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Adaptation of the Small Intestine during Pregnancy and Lactation in the Rat

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During pregnancy and lactation in the rat the small intestine in general and the mucosal epithelium in particular gain weight. The specific activities of sucrase, lactate dehydrogenase and succinate–tetrazolium reductase remain constant and those of glucose 6-phosphate dehydrogenase and isocitrate dehydrogenase increase. There is no evidence that the reported decrease in absorption per unit area or weight of mucosal epithelium during pregnancy and lactation is due to decreases in enzyme activities within the epithelium. The pattern of enzyme change shows that the response of the gut to the stimuli of pregnancy and lactation must be a complex one, possibly involving increases in the specific activities of some enzymes.

The small intestine of rat and mouse increases in weight and in length during pregnancy and lactation (Fell *et al.*, 1963; Ajam, 1975; Cripps & Williams, 1975). There is hyperplasia of the mucosal epithelium and an increase in the total absorptive capacity of the gut. Presumably these changes ensure that the intestine is able to deal with the increased dietary intake associated with pregnancy and lactation (to peak values of 150 and 400% respectively of those measured with control rats). Despite the increase in total absorptive capacity, the absorptive capacity per unit area of intestine in the rat falls for both monosaccharides and amino acids (Craft, 1970; Cripps & Williams, 1975). The decrease, which is significant in lactation and is marginal in pregnancy, could be due to decreased efficiency of the enterocytes. Cairnie & Bentley (1967) noted that the turnover times of cells in the mucosal epithelium of lactating rats is less than that in virgin animals, despite the greater length of the villi. Consequently the epithelium could contain a greater proportion of immature cells. Alternatively (or in addition) the enlargement of the gut could be an adaptation not only to increased food intake but to other increased metabolic demands during lactation (Fell *et al.*, 1963).

Little work has been reported on enzyme changes in the mucosal epithelium of pregnant and lactating animals. Rolls (1975) found that the specific activities of rat intestinal dipeptidases increased a little in pregnancy and still further in lactation. Burdett *et al.* (1978) surveyed changes in the activities of a number of enzymes using histochemical techniques. They agreed with the conclusion of Rolls (1975) that the pattern of change was too complex to be the result of simple hyperplasia, and probably represented adaptation of the enterocytes.

The purpose of the present study was to investigate changes in the specific activities of some of the enzymes involved in energy production within the enterocyte. This would test biochemically the conclusions of our histochemical analysis (Burdett *et al.*, 1978) and determine whether the decreased absorptive capacity per unit area of epithelium was due to enzyme changes within it.

Experimental

Materials

Tetrazolium [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride], sucrose (Aristar) and propan-2-ol (AnalaR) were purchased from BDH, Speke, Liverpool, U.K. Bovine serum albumin (Cohn fraction V), lactate, glucose 6-phosphate, isocitrate and succinate were obtained from Sigma London Chemical Co., Poole, Dorset, U.K. Glucose oxidase kits for glucose measurement, NADH, $NADP^+$ and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) were obtained from Boehringer Corp. (London) Ltd., Lewes, East Sussex, U.K. Ethyl carbamate was purchased from Fisons Scientific Apparatus, Loughborough, Leics., U.K.

Animals

Virgin female, pregnant and lactating Sprague–Dawley rats were obtained from our closed colony which is maintained on a 12h light/12h dark regime (light 08:00–20:00h). The colony was maintained on the standard Porton Mouse Diet (supplied by Oakes of Congleton, Cheshire, U.K.). Pregnant animals were primiparous and 18–20 days pregnant. Lactating rats were used 18–20 days after delivery (i.e. 1–3 days before the litters are normally weaned). These times were selected because they are the periods of maximum food intake during pregnancy and lactation (Rolls,

1975; Cripps & Williams, 1975). The controls were mature virgin rats selected to be age-related to the pregnant and lactating groups. Food and water were freely available up to the time the experiments were started, which was standardized at 10:30–11:00h each day to minimize effects due to circadian changes.

