxine (□) and tetragastrin (○), respectively. Hydrocortisone-induced increases in IF (A) and Pg (B) mRNA levels (dotted lines) were expressed as a % of the ratio to β-actin mRNA at the 5th d after the administration. Each value is the mean of 4 experiments.

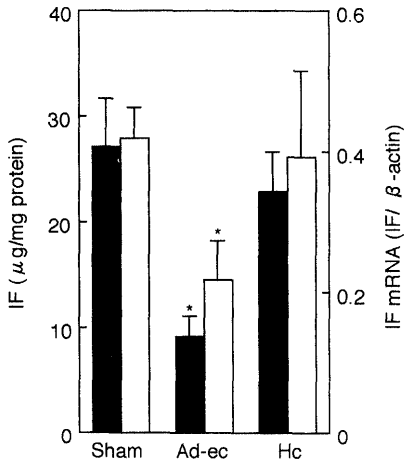

Fig. 3. Effects of Hydrocortisone on IF Content and IF mRNA Level in the Adrenalectomized Adult Rat Stomachs

IF content in the stomachs (closed column) was expressed as IF (μg) per protein (mg) in the supernatant of the homogenate, and mRNA levels (open column) were done as the ratio to β-actin mRNA on the same lane after electrophoresis. Symbols represent sham operated (Sham), adrenalectomized (Ad-ec) and hydrocortisone administrated (Hc) rats, respectively. Each value is the mean±S.E. of 3 experiments. Significantly different ($*p<0.05$, $**p<0.01$) from sham operated rats.

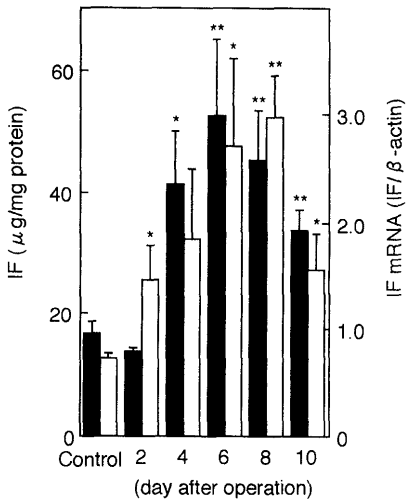

Fig. 4. Influence of Partial Hepatectomy on IF Content and IF mRNA Levels in Rat Stomachs

Closed and open columns represent IF content and mRNA levels in the stomachs, respectively. Each value is the mean±S.E. of those from 3—5 rats. Significantly different (*$p<0.05$, **$p<0.01$) from normal rats.

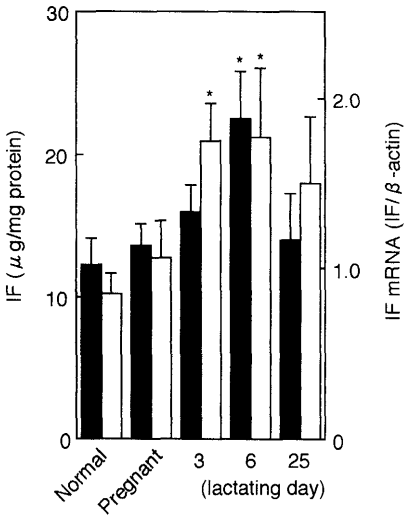

Fig. 5. IF Content and IF mRNA Levels in Pregnant and Lactating Rats

Closed and open columns represent IF content and mRNA levels in the stomachs, respectively. Each value is the mean±S.E. of those from 3—6 rat stomachs. Significantly different (*$p<0.05$) from adult female rats.

Influences of Partial Hepatectomy, Pregnancy and Lactation on IF Content and IF mRNA Level in the Rat

During regeneration of the liver after partial hepatectomy, the IF content and IF mRNA level in the stomach were measured for 10 d. The weight of the liver recovered to the previous level by the 6th d after the operation, whereas the increase in IF content doubled by the 6th d, and the IF mRNA level increased 170% at the 8th d (Fig. 4). At the 10th d, even after recovery of the liver weight, the IF content and IF mRNA level still remained high.

IF and IF mRNA in pregnant and lactating rats were measured (Fig. 5). The IF content and IF mRNA level in pregnant rats (15th—20th d) were almost similar to those in non-pregnant rats. During the lactating period, the IF content and IF mRNA level increased to 184% and 207% of the control at the 6th d after delivery, respectively. Then, at the 25th d, after a lactation period, IF and IF mRNA fell to levels statistically insignificant compared with normal ones.

