Correlative Study of Hydrochloric Acid, Pepsin, and Intrinsic Factor Secretion in Newborns and Infants

MONA AGUNOD, MD, NOBUO YAMAGUCHI, MD, RAFAEL LOPEZ, MD, A. LEONARD LUHBY, MD, and GEORGE B. JERZY GLASS, MD

The developmental patterns of secretion of hydrochloric acid, pepsin, and intrinsic factor were studied, following Histalog stimulation, in gastric juice of infants 12 hr to 3 months old. In the first day of life, the outputs of hydrochloric acid, intrinsic factor, and pepsin were very low, but in no instance was achlorhydria found. The outputs of these three glandular secretory components then showed a gradual increase until the third week after birth, followed by a short decline in the third to fourth week, and a second sustained rise continuing into the second and third month of life.

A good correlation was observed between the outputs of hydrochloric acid and intrinsic factor in gastric juice of the neonate following Histalog stimulation; and a somewhat poorer correlation between that of intrinsic factor and pepsin.

When secretory outputs were recalculated for body weight, those of intrinsic factor in infants 2–3 months old were at levels comparable to that observed in older children and adults; those of hydrochloric acid, at the lower range of normal, or slightly below adult levels; and that of pepsin, well below adult values.

On serial determinations, one infant showed intrinsic-factor activity, throughout the period of study, that was well below that of other infants of comparable age. The possible relationship of this case to juvenile pernicious anemia is discussed.

THERE HAVE BEEN relatively few studies of the secretory activity and the mutual correlation of the various cellular elements of the gastric mucosa in the normal newborn. Most studies of gastric secretion in infancy have centered only on acid secretion,[1–4] and only a limited number have been performed under conditions approaching maximal stimulation.

In 1948 and 1959, Luhby[5,6] reported on the intrinsic factor (IF) secretion

From the Sections of Gastroenterology, Department of Medicine, and of Hematology, Department of Pediatrics, New York Medical College, New York.

Supported in part by Research Grants-in-Aid AM-00068 and AM-09701 from the National Institute of Arthritis and Metabolic Diseases; HD-01162 from the National Institute of Child Health and Human Development; and CA-08251 from the National Cancer Institute, National Institutes of Health, US Public Health Service.

Address for reprint requests: Dr. G. B. J. Glass, New York Medical College, Flower and Fifth Avenue Hospitals, New York, NY 10029.

in megaloblastic anemia of infancy, and in 1954, data were presented on the gastric mucoprotein and hydrochloric acid (HCl) secretion in infants and young children.[7]

As far as we can determine, no study has been published on pepsin secretion in normal infants born at term except for the work of Grayzel and co-workers[8] who reported on plasma pepsinogen levels in the newborn. More recently, Rodbro *et al*[9, 10] reported on acid, pepsin, and IF secretion in a group of children 9–30 months old; but no comprehensive study of gastric secretory activity in newborns and young infants has been published.

In this study we have attempted to correlate the developmental patterns of fundic glandular secretions as assayed after Histalog stimulation in newborns and infants. Since in man, HCl and IF appear to be derived from parietal cells,[11] though differing probably in the mechanisms of their secretions,[12] the developmental patterns of these secretory products may throw some light on the physiology of parietal cell secretion. This information may also be of significance in the better understanding of the development of juvenile pernicious anemia, in which there is an ever-growing interest.[13]

MATERIAL AND METHODS

Subjects

Babies selected for the study were born at term of uncomplicated pregnancies, and were normal on physical examination, with a minimum weight of 2.8 kg. Mothers had received no drugs during their pregnancies other than the usual iron and vitamin supplements. Blood counts done on mother and infant at the time of birth were normal.

After informed consent had been obtained from the parents, 12 infants were intubated. In the 10 infants that comprise the 1-day-old group, initial gastric aspiration was done 12–24 hr after birth. In 8 infants, two or more gastric aspirations were done throughout the study for a total of 26 gastric juice collections. In 4 others, only a single gastric collection was possible. For comparison, a 4-year-old and 9-year-old child were included in the study.

Collection of Gastric Juice

The gastric juice was collected through a plastic nasogastric tube by intermittent manual suction. An initial 10-min collection was obtained several hours after the last feeding and was discarded. Following this a 20-min basal secretion was collected. Since the majority of the basal collections yielded a thick mucoid material in an amount insufficient for the assays, the data pertaining to these basal specimens were omitted.

Histalog Stimulation

Histalog (1.0 mg/kg body weight) was injected subcutaneously or intramuscularly and the gastric secretion was collected, as completely as possible over the following 1-hr period by aspiration. The collection was placed in a beaker immersed in an ice-water bath until processed in the laboratory. The augmented dose of Histalog, chosen initially as 1.5 mg/kg[14] resulted in flushing, irritability, and borborygmi. When reduced to 1.0 mg/kg it was better tolerated.

The post-Histalog specimens collected for 1 hr were pooled and spun immediately at 27,000 g for 20 min in a refrigerated centrifuge. The supernatant gastric juice was separated by micropipet from the mucus residue, and the volumes of both were measured. An aliquot of the supernatant was immediately neutralized to pH 7.4, and both native and neutralized samples were then stored at −20° C until analyzed.

