JCI The Journal of Clinical Investigation

Development of Intestinal Adenyl Cyclase and Its Response to Cholera Enterotoxin

Richard J. Grand, … , Frank M. Torti, Stephanie Jaksina

J Clin Invest. 1973;52(8):2053-2059. https://doi.org/10.1172/JCI107389.

Research Article

Adenyl cyclase activity in intestinal membranes has been studied during development in the rabbit fetus from fetal day 17 to 10 days postnatally and in the human fetus from the 10th to the 17th wk of gestation. In the rabbit, the enzyme was already present by fetal day 17 and showed a fourfold peak rise in specific activity by 22 days. By 28 days, the specific activity had fallen toward adult levels and remained constant throughout gestation and the 1st wk of life. Fluoridestimulated activity showed a similar curve, and was 2.5-5 times the basal values. Activities in jejunum and ileum were comparable at all time points studied. Phosphodiesterase activity did not change during gestation. When fetal intestinal segments were incubated in vitro with purified cholera enterotoxin, adenyl cyclase activity in subsequently prepared membranes was increased two- to threefold. This level was not regularly further elevated by fluoride ion. Lithium ion inhibited both the basal and fluoride-stimulated enzyme activity in membranes prepared from rabbit fetuses at term. Lactase activity (reflecting the development of the microvilli) in either whole intestinal homogenates or in the membrane fractions showed a differnet pattern of development, with a rise beginning on fetal day 24 and a plateau just after birth. In intestinal membranes prepared from human fetuses, the activity of both basal and fluoride-stimulated adenyl […]

Development of Intestinal Adenyl Cyclase and Its Response to Cholera Enterotoxin

RICHARD J. GRAND, FRANK M. TORTI, and STEPHANIE JAKSINA

From the Department of Pediatrics, Harvard Medical School, and the Department of Medicine (Division of Clinical Nutrition), the Children's Hospital Medical Center, Boston, Massachusetts 02115

ABSTRACT Adenyl cyclase activity in intestinal membranes has been studied during development in the rabbit fetus from fetal day 17 to 10 days postnatally and in the human fetus from the 10th to the 17th wk of gestation. In the rabbit, the enzyme was already present by fetal day 17 and showed a fourfold peak rise in specific activity by 22 days. By 28 days, the specific activity had fallen toward adult levels and remained constant throughout gestation and the 1st wk of life. Fluoride-stimulated activity showed a similar curve, and was 2.5–5 times the basal values. Activities in jejunum and ileum were comparable at all time points studied. Phosphodiesterase activity did not change during gestation. When fetal intestinal segments were incubated in vitro with purified cholera enterotoxin, adenyl cyclase activity in subsequently prepared membranes was increased two- to threefold. This level was not regularly further elevated by fluoride ion. Lithium ion inhibited both the basal and fluoride-stimulated enzyme activity in membranes prepared from rabbit fetuses at term. Lactase activity (reflecting the development of the microvilli) in either whole intestinal homogenates or in the membrane fractions showed a different pattern of development, with a rise beginning on fetal day 24 and a plateau just after birth. In intestinal membranes prepared from human fetuses, the activity of both basal and fluoride-stimulated adenyl cyclase tripled from the 10th to the 17th wk of gestation. The data both in the rabbit and in man show that intestinal adenyl cyclase is capable of responding to cholera enterotoxin quite early in gestation. In the rabbit, this occurs before the time of appearance or villi or of an enzyme marker (lactase) for microvilli. The results support the concept that adenyl cyclase is present in plasma membrane other than the brush border.

INTRODUCTION

Cyclic AMP has been shown to mediate the secretory response of the small intestine to cholera enterotoxin (1–6). It has also been suggested that enterotoxins elaborated by bacteria associated with other forms of diarrhea may exert their effects by stimulating cyclic AMP production (7, 8). Indeed, this concept is supported by the recent recognition that enterotoxin from certain strains of *Escherichia coli* stimulates adenyl cyclase activity in rabbit ileal mucosa (9).

Clinically, cholera is rare in infants younger than 1 yr of age, presumably because of passively acquired immunity and repeated, clinically insignificant exposure to the vibrio (10). Nevertheless, studies of infants of this age have failed to reveal circulating agglutinating or vibriocidal antibodies (11). No experimental data are available regarding the response of the human intestine to cholera enterotoxin in this age group. However, failure of the neonatal intestine to produce a secretory response to the toxin has been reported in some animals (12, 13). (The levels of cyclic AMP have not been measured.) If this is the explanation for the absence of cholera in this age group in humans, it is of special interest, since infants are clearly susceptible to the effects of *E. coli* enterotoxin (8).

