Interrelationship of Glutathione–Cystine Transhydrogenase and Glutathione Reductase in Developing Rat Intestine

By BEATRICE STATES and STANTON SEGAL

Division of Biochemical Development and Molecular Diseases, Children's Hospital of Philadelphia, and Departments of Pediatrics and Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pa. 19146, U.S.A.

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1. Glutathione reductase and glutathione–cystine transhydrogenase activity in supernatant fractions of whole homogenates and homogenates of mucosal and muscular layers were determined in developing rat intestine after determination of the optimum conditions for assay of the two enzymes. In jejunum from adult rat, the K_m values for GSSG reductase and GSH–cystine transhydrogenase activities were 0.25 mM-GSSG and 0.23 mM-cystine respectively. 2. The two activities could be differentiated by stability studies since GSSG reductase was stable at 60°C for 10 min and could be stored at 4°C for 24 h without loss of activity. GSH–cystine transhydrogenase, on the other hand, was denatured at 60°C and completely inactive after 24 h storage at 4°C. 3. Based on calculations of total activities, both enzymes increased from the eighteenth day until the animals were young adults. 4. Total GSSG reductase activity increased at a greater rate with age than total GSH–cystine transhydrogenase activity as evidenced by activity ratios for GSH–cystine transhydrogenase/GSSG reductase of 0.44 and 0.12 in ileum from suckling and adult rats respectively, and 0.31 and 0.24 in jejunum from suckling and adult rats respectively. 5. In mucosa from adult rats GSSG reductase was more active in the ileum than in the jejunum, whereas GSH–cystine transhydrogenase activity was higher in the jejunum. 6. GSH–cystine transhydrogenase was active only in the muscle cells of the ileum of 7-day-old rats but became localized primarily in the mucosal layer in the adult rat. However, GSSG reductase activity was distributed evenly between the two layers throughout the intestine.

Racker (1955) reported the presence of an enzyme in liver and yeast which, when coupled with GSSG reductase and a GSH-regenerating system, was capable of reducing homocystine to homocysteine. Since that time several enzymes have been found that require GSSG reductase, NADPH and GSH, which are instrumental in thiol–disulphide exchange. For example, reports have appeared in the literature on the presence of a thiol–disulphide transhydrogenase in yeast (Nagai & Black, 1968), on the reduction of a L-cysteine–glutathione mixed disulphide in rat liver (Eriksson & Mannervik, 1970), on the characterization of a CoA–GSSG reductase in yeast (Ondarza *et al.*, 1969), and on partial purification of an enzyme from bovine kidney which participates in CoA–GSSG thiol–disulphide interchange (Chang & Wilken, 1966). Wendell (1968) studied the distribution of GSSG reductase and detected GSH–cystine transhydrogenase activity in rat kidney and intestine. However, the determination of GSH–cystine transhydrogenase activity in the crude soluble fraction of rat intestinal mucosa in the absence of exogenous yeast GSSG reductase was reported for the first time from this laboratory (States & Segal, 1969b). We

found that this fraction contained high GSSG reductase activities. Recently, enzymes with thiol–disulphide transhydrogenase activity have been separated from ones with disulphide reductase activity (Eriksson & Mannervik, 1970; Tietze, 1970) and GSSG reductase has been purified from human erythrocytes (Staal *et al.*, 1969; Icen, 1967), rat liver (Mize *et al.*, 1962) and yeast (Black & Hudson, 1961; Massey & Williams, 1965; Mavis & Stellwagen, 1968). Only one report, however, appears in the literature on changes in activities of either enzyme with development (Pinto & Bartley, 1969). These investigators studied GSSG reductase activity with development in rat liver and the effects of dietary changes and sex differences on enzyme activities. They coupled reductase activity with glutathione peroxidase activity.

Studies of development of GSH–cystine transhydrogenase activity, in particular, take on added importance in view of findings by States & Segal (1968) and Segal & Smith (1969) that cystine when transported into cells is recovered intracellularly as cysteine at all stages of development of rat intestine and kidney. The experiments described in the present paper were designed to study the kinetic properties

of GSSG reductase and GSH-cystine transhydrogenase to correlate the activities of the two enzymes with development of the jejunum and ileum and to determine whether or not these enzymes were localized in the muscular and/or mucosal layers of the developing rat intestine.

