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CHARACTERIZATION OF SOLUBLE GLUTATHIONE TRANSFERASE ACTIVITY IN RESTING MONONUCLEAR LEUKOCYTES FROM HUMAN BLOOD

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Abstract—Glutathione transferase activity in the soluble fraction of resting human mononuclear leukocytes was measured and characterized using [^{3}H] *trans*-stilbene oxide as a substrate. Because of the low activity of this enzyme in these cells, a published assay procedure developed for rodent liver was slightly modified to improve its sensitivity: (1) the substrate was highly radiolabeled (2 Ci/mmole) and carefully purified, and (2) the incubation time was extended to 30–60 min. The activity measured was linear with cell density up to at least 6 million cells.

Soluble glutathione transferase activity measured in this manner has a pH optimum around 7.4 and an optimal temperature of 40°. This activity could be measured in lymphocytes, monocytes, granulocytes, erythrocytes and platelets, but not in plasma. From these measurements it could be calculated that lymphocytes account for somewhat more than half of the total activity in the mononuclear leukocyte fraction and that monocytes account for the rest.

The intraindividual variation in soluble glutathione transferase activity towards *trans*-stilbene oxide in the mononuclear leukocyte fraction from different subjects was only about 10%, whereas the interindividual variation in this same activity was 15-fold. An explanation for this relatively large interindividual variation is now being sought.

Most toxic, mutagenic and carcinogenic compounds undergo biotransformation either to a biologically more active (i.e. via phase I, mixed function oxygenases) or to an inactive form (i.e. via phase II, conjugation reactions). Because of the central importance of drug-metabolizing systems in determining tissue responses to xenobiotics (for a review, see ref. [1]), there is at present a rapidly growing interest in characterizing these systems in human blood. This interest undoubtedly relates to the fact that circulating blood is one of the few human tissues that can be obtained without undue stress or damage to the individual; moreover blood cells such as mononuclear leukocytes have been widely used in studies of human risk to genotoxic exposures [2].

In the early 70s, a large number of studies were directed towards measuring the level and inducibility of benzo[*a*]pyrene monooxygenase activity in human lymphocytes [3–6], and the relationship to lung cancer [6–10]. At the same time, relatively little is known about other drug-metabolizing enzymes in human lymphocytes. The detoxication of reactive xenobiotic metabolites, especially by epoxide hydrolases and glutathione transferases, might well have equally profound effects on the toxicity and genotoxicity of these compounds as does the actual formation of reactive intermediates via the cytochrome P-450 system. In addition, since there are many different isoenzymes of cytochrome P-450, benzo[*a*]pyrene monooxygenase activity might not reflect the rate of metabolism of other xenobiotics via the cytochrome P-450 system. Finally, the levels of co-factors and of other enzymes responsible for producing these co-factors is also important in determining the rate and nature of xenobiotic metabolism by human lymphocytes.

For these reasons we have begun a series of characterizations of different drug-metabolizing systems in human leukocytes. In the present study we have examined soluble glutathione transferase activity in various blood cell types and in particular resting mononuclear leukocytes. Previously, only a few reports on the level of glutathione transferase activity in human leukocytes have appeared [11, 12], and also on epoxide hydrolase [13].

MATERIALS AND METHODS

Chemicals. ^{3}H-*cis*- and *trans*-Stilbene oxides of high radiospecificity (2 Ci/mmole) were synthesized according to a published procedure [14]. Unlabeled *cis*-stilbene oxide was also synthesized using the same method. These synthesized products were further purified by recrystallization and their identity confirmed by NMR spectroscopy and melting point determinations.

trans-Stilbene oxide was obtained from EGA-Chemie (Steinheim/Albuch, F.R.G.), and reduced glutathione from Sigma Chemical Co. (St. Louis, MO). Other chemicals used were of reagent grade and purchased from common commercial sources.

