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

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(Received 23 October 1972)

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 homocysteine 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 transhydro- 
genase 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 develop-
ning 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 
ine 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]Cys-
tine 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. Ninyhydrin (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 0.15M-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 
(jejenum) 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 0.25M-D-mannitol– 
2.5mM-EDTA buffer adjusted to pH 7.4 with NaOH 
at a ratio of 1g wet wt. of tissue/14ml 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 7ml Dounce homo-
genizer made by Kontes Glass Co., Vineland, N.J., 
U.S.A. Twenty strokes with a pulley-driven Corning– 
TenBroek 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 105000g-
supernatant fractions were assayed for protein by the 
metho of Lowry et al. (1951) with bovine 
serum albumin as standard, before assays of GSSG 
reductase and GSH–cystine transhydrogenase activ-
ities. 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 340nm by following the dis-
appearance 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 30s intervals. 
The complete assay system, unless otherwise noted in 
the text, consisted of 1ml of 0.1m-potassium phos-
phate buffer, pH 7.4, containing 2mM-EDTA, 
0.050m-NADPH, 0.50mM-GSSG and approx. 80μg 
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 
5min. The amount of NADPH oxidized (μmol/ml 
per min per mg of protein), after subtraction of control 
values, served as the basis for calculation of the μm-
GSSG reduced/min per mg of protein according to the 
equation:

\[
\text{GSSG} + \text{NADPH} + \text{H}^+ \xrightarrow{\text{GSSG reductase}} 2 \text{GSH} + \text{NADP}^+
\]

Determination of GSH–cystine transhydrogenase activity

The routine incubation mixture consisted of 0.10ml 
of enzyme preparation containing 75–110μg of pro-	ein in 0.10ml of 0.1m-potassium phosphate buffer, 
\(\text{pH} 6.5\), containing final concentrations of 
2mM-EDTA, 50μm-NADPH, 0.225mM-GSH and 17.1m-
L-[35S]cystine (approx. 0.2μCi of \(35\)S). Incubations 
were for 6min at 25°C. The reaction was stopped by 
addition of 0.2ml of 2.5mM-N-ethylmaleimide 
prepared in 0.1m-potassium phosphate buffer, \(\text{pH} 6.5\). 
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 x μM original substrate after subtraction of control values. GSH-cystine transhydrogenase activity was calculated as μM-cysteine reduced/min per mg of protein. Total activity is defined as specific activity x 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 particular 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. 24-27μM-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 μg 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 μg 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 Kₘ value was 0.25 mM-GSSG.

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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 concentrations, 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

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.

$$\text{GSH–cystine transhydrogenase} \quad \text{Cys} + 2 \text{GSH} \xrightarrow{\text{GSH reductase}} 2 \text{CysH} + \text{GSSG}$$

$$\text{GSSG} + \text{H}^+ + \text{NADPH} \xrightarrow{\text{GSSG reductase}} 2 \text{GSH} + \text{NADP}^+$$

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 (μM/min). Below 40 μg of supernatant protein from jejunum from adult rats, non-enzymatic reduction of cystine equalled enzymic reduction. The large correction for non-enzymatic 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 homogenized 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 ± 1.84 μM-GSSG reduced/min per mg of protein on the seventh day to 25.66 ± 2.50 μM-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 ± 3.54 μM/GSSG reduced/min per mg of protein, a value not significantly different from that in the adult of 38.55 ± 2.37 μM-GSSG reduced/min per mg of protein, i.e. $P = 0.30$.

In jejunal mucosa, GSSG reductase activity remained constant at 14–15 μM-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 ± 1.12 μM-GSSG reduced/min per mg of protein to 25.18 ± 2.68 μM-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 ± 5.0 μM-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 ± 1.15 μM-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 ± 1.05 μM-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 ± 0.71 μM-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 ± 0.72 μM-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 ± 1.51 μM-cystine reduced/min per mg of protein respectively. Also, as shown in the ileal mucosa, the
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 ± 1.36 μM-cystine reduced/min per mg of protein in the 28-day-old rat compared with 12.55 ± 0.56 μM-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
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 μM-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 birth), the total activity of the enzyme 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 (Ioen, 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 cysteine 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.

This study was supported by Grant AM13900 from the National Institutes of Health, Bethesda, Md., U.S.A.

\[ \text{Scheme 1. Interrelationship between GSH–cystine transhydrogenase and GSSG reductase activities} \]
References


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