Preparation of mucosal epithelium for analysis

Rats were anaesthetized by the intraperitoneal injection of ethyl carbamate and then killed by cervical fracture. The whole of the jejunum and ileum was removed, cut free of mesentery and then rinsed with physiological saline (0.154M-NaCl) at 37°C to flush out the luminal contents. The duodenum was not included in the study because the activity there of sucrase, one of the enzymes selected for study, is zero. The intestine was allowed to hang under its own weight at 22°C in order to determine its length. In the first few minutes after death the intestine relaxed somewhat and the length recorded by this procedure is undoubtedly greater than that *in vivo*. Nevertheless, the procedure was sufficiently uniform to allow comparisons to be made within the study. The intestine was weighed, and then used in one length or divided into three equal lengths designated as jejunum, proximal and distal ileum. Each length was ligated at one end, filled with a solution of 10mM-EDTA in sodium/potassium phosphate buffer, pH7.0 [essentially the method of Evans *et al.* (1971)] and then ligated at the other end. After incubation of the gut in oxygenated physiological saline at 37°C for 15min, the EDTA/buffer was discarded. The intestinal lumen was filled with physiological saline into which the mucosal epithelium was detached by gentle manipulation of the intestinal wall. This procedure removes all of the epithelium on the crest of the villus, but leaves the crypts and villus cores intact (Evans *et al.*, 1971). The residual intestinal wall was reweighed, and the wet weight of the mucosal epithelium obtained by difference. The suspensions of epithelial cells were centrifuged at 22°C for 5min at $500g_{av.}$. The supernatant was removed from the packed cells and kept at 4°C until required. Packed cells were resuspended in physiological saline and kept at 4°C while being homogenized by ultrasonication for a total of 5min in an MSE ultrasonicator (21–22kHz). Samples of diluted mucosal homogenates and of packed-cell supernatants were used for protein and enzyme assays. In some instances (see below) enzyme activities were measured on the supernatant obtained by centrifuging the mucosal homogenates at $17000g_{av.}$ for 20min at 0°C. Enzyme activities did not change during the 5–6h needed to complete the analyses.

Protein assay

The method of Gornall *et al.* (1949) was calibrated with bovine serum albumin that had been dried in a vacuum desiccator to constant weight. The only modification was to centrifuge each sample after colour development for 3min at $500g_{av.}$ to remove the slight residual turbidity produced in intestinal samples by insoluble glycoproteins.

Enzyme assays

Enzymes were assayed in duplicate at 30°C under conditions of initial velocity.

Lactate dehydrogenase (L-*lactate–NAD*$^+$ *oxidoreductase; EC* 1.1.1.27). This was measured in the supernatants from packed cells and in the sonicated homogenates by the method of Wróblewski & LaDue (1955). One unit of enzyme activity catalyses the formation of 1μmol of NAD^+/min. The specific activities in the Results section are expressed as units/mg of protein.

Isocitrate dehydrogenase [*threo*-D$_s$-*isocitrate–NADP*$^+$ *oxidoreductase* (*decarboxylating*); *EC* 1.1.1.42]. This was measured by the method of Ochoa (1955) in the packed-cell supernatants and in the $17000g_{av.}$ supernatants of mucosal homogenates. One unit of enzyme activity catalyses the formation of 1μmol of NADPH/min. The specific activities in the Results section are expressed as munits/mg of protein.

