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The Use of Poly R-478 as a Marker to Determine Gastric Emptying and Intestinal Propulsive Motility in Suckling Rats

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Summary

During our studies on gastrointestinal motility in suckling rats using ^{51}Cr or ^{51}Cr-EDTA as markers, we noticed that these markers – in contrast to studies in adult rats – "adhered" to the gastrointestinal wall of sucklings. We therefore decided to test the use of another non-absorbable marker Poly R-478 (an acetylated anthrapyridone chromophore linked to an polyamino-ethylene-sodium ethylene sulfonate copolymer backbone developed by the Dynapol Corporation (Palo Alto, CA). This new method has appeared to be useful.

Key words

Gastric emptying – Intestinal propulsive motility – Suckling rats – Gastrointestinal motility – New method

Introduction

Gastrointestinal motility increases during perinatal development. These changes have been well documented in various experimental mammals (Ruckebush 1986) and in humans (Koshtoyantz and Mitropolitanskaya 1934, Tornwall *et al.* 1958, McLain 1963, Bisset *et al.* 1988, Tomomasa *et al.* 1985, 1993, Ittman *et al.* 1992, Berseth and Nordyke 1993; for recent reviews, see Boyle 1992 and Koldovský 1992). There are only a few studies exploring the immediate and maturational role of various factors in the development of these functions (in humans: Berseth and Nordyke 1993, Morriss *et al.* 1986, Tomomasa *et al.* 1993; in dogs: Malloy *et al.* 1979; in rats: Jiang *et al.* 1991, Tomomasa *et al.* 1991).

Several studies in rats have shown that gastric emptying is slower in sucklings than in weanlings and adults (Heller 1963, Behnke *et al.* 1966, Lorenz 1985, Jiang *et al.* 1991). Small intestinal propulsive motility measured with a mixture of charcoal and gummi arabicum was also found to increase postnatally (Koldovský *et al.* 1963). Since ^{51}Cr or ^{51}Cr-EDTA were successfully used as markers to determine the rate of gastric emptying in adult rats (Porreca and Burks 1983) and can be added to liquid diets including milk, we have decided to use this technique in suckling rats (Jiang *et al.* 1991). In our studies, we have seen that – in contrast to adult rats – both ^{51}Cr and ^{51}Cr-EDTA adhered to and/or were taken up by the small intestinal wall (unpublished data). We therefore tested another non-absorbable marker Poly R-478 recently used in absorption studies in suckling rats from isolated intestinal loops *in vivo* (Stahl *et al.* 1991) and *in vitro* (Rao *et al.* 1991). Poly R-478 is an acetylated anthrapyridone chromophore linked to an polyamino-ethylene-sodium ethylene sulfonate copolymer backbone.

Methods

Poly R-478 (an acetylated anthrapyridone chromophore linked to an polyamino-ethylene-sodium ethylene sulfonate copolymer backbone) was

purchased from Sigma Chemical Co., St. Louis, MO USA.

Animals. Twenty-eight Sprague-Dawley 12-day-old rats weighing 26.8±0.81 g (mean ± S.E.M.) originating from 7 litters were obtained from our breeding colony. The size of litters was adjusted to 10 pups on day 2 postnatally. Since intralitter variation is smaller than interlitter variation, rats from each litter were distributed into various experimental groups. Pups were removed from the mother 15 hours before the experiment, and kept in cages with half of the cage resting on a heating pad to maintain body temperature. The experiments were performed between 0900 and 1100 h.

One hundred μl of a 5 % solution of Poly R-478 in double distilled water was administered by gastric intubation. Animals were decapitated at 1 minute and 1, 2, 4 and 7 hours after Poly R-478 feeding, and the stomach and small and large intestine were removed. The small intestine was cut into 6 segments of equal length. Segments were numbered from 1 (proximal) to 6 (distal), and all samples were flushed with saline up to the final volume of 6 ml at room temperature. Samples from the wall as well as lumen were collected and measured separately. Both the stomach flush and wall and the intestinal flush and wall were placed into test tubes and Poly R-478 was determined by colorimetric assay (see below). Data for each segment (content in flush = f, sum of content in flush and wall = s) were expressed as a percentage of the sum of total amount found in the appropriate "compartment" (i.e. sum of wall and luminal content = S or sum of the total fluid content = F) of the gastrointestinal tract.

