neutral-type GST in human mononuclear leucocytes and demonstrates the existence of polymorphism. This method should prove valuable during further investigations into the expression of neutral GST in populations that are at risk from chemical carcinogenesis.

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Effect of age on the sinusoidal release of hepatic glutathione from the perfused rat liver*†

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GSH plays a critical role in cellular detoxification and defense. Recently, a complex interorgan process of regulation of GSH has been proposed [see, for example, Ref. 1]. According to this view, the liver releases GSH into the circulation by a carrier-mediated process [2, 3], accounting for nearly all release of GSH into plasma [4]. The circulating GSH is then cleared by the kidneys and other organs [5]. Thus, the liver plays a central role in the homeostasis of GSH.

The rate of hepatic GSH turnover has been reported to decrease with age [6]. However, while it has been estimated that the sum of sinusoidal and biliary release accounts for total hepatic GSH turnover [7], this relationship has not been explored over the whole range of ages used in the turnover studies. Besides, in these studies, similar to all earlier ones [1, 8], sinusoidal release was estimated to account for 80–90% and the biliary release for 10–20% of the total hepatic release. This contention is now under major revision due to findings in two recent independent studies [9, 10]. These studies, using retrograde biliary infusions of AT-125, a potent inhibitor of γ-glutamyltransferase, have documented substantial hydrolysis of GSH in bile. Thus, all previous studies, not using this agent, have underestimated biliary GSH release, which may actually account for up to 50% of total hepatic GSH release. In addition, one study [9] has shown that, while biliary GSH release remains constant in immature rats, it increases linearly (by about 6-fold) in the range of 180–300 g body weight. Thus, for total hepatic turnover to decline with age, the sinusoidal release will have to drop sharply to compensate for the rising biliary GSH.

The evidence reviewed above points to the fact that the sinusoidal GSH release may not quantitatively account as the major (i.e. ≈ 80%) component of total hepatic GSH turnover. Instead, it may account for no more than 50% of total turnover. Nevertheless, it still remains a significant component of hepatic GSH release and turnover. Therefore, it is important to determine its relationship to age. Our studies were designed to delineate this relationship.

Methods

Livers of male, Sprague–Dawley rats, 85–350 g body weight, fed *ad lib.*, were perfused *in situ*, single-pass with Krebs bicarbonate buffer gassed with 95% O_2/5% CO_2, pH 7.4 at 37°, as described earlier [2]. After an initial 10- to 15-min equilibration period, perfusates were sampled at 5-min intervals for 30–60 min. The rate of sinusoidal GSH release [nmol · min^{-1} · (g liver)$^{-1}$], measured as the product of perfusate GSH concentration, determined by the method of Tietze [11], and the perfusion rate (kept constant in each perfusion) remained at steady state throughout each and every perfusion (coefficient of variation ≤ 10%). As before

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† In our previous publications, we used efflux for the process we refer to as release here. The two terms refer to the same process in our papers.

Table 1. Data showing uniformity of the monitored perfusion variables among different body weight groups

N	Body weight (g)	Liver weight (g)	Liver weight/ Body weight (%)	Perfusion rate [ml·min^{-1}·(g liver)$^{-1}$]	Perfusion pressure (cm water)	O_2 uptake [μmol·min^{-1}·(g liver)$^{-1}$]
12	104 ± 1	3.84 ± 0.07	3.72 ± 0.06	4.2 ± 0.2	3.6 ± 0.3	2.1 ± 0.1
9	163 ± 2	6.24 ± 0.11	3.83 ± 0.09	3.7 ± 0.1	4.2 ± 0.2	2.2 ± 0.1
14	207 ± 2	8.16 ± 0.23	3.94 ± 0.10	3.9 ± 0.1	4.9 ± 0.7	2.2 ± 0.1
34	253 ± 2	9.32 ± 0.21	3.69 ± 0.08	3.8 ± 0.1	4.3 ± 0.5	2.1 ± 0.1
8	286 ± 2	10.1 ± 0.14	3.55 ± 0.05	3.8 ± 0.1	4.8 ± 1.2	2.4 ± 0.2
7	317 ± 6	10.8 ± 0.29	3.42 ± 0.09	3.9 ± 0.2	6.5 ± 1.1	2.2 ± 0.2

[2], liver viability was ascertained by continuous monitoring of O_2 uptake, perfusion pressure, and absence of release of cytosolic GSH *S*-transferase into the perfusate throughout each perfusion. Concentration of hepatic GSH (μmol/g) was measured at the end of each perfusion by the method of Owens and Belcher [12], after homogenization of the livers and precipitation of proteins [2]. These concentrations were insignificantly (≈5–10%) lower than unperfused livers from the same population of rats.

