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Degradation of Glucose-Metabolizing Enzymes in the Rat Small Intestine during Starvation

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1. The degradation rates and half-lives of hexokinase, 6-phosphogluconate dehydrogenase, lactate dehydrogenase, pyruvate kinase, glucose 6-phosphate dehydrogenase, phosphoglycerate kinase and aldolase were calculated from measurements of the decline in activities of these enzymes in rat small intestine during starvation. 2. The half-lives of the enzymes are: hexokinase, 5.7h; 6-phosphogluconate dehydrogenase, 7.6h; glucose 6-phosphate dehydrogenase, 6.0h; pyruvate kinase, 8.9h; lactate dehydrogenase, 8.7h; phosphoglycerate kinase, 8.7h; aldolase, 5.1h. 3. The significance of the results is discussed with respect to the regulation of enzyme concentrations in response to changes in diet.

It is well established that the enzyme content of intestinal mucosa is affected by diet. For instance, the activities of hexokinase (EC 2.7.1.1) (Srivastava *et al.*, 1968), glucose 6-phosphate dehydrogenase (EC 1.1.1.49), 6-phosphogluconate dehydrogenase (EC 1.1.1.44) (Srivastava & Hübscher, 1966) and pyruvate kinase (EC 2.7.1.40) (Anderson & Zakim, 1970) decline in starvation. In a constant cell population a decrease in enzyme content relative to cell number may be a result either of a decrease in the rate of synthesis or of an increase in the rate of degradation of the enzyme or both. In the intestine changes in enzyme content are often a reflection of changes of cell population. Hence experiments *in vivo* investigating the synthesis and degradation rates of enzymes must be confined to relatively short time-periods to ensure minimum changes in cell population.

The object of the present work was to determine the half-lives of certain glucose-metabolizing enzymes in the small intestine. The half-lives of intestinal enzymes must be considered within the context of epithelial cell turnover, since the life-span of a villus cell is only 1.3 days (Bertalanffy, 1960; Creamer, 1967). If the half-lives of the enzymes studied in the the small intestine are comparable with those observed for the same enzymes in other tissues, then cell turnover would be more rapid than enzyme turnover. For example, the half-life of hepatic pyruvate kinase is 24h (Freedland, 1968), that of hepatic aldolase (EC 4.1.2.13) is 4.9 days (Kuehl & Sumsion, 1970) and that of hepatic glucose 6-phosphate dehydrogenase is 37h (Szepesi & Freedland, 1971) to 69h (Rudack *et al.*, 1971*a*). However, it is known that the degradation rate of an enzyme may vary depending on the tissue of origin, e.g. the half-life of lactate dehydrogenase isoenzyme 5 from rat heart is 46 days, from rat liver is 16 days and from muscle is 31 days (Fritz *et al.*, 1969). The finding that the half-life of intestinal disaccharidase is 11.5h (James *et al.*, 1971) indicates that the amounts of intestinal enzymes may fluctuate in a particularly dynamic way.

The method used to find the degradation rates and half-lives of the enzymes involved the measurement of the decay of enzyme activities from an original steady state to a new steady state under changing dietary conditions (Schimke, 1970).

Materials and Methods

Animals

Male Wistar rats weighing between 175 and 225g were obtained from the Breeding Unit, Nottingham School of Agriculture, Sutton Bonington, Leics., U.K. The animals were fed on rat cake, containing 20% fat, provided by the Breeding Unit, and during starvation they were kept in metabolic cages to minimize coprophagy.

Chemicals

Glycerate 3-phosphate, fructose 1,6-diphosphate, phosphoenolpyruvate, pyruvate, ATP and ADP (sodium salts), $NADP^+$, NAD^+, NADH, glyceraldehyde 3-phosphate dehydrogenase (70i.u./mg of protein), glucose 6-phosphate dehydrogenase (140i.u./mg of protein), 6-phosphogluconate dehydrogenase (12i.u./mg of protein), lactate dehydrogenase (360i.u./mg of protein), aldolase (9i.u./mg of protein) and a mixture of glycerol 1-phosphate dehydrogenase and triose phosphate isomerase (ratio of specific activities, 1:6) were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K.