Colorimetric assay of Poly R-478 This was performed as described by Stahl *et al.* (1991) with small modifications. Flush samples were homogenized for 10 seconds by Polytron (Brinkmann Instruments Inc., Westbury, NY, USA) using a PTA 10S generator at speed 6. Wall samples were homogenized in 2 ml of saline for 30 seconds. One half millilitre aliquots (in duplicates) of each homogenate from flush and wall were placed into 12 x 75 mm test tubes containing 1.5 ml 1 N KOH, vortexed, capped and left at room temperature for at least 12 hours. The samples were spun for 10 min at 3000 rpm in a Sorvall RT6000 Refrigerated Centrifuge (Dupont, Wilmington, Delaware, USA) and the supernatants were decanted into test tubes. The supernatants were read against a KOH blank at 515 nm on a Spectronic 1001 spectrophotometer (Milton Roy Co, Rochester, NY, USA). Sample blanks for flush and wall were prepared identically without Poly R-478. Several standard curves were used.

(a) Standard curve: a solution of Poly R-478 in double distilled water was diluted with 1 N KOH to give concentrations of 1–10 mg/dl, the optical density units (OD) at 515 nm were in the range of 0–1.2.

(b) Standard curve with homogenates was done as (a); 25 % of the mixture was replaced by samples of flush or of wall ; final concentrations were 1–7 mg/dl. These standard curves were somewhat higher than (a); the colour of homogenates from flush (yellowish) or wall (cloudy) slightly interfered with the colour of the dye at 515 nm.

(c) Internal standard: One hundred μl of Poly R-478 5 % solution in a test tube were mixed with 6 ml of 0.9 % NaCl and the samples were processed in the same way as the experimental samples.

(d) Regular blank: 1 N KOH.

(e) Sample blank: Material (flush or wall) from one rat was always used for a sample blank that was processed in the same manner as the experimental samples to correct for possible interference by the colour of the tissue. This sample was also used for (b).

All samples ranged from 0 to 0. 8 OD units.

Calculations: A) the recovery of the dye amount per rat was calculated from (a), (e) and (d). B) the recovery per segment and amounts per segment were calculated from (b), (e) and A).

Statistics. Statistical analysis of the results was performed by one-way ANOVA followed by Fisher PLSD using the statistical program Statview v. 4.01 (Abacus Concepts Inc., Berkeley, CA). Value of $p<0.05$ was considered significant. All data in the figures are means ± S.E.M.

Results

As described in the Methods, data for each segment (content in flush = f, sum of content in flush and in wall = s) were expressed as a percentage of the sum of total amount found in the appropriate "compartment" (i.e. sum of wall and luminal content = S or sum of the total fluid content = F) of the gastrointestinal tract. Since expression of results as f/F and s/S ratio gave the same results, only values of s/S and f/S are given.

In control animals (N=3) killed at time zero, Poly R-478 was detected only in the stomach. The recovery of Poly R-478 was high. Nevertheless, the f/S value (93.6±1.5 %) was slightly, but significantly lower than the s/S value (98.7±0. 32 %).

Data obtained at 1, 2, 4 and 7 hours (Figs 1–4) show that the amount of the Poly R-478 in the stomach decreases with time; higher values being found in the distal segments. Very little of Poly R-478 adheres to and/or is taken up by the gastrointestinal wall during the first hour. This process increases with time; at 7 hours, a large discrepancy appears between f/S and s/S values. The distal shift is demonstrated in detail in Fig. 5 that depicts the increase of Poly R-478 in segment 5 with time.