Glucose 6-phosphate dehydrogenase (D-*glucose 6-phosphate–NADP*$^+$ 1-*oxidoreductase*; *EC* 1.1.1.49). This was determined by the method of Lohr & Waller (1965), except that the concentration of $NADP^+$ was doubled. One unit of enzyme activity catalyses the formation of 1μmol of NADPH/min. The enzyme was assayed in both the $17000g_{av.}$ supernatant and the packed-cell supernatant. Glucose 6-phosphate dehydrogenase activity was also measured in random samples of mucosal homogenates from virgin, pregnant and lactating rats in the presence of excess 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate–$NADP^+$ oxidoreductase; EC 1.1.1.44). Since there was no increase in NADPH production, the intestinal samples had sufficient 6-phosphogluconate dehydrogenase activity to oxidize the 6-phosphogluconic acid produced from glucose 6-phosphate. The rate of reduction of $NADP^+$ was due therefore to the combined activities of the two dehydrogenases, and the calculation of glucose 6-phosphate dehydrogenase activity was corrected accordingly. The specific activities in the Results section are expressed as munits/mg of protein.

Succinate–tetrazolium reductase. Succinate–tetrazolium oxidoreductase (EC 1.3.99.1) was assayed by an unpublished method devised by K. V. Rowsell. An assay medium of 65mM-sodium phosphate buffer, pH7.0, 15mM-sodium succinate and 0.4mM-tetrazolium was preincubated at 30°C for 5min, and the reaction was started by adding sonicated mucosal homogenate (total volume of assay 12ml). Samples (3ml) were transferred at 0, 10 and 20min into 10ml

of propan-2-ol, which both stopped the reaction and dissolved the product, formazan. The protein sediment was removed by centrifugation at $500g_{av.}$ for 5 min, and the A_{490} was measured against propan-2-ol [molar absorption coefficient at 490 nm of pure recrystallized formazan dissolved in propan-2-ol is 2.238×10^4 litre·mol^{-1}·cm^{-1} (K. V. Rowsell, unpublished work)]. Control assays that contained no succinate were used. One unit of enzyme activity catalyses the formation of 1 μmol of formazan/min. The specific activities in the Results section are expressed as munits/mg of protein.

Sucrase. The activity of sucrase (β-D-fructofuranoside fructohydrolase; EC 3.2.1.26) was measured by the method of Hübscher *et al.* (1965), by using homogenates that had not been dialysed. Glucose was assayed with commercial glucose oxidase kits to which 0.5 M-Tris/HCl buffer, pH 7.0, was added to inhibit fungal disaccharidase (Dahlquist, 1964). One unit of enzyme activity liberates 1 μmol of glucose/min. The specific activities in the Results section are given as munits/mg of protein.

Calculation of results

The specific activities of enzymes were calculated with reference to the protein content of the mucosal epithelium. Since cytoplasmic enzymes, such as lactate dehydrogenase, isocitrate dehydrogenase and glucose 6-phosphate dehydrogenase, leak from the enterocytes during their removal from the intestinal wall (Evans *et al.*, 1971), their activities in the mucosal homogenates and packed-cell supernatants were added together. Similarly, the protein content of the packed-cell supernatants was added to that in the mucosal homogenates in the calculation of results.

Since succinate–tetrazolium oxidoreductase and sucrase did not leak from the enterocytes (less than 2% of total activity was found in the packed-cell supernatant), these enzymes were routinely assayed in the sonicated mucosal homogenates only. The differences between the values for control, pregnant and lactating rats were investigated for significance by using Student's t test.

Results and Discussion

The small intestine increases significantly in both length and weight during pregnancy and lactation (Table 1), and the greater increase in weight confirms the observations of Ajam (1975) that the intestinal wall becomes thicker. The mucosal epithelium makes a substantial contribution to the increase in gut weight, with 30–40% gains in wet weight and protein in pregnancy and 140–150% during lactation. Fell *et al.* (1963) have demonstrated that the increases in mucosal epithelium are the combined result of hyperplasia and hypertrophy. These changes are accompanied by small but significant increases in the specific activities of dipeptidases in both pregnant and lactating rats (Rolls, 1975). Our histochemical observations (Burdett *et al.*, 1978) agree in general with this increased specific activity of dipeptidase in pregnancy. In lactating rats, however, we noted decreases in activity towards the values seen in virgin controls. The biochemical analysis in this study differs from our histochemical data in that the specific activities of lactate dehydrogenase, succinate–tetrazolium oxidoreductase and sucrase (Table 2) were constant from virgin to pregnant and lactating rats. Furthermore, although the specific activities of glucose 6-phosphate dehydrogenase and isocitrate dehydrogenase increased in pregnancy, neither enzyme decreased in activity in lactation. In fact, isocitrate dehydrogenase increased still further in lactation, though the change was not quite significant at the $P<0.05$ level. The increases recorded by Rolls (1975) in the specific activity of glycyl-L-leucine dipeptidase (EC 3.4.13.2) during pregnancy and lactation in