Results and discussion

Table 1 shows the important perfusion variables grouped according to ascending ranges of body (and liver) weights. As can be seen, perfusion rate, pressure, and O_2 uptake were uniform and in an acceptable range for all groups. On the other hand, while liver GSH concentrations of different age groups remained in the range of normal, *ad lib.*-fed levels, the sinusoidal rates of GSH release [both mass, nmol·min^{-1}·(g liver)$^{-1}$ and fractional, percent/hr, computed as the ratio of mass release to liver GSH concentration] declined with age (Fig. 1). The decline of the mass release rate appeared to be linear. A negative slope equal to 6.4 ± 0.4 (mean ± SEM) nmol·min^{-1}·(g liver)$^{-1}$ per 100 g body weight was resolved by linear regression analysis ($r = 0.99$). The decline of the fractional rate of release was not linear over the range of body weights studied. It had a steeper slope at the lower end of the body weight range, converging to a slope equal to that of the mass release data at higher weight levels.

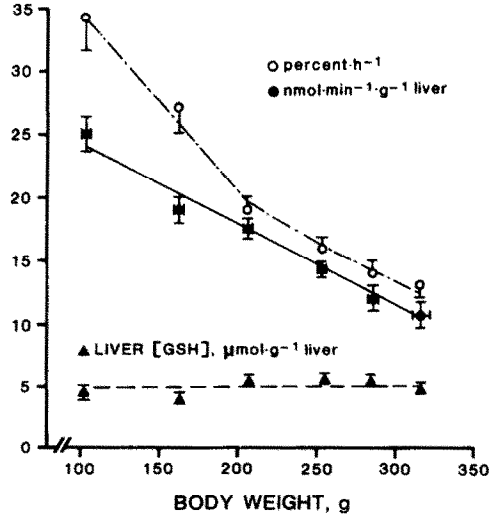

Fig. 1. Relationship of sinusoidal GSH release to body weight (age) in the perfused rat liver. Units are defined in the figure for fractional (○) and mass release (●) as well as for liver GSH concentration (▲). Symbols represent mean values; bars, SEM. The number of animals in each group is given in Table 1.

In our earlier studies we had perfused livers from rats fed *ad lib.*, with body weights in the range of 200–300 g, to define the saturable kinetics of GSH release as a function of liver GSH concentration [2]. Even in this rather narrow range of body weights, there was a hint of dependence of the rate of sinusoidal GSH release on body weight for livers having similar GSH concentrations. Thus, we decided to study the relationship of GSH release to body weight in more detail in livers with normal (fed *ad lib.*) levels of GSH over a wider range of body weights. Our present results show unequivocally that there was an age-dependent decrease in both mass rates [nmol·min^{-1}·(g liver)$^{-1}$] and fractional (percent/hr) rates of GSH release. Therefore, investigators studying hepatic GSH release or transport will need to consider this fact in their experimental designs and interpretations of results.

Lauterburg *et al.* [6], using their acetaminophen probe, have reported an age-dependent decrease in the total fractional rate of hepatic GSH turnover in rats. Their measured fractional rates decreased linearly from ≈55 to ≈35 percent/hr over a 100–300 g body weight range. Thus, their data defined a negative slope for this dependence of ≈10 percent/hr per 100 g body weight. Our data show a declining fractional rate of release from ≈35 to ≈15 percent/hr over a 100–300 g body weight range (with a nonlinear relationship, beginning and ending with negative slopes of ≈15 and ≈6 percent/hr per 100 g body weight). Although qualitatively similar to their data with increasing age, our measured fractional rates of release account for only ≈60 to ≈40% of the total fractional turnover rates reported by Lauterburg *et al.* [6].

Thus, our results are consistent with two aspects of the recent findings [9, 10]. First, the sinusoidal GSH release, at best, accounted for no more than 60% of the reported turnover rates, rather than the earlier estimates of ≈80%. Second, the contribution of sinusoidal GSH release to total turnover declined with age to as low as ≈40% for animals weighing 300–350 g. Hence, although falling GSH release contributed significantly to the decline in GSH turnover with age, it is doubtful that it completely accounted for the latter. Biliary GSH release is very low in immature rats, but it increases linearly with body weights above 180 g [9]. Therefore, as sinusoidal GSH release declined with age, biliary release apparently became a more important component of total hepatic GSH turnover.