Titratable Acidity

Acidity was measured automatically by electrometric titration to pH 7.4, and pH measurements were done in the electrometric cell. The acid output was calculated from the 1-hr volume of gastric juice and the acid concentration.

IF Assay

The assay was performed: (1) By charcoal radioimmunoassay using the Ardeman-Chanarin[15] and Gottlieb *et al*[16] methods modified in our laboratory,[17] and then calculating the concentration and the hourly output of IF-bound B_{12}, and its ratio to totally bound B_{12} in gastric juice. (2) On guinea pig intestinal mucosa homogenates (GPIMH) [18–20] by the technic used in our laboratory.[19, 20] The uptake of B_{12} by the homogenates was calculated in picograms, and its increase over the saline control due to IF present in the gastric juice was expressed as percent enhancement.

Pepsin Activity

At pH 2.0 pepsin activity in the gastric juice was measured by a modification of the Klotz-Duvall method[21] using 1/50 or 1/100 dilutions of gastric juices in Sörensen's glycine-HCl buffer, 0.1 M, pH 1.8. To 5 ml of the diluted gastric juice, 2 ml of a 1:1 mixture of ^{131}I-albumin* (specific activity 0.03 μc per ml) and 6% bovine albumin solution† were added. The mixture was incubated at 37° C for 30 min in a water bath; 1.0 ml of a 50% trichloracetic acid was added, and the tubes were centrifuged at 2000 rpm for 30 min. The supernatants were decanted and counted in a well-type NaI scintillation counter. Pepsin activity at pH 2.0 was calculated from a standard curve of 3-times crystallized bovine pepsin‡ at various dilutions, and expressed as milligrams pepsin-equivalent per milliliter gastric juice (mg PE/ml). Hourly out-

*Squibb & Sons, New York, NY.
†Armour Pharmaceutical Co, Chicago, Ill.
‡National Laboratories.

put of pepsin was calculated by multiplying this figure by the 1-hr output of gastric juice after Histalog stimulation.

RESULTS

Overall results are presented in Table 1. Changes occurring in a single individual (from birth to 67 days) are illustrated in Fig 1 and 2, while the means drawn from all the cases are shown in Fig 3 and 4.

Output of Gastric Juice

The 1 hr outputs of gastric juice after histalog stimulation, remained at relatively constant but low levels from birth to the age of 3 weeks, followed first by a transient drop and then a sharp rise to a mean of 13.4 ml at about 3 months (Table 1).

Hydrochloric Acid in Gastric Juice

Only traces of acid could be demonstrated by electrometric titration at birth, with a mean titratable acidity of 3.3 mEq/liter, and a pH ranging from 2.5 to 5.5 (median 3.7).

Mean HCl concentration after Histalog injection showed a steady increase from the mean value of 8.1 in the first 24 hr to that of 34.8 mEq/liter at about 3 months of age (Table 1). This pattern was seen in 4 of the 5 infants serially observed. There was, however, a transient drop in HCl concentration between the second and fourth week after birth in all cases, which was associated with a decrease in the volume of gastric secretion. In one infant (Baby R), the concentration of acid remained low throughout the study.

Mean HCl output per hour after Histalog (Table 1 and Fig 3) was markedly reduced initially (0.03 mEq at birth, 0.06 mEq in the first week, and 0.19 mEq at the third week). At the end of the fourth week, the mean acid output showed a marked decrease in all 5 infants on whom serial observations were carried out, to an average value of 0.08 mEq. At 3 months, however, a definite increase in the mean acid output was noted again. This value of 0.47 mEq/hr was still low, compared with that in the two 4- and 9-year-old children, in whom the acid output was on the average 10 times higher.

It should be mentioned that 2 of the 10 gastric juices collected after birth showed the presence of an elevated amount of free acid in the first day after birth (0.22 and 0.17 mEq/hr, respectively). By the eleventh day, however, the HCl outputs in both cases were close to the levels seen in other infants of the same age group (Table 1).

When the acid output was calculated per weight (kilogram) of the infants, the general appearance of the acid output curve over the period of the first 3 months showed a similar trend with that discussed above (Fig 4).

TABLE 1. GASTRIC SECRETION AND CONCENTRATION OF IF AND NON-IF BINDERS IN GASTRIC JUICE OF INFANTS AND CHILDREN AS COMPARED TO ADULTS