Table 1. *Changes in the small intestine in pregnancy and lactation*

The methods used are described in the Experimental section. The wet weight of mucosal epithelium was obtained as the difference between the weight of the intact small intestine and that of the residual tissue after the mucosal epithelium had been removed. The results are expressed as mean values ± S.E.M. for the numbers of rats given in parentheses.
* Difference between pregnancy and lactation has $P<0.001$.

		Pregnancy		Lactation	
	Value in virgin controls	Value	Percentage change over control value	Value	Percentage change over control value
Gut length (cm)	104 ± 1.1 (11)	118 ± 2.3 (11)	+13 $P<0.001$	148 ± 3.4 (10)	+42 $P<0.001$*
Gut wet weight (g)	9.3 ± 0.3 (10)	13.1 ± 0.4 (11)	+41 $P<0.001$	17.8 ± 0.9 (10)	+92 $P<0.001$*
Protein in mucosal epithelium (mg)	405 ± 2 (11)	561 ± 4 (11)	+38 $P<0.03$	1023 ± 5 (11)	+153 $P<0.001$*
Wet weight of mucosal epithelium (g)	2.7 ± 0.2 (10)	3.6 ± 0.4 (10)	+33 $P<0.05$	6.5 ± 0.4 (10)	+141 $P<0.001$*

Table 2. *Changes in specific activities of enzymes in the mucosal epithelium of the whole small intestine*
The assay methods and the units used are described in the Experimental section. The results are mean values ± S.E.M. for numbers of rats given in parentheses. * Difference between pregnancy and lactation is not significant (NS).

		Pregnancy		Lactation	
	Control specific activity	Specific activity	Percentage change over control values	Specific activity	Percentage change over control values
Lactate dehydrogenase	2.46 ± 0.2 (10)	2.3 ± 0.24 (7)	−6.5 NS	2.74 ± 0.07 (7)	+11 NS*
Succinate–tetrazolium reductase	1.61 ± 0.17 (11)	1.25 ± 0.12 (9)	−22 NS	1.29 ± 0.14 (9)	−20 NS*
Glucose 6-dehydrogenase	10.4 ± 0.6 (9)	14.3 ± 1.0 (10)	+37.5 $P<0.003$	13.5 ± 0.82 (9)	+30 $P<0.004$*
Isocitrate dehydrogenase	36.7 ± 2.3 (3)	54.8 ± 5.5 (5)	+49 $P<0.02$	72.9 ± 7 (3)	+99 $P<0.02$*
Sucrase	93 ± 7.6 (18)	106 ± 9.3 (13)	+13.6 NS	89 ± 8.3 (11)	−4 NS*

Hooded Norwegian rats were approx. 20 and 30% respectively and therefore of the same order as those recorded here.

The protein content and wet weight of mucosal epithelium increase in parallel (Table 1). Consequently if the specific activities of the enzymes are calculated on the basis of epithelial weight, comparison between the control, pregnant and lactating rats is the same as that shown in Table 2.

We did not routinely assay DNA, but a preliminary investigation gave results that agreed with the report by Ajam (1975) that in mice the protein and DNA also increased at approximately the same rate during pregnancy and lactation. This is to be expected if the major adaptive change in the epithelium is hyperplasia.

