0031-3998/86/2008-0793$02.00/0
PEDIATRIC RESEARCH

Vol. 20, No. 8, 1986
Printed in U.S.A.

Effect of Nutritional Rehabilitation on the Development of Intestinal Brush Border Disaccharidases of Postnatally Malnourished Weanling Rats

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ABSTRACT. The reversibility of the effects of postnatal malnutrition on the intestinal brush border enzymes and somatic and intestinal weights were examined using either *ad libitum* or restricted feedings. Malnutrition was induced in the immediate postnatal period by expanding newborn rat litters to 20 pups/dam. At 21 days of age, malnourished pups exhibited significantly decreased body and intestinal weights as compared to those from control litters. Malnourished pups also had significantly elevated lactase specific activities whereas sucrase and maltase activities were not affected in the proximal small intestine. With subsequent nutritional rehabilitation by an *ad libitum* (food available 24 h/day) or restricted feeding regimen (food available 2 h/day), body and intestinal weights remained significantly depressed by 56 days in malnourished as compared to control animals. Rats on 2-h feedings consumed approximately 35% of the food consumed by their *ad libitum*-fed counterparts. Comparison of the ratio of weight gained to the amount of food consumed did not demonstrate a greater food efficiency with any particular feeding pattern. With *ad libitum* or restricted feedings, lactase specific activity in the proximal segment attained control values by 14 days. Restricted feedings resulted in an apparent elevation of specific activity of sucrase and of maltase, when rats were sacrificed at one chosen time point. Multiple time studies in a 24-h cycle showed that maximal elevations in enzyme activities were associated with feeding time. There were no significant differences in mean specific daily enzyme activities between the two feeding regimens. Restricted feedings show no advantage in enzyme efficiency or in promoting the rate of recovery of the intestine after postnatal malnutrition. (*Pediatr Res* 20: 793–797, 1986)

Malnutrition in the immediate postnatal period has been shown to cause marked alterations in small intestinal physiology, structure, and brush border enzymes. In a previous study (1), malnutrition induced by the litter expansion technique resulted in reduction in body weight, intestinal weight and DNA content of pups at weaning. Brush border disaccharidases were also affected. Specific activities of sucrase and maltase were lower and lactase was higher than controls. These results have been viewed as a delay in the "developmental clock" which is taken to be an inherent timing mechanism that determines the expression of the enzymes in question. Malnourished pups appear to resemble a lesser developed animal in terms of intestinal brush border disaccharidase activities.

Whether the observed changes in the small intestine following immediate postnatal malnutrition are permanent or reversible with subsequent nutritional rehabilitation has not been examined. Furthermore, investigation into different modes of nutritional rehabilitation might reveal advantages associated with a particular feeding pattern.

Previous workers have demonstrated that restricted feedings induce a more efficient absorption and metabolism of nutrients. Adult animals receiving restricted feeding schedules exhibit higher levels of disaccharidases (2, 3). They have been shown to possess higher levels of hepatic and adipose tissue enzymes involved with lipogenesis (4, 5) and increased absorption of glucose (6–9). Such a feeding mode thus may be advantageous in promoting the recovery of small intestinal enzymes following a period of malnutrition.

However, in our study comparing recovery of the pancreas in malnourished pups after *ad libitum* or restricted feedings, we showed that restricted feeding did not, but *ad libitum* feeding did lead to complete recovery of various pancreatic parameters measured (10). The present study was designed to look at the recovery of the small intestine following immediate postnatal malnutrition under settings similar to the restricted and *ad libitum* feeding model used for the pancreatic studies.

MATERIALS AND METHODS

Animals and feeding protocol. An expanded litter model similar to the one reported previously was used to induce immediate postnatal malnutrition (1, 10). Pregnant Sprague Dawley rats were obtained commercially (Charles River). Normal litters delivering within a 12- to 16-h time period were redistributed such that control litters consisted of 12 pups per dam whereas experimental litters consisted of 20 pups per dam. Both control and experimental litters were maintained in the same environment with stock feeding and water given *ad libitum* for the first 21 days.