Materials and Methods

Animals

Sprague-Dawley female rats, 12–14 days pregnant, were obtained from Charles River Breeding Farms, Wilmington, Mass., U.S.A. Litters were adjusted to nine animals 2 days after birth. Pups were weaned after 22 days and were fed a Purina rat-chow diet and water *ad libitum*. The number of animals used in each experiment depended on their size, nine to twelve 4-day-old rats and three 21-day-old rats being used.

Chemicals

L-Cystine, cysteine hydrochloride, NADPH, reduced glutathione (GSH), oxidized glutathione (GSSG) and *N*-ethylmaleimide were purchased from Calbiochem, Los Angeles, Calif., U.S.A. L-[³⁵S]Cystine with a specific radioactivity of 47.5 mCi/mmol was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Liquifluor was purchased from New England Nuclear Corp., Boston, Mass., U.S.A. Thin-layer cellulose plates were obtained from Eastman-Kodak Co., Rochester, N.Y., U.S.A. Ninhydrin (0.2%) in butan-1-ol was a Mann Spraytec Reagent produced by Mann Research Laboratories Inc., subsidiary of B-D Laboratories Inc., New York, N.Y., U.S.A. All other reagents used were A.R. grade purchased from Fisher Scientific Co., King of Prussia, Pa., U.S.A.

Experimental procedure

Animals were killed by decapitation. The intestine was rinsed with ice-cold 0.15 M-KCl by inserting a polyethylene tube attached to a syringe into the duodenum. The entire small intestine was removed from the animal and laid on a clean towel resting on cracked ice. Since the duodenum was not a significant site of either active cystine transport or reduction, it was discarded and the rest of the small intestine was divided into three equal lengths. The proximal (jejunum) and distal (ileum) parts were rapidly and carefully opened to expose the mucosa. The opened portions were scraped just hard enough not to disrupt the integrity of the muscular layers. The mucosal and muscular layers, whole jejunum and whole ileum were weighed and added to cold 0.25 M-D-mannitol-2.5 mM-EDTA buffer adjusted to pH 7.4 with NaOH

at a ratio of 1 g wet wt. of tissue/14 ml of medium. The mucosal and muscular layers and whole jejunum and ileum from suckling rats were homogenized by using ten strokes with a glass pestle having a 'large' clearance followed by five strokes with a glass pestle having a 'small' clearance in a 7 ml Dounce homogenizer made by Kontes Glass Co., Vineland, N.J., U.S.A. Twenty strokes with a pulley-driven Corning-TenBroeck homogenizer were used to rupture the cells of the whole jejunum and ileum from adult rat. The latter homogenization and centrifugation were by the method of Rodgers *et al.* (1967). The 105 000g-supernatant fractions were assayed for protein by the method of Lowry *et al.* (1951) with bovine serum albumin as standard, before assays of GSSG reductase and GSH-cystine transhydrogenase activities. All assays were performed on freshly prepared supernatant fractions.

Determination of GSSG reductase activity

The enzyme activity was assayed in a Beckman DU spectrophotometer at 340 nm by following the disappearance of NADPH with an attached Gilford automatic recorder. The recorder was calibrated so that 0.500 absorbance unit represented full-scale deflexion. Readings were recorded at 30 s intervals. The complete assay system, unless otherwise noted in the text, consisted of 1 ml of 0.1 M-potassium phosphate buffer, pH 7.4, containing 2 mM-EDTA, 0.05 mM-NADPH, 0.50 mM-GSSG and approx. 80 μ g of protein. Initial readings were recorded and cuvettes with boiled-tissue extracts and a reagent blank were run simultaneously. The reaction at 25°C was started by adding GSSG and was followed for at least 5 min. The amount of NADPH oxidized (μ mol/ml per min per mg of protein), after subtraction of control values, served as the basis for calculation of the μ M-GSSG reduced/min per mg of protein according to the equation:

Determination of GSH-cystine transhydrogenase activity

The routine incubation mixture consisted of 0.10 ml of enzyme preparation containing 75–110 μ g of protein in 0.10 ml of 0.1 M-potassium phosphate buffer, pH 6.5, containing final concentrations of 2 mM-EDTA, 50 μ M-NADPH, 0.225 mM-GSH and 0.17 mM-L-[³⁵S]cystine (approx. 0.2 μ Ci of ³⁵S). Incubations were for 6 min at 25°C. The reaction was stopped by addition of 0.2 ml of 2.5 mM-*N*-ethylmaleimide prepared in 0.1 M-potassium phosphate buffer, pH 6.5. The mixture was mixed immediately to form the *N*-ethylmaleimide adducts of GSH and cysteine.

Samples were deproteinized with 0.1 ml of 50% (w/v) trichloroacetic acid and spun for 20 min at 1000g in an International centrifuge. Supernatants were transferred to small tubes and unchanged *N*-ethylmaleimide and trichloroacetic acid were removed by extracting three times with 1 ml portions of diethyl ether. All assays were run in quadruplicate with duplicate boiled-tissue controls. Chromatography to separate glutathione-*N*-ethylmaleimide, cysteine-*N*-ethylmaleimide and cystine was carried out in solvent B4 (butan-1-ol – pyridine – acetic acid – water, 3:2:0.6:1.5, by vol.) as described by States & Segal (1969a).

Standards of cystine, glutathione-*N*-ethylmaleimide and cysteine-*N*-ethylmaleimide were chromatographed on each thin-layer plate and were located by staining with 0.2% ninhydrin in butan-1-ol followed by air-drying. Sections of the chromatographed samples comparable in position on the plate with the three standards were placed in vials containing 3 ml of phosphor scintillator (Liquifluor). Radioactivity was determined by counting in a Packard liquid-scintillation counter. Reduction of cystine to cysteine was based on the percentage of total radioactivity recovered in cysteine-*N*-ethylmaleimide $\times \mu\text{M}$ original substrate after subtraction of control values. GSH-cystine transhydrogenase activity was calculated as μM -cystine reduced/min per mg of protein. Total activity is defined as specific activity $\times$ total amount of protein (mg). The total amount of protein (mg) was based on total wet wt. of jejunum and total wet wt. of ileum as described by Herbst & Sunshine (1969), calculated by multiplication of the mg of protein/mg wet wt. by the total wet wt.

The activity of GSSG reductase has been amply demonstrated to reside in the soluble supernatant fraction of adult cells (Staal *et al.*, 1969; Icen, 1967; Mize *et al.*, 1962; Black & Hudson, 1961; Mavis & Stellwagen, 1968; Massey & Williams, 1965). States & Segal (1969b) showed that GSH-cystine transhydrogenase activity also was located primarily in the supernatant fraction of intestinal mucosa of adult rats. We determined that both activities are absent in particulate fractions of jejunum and ileum of 14-day-old rats and have therefore analysed activity only in the 105000g supernatant in our developmental studies.

Storage of the 105000g-supernatant fractions from jejunum from adult rats for 24 h at 4°C or at -20°C resulted in complete loss of GSH-cystine transhydrogenase activity but no decrease in GSSG reductase activity. However, the latter activity had dropped after 48 h of cold storage of the crude extract to 30–50% of the original values. Heating of the 105000g-supernatant fractions for 2–5 min at 60°C followed by centrifugation in the cold at 12000g for 10 min also caused complete inactivation of GSH-cystine transhydrogenase with no appreciable loss

in GSSG reductase activity, i.e. 24–27 μM -GSSG reduced/min per mg of protein after boiling.

Results

Studies of optimal assay conditions

Determination of GSSG reductase activity. To establish optimum conditions for assay of GSSG reductase activity, the velocity of the reaction with regard to substrate concentrations and amounts of enzyme protein was investigated. The results are shown in Figs. 1(a) and 1(b). Fig. 1(a) shows the linearity of the reaction with 0.02–0.12 mg of protein from extracts from intestines of newborn and adult rats. In all further studies, the amount of enzyme used in each assay was approx. 0.08 mg of protein.