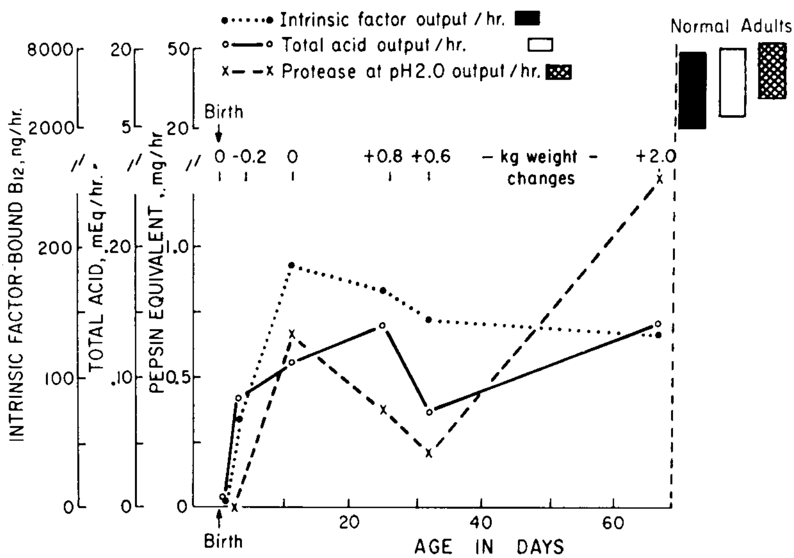
	Values at various ages							
	1 day	*3–8 days*	*10–11 days*	*14–17 days*	*25–32 days*	*67–110 days*	*4–9 yr*	*Adults* [13,19]
GASTRIC SECRETION								
Cases (No.)	10	7	5	4	3	4	2	—
Mean Weight (kg)	3.4	3.3	3.0	3.4	3.9	4.9	—	70.0
Gastric juice (ml)								
Mean	3.3	3.7	4.0	6.4	3.1	13.4	42.5	143.2
Range	0.8–9.3	1.3–4.7	2.0–5.4	1.5–12.0	3.0–3.2	7.1–19.9	35.0–50.0	—
Titratable acid								
Concentration (mEq/liter)								
Mean	8.1	14.4	34.4	26.7	26.4	34.8	114.2	91.2
Range	4.2–16.7	9.2–26.0	20.6–55.0	15.4–35.0	10.0–45.3	19.6–50.0	111.0–117.5	50.1–132.3
Output (mEq/hr)								
Mean	0.03	0.06	0.12	0.19	0.08	0.47	4.88	13.06
Range	0.01–0.09	0.01–0.12	0.11–0.15	0.04–0.37	0.03–0.14	0.14–0.65	3.89–5.88	7.17–18.94
Output (mEq/hr/kg)								
Mean	0.01	0.02	0.04	0.05	0.02	0.10	—	0.19
Range	0–0.03	0–0.03	0.04–0.05	0.01–0.09	0–0.06	0.03–0.14	—	0.10–0.27
Intrinsic factor								
Concentration (ng B_{12}/ml)								
Mean	6.8	20.2	36.6	28.7	38.4	33.0	86.3	57.9
Range	3.0–15.3	4.7–31.0	15.2–59.4	23.4–34.2	14.5–53.5	18.7–59.9	74.4–98.2	14.7–98.2
Output (ng B_{12}/hr)								
Mean	22.5	78.0	156.8	172.4	118.0	430.5	3757.0	4131.0
Range	4.6–79.1	6.1–127.7	30.4–243.5	51.3–280.8	46.4–165.9	132.8–658.9	2604.0–4910.0	1970.0–7613.0

Output (ng B_{12}/hr/kg)								
Mean	7.1	24.1	51.7	48.3	31.2	90.0	—	118.4
Range	1.2–25.4	1.7–45.6	11.3–81.2	17.1–74.1	10.8–43.6	26.6–146.4	—	28.1–108.8
Pepsin								
Concentration (PE mg/ml)								
Mean	0.04	0.05	0.12	0.14	0.10	0.12	0.45	0.29
Range	0.07	0–0.09	0.09–0.14	0.12–0.16	0.07–0.12	0.06–0.18	0.41–0.50	0.23–0.36
Output (PE mg/hr)								
Mean	0.18	0.21	0.46	0.88	0.32	1.34	18.5	41.9
Range	0–0.53	0.11–0.44	0.29–0.67	0.20–1.46	0.21–0.38	1.25–1.44	14.2–29.8	32.7–51.0
Output (PE ng/hr/kg)								
Mean	0.04	0.06	0.15	0.24	0.08	0.28	—	0.60
Range	0–0.19	0–0.12	0.11–0.22	0.06–0.36	0.06–0.10	0.23–0.32	—	0.47–0.73
IF AND NON-IF BINDERS								
IF bound B_{12} (ng/ml)								
Mean	6.8	20.2	36.6	28.7	38.4	33.0	86.3	57.9
Range	3.0–15.0	4.7–31.0	15.2–59.4	23.4–34.2	14.5–53.5	18.7–59.9	74.4–98.2	14.7–98.2
Non-IF bound B_{12} (ng/ml)								
Mean	76.9	89.2	87.6	75.0	94.1	60.6	21.9	15.7
Range	35.9–155.5	81.2–100.0	73.3–104.3	53.2–99.9	91.8–95.4	42.5–79.9	13.0–38.0	8.3–21.0
Total bound B_{12} (ng/ml)								
Mean	83.7	109.4	124.2	103.7	132.5	93.6	108.2	73.6
Range	38.9–170.8	97.0–128.7	110.6–135.3	76.6–134.1	109.6–149.6	66.2–122.6	105.2–111.2	65.7–106.5
IF bound B_{12}: total bound B_{12}								
Mean	8.0	17.8	29.2	27.9	27.7	34.4	79.5	78.7
Range	4.2–14.1	4.7–26.7	12.7–43.9	24.2–31.4	13.2–36.8	18.9–48.9	70.7–88.3	68.1–92.6
GPIMH assays (% enhancement over control)*								
Mean	6	65	65	135	115	103	192	196
Range	−48 to 86	−28 to 109	−23 to 140	97–166	26–203	54–206	185–200	156–236

*Decrease of uptake is shown by minus sign.

