Comparison of Renal and Hepatic Glutathione *S*-Transferases in the Rat*

By NEIL KAPLOWITZ,† GIL CLIFTON, JOHN KUHLENKAMP and JOHN D. WALLIN
Clinical Investigation Center, Naval Regional Medical Center, Oakland, CA 94627, U.S.A.

(Received 16 February 1976)

Renal and hepatic GSH (reduced glutathione) *S*-transferase were compared with respect to substrate and inhibitory kinetics and hormonal influences *in vivo*. An example of each of five classes of substrates (aryl, aralkyl, epoxide, alkyl and alkene) was used. In the gel filtration of renal or hepatic cytosol, an identical elution volume was found for all the transferase activities. Close correspondence in K_m values was found for aryl-, epoxide- and alkyl-transferase activities, with only the aralkyl activity significantly lower in kidney. Probenecid and *p*-aminohippurate were competitive inhibitors of renal aryl-, aralkyl-, epoxide- and alkyl-transferase activities and inhibited renal alkene activity. Close correspondence in K_i values for inhibition by probenecid of these activities in kidney and liver was found. In addition, furosemide was a potent competitive inhibitor of renal alkyl-transferase activity. Hypophysectomy resulted in significant increases in aryl-, aralkyl-, and epoxide-transferase activities in liver and kidney. The hypophysectomy-induced increases in renal aryl- and aralkyl-transferase activities (approx. 100%) were more than twofold greater than increases in hepatic activities (approx. 40%). Administration of thyroxine prevented the hypophysectomy-induced increase in aryltransferase activity in both kidney and liver. The renal GSH *S*-transferases, in view of similarities to the hepatic activities, may play a role as cytoplasmic organic-anion receptors, as previously proposed for the hepatic enzymes.

The hepatocyte and kidney-tubular cell share the capacity to transport organic anions at physiological pH values. Organic anions are delivered to these cells principally bound to soluble plasma protein carriers such as albumin. In the translocation of these substances from blood to bile or urine, it is reasonable to suppose that cytosolic protein carriers may exist with an intracellular role comparable with that of albumin in plasma. Such a cytosolic transport protein has been identified in liver and was originally called 'Y protein', a designation which refers to organic-anion binding to the 45000-mol.wt. protein fraction in gel filtration (Levi *et al.*, 1969). Subsequently, a single protein was purified from the Y-protein fraction and was designated 'ligandin' (Litwack *et al.*, 1971). Ligandin binds a variety of organic anions which are selectively extracted from plasma by the liver (Litwack *et al.*, 1971). Ligandin

has been identified as one (GSH† *S*-transferase B) of a family of hepatic cytosol detoxifying enzymes, the GSH *S*-transferases (Habig *et al.*, 1974a). Therefore it is believed that it functions both as an organic-anion binding receptor and as an enzyme (Habig *et al.*, 1974a). However, ligandin is not unique as an organic-anion receptor and may share this role with the other GSH *S*-transferases, all of which bind organic anions (Kaplowitz *et al.*, 1975b).

GSH *S*-transferase activities have been identified also in rat kidney and studied with respect to drug induction and sex differences (Clifton *et al.*, 1975b). Ligandin has been shown to be immunologically identical in rat liver and kidney (Kirsch *et al.*, 1975), but no comparison of the catalytic properties of this protein in the two organs was made. The present paper describes studies in the rat designed to compare the GSH *S*-transferases of liver and kidney with respect to Michaelis-Menten kinetics, inhibitory kinetics, and hormonal influences such as hypophysectomy, after which hepatic Y protein is known to increase (Reyes *et al.*, 1971).

Part of this work was presented at the Western

* Reprint requests should be addressed to the Publications Office, Clinical Investigation Center, Naval Regional Medical Center, Oakland, CA 94627, U.S.A.

† Present address: Veterans Administration, Wadsworth Hospital Center, Wilshire and Sawtelle Boulevards, Los Angeles, CA 90073, U.S.A.

‡ Abbreviation: GSH, reduced glutathione.

Section, American Federation for Clinical Research, Carmel, CA, U.S.A. (Clifton *et al.*, 1975a).