Isolation of blood components. Routinely, the

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mononuclear fraction was isolated from the whole blood of apparently normal individuals by the standard procedure of Isopaque–Ficoll gradient centrifugation [15]. However, in order to determine the contributions of the various blood cell components to the total activities of this fraction, individual blood cell types were also isolated in several experiments.

The mononuclear leukocyte fraction was rapidly depleted of monocytes by passage through a column of gelatin beads in the presence of fresh plasma and heparin [16]. Lymphocytes could be eluted from this column simply by washing with the elution buffer (Hanks' Eagle + 10% plasma + 50 IE heparin/ml), whereas monocytes were subsequently eluted using 50 mM EDTA in phosphate-buffered saline. The first fraction obtained in this manner contained 95% lymphocytes, whereas the EDTA-containing fraction was 50–70% monocytes.

Granulocytes were isolated from the pellet obtained after Isopaque–Ficoll gradient centrifugation by adding an equal volume of 2% Dextran T 500 in 0.15 M NaCl. After standing at room temperature for 30 min the erythrocytes sedimented from this solution. The granulocytes remaining in suspension were about 90% pure.

Finally, platelets were isolated from platelet-rich plasma by differential centrifugation at 400 *g* for 20 min as previously described [17].

Preparation of sonicates and the soluble fraction from mononuclear leukocytes. All of the blood components, with the exception of plasma, were sonicated for 5–10 sec at 0–4° with a Branson sonifier at a setting of 1.5 A. This procedure was found to disrupt all of the cells without denaturing the activity under investigation. Subsequently, the sonicate obtained from mononuclear leukocytes was centrifuged at 105,000 *g* for 60 min to obtain a pellet and a supernatant. The supernatant was used as the soluble fraction. In the case of the other blood cells whole sonicates were used to assay for glutathione transferase activity.

Enzyme assay. In order to increase the sensitivity of a reported radiometric assay procedure for glutathione transferase activity [14] highly labeled *cis*- and *trans*-stilbene oxides were used as the substrates (see above). In addition, these substrates were also carefully purified from the stock solution (toluene) using thin-layer chromatography plates (Silica gel GF_{254}) developed in a solvent system of hexane–ethylacetate (95:2) and redissolved in ethanol shortly before use in order to keep the background as low as possible. Routinely, 2–3 million cells disrupted by sonication were used to measure soluble glutathione transferase activity in a total volume of 100 μl. However, since this activity was very low with *cis*-stilbene oxide as substrate (1–2 pmol conjugate formed/min/10^7 cells) compared to the activity obtained when using *trans*-stilbene oxide as substrate (10–100 pmol conjugates formed/min/10^7 cells), further characterization was performed with *trans*-stilbene oxide.

Optimal soluble glutathione transferase activity could be measured in 250 mM Tris–HCl, pH 7.2, with 90 μM *trans*-stilbene oxide (see also Results). After addition of the substrate in 1 μl ethanol the reaction mixture was incubated for 30–60 min at 37°. The assay was terminated by adding 200 μl hexanol, followed by vigorous shaking and then centrifuging in a desk centrifuge to obtain phase separation. It has been previously determined [14] that 99.8% of the unreacted substrate as well as the diol formed by soluble epoxide hydrolase were thus extracted into the organic phase while 99.6% of the conjugate formed remained in the aqueous phase. Aliquots of the aqueous phase were scintillation counted and corrected for quenching. The assay was routinely performed in duplicate and in one experiment involving 12 sets of duplicates, the values were found to differ from one another by an average of 5.9%.

Measurement of protein was achieved using the method of Lowry *et al.* [18] with bovine serum albumin as the standard. The whole sonicate from 10 million cells was found to contain approximately 0.8 mg protein.