Intrinsic Factor

IF was present only in traces (below 6 ng/ml IF-bound B_{12}) in 6 of 10 infants at birth, and in the remaining infants it was below 15 ng/ml (mean 6.8 ng/ml). It was found in small quantities by charcoal immunoassay (mean 20.2 ng/ml) in the gastric juices of all infants 3 days after birth (Table 1). By Day 10–11, all of the gastric juices showed IF concentrations in the range



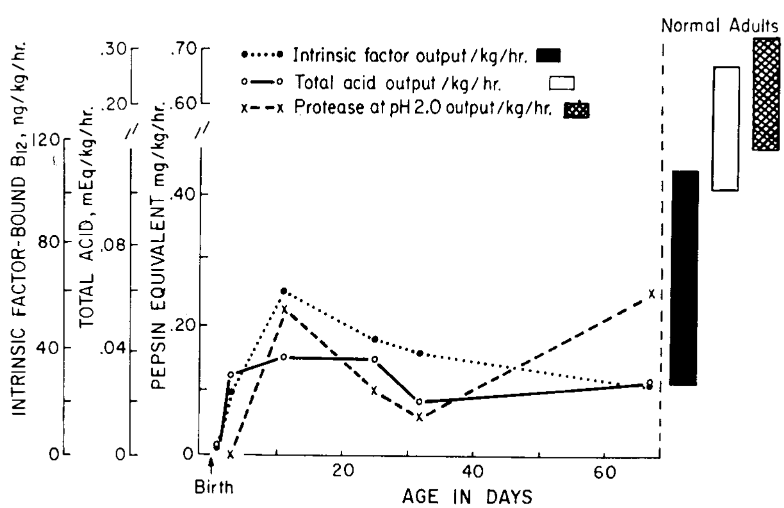
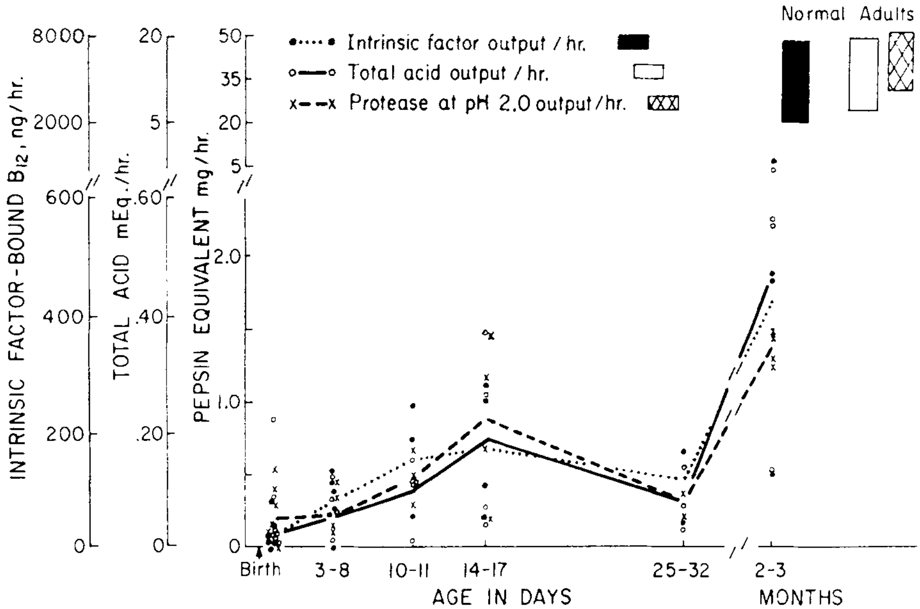


Fig 1 (*top*). Gastric secretion in Infant V. **Fig 2** (*bottom*). Gastric secretion in Infant V corrected for body weight.



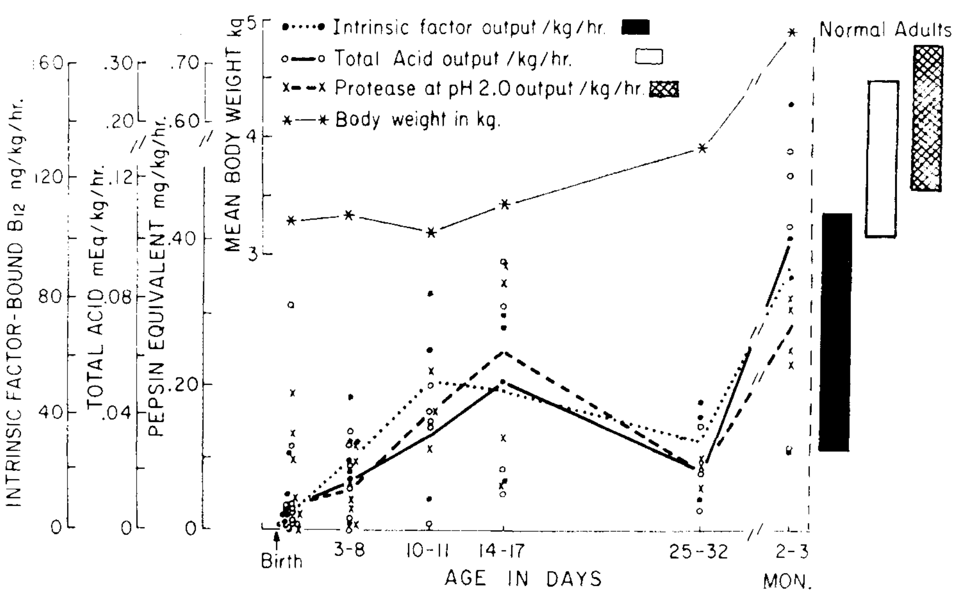


Fig 3 (*top*). Gastric secretion in newborns and infants. **Fig 4** (*bottom*). Gastric secretion in newborns and infants corrected for body weight.

