Biochem. J. (1980) **189**, 135–142
Printed in Great Britain

The Presence and Longitudinal Distribution of the Glutathione *S*-Transferases in Rat Epididymis and Vas Deferens

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(*Received 5 December 1979*)

The presence of the glutathione *S*-transferases, enzymes that catalyse the conjugation of glutathione with a variety of compounds, is reported here, for the first time, in the mammalian epididymis–vas deferens. These glutathione *S*-transferases, approx. 50% of those from rat liver on a per-mg-of-protein basis, are resolved by isoelectric focusing into six peaks, each with a characteristic isoelectric point and substrate specificity. By these same criteria, the first three peaks (pI 8.9, 8.2 and 7.8) can be identified as transferases B, A and C respectively. The fifth peak (pI 7.2) may correspond to transferase M; the fourth (pI 7.5) and sixth (pI 7.0) peaks do not correspond to previously described transferases. The distribution of transferase activity towards any one substrate studied differs in sequential sections of the epididymis and vas deferens; in addition, the longitudinal-distribution pattern differs for each of the three substrates studied. Isoelectric focusing of the cytosol fractions of the different sections further substantiates these observations. The potential significance of these enzymes and of their distribution in terms of epididymal function, maturation of spermatozoa, is discussed.

The glutathione *S*-transferases (EC 2.5.1.18) are a family of enzymes catalysing the nucleophilic attack by the thiol group of glutathione on compounds with an electrophilic site such as epoxides and halogenonitrobenzenes (Boyland, 1971; Jakoby *et al.*, 1976*a*). Various glutathione *S*-transferases (transferases AA, A, B, C, E) have been purified to homogeneity from rat liver (Fjellstedt *et al.*, 1973; Habig *et al.*, 1974; Pabst *et al.*, 1974; Askelöf *et al.*, 1975; Habig *et al.*, 1976). Except for transferases A and C, each is distinguishable both by immunological methods and by amino acid composition. Each transferase has a distinct isoelectric point (ranging between pI 7.0 and 10.0), thus allowing their resolution by isoelectric focusing or ion-exchange chromatography (Habig *et al.*, 1974; Jakoby *et al.*, 1976*a*,*b*; Hales *et al.*, 1978). Their specificities toward electrophilic substrates are broad and overlapping, yet distinctive for each form of the enzyme. Glutathione *S*-transferase B, the predominant transferase in rat liver has also been identified as ligandin (Litwack *et al.*, 1971), as a 3-oxo steroid Δ^5–Δ^4-isomerase (EC 5.3.3.1) (Benson *et al.*, 1977) and as a non-selenium-containing glutathione peroxidase (Burk *et al.*, 1977; Prohaska & Ganther, 1977). The precise biological role of this family of enzymes remains unresolved.

The glutathione *S*-transferases have been studied in some reproductive tissues of the rat. In the female rat, ovarian glutathione *S*-transferase activity toward styrene oxide (on a per-mg-of-protein basis) is 67% of that of the liver (Mukhtar *et al.*, 1978*a*). In the male rat, testicular glutathione *S*-transferase activities toward styrene oxide, 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene and *trans*-4-phenylbut-3-en-2-one vary from 40 to 90% of the hepatic activity (per mg of protein) toward these substrates (Mukhtar *et al.*, 1978*b*). Very low (less than 5%) glutathione *S*-transferase activity towards styrene oxide, benzo[*a*]pyrene 4,5-oxide and 1-chloro-2,4-dinitrobenzene has also been found in rat spermatozoa and human semen (Mukhtar *et al.*, 1978*c*). Although transferase activity is relatively high in both ovaries and testes, the functional significance of these proteins in the gonads is unknown. It has been suggested that the transferases perform an important protective role in preventing damage to germ cells by xenobiotic compounds (Mukhtar *et al.*, 1978*c*). To our knowledge, no-one has looked for glutathione *S*-transferase activity in other organs of the male reproductive tract, and in particular not in the epididymis.