Fig. 1(b) is a Lineweaver-Burk plot of enzyme activity in the 105000g-supernatant fraction from jejunum from adult rats with different concentrations of GSSG. The calculated K_m value was 0.25 mM-GSSG.

Determination of GSH-cystine transhydrogenase

Fig. 1. Determination of GSSG reductase activity

For details see the text. (a) shows the linearity of reaction rate at various protein concentrations of the 105000g-supernatant fraction from homogenates of: ●, adult rat ileum; ▲, adult rat jejunum; ○, newborn (12-day-old) rat ileum; △, newborn (12-day-old) rat jejunum. (b) Lineweaver-Burk plot of GSSG reductase activity in the 105000g-supernatant fraction from jejunum from adult rat at various GSSG concentrations.

activity. The spontaneity of cystine reduction in the presence of GSH, the solubility of L-cystine by itself (Jocelyn, 1967) and the rapid auto-oxidation of cysteine (Gorin & Doughty, 1968) prompted studies to determine the optimum cystine and GSH con-

centrations, the most advantageous amounts of enzyme protein, and the linearity of the rate of enzymic cystine reduction in the presence only of endogenous GSSG reductase in the tissue extract. Fig. 2 shows the results of such experiments. Lineweaver-Burk plots

Fig. 2. Determination of GSH-cystine transhydrogenase activity in the 105000g-supernatant fraction from jejunum from adult rat

For details see the text. (a) Lineweaver-Burk plot at various concentrations of cystine with 0.225 mM-GSH present. (b) Lineweaver-Burk plot at various concentrations of GSH with 0.17 mM-cystine present. (c) shows the effects of enzyme protein concentrations on GSH-cystine transhydrogenase activity. (d) shows the rate of reduction of cystine by GSH-cystine transhydrogenase assayed at 2 min intervals over a 12 min period.

of GSH-cystine transhydrogenase activities from results obtained by using various cystine concentrations with 0.225 mM-GSH present, and various GSH concentrations with 0.17 mM-cystine present, are shown in Figs. 2(a) and 2(b) respectively. Based on Fig. 2(a), the calculated K_m value for GSH-cystine transhydrogenase in a crude extract from jejunum of adult rat is 0.23 mM-cystine with a V_{max} of 0.023 mM-cystine reduced/min per mg of protein. The concentration of GSH giving one-half maximum velocity with a substrate concentration of 0.17 mM-cystine, calculated from the Lineweaver-Burk plot depicted in Fig. 2(b), is 1.67 mM-GSH.

In view of the above results, the limited solubility of L-cystine at pH 6.5 and the necessity to readjust the pH of the incubation media if higher concentrations of a stock solution of cystine solubilized with 0.5 M-NaOH were added, the following concentrations were used in all further assays: 0.17 mM-L-cystine, close to the value giving one-half maximum velocity, and 0.23 mM-GSH, which would act only in the capacity of a cofactor, i.e.

and would lead to no excessive spontaneous reduction of cystine.

Fig. 2(c) shows the effects of enzyme protein concentration on the amount of cystine reduced ($\mu\text{M}/\text{min}$). Below 40 μg of supernatant protein from jejunum from adult rats, non-enzymic reduction of cystine equalled enzymic reduction. The large correction for non-enzymic reduction prevents accurate determination of the enzymic reduction and is responsible for the line in Fig. 2(c) at values below 40 μg not going through the origin. Under the assay conditions the rate of reaction remained constant from 70–110 μg of protein and, though not shown in Fig. 2(c), steadily decreased above 110 μg of protein. Extrapolation of the second linear portion of the curve (70–110 μg of protein) to zero protein concentration places the line through the origin. Fig. 2(d) shows the linearity of the rate of reaction for the first 10 min of assay. Therefore, based on these results, an incubation time of 6 min and 75–100 μg of protein were selected in studies of enzyme development in rat intestine.