Because very little is known about the activity of adenyl cyclase in the intestine of the fetus or newborn, experiments were undertaken to establish the pattern of development of this enzyme in the rabbit, and in man, and to relate the appearance of activity to sensitivity to cholera enterotoxin. The data show that the

Presented in part at the meeting of the Society for Pediatric Research, Washington, D. C., 25 May 1972, and published in abstract form (*Pediatr. Res.* 1972. **6**: 374.).

Dr. Grand is a recipient of an Academic Career Development Award (5-KO7-AM44590) from the National Institute of Arthritis, Metabolism, and Digestive Diseases.

Received for publication 27 November 1972 and in revised form 5 April 1973.

response to the toxin is a characteristic of the enzyme early in development and is of a magnitude comparable with that seen during maturity.

METHODS

Animals and tissue preparation. Fetuses were obtained from timed pregnancies in New Zealand white rabbits. Only the second through fifth pregnancies in a given doe were utilized. Does were anesthetized with sodium pentobarbital and fetuses rapidly delivered by cesarean section. There were approximately 8–10 fetuses per litter. Human fetal intestine was obtained during therapeutic abortions. Fetal intestine was quickly stripped away from the serosa. Histological examination of the resulting tissue revealed that it represented intact mucosa and approximately one-half the thickness of the muscularis. All further procedures were as performed by Kimberg, Field, Johnson, Henderson, and Gershon (3), with modifications as described below. The intestine thus obtained was quickly rinsed in iced saline, gently blotted and weighed; it was then minced on an iced square of aluminum foil and homogenized in 50 vol of 20 mM glycylglycine buffer, pH 7.8, containing 0.25 M sucrose and 1 mM $MgSO_4$, using a glass homogenizer with three passes of a Teflon pestle. A pellet was sedimented at 2,000 *g* for 10 min and washed twice and resuspended in 20 mM glycylglycine buffer, pH 7.8 containing 1 mM $MgSO_4$ (3). The final pellet was suspended by vortexing in the same buffer in a volume equivalent to twice the weight of the original intestine. This suspension usually contained 6.0–15 mg of protein per ml, as determined by the method of Lowry, Rosebrough, Farr, and Randall (14).

Enzyme assays. Adenyl cyclase activity was measured exactly as described by Kimberg et al. (3) appropriately modified for use with a smaller quantity of enzyme protein. The incubation mixture contained 50 mM Tris-HCl buffer at pH 7.4, 5 mM $MgCl_2$, 10 mM caffeine, 2 mM ATP, 20 μCi/ml of [8-^{14}C]ATP and an ATP-regenerating system composed of 10 mM phosphoenolpyruvate and 250 μg/ml of pyruvate kinase. When NaF was used in an assay, the concentration in the final mixture was 10 mM. Including additives, the final volume of the incubation mixture was 50 μl. The usual concentration of enzyme was 0.1–0.3 mg of protein per assay. Because of the small volume of incubation mixture, after additions had been made to the assay tubes (10 × 75 mm) they were centrifuged at 4°C for 1 min at 200 *g* before enzyme was introduced. Incubation (performed in triplicate) was begun by adding 20 μl of the enzyme suspension and was continued for 10 min at 37°C. Immediately before terminating the reaction, 0.2 ml of a solution containing 0.6 mg/ml of cyclic 3′,5′-AMP (cyclic AMP) and 1.25 μCi/ml of [^{3}H]cyclic AMP was added to each tube. The reaction was then stopped by boiling for 3 min. Further extraction procedures, counting and calculations, were exactly as described by Kimberg et al. (3). A Packard scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.) was used for counting the samples.

Cyclic nucleotide phosphodiesterase activity was measured by a technique which assayed the disappearance of [^{3}H]-cyclic AMP during conversion to adenosine 5′-monophosphate. The incubation mixture contained concentrations of Tris-HCl buffer, pH 7.4, $MgCl_2$ and caffeine identical with those in the mixture for the adenyl cyclase assay. In addition, the mixture contained 2.0 μM cyclic AMP and 0.4 μCi/ml of [^{3}H]cyclic AMP. The final volume of the reaction mixture was 0.25 ml per assay. The enzyme protein initiating the reaction was between 1.2–2.0 mg per assay. Further procedures were as described by Kimberg et al. (3).