Tritiated thymidine was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Crystalline bovine serum albumin (fraction V, fatty acid-poor) was obtained from Miles Laboratories Inc., Kankakee, Ill., U.S.A. *N*-Acetylcysteine and DNA were purchased from Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K. and dithiothreitol from Koch–Light, Colnbrook, Bucks., U.K. All other chemicals were of A.R. grade.

Enzyme assays

Hexokinase, pyruvate kinase and lactate dehydrogenase activities were assayed by the method described by Srivastava *et al.* (1968). Glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities were assayed by the method described by Srivastava & Hübscher (1966). Phosphoglycerate kinase activity (EC 2.7.2.3) was assayed by the method of Bücher (1955).

Aldolase activity was assayed by a modification of the method of Taylor (1955). The assay system contained: glycine buffer, pH7.6 (90mM); NAD^+ (0.2mM); *N*-acetylcysteine (4mM); fructose 1,6-diphosphate (20mM); 10μg of protein of a mixture of glycerol 1-phosphate dehydrogenase and triose phosphate isomerase; 0.1–0.2mg of protein of the preparation to be assayed, in a final volume of 1ml. The reaction was started by the addition of substrate.

All enzyme assays were performed at two enzyme concentrations at 30°C. The change in NADH concentration was followed spectrophotometrically at 340nm. Enzyme activities are expressed as μmol of substrate changed/min per μmol of DNA phosphorus.

Determination of protein and DNA

Protein and DNA concentrations were determined by the method described by Hübscher *et al.* (1965).

Radioautography

Eight rats were starved for 12h and then injected intraperitoneally with 0.65μCi of [U-^{3}H]thymidine/kg body wt. Four of the animals were killed after 12h and four were killed after 36h. Transverse sections of the intestine were prepared and radioautography was carried out.

Preparation of tissue

The animals were killed by cervical dislocation and the small intestine was removed, irrigated with 0.9% NaCl and scraped with a wooden spatula (Hübscher *et al.*, 1965). The scrapings were homogenized in 0.14M-KCl containing 1mM-dithiothreitol and 5mM-Tris–HCl buffer, pH7.4, by using a Potter–Elvehjem homogenizer [580rev./min; clearance 0.0165cm (0.0065in)]. Samples of the total homogenate were assayed for enzyme activities.