The epididymis is the site of spermatozoal maturation (Bedford, 1966; Orgebin-Crist, 1967; Blaquier *et al.*, 1972). Proteins in mammalian

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spermatozoal nuclei and tails contain an unusually high concentration of cysteine, and it has been suggested that disulphide cross-links are formed after spermiation during passage through the epididymis, presumably to stabilize nuclear chromatin (Calvin & Bedford, 1971). Epididymal thiol oxidase may, according to Chang & Morton (1975), protect spermatozoa from damage by physiological concentrations of thiol compounds such as glutathione and cysteine. Enzymes of the γ-glutamyl cycle are also active in the epididymis (DeLap *et al.*, 1977). Concentrations of γ-glutamyl transpeptidase (EC 2.3.2.2) are higher in epididymis than in other rat tissues studied, with the exception of the kidney. This enzyme apparently also functions as a glutathione oxidase, catalysing the conversion of reduced glutathione into oxidized glutathione (Tate *et al.*, 1979; Tate & Orlando, 1979). In spermatozoa obtained from the vas deferens or from semen, it has been estimated that the concentration of glutathione is approx. 0.3 mM (Li, 1975), and an association of spermatozoa with glutathione reductase, glutathione peroxidase and glutathione transferase activities has been reported (Li, 1975; Mukhtar *et al.*, 1978*c*). Although spermatozoa undergo changes in their reduced-thiol content during epididymal transit and some enzyme activities are found in spermatozoa, few studies have evaluated the role(s) of enzymes that alter the balance between reduced and oxidized glutathione within the epididymis.

The present report focuses on the glutathione *S*-transferases in rat epididymis and vas deferens. The presence of glutathione *S*-transferase activity toward three substrates, 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene and *trans*-4-phenylbut-3-en-2-one, along rat epididymis–vas deferens is demonstrated. These substrates were chosen because the purified hepatic transferases have differential activity toward them. By using resolution by isoelectric focusing, the composition of the family of glutathione *S*-transferases along these tissues was studied.

Experimental

Animals and tissue preparations

Adult male Sprague–Dawley rats (Canadian Breeding Farm) weighing 250–300 g were maintained on a 14 h-light/10 h-dark cycle and received water and chow *ad libitum*. Animals were decapitated; both epididymides and vasa deferentia were removed, dissected free of fat and blotted. Each tissue was cut into eight sections, as shown in Fig. 1. The sections were weighed, and one tissue from each animal was prepared for the determination of spermatozoal content (see below), and the other was homogenized (1:10, w/v) in a Krebs–Ringer phosphate buffer and the cytosol fraction was prepared as previously described (Robaire, 1979). For isoelectric focusing this method was modified. The tissues were homogenized (1:3, w/v) in 10 mM-sodium phosphate buffer, pH 7.0, containing 20% (v/v) glycerol. The differences in the two buffers did not result in a significant change in enzymic activities.

Glutathione S-transferase assays

Assay conditions for measurement of the conjugation of 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene (Eastman Kodak Co., Rochester, NY, U.S.A.) and *trans*-4-phenylbut-3-en-2-one (Aldrich Chemical Co., Milwaukee, WI, U.S.A.) with glutathione were identical with those of Habig *et al.* (1974). Assays were conducted at room tempera-

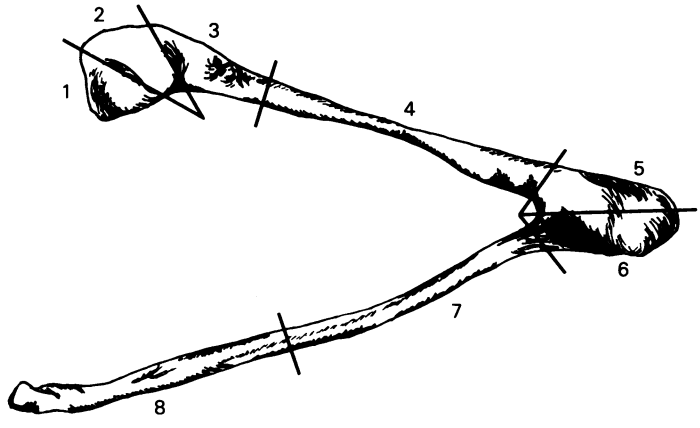

Fig. 1. *Epididymis–vas deferens tissue sections*
Epididymis–vas deferens was divided into eight sections: caput epididymis (sections 1, 2 and 3), corpus epididymis (section 4), cauda epididymis (sections 5 and 6) and vas deferens (sections 7 and 8).

ture (21–23°C) and were followed with a Beckman model 35 recording spectrophotometer. Reaction rates were linear with protein concentration and time for at least 2 min.

