The Distinction Between γ -Glutamylhydroxamate Synthetase and L-Glutamine-Hydroxylamine Glutamyltransferase Activities in Rat Tissues

STUDIES *IN VIVO*

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The formation of γ -glutamylhydroxamate by homogenates under optimum assay condition showed an inconstancy in the ratios of the enzyme activities utilizing L-glutamate and ATP (γ -glutamylhydroxamate synthetase) and L-glutamine and ADP (L-glutamine-hydroxylamine glutamyltransferase) in a number of normal and neoplastic rat tissues. Although γ -glutamylhydroxamate synthetase activities in adult livers and kidneys were identical in males and females, L-glutamine-hydroxylamine glutamyltransferase activities in the organs of females were significantly lower. The developmental formations of the two activities in liver, kidney, brain and muscle were not simultaneous. The L-glutamine-hydroxylamine glutamyltransferase activity in foetal liver or neonatal kidney could be prematurely evoked by thyroxine, but the γ -glutamylhydroxamate synthetase activity remained unchanged. Injections of cortisol also had dissimilar effects on the two activities in thymus and hepatomas. The discrepant tissue distribution, asynchronous developmental formation and differential response to several hormonal stimuli provide evidence *in vivo* that the two activities are not catalysed by the same protein.

The preceding paper demonstrated by experiments *in vitro* that γ -glutamylhydroxamate formation by the γ -glutamylhydroxamate synthetase (EC 6.3.1.2) and L-glutamine-hydroxylamine glutamyltransferase reactions was not catalysed exclusively by the same protein (Herzfeld, 1973*b*). The synthesis of L-glutamine was closely associated with the GS enzyme† reaction but not with the GT enzyme reaction. In tissues actively hydrolysing L-glutamine, the assay of the activated glutamine moiety as the γ -glutamylhydroxamate is probably a more accurate quantitative estimate of glutamine formation in the tissue than is the isolation of L-glutamine itself.

The present paper discusses some characteristics inherent in a number of crude tissue preparations, which corroborate some of the findings *in vitro* (Herzfeld, 1973*b*). If both activities were catalysed by the same enzyme protein their ratios should be constant in different tissues (Mezl & Knox, 1972); their developmental formation in the same tissue should be coincident and their responses to hormonal stimuli should be identical. The results of the present study show that none of these criteria have been met when both GS enzyme and GT enzyme reactions were measured in the same tissue.

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† Abbreviations: GS enzyme, γ -glutamylhydroxamate synthetase; GT enzyme, glutamyl transferase (L-glutamine-hydroxylamine glutamyltransferase).

Methods and Materials

Normal tissues were from inbred male Kx rats (formerly described as NEDH rats) between 56 and 90 days old. Foetal ages were determined from the correlation between body weight and age (Gonzalez, 1932) in time-mated Kx rats. The characteristics of growth of Morris hepatomas and kidney tumours in male Buffalo rats (Morris, 1966), mammary tumours 1C, 5A, 3230AC and 7A in male Fischer rats and Walker 256 tumours in Kx male rats have been previously described (Knox *et al.*, 1970). Rhabdomyosarcoma NS104 was obtained from the Mason Institute, Worcester, Mass., U.S.A. The MK tumours (Morris, 1966) were obtained from Dr. H. P. Morris (Howard University, Washington, D.C., U.S.A.) and transplanted in the Cancer Research Institute animal colony into Buffalo rats.

All injections were intraperitoneal. Foetal animals were injected *in utero* (Greengard & Dewey, 1967); the indicated hormone was given to each foetus in one horn while foetuses in the other horn, injected with 0.9% NaCl, served as controls. Ovariectomies were performed by the procedure described by Ingle & Griffith (1942) at the age of 32 days.

Freshly excised tissues were disrupted in 9 vol. of 0.25M-sucrose or water in glass-Teflon homogenizers. For muscle, heart and mammary gland conical all-glass homogenizers were used. There was no difference in GS enzyme or GT enzyme activities whether homogenates were prepared in water or sucrose.