Experimental and Results

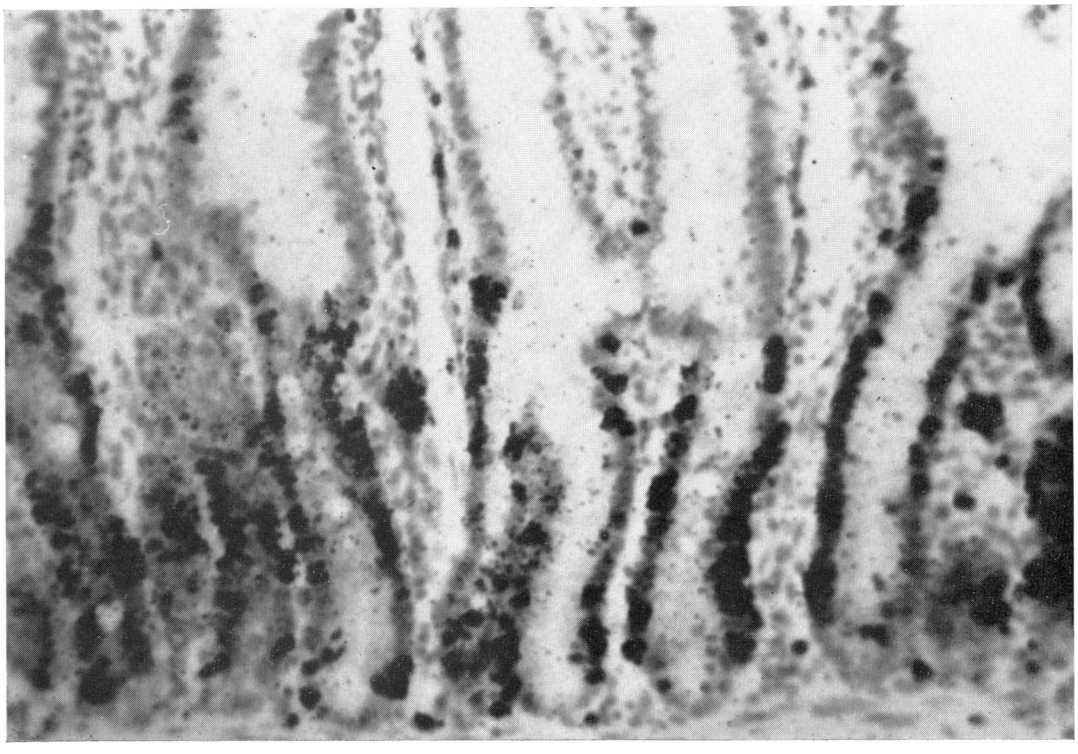
To minimize variation in enzyme concentrations caused by circadian rhythms all animals were killed between 07:00h and 08:00h. The decline in enzyme activity in starvation was measured from 24 to 60h rather than from 0 to 36h for two reasons. First, any measurement of enzyme turnover in the intestine is complicated by the continual replacement of epithelial cells. Starvation is known to slow epithelial cell replacement (Clarke, 1970). Therefore the change in cell population in a given period of time would be less later in starvation. Secondly, rats vary in their feeding and stomach emptying times and therefore removal of food is not synonymous with starvation. During the initial stages of starvation these factors would tend to obscure any changes in enzyme concentrations in the small intestine.

Turnover of the cell population during starvation was measured by radioautography. These experiments showed that after 24h starvation, i.e. 12h after administration of [U-^{3}H]thymidine, labelled nuclei were just beginning to ascend the villi (Plate 1). After 60h starvation labelled nuclei had reached the tips of the villi (Plate 2), indicating that over the time-period investigated all the epithelial cells were shed. Almost none of the lamina propria was renewed. If the volume of the lamina propria is taken as 25% of the total mucosal tissue (Jones, 1972) then approx. 75% of the total mucosal tissue was replaced in 36h.

The activities of hexokinase, 6-phosphogluconate dehydrogenase, lactate dehydrogenase, pyruvate kinase, glucose 6-phosphate dehydrogenase, phosphoglycerate kinase and aldolase were measured after 24, 27, 29, 32, 36, 42 and 60h of starvation. The decline in activity of the enzymes relative to DNA phosphorus is shown in Figs. 1(*a*)–1(*g*). All the enzyme activities tended towards a new steady-state value. To ensure that the decline was not due to the production of an inhibitor or the loss of an activator samples of tissue from an animal starved for 24h and an animal starved for 60h were mixed. For all enzymes the activity in the mixed sample was the same as the mean of the activity in the two separate samples.