Materials and Methods

Animals and preparation of cytosol

Male Sprague-Dawley rats (160–180 g) were killed after light ether anaesthesia. Hypophysectomized, adrenalectomized and gonadectomized male rats were obtained from Simonsen Laboratories, Gilroy, CA, U.S.A. All animals were maintained on usual laboratory chow and drinking water for 2 weeks before killing. Certain animals received L-thyroxine (Sigma Chemical Co., St. Louis, MO, U.S.A.) intraperitoneally in a daily dose of 14 µg/100 g body wt. The livers and kidneys were perfused *in situ* with 0.25 M-sucrose containing sodium phosphate buffer, 0.01 M, pH 7.4, at 4°C. Homogenates (20%, w/v) were prepared in 0.01 M-sodium phosphate buffer, pH 7.4, and centrifuged for 10 min at 500g followed by 60 min at 105 000g in a Beckman L2-65B ultracentrifuge (Beckman Instruments, Fullerton, CA, U.S.A.). The supernatants (cytosol) were carefully pipetted after removal of the lipid layer by suction.

Gel-filtration experiments

Gel filtration with columns (38 cm × 2.5 cm) containing Sephadex G-75 or G-100 (Pharmacia, Uppsala, Sweden), flow rate 22–24 ml/h (10 fractions/h), was performed by using 0.01 M-sodium phosphate buffer, pH 7.4, as mobile phase in a pump-driven upward-flow system at 4°C. To 4.0 ml of cytosol was added 1.0 µCi of [[2-³H]Gly]glutathione [³H]GSH; New England Nuclear Corp., Boston, MA, U.S.A.; 250 mCi/µmol), 5.0 µCi of [[2-³H]Gly]*p*-aminohippuric acid (Amersham/Searle, Arlington Heights, IL, U.S.A.; 149 µCi/mmol) or 5.0 µCi of [³⁵S]furosemide (a gift from Hoechst Pharmaceuticals, Somerville, NJ, U.S.A.; 662 µCi/mmol).

Determination of enzymic activities

The GSH *S*-transferase activities were measured in cytosol and column fractions by using one example of five classes of substrates and previously described techniques (Kaplowitz *et al.*, 1975a; Pabst *et al.*, 1974; Habig *et al.*, 1974b): 3,4-dichloronitrobenzene (GSH *S*-aryl-), *p*-nitrobenzyl chloride (*S*-aralkyl-), 1,2-epoxy-(3-*p*-nitrophenoxy)propane (*S*-epoxide-), ethacrynic acid (*S*-alkene-), and [¹⁴C]methyl iodide (*S*-alkyl-transferase). The latter was obtained from New England Nuclear Corp. (4.86 mCi/mmol). All assays used excess of GSH (10 mM), except that for alkenetransferase activity, which used a lower GSH concentration (0.25 mM). Non-enzymic interaction of substrates was measured by using the same assay conditions without cytosol and was subtracted from reaction rates with cytosol. The enzymic reactions

were linear with respect to time and protein concentrations. The substrates for enzymic reactions were obtained from Aldrich Chemical Co., Milwaukee, WI, U.S.A. GSH was obtained from Sigma. Furosemide was a gift from Hoechst Pharmaceuticals and ethacrynic acid was a gift from Merck, Sharp and Dohme, West Point, PA, U.S.A. Ovalbumin (mol. wt. 45 000) was obtained from Pharmacia, Piscataway, NJ, U.S.A. Probenecid [4-(dipropylsulphamyl)-benzoic acid] and *p*-aminohippuric acid were obtained from Sigma and Aldrich respectively.

Enzyme kinetics

Each activity in kidney and liver was determined over a range of substrate concentrations. Data were expressed by the method of Lineweaver & Burk (1934). The Michaelis constant (K_m) was calculated from the abscissa intercept of plots of least-squares-regression equations. An accurate determination of K_m for ethacrynic acid substrate could not be obtained because of the limiting GSH concentration required to decrease the rate of the non-enzymic reaction.

Inhibitory kinetics were investigated by using three substrate concentrations and a range of inhibitor concentrations. The data were expressed by the method of Dixon (1953). The inhibitor constants were calculated from the least-squares-regression equations and represent the mean intersection of three lines.