RESULTS

Assay procedure for soluble glutathione transferase activity

The assay procedure utilized here is essentially that reported by Gill *et al.* [14]. One of the advantages of using *cis*- and *trans*-stilbene oxide as substrates for soluble glutathione transferase is that the non-enzymatic conjugation with reduced glutathione, i.e. the background, is relatively low. The background obtained by incubating *cis*- or *trans*-stilbene oxides in combination with boiled fractions for 1 hr was approximately 0.3% and 1.0% of the total radioactivity respectively. If the boiled fraction was omitted in this control, the background was at least doubled. Consequently, as little as 30 pmol conjugates formed from *cis*-stilbene oxide and 70 pmol conjugates formed from *trans*-stilbene oxide during 1 hr incubation could be detected at the concentrations of these substrates used. Thus, by using these substrates at a high radiospecificity in well-purified form, together with long incubation times linear up to 60 min (Fig. 1), it proved possible to measure soluble glutathione transferase activity with relatively small numbers of freshly isolated human

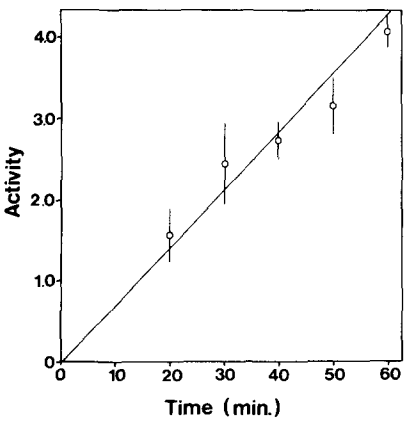

Fig. 1. Linearity of soluble glutathione transferase activity of human mononuclear leukocytes with time. Activity = nmole conjugate formed per 10^7 cells. The points and bars represent the means and average deviations, respectively, of three different determinations.

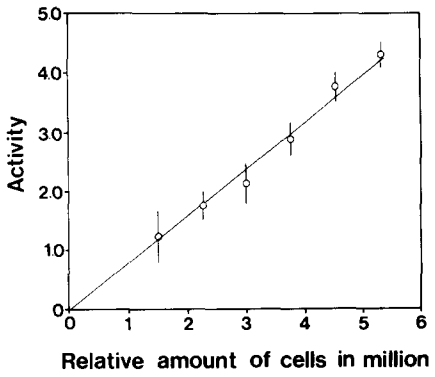

Fig. 2. Linearity of soluble glutathione transferase activity of human mononuclear leukocytes on the relative amount of cells. Activity = pmol conjugate formed per min. The points and bars represent the means and average deviations, respectively, of three different determinations.

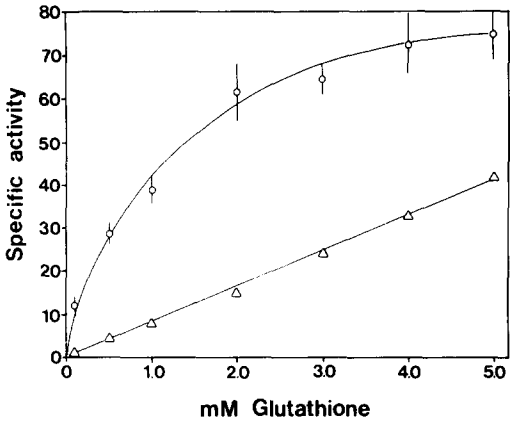

Fig. 3. Dependence of the soluble glutathione transferase activity of human mononuclear leukocytes on the concentration of reduced glutathione. Specific activity = pmole conjugate formed per min per 10^7 cells. The points and bars represent the means and average deviations, respectively, of three different determinations. Δ, Non-enzymatic conjugation with glutathione.

mononuclear leukocytes. The activity was linear with cell number up to at least 5 million cells (Fig. 2).

In order to test the possibility that the activity of soluble glutathione transferase is affected by sonication of the mononuclear leukocytes, the enzyme activity was measured in intact cells in one experiment. The activities measurable in this case were the same as those obtained with the disrupted preparation.