Calculation of degradation constants and half-lives of the enzymes

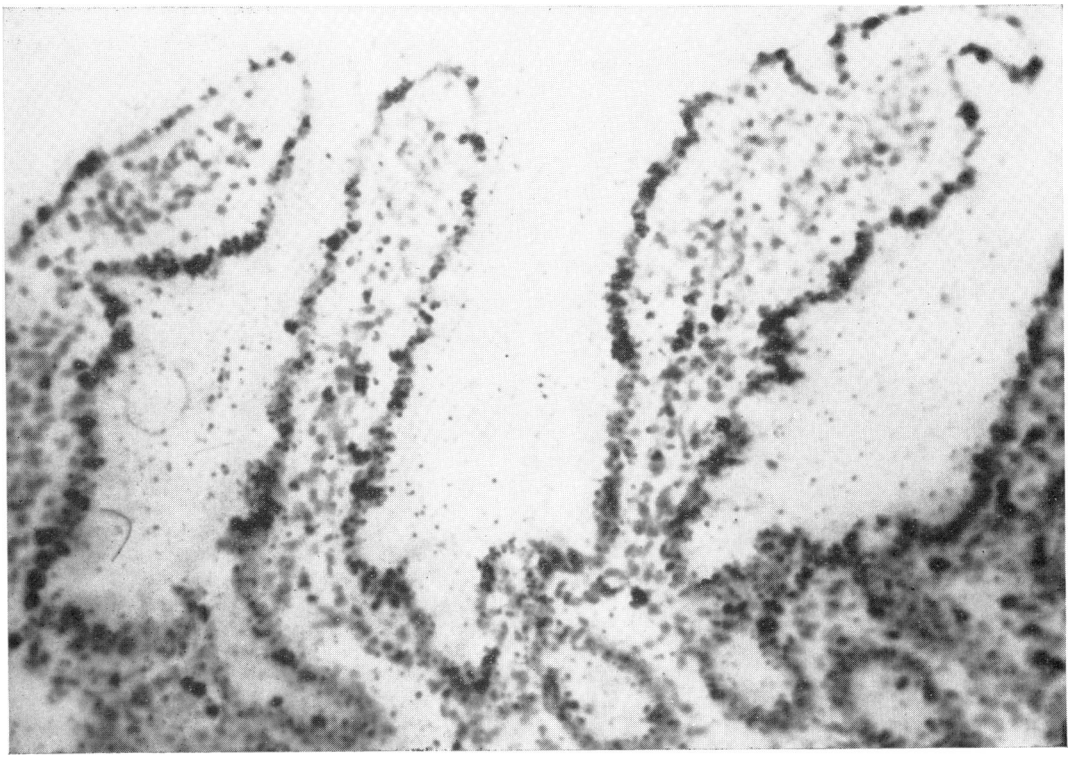
Decline in enzyme concentration in a tissue is an exponential function of the rate of degradation and a linear function of the rate of synthesis of the enzyme (Schimke, 1970).



EXPLANATION OF PLATE 1

Radioautography of sections of small intestine taken from rats starved for 24h

For details see the text. [U-^{3}H]Thymidine was injected intraperitoneally 12h before death.



EXPLANATION OF PLATE 2

Radioautography of sections of small intestine taken from rats starved for 60*h*

For details see the text. [U-^{3}H]Thymidine was injected intraperitoneally 48h before death.