Inhibition experiments were also performed by using [2-¹⁴C]ethacrynic acid (a gift from Merck, Sharp and Dohme Research Laboratories, Rahway, NJ, U.S.A.; 16 µCi/mg) as substrate. Duplicate 1 ml reaction mixtures contained 0.2 mM-ethacrynic acid (0.3 µCi), 0.25 mM-GSH, 0.1 M-sodium phosphate buffer, pH 6.5, and inhibitor. Renal cytosol (50 µl) was added to initiate the reaction at 37°C, and the reaction was stopped after 30 s by adding 1.0 ml of methanol and placing the sample on ice. Immediately, 25 µl was spotted on a pre-coated silica-gel t.l.c. plate (0.2 mm thickness) (Quanta gram, Quantum Industries, Fairfield, NJ, U.S.A.) and run overnight in a tank containing a butanol/acetic acid/water (4:1:2, by vol.) solvent system. Sections (1 cm wide) of the dried plates were scraped off and placed in counting vials to which were added 1.0 ml of water and 10 ml of Aquasol-II (New England Nuclear Corp.). Samples were counted for radioactivity in a Packard Tri-Carb liquid-scintillation spectrometer. The proportion of radioactivity (counts) running as the ethacrynic acid-GSH conjugate was calculated.

Protein concentration was determined by the method of Lowry *et al.* (1951), with bovine albumin (Sigma) as standard.

Statistical analyses involving comparison of unpaired groups of data used Student's *t* test (Batson, 1956).

Results

Identification of GSH S-transferases in gel filtration

Gel filtration (on Sephadex G-100) was applied with kidney cytosol, and each fraction was assayed for five enzyme activities and binding of [^3H]GSH. An identical elution volume (90ml) was found for each activity and [^3H]GSH. Under the same column conditions, an identical elution volume (90ml) was found for the hepatic activities and ovalbumin (mol.wt. 45000). By using renal cytosol [^3H]p-aminohippuric acid and [^{35}S]furosemide bound to the same gel filtration fractions that exhibited GSH S-transferase activity.

Comparison of K_m values for renal and hepatic GSH S-transferases

K_m values for the four activities in liver and kidney listed in Table 1 represent means $\pm$ S.E.M. of determinations with renal or hepatic cytosol from four rats. No significant difference in K_m between kidney and liver was found for aryl-, epoxide, or alkyl-transferase activity. The K_m for renal aralkyl-transferase activity, however, was significantly lower than the hepatic value ($P < 0.005$).

Table 1. Comparison of Michaelis constants for renal and hepatic glutathione S-transferases

See the text for experimental details. N.S., not significant ($P > 0.05$).

Transferase activity	K_m (mM)		
	Kidney	Liver	
Aryl	3.1 $\pm$ 0.1	3.2 $\pm$ 0.6	N.S.
Epoxide	0.69 $\pm$ 0.02	0.73 $\pm$ 0.03	N.S.
Aralkyl	0.74 $\pm$ 0.03	1.15 $\pm$ 0.05	$P < 0.050$
Alkyl	2.4 $\pm$ 0.4	2.8 $\pm$ 0.6	N.S.

Inhibition by organic anions

Inhibitory kinetics of GSH S-transferases by organic anions were studied by using kidney supernatant as the source of enzyme activity. Both probenecid and p-aminohippurate were competitive inhibitors of all GSH S-transferase activities studied. The inhibitor constants for the competitive inhibition of the GSH S-transferases by probenecid and p-aminohippurate are given in Table 2. On the basis of the 2- to 40-fold lower K_i values, probenecid was a more potent inhibitor than p-aminohippurate. As a typical example, the competitive inhibition of GSH S-epoxidettransferase activity by probenecid is shown as a Dixon plot in Fig. 1. As indicated in Table 2, a close correspondence in K_i values for the competitive inhibition by probenecid of the hepatic and renal GSH S-transferases was found.

Inhibition of renal GSH S-alkenetransferase activity by organic anions was examined by using [^{14}C]ethacrynic acid as substrate. The spectrophotometric assay was not used because of inhibitor interference and therefore a detailed kinetic study was not made. As indicated in Table 3, both probenecid and p-aminohippurate inhibited this GSH S-alkenetransferase activity. However, a more than 12-fold higher concentration of p-aminohippurate than probenecid was required to produce approx. 50% inhibition of the enzymic reaction.

Examination of kinetics by using furosemide as inhibitor was hampered by its strong interference with the spectrophotometric enzyme assays. Therefore only the inhibition of renal GSH S-alkyltransferase ([^{14}C]methyl iodide as substrate) was studied in detail. Furosemide was found to be a potent competitive inhibitor of this activity, with a K_i value (0.19mM) one order of magnitude lower than that found for the probenecid inhibition (cf. Table 2).