Isoelectric focusing in polyacrylamide gels

Isoelectric focusing in polyacrylamide disc gels was performed as described by Wrigley (1972). The Ampholine mixture (Hales *et al.*, 1978) consisted of: 25% (v/v) pHisolyte 2–11 (40%, w/v, solution), 25% (v/v) pHisolyte 8–10 (40%, w/v, solution) and 50% (v/v) pHisolyte 9–11 (20%, w/v, solution; Brinkmann Instruments, Westbury, NY, U.S.A.). Gels (10 cm) were chemically polymerized with ammonium persulphate and *NNN'N'*-tetramethylethylenediamine as catalysts. Samples (300 μl) of cytosol prepared from individual epididymal sections (Fig. 1) from a single rat were applied on top of the gels. Electrofocusing was carried out overnight at a maximum current of 1 mA/gel. After focusing, gels were cut into 0.3 cm pieces and extracted into 500 μl of 0.1 M-potassium phosphate, pH 6.5. Extracts were assayed for transferase activity as described above. One gel in each set was cut into 0.3 cm pieces and extracted into 500 μl of deionized water. The pH of each gel piece was measured with a pHM 62 pH-meter (Radiometer) and a combination micro-electrode.

Protein assays

Protein concentrations were measured by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

Tissue content of spermatozoa

Heads of spermatozoa in a tissue homogenate were counted with a haemocytometer by the method of Robb *et al.* (1978). Each value represents the mean of at least four determinations for each section of each animal. Number of spermatozoa per mg of tissue was obtained by dividing the mean number of spermatozoa per section by the mean tissue weight.

Statistical analysis

Statistical evaluation was done by using Student's *t* test and analysis of variance as described by Snedecor & Cochran (1967).

Results

Content of spermatozoa in epididymis–vas deferens

The section wet weights, numbers of spermatozoa per section and numbers of spermatozoa per mg of tissue are shown in Table 1. Spermatozoal content, expressed per section or per mg of protein, increases along the epididymis, with a sharp peak in the distal section of the cauda epididymis (Fig. 1, section 6). This is consistent with the hypothesis that the caput epididymis functions as the site for spermatozoal maturation and the cauda epididymis as that for spermatozoal storage (Orgebin-Crist *et al.*, 1975).

Glutathione S-transferase activities in epididymis and vas deferens

The glutathione *S*-transferase activities toward 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene and *trans*-4-phenylbut-3-en-2-one in sections of the rat epididymis (sections 1–6) and vas deferens (sections 7 and 8) are presented in Table 2. Glutathione *S*-transferase activity toward all three substrates is present in the rat epididymis (sections 1–6). Activity toward 1,2-dichloro-4-nitrobenzene and *trans*-4-phenylbut-3-en-2-one was below detect-

Table 1. *Spermatozoal content of epididymis–vas deferens sections*

The epididymis and vas deferens were cut into sections as illustrated in Fig. 1. The sections were weighed and the numbers of spermatozoa per section and per mg of tissue were determined as described in the text. Values are means ± S.E.M. for tissue sections from five animals.

Section	Weight (mg)	10^{-5} × No. of spermatozoa per section	10^{-5} × No. of spermatozoa per mg of tissue
Caput epididymis			
1	71 ± 4	154 ± 16	2.2
2	87 ± 9	296 ± 30	3.4
3	92 ± 10	485 ± 51	5.3
Corpus epididymis			
4	84 ± 8	400 ± 25	4.8
Cauda epididymis			
5	113 ± 4	494 ± 59	4.4
6	182 ± 19	1249 ± 116	6.9
Vas deferens			
7	41 ± 3	32 ± 5	0.8
8	66 ± 3	20 ± 5	0.3

Table 2. *Glutathione S-transferase activity of epididymis–vas deferens sections*

Enzymic activities, expressed in nmol/min per mg of cytosol protein, were assayed as described in the text. Values are means ± S.E.M. for five animals. Activity toward 1,2-dichloro-4-nitrobenzene and *trans*-4-phenylbut-3-en-2-one was not detectable (nd) in vas deferens (sections 7 and 8).