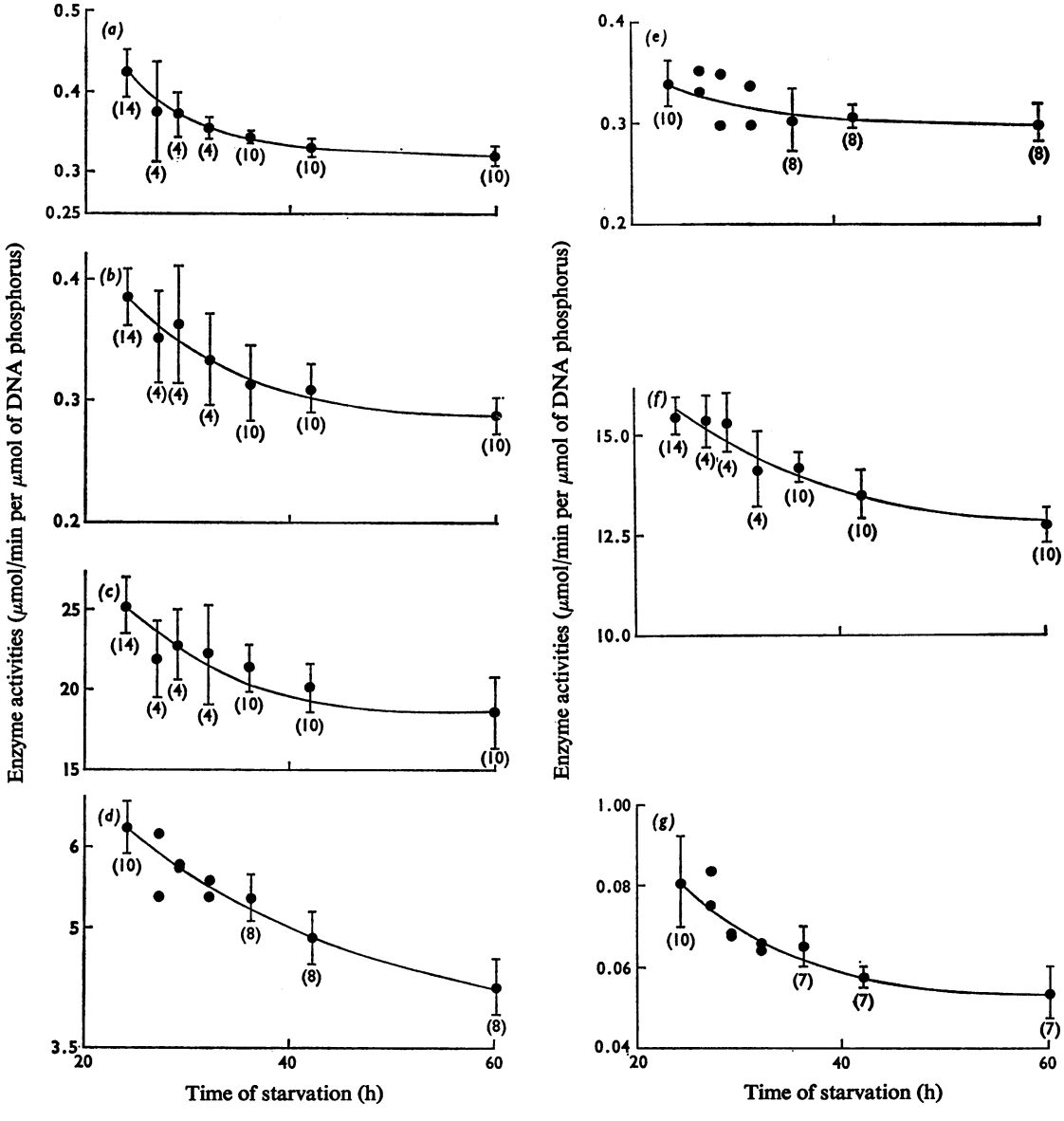


Fig. 1. *Decline of enzyme activities in the small intestine on starvation*

For details see the text. Values in parentheses denote the number of observations at each time-point. Results are means±2×S.E.M. (*a*) Hexokinase; (*b*) 6-phosphogluconate dehydrogenase; (*c*) lactate dehydrogenase; (*d*) pyruvate kinase; (*e*) glucose 6-phosphate dehydrogenase; (*f*) phosphoglycerate kinase; (*g*) aldolase.

Haining (1971) has described a general solution of the kinetics of enzyme regression:

$$[E_t] = \frac{k_s}{k_d} + \left([E'] - \frac{k_s}{k_d}\right) e^{-k_d t} \quad (1)$$

where $[E_t]$ is the enzyme concentration at time t, k_s is the rate of synthesis (assumed to be zero order), k_d is the first-order rate constant for degradation and $[E']$ is the maximum inducible enzyme concentration as delimited by the ratio k_s'/k_d, where