Lactase activity was determined by a modification (15) of the method of Messer and Dahlqvist (16). Assays were performed both on the membrane fraction used for the adenyl cyclase determinations and on mucosal homogenates prepared in 30 vol of 5 mM EDTA, pH 7.4. Results were

TABLE I

Activity of Adenyl Cyclase in Rabbit Jejunum and Ileum

	Whole jejunal homogenate		Membrane fraction			
			Jejunum		Ileum	
Age of animals	Basal	Flouride	Basal	Flouride	Basal	Flouride
days	*nmol cAMP formed/min per mg protein*					
Fetus						
17	0.015	0.084	0.029	0.107	—	—
22	0.027	0.105	0.120	0.324	0.094	0.254
25	—	—	0.084	0.236	0.076	0.190
28	0.016	0.059	0.028	0.142	—	—
Newborn						
5	—	—	0.021	0.150	0.020	0.101
Adult						
62	—	—	0.009	0.040	0.004	0.020

Pooled jejunal membranes prepared from one entire litter (8–10 animals) were studied at each time point during development; adult values are the average of three separate experiments in mature rabbits (jejunum), or a single animal (ileum).

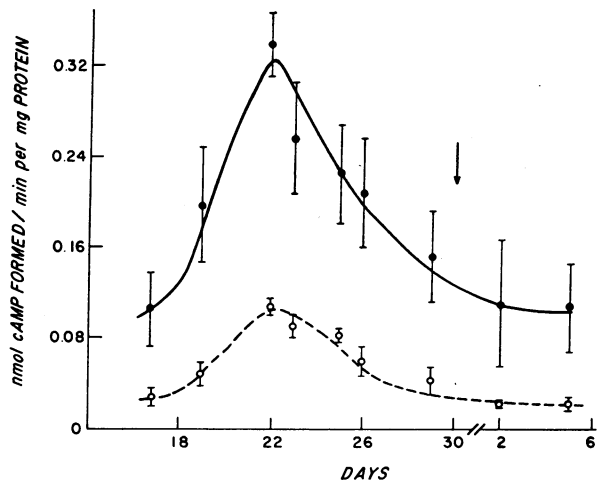

FIGURE 1 Developmental pattern of rabbit jejunal adenyl cyclase activity expressed as a function of gestational age. Pooled jejunal membranes from a full litter (8–10 animals) were used in each experiment and three separate experiments were performed at each time point. Results shown are means ±SEM. ○, Basal enzyme activity, ●, fluoride-stimulated enzyme activity. The arrow indicates the day of birth.

expressed as nmoles of glucose liberated/minute per milligram protein.

Incubations with cholera enterotoxin. Intestinal mucosa was obtained as described above. Approximately 75 mg of minced tissue was added to 25-ml Ehrlenmeyer flasks containing 3.0 ml of Medium 199 (Microbiological Associates, Inc., Bethesda, Md.) and 1.0 μg/ml of highly purified cholera enterotoxin.[1] Control flasks contained no enterotoxin. Flasks were gassed with 95% O_2-5% CO_2, sealed, and incubated at 37°C in a Dubnoff metabolic shaker (80 rpm). After 90 min, flasks were chilled, the contents rapidly transferred to homogenizing tubes, and centrifuged in the cold at 600 *g* for 3 min to sediment the tissue. Homogenization and further procedures were as described above.

Materials. Pyruvate kinase (type II) and phosphoenolpyruvate were obtained from Sigma Chemical Co. (St. Louis, Mo.). ATP was a product of Worthington Biochemical Corp. (Freehold, N. J.). Cyclic AMP and [^{3}H]cyclic AMP (14.5 Ci/mmol) were purchased from Schwarz/Mann (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.) and [8-^{14}C]ATP (44.9 or 52 mCi/mmol) from New England Nuclear (Boston, Mass.).

RESULTS

The activity of adenyl cyclase in the jejunum and ileum of fetal and newborn rabbits at selected time points is shown in Table I. In the membrane fractions, basal- and fluoride-stimulated enzyme activities were comparable at both sites, with a maximum value at 22 days of gestation and a fall thereafter as gestational age increased. Fluoride-stimulated activity was 2.5–7 times the basal values. By contrast, the adenyl cyclase activity of the crude jejunal homogenates did not change as markedly, showing a level on day 22 less than twice that on days 17 and 28. Because the large quantity of adherent meconium interfered with uniform preparation of fetal ileal membranes, all further experiments employed the jejunum only.