of 15–60 (mean 37 ng/ml)—ie, only slightly below those observed in our laboratory in the gastric juices of normal adults (15–98, mean 58 ng/ml).[15] The concentration of IF remained approximately at this level until the third month.

In each gastric juice, we also calculated the ratio of IF-bound B_{12} to totally bound B_{12} (Table 1). While in 2 children, 4 and 9 years of age, this ratio was above 68% (which is the normal value for adults), it was exceedingly low at birth (mean 8%). There was then a gradual increase to the end of the third month, when it reached 34.4%. At this time, it was in the range seen in adult patients with mild or moderate gastric, atrophic lesions.[17] Thus, the contribution of IF to the total Vitamin B_{12} binding in infants is very small at birth, and although it gradually increases (Table 1), it still lags behind that seen in normal adults. In one infant (Baby R), this ratio was markedly depressed (below 15%) throughout the entire observation period of 1 month.

We have also determined the hourly output of IF after Histalog stimulation (Table 1 and Fig 3). This was markedly lowered throughout the first 3 months of infancy mainly because of the low output of gastric juice. In normal adults the IF output was in the range of 1970–7613 ng B_{12} per hour; and in the two older children (4 and 9 years of age) 2604 and 4910 ng/hr. Yet in infants 1–32 days old, it was below 280 ng/hr, and in the 2- to 3-month-old babies, it was below 660 ng B_{12} per hour (Table 1). The rates of this increase differed in the individual infant. A transient drop at the end of the first month could be detected in all infants on whom we were able to carry out serial observations (Table 1, Figs 1 and 3).

When IF output was calculated in terms of body weight (ie, in nanograms B_{12} bound to IF per hour per kilogram), by the end of the second week of life, it averaged about two times less than in adults with normal gastric mucosa, but approximated their levels during the third month of life (Table 1 and Fig 4). Only in Baby R did the output of IF remain much below normal levels throughout the observation period (below 10 ng/hr/kg as compared to the mean value of 42 ng/hr/kg at the end of the first month in other infants.)

In several infants, the IF assay in gastric juice was done by two methods (Fig 5). As in the adults[13] whenever the GPIMH assays demonstrated IF activity in the gastric juice (percent enhancement of the uptake above 50%), the ratio of IF-bound to total bound B_{12} in the gastric juice was over 15%. This was seen in all babies older than 3 days (with the one exception mentioned above). On the other hand, in the first 24 hr, only 1 of 10 babies demonstrated IF activity on GPIMH, and none had the ratio of IF-bound to totally bound B_{12} in gastric juice above 15%.

Peptic Activity

Only traces of proteolytic activity at pH 2.0 were detected in gastric juice collected 1 day after birth. In the seven infants in whom serial determinations were carried out, there was a very slow increase in pepsin concentration from birth to 3 months, with a transient drop (in 3 infants) occurring at the end of the first month (Table 1).

The mean pepsin output 1 day after birth was 0.18 mg PE/hr, gradually increasing to 1.34 mg PE/hr at 3 months. For comparison, it should be noted that in the 4- and 9-year-old children included in the series, the mean output

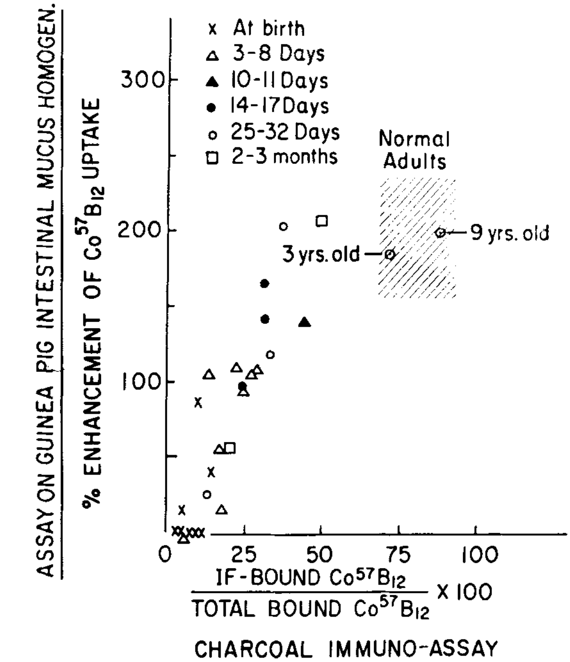


Fig 5. Intrinsic factor in gastric juice of newborns and infants as assayed by two methods.