Enzyme assays, in homogenates were performed as described previously (Herzfeld, 1973b). GS enzyme incubation mixtures always contained an ATP-generating system; GT enzyme assays were done with 10mM-Mn²⁺. Enzyme activities are expressed as units ($\mu\text{mol/min at } 37^\circ\text{C}$)/g of tissue. The statistical significance of the values was established by evaluation of the results by Student's *t* test. A *P* value less than 0.01 was considered significant.

Results

Tissue distribution

A comparison of GS enzyme and GT enzyme activities in a variety of normal and neoplastic tissues is shown in Table 1. Enzyme activities, in units/g of tissue, are expressed relative to those in normal adult

male liver. One reference unit (R.U.) equals the enzyme activity (units/g) found in adult male liver. A number of normal tissues, e.g. spleen, brain, kidney and liver of males and lactating mammary gland, contained essentially the same relative amounts of GS enzyme and GT enzyme activities. These tissues are separated by a broken line in Table 1 from the next group of normal adult tissues in which the two activities did not parallel each other. In this group the GS enzyme and GT enzyme activities (R.U.) in each tissue were significantly different ($P < 0.01$). At one extreme, retina exhibited much higher GT enzyme activity than any other tissue (nearly twice that of standard liver) but a GS enzyme activity close to the median value for all tissues. Thymus, placenta, heart, skeletal muscle and pancreas had only marginally measurable GT enzyme activity but considerable

Table 1. Tissue distribution of γ -glutamylhydroxamate synthetase (GS enzyme) and L-glutamine-hydroxylamine glutamyltransferase (GT enzyme) activities in normal and neoplastic adult tissues

In the first two columns enzyme activities (units/g) are expressed (as means $\pm$ s.d.) in reference units (R.U.) relative to the activities in male adult liver. The absolute activities found in the reference tissue are shown in brackets (units/g $\pm$ s.d.). The ratios of GT enzyme/GS enzyme activities (third column) were calculated from the means of the absolute enzyme activities. The number of animals assayed are given in parentheses. The division of the Table into groups is explained in the text.

Normal tissues	Enzyme activities		Ratio [calculated from enzyme activities (units/g)] of GT enzyme/GS enzyme
	GS enzyme (R.U.)	GT enzyme (R.U.)	
Liver (male) (26)	1.0 [4.4 $\pm$ 1.1]	1.0 [200 $\pm$ 32]	45
Spleen (4)	0.98 $\pm$ 0.3	1.09 $\pm$ 0.1	51
Brain (16)	0.71 $\pm$ 0.16	0.85 $\pm$ 0.13	55
Kidney (male) (4)	0.61 $\pm$ 0.12	0.68 $\pm$ 0.18	51
Mammary gland, lactating, 5th-11th day (3)	0.23 $\pm$ 0.07	0.30 $\pm$ 0.08	59
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Skeletal muscle (8)	2.27 $\pm$ 0.78	0.08 $\pm$ 0.02	2
Liver (female) (5)	0.96 $\pm$ 0.07	0.57 $\pm$ 0.23	27
Heart (8)	0.80 $\pm$ 0.16	0.03 $\pm$ 0.03	2
Kidney (female) (5)	0.54 $\pm$ 0.2	0.33 $\pm$ 0.1	28
Retina (6 pools)	0.48 $\pm$ 0.1	1.78 $\pm$ 0.15	168
Placenta, 17th-22nd day (7)	0.40 $\pm$ 0.01	0.09 $\pm$ 0.06	10
Lung (4)	0.37 $\pm$ 0.25	0.07 $\pm$ 0.01	9
Thymus (4)	0.30 $\pm$ 0.05	0.05 $\pm$ 0.03	8
Pancreas (3)	0.29 $\pm$ 0.02	0.00 $\pm$ 0.01	∞
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Neoplasias			
Tumours MK-2, MK-3 (3)	0.44 $\pm$ 0.05	0.39 $\pm$ 0.21	40
Hepatoma 7800 (5)	0.35 $\pm$ 0.05	0.5 $\pm$ 0.2	67
Tumour NS104 (3)	0.10 $\pm$ 0.01	0.10 $\pm$ 0.005	51
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Mammary tumour 1C (3)	0.70 $\pm$ 0.09	0.20 $\pm$ 0.001	15
Mammary tumour 3230AC (5)	0.36 $\pm$ 0.16	0.05 $\pm$ 0.02	7
Walker 256 tumour (3)	0.32 $\pm$ 0.11	0.08 $\pm$ 0.01	11
Mammary tumour 5A (4)	0.23 $\pm$ 0.07	0.03 $\pm$ 0.025	6
Mammary tumour 7A (3)	0.20 $\pm$ 0.02	0.10 $\pm$ 0.03	25