DISCUSSION

In addition to IF, many substances which bind to $V.B_{12}$ are known: haptocorrin (R binder), a carrier in the stomach mainly secreted from the salivary gland, transcobaramine, a carrier in the blood and cells, and enzymes whose coenzyme is $V.B_{12}$. Although they have $V.B_{12}$ binding sites and their DNA sequences resemble each other,[12] the antisense oligodeoxynucleotide of IF used is hybridized only to the RNA from the stomach, making is specific for IF. The specificity of Pg antisense oligodeoxynucleotide has already been confirmed in our previous paper.[13]

We precisely measured IF and Pg content and their mRNA levels from birth for about one month. We obtained results similar to those reported previously by Dieckgraefe, *et al.*[4] in which an increase in IF mRNA level started during days 12—20 . In this paper we confirmed that increases in IF content and IF mRNA level began from day 13, and those in Pg and Pg mRNA did from day 16. For the question of how postnatal rats absorb $V.B_{12}$ before the appearance of IF, there was a report of neonatal rats freely absorbing $V.B_{12}$ from the milk without IF.[14] Since it was thought that the appearance of IF coincided with accomplishments of the gastrointestinal tract, the next question, what induced the expression of IF mRNA, arose. Taking into consideration the report that an increase in circulating corticosteroid began at around day 12,[5] it was thought that the expression of IF mRNA was closely related to corticosteroid secretion. Thus, we examined the effect of hydrocortisone on the appearance of IF and Pg and their mRNA. When hydrocortisone (50 mg/kg) was subcutaneously injected in 5-d-old rats, in which the IF content and IF mRNA levels had stayed low, increases in IF content and IF mRNA level began within 2 d, and they persisted at high levels thereafter. Thyroxine and tetragastrin, although thyroxine was known as a regulating factor of gene expression[15] and gastrin stimulated cell proliferation in the gastrointestinal tract,[16] did not exhibit any influence on the expression of IF mRNA in the rats. In addition to the report of Dieckgraefe, *et al.*, there was also a report that the appearance of the gastrin receptor was later than that of IF,[17] so tetragastrin could not act on 5-d-old rats. Even though both IF and Pg are synthesized and secreted from chief cells in the rats, it seems they are under different regulatory mechanisms.

In adult rats, adrenalectomy led to decreases in IF content and IF mRNA level, and the decreases were recovered by hydrocortisone administration. These results suggest that corticosteroid-induced IF was not accompanied with growth but by the effect of corticosteroid itself. However, the glucocorticoid responsive element has not yet been found upstream of the IF DNA sequence.[7] Because it has been reported that mesenchymal cells play an important role in the differentiation of chief cells,[18] the possibility that hydrocortisone causes an irreversible event on certain cells is also possible. Further estimation of the upstream portion of the IF gene or research on cell–cell interaction might be the key to solving this problem.

In this paper we chose two kinds of the increased need for $V.B_{12}$: partial hepatectomy and the reproductive process. $V.B_{12}$ is stored in the liver, and is provided according to re-

quirement. We surgically removed 2/3 of the liver so as to produce an increased need for $V.B_{12}$ from two points of view: removal of $V.B_{12}$ preservation and an increased need for regeneration of the liver as the coenzyme. IF content and IF mRNA level markedly increased during regeneration of the liver after partial hepatectomy. These results showed that the artificially created increased need for $V.B_{12}$ also induced enhancement of IF and IF mRNA biosynthesis.

We examined IF and IF mRNA in pregnant and lactating rats as a physiological increased need for $V.B_{12}$. No increases in IF content and IF mRNA level in pregnant (15th—20th d) rats were seen, whereas in lactating ones (3rd and 6th d) remarkable increases occurred. A similar phenomenon was obtained in the serum level of Pg in our previous paper.[19] IF content and IF mRNA level in female rats were lower than those in male rats, as shown in the results. These results showed that sufficient amounts of $V.B_{12}$ to supply their fetus might be reserved in a maternal $V.B_{12}$ pool or recovered by an ordinary secreted amount of IF for the absorption of $V.B_{12}$ in pregnant rats. On the other hand, lactating rats surely needed excess $V.B_{12}$ to supply their postnatal rats with milk containing it and to restore depleting supplies. It was thought that the supply of $V.B_{12}$ to their postnatal rats would lead to a severe shortage of it, because $V.B_{12}$ was out of the system of recycling in the mother's bodies.

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