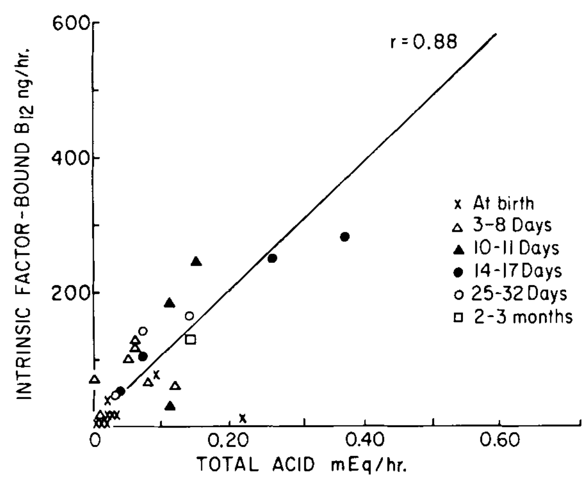


Fig 6. Correlation of output of intrinsic factor and total acid in gastric juice of newborns and infants.

of pepsin was 10–20 times greater than at the end of 2–3 months, while in adults with normal gastric mucosa on biopsy, using the same method, the mean pepsin output was about 30 times greater (Table 1 and Fig 3).

When the mean pepsin values were calculated per kilogram body weight,

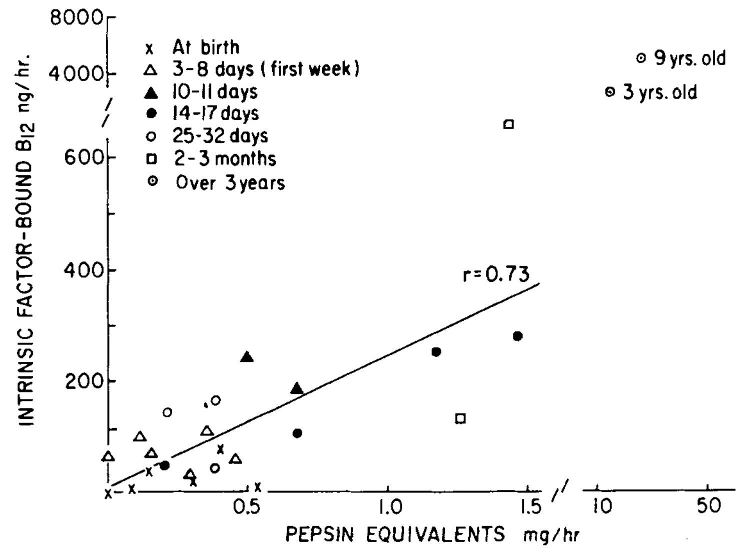


Fig 7. Correlation of output of intrinsic factor and protease activity at pH 2.0 in gastric juice of newborns and infants.

the mean output of the proteolytic enzymes in 3-month-old babies was about half that in adults with normal gastric mucosa—0.28 mg PE/kg/hr vs 0.60 mg PE/kg/hr (Table 1 and Fig 4).

Correlation Between Acid, IF, and Pepsin Secretion

We observed a linear relationship between the outputs of HCl and that of IF in infants, with a coefficient of correlation $r = 0.88$ (Fig 6). This degree of correlation was similar to that observed in adults by Ardeman and Chanarin[22] and Rodbro *et al.*[23]

A similar but somewhat poorer correlation ($r = 0.73$) was noted between the outputs of IF and that of pepsin (Fig 7).

DISCUSSION

In our studies the secretory activity of the fundic glands at birth was very low. While the rate of increase in activity varied from infant to infant, the general trend was that of progressive increase during the first 2–3 weeks of life, followed by a transient drop in all three parameters of secretory activity of fundic glands at the end of the first month. Then, the secretory activity increased and showed a steady rise to the age of 3 months in the majority of infants studied.

Although achlorhydria in the newborn has been noted,[2] with the exception of one infant (Baby R) who had no detectable acid in gastric juice up to 8 days after birth, we have not observed achlorhydria in any of our infants, either at birth or later.

Previous investigators have suggested the possibility that low acid levels at birth may be artifactual in that the ingestion of amniotic fluid by the infant at birth would buffer the acid secreted. In order to obviate this possible artifact, as well as to avoid the disadvantages of preparatory saline gastric washing, we delayed gastric aspiration until a minimum of 12 hr after birth.

To the contrary, several investigators have noted high acid concentrations in 1-day-old infants. We observed a similar relatively high level only in 2 of the 10 one-day-old infants in our series. In one of them the acid concentration was surprisingly high from the first day (57.7 mEq/liter) and remained elevated (55 mEq/liter) for the first 2 weeks of life. Only subsequently did acid concentration fall to a level comparable with other infants in the same age group. Despite the relatively high initial acid concentration, however, acid output remained well below values seen in the children 4 and 9 years old.