amounts of GS enzyme activity. The GS enzyme content of liver and kidney in females was the same as in males but their GT enzyme activities were about one-half those in the male organs. Unlike Wu (1963b) and Iqbal & Wu (1971), we found high GS enzyme activity in skeletal muscle and some GS enzyme activity in heart coexisting with very low GT enzyme activity. It was observed previously (Herzfeld, 1973b) that in the absence of an ATP-generating system, no significant GS enzyme activity could be detected in muscle (<0.1 R.U.). Neoplastic tissues are grouped similarly to the normal ones: for tumours above the broken line in Table 1, GS enzyme and GT enzyme activities deviated from the standard tissue in constant proportion whereas tumours listed below the broken line showed relatively greater diminution of GT enzyme than of GS enzyme activity. Mammary tumour 1C, for example, had 70% of the GS enzyme activity found in liver but only 20% of its GT enzyme activity. In all tumours with disparate GS enzyme and GT enzyme activities, relatively high GS enzyme activity was maintained whereas that of GT enzyme was decreased noticeably. With the exceptions of hepatoma 7800 and tumour NS104, the tumours, representing widely different growth rates [b_{vol} , (change of log volume in ml/day) measured in six or more tumours in the generation analysed was 0.047–0.238] (Herzfeld & Knox, 1972) retained GS enzyme values as high as those found in their cognate tissues although their GT enzyme activities (again excepting that of tumour NS104) were lowered significantly.

The ratios between GT enzyme and GS enzyme activities (Table 1, last column), calculated from the absolute activities in each tissue, also show clearly the disparity between the activities of the two enzymes in some tissues. Among both normal and neoplastic tissues there were some in which the ratio was constant (about 50 times more GT enzyme activity than GS enzyme activity); there were, however, many tissues in which no such constancy of ratio between activities was obtained. It was shown previously (Table 4 of Herzfeld, 1973b) that some GT enzyme activity remains associated (in a constant ratio) with the GS enzyme activity during extraction and partial purification of the GS enzyme from liver homogenates. In assays of homogenates, this activity (8.5 times the GS enzyme activity) is always determined irrespective of any additional 'independent' GT enzyme activity the tissue might have. It is plausible to correct the GT enzyme values obtained in homogenates (Table 1) for this fraction of GT enzyme undissociable from GS enzyme activity (units of total GT enzyme/g minus $8.5 \times$ units of GS enzyme/g). Such revision gives a preliminary estimate of the 'independent' GT enzyme activity. It is slightly lowered from the values in Table 1, and the lowest values become zero.

Developmental formation

This inconstancy of the ratios in the majority of adult normal and neoplastic tissues prompted us to look more closely at the developmental formation of the two activities in several tissues. Fig. 1 shows the developmental formation of GS enzyme and GT enzyme in some rat tissues. All points show the enzyme activities as percentages of the respective activities in the adult. In liver and kidney GS enzyme activities (Fig. 1a) increased distinctly during late gestation, reached a transitory peak (32–55% of adult values) by the fifth postnatal day and attained the adult enzyme complement between the 12th and 21st day. GS enzyme activity in kidney was at the adult value by the 17th day. A transient neonatal peak in activity (between birth and day 5) had been observed previously in liver (Knox *et al.*, 1971; Wu, 1964) and in brain (Wu, 1964). Our results (not shown) for the developmental formation of GS enzyme in brain closely followed that in kidney. Muscle, in contrast to the other tissues, had low GS enzyme activity until the 17th day; its increase toward adult values had begun by the 21st day.