In summary, we found that sinusoidal GSH release from the perfused rat liver decreased with age (body weight range = 85–350 g). This decrease occurred in the presence of liver GSH concentrations that remained in the normal range (≈4–7 μmol/g liver). Starting with ≈25 nmol·min^{-1}·(g liver)$^{-1}$ for rats weighing ≈100 g, the mass transport rate of release fell as a single straight line with a slope of 6.4 nmol·min^{-1}·(g liver)$^{-1}$ per 100 g body weight. On the other hand the fractional rate of GSH release (percent/hr) had a steeper slope of decline for the younger animals (up to ≈200 g) but began to parallel the decline in the mass rate of release for older animals.

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Relationship between binding and action of different prostaglandins in rat adipocytes with special reference to PGE_2 and PGI_2

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Prostaglandins (PGs) of the E-series (PGE_1 and PGE_2) have previously been shown to be potent inhibitors of stimulated cAMP accumulation and lipolysis both in rat [1–3] and human adipocytes [4–5]. These effects of PGEs are initiated by a binding reaction between PGE and a PG receptor located on the plasma membrane of the adipocytes [2–4, 6].

Recent studies by Axelrod *et al.* [7, 8] indicate that the only PGs that are produced in considerably sufficient amounts in rat adipocytes are PGE_2 and prostacyclin (PGI_2). In these studies and from our own study (to be published) it is shown that in adipocytes PGI_2 actually is produced in 2–4 times higher amounts than PGE_2. Although PGI_2 is produced in excess of PGE_2 the effect of PGI_2 on adipocyte metabolism has been much less studied. It has recently been shown that PGI_2 may have biphasic effects on the adenylate cyclase (AC) activity [9] and on lipolysis [10]. At low concentrations (nanomolar) PGI_2 enhanced the cAMP and the lipolytic responses. Whereas at higher concentrations (micromolar) PGI_2 had a similar effect to PGE_2 which is an inhibition of the AC complex and lipolysis [9, 10].

The present study was undertaken to determine if the effect of PGE_2 and PGI_2 were mediated by the same or by different receptors. In addition, the existence of other PG receptors in isolated rat adipocytes was also examined. The binding studies were related to the biological actions (antilipolysis) of these PGs.

Materials and methods

[^{3}H]PGE_2 (140–170 Ci/mmol), [^{3}H]PGI_2 (12.2 Ci/mmol), [^{3}H]$PGF_{2\alpha}$ (177 Ci/mmol) and [^{3}H]PGD_2 (131 Ci/mmol) were from New England Nuclear (Dreieich, F.R.G.). [^{3}H]PGA_2 (140 Ci/mmol) was from Amersham (U.K.). All other reagents were from Sigma (St Louis, MO).

Adipocytes from male Wistar rats were isolated as previously described [3, 4] with minor modifications since adenosine was added to the isolation buffer at a final concentration of 200 nmol/l in accordance to a recent report by Honnor *et al.* [11]. The adipocytes were then resuspended in a 10 mmol/l Hepes buffer (pH 7.4) [3, 4]. However, in studies with PGI_2, the pH of the incubation buffer was changed to 8.5 (at 37°) as described by Gaion *et al.* [11]. The alkanization of the incubation buffer was performed in an attempt to reduce the degradation of PGI_2, since PGI_2 is very labile under physiological conditions ($t^{1/2}$ = 3–5 min) [10, 12, 13].

Glycerol, cAMP and PG binding were determined under similar conditions. Adenosine deaminase (ADA) was present in a final concentration of 0.5 U/ml both in lipolysis and binding assays and these studies were performed for 60 min at 37° (in the PGI_2 studies the incubations were performed for 15 min). The contents of cAMP in adipocytes were determined after stimulation for 10 min with isoproterenol (500 nmol/l) by radioimmunoassay using an acetylation procedure [14].

PGE_2 binding sites in adipocytes were determined by [^{3}H]PGE_2 at a final concentration of 1–2 nmol/l. The binding affinity, expressed as the half-maximal inhibitory concentration (ED_{50}) of PGs, was determined from computer analysis of the individual competition curves. In some experiments the total binding capacity (B_{max}) and the binding affinity (K_d) were determined from Scatchard analysis [15] of the binding data.

Results and discussion

The potency order of several PGs for inhibition of PGE_2 binding was comparable with their antilipolytic potencies, with $PGE_2 \geq PGE_1 \gg PGI_2 \geq PGF_{2\alpha} \gg PGA_1 > PGD_2 >$ 6-keto-$PGF_{1\alpha}$ > arachidonic acid (Table 1). The K_d of the [^{3}H]PGE_2 receptor binding was 1.3 nmol/l (estimated from Scatchard plot). Thus, these PGE_2 binding sites had about 50 times higher affinity for PGs of the E-series than for the next group of PGs which includes PGI_2 and $PGF_{2\alpha}$ (Table 1). Except from PGI_2 no other prostaglandins