The specific-activity changes for glucose 6-phosphate dehydrogenase and isocitrate dehydrogenase are modest. However, with the increase in total protein within the mucosal epithelium the total activities for glucose 6-phosphate dehydrogenase in pregnancy and lactation are 2 times and 3 times the control values respectively, and for isocitrate dehydrogenase the corresponding values are 2 times and 5 times the control values respectively.

In our histochemical analysis the changes observed for succinate–tetrazolium reductase and lactate dehydrogenase were greater in the ileum than in the jejunum, though the enzyme activities were lower in the ileum. In view of this and to ensure that small but significant changes in particular regions of the intestine were not being masked by the procedure of pooling the entire mucosal epithelium, the intestine was divided into three equal lengths corresponding approximately to the jejunum and the proximal and distal ileum. The recoveries of protein (Table 3) indicate that mucosal growth was enhanced during pregnancy and lactation, with the greatest increase occurring in the jejunum. It was to be expected that the recovery of protein from the distal ileum of control rats would be lower than that from the upper intestine. Ileal villi are smaller than those in the upper gut and therefore require less mucosal epithelium to cover them. The major nutrients are absorbed in the upper intestine and in many respects the lower intestine appears to act as part of a large functional reserve of absorptive capacity. The increases in gut and mucosal weights and in protein during pregnancy suggest that the functional reserve in virgin rats is not adequate to cope with the 50% increase in dietary

Table 3. *Changes during pregnancy and lactation in the protein content of mucosal epithelium from three segments of small intestine*
The methods used are described in the Experimental section. The results are expressed as mean values ± S.E.M. for the numbers of rats given in parentheses. * Difference between pregnancy and lactation has $P<0.01$.

		Pregnancy		Lactation	
	Virgin control (mg of protein)	Protein content (mg)	Percentage increase over control value	Protein content (mg)	Percentage increase over control value
Jejunum	168 ± 2.9 (7)	263 ± 19 (6)	57 $P<0.01$	437 ± 33 (7)	160 $P<0.0002$*
Proximal ileum	150 ± 8.4 (7)	226 ± 12 (6)	51 $P<0.0005$	352 ± 28 (7)	134 $P<0.003$*
Distal ileum	109 ± 8.8 (7)	148 ± 6.8 (6)	36 $P<0.003$	245 ± 27 (7)	125 $P<0.001$*

Table 4. *Specific activities of enzymes in the jejunum of virgin, pregnant and lactating rats*

The details of the assays and of the units used are described in the Experimental section. The results are mean values ± S.E.M. for the numbers of rats given in parentheses. *Difference between pregnant and lactating not significant (NS). †Difference between pregnant and lactating has $P<0.05$.

	Lactate dehydrogenase		Succinate–tetrazolium reductase		Glucose 6-phosphate dehydrogenase		Isocitrate dehydrogenase		Sucrase	
	Specific Activity	Percentage change over control	Specific activity	Percentage change over control	Specific activity	Percentage change over control	Specific activity	Percentage change over control	Specific activity	Percentage change over control
Virgin controls	3.1 ± 0.4 (4)	—	2.0 ± 0.3 (7)	—	11.5 ± 0.7 (6)	—	39.6 ± 3.5 (4)	—	134 ± 15 (14)	—
Pregnant	3.5 ± 0.4 (3)	+11 NS	1.7 ± 0.15 (15)	−15 NS	13.8 ± 1.0 (7)	+19 $P<0.05$	54.9 ± 5.3 (5)	+39 $P<0.02$	146 ± 18 (7)	+9 NS
Lactating	3.5 ± 0.2 (4)	+11 NS	1.7 ± 0.2	−15 NS	15.3 ± 1.4 (6)	+33 $P<0.03$*	70.3 ± 5.2 (3)	+77 $P<0.005$†	125 ± 15 (6)	−7 NS

Table 5. *Specific activities of enzymes in the proximal ileum of virgin, pregnant and lactating rats*

The details are as for Table 4.