The developmental accretion of GS enzyme activity in liver and kidney (and brain) may be contrasted with that of GT enzyme in the same tissues. Fig. 1(b) shows that during the first two postnatal weeks the activity of GT enzyme in liver and kidney as well as brain (not shown) remained consistently low; there was no neonatal increase. GT enzyme activity began to accumulate significantly in the third postnatal week. Adult activities were reached somewhat earlier in liver (23 days after birth) than in kidney. The developmental formation of GT enzyme in muscle is not shown in Fig. 1(b); as shown in Table 1, even adult muscle had very low activity which was the same between the second and 21st days.

Hormonal effects

Disproportionality in the distribution of GS enzyme and GT enzyme activities in adult tissues and discrepant developmental formation of GS enzyme and GT enzyme provided support for the differences found *in vitro* between the GS enzyme and GT enzyme reactions (Herzfeld, 1973b). The significance of the findings shown in Table 1 and Fig. 1 could, however, be further substantiated by application of physiological stimuli which can distinguish GS enzyme and GT enzyme activities *in vivo* by altering the value of only one of the enzyme activities. Animals at various ages were therefore treated with hormonal stimuli known to enhance the developmental formation of some enzymes in liver or kidney (Greengard & Dewey, 1968; Herzfeld & Knox, 1968; Herzfeld & Greengard, 1971), or to change activities in tissues of adults (Herzfeld & Knox, 1968; Greengard & Machovich, 1972).

Fig. 1. Developmental formation of γ -glutamylhydroxamate synthetase (GS enzyme) (a) and L-glutamine-hydroxylamine glutamyltransferase (GT enzyme) (b) in rat tissues

Enzyme activities (units/g) are expressed as percentages of those in the adult tissues: $\circ$, liver; $\bullet$, kidney; $\triangle$, muscle. Absolute values for GS enzyme and GT enzyme activity (units/g) in adult male tissues were 4.4 ± 0.8 and 200 ± 32 (liver), 2.9 ± 0.12 and 137 ± 37 (kidney), and 10 ± 3.4 and 16 ± 5 (muscle) (see also Table 1). Points are means of values from three to ten postnatal animals (± 1 s.d.), from three or four foetal litters (± 1 s.d.) or averages of two or three pools from a single foetal litter. B indicates birth.

Table 2 summarizes some of the results obtained by injection of thyroxine into foetal animals. An injection of thyroxine into foetal animals did not increase the GS enzyme activity in liver but did evoke GT enzyme activities not normally observed in liver until many days after birth. Thyroxine injected 5 days after birth no longer stimulated GT enzyme accumulation in liver. Injections of cortisol acetate into foetal, neonatal or normal adult animals had no significant effect on either GS enzyme or GT enzyme activities in liver.

The present observations on the effects of cortisol on two abnormal variants of hepatic tissue, host liver

and hepatoma 7800, confirmed and expanded the findings by Wu & Morris (1970) that multiple injections of cortisol increased the activity of GS enzyme in hepatoma 7800. Two injections of 2.5 mg of cortisol/100 g body wt. doubled the activity of GS enzyme in the tumours whereas GT enzyme activity measured in the same homogenates, quadrupled. Somewhat less striking results were obtained with hepatoma 7777 (A. Herzfeld, unpublished work). Like Wu & Morris (1970), we found no important changes in GS enzyme activities in livers of hepatoma-bearing rats with or without cortisol treatment. GT enzyme activity, however, was depressed in host

Table 2. Effects of hormones on γ -glutamylhydroxamate synthetase (GS enzyme) and L-glutamine-hydroxylamine glutamyltransferase (GT enzyme) activities in normal and neoplastic tissues

Thyroxine (1 μ g/foetus or postnatal animal) or cortisol acetate (2.5 mg/100 g body wt.) were injected intraperitoneally 18 h before assay (prenatally) or 42 and 18 h before assay (postnatally). To induce thymus involution normal adult males were injected with 5 mg of cortisol/100 g body wt. twice daily for 2 days before assay. Thymus weights decreased by approx. 50% during the 2 days of cortisol treatment. Tumour weights in both untreated and cortisol-treated animals ranged from 0.5 to 1.6 g. Female animals were ovariectomized at the age of 32 days and assays were done between the 54th and 56th day. The number of litters or individual animals tested is given in parentheses. The values of activities are means $\pm$ s.d.