The full spectrum of the development of adenyl cyclase activity is shown in Fig. 1, plotted as a function of gestational age. Similar curves were obtained when the data were plotted against crown-rump (C-R) length. In the youngest rabbit fetuses studied (17 day, 2.0 cm C-R length) enzyme activity was nearly equal to that at 5 days of age. Basal activity then rose progressively, reaching a peak on the 22nd day of gestation (5.2 cm C-R length), and fell thereafter to a plateau at the time of delivery (a level that later diminished slowly toward adult values [Table I]). Fluoride-stimulated enzyme activity showed a similar curve with a peak at 22 days and levels 2.5–5 times the basal values.

Because of the possibility that these changes in adenyl cyclase activity (measured by the accumulation of labeled cyclic AMP) were merely reflections of changes in the levels of cyclic nucleotide phosphodiesterase recoverable in the membranes at various stages of development, the activity of the latter enzyme was measured (Table II). Although phosphodiesterase activity was slightly higher after birth, at no time during development was the activity variable enough to explain the observed changes in adenyl cyclase activity.

The effects of cholera enterotoxin on adenyl cyclase activity during development are shown in Table III.

TABLE II
Residual Phosphodiesterase Activity in Fetal Jejunal Membranes

Age of animals	Disappearance of cAMP
days	*nmol/min per mg protein*
Fetus	
19	0.008
23	0.002
26	0.007
Newborn	
6	0.011
Adult	
65	0.010

Pooled jejunal membranes prepared from one entire litter (8–10 animals) were studied at each time point during development, but only a single adult was utilized.

[1] Lot 1071. Prepared under contract for the National Institute of Allergy and Infectious Diseases (NIAID) by R. A. Finkelstein, Ph.D., The University of Texas Southwestern Medical School, Dallas, Tex.; essentially according to procedure described in *J. Infect. Dis.* 1970. **121** (Suppl.): S 63.

TABLE III

Effect of In Vitro Incubation with Cholera Enterotoxin on Adenyl Cyclase Activity during Development

Age of animals	Control		Cholera enterotoxin	
	Basal	Fluoride	Basal	Fluoride
days	*nmol cAMP formed/min per mg protein*			
Fetus				
17	0.023	0.072	0.079	0.072
19	0.030	0.093	0.092	0.117
26	0.078	0.203	0.148	0.254
Newborn				
4	0.023	0.093	0.104	0.123

Jejunal mucosa obtained from 8 to 10 fetuses at each time point was minced and incubated for 90 min in the absence (control) or presence of enterotoxin (1 μg/ml). Membranes were subsequently prepared as described under Methods.

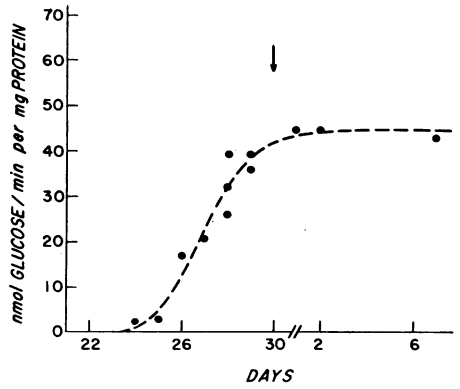

FIGURE 2 Developmental pattern of rabbit jejunal lactase activity expressed as a function of gestational age. Each point represents an homogenate prepared from the pooled jejuna of one complete litter (8–10 animals). The arrow indicates the day of birth.

At all time points studied, the toxin increased adenyl cyclase activity two- to fivefold over the basal levels. The addition of fluoride ion to the membrane preparation was inconstantly associated with further elevations of adenyl cyclase activity over the level achieved with enterotoxin alone.