The soluble glutathione transferase activity of both disrupted and whole cells seems to be reasonably stable during storage at −20°. During a week of such storage the activity of disrupted cells decreased 15.7% (mean of three determinations), whereas the activity of whole cells decreased 11.8% (mean of three determinations). Little additional change in these activities could be detected after a month of storage in the freezer (12–17% of the total activity).

Kinetic parameters

Soluble glutathione transferase activity towards *trans*-stilbene oxide was measured as a function of the concentration of reduced glutathione in order to determine the lowest concentration at which the enzyme(s) involved is saturated. By using a concentration that just saturates the activity, the background non-enzymatic conjugation can be kept as low as possible. As can be seen from Fig. 3, the enzyme(s) seems to have half-maximal activity with 1 mM reduced glutathione and a concentration of 4 mM reduced glutathione seems to be appropriate for this assay.

At this concentration of glutathione, an apparent K_m for *trans*-stilbene oxide of 35–75 μM and a V_{max} of 50–100 pmol/min/10^7 cells were determined using the soluble fractions from mononuclear leukocyte fractions pooled from different donors. The reason for this relatively large variation in the kinetic parameters of the soluble glutathione transferase activity in these cells remains unclear (see also below).

Dependence of soluble glutathione transferase activity on pH

Soluble glutathione transferase activity measured with *trans*-stilbene oxide as substrate was examined over a wide range of pH in mononuclear leukocytes (Fig. 4). The activity demonstrates a broad optimal pH between 7.0 and 7.8 and no buffer effect could be observed between 50 and 250 mM which is consistent with findings with other species as well [19]. As also shown in this figure the non-enzymatic conjugation with reduced glutathione rapidly increases at pH's above 7.5. Routinely a pH of 7.2 was chosen for this assay.

Dependence of soluble glutathione transferase activity upon temperature

The optimal temperature for this enzyme in human mononuclear leukocytes is close to 40° (Fig. 5).

The level of soluble glutathione transferase activity in individual blood components

As shown in Table 1, all blood components exam-

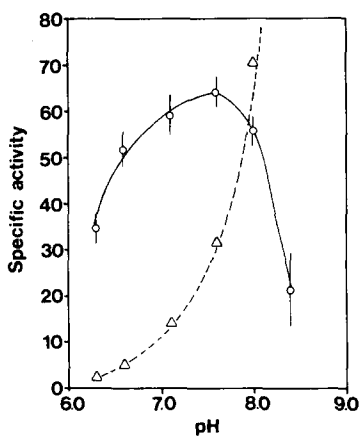

Fig. 4. Dependence of soluble glutathione transferase activity of human mononuclear leukocytes on pH. Specific activity = pmole conjugate formed per min per 10^7 cells. 250 mM sodium phosphate buffer was used. The points and bars represent the means and average deviations, respectively, of three different determinations. Δ, Non-enzymatic conjugation with glutathione.

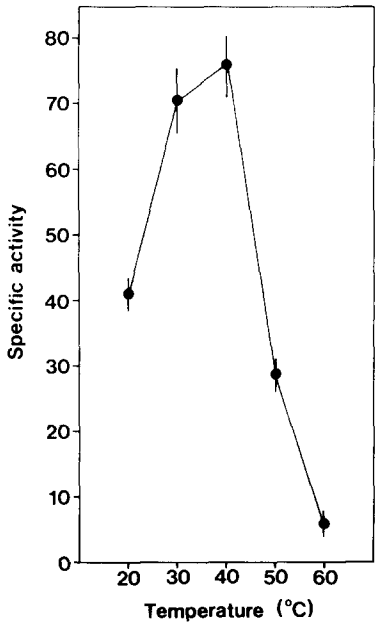

Fig. 5. Dependence of the soluble glutathione transferase activity of human mononuclear leukocytes on temperature. Specific activity = pmole conjugate formed per min per 10^7 cells. The points and bars represent the means and average deviations, respectively, of three different determinations.

