Effect of the type of diet on the distribution of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in rat small intestine

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Summary. The jejunoileal gradient for the HMG-CoA reductase activity in the microsomal fraction of the epithelial cells of the small intestine of rats given commercial pellets was reserved within a few days after changing the ration to a semipurified diet. The response of the reductase was essentially the same in villus and crypt cells.

The ileum has long been recognized as the major site of intestinal cholesterogenesis[1-5], and this is perhaps true for animals given a commercial stock diet (pellet or chow). Feeding a sucrose-enriched semipurified diet to rats results in elimination of the jejunoileal gradient for cholesterol synthesis[6]. The specific activity of microsomal HMG-CoA reductase [E.C. 1.1.1.34] is also significantly higher in the proximal than in the distal half of the small intestine of rats given semipurified diets[7].

Dietary factors that cause such modifications in the distribution of sterogenic activities are not clear at present. Holt et al.[6] have indicated that the type of carbohydrate may be a factor responsible for changing the gradient in rats. Dietary fats also appear to modify the heterogenous distribution of the reductase activity when cholesterol is fed simultaneously[8]. In addition, a large difference in the content and composition of fibre between laboratory chow and semipurified diet hitherto used may be the cause of the difference in cholesterol metabolism in rats given these diets[9,10].

However, because of differences in the experimental conditions and of the limited information available, time required for manifesting the diet-dependent change is uncertain. In the present study, the effect of substitution of a semipurified diet for commercial pellet on the microsomal HMG-CoA reductase activity in villus and crypt cells of the jejunum and ileum of rats was studied at timed intervals.

Methods and materials. Male Wistar rats, weighing about 150 g, grown on commercial pellet (type NMF, Oriental Yeast Co, Tokyo) were used. The animals were then fed ad libitum with semipurified diet, the composition of which in percent was[7,11]: casein 20, corn oil 5, mineral mixture 4, vitamin mixture 1, choline chloride 0.15, cellulose powder 4 and sucrose to 100. Since the diurnal variation in the intestinal reductase activity was small in its magnitude, rats were maintained on the usual light cycle (lights on 06.00 h to 18.00 h) and killed by decapitation between 09.00 and 10.00 h.

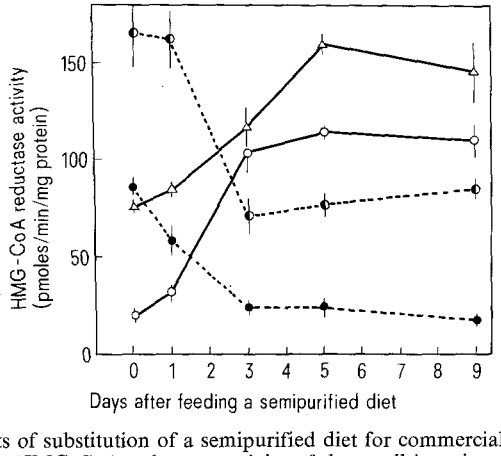
The small bowel distal to the ligaments of Treitz was removed and flushed gently with ice-cold 0.9% NaCl containing 1 mM dithiothreitol. The upper half (jejunum) and lower half (ileum) were separately scraped with a microscopic slide glass below 4 °C to obtain mucosal samples[7,12]. The edge of the slide glass was sharpened and polished in order to minimize the damage of mucosal cells during scraping. The isolation of microsomes and the assay for HMG-CoA reductase in the presence of trypsin inhibitor were performed as previously reported in detail[7,13,14].

Results and discussion. The time course of the change in the distribution of the reductase activity in villi and crypts along the small intestine was studied by following up the effect of substitution of a semipurified diet for commercial pellet. As the figure illustrates, there were already signs of the reversal of the jejunoileal gradient after feeding the semipurified diet for 1 day. The reversal was completed in 3-5 days and the activity became significantly higher in the jejunum than in the ileum. The activity pattern in villus and crypt cells showed a similar pattern. There was obviously the same pattern of the reductase distribution even when total activities in these cell populations were calculated on the basis of microsomal protein recovered.

These rapid modifications in the distribution of the reductase activity may be relevant to a very fast renewal rate of the epithelial cells owing to their continuous desquamation into the lumen. Dietary ingredient(s) appears to influence the proliferation of mucosal cells of the jejunum and ileum differently. In this connection, Vahouny et al.[15] have recently suggested that fibres influence the surface and ultrastructure of the gastrointestinal tract even after short-term (for 3 days) feeding of fibre. Brown et al.[16] have also reported the specific effect of pectin on the structure and function of the rat small intestine.

Commercial pellet used in this experiment was labelled to contain 4% crude fibre. When analyzed, it contained about 30% dietary fibre (neutral detergent fibre[7,17]), the amount being more than 7-fold the fibre content of the semipurified diet, 4% cellulose. Data supporting the observation that the type and amount of fibres are at least partly responsible for the observed reversal of the reductase gradient are accumulating in our associated experiments in which different levels of neutral detergent fibre or cellulose were fed to the rat for 7 days.