Tissue	Age	Enzyme activities (units/g)					
		GS enzyme			GT enzyme		
		No treatment	+Thyroxine	+Cortisol	Ovariectomy	No treatment	+Thyroxine +Cortisol Ovariectomy
Hepatic tissues	(3)	1.1 $\pm$ 0.02	1.15 $\pm$ 0.02	1.0 $\pm$ 0.2		19 $\pm$ 4	47 $\pm$ 6 25 $\pm$ 14 —
	(3)	1.6 $\pm$ 0.05	1.5 $\pm$ 0.3	—		30 $\pm$ 6	89 $\pm$ 15 —
	(10)	1.5 $\pm$ 0.15	1.65 $\pm$ 0.2	1.55 $\pm$ 0.15		54 $\pm$ 12	67 $\pm$ 7 58 $\pm$ 7 —
	(female) (5)	4.25 $\pm$ 0.3	—	—	3.7 $\pm$ 0.7	103 $\pm$ 46	— 172 $\pm$ 20
	(male) (13)	4.3 $\pm$ 0.5	—	4.6 $\pm$ 0.5	—	191 $\pm$ 32	183 $\pm$ 33
Hepatoma 7800	(host) (3)	5.2 $\pm$ 1.1	—	5.0 $\pm$ 1.0		121 $\pm$ 8	174 $\pm$ 8
	(3)	1.4 $\pm$ 0.2	—	3.4 $\pm$ 0.6		71 $\pm$ 24	283 $\pm$ 39
Kidney	—3	—	—	—		6.0	8.0
	+6	1.5 $\pm$ 0.2	1.5 $\pm$ 0.1	1.5		20 $\pm$ 7	55
	(6)	2.38 $\pm$ 0.8				66 $\pm$ 20	
(female)	Adult	2.70 $\pm$ 0.46			2.9 $\pm$ 0.3	136 $\pm$ 26	117 $\pm$ 10
	(5)						
	(male) (4)						
Thymus	Adult	1.4 $\pm$ 0.2		2.5 $\pm$ 0.2		7 $\pm$ 3	43 $\pm$ 5
	(6)						

livers and restored to normal by treatment with cortisol.

As shown in Table 1, the GS enzyme activities in livers of male and female adults were the same whereas GT enzyme activities in females were significantly lower than in males. The activities of at least two other transferases, ornithine aminotransferase (EC 2.6.1.13) (Herzfeld & Knox, 1968; Herzfeld & Greengard, 1969) and aspartate aminotransferase (EC 2.6.1.1) (Herzfeld & Greengard, 1971) in liver were decreased by the administration of oestrogen to male animals. We therefore examined the GS enzyme and GT enzyme activities in livers of rats ovariectomized shortly before the onset of sexual differentiation. As expected, no change occurred in the GS enzyme activities, but livers of the ovariectomized females showed GT enzyme activities nearly equal to those found in males. In summary, then, in hepatic tissues, whether during normal development, in host liver or in hepatoma, GT enzyme and GS enzyme activities showed a quantitatively dissimilar response to thyroxine, cortisol or ovariectomy.

Both enzyme activities were measured in the kidneys of some of the same animals treated with hormones. In foetal rats, GT enzyme activity was not increased in kidney by thyroxine injections, whereas at an early postnatal age injection of either thyroxine or cortisol evoked significant increases in GT enzyme activity but none in GS enzyme activity. As in livers of females, ovariectomy restored the GT enzyme activities in kidney to those normally found in males. Thus the lower GT enzyme activities normally found in liver and kidney of females may result from a suppressive effect of oestrogen on GT enzyme activity specifically.