	Lactate dehydrogenase		Succinate–tetrazolium reductase		Glucose 6-phosphate dehydrogenase		Isocitrate dehydrogenase		Sucrase	
	Specific activity	Percentage change over control	Specific activity	Percentage change over control	Specific activity	Percentage change over control	Specific activity	Percentage change over control	Specific activity	Percentage change over control
Virgin controls	2.7 ± 0.6 (4)	—	1.5 ± 0.2 (7)	—	9.5 ± 0.6 (6)	—	32.5 ± 2 (4)	—	119 ± 12 (14)	—
Pregnant	2.7 ± 0.1 (3)	0	1.4 ± 0.2 (5)	−7 NS	12.5 ± 0.4 (6)	+31 $P<0.002$	52.3 ± 7 (4)	+64 $P<0.02$	167 ± 22 (7)	+40 $P<0.05$
Lactating	2.7 ± 0.2 (4)	0	1.2 ± 0.2 (6)	−20 NS	12.2 ± 1.2 (6)	+28 $P<0.05$*	77.9 ± 15 (3)	+140 $P<0.05$*	102 ± 23 (6)	−14 NS

Table 6. *Specific activities in the distal ileum of virgin, pregnant and lactating rats*
The details are as for Table 4.

	Lactate dehydrogenase		Succinate–tetrazolium reductase		Glucose 6-phosphate dehydrogenase		Isocitrate dehydrogenase		Sucrase	
	Specific activity	Percentage change over control	Specific activity	Percentage change over control	Specific activity	Percentage change over control	Specific activity	Percentage change over control	Specific activity	Percentage change over control
Virgin controls	1.2±0.1 (3)	—	1.6±0.3 (7)	—	10.8±0.3 (6)	—	40.6±6.7 (4)	—	21.3±3 (13)	—
Pregnant	1.2±0.1 (3)	0	1.1±0.3 (5)	−30	12.4±1 (7)	+15	59.8±7.7 (5)	+47	43±11 (6)	+103
				NS		NS		$P<0.05$		$P<0.05$
Lactating	1.4±0.1 (4)	17	1.4±0.1 (6)	−13	13.5±0.8 (6)	+25	67.1±10 (3)	+65	39 ±19 (6)	+84
		NS		NS		$P<0.01$*		$P<0.05$*		NS*

intake recorded in the final stage of pregnancy. Furthermore the mucosal adaptation to pregnancy and lactation appears to be greater in the jejunum than in the ileum.

Both lactate dehydrogenase and succinate–tetrazolium oxidoreductase (Tables 4, 5 and 6) were distributed along the intestine of control animals in a way that is consistent with the established pattern of lactate formation and O_2 uptake in the rat intestine (Sherratt, 1968). The specific activity of neither enzyme changed during pregnancy and lactation. Insofar as the specific activities of two enzymes can indicate the flux through particular metabolic pathways, there is no suggestion that the balance of energy production by glycolysis and the citric acid cycle is radically changed, despite marked mucosal growth. However, glucose 6-phosphate dehydrogenase and isocitrate dehydrogenase (Tables 4, 5 and 6) increased in specific activity in each section of the small intestine during pregnancy. For isocitrate dehydrogenase, there is some evidence of a further increase during lactation, though the increase only reached significance in the jejunum (Table 4). In neither instance is the change particularly large. It remains to be seen whether increases of this order cause, or simply reflect, modifications in the metabolism of the enterocyte during pregnancy and lactation. Both enzymes are involved in NADPH production, which could be used for the biosynthesis of lipid. It would be interesting to see if the small intestine of pregnant and lactating rats can synthesize lipid at rates significantly in excess of those in control virgin animals. Fell *et al.* (1963) postulated that changes in the gut during lactation could be an adaptation to the metabolic demands of lactation other than those simply of increased food intake. It is not unreasonable to expect that some of the metabolic capacity of the gut is used for tasks other than the absorption of nutrients.