Neither probenecid, p-aminohippurate, nor furosemide was a substrate for enzymic activity when incubated for 60 min *in vitro* with cytosol and GSH.

Table 2. Inhibition of glutathione S-transferase by organic anions

See the text for experimental details. No significant difference in K_i was found for probenecid inhibition of renal and hepatic activities. All the inhibition reactions studied were found to be competitive. N.D., not done owing to p-aminohippurate interference with spectrophotometric assay. Values are means $\pm$ S.E.M. for three experiments.

Inhibitor ... Transferase activity	K_i (mM)		
	Kidney		Liver
	p-Aminohippurate	Probenecid	Probenecid
Aryl	16.3 $\pm$ 1.2	7.9 $\pm$ 0.8	9.2 $\pm$ 0.7
Aralkyl	N.D.	4.4 $\pm$ 0.6	3.1 $\pm$ 0.6
Epoxide	44.7 $\pm$ 5.4	1.0 $\pm$ 0.1	0.99 $\pm$ 0.05
Alkyl	10.6 $\pm$ 2.2	1.5 $\pm$ 0.2	1.5 $\pm$ 0.1

Fig. 1. Dixon plot showing competitive inhibition of renal GSH *S*-epoxidettransferase activity by probenecid

Reaction mixtures (2.05 ml) contained 1,2-epoxy-3-(*p*-nitrophenoxy)propane (0.3, 0.5 or 0.7 mM), GSH (10 mM) and probenecid (0–5 mM) in 0.1 M-sodium phosphate buffer, pH 6.5, at 37°C. The reaction was initiated by the addition of 200 μ l of renal cytosol. The reciprocal of the initial rate ($1/v$) is expressed as arbitrary units of activity derived from changes in E_{360} . The K_i value (1.07 mM) was obtained as the mean intersection between the three lines (1.10, 1.06 and 1.04 mM). For substrate concentrations 0.3, 0.5, and 0.7 mM the respective lines are $y = 3.13x + 11.33$, $1.66x + 9.71$ and $1.9x + 9.17$ ($r = 0.99$ for each).

Table 3. Inhibition of enzymic ethacrynic acid–glutathione conjugation

See the text for experimental details. Non-enzymic glutathione conjugation of ethacrynic acid was 4%, cf. 10% enzymic conversion in the absence of inhibitor.

Inhibitor	Concentration (mM)	Inhibition (%)
<i>p</i> -Aminohippurate	9.5	38
	24	54
	48	81
Probenecid	0.95	19
	1.9	53
	2.9	82

Effect of hypophysectomy on renal and hepatic GSH *S*-transferases

The GSH *S*-transferases were measured in groups of eight hypophysectomized and eight sham-operated control rats 14 days after surgery. No change in liver wt./body wt. or kidney wt./body wt. ratios was found after surgery. Significant increases in both renal and hepatic aryl-, aralkyl-, and epoxide-transferase activities ($P < 0.01$ for hepatic aryltransferase; $P < 0.001$ for the others) were found after hypophysectomy (Fig. 2). Increases in aryl- and aralkyl-transferase activities, however, were significantly greater (more than twofold) in the kidney ($P < 0.001$) than in liver, whereas the increase in epoxidettransferase activity was comparable in the two organs. There was no significant change in alkyl- and alkene-transferase activities in either organ. Groups of six animals that had been subjected to gonadectomy or adrenalectomy showed no significant change in hepatic or renal GSH *S*-transferases activities compared with sham-operated controls assayed after a 14-day interval. Thyroxine was administered daily to eight sham-operated controls and eight hypophysectomized rats from 8–14 days after surgery by the procedure of Reyes *et al.* (1971). The hypophysectomy-induced increase in hepatic and renal aryltransferase activity was prevented by thyroxine administration so that activities were the same in both groups. Thyroxine did not, however, prevent the hypophysectomy-induced increases in aralkyl- and epoxide-transferase activities in kidney and liver. Thyroxine administration to controls did not alter the transferase activities.