At 21 days of age, randomly selected pups from both groups were sacrificed by cervical dislocation. The entire intestine was resected and weighed. Mucosal scrapings from the entire luminal surface of the first 15 cm was collected with a spatula and frozen

Received April 29, 1985 accepted April 7, 1986.
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This study was supported by NIH Grant 12585, Bio-Medical Research Support Grant RR-05493-18, and by the Ralph Hochstetter Medical Research Advance in honor of Dr. Henry C. and Bertha H. Buswell, State University of New York at Buffalo.

at −20° C until further biochemical determination could be performed. Remaining pups from both control and malnourished groups were then subdivided and weaned onto an *ad libitum* (food available for 24 h) or restricted (food available for 2 h, 0800–1000 h) feeding schedule. There were four subgroups of animals: 1) control, fed *ad libitum*; 2) control, restricted feedings; 3) malnourished, fed *ad libitum*; and 4) malnourished, restricted feedings. Thereafter, at weekly intervals until 56 days of age, rats from each group were sacrificed at 0800–1000 h after an overnight fast. The intestinal mucosa from the first 15 cm was also collected and frozen at −20° C for later enzyme determinations. The body weight and intestinal weight for each animal were recorded.

In a separate experiment, groups of animals received the same treatment during lactation and after weaning, throughout 56 days of age. Food intakes were determined daily after weaning for these animals in both the *ad libitum* and the 2-h feeding groups. Body weights were obtained weekly.

Twenty-four h assessment of disaccharidase activities. In order to assess the effect of restricted feedings on the daily pattern of dissacharidase activities, previously malnourished animals (49 days of age), receiving either *ad libitum* or restricted feedings, were sacrificed at 4-h intervals over a 24-h period. For this part of the experiment, the feeding time was shifted from 0800–1000 h to 1600–1800 h. Animals were treated as above and the proximal 15 cm intestinal specimens were similarly analyzed. The mean daily specific activities for each enzyme were calculated by averaging the values obtained at each time point according to the method of Stevenson and Fierstein (3).

Preparation of intestinal homogenate and enzyme measurement. The frozen mucosa was weighed, diluted with ice cold water, and homogenized in a Potter-Elvehjem glass homogenizer. The homogenate was then used for enzyme determinations. The disaccharidases, lactase (E.C. 3.2.1.23), sucrase (E.C. 3.2.1.26) and maltase (E.C. 3.2.1.20), were assayed according to the method of Dahlqvist (1) by incubating aliquots of the homogenates with the appropriate substrate in a malate buffer at pH 6.4, except for lactase where the buffer was at pH 5.6. Liberated glucose was determined enzymatically, using reduced nicotinamide-adenine dinucleotide formation as the index of activity (11). Units are expressed as micromoles of glucose formed per minute per gram protein. Total protein was measured according to the Lowry method, using the Folin phenol reagent (12, 13).

Statistics. Statistical evaluation was performed by ANOVA. The difference between groups was further evaluated by Tukey's test with $p < 0.05$ considered significant.

RESULTS

Growth parameters. At 21 days of age, the mean body weight for malnourished animals was significantly less ($p < 0.0005$) than control animals of the same age (Fig. 1). Similarly, mean intestinal weight was also significantly diminished ($p < 0.0025$). Both body and intestinal weights were approximately 70% of the control pups.

With subsequent nutritional rehabilitation, either by *ad libitum* or restricted feedings, mean body and intestinal weights increased. The gain in weights of the body and intestine of *ad libitum* fed, previously malnourished animals paralleled that of the *ad libitum*-fed controls. Thus, the effects of prior malnutrition were still evident at 35 days of nutritional rehabilitation (56 days of age) in that the previously malnourished animals still showed significantly lower body weights ($p < 0.025$) and intestinal weights ($p < 0.05$) (Fig. 1). The differences in weights between previously malnourished and control groups were even more striking in the animals on restricted feedings. At day 56, the body weights of the previously malnourished restricted group were only 52% of the restricted controls, whereas the *ad libitum*-fed previously malnourished group reached 84% of the continuously fed controls.