Tissue section (see Fig. 1)	Glutathione *S*-transferase activity		
	1-Chloro-2,4-dinitrobenzene	1,2-Dichloro-4-nitrobenzene	*trans*-4-Phenylbut-3-en-2-one
1	535.7 ± 41.9	19.9 ± 2.3	2.04 ± 0.34
2	738.5 ± 50.6	25.9 ± 2.9	3.25 ± 0.31
3	819.4 ± 66.4	37.4 ± 3.7	2.85 ± 0.07
4	797.7 ± 37.3	32.6 ± 2.8	3.31 ± 0.30
5	674.9 ± 56.5	30.7 ± 7.2	3.15 ± 0.24
6	507.7 ± 49.2	24.9 ± 1.7	3.05 ± 0.29
7	295.3 ± 34.7	nd	nd
8	271.3 ± 37.7	nd	nd

able limits in vas deferens when the final assay volume was 1 ml; low activity was detectable, however, when the assay volume was decreased to 0.5 ml and a 1:3 (w/v) tissue homogenate was prepared (isoelectric-focusing studies).

Along the rat epididymis and vas deferens, transferase activity toward 1-chloro-2,4-dinitrobenzene increased from 536 ± 42 nmol/min/mg of protein (mean ± S.E.M., $n = 5$) in section 1 to 819 ± 66 nmol/min per mg in section 3 and then diminished to 271 ± 38 nmol/min per mg in section 8. Transferase activity toward 1,2-dichloro-4-nitrobenzene increased from 19.9 ± 2.3 nmol/min per mg of protein in section 1 to 37.4 ± 3.7 nmol/min per mg in section 3 and then diminished to 24.9 ± 1.7 nmol/min per mg in section 6. Transferase activity toward *trans*-4-phenylbut-3-en-2-one increased from 2.04 ± 0.34 nmol/min per mg in section 1 to 3.25 ± 0.31 nmol/min per mg in section 2; then, in contrast with activity toward 1-chloro-2,4-dinitrobenzene or 1,2-dichloro-4-nitrobenzene, activity toward *trans*-4-phenylbut-3-en-2-one did not change significantly further along in the epididymis.

The conjugation of 1-chloro-2,4-dinitrobenzene with glutathione proceeds rapidly in the presence of transferases AA, A, B and C, whereas the conjugation of 1,2-dichloro-4-nitrobenzene proceeds rapidly with only transferases A and C (Jakoby *et al.*, 1976*a*). The discrimination by glutathione *S*-transferase B between these two substrates provides a qualitative measure of the concentration of transferase B relative to the other transferases (Hales & Neims, 1976). The ratio of glutathione *S*-transferase catalytic activities toward 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene is 22 in male-rat liver, where glutathione *S*-transferase B represents 48% of the activity toward 1-chloro-2,4-dinitrobenzene (Hales & Neims, 1976). This ratio is higher (27–29) in caput epididymis sections 1 and 2 than in cauda epididymis (20–22) sections 5 and 6. This difference indicates that there may be a higher proportion of glutathione *S*-transferase B in the caput epididymis. The conjugation of *trans*-4-phenylbut-3-en-2-one with glutathione is catalysed preferentially by transferase C (Jakoby *et al.*, 1976*a*) and perhaps by transferase M (Jaeger, 1979). Activity toward this substrate remains constant; thus the concentration of these enzymes may not change dramatically along the epididymis.

Transferase activity toward all three substrates is lower in the vas deferens than in the caput, corpus or cauda epididymis.

All glutathione *S*-transferase activities described above are given for the cytosol fraction of each tissue section. The cytosol activities represent 85–92% of the total homogenate activities in all sections of the epididymis–vas deferens. The remaining enzymic activities are found in the crude nuclear fraction (1000 *g* × 10 min pellet). Spermatozoa, extruded from tissue slices by positive pressure, have enzymic activities which are sufficient to account for those found in the crude nuclear fraction (8–15% of the total activities in the homogenate).

Isoelectric-focusing pattern of glutathione S-transferase activities

To determine the forms of glutathione *S*-transferase present in rat epididymis and vas deferens and to study the distribution of these forms along the epididymis, cytosol from sections 1, 3, 6 and 7 from single rats ($n = 5$) was electrofocused on polyacrylamide gels. Representative profiles of the focused glutathione *S*-transferase activities toward 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene and *trans*-4-phenylbut-3-en-2-one are shown in Fig. 2. Six major peaks of transferase activity (pI 8.9, 8.2, 7.8, 7.5, 7.2 and 7.0) were resolved in section 1. A comparison of the isoelectric points of these peaks in section 1 with the