To test whether the hormonal treatments used induced increases in the activities of GS enzyme or GT enzyme in other tissues, we examined the effects of cortisol on the activities of these enzymes in adult thymus and in mammary tumour 5A. We also compared the enzyme activities in brains of foetal and young postnatal animals when untreated or injected with thyroxine or cortisol. Some changes in enzyme activities in thymus during involution caused by injections of cortisol have recently been described (Greengard & Machovich, 1972). Table 2 illustrates that both GS enzyme and GT enzyme activities were raised in thymus of rats injected with cortisol but that the increases were quantitatively dissimilar: GS enzyme activity doubled whereas that of GT enzyme increased sixfold in the same animals. GS enzyme and GT enzyme activities in liver were unchanged by the same cortisol treatment. Injections of cortisol for 2 days also were ineffective in increasing either enzyme activity in mammary tumour 5A, and injections of neither thyroxine nor cortisol raised GS enzyme or GT enzyme activities in brain of foetal or neonatal animals.

The results presented in Table 2 indicate that, whenever a hormone stimulated or suppressed GS enzyme or GT enzyme activity, the GT enzyme activity was more sensitive to its action; if both enzyme activities were altered there was a quantitative dissimilarity between GS enzyme and GT enzyme responses to the stimulus. During foetal and neonatal development thyroxine, but not cortisol, enhanced the premature formation of GT enzyme but not of GS enzyme in liver. In kidney, either hormone accelerated postnatal accretion of GT enzyme but not of GS enzyme. Injections of cortisol raised the GT enzyme activity in host liver as well as in the hepatomas themselves: the increase in GS enzyme activity was quantitatively lower and restricted to the hepatomas. Cortisol also had selective and disproportionate effects on GS enzyme and GT enzyme activities in liver and thymus of the same adult animal.

Discussion

Many of our results are in accord with those reported in the literature. Values for GS enzyme activity, for example, obtained by Wu (1963a,b) and others (Raina & Ramakrishnan, 1964; Berl, 1966; Knox *et al.*, 1971) for liver, brain and kidney are of the same order of magnitude as those determined in the present study. The discrepancy between values reported by others (Wu, 1963b; Iqbal & Ottaway, 1970) for muscle and heart and the present observations can be partially accounted for by the modifications made in the assay procedure. We, too, could find essentially no glutamine synthetase activity in rat muscle when, like the previous authors, we relied on added ATP alone (Herzfeld, 1973b); only when an ATP-generating system was included in the incubation mixture could we obtain high γ -glutamylhydroxamate accumulation. Although not tested by us, heart may be another tissue exhibiting such a stringent requirement for a ATP-generating system.

GT enzyme activities reported in the literature are difficult to compare with our values since the reported assays were usually done under suboptimum conditions (Raina & Ramakrishnan, 1964; Moscona *et al.*, 1968; Reif-Lehrer & Amos, 1968; Chader, 1971) or are expressed in terms not convertible into enzyme units (Reif-Lehrer & Amos, 1968). When comparisons can be made, however, our values for enzyme activities agree fairly well with those reported (Berl, 1966; Chader, 1971), but ours are, in general, somewhat higher.

The results indicate that the GS enzyme and GT enzyme reactions are not catalysed exclusively by the same enzyme. This conclusion was first suggested by the remarkably wide range found in the ratios between the two activities in different tissues. In liver, some of the GT enzyme activity remained firmly associated with the partially purified fraction cata-

lysing the GS enzyme reaction (Herzfeld, 1973*b*) at a constant GT enzyme/GS enzyme activity ratio of 8.5. The major portion of the GT enzyme activity appeared to be independent of GS enzyme activity. Until the ratios of 'GS enzyme-associated' and 'independent' GT enzyme activities are determined in tissues other than liver, we can tentatively correct the total GT enzyme activities, measured in homogenates of these tissues, for the 'GS enzyme-associated' form (total GT enzyme activity in units/g minus $8.5 \times$ GS enzyme activity in units/g) on the basis of our findings in liver. These calculations do not alter the grouping shown in Table 1 but they suggest that although heart, placenta, pancreas, muscle, thymus and some mammary tumours clearly contain GS enzyme activity, they lack the independent GT enzyme activity.