Changes in specific activities in intestine of developing rat

Development of GSSG reductase activity. Fig. 3 shows the changes in specific activity with development in the 105000g-supernatant fraction of homo-

genized mucosa from the ileum and jejunum. GSSG reductase activity is present at all stages of development investigated. In ileal mucosa, however, the specific activity does increase from a value of $16.4 \pm 1.84 \mu\text{M-GSSG}$ reduced/min per mg of protein on the seventh day to $25.66 \pm 2.50 \mu\text{M-GSSG}$ reduced/min per mg of protein on the twelfth postnatal day. The activity of the enzyme does not appear to change again until just before the period of weaning, i.e. 21 days, when the specific activity was $33.88 \pm 3.54 \mu\text{M-GSSG}$ reduced/min per mg of protein, a value not significantly different from that in the adult of $38.55 \pm 2.37 \mu\text{M-GSSG}$ reduced/min per mg of protein, i.e. $P = 0.30$.

In jejunal mucosa, GSSG reductase activity remained constant at $14\text{--}15 \mu\text{M-GSSG}$ reduced/min per mg of protein from day 7 to day 18. From the eighteenth day to the twenty-first day after birth, the specific activity increased from $15.0 \pm 1.12 \mu\text{M-GSSG}$ reduced/min per mg of protein to $25.18 \pm 2.68 \mu\text{M-GSSG}$ reduced/min per mg of protein respectively. As in the ileal mucosa, by 21 days the specific activity of the enzyme in the mucosa of the jejunum was comparable with that in adult rats of $31.0 \pm 5.0 \mu\text{M-GSSG}$ reduced/min per mg of protein.

Development of GSH-cystine transhydrogenase. Fig. 3 shows that no GSH-cystine transhydrogenase activity was found in the ileal mucosa of the 7-day-old rat. To test for the presence of an inhibitor of the enzyme at this stage of development, an extract of ileal mucosa from 7-day-old rats was mixed with an extract from the mucosa of the jejunum from adult rats at a 1:1 ratio. The mixed sample contained 95% of the GSH-cystine transhydrogenase activity in the jejunum from adult rat alone. As the ileum developed, however, the specific activity of the enzyme increased rapidly in the mucosa from no activity in the 7-day-old suckling rat to $6.69 \pm 1.15 \mu\text{M-cystine}$ reduced/min per mg of protein in the 14-day-old rat. By the eighteenth day, the specific activity had increased to $9.65 \pm 1.05 \mu\text{M-cystine}$ reduced/min per mg of protein. After weaning, the specific activity of the enzyme in the ileal mucosa of the 28-day-old animal decreased to $6.67 \pm 0.71 \mu\text{M-cystine}$ reduced/min per mg of protein, the value found in the 14-day-old suckling rat. By the time the animals were 35–42 days old, however, the specific activity of this thiol-disulphide-exchanging enzyme had reached its highest value of $12.99 \pm 0.72 \mu\text{M-cystine}$ reduced/min per mg of protein in the mucosa of the ileum.

The development of specific activity of GSH-cystine transhydrogenase in the mucosa of the jejunum was not as striking as in ileal mucosa. However, significant increases in specific activity did occur in the jejunal mucosa between the fourteenth and eighteenth days from 6.93 ± 0.14 to $13.87 \pm 1.51 \mu\text{M-cystine}$ reduced/min per mg of protein respectively. Also, as shown in the ileal mucosa, the

Fig. 3. Changes in specific activity of GSSG reductase (■) and GSH-cystine transhydrogenase (▨) with development in the high-speed-supernatant fractions of the mucosa from the ileum (a) and jejunum (b)

For details see the text. Values are means $\pm$ S.E.M. with the numbers of determinations in parentheses. The significance is based on probabilities (P) as determined by Student's t test distribution by the small-sample method which applies to the Bessel correction. For GSSG reductase activity: in ileal mucosa from: 7- and 12-day-old rats, $P < 0.02$; 7-day-old and adult rats, $P < 0.02$; 12-day-old and adult rats, $P < 0.01$; in jejunal mucosa from: 7- and 21-day-old rats, $P < 0.01$; 7-day-old and adult rats, $P < 0.01$; 18- and 21-day-old rats, $P < 0.02$; 18-day-old and adult rats, $P < 0.02$. For GSH-cystine transhydrogenase activity: in ileal mucosa from: 12- and 18-day-old rats, $P < 0.001$; 12-day-old and adult rats, $P < 0.001$; in jejunal mucosa: 7- and 18-day-old rats, $P < 0.01$; 7- and 28-day old rats, $P < 0.01$; 7- and 21-day-old rats, $P < 0.001$; 7-day-old and adult rats, $P < 0.001$; 12- and 18-day-old rats, $P < 0.01$; 12- and 21-day-old rats, $P < 0.01$; 12-day-old and adult rats, $P < 0.001$.