ined, with the exception of plasma, demonstrated glutathione transferase activity towards *trans*-stilbene oxide. This activity per monocyte was more than twice as great as the corresponding activity per lymphocyte, which was in turn more than 40% greater than the granulocyte activity. Although the lymphocytes account for most of the glutathione transferase activity in the total mononuclear cell fraction, the monocytes also make a highly significant contribution. The erythrocytes were routinely prepared in a manner which would result in as much as 1% contamination by granulocytes, which could thus account for the total activity in these preparations. However, even after three additional sedimentations through 2% solution of Dextran no loss of the activity of the erythrocyte preparation could be observed, suggesting that the activity measured actually belongs to this cell type.

Inter- and intraindividual variation in soluble glutathione transferase activity of human mononuclear leukocytes

In Table 2 the intraindividual variations in soluble glutathione transferase activity in mononuclear leukocytes from three different subjects is presented. As indicated, the mean intraindividual variation of four different measurements during an 18-day period was about 10%. At the same time, an interindividual variation of 14-fold was found in these three subjects.

In another experiment, the interindividual variation was investigated. As can be seen in Table 3, there are two different groups of individuals, one with high glutathione transferase activity towards *trans*-stilbene oxide and another group with about 18-fold lower activity. This variation cannot be explained by the fact that the distribution of monocytes and lymphocytes may vary in different individuals (see Table 1).

DISCUSSION

Because of the central importance of drug-metabolizing systems in the detoxification of DNA-damaging metabolic intermediates such as epoxides, it would be useful to quantify the relative activities of drug-metabolizing enzymes in individual human beings. Such knowledge will probably prove to be useful in a number of different respects, including determining the dosage of a medicine which a patient should receive, monitoring the exposure of a given population to xenobiotics in the work or general environment, predicting the susceptibility of an individual to the toxic and genotoxic effects of different substances, etc.

A number of experimental approaches designed

Table 1. Specific activity of soluble glutathione transferase in different blood components and their contribution to the total activities in the mononuclear leukocyte fraction and in whole blood

Component	Activity per 10^7 cells*	% of cells present in total mononuclear cell fraction	% contribution to total activity in mononuclear cell fraction
Lymphocytes	104.2 ± 1.0	75–80	58–62
Monocytes	225.6 ± 10.8	20–25	34–42
Granulocytes	73.0 ± 5.8	0–3	0–2
	0.32		
Erythrocytes		0	0
	0.43		
Platelets		0	0–8†
Plasma	n.d.‡	0	0

* The activity is expressed in pmole conjugate formed per min ± average deviation (three determinations) in one individual.

† The presence of platelets in a normal mononuclear cell fraction is about 20–40 platelets per mononuclear cell.

‡ n.d. = not detectable.

Table 2. Intraindividual variations of soluble glutathione transferase activity in fresh resting human mononuclear leukocytes. Cells were prepared and sonicated and glutathione transferase activity measured as described in Materials and Methods

Sampling time	Soluble glutathione transferase activity* Subject No. I	II	III
day 0	155.5	9.38	11.47
day 6	146.5	6.10	10.70
day 12	146.8	9.85	15.12
day 18	148.5	8.91	12.50
Mean ± S.D.	149.3 ± 3.6	8.56 ± 1.46	12.45 ± 1.67
Coefficient of variation (%)	2.4	17.1	12.4
Mean of coefficients of variation (%)		10.6	

* The activity is expressed in pmole of conjugate formed per min per 10^7 cells.

to investigate the genotoxic response of an individual to xenobiotics—including studies of chromosome breakage, sister chromatid exchange, single strand breaks in DNA, binding of xenobiotics and/or their metabolites to DNA, and DNA repair—are being applied to human circulating mononuclear leukocytes, which can be easily obtained without damage or pain. In our opinion an extensive, thorough characterization of the drug-metabolizing systems in lymphocytes from individuals in various life situations would provide highly valuable information which would complement and facilitate the understanding of data obtained from these other approaches.