Although the intestine's contribution, both to overall synthesis and to plasma input of cholesterol, appears significant in rats and monkeys[1,5,18,19], recent study of Angel and Bray[20] with humans indicates that the adipose tissue seems to be more important than the intestine; the intestinal mucosa has a minor role. However, the significance of the present study lies in the fact that the dietary modification of inter-tissue distribution of cholesterogenic activity in the



Effects of substitution of a semipurified diet for commercial pellet on the HMG-CoA reductase activity of the small intestine of rats. Values are mean ±SE of 3-5 rats. The reductase activity was expressed as pmoles mevalonate formed per min per mg microsomal protein. ○——○, Jejunal villi; △——△, jejunal crypts; ●——●, ileal villi; ◐——◐, ileal crypts.

intestine is a quick process and that this system is perhaps an appropriate model for dietary regulation of cholesterol synthesis as suggested previously[7].

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The possibility of centrifugal projections to the retina in the rat

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Summary. In rats, glutamic acid decarboxylase activity increased in the proximal portion of the optic nerve after its ligation, whereas the activities of choline acetyltransferase and tyrosine hydroxylase remained constant. Possible centrifugal neurons to the retina are GABAergic.

In 1889 Ramón y Cajar speculated that the centrifugal axons through the optic nerve in pigeons terminated the amacrine cells in the retina[1], and thereafter the existence of centrifugal optic fibres in the mammal has been in dispute[2-10]. To analyze the possibility of centrifugal projections to the retina in the rat, I studied the activities of enzymes relating to the synthesis of putative transmitter substances, namely glutamic acid decarboxylase (GAD) for GABA, choline acetyltransferase (ChAc) for acetylcholine and tyrosine hydroxylase (TH) for dopamine.

Materials and methods. Adult male Wistar rats, weighing 180–220 g, were used. Ligation was performed on the optic nerves bilaterally and intraorbitally under ether narcosis. On the 1st, 2nd, 3rd, 4th and 7th days after the ligation, the ligated animals were decapitated under ether narcosis and the proximal portion of the optic nerves was taken; sheaths and blood vessels were eliminated. Tissue specimens thus dissected out were weighed and homogenized with 50 µl of 0.1% Triton X-100 solution. The activity of GAD in the tissues was analyzed by a radioactive $^{14}CO_2$ trapping method using [1-^{14}C] DL-glutamic acid[11] (New England Nuclear, sp. act. 47.25 mCi/mmole). ChAc activity was assayed by kalignost extraction of radioactive acetylcholine formed by a reaction with [acetyl-^{3}H] acetyl coenzyme A[12] (New England Nuclear, sp. act. 2.1 Ci/mmole). The activity of TH was determined by a radioactive $^{14}CO_2$ trapping method using [1-^{14}C] L-tyrosine[13,14] (New England Nuclear, sp. act. 53.6 mCi/mmole). The analysis of GAD, ChAc and TH on each piece of tissue was done in duplicate and the mean of the 2 trials used in subsequent calculation. If the results of the duplicate analyses were not reasonably close, within 5%, the results were discarded.

Results. Experimental animals with their optic nerves ligated showed practically no atrophy of their optic nerves by the 7th day after ligation. The putative transmitter-synthesizing enzyme activities in the optic nerve of the rats after ligation are shown compared to those of normal rats in the table. No significant change in ChAc and TH activities was

Effect of optic nerve ligation on GAD, ChAc and TH activities. Values are expressed as nmole product formed/mg wet wt/h. Number of experiments are shown in parentheses. Each value is the mean of duplicate determination ± SD

Days after ligation		GAD	ChAc	TH
1	Control	4.42±0.15 (3)	0.24±0.11 (3)	0.014±0.003 (3)
	Ligation	5.99±0.42 (8)*	0.27±0.10 (4)	0.012±0.005 (4)
2	Control	4.48±0.04 (3)	0.30±0.10 (3)	0.012±0.006 (3)
	Ligation	6.55±0.48 (6)*	0.32±0.09 (4)	0.010±0.006 (4)
3	Control	4.34±0.25 (3)	0.31±0.11 (3)	0.008±0.003 (3)
	Ligation	4.45±0.26 (8)	0.29±0.09 (4)	0.010±0.005 (4)
4	Control	4.49±0.22 (4)	0.33±0.07 (3)	0.009±0.005 (3)
	Ligation	5.63±0.51 (16)*	0.29±0.10 (4)	0.010±0.005 (4)
7	Control	4.66±0.47 (3)	0.26±0.13 (3)	0.011±0.005 (3)
	Ligation	6.06±0.47 (10)*	0.22±0.12 (4)	0.013±0.005 (4)

* Difference from the control value is significant at $p < 0.001$.