Food intake. Food intakes for rats in the four dietary treatment groups are shown in Figure 2. Determinations were made on six animals for each group. Food intake was consistently higher in rats fed *ad libitum* than in rats restricted to 2-h feedings, with rats on 2-h feeds eating approximately 35% of the amount of food consumed by *ad libitum*-fed animals, in both control and previously malnourished groups (Table 1). In addition, there was no consistent indication of improved feed efficiency, as estimated

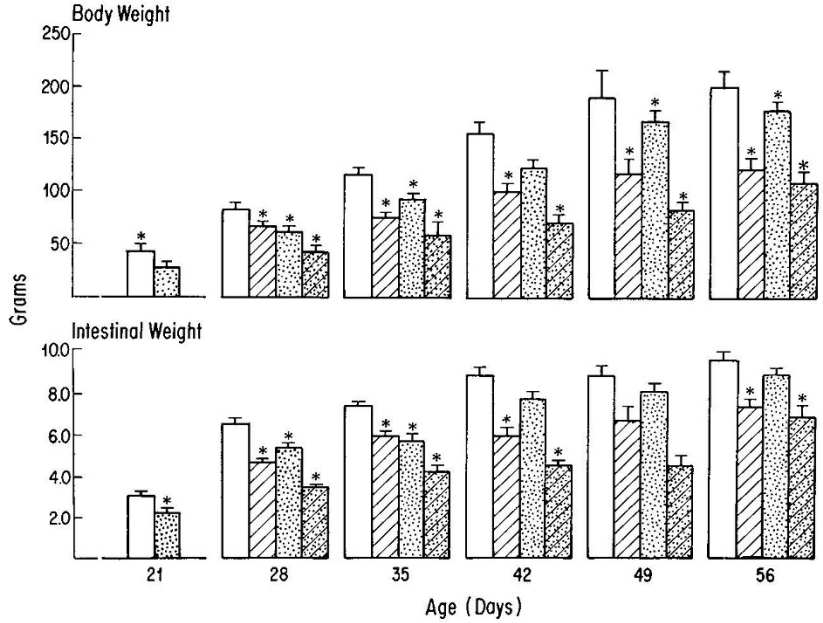

Fig. 1. Effect of postnatal malnutrition and subsequent nuritional rehabilitation by either *ad libitum* or restricted feedings on body weight and intestinal weight. *Open bars*—control, *ad libitum* (food available 24 h). *Open slashed bars*—control restricted feedings (food available 0800–1000 h). *Shaded bars*—malnourished, *ad libitum* feedings (food available 24 h). *Shaded/slashed bars*—malnourished, restricted feedings (food available 0800–1000 h). Mean values ± SEM are depicted. * values are significantly less ($p < 0.05$) than the control group receiving *ad libitum* feedings.

by the ratio of weight gain to amount of food consumed, in any one group (Fig. 3). There was no effect of prior nutritional status on food intake after weaning.

Intestinal enzymes. Pups malnourished for the first 21 days after birth had significantly elevated ($p < 0.05$) lactase specific activities (Fig. 4) in the proximal intestinal segment examined. The specific activities of sucrase and maltase were not significantly altered compared to controls. With subsequent nutritional rehabilitation, lactase specific activity declined to levels comparable to controls after 14 days on the new feeding regimen (35 days of age). No discernable differences were noted between feeding patterns for the remainder of the experiment.

Sucrase and maltase activities of restricted animals of both control and previously malnourished groups (groups 2 and 4) became significantly elevated as compared to their *ad libitum*-fed counterparts (Fig. 4). Examination of activities in the previously malnourished and then restricted group over a 24-h period (Figs. 5 to 7) revealed that the elevations were confined only to the feeding period (1600–1800 h). Previously malnourished and then *ad libitum*-fed animals exhibited maximal elevations in enzyme activities in the early morning hours, but the average daily specific activities of restricted fed animals were not significantly different from those of their *ad libitum*-fed counterparts (Figs. 5 to 7).