Miller,[2] in his study of acid secretion under fasting and postfeeding conditions, noted a decrease in acid appearing about the tenth day of life. He ascribed this phenomenon to the possible effects of "some gastrogenic hormone . . . supplied to the infant in utero, either from the mother, or the placenta." This initial high acid level, followed by its transient decrease, and a similar decrease in HCl, IF, and pepsin secretion occurring in our series during the middle of the first month of life, may perhaps be related to the transplacental transfer of the maternal gastrin to the infant, its circulation in the first few days of life, as well as the exhaustion of its effects after 2–3 weeks. Initial high acid output shortly after birth might be attributed to a direct secretory effect of maternal gastrin on gastric glandular tissue. However, in Miller's study as well as in our series, this transient decrease did not occur until the middle of the first month of life. We would have to consider, therefore, not only a direct stimulatory effect of maternal gastrin on parietal cell secretion at birth, but in addition, a stimulating effect of the maternal circulating gastrin on the growth and development of fundic glands in the newborn which then becomes exhausted. This stimulatory effect of gastrin on the growth of gastric glands has been reported in animal experiments.[24]

In earlier studies,[7] the concentrations of HCl reached by the newborn infants were at almost adult levels, but the total gastric juice volumes were one-tenth of adult levels in the newborn and did not approach adult volumes until 5–6 years of age. In our current observations, the acid output in the first month remained well below adult levels, due to the marked difference in volume of gastric juice secreted and lower acid concentrations.

Ghai *et al*[25] reported a significant correlation ($r = 0.81$) between acid output after augmented histamine stimulation, and body weight of 16 young normal subjects (mean age of 16 years), with a mean maximal acid output of 2.02 mEq/10 kg/hr. Rodbro[23] obtained a similar figure of 2.18 mEq/10 kg/hr in children with an average age of 20 months. In our series, the mean acid output at birth was only 0.15 mEq/10 kg/hr; in the first month it was 0.31 mEq/10 kg/hr, and in 3-month-old babies, 1.22 mEq/10 kg/hr. In adults with normal gastric biopsy specimens, acid output ranged from 1.02 to 2.71 mEq/10 kg/hr.[17, 22]

Thus, although mucosal volume is related directly to stomach size and body weight, and though a close relationship exists between mucosal volume and parietal cell mass,[26] this does not hold true for the acid output of infants below 3 months of age (Fig 2 and 4, and Table 1). The acid secretion in the first months of life appears to be a function of age rather than weight, and lags behind the standards which apply to the later stages of development.

The same is true for IF. IF was not detectable during the first 24 hr, as assayed on GPIMH. By the second week, it was present in five of seven gastric juices, both on charcoal immunoassay, and on GPIMH.

In all infants at the age of 2–3 months, with the exception of one (Baby R), the hourly output of IF after Histalog stimulation (when calculated per kilogram of body weight), was within the range of our 2 older children and comparable to adult normals. The single exception (Baby R) had a markedly low level of both HCl and IF output at birth and throughout the period of the study; pepsin output was likewise low when compared to infants in the same age groups until the age of 1 month, when pepsin output per kilogram body weight became comparable to similar-aged infants. This raised the possibility that this infant represented a prospective candidate for juvenile form of pernicious anemia, due to a developmental defect in the gastric mucosa, or an early atrophic lesion which will be further studied.

McIntyre *et al*[27] estimated that approximately 40 ng of B_{12} may be the daily requirement for a 3-kg baby. Our data listed in Table 1 indicate that by the second week, the average infant has an IF output sufficient, in spite of its low level, to ensure absorption of an adequate amount of Vitamin B_{12}.

In megaloblastic anemia of infancy, subsequently known to be due to folate deficiency, Luhby[6, 7] showed that IF output was high. When ground beef was added to the infant's gastric juice after histamine stimulation, there was sufficient IF in 30 ml of the gastric juice aspirated from a 7-month-old infant with megaloblastic anemia in relapse, to produce a classic reticulocyte response in an adult with pernicious anemia in relapse, as described in the original Castle[28] experiment.

Pepsin secretion in infants showed a wider range of variation than that of HCl and IF (Fig 3). This scatter of peptic activity became less pronounced in the older infants. However, in spite of the progressive rise in pepsin levels with increasing age, pepsin output at the age of 3 months remaind well below adult values, even when calculated on a weight basis. In the study by Grayzel *et al*[8] of plasma pepsinogen levels in the newborn, a higher level was reported in the first week of life as compared to levels found in children 3 weeks and older. We have found a somewhat similar pattern in the present study of pepsin output in gastric juice where, at the age of 2 weeks, pepsin output per kilogram body weight was higher than in any other previous period or even at the age of 4 weeks. At the age of 3 months, pepsin output was only slightly higher (0.28/mg/hr/kg) and remained well below the levels seen in older normal children and normal adults.

Closely related to pepsin,[29] glandular mucoprotein fraction showed similar patterns in our earlier studies.[7] In 8 normal newborn infants, the fasting glandular mucoprotein levels were 15–26 mg/100 ml, while in 10 older infants and children ranging in age from 2 months to 8 years, they ranged from 48 to 110 mg/100 ml. The higher levels usually were found after 1 year of age. Although pepsin is needed for the curdling of lactalbumin, it is possible that the induction of pepsin production in infants is retarded until more protein is included in the diet at the later stage of infancy.