Field, Fromm, Al-Awqati, and Greenough have shown that in the presence of cholera enterotoxin in vitro short circuit current is increased only after a lag period of about 30 min (6), and recent experiments have demonstrated that a similar lag exists in the rise of intracellular cyclic AMP concentrations.[2] In the present studies, fetal intestinal membranes obtained on day 22 of gestation and incubated with cholera enterotoxin as described under Methods (except with additional samples assayed at earlier time points) showed a lag period of 30 min before the rise in intracellular cyclic AMP concentrations and then the expected response by 90 min: (control tissues incubated without enterotoxin: 6.67 nmol/mg protein; tissues incubated with enterotoxin: 21.36 nmol/mg protein).[3]

TABLE IV

Effect of Lithium on Adenyl Cyclase Activity

Experimental conditions	cAMP produced	
	Basal	Fluoride
	nmol/min per mg protein	
Control	0.016±0.001	0.082±0.002
NaCl, 60 mM	0.018±0.001	0.080±0.002
KCl, 60 mM	0.015±0.001	0.078±0.002
LiCl, 60 mM	0.009±0.001*	0.021±0.002‡

Membranes were prepared from one complete litter at term, and incubated in various test solutions with and without NaF (10 mM).

* $P < 0.02$ compared with control.

‡ $P < 0.01$ compared with control.

Because of the reported effects of lithium ion on intestinal function (18) it was of interest to study the inhibition of adenyl cyclase activity produced by this ion in fetal intestinal membranes (Table IV). At concentrations known to inhibit the enzyme in the thyroid (19), fetal intestinal adenyl cyclase activity was significantly reduced and the fluoride-stimulated rise was inhibited.

In an effort to obtain information regarding the site of localization of fetal adenyl cyclase, the timing of the appearance of the enzyme was compared with that for lactase (Fig. 2), whose developmental pattern in the rabbit and relationship to the brush border of the intestinal epithelial cell has been well-established. As shown previously by others (20) and in the present study, lactase activity first appeared on day 24 of gestation and rose progressively with increasing fetal age, reaching a peak and plateau just after birth (Fig. 2). The patterns were the same when the whole homogenate (Fig. 2) was assayed as when the membranes used for the adenyl cyclase determination were assayed (although in the latter preparation the specific activity was regularly twofold higher). This peak in lactase activity contrasts with that in adenyl cyclase activity occurring much earlier (Fig. 1) and suggests that the two enzymes are localized at separate sites in the epithelial cell.

[2] Field, M. Personal communication.

[3] Assays of tissue cyclic AMP content performed by the Gilman binding assay (17) in the laboratory of Dr. Michael Field.

The pattern of development of adenyl cyclase in normal human fetuses is shown in Fig. 3. The average basal activity rose more than twofold from approximately the 10th to the 17th wk of gestation (4.4–12.3 C-R length). Fluoride-stimulated activity rose concomitantly and ranged from 3–10 times the basal values. When human fetal intestine was incubated in vitro with cholera enterotoxin (Table V) a similar result was obtained as with rabbit intestine. In human ileum the toxin produced a twofold rise in basal adenyl cyclase activity; the addition of fluoride did not produce a further increase in activity over the control.

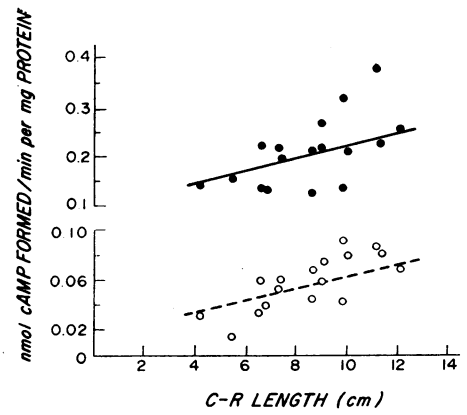

FIGURE 3 Developmental pattern of human jejunal adenyl cyclase activity expressed as a function of C-R length. Each point represents data obtained from the jejunal membranes of a single fetus. ○, Basal enzyme activity; ●, fluoride-stimulated activity. Each calculated regression line has a positive slope which is statistically significant ($P < 0.01$, basal enzyme activity; $P < 0.05$, fluoride-stimulated activity.)

DISCUSSION

Although studies of the development of adenyl cyclase activity in animal brain (21), liver (22, 23), muscle, and kidney (23) are available, no data have been presented heretofore concerning either the developmental pattern of adenyl cyclase in the intestine or the effect of cholera enterotoxin on the intestine of the fetus or newborn.