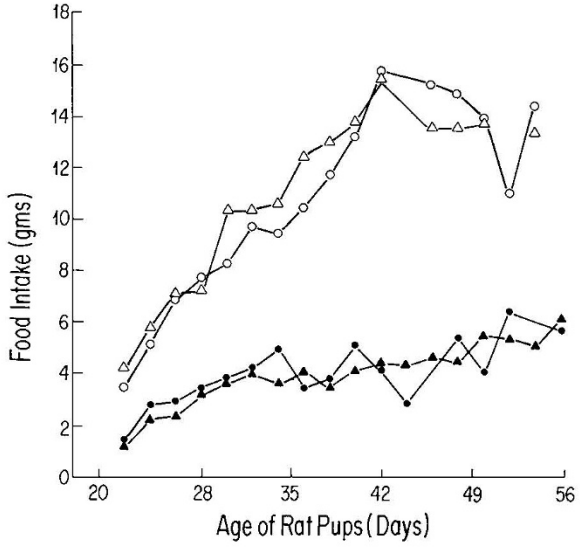

Fig. 2. Food intake measured in grams of food consumed per animal. Symbols represent control (△), *ad libitum* feedings (food available 24 h), and (▲) restricted feedings (food available 0800–1000 h); malnourished (○), *ad libitum* feedings (food available 24 h), and (●), restricted feedings (food available 0800–1000 h). The statistical variations have been omitted to enhance visual clarity. Values for restricted groups are significantly less ($p < 0.05$) than the control group receiving *ad libitum* feedings at all time points.

Table 1. *Food intake**

Age	Controls	Malnourished
28	13.8/40.8 = 0.34	12.9/39.2 = 0.33
35	25.1/71.7 = 0.35	26.5/62.8 = 0.42
42	28.9/92.6 = 0.31	28.9/86.3 = 0.33
49	32.1/104.3 = 0.31	28.2/115.1 = 0.25
56	33.6/90.8 = 0.37	42.7/94 = 0.45
	x = 0.34	x = 0.36

* Ratio of 2-h feeds/*ad libitum* feeds in mean number of grams of food consumed per animal per day ($n = 6$ per group).

DISCUSSION

Gastrointestinal enzymes appear to be exquisitely sensitive to a number of environmental influences. Viral infections (14), intrauterine growth retardation (15), bacterial overgrowth (16, 17), and malnutrition (18) have all been associated with alterations in gastrointestinal enzyme activities. Further, environmental influences may have different effects on enzymatic activities depending on the phase in the life cycle during which they are imposed. Information regarding the reversibility of the enzyme changes induced in the gastrointestinal tract by environmental influences is scanty. In the rat, we have demonstrated that recovery from malnutrition with restricted feedings results in pancreatic enzyme levels that are delayed (amylase), or permanently retarded (lipase), whereas the same enzymes recovered completely and rapidly with *ad libitum* feedings (10).

Results from the present study confirm our previous observation (1) that malnutrition induced in the immediate postnatal period prevents the normal decline in lactase activity that is usually seen at the time of weaning. Our findings indicate that the decline in lactase activity is rapidly achieved with subsequent nutritional rehabilitation by either *ad libitum* or restricted feedings. This decline in lactase was seen 14 days after refeeding. Sucrase and maltase activities were, however, not affected significantly by malnutrition in the present study.

Restricted feedings were associated with an apparent increase in α-glucosidase activities in both control and malnourished

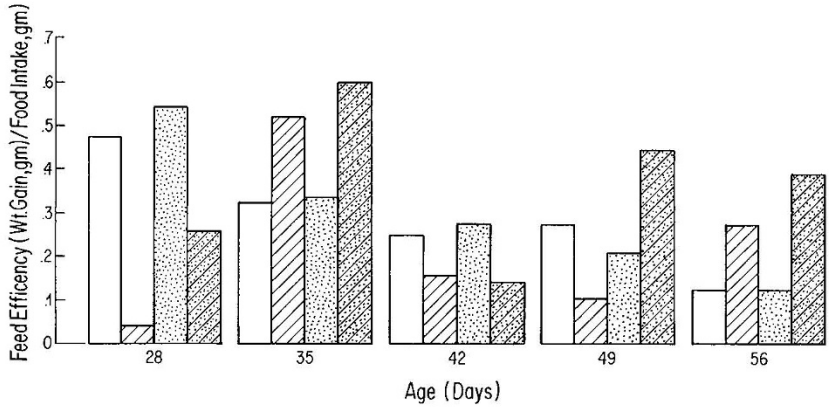

Fig. 3. Feeding efficiency, as measured in weight gain achieved during each 7-day interval per gram of food intake. *Symbols* are the same as Figure 1; values represent mean of six determinations per group.