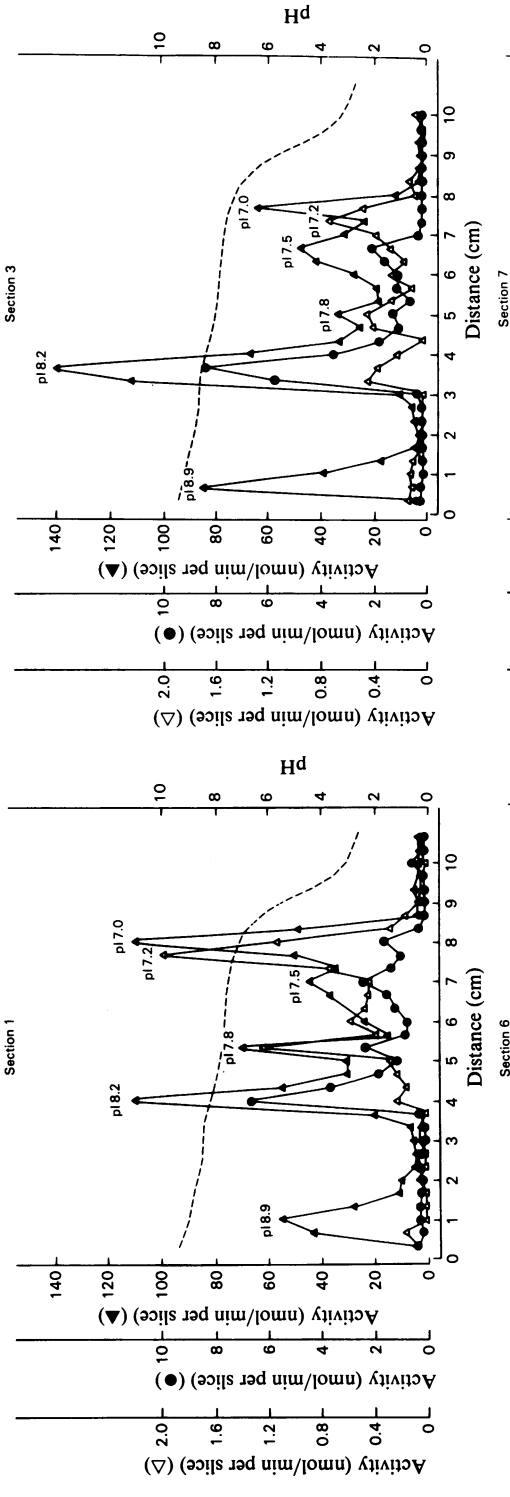

Fig. 2. *Isoelectric-focusing patterns of glutathione S-transferase activity in cytosol from epididymis–vas deferens sections*
Cytosol from rat epididymis–vas deferens sections (300 µl) prepared as described in the text was applied to 10 cm polyacrylamide gels, containing the described Ampholine mixture, and focused overnight at an initial current of 1 mA/gel. The pH (– – –) of each gel piece (0.3 cm) was measured. Enzyme activity (nmol/min per gel slice) toward 1-chloro-2,4-dinitrobenzene (▲), 1,2-dichloro-4-nitrobenzene (●) and *trans*-4-phenylbut-3-en-2-one (△) was eluted and assayed as described in the text.

isoelectric points of the peaks of transferase activities after focusing sections 3, 6 and 7 revealed that the transferases in all sections had the same isoelectric points (Table 3). Epididymal transferase peaks with pI 8.9, 8.2 and 7.8 correspond to hepatic transferases B, A and C respectively on the basis of substrate specificity and relative isoelectric point. The transferase with pI 7.2 may correspond to transferase M. Epididymal transferase peaks of pI 7.5 and 7.0 do not seem to correspond to previously described transferases.

The various transferase peaks present in each section of the epididymis–vas deferens have been quantified by measuring peak height (nmol/min per gel piece) in the five replicates. This is illustrated in Fig. 3. There is less transferase activity toward all three substrates in all isoelectric-focused peaks in vas deferens (section 7) as compared with epididymal sections 1, 3 or 6. Transferase peak pI 7.5 is not present in vas deferens. Transferase B (pI 8.9) increases in section 3, as was predicted by the ratio of activity toward 1-chloro-2,4-dinitrobenzene to that toward 1,2-dichloro-4-nitrobenzene. All other peaks decrease or are not altered in sequential sections along the epididymis–vas deferens as compared with section 1 (caput epididymis).