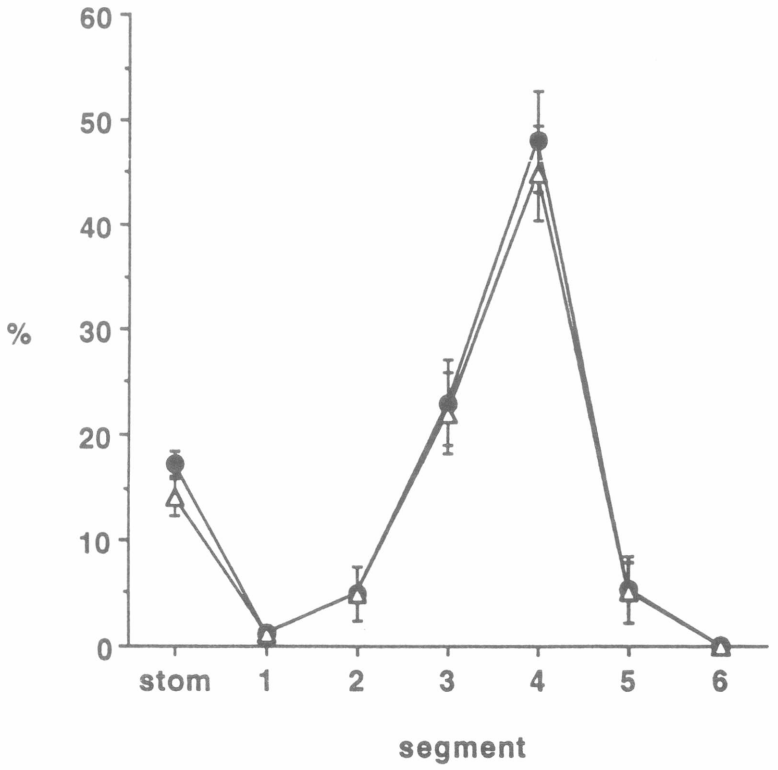

Fig. 1
Distribution of Poly R-478 along the gastrointestinal tract (GIT) at 1 hour after its administration. Vertical axis: Total amount (closed circle) or amount in flush (triangle) of Poly R-478 detected in segment expressed as % of the total Poly R-478 in the entire GIT. Horizontal axis: Stom = stomach; numbers denote segments of the small intestine. No counts were found in the caecum or colon. Symbols denote means ± S.E.M.; N=8.

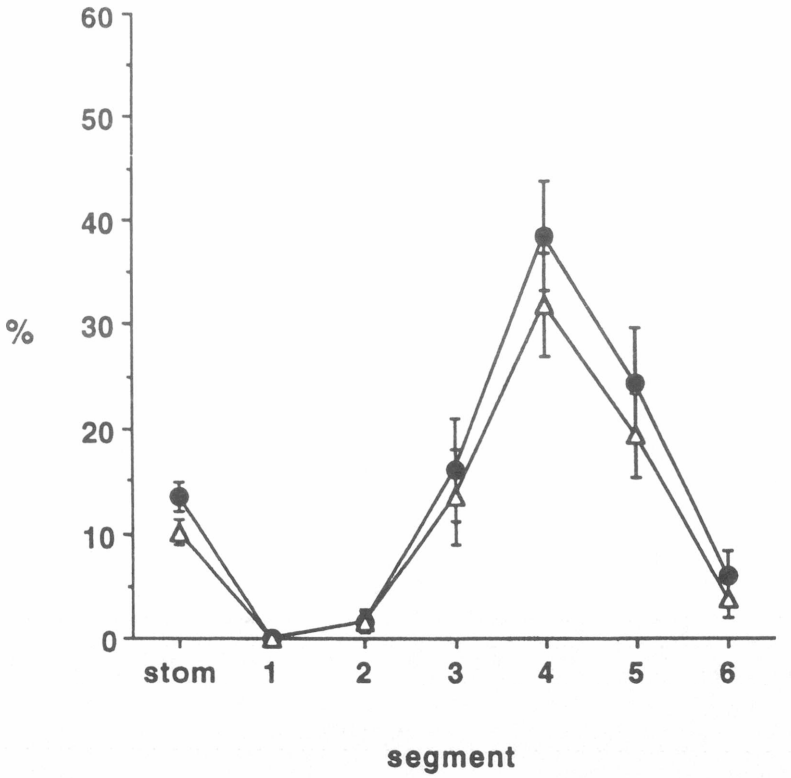

Fig. 2
Distribution of Poly R-478 along the gastrointestinal tract at 2 hours after its administration. Same arrangement as in Fig. 1; N=11.