During development the accretion of GT enzyme activity lags behind the accumulation of GS enzyme activity in liver, kidney and brain. Several enzymes whose developmental histories have been studied in several tissues in detail (Knox *et al.*, 1970; Greengard *et al.*, 1970; Herzfeld & Greengard, 1971; Machovich & Greengard, 1973; Herzfeld, 1973*a*; Snell & Walker, 1972*a,b*) exhibit, around the fifth postnatal day, a peak that either exceeds the normal adult value or is a transient intermediate rise, followed, in the third postnatal week, by a second and often major increase to adult values. A similar developmental course occurs for GS enzyme activity in liver, kidney and brain, and was previously observed (Wu, 1964; Knox *et al.*, 1971). For GT enzyme no significant increases in enzyme activities occur at all until the third week. These formational discrepancies suggest that different physiological stimuli may regulate the emergence of these enzymes.

The specific evocation of GT enzyme activity by thyroxine in livers of foetal rats and in kidneys of neonatal animals without coincident changes in GS enzyme activities strongly indicates that the two enzyme activities are indeed regulated by different physiological stimuli and therefore cannot represent two aspects of a single overall reaction. The sex difference exhibited by GT enzyme but not by GS enzyme activities in liver and kidney, which is overcome by ovariectomy, also indicates that the enzyme activities may be controlled independently.

The effect of cortisol on GS enzyme and GT enzyme activities in adult thymus, leading to a doubling of GS enzyme activity and a sixfold increase in GT enzyme activity, is worth noting for two reasons: cortisol, though in some circumstances enhancing both enzyme activities (cf. hepatoma 7800), alters their activities in a quantitatively dissimilar manner; and secondly, the augmentation of GS enzyme and GT enzyme activities in thymus and hepatoma 7800 is one of the few examples in which enzyme activities are increased in a tumour or in an involuting tissue

(Wu & Morris, 1970; Wu *et al.*, 1971; Greengard & Machovich, 1972) by such a treatment.

The relative preservation of GS enzyme activity in mammary tumours, accompanied by a consistent loss of GT enzyme activity as compared with liver, suggests that the GS enzyme reaction, but not the GT enzyme reaction, may measure a function physiologically important for tumours. Since glutamine formation may be considered a universally required activity, it is not surprising to find no tissue devoid of GS enzyme activity. The virtual absence of GT enzyme activity from some normal tissues and its general loss in tumours can be interpreted to mean that whatever function GT enzyme serves in a tissue, is not as vital for tumour growth as is the function of GS enzyme. It would seem to be characteristic of highly differentiated tissue states. Other tissues, less differentiated than normal adult organs, also show low GT enzyme activity. The more restricted range of GS enzyme and GT enzyme activities in tumours and foetal tissues (0.1–0.7 R.U. for GS enzyme and 0.03–0.5 R.U. for GT enzyme) when compared with the wide spread in well-differentiated tissues (0.23–2.27 R.U. for GS enzyme and 0–1.68 R.U. for GT enzyme) is yet another example supporting the generalization that less differentiated tissues, e.g. tumours, tend to resemble each other and foetal tissues more closely than they do more differentiated ones (Greenstein, 1954; Knox, 1972).

The variations in the distribution of GS enzyme and GT enzyme activities in different tissues, their asynchronous developmental formation, their differential premature evocation by thyroxine and their dissimilar response to cortisol in hepatomas and in thymus provide further evidence that GS enzyme and GT enzyme activities are not catalysed exclusively by the same protein.

The functional significance of each regulatory change in activity is not obvious, but the observed changes are at least consistent with the putative role of GS enzyme in glutamine synthesis and of GT enzyme in some as yet undescribed metabolic function of highly differentiated tissues.

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