In the mature intestine, the role of cyclic AMP in mediating the effects of cholera enterotoxin has been established, and the biochemical events triggered by the cyclic AMP have been reviewed recently (3, 4, 6). Application of the enterotoxin in vitro to the luminal surface of the small intestine produces (after a lag of 30 min) an increase in mucosal adenyl cyclase activity and a gradual rise in short circuit current and in intracellular concentrations of cyclic AMP. This is followed by an inhibition of sodium transport from mucosa to serosa and marked stimulation of chloride secretion from serosa to mucosa. The secretory changes are identical to those produced when cyclic AMP is applied directly to the mucosal surface (4).

The current studies document that the small intestine contains adenyl cyclase activity very early in development in both the rabbit and man, and show that the pattern of maturation is identical in the presence or absence of sodium fluoride. Since the sharp rise at day 22 in the rabbit is in the specific activity of the enzyme in the membrane fraction, but not in the homogenate, it suggests that there is considerable enrichment of the membrane preparations with increasing quantities of the enzyme from day 17 to 22. While the possibility of the presence of varying quantities of muscularis in the membrane preparations must be considered as a source of nonmucosal adenyl cyclase activity, the contamination could not have been sufficient to account for the fivefold difference between peak activity and that before and after the peak (Fig. 1).

The end of the second trimester is a critical point in the differentiation of structure and function in the rabbit intestine (24). Before day 21, the luminal surface of the small intestine is smooth, with only some longitudinal folds; the mucosa is composed of a double layer of epithelial cells. No villi or crypts are present, and the mature microvillus membrane has not formed. During the next 9 days, an enormous increase in surface area occurs due to the formation of villi, the proliferation of the microvilli, and increases in the length and diameter of the intestine. On day 22 villi begin to appear; from day 23 to 25 distinct villi develop; on day 25 primordial crypts appear, and the luminal surface of the villus epithelial cells contains short and irregularly spaced microvilli. Over the next 5 days, the intestine assumes a nearly mature appearance: villi become tall and straight, the microvilli increase in number,

TABLE V

Effect of In Vitro Incubation with Cholera Enterotoxin on Adenyl Cyclase Activity in Human Fetal Ileum

Fetal age	Experimental condition	Adenyl cyclase activity	
		Basal	Fluoride
cm		*nmol cAMP formed/ min per mg protein*	
8.9	Control	0.048	0.145
	Cholera enterotoxin	0.099	0.102
13.5	Control	0.056	0.180
	Cholera enterotoxin	0.100	0.157

Minced mucosa from each fetus was incubated for 90 min in the absence (control) and presence of cholera enterotoxin (1 μg/ml), and membranes subsequently prepared as described under Methods.

lengthen, and are regularly spaced, but the crypts are still less deep than in the adult (24). Biochemical differentiation also occurs during this period. The absorption of α-methyl glucoside against a concentration gradient, which is just barely detectable at 22 days' gestation, increases eightfold toward term, as does the rate of absorption of valine. Methionine absorption increases fourfold (24). Coincidental with the appearance of the microvilli, lactase activity becomes detectable, but its peak does not occur until just after birth (20, and see Fig. 3).

As shown in the present studies, the appearance of adenyl cyclase activity occurs much earlier than that of lactase, and the activity peak of adenyl cyclase at day 22 is well in advance of the structural and functional differentiation described above. Although there are no detailed studies of the rabbit fetal intestine before day 22, extrapolation to this time point from data obtained in the rat (25) would suggest that the period up to the appearance of the villi is a time of active cell proliferation (although not of differentiation). In the rat, for example, the activities of aspartate transcarbamylase and thymidine kinase are at a peak just before or at the appearance of the microvilli, and then fall abruptly to adult levels at the time of birth (25). At this time, cell proliferation becomes limited to the crypt region (26). Since, in the rabbit, adenyl cyclase activity peaks before the appearance of villi and before the appearance of lactase (an enzyme marker for the microvilli), and since adenyl cyclase activity then falls while lactase activity is rapidly rising, and while, morphologically, the microvilli are rapidly increasing in number and complexity, it seems reasonable to conclude that the two enzymes are developing in separate sites, and that adenyl cyclase is probably not localized to the brush border. If this is so, the rise in adenyl cyclase activity before day 22 must correlate with cell proliferation, whereas the fall in activity after day 22 must represent a sizable and increasing dilution of the crude membrane preparation by adenyl cyclase-poor membranes, very likely the microvillus membranes.