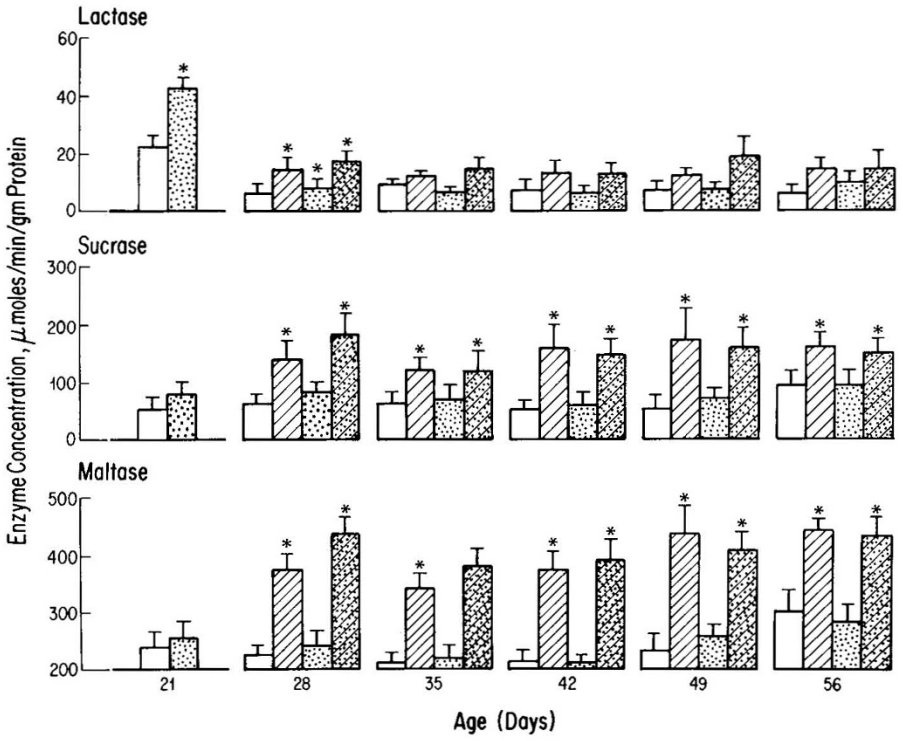

Fig. 4. Effect of postnatal malnutrition and subsequent nutrtional rehabilitation by either *ad libitum* or restricted feedings on the specific activities of lactase, sucrase, and maltase in the first 15-cm segment of the small intestine. *Symbols* are the same as in Figure 1.

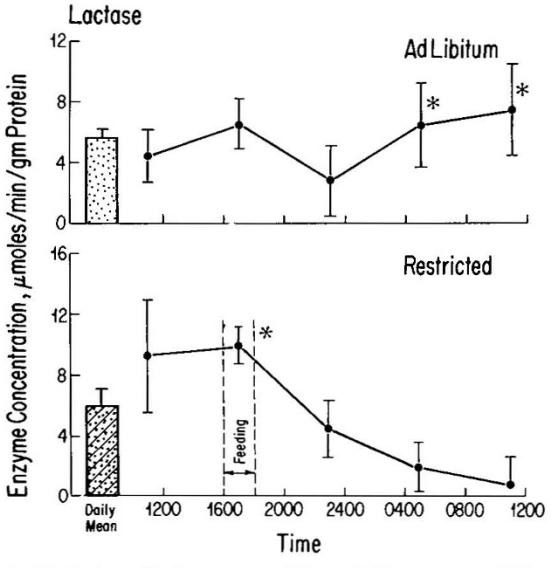

Fig. 5. Variations in lactase specific activities over a 24-h period during either *ad libitum* (*upper portion*) or restricted feedings (*lower portion*) in the first 15-cm segment of the small intestine of 49-day-old postnatally malnourished rats. Mean daily value was calculated according to data in Reference 3. Mean values ± SEs are depicted. * Values are significantly less ($p < 0.05$) than the alternative feeding group at the specified time period.