Discussion

The GSH *S*-transferases are a group of soluble enzymes (mol.wt. 45000) which catalyse the detoxification of electrophilic drugs and carcinogens through conjugation with nucleophilic GSH (Boylard & Chasseaud, 1969; Habig *et al.*, 1974b; Pabst *et al.*, 1974; Kaplowitz *et al.*, 1975a). In the liver, four enzymes have been identified, referred to as glutathione *S*-transferases A, B, C and E (Habig *et al.*, 1974b; Pabst *et al.*, 1974). Overlap has been found in the ability of the individual enzymes to catalyse reactions with the different substrate classes. However, transferase E alone seems to have epoxide-transferase activity (Habig *et al.*, 1974b), and transferase B, which is identical with ligandin, has the bulk of activity towards ethacrynic acid (alkene substrate) (Habig *et al.*, 1974b). According to these workers, although transferase E has higher activity towards methyl iodide (alkyl substrate), the more abundant transferase B accounts for the bulk of activity towards this substrate in liver.

Transferases A and C, which are immunologically related, share virtually all activity towards 3,4-

Fig. 2. Effect of hypophysectomy on hepatic and renal GSH S-transferase specific activities

* Indicates a significant difference ($P < 0.001$) between the change in enzyme activity per mg of protein in kidney compared with liver. S.E.M. values are indicated. For experimental details, see the Materials and Methods section. N.S., not significant.

dichloronitrobenzene (aryl substrate) (Habig *et al.*, 1974b; Pabst *et al.*, 1974). Activity towards aralkyl substrates, represented by *p*-nitrobenzyl chloride, is low for transferase B but does reside in all four proteins (Habig *et al.*, 1974b), although its hepatic induction by phenobarbital seems to parallel increases in aryltransferase activity (Kaplowitz *et al.*, 1975a).

The GSH S-transferase activities for the above substrate classes have also been identified in the kidney (Clifton *et al.*, 1975a,b). The specific activities of the GSH S-transferases in kidney as compared with liver for aryl, aralkyl, epoxide, alkyl and alkene substrates are 2, 17, 68, 92 and 109% respectively (Clifton *et al.*, 1975b). Purification of all enzyme proteins in kidney responsible for these activities has not been accomplished. In gel filtration, the elution volume of the enzyme activities is the same for kidney or liver. Further, ligandin (transferase B) has been identified by an immunofluorescence technique in the proximal tubular cell and is immunologically identical with liver ligandin (Kirsch *et al.*, 1975). Thus there is little doubt that the group of enzymes present in the kidney is closely related to those in the liver. The similarity between the enzymes in both organs is now further supported by comparisons of substrate-dependence and response to inhibitors, and the effects of hypophysectomy.

Close correspondence in K_i values for probenecid inhibition of both hepatic and renal activities was noted. In addition, apparent K_m values were found

to be very close for all but the aralkyltransferase activity, which had a K_m significantly different in the two organs. The finding of a difference in the activity with *p*-nitrobenzylchloride is particularly noteworthy, because of the previously reported selective induction of this renal activity by phenobarbital in the rat (Clifton *et al.*, 1975b).

We have previously described sex differences in activities of both hepatic and renal GSH S-transferases (Clifton *et al.*, 1975b; Kaplowitz *et al.*, 1975a). The effect of hypophysectomy to increase certain of these enzyme activities in both kidney and liver further emphasizes the complex hormonal regulation of this enzyme system. Measurement of these activities after adrenalectomy and gonadectomy failed to reproduce the increases in enzyme specific activities seen with hypophysectomy. In addition, the administration of thyroxine by the same procedure shown to affect Y protein (Reyes *et al.*, 1971) blocked only the increase in renal and hepatic aryltransferase activity, not affecting the hypophysectomy-induced increases in epoxide- or aralkyl-transferase activities. These observations, in addition to contrasting the renal and hepatic responses to changes in hormonal milieu, may help clarify previous studies of the hormonal influences on hepatic Y protein. Hepatic Y protein, characterized by sulphobromophthalein binding in gel filtration of the cytosol, has been reported to increase in response to hypophysectomy, and this effect has been blocked by thyroxine replacement

(Reyes *et al.*, 1971). The gel-filtration fractions exhibiting sulphobromophthalein binding, however, have been shown to contain all of the GSH *S*-transferases (Kaplowitz *et al.*, 1975b). The effect of hypophysectomy on content of ligandin (GSH *S*-transferase B), which has activity with iodomethane and the bulk of activity with ethacrynic acid but trace activity for 3,4-dichloronitrobenzene, has not been reported. In the present studies, the lack of response to hypophysectomy for the hepatic and renal alkyl-transferase and alkenettransferase activities strongly suggests that, in fact, ligandin is not affected by hypophysectomy and/or thyroxine administration. All the present findings are consistent with the suggestion that the previously reported increase in Y protein after hypophysectomy may reflect enhanced sulphobromophthalein binding as a result of a relatively specific increase, namely, in the enzyme(s) having the bulk of *S*-aryltransferase activity.