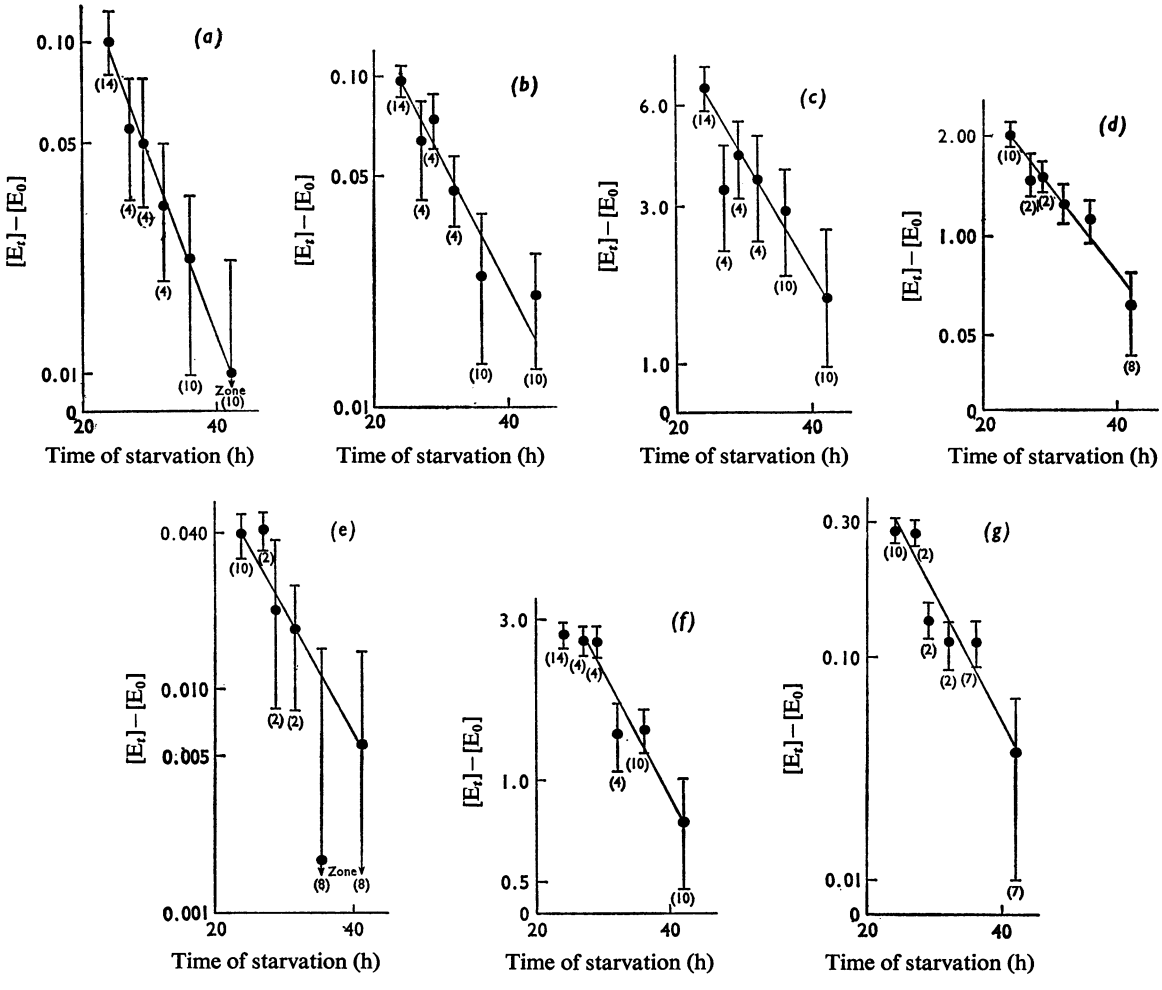


Fig. 2. *Rate of change of enzyme concentrations in the small intestine on starvation*

For details see the text. $[E_t]$ is the enzyme concentration (expressed as μmol of substrate changed/min per μmol of DNA phosphorus) at time t; $[E_0]$ is the enzyme concentration at 60h ($n = 10$). The term $([E_t]-[E_0])$ is calculated from the results in Fig. 1. Values in parentheses denote the number of observations at each time-point. The mean values of $([E_t]-[E_0])\pm 2\times$ S.E.M. are given. In some cases the mean value $-2\times$ S.E.M. gives a value less or equal to zero. This is symbolized by an arrow. (*a*) Hexokinase; (*b*) 6-phosphogluconate dehydrogenase; (*c*) lactate dehydrogenase; (*d*) pyruvate kinase; (*e*) glucose 6-phosphate dehydrogenase; (*f*) phosphoglycerate kinase; (*g*) aldolase.