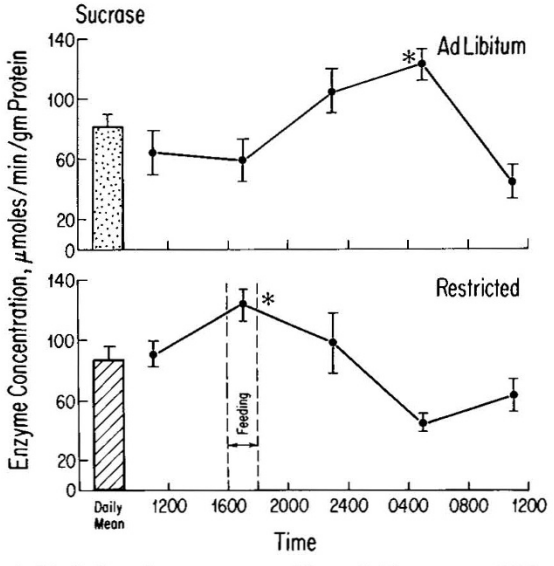

Fig. 6. Variations in sucrase specific activities over a 24-h period during either *ad libitum* (*upper portion*) or restricted feedings (*lower portion*) in the first 15 cm of the small intestine of 49-day-old postnatally malnourished rats. Mean value was calculated according to data in Reference 3. *Symbols* are the same as in Figure 5.

groups. Elevation in both sucrase and maltase specific activities in these animals achieved levels approaching twice that of the corresponding *ad libitum*-fed groups. Such elevations in α-glucosidase activities have been previously demonstrated in adult animals receiving (2, 3) restricted feedings and our results seem to confirm these observations in younger animals as well. However, since rats have been found to exhibit a diurnal cycle of activity of disaccharidases which are closely associated with feeding times, the observed elevated maltase and sucrase may reflect the time of feeding and sacrificing of the restricted-fed animals. The results of the 24-h experiment confirms this notion in that the elevations in activities were found to be centered about the time of feeding. This finding is similar to that demonstrated by Stevenson and Fierstein (3) for adult rats, as elevated activities seem to be cued specifically to the anticipation of food.

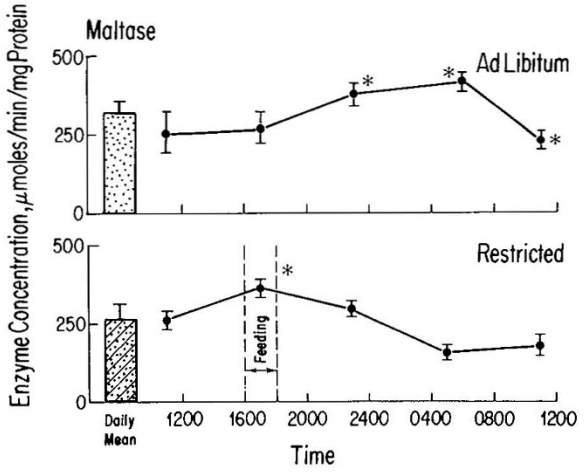

Fig. 7. Variations in maltase specific activities over a 24-h period during either *ad libitum* (*upper portion*) or restricted feedings (*lower portion*) in the first 15-cm segment of the small intestine of 49-day-old postnatally malnourished rats. Mean daily value was calculated according to data in Reference 3. *Symbols* are the same as in Figure 5.

Thus, elevations of α-glucosidase activities found in the restricted fed groups were not sustained throughout the 24-h period. The average daily activity provides a useful measurement to compare the two groups. Stevenson and Fierstein (3) found a correlation of monosaccharide transport activity associated with elevation in average daily disaccharidase activity. However, the mean daily specific activities as calculated in the present study showed no difference between restricted and *ad libitum*-fed groups. Restricted feedings, therefore, did not lead to an increase in total daily disaccharidase activities and should not offer any advantage in accelerating the recovery from malnutrition. This was confirmed by the observation that the body weight of previously malnourished animals receiving restricted feedings remained significantly depressed below that of their *ad libitum*-fed counterparts even at 56 days of age.

Malnutrition in the immediate postnatal period thus led to changes in intestinal brush border enzyme activities that are reversible following nutritional rehabilitation. The stunted growth in body weight and intestinal weight is, however, irreversible even after 7 wk of refeeding. Food consumption was significantly less and an increased efficiency in food utilization was not demonstrated in animals receiving 2-h feeding. *Ad libitum* feeding would thus still be the mode of choice for rehabilitation of small intestinal enzyme changes following a period of malnutrition. These findings are in contrast to Hollifield and Parson (19, 20) who demonstrated greater weight gain and food consumption in restricted fed animals, but supportive of the findings of Leveille (21).

Acknowledgment. The authors thank Mrs. Sharon Sullivan for her excellent secretarial assistance.

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