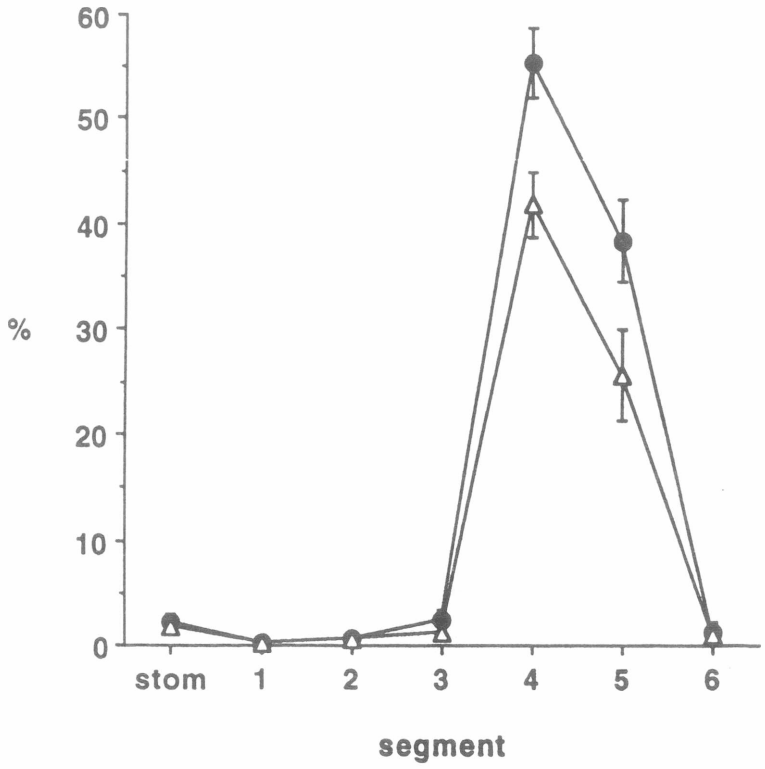

Fig. 3
Distribution of Poly R-478 along the gastrointestinal tract at 4 hours after its administration. Same arrangement as in Fig. 1; N=5.

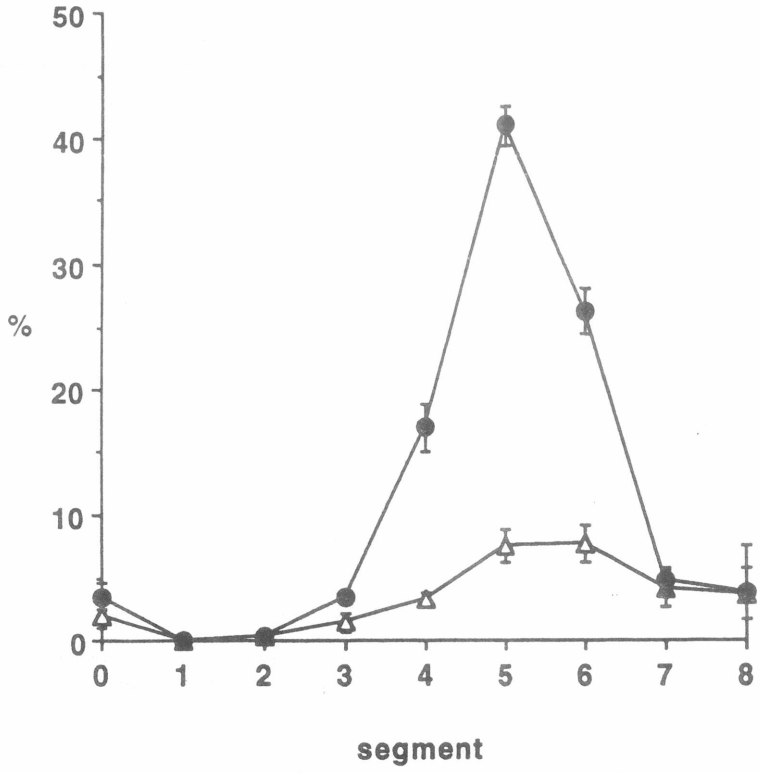

Fig. 4
Distribution of Poly R-478 along the gastrointestinal tract at 7 hours after its administration. Same arrangement as in Fig. 1, except that number 7 and 8 represent caecum and colon values, respectively; N=4.