Relative glutathione *S*-transferase activity can be expressed as a ratio of activity (peak height) in any single peak to the sum of activities for any given

Table 3. *Isoelectric points of the glutathione S-transferase peaks resolved by isoelectric focusing*
The pH of each gel piece where glutathione *S*-transferase activity reaches a peak was determined as described in the text. Isoelectric points are means ± S.E.M. for five cytosol preparations isoelectrofocused in separate experiments.

Peak of enzyme activity	Tissue section ...	Isoelectric point 1	3	6	7
1		8.91 ± 0.03	8.91 ± 0.08	8.91 ± 0.06	8.85 ± 0.04
2		8.21 ± 0.08	8.29 ± 0.12	8.22 ± 0.07	8.04 ± 0.07
3		7.84 ± 0.11	7.83 ± 0.13	7.74 ± 0.11	7.73 ± 0.27
4		7.46 ± 0.13	7.39 ± 0.15	nd*	nd*
5		7.18 ± 0.14	7.17 ± 0.21	7.17 ± 0.13	7.21 ± 0.16
6		6.98 ± 0.16	6.99 ± 0.23	6.99 ± 0.13	7.20 ± 0.24

* For these sections no discernible (nd) peak of transferase activity was observed in this pH range.

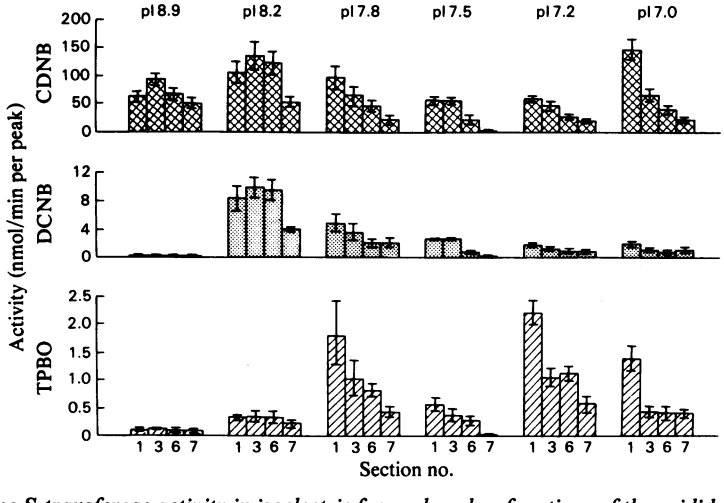

Fig. 3. *Glutathione S-transferase activity in isoelectric focused peaks of sections of the epididymis–vas deferens*
Glutathione *S*-transferase activity for each isoelectric-focused peak was quantified by measuring peak height (nmol/min per gel piece). Values are means ± S.E.M. for five replicates. Enzyme activity per peak toward 1-chloro-2,4-dinitrobenzene (CDNB), 1,2-dichloro-4-nitrobenzene (DCNB) and *trans*-4-phenylbut-3-en-2-one (TPBO) was obtained from isoelectric-focused polyacrylamide gels as described in the text.

substrate and tissue section. By using this type of analysis, it is apparent that although the relative activity of some transferases toward 1-chloro-2,4-dinitrobenzene increases from the caput epididymis to the vas deferens [transferase B (pI 8.9), from 12 to 32%; transferase A(pI 8.2), 20 to 33%], the activity of other transferases remains fairly constant [transferase C (pI 7.8), and transferase with pI 7.2] and the activity of others (transferases with pI 7.5 and 7.0) decreases markedly. Analysis of the transferase activities towards 1,2-dichloro-4-nitrobenzene and *trans*-4-phenylbut-3-en-2-one further support these conclusions.