specific activity in the mucosa of the jejunum remained high during the eighteenth to twenty-first day after birth. After weaning, the specific activity of the enzyme was found to stabilize at a value not significantly different from that in the adult jejunal mucosa, i.e. $11.50 \pm 1.36 \mu\text{M}$ -cystine reduced/min per mg of protein in the 28-day-old rat compared with $12.55 \pm 0.56 \mu\text{M}$ -cystine reduced/min per mg of protein in the jejunum of adult rat.

Localization of activities during development. Table 1 records the specific activities of GSH-cystine transhydrogenase in the high-speed-supernatant fractions from the isolated mucosal and muscular layers of the jejunum and ileum. GSSG reductase activity is not included because this enzyme was distributed equally between the two layers throughout development. Table 1 shows that although GSH-cystine transhydrogenase activity is not detected in the mucosal

Table 1. *Specific activity of GSH-cystine transhydrogenase in high-speed-supernatant fractions of mucosal and muscular layers of jejunum and ileum*

For details see the text. Values of GSH-cystine transhydrogenase activity are calculated as μM -cystine reduced/min per mg of protein $\pm$ S.E.M., with the numbers of determinations in parentheses.

Age (days)	Jejunum		Ileum	
	Mucosa	Muscle	Mucosa	Muscle
7	5.40 ± 0.73 (7)	8.91 ± 0.59 (7)	0.00 (6)	6.21 ± 0.61 (6)
12	7.64 ± 0.60 (9)	7.96 ± 1.12 (9)	2.93 ± 1.11 (8)	9.59 ± 0.67 (8)
18	13.87 ± 1.51 (3)	7.32 ± 0.69 (3)	9.65 ± 1.05 (5)	10.39 ± 0.73 (5)
28	11.50 ± 1.36 (3)	8.06 ± 2.00 (3)	6.67 ± 0.71 (4)	3.64 ± 1.60 (4)
Adult	12.55 ± 0.56 (14)	7.69 ± 2.40 (14)	12.99 ± 0.72 (6)	3.33 ± 0.60 (6)

layer of the ileum of the 7-day-old suckling rat, it is present in the high-speed-supernatant fraction from the muscular layer. In fact, until the eighteenth day after birth, the enzyme has a higher specific activity in this fraction than in the supernatant fraction of the mucosa. After weaning, however, there is a shift in the locale of the higher specific activity of the enzyme from the muscular to the mucosal layer of the ileum. The specific activity of the enzyme in the mucosa continues to increase until, in the ileum from adult rat, it is approximately twice that of the 28-day-old rat. In the muscular layer of the ileum from adult rat, the specific activity of the enzyme remains at approximately one-third that found in the ileal muscle of the 12- to 18-day-old suckling rat.

In the jejunum, the specific activity of GSH-cystine transhydrogenase in the muscular layer did not change with development (approx. $8 \mu\text{M}$ -cystine reduced/min per mg of protein). Also, there were no significant differences in specific activities between the muscular and mucosal layers.

Comparative studies of total activities

For comparative purposes, the total activities of GSSG reductase and GSH-cystine transhydrogenase in the supernatant fractions of homogenates of jejunum and ileum were calculated (see the Materials and Methods section) for the entire length of these segments. Fig. 4 shows that initially in suckling rats, there is no significant difference in total GSSG reductase activity in the ileum and jejunum but by the twenty-first to twenty-eighth day after birth, total activity in the ileum surpassed that in the jejunum by 1.5 to 2 times. In the adult rat, activity in the ileum still exceeded the activity in the jejunum, although to a lesser degree, i.e. 1.25 times.