Here we present our investigation characterizing soluble glutathione transferase activity in these cells. These enzymes can, like epoxide hydrolases, detoxify reactive xenobiotics and/or metabolic intermediates, most often formed via the cytochrome P-450 system, and are also sometimes involved in the production of even more reactive intermediates. We have shown that soluble glutathione transferase activity can easily and routinely be measured in the soluble fraction from freshly isolated mononuclear leukocytes, especially with *trans*-stilbene oxide as substrate. This fraction can be stored for at least a month in the freezer without losing more than about 10% of its glutathione transferase activity. We have also defined the optimal conditions with regards to reduced glutathione concentration, pH and temperature which should be used in assaying this activity in resting human mononuclear leukocytes.

Table 3. Interindividual variations of soluble glutathione transferase activity in fresh resting human mononuclear leukocytes. Cells were prepared and sonicated and glutathione transferase activity measured as described in Materials and Methods

Subject No.	Activity*	Subject No.	Activity
2	159	1	4.0
4	110	15	11.7
9	136	20	8.1
I	149	22	9.6
		24	9.2
		26	1.9
		27	5.6
		28	7.5
		29	5.8
		II	8.6
		III	12.5
Mean	139		7.7

* The activity is expressed in pmole of conjugate formed per min per 10^7 cells.

It is often assumed that the properties of mononuclear leukocytes isolated from human blood are also those of circulating human lymphocytes. However, as can be seen in Table 1, this fraction contains a number of different blood components, chiefly lymphocytes and monocytes. Consequently, we measured the soluble glutathione transferase activity in individual blood components, partially because these data are in themselves of interest and partially to ascertain to what extent the glutathione transferase activity of the human mononuclear leukocyte fraction can be taken to reflect this same activity in circulating human lymphocytes.

Cytosolic glutathione transferase activity has been found in virtually all the cell types in humans where it has been looked for—including liver, kidney, lung and placenta [20–24]. It is therefore not surprising that we detected such activity in all the different blood cells examined here, i.e. lymphocytes, monocytes, granulocytes, erythrocytes and platelets. Indeed, other groups have also observed glutathione transferase activity in erythrocytes and have even isolated some of the proteins which catalyze this activity [25]. In the mononuclear leukocyte fraction lymphocytes account for more than 50% of the total glutathione transferase activity towards *trans*-stilbene oxide, but monocytes also make a very significant contribution (34–42%).

Differences in the (uninduced) benzo[*a*]pyrene monooxygenase activity in circulating lymphocytes from different individuals may be as much as 10-fold [4, 5, 26]. Such interindividual differences may result both from hereditary, environmental and/or methodological factors and may have profound influences on the metabolism and, thereby, on the toxicity and

genotoxicity of different xenobiotics. More problematic is the observation that the benzo[*a*]pyrene monooxygenase activity in circulating lymphocytes from the same individual at different points of time also demonstrates differences [4]. Whether such intraindividual differences result from environmental, hormonal or other factors, or whether they are at least partially due to methodological difficulties (including the fact that these cells must be mitogen-stimulated before assay) is not yet clear.

Glutathione transferase activity towards *trans*-stilbene oxide in mononuclear leukocyte fractions from different individuals varies widely, at least as much as 18-fold. We are presently investigating the possibility that this large interindividual variation may reflect genetic polymorphism resembling that seen with acetylation. Oesch and coworkers [27] did not observe such large interindividual differences in the glutathione transferase activity of cultured human fibroblasts, but this may simply reflect that use of a different substrate, a different cell type, or even the loss of such differences upon growing cells in cultures.

Our next immediate goal is to use the assay procedure and characterization reported here as a basis for screening this activity in a large number of individuals in various life situations. It is our hope that we can thus explain why the interindividual variation in this activity is so great and perhaps even supply information which can help individuals to avoid the toxic and genotoxic effects of xenobiotics.