Further evidence that adenyl cyclase is not limited to the brush border can be obtained from the present studies of the effects of cholera enterotoxin. The responses of rabbit fetal intestine to this agent (Table III) suggest that the enzyme is fully differentiated early in development (with characteristics comparable with those of the mature enzyme), and that the villi and microvilli do not mediate the responses of adenyl cyclase to the toxin, because, from the 17th to 22nd days of gestation, neither villi nor microvilli are present, and toxin-stimulated increases in adenyl cyclase activity and intracellular concentrations of cyclic AMP can be obtained. In the human fetal intestine, similar segregation of the responses to cholera enterotoxin and morphological development was not possible because villi, microvilli, and enzyme markers for the microvillus membrane (27) were already well-developed in the 4.5 cm fetus. At present, the specific membrane locus for adenyl cyclase is unknown and definitive evidence for the cell population particularly affected by the toxin is not available. It is possible that there are a finite number of sites on the luminal surface (e.g., in the intervillous spaces) that are receptors for cholera enterotoxin or loci for adenyl cyclase (and the progressive reduction in adenyl cyclase activity, which occurs as the villi and microvilli develop, is compatible with such an hypothesis). However, specific loci on the lateral cell membrane may also exist, as recently postulated by Parkinson, Ebel, DiBona, and Sharp (28).

The sensitivity of adenyl cyclase to lithium ion has been shown in other tissues (19, 29). In the present studies, lithium markedly reduced basal adenyl cyclase activity and inhibited the expected fluoride-stimulated increase. Preliminary experiments in this laboratory reveal that preincubation of intestinal mucosa with lithium chloride does not inhibit the subsequent effect of cholera enterotoxin on intestinal membranes, but in the thyroid, it has been shown that lithium ion prevents the thyroid-stimulating hormone (TSH) stimulated rise in adenyl cyclase activity (19), while in adipose tissue, basal, ACTH-stimulated, and fluoride-stimulated adenyl cyclase activities are reduced by the ion (29). Appropriate further experiments in fetal intestine are currently in progress.

The present studies demonstrate that the sensitivity of intestinal adenyl cyclase to cholera enterotoxin is present early in development both in the rabbit and in man. Thus, despite data obtained in the mouse (13) and rat (12), it would seem apparent that the failure of human infants to develop cholera cannot be due merely to alterations in the generation of cyclic AMP.

ACKNOWLEDGMENTS

The authors are indebted to Doctors Michael Field and Daniel V. Kimberg for critical review of work in progress; to Dr. Shirley G. Driscoll, Boston Hospital for Women, for preparing the human fetuses; to Dr. Yvonne Bishop for statistical analyses; and to Mss. Marci Moore, Antonia Henderson, and Natalie Daniels for expert technical assistance.

These studies were supported by U. S. Public Health Service Grant, AM 14523, from the National Institute of Arthritis, Metabolism, and Digestive Diseases.

REFERENCES

1. Schafer, D. E., W. D. Lust, B. Sircar, and N. D. Goldberg. 1970. Elevated concentration of adenosine 3′: 5′-cyclic monophosphate in intestinal mucosa after treatment with cholera toxin. *Proc. Natl. Acad. Sci. U. S. A.* **67**: 851.