In comparing the total activities of GSH-cystine transhydrogenase, no significant differences existed between the developing jejunum and ileum. However, after the point of intestinal maturity (18 days after

Fig. 4. Total activity (μM -substrate reduced/min per mg of protein $\times$ total mg of protein) of GSSG reductase ($\circ$, $\bullet$) and GSH-cystine transhydrogenase (Δ , $\blacktriangle$) in the 105000g-supernatant fractions from the homogenates of rat ileum ($\circ$, Δ) and jejunum ($\bullet$, $\blacktriangle$)

For details see the text. Values are means $\pm$ S.E.M. of six determinations on days 4, 7, 18, 21 and 42, four determinations on day 35, seven determinations on day 14, and nine determinations on day 12.

birth), the total activity of the enzymes slowly increased in the jejunum compared with that in the ileum to a point where it was approximately 1.5 times greater in jejunum from adult rat.

Calculations of the ratios of total GSH-cystine transhydrogenase activity/total GSSG reductase activity in the supernatant fractions of the homogenized jejunum show no significant differences during development, i.e. about 0.32, with the overall change in ratios from suckling to adult rats being only 0.32 to 0.29. Ratios of total activities in the ileum, however, change from 0.44 in the 4-day-old rat to 0.12 in the adult rat, a 3.5-fold difference.

Comparison of the total activities of entire gut segments in the adult rat shows that total GSSG reductase activity is four times (jejunum) to eight times (ileum) greater than total GSH-cystine transhydrogenase activity.

Discussion

The existence in the high-speed-supernatant fraction of rat intestine of a heat-stable enzyme which reduces GSSG in the presence of NADPH and a heat-labile enzyme responsible for thiol-disulphide interchange parallels the findings by Tietze (1970) of a comparable disulphide-reducing system in rat liver. The heat-stable GSSG reductase investigated in the present study has many of the characteristics of its counterparts isolated from human erythrocytes (Icen, 1967), from rat liver (Tietze, 1970) and from rat kidney (Racker, 1955). The heat-labile GSH-cystine transhydrogenase, on the other hand, has many of the properties of GSH-disulphide transhydrogenase of rat liver (Tietze, 1970) and GSH-homocystine transhydrogenase of rat liver and kidney (Racker, 1955).

The question of the presence in rat intestine of either one enzyme with two active sites or the presence of two distinct enzymes cannot be answered unequivocally by the present study. However, the greater localization of GSH-cystine transhydrogenase in the mucosal layer of intestine from adult rat, together with different sites of greater total activity in the jejunum and ileum (GSSG reductase in ileum, GSH-cystine transhydrogenase in jejunum), suggest that two distinct enzymes are involved in this thiol-disulphide-interchange system. In either case, the close interrelationship between the two enzymic activities conforms with the mechanisms of many disulphide-reducing systems found in rat liver and other mammalian tissues (Scheme 1). The increases in specific activities of GSSG reductase and GSH-cystine transhydrogenase in the period of weaning are similar to patterns of development for a

number of other functions of the small intestine (Koldovsky, 1970). Such increases may be related to various factors such as dietary changes (Koldovsky *et al.*, 1963) and the effects of hormones (Koldovsky *et al.*, 1965-66; Pelichova *et al.*, 1967; Herbst & Sunshine, 1969). However, reports in the literature describe only the effects of diet on GSSG reductase activity. A direct correlation between increased activities of rat liver GSSG reductase and weaning on to high-carbohydrate diets has been demonstrated (Pinto & Bartley, 1968, 1969). Also significant increases in glycolytic activity in rat intestinal mucosa at the time of weaning (Srivastava & Hubscher, 1968), together with the role of GSSG reductase in regeneration of the NADP^+ essential to the pentose monophosphate pathway (Hosoda & Nakamura, 1970), may be related to the pronounced increase in activities of GSSG reductase within the intestine at the time of weaning.

Several observations indicate that GSH-cystine transhydrogenase may be the enzyme instrumental in the intracellular reduction of exogenous cystine to cysteine that occurs with entrance of cystine into cells. The findings in the present study of increased activities of intestinal GSH-cystine transhydrogenase about the twenty-first day after birth follows the pattern of increased transport of cystine into jejunal segments (States & Segal, 1968).