Discussion

Glutathione S-transferase activity toward 1-chloro-2,4-dinitrobenzene, on a per-mg-of-protein basis, in sections 1, 3 and 6 of the epididymis is 55, 83 and 52% of transferase activity toward this substrate in liver (Hales *et al.*, 1978). Activity in these three sections toward 1,2-dichloro-4-nitrobenzene is 45, 85 and 57%, respectively, of the hepatic activity toward this substrate (Hales *et al.*, 1978). In contrast with these high activities, activity toward *trans*-4-phenylbut-3-en-2-one is relatively low, representing 12, 17 and 18% of the liver activity toward this substrate (Hales *et al.*, 1978). Glutathione *S*-transferase B, the predominant hepatic transferase, has been estimated to make up 3–5% of male liver cytosol protein (Hales & Neims, 1976), with the family of glutathione *S*-transferases adding up to approx. 10% of cytosol protein in normal rat liver (Jakoby *et al.*, 1976*b*). If the specific activities of the epididymal transferases B, A and C are the same as those purified from liver, it would appear that these glutathione *S*-transferases may comprise 5–8% of epididymal cytosol protein. This is interesting, as the epididymis, unlike the liver, is not thought to have a major involvement in drug metabolism or mercapturic acid synthesis. The glutathione *S*-transferases may serve as transferases to protect the epididymis and maturing spermatozoa from electrophiles. Alternatively, these enzymes may catalyse a reaction completely distinct from glutathione conjugation (e.g. steroid isomerization) in this tissue.

Glutathione *S*-transferase activity toward 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene is differentially distributed along the epididymis–vas deferens, with the highest activity found in the distal caput epididymis and lowest in the cauda epididymis and vas deferens. Activity toward *trans*-4-phenylbut-3-en-2-one does not change significantly along the epididymis, but is low or non-detectable in vas deferens.

Three of the peaks of transferase activity resolved by isoelectric focusing of epididymal cytosol appear identical with liver transferases B (pI 8.9), A (pI 8.2) and C (pI 7.8) on the basis of isoelectric point and substrate specificity (Hales *et al.*, 1978). Epididymal transferase peaks of pI 7.5 and 7.0 do not correspond to described hepatic transferases. The transferase of pI 7.2 may be the same as the previously described renal transferase of pI 7.0 (Hales *et al.*, 1978) and hepatic transferase of pI 6.6. Experiments (Jaeger, 1979) with the renal transferase of pI 7.0 led to the suggestion that this protein may correspond to the hepatic glutathione *S*-transferase M purified by Gillham (1973).

The data in Fig. 3 show that there is less of each transferase peak in vas deferens than in the epididymis. The peak of pI 7.5 is not present in vas deferens. In the epididymis, transferase B (pI 8.9) activity is higher in section 3 than in section 1, as predicted from the ratio of activity toward 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene (Table 2). The other transferases either do not change or decrease in successive sections of the epididymis. Expressed as the relative contribution of each peak to the sum of activities for a given substrate, two transferases increase (transferases B and A), two transferases remain constant (transferases C and pI 7.2) and two others decrease (transferases pI 7.5 and 7.0) along the epididymis–vas deferens, with respect to caput epididymis (section 1). It should be noted that this analysis reflects only the major isoelectrofocused peaks of transferase activity and does not take into account the basal activity that is distributed throughout the gel.

The nature and biological significance of the balance between reduced and conjugated thiol groups and of the different enzymes that can alter this balance remains unresolved. However, in the present paper it has been established that there is high glutathione *S*-transferase activity (50% on a per-mg-of-protein basis of that found in the liver, the tissue having the highest specific activity in the body; Boyland, 1971) in the epididymis–vas deferens, that a large number of transferases exist in this tissue and that there is a differential localization of these enzymes along the epididymis–vas deferens. Furthermore, it is apparent that the activities in sequential sections reflect neither the amount of spermatozoa present (Table 1) nor the activities of either of the two major epididymal androgen-metabolizing enzymes, steroid Δ^4-5α-reductase and 3α-hydroxy steroid dehydrogenase (Robaire *et al.*, 1977; B. Robaire & C. Hachey, unpublished work). It is noteworthy, however, that section 3 is the site where a high proportion of spermatozoa gain fertilizing capacity (maturation) (Orgebin-Crist *et al.*, 1975). The hormonal regulation of this family of enzymes may thus be of key significance in understanding the process of spermatozoal maturation.

This study was supported by the Medical Research Council of Canada, the Fraser Memorial Fund of the Royal Victoria Hospital and McGill University. B. F. H. is the recipient of a fellowship from the Conseil de la Recherche en Santé du Québec and B. R. is a Scholar of The Medical Research Council of Canada.