REFERENCES

1. Hess, A. F. The gastric secretion of infants at birth. *Amer J Dis Child 6:*264, 1913.
2. Miller, R. A., Observations on the gastric acidity during the first month of life. *Arch Dis Child 16:*22, 1941.
3. Ebers, D. W., Smith, D. I., and Gibbs, G. E. Gastric acidity on the first day of life. *Pediatrics 18:*800, 1956.
4. Avery, G. B., Randolph, J. G., and Weaver, T. Gastric acidity in the first day of life. *Pediatrics 37:*1005, 1966.
5. Luhby, A. L. Studies of extrinsic-intrinsic factor relationships in megaloblastic anemia of infancy. *Ohio State Health Center J 2:*35, 1948.
6. Luhby, A. L. Megaloblastic anemia of infancy. *J Pediat 54:*617, 1959.
7. Luhby, A. L., Glass, G. B. J., and Slobody, L. B. Studies of the gastric mucoprotein secretion in infants and children. *AMA J Dis Child 88:*517, 1954.
8. Grayzel, H. G., Elkan, B., Moghazeh, M., Schneck, L., and Garza, S. Plasma pepsinogen levels in the newborn. *Amer J Dis Child 103:*759, 1962.
9. Rodbro, P., Krasilnikoff, P. A., and Christiansen, P. M. Parietal cell secretory function in early childhood. *Scand J Gastroent 2:*209, 1967.
10. Rodbro, P., Krasilnikoff, P. A., and Bitsch, V. Gastric secretion of pepsin in early childhood. *Scand J Gastroent 2:*257, 1967.
11. Hoedemaeker, P. J. Investigations of the Site of Production of Castle's Gastric Intrinsic Factor. Thesis, University of Groningen, 1965.
12. Glass, G. B. J. Gastric intrinsic factor and its function in the metabolism of vitamin B_{12}. *Physiol Rev 43:*529, 1963.
13. Spurling, C. L., Sacks, M. S., and Jiji, R. M. Juvenile pernicious anemia. *New Eng J Med 271:*995, 1964.
14. Kirsner, J. B., and Ford, H. The gastric secretory response to Histalog. *J Lab Clin Med 46:*307, 1955.
15. Ardeman, S., and Chanarin, I. A method for the assay of human gastric intrinsic factor and for the detection and titration of antibodies against intrinsic factor. *Lancet 2:*1350, 1963.
16. Gottlieb, C., Lau, K. S., Wasserman, L. R., and Herbert, V. Rapid charcoal assay for

intrinsic factor (IF), gastric juice unsaturated B_{12} binding capacity, antibody to IF, and serum unsaturated B_{12} binding capacity. *Blood 25:*875, 1965.
17. YAMAGUCHI, N., and GLASS, G. B. J. The determination of intrinsic factor in gastric secretory analysis. *Ann NY Acad Sci 140:*924, 1967.
18. SULLIVAN, L. W., HERBERT, V., and CASTLE, W. B. *In vitro* assay for human intrinsic factor. *J Clin Invest 42:*1443, 1963.
19. CASTRO-CUREL, Z., and GLASS, G. B. J. Assay of intrinsic factor activity on guinea pig intestinal mucosa homogenate. *Proc Soc Exp Biol Med 112:*715, 1963.
20. CASTRO-CUREL, Z., and GLASS, G. B. J. Comparative study of IF activity of gastric materials by urinary excretion test *in vivo* and on guinea pig intestinal mucosa homogenates *in vitro. Clin Chim Acta 9:*317, 1964.
21. KLOTZ, A. P., and DUVALL, M. R. The laboratory determination of pepsin in gastric juice with radio-iodinated albumin. *J Lab Clin Med 50:*753, 1957.
22. ARDEMAN, S., CHANARIN, I., and DOYLE, J. C. Studies on secretion of gastric intrinsic factor in man. *Brit Med J 2:*600, 1964.
23. RODBRO, P., and CHRISTIANSEN, P. M. Quantitative estimation of parietal cell secretory function in man. *Scand J Gastroent 1:*292, 1966.
24. CREAN, G. P. Experimental hyperplasia of the gastric mucosa. British Society of Gastroenterology, Dublin, November 1967.
25. GHAI, P., SINGH, M., WALIA, B. N. S., and GADEKAR, N. G. An assessment of gastric acid secretory response with 'maximal' augmented histamine stimulation in children with peptic ulcer. *Arch Dis Child 40:*77, 1965.
26. CREAN, G. P. "Observations on the Regulation of the Growth of the Gastric Mucosa." In *Gastric Secretion: Mechanisms and Control* (Proceedings of a Symposium, University of Alberta, Canada), Shnitka, T. K., Gilbert, J. A. L., and Harrison, R. C., Eds. Pergamon, New York, 1967, p. 33.
27. MCINTYRE, P. A., HAHN, R., CONLEY, C. L., and GLASS, G. B. J. Genetic factors in predisposition to pernicious anemia. *Bull Hopkins Hosp 104:*309, 1959.
28. CASTLE, W. B. Observations on the etiologic relationship of achylia gastrica to pernicious anemia. I. The effect of the administration in patients with pernicious anemia of the contents of the normal human stomach recovered after the ingestion of beef muscle. *Amer J Med Sci 178:*748, 1929.
29. GLASS, G. B. J. Current status of the "glandular mucoprotein" and "mucoproteose" fractions of the gastric mucin: A review of 15 years' progress in this area. *Ann NY Acad Sci 140:*804, 1967.