The importance of GSH-cystine transhydrogenase in cystine reduction takes on new meaning when one considers that this enzyme may be essential in maintaining the intracellular environment in its proper state. In human cystine-storage disease, there appears to be a failure in maintenance of the proper reductive state of cystine. It is known that in this disease there is a large accumulation of cystine in the intestine (Morecki *et al.*, 1968), and analysis of these two enzymes under proper assay and experimental conditions would appear to be an important undertaking.

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Scheme 1. Interrelationship between GSH-cystine transhydrogenase and GSSG reductase activities

References

- Black, S. & Hudson, B. (1961) *Biochem. Biophys. Res. Commun.* **5**, 135-138
- Chang, S. H. & Wilken, D. R. (1966) *J. Biol. Chem.* **241**, 4251-4260
- Eriksson, S. A. & Mannervik, B. (1970) *FEBS Lett.* **7**, 26-28
- Gorin, G. & Doughty, G. (1968) *Arch. Biochem. Biophys.* **126**, 547-551
- Herbst, J. J. & Sunshine, P. (1969) *Pediat. Res.* **3**, 27-33
- Hosoda, S. & Nakamura, W. (1970) *Biochim. Biophys. Acta* **222**, 53-64
- Icen, A. (1967) *Scand. J. Clin. Lab. Invest.* **96**, 7-67
- Jocelyn, P. C. (1967) *Eur. J. Biochem.* **2**, 327-331
- Koldovsky, O. (1970) in *Physiology of the Perinatal Period* (Stave, U., ed.), pp. 379-415, Appleton-Century-Crofts, New York
- Koldovsky, O., Danysz, J., Faltova, E. & Hahn, P. (1963) *Physiol. Bohemoslov.* **12**, 208-212
- Koldovsky, O., Heringova, A., Hoskova, J., Jirsova, V., Noack, R., Friedrich, M. & Schenck, G. (1965-66) *Biol. Neonatorum* **9**, 33-43
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Massey, V. & Williams, C. H., Jr. (1965) *J. Biol. Chem.* **240**, 4470-4480
- Mavis, R. D. & Stellwagen, E. (1968) *J. Biol. Chem.* **243**, 809-814
- Mize, C. E., Thompson, T. E. & Langdon, R. G. (1962) *J. Biol. Chem.* **237**, 1596-1600
- Morecki, R., Paunier, L. & Hamilton, J. R. (1968) *Arch. Pathol.* **86**, 297-307
- Nagai, S. & Black, S. (1968) *J. Biol. Chem.* **243**, 1942-1947
- Ondarza, R. N., Abney, R. & Lopez-Colome, A. M. (1969) *Biochim. Biophys. Acta* **191**, 239-248
- Pelichova, H., Koldovsky, O., Heringova, A., Jirsova, V. & Kraml, J. (1967) *Can. J. Biochem.* **45**, 1375-1384
- Pinto, R. E. & Bartley, W. (1968) *Biochem. J.* **109**, 34p
- Pinto, R. E. & Bartley, W. (1969) *Biochem. J.* **112**, 109-115
- Racker, E. (1955) *J. Biol. Chem.* **217**, 867-874
- Rodgers, J. B., Riley, E. M., Drummey, G. D. & Isselbacher, K. J. (1967) *Gastroenterology* **53**, 547-556
- Segal, S. & Smith, I. (1969) *Proc. Nat. Acad. Sci. U.S.* **63**, 926-933
- Srivastava, L. & Hubscher, G. (1968) *Biochem. J.* **110**, 607-608
- Staal, G. E. J., Visser, J. & Veeger, C. (1969) *Biochim. Biophys. Acta* **185**, 39-48
- States, B. & Segal, S. (1968) *Biochim. Biophys. Acta* **163**, 154-162
- States, B. & Segal, S. (1969a) *Anal. Biochem.* **27**, 323-329
- States, B. & Segal, S. (1969b) *Biochem. J.* **113**, 443-444
- Tietze, F. (1970) *Biochim. Biophys. Acta* **220**, 449-462
- Wendell, P. L. (1968) *Biochim. Biophys. Acta* **159**, 179-181