References

Askelöf, P., Guthenberg, C., Jakobson, I. & Mannervik, B. (1975) *Biochem. J.* **147**, 513–522

Bedford, J. M. (1966) *J. Exp. Zool.* **163**, 319–330

Benson, A. M., Talalay, P., Keen, J. H. & Jakoby, W. B. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 158–162

Blaquier, J. A., Cameo, M. S. & Burgos, M. H. (1972) *Endocrinology* **90**, 839–842

Boyland, E. (1971) in *Handbook of Experimental Pharmacology* (Brodie, B. B. & Gillette, J. R., eds.), vol. 28, pp. 584–608, Springer-Verlag, Berlin

Burk, R. F., Lawrence, R. A. & Parkhill, L. K. (1977) *Gastroenterology* **73**, A-17/1215

Calvin, H. I. & Bedford, J. M. (1971) *J. Reprod. Fertil., Suppl.* **13**, 65–75

Chang, T. S. K. & Morton, B. (1975) *Biochem. Biophys. Res. Commun.* **66**, 309–315

DeLap, L. W., Tate, S. S. & Meister, A. (1977) *Life Sci.* **20**, 673–680

Fjellstedt, T. A., Allen, R. H., Duncan, B. K. & Jakoby, W. B. (1973) *J. Biol. Chem.* **248**, 3702–3707

Gillham, B. (1973) *Biochem. J.* **135**, 797–804

Habig, W. H., Pabst, M. J. & Jakoby, W. B. (1974) *J. Biol. Chem.* **249**, 7130–7139

Habig, W. H., Pabst, M. J. & Jakoby, W. B. (1976) *Arch. Biochem. Biophys.* **175**, 710–716

Hales, B. F. & Neims, A. H. (1976) *Biochem. J.* **160**, 223–229

Hales, B. F., Jaeger, V. & Neims, A. H. (1978) *Biochem. J.* **175**, 937–943

Jaeger, V. (1979) Ph.D. Thesis, McGill University, Montreal

Jakoby, W. B., Habig, W. H., Keen, J. H., Ketley, J. N. & Pabst, M. J. (1976*a*) in *Glutathione: Metabolism and Function* (Arias, I. M. & Jakoby, W. B., eds.), pp. 189–211, Raven Press, New York

Jakoby, W. B., Ketley, J. N. & Habig, W. H. (1976*b*) in *Glutathione: Metabolism and Function* (Arias, I. M. & Jakoby, W. B., eds.), pp. 213–223, Raven Press, New York

Li, T.-K. (1975) *Biol. Reprod.* **12**, 641–646

Litwack, G., Ketterer, B. & Arias, I. M. (1971) *Nature (London)* **234**, 466–467

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Mukhtar, H., Philpot, R. M. & Bend, J. R. (1978*a*) *Biochem. Biophys. Res. Commun.* **81**, 89–98

Mukhtar, H., Lee, I. P., Foureman, G. L. & Bend, J. R. (1978*b*) *Chem.-Biol. Interact.* **22**, 153–165

Mukhtar, H., Lee, I. P. & Bend, J. R. (1978*c*) *Biochem. Biophys. Res. Commun.* **83**, 1093–1098

Orgebin-Crist, M.-C. (1967) *Nature (London)* **216**, 816–818

Orgebin-Crist, M.-C., Danzo,, B. J. & Davies, J. (1975) in *Handbook of Physiology* (Hamilton, D. W. & Greep, R. O., eds.), section 7, vol. 5, pp. 319–338, American Physiology Society, Washington D.C.

Pabst, M. J., Habig, W. H. & Jakoby, W. B. (1974) *J. Biol. Chem.* **249**, 7140–7150

Prohaska, J. R. & Ganther, H. E. (1977) *Biochem. Biophys. Res. Commun.* **76**, 437–445

Robaire, B. (1979) *Can. J. Physiol. Pharmacol.* **57**, 998–1003

Robaire, B., Ewing, L. L., Zirkin, B. R. & Irby, D. C. (1977) *Endocrinology* **101**, 1379–1390

Robb, G. W., Amann, R. P. & Killiam, G. J. (1978) *J. Reprod. Fertil.* **54**, 103–107

Snedecor, G. W. & Cochran, W. G. (1967) *Statistical Methods*, 6th edn., Iowa State University Press, Ames, Iowa

Tate, S. S. & Orlando, J. (1979) *J. Biol. Chem.* **254**, 5573–5575

Tate, S. S., Grau, E. M. & Meister, A. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2715–2719

Wrigley, C. W. (1972) *Methods Enzymol.* **22**, 559–564