Riecken *et al.* (1965) found an increase in the specific activity of certain jejunal dehydrogenases in rats whose absorptive capacity had been increased by bulk feeding. They suggested that enhanced absorption was related to the increased enzyme activity. None of the enzymes included in our study decreased in specific activity, though the absorptive capacity (i.e. amount of nutrient absorbed per unit area of mucosal epithelium) decreases in pregnant and lactating rats. If diminished enzyme activity is responsible for decreased absorptive capacity, it does not appear to be associated with the brush-border (sucrase), mitochondrial (succinate–tetrazolium reductase) or cytosolic enzymes (lactate dehydrogenase, glucose 6-phosphate dehydrogenase and isocitrate dehydrogenase) selected for this investigation.

The nature of the adaptation shown by the intestine during lactation has been investigated by Cairnie & Bentley (1967) and by Elias & Dowling (1974). Where-

as increased food consumption, and thereby increased luminal nutrition, seems to stimulate intestinal mucosal hyperplasia in conditions such as adaptation to cold, endocrine changes are implicated in lactation, and luminal nutrition is not. Cairnie & Bentley (1967) concluded that the mucosal epithelium of rats in mid-lactation had both a decreased turnover time and an increased cell population. This must mean an increased proportion of immature cells in the epithelium, which should be reflected in changes in specific activities of at least some enzymes. The reasons for this assertion are that normally, as enterocytes migrate from the crypts and up the villus, their enzyme activities change. de Both & Plaisier (1974) reported that the specific activities of α-glucosidase (EC 3.2.1.20), lactate dehydrogenase and, to a lesser extent, glucose 6-phosphate dehydrogenase increase as enterocytes mature (i.e. move nearer the villus tip), whereas, by contrast, $NADP^+$-dependent isocitrate dehydrogenase has a fairly constant specific activity. Succinate dehydrogenase has a much higher specific activity in the villus than in the crypts, and mitochondrial enzymes generally have been reported to exhibit differential development as migration of enterocytes proceeds from the crypts (Iemhoff & Hülsmann, 1971). If intestinal adaptation in pregnancy and lactation is controlled by enteroglucagon or some other hormones (Elias & Dowling, 1974), the response must be a complex one. The results obtained by de Both & Plaisier (1974) on normal, germ-free and X-irradiated rats show that a simple increase in the rate of cell proliferation would decrease the specific activities of lactate dehydrogenase and glucose 6-phosphate dehydrogenase, whereas that of the $NADP^+$-dependent isocitrate dehydrogenase would remain unchanged. The pattern of change observed here is quite different from that which can be predicted from the data of de Both & Plaisier (1974). We conclude therefore that either the cell population kinetics in their Glaxo–Wistar rats and our Sprague–Dawley rats are different or the response of the gut during pregnancy and lactation is to produce more cells and to increase the activities of specific enzymes. The latter explanation seems more likely. Since glucose 6-phosphate dehydrogenase and isocitrate dehydrogenase exist in multiple forms, it would be of interest to see if the relative proportions of the isoenzymes in control animals change in pregnancy and lactation. One objective in measuring enzyme activities was to determine if there were decreases during pregnancy and lactation that could be causally related to the diminution in absorptive capacity. If microvillus development was less complete because of an increased rate of cell proliferation, there would be less surface area available per enterocyte for absorption. Sucrase, a microvillus enzyme of great importance in the terminal stage of carbohydrate digestion and absorption, showed no decrease in specific activity (Tables 4, 5 and 6). In fact its activity in the ileal mucosa increased during pregnancy, although the increase was significant only at the $P<0.05$ level (Table 6). It seems probable therefore that changes in the specific activity of sucrase do not account for variations in the absorptive capacity for carbohydrate.

We conclude that if there are enzymes whose activities limit the efficiency of intestinal absorption as the intestine adapts during pregnancy and lactation, they do not include the enzymes selected for this investigation.

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