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REFERENCES

1. W. S. Jakoby (Ed.), *Enzymatic Basis of Detoxication*, Vols 1–2. Academic Press, New York (1980).
2. D. J. Kilian and D. Picciano, in *Chemical Mutagens: Principles and Methods for their Detection* (Ed. A. Hollander), Vol. 4, p. 321. Plenum Press, New York (1976).
3. J. P. Whitlock Jr, H. L. Cooper and H. V. Gelboin, *Science* **177**, 618 (1972).
4. G. Kellermann, E. Cantrell and C. R. Shaw, *Cancer Res.* **33**, 1654 (1973).
5. G. Kellermann, M. Luyten-Kellermann and C. R. Shaw, *Am. J. Hum. Genet.* **25**, 327 (1973).
6. G. Kellermann, C. R. Shaw and M. Luyten-Kellermann, *New Engl. J. Med.* **289**, 934 (1973).
7. H. A. Guirgi, H. T. Lynch, T. Mate, R. E. Harris, F. Wells, L. Caha, J. Andersson, K. Maloney and L. Rankin, *Oncology* **33**, 105 (1976).
8. A. E. H. Emery, R. Anand, D. Danford, W. Duncan and L. Paton, *Lancet* **i**, 470 (1978).
9. E. Trell, R. Korsgaard, B. Hood, P. Kitzing, G. Nordén and B. G. Simonsson, *Lancet* **ii**, 140 (1976).
10. R. E. Kouri, C. E. McKinney, D. J. Slomiany, D. R. Snodgrass, N. P. Wray and T. L. McLemore, *Cancer Res.* **42**, 5030 (1982).
11. N. Kaplowitz, C. Spina, M. Graham and J. Kuhlenkamp, *Biochem. J.* **169**, 465 (1978).
12. N. Singh and J. Clausen, *Cancer Lett.* **13**, 53 (1981).
13. H. Glatt, E. Kaltenbach and F. Oesch, *Cancer Res.* **40**, 2552 (1980).
14. S. S. Gill, K. Ota and B. D. Hammock, *Analyt. Biochem.* **131**, 273 (1983).
15. A. Böyum, *Scand. J. clin. Lab. Invest.* **21** (Suppl.), 97 (1968).
16. H. O. Sjögren, K. Nilsson and B. Axelsson, *J. Immunol. Meth.* **56**, 285 (1983).
17. R. W. Pero and C. Vopat, *Carcinogenesis* **2**, 1103 (1981).
18. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
19. W. H. Habig, M. J. Pabst and W. B. Jakoby, *J. biol. Chem.* **249**, 7130 (1974).
20. K. Kamisaka, W. H. Habig, J. N. Kettley, I. M. Arias and W. B. Jakoby, *Eur. J. Biochem.* **60**, 153 (1975).
21. K. Koskelo and E. Valmet, *Scand. J. clin. Lab. Invest.* **40**, 179 (1980).
22. K. Koskelo, E. Valmet and R. Tenhunen, *Scand. J. clin. Lab. Invest.* **41**, 683 (1981).
23. G. Fleischner, J. Robbins and I. M. Arias, *J. clin. Invest.* **51**, 677 (1972).
24. G. Polidoro, C. Di Ilio, G. DelBoccio, P. Zulli and G. Federici, *Biochem. Pharmac.* **29**, 1677 (1980).
25. C. J. Marcus, W. H. Habig and W. B. Jakoby, *Archs Biochem. Biophys.* **188**, 287 (1978).
26. H. L. Gurtoo, J. Minowada, B. Paigen, N. B. Parker and N. T. Hayner, *J. Natl. Cancer Inst.* **59**, 787 (1977).
27. F. Oesch, F. Tegtmeyer, F.-V. Kohl, H. Rüdiger and H. R. Glatt, *Carcinogenesis* **1**, 305 (1980).