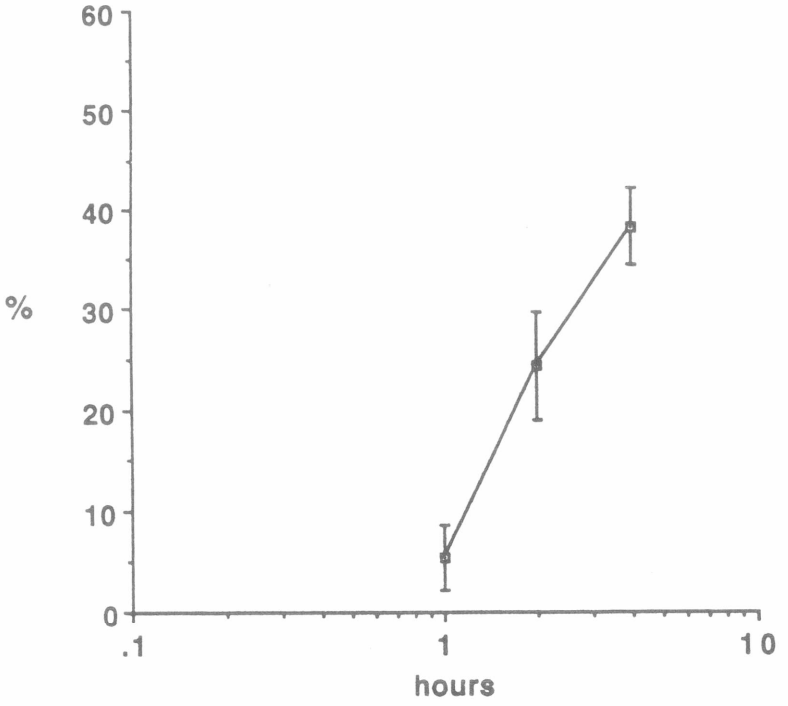

Fig. 5
Total amount (closed circle) of Poly R-478 detected in segment 5 expressed as % of the total Poly R-478 content in the gastrointestinal tract. Vertical axis: % of total amount in segment 5. Horizontal axis: hours after administration of Poly R-478. Symbols denote means ± S.E.M.; N=8, 11 and 5, respectively.

Discussion

Using Poly R-478 as a marker, we were able to quantitate the gastric evacuation and intestinal propulsive motility in suckling rats. When total amount values (s/S) are used, the determinations within the first two hours are very reliable. Prolongation of the experiments demonstrated an increase of the adhesion and/or uptake of Poly R-478. The mechanisms of the adhesion and/or uptake of the marker Poly R-478 and other markers (^{51}Cr or ^{51}Cr-EDTA, unpublished data) by the gastrointestinal wall of sucklings were not studied, but we speculate that unspecific uptake of macromolecules by the small intestine of developing rodents is involved (Brambell 1970). The effect of this adhesion/uptake can be "cancelled" by calculation of the f/F ratio.

Thus we conclude that Poly R-478 can be used in developmental studies of regulation of gastrointestinal motility in suckling rats; only determination of Poly R-478 in the flush of the small intestine is sufficient. One hour duration of the experiment appears to be the best. Nevertheless, as a word of caution, we recommend that in preliminary experiments Poly R-478 should also be determined in the wall to avoid possible artifacts. It might be possible that dietary or hormonal manipulation might influence the process(es) of adhesion and/or uptake of Poly R-478. We speculate that precocious maturation might lead to a decrease of these processes.

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