k_s' is a higher synthetic rate found in the induced steady state. In the non-induced steady state:

$$\frac{k_s}{k_d} = [E_0] \qquad (2)$$

where $[E_0]$ is the lower steady-state enzyme concentration. Therefore:

$$[E_t]-[E_0] = ([E']-[E_0])\,e^{-k_d t} \qquad (3)$$

and the quantity $[E_t]-[E_0]$ changes exponentially with time. If the term $\log([E_t]-[E_0])$ is plotted against time, a straight line is obtained, the slope of which gives the degradation constant, k_d.

The results of the experiments described were plotted according to eqn. (3) (Figs. 2*a*–2*g*). Enzyme activity was taken as the measure of enzyme concentration. The enzyme activity at 60h starvation was taken as $[E_0]$, the preinduction steady-state

Table 1. *Half-lives of some intestinal enzymes*

The half-lives were obtained by using eqn. (4), i.e. $t_{\frac{1}{2}} = \ln 2/k_d$ (see the text).

Enzyme	k_d (h^{-1})	$t_{\frac{1}{2}}$ (h)
Hexokinase	0.1225	5.66
Aldolase	0.137	5.06
Phosphoglycerate kinase	0.0859	8.69
Pyruvate kinase	0.0778	8.91
Lactate dehydrogenase	0.0800	8.66
Glucose 6-phosphate dehydrogenase	0.115	6.02
6-Phosphogluconate dehydrogenase	0.0907	7.64

enzyme concentration (see Figs. 1*a*–1*g*). Use of eqn. (3) presumes that after removal of the stimulus causing the induced steady-state concentration (feeding) the enzyme concentrations decline in a way determined by constants of enzyme degradation and synthesis characteristic of the non-induced steady state (starvation).

The term $\log([E_t]-[E_0])$ is plotted for each enzyme against time in Figs. 2(*a*)–2(*g*), and the apparent degradation constant, k_d, for each enzyme determined. The half-life of the enzymes were determined from the degradation constant by using the equation:

$$t_{\frac{1}{2}} = \ln 2/k_d \qquad (4)$$

and are listed in Table 1.

Discussion

In the fed rat the epithelial cells of the intestine differentiate in the crypts of Lieberkühn, move up the villi and are shed within 1–3 days. During starvation the life of the epithelial cells is longer. Therefore slow changes, over a period of days, in the enzyme content of the epithelium may be mediated through changes in the cell population. The values of the half-lives of the enzymes studied in this work indicate that there is considerable enzyme turnover within the limited lifetime of the epithelial cells.

It may be argued that, since all the enzymes showed relatively similar apparent half-lives, the turnover of the epithelium was responsible for the decline in enzyme activities rather than degradation of the individual enzymes. However, the results in Figs. 1(*a*)–1(*g*) show that after 42h starvation all the enzymes were approaching the new steady state. If the decline in enzyme activity were due to the replacement of the original cell population by a population of cells with a lower enzyme content, it should have continued for a longer period of time until all the original cell population had been replaced. Since the enzyme activities are expressed relative to the DNA phosphorus content of the cells, the decline in activities are not caused by a fall in cell number.

The degradation rates of the enzymes were those obtaining in a state of starvation. It has been shown that changes in the dietary regime may alter the rate of degradation of enzymes. Rudack *et al.* (1971*a*,*b*) showed that the half-life of hepatic glucose 6-phosphate dehydrogenase in rats fed with a pellet diet was five times higher than in rats fed on a high-carbohydrate diet, and that the half-life of 6-phosphogluconate dehydrogenase was eight times higher. It is possible that the degradation rates of the intestinal enzymes change with different diets. This could be estimated by measuring the increase in enzyme activities on re-feeding with various diets (Schimke, 1970). That the half-lives of the enzymes are so short, at least in starvation, implies that the content of a specific enzyme may vary within the life-span of the epithelial cell.

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