Our observations are not entirely consistent, however, with the existence of only four GSH *S*-transferase enzymes as characterized by Habig *et al.* (1974b). As a consequence of hypophysectomy, epoxide activity (associated almost exclusively with transferase E) is increased but not alkyl activity (transferases E and B). These observations would suggest that transferase E is heterogeneous; that the epoxide and alkyl activities attributed to transferase E belong to different enzymes.

The binding to the enzymes of organic anions that are not substrates has been demonstrated at least qualitatively; probenecid, *p*-aminohippurate and furosemide are competitive inhibitors of the transferases. The hepatic activities are also inhibited by bilirubin and Indocyanine Green (Kaplowitz *et al.*, 1975b). As previously demonstrated for the liver (Kaplowitz *et al.*, 1975b), it is likely that several different glutathione *S*-transferase enzymes bind organic anions. The more potent inhibition by probenecid as compared with *p*-aminohippurate parallels evidence *in vitro* regarding the relative rate of renal uptake of the two compounds (Huang & Lin, 1965; Park *et al.*, 1971). The identification of ligandin in the proximal tubule (Kirsch *et al.*, 1975) is consistent with

a physiological role of the transferases in organic-anion transport in this portion of the nephron.

This study was supported through funds provided by the Bureau of Medicine and Surgery, Navy Department, for CI 5-48-498C. The opinions and assertions contained herein are those of the authors and are not to be construed as official or as reflecting the views of the Navy Department or the naval service at large.

References

- Batson, H. C. (1956) *An Introduction to Statistics in the Medical Sciences*, Chicago Medical Book Co., Chicago
- Boylard, E. & Chasseaud, L. F. (1969) *Adv. Enzymol. Relat. Areas Mol. Biol.* **32**, 172-219
- Clifton, G., Kaplowitz, N., Kuhlenskamp, J. & Wallin, J. D. (1975a) *Clin. Res.* **23**, 120A
- Clifton, G., Kaplowitz, N., Wallin, J. D. & Kuhlenskamp, J. (1975b) *Biochem. J.* **150**, 259-262
- Dixon, M. (1953) *Biochem. J.* **55**, 170-171
- Habig, W. H., Pabst, M. J., Fleischner, G., Gatmaitan, Z., Arias, I. M. & Jakoby, W. B. (1974a) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3879-3882
- Habig, W. H., Pabst, M. J. & Jakoby, W. B. (1974b) *J. Biol. Chem.* **249**, 7130-7139
- Huang, K. C. & Lin, D. S. (1965) *Am. J. Physiol.* **208**, 391-396
- Kaplowitz, N., Kuhlenskamp, J. & Clifton, G. (1975a) *Biochem. J.* **146**, 351-356
- Kaplowitz, N., Kuhlenskamp, J. & Clifton, G. (1975b) *Proc. Soc. Exp. Biol. Med.* **149**, 234-237
- Kirsch, R., Fleischner, G., Kamisaka, K. & Arias, I. M. (1975) *J. Clin. Invest.* **55**, 1009-1019
- Levi, A. J., Gatmaitan, Z. & Arias, I. M. (1969) *J. Clin. Invest.* **48**, 2156-2167
- Lineweaver, H. & Burk, D. (1934) *J. Am. Chem. Soc.* **56**, 658-666
- 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
- Pabst, M. J., Habig, W. H. & Jakoby, W. B. (1974) *J. Biol. Chem.* **249**, 7140-7150
- Park, Y. S., Yao, H. S. & Hong, S. K. (1971) *Am. J. Physiol.* **220**, 95-99
- Reyes, H., Levi, A. J., Gatmaitan, Z. & Arias, I. M. (1971) *J. Clin. Invest.* **50**, 2242-2252