2. Sharp, G. W. G., and S. Hynie. 1971. Stimulation of intestinal adenyl cyclase by cholera toxin. *Nature (Lond.)*. **229**: 266.
3. Kimberg, D. V., M. Field, J. Johnson, A. Henderson, and E. Gershon. 1971. Stimulation of intestinal mucosal adenyl cyclase by cholera enterotoxin and prostaglandins. *J. Clin. Invest.* **50**: 1218.
4. Field, M. 1971. Intestinal secretion: effect of cyclic AMP and its role in cholera. *N. Engl. J. Med.* **284**: 1137.
5. Chen, L. C., J. E. Rohde, and G. W. G. Sharp. 1972. Properties of adenyl cyclase from human jejunal mucosa during naturally acquired cholera and convalescence. *J. Clin. Invest.* **51**: 731.
6. Field, M., D. Fromm, Q. Al-Awqati, and W. B. Greenough, III. 1972. Effect of cholera enterotoxin on ion transport across isolated ileal mucosa. *J. Clin. Invest.* **51**: 796.
7. Pierce, N. F., and C. K. Wallace. 1972. Stimulation of jejunal secretion by a crude Escherichia coli enterotoxin. *Gastroenterology.* **63**: 439.
8. Gorbach, S. L., and C. M. Khurana. 1972. Toxigenic *Escherichia coli:* a cause of infantile diarrhea in Chicago. *N. Engl. J. Med.* **287**: 791.
9. Evans, D. J., Jr., L. C. Chen, G. T. Curlin, and D. G. Evans. 1972. Stimulation of adenyl cyclase by *Escherichia coli* enterotoxin. *Nat. New Biol.* **236**: 137.
10. Carpenter, C. C. J., Jr., and N. Hirschorn. 1972. Pediatric cholera: current concepts of therapy. *J. Pediatr.* **80**: 874.
11. Mosley, W. H., A. S. Benenson, and R. Barui. 1968. A serological survey for cholera antibodies in rural East Pakistan. *Bull. WHO.* **38**: 327.
12. Bayless, T. M., E. Luebbers, and H. L. Elliott. 1971. Immature jejunal crypts: absence of response to stimulus for fluid secretion. *Gastroenterology.* **60**: 762. (Abstr.)
13. Dean, A. G., Y. C. Ching, R. G. Williams, and L. B. Harden. 1972. Test for *Escherichia coli* enterotoxin using infant mice: application in a study of diarrhea in children in Honolulu. *J. Infect. Dis.* **125**: 407.
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**: 265.
15. Grand, R. J., D. A. Chong, and K. J. Isselbacher. 1972. Intracellular processing of disaccharidases: the effect of actinomycin-D. *Biochim. Biophys. Acta.* **261**: 341.
16. Messer, M., and A. Dahlqvist. 1966. A one-step ultramicro method for the assay of intestinal disaccharidases. *Anal. Biochem.* **14**: 376.
17. Gilman, A. G. 1970. A protein binding assay for adenosine 3':5'-cyclic monophosphate. *Proc. Natl. Acad. Sci. U. S. A.* **67**: 305.
18. Schou, M., A. Amdisen, and K. Thomsen. 1968. Clinical and experimental observations concerning the absorption and elimination of lithium and on lithium poisoning. *Acta Psychiatr. Scand. Suppl.* **203**: 153.
19. Wolff, J., S. C. Berens, and A. B. Jones. 1970. Inhibition of thyrotropin-stimulated adenyl cyclase activity of beef thyroid membranes by low concentration of lithium ion. *Biochem. Biophys. Res. Commun.* **39**: 77.
20. Doell, R. G., and N. Kretschmer. 1962. Studies of small intestine during development. I. Distribution and activity of β-galactosidase. *Biochim. Biophys. Acta.* **62**: 353.
21. Schmidt, M. J., E. C. Palmer, W. D. Dettbarn, and G. A. Robison. 1970. Cyclic AMP and adenyl cyclase in the developing rat brain. *Dev. Psychobiol.* **3**: 53.
22. Bär, H. P., and P. Hahn. 1971. Development of rat liver adenyl cyclase. *Can. J. Biochem.* **49**: 85.
23. Hommes, F. A., and A. Beere. 1971. The development of adenyl cyclase in rat liver, kidney, brain and skeletal muscle. *Biochim. Biophys. Acta.* **237**: 296.
24. Deren, J. J., E. W. Strauss, and T. H. Wilson. 1965. The development of structure and transport systems of the fetal rabbit intestine. *Dev. Biol.* **12**: 467.
25. Sunshine, P., J. J. Herbst, O. Koldovsky, and N. Kretschmer. 1971. Adaptation of the gastrointestinal tract to extrauterine life. *Ann. N. Y. Acad. Sci.* **176**: 16.
26. Hermos, J. A., M. Mathan, and J. S. Trier. 1971. DNA synthesis and proliferation by villous epithelial cells in fetal rats. *J. Cell Biol.* **50**: 255.
27. Dahlqvist, A., and T. Lindberg. 1966. Development of the intestinal disaccharidase and alkaline phosphatase activities in the human feotus. *Clin. Sci. (Oxf.).* **30**: 517.
28. Parkinson, D. K., H. Ebel, D. R. DiBona, and G. W. G. Sharp. 1972. Localization of the action of cholera toxin on adenyl cyclase in mucosal epithelial cells of rabbit intestine. *J. Clin. Invest.* **51**: 2292.
29. Birnbaumer, L., S. L. Pohl, and M. Rodbell. 1969. Adenyl cyclase in fat cells. I. Properties and the effects of adrenocorticotropin and fluoride. *J. Biol. Chem.* **244**: 3468.