PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 169, 463–469 (1982)

The Glyoxalase System in Rat Blood[1] (41376)

RICHARD B. BRANDT, MICHAEL G. WATERS, DAVID J. MURON, AND
MICHAEL H. BLOCH

*Department of Biochemistry, and MCV/VCU Cancer Center, Medical College of Virginia,
Virginia Commonwealth University, Richmond, Virginia 23298*

Abstract. The 3-carbon keto-aldehyde, methylglyoxal (MeG), inhibits growth in bacterial and mammalian systems. The glyoxalase enzyme system catalyzes the catabolism of the α,β-dicarbonyl to form D-lactate with glutathione (GSH) as a cofactor. Glyoxalase I (*S*-lactoyl-glutathione methylglyoxal-lyase, isomerizing; EC 4.4.1.5) and glyoxalase II (*S*-2-hydroxyacylglutathione hydrolase; EC 3.1.2.6) are present in mammalian tissues and have a high specific activity in red blood cells. In order to determine possible changes in the glyoxalase system with growth, the various components of the glyoxalase system were measured in the blood of male Sprague–Dawley rats aged 20 to 89 days. Glyoxalase I and II kinetic parameters were determined in potassium phosphate buffer at pH 6.8. Glutathione reductase activity, blood concentration of GSH and protein, plasma concentration of D-lactate, and hematocrit were also measured. The mean V for glyoxalase I was 81 μmole·min^{-1}·ml^{-1} of red blood cells as compared to the glyoxalase II catalysis of *S*-lactoyl-glutathione (SLG) to D-lactate of 48 μmole·min^{-1}·ml^{-1}. The mean K_m values for MeG and SLG were 0.9 and 1.2 m*M*, respectively. The K_m and V for the glyoxalase enzymes were not age dependent in the growing animal when controlled for changes in hematocrit and protein. Only D-lactate plasma concentration showed a positive correlation with age ($P < 0.005$) when hematocrit and protein changes were controlled. The increase in D-lactate may indicate an increased formation of MeG with potential for cellular inhibition.

The research of Szent-Gyorgyi and others has shown that the 3-carbon keto-aldehyde, methylglyoxal (MeG), inhibits growth in bacterial and mammalian systems (1, 2). The glyoxalase enzyme system shown in Fig. 1 catalyzes the formation of D-lactate from the cytotoxic α,β-dicarbonyl with glutathione (GSH) as a coenzyme (3). Glyoxalase I (*S*-lactoyl-glutathione methyl-glyoxal-lyase, isomerizing; EC 4.4.1.5) and glyoxalase II (*S*-2-hydroxyacylglutathione hydrolase; EC 3.1.2.6) are ubiquitous in mammalian tissues and have a high specific activity in red blood cells (4). The substrate for the system is the nonenzymatically formed hemimercaptal from MeG and GSH (5). The synthesis of MeG in mammalian tissues appears to be mainly nonenzymatic (6, 7) although a methylglyoxal synthase (EC 4.2.99.11) has been isolated from *Escherichia coli* (8) and enzymatic formation of MeG from dihydroxyacetone phosphate in rat liver homogenates has recently been reported (9). D-Lactate formation, presumably through MeG, occurs in human blood *in vitro* when glycolysis is inhibited or by addition of MeG (10).

The activity of the glyoxalase enzyme system changes under rapid growth conditions such as liver regeneration and cancer. Liver GSH and glyoxalase I activity double 12 hr after partial or sham hepatectomy (11), while hepatic glyoxalase I and II activities decrease in tumor-bearing mice (12, 13). Molecular weight modification of glyoxalase I also occurs in tumor-bearing animals without any change in kinetic properties (14). In the present study, changes in the glyoxalase system with normal growth and maturation were determined. The various components of the glyoxalase system including glyoxalase I and II, the cofactor GSH, and the product D-lactate were measured in the blood of rats aged 20 to 89 days.

---

[1] A preliminary report of this work was presented at the 1981 meeting of FASEB in Atlanta, Ga. An abstract appeared in Fed Proc 40:555, 1981.

0037-9727/82/040463-07$01.00/0

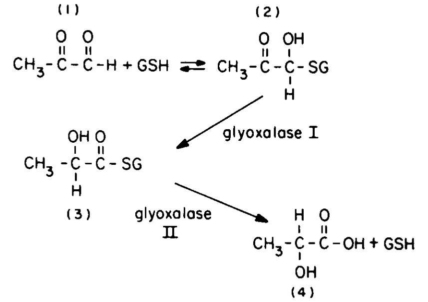

FIG. 1. Methylglyoxal and the glyoxalase enzyme system. (1) Methylglyoxal; (2) nonenzymatically formed hemimercaptal; (3) *S*-lactoyl glutathione; (4) D-lactic acid.

Materials and Methods. *Reagents.* Except as noted, any chemicals used were obtained from Sigma Chemical Company. Gallard-Schlesinger metaphosphoric acid sticks contained approximately 60% HPO_3 and 40% $NaPO_3$. Hydrazine (95%) was from Eastman Chemicals. MeG was steam distilled and its concentration determined using the Friedemann method (15). *S*-Lactoyl-glutathione (SLG), the substrate for glyoxalase II, was prepared by adding 12 ml of potassium phosphate buffer, pH 6.8, 0.1 *M* to 24 μmole of the Ba salt of SLG, adding 75 μmole of Na_2SO_4, mixing, and centrifugation at 12,000*g* for 10 min. The supernatant fluid was used as the substrate stock solution and was about 2.0 m*M*. The actual concentration of SLG was determined after dilution in phosphate buffer by SLG molar absorptivity of 3.37 $mM^{-1} \cdot cm^{-1}$ at 240 nm (16). GSH for glyoxalase I activity determination was prepared at 0.1 *M* with the pH adjusted to 6.8.

Samples. All rats used in this study were Sprague–Dawley males from Flow Laboratory (Dublin, Va.). The rats were housed in an animal room which had thermostatically controlled temperature (22–26°) on a 12 hr of light schedule. Each shipment of rats was kept in the animal room for at least a week before using in the study. Ralston Purina rodent laboratory chow and water were provided *ad libitum*. Food was withdrawn in the afternoon before a rat was to be used the next morning. All rats used were decapitated between 10 and 11 AM. Blood for analysis was collected in 103 × 29-mm polypropylene centrifuge tubes containing 100 USP units of sodium heparin. Hematocrit was determined using Dade microhematocrit capillary tubes in a clinical centrifuge. Using 0.5 ml of blood, hemolysates were prepared for the measurement of GSH, glyoxalase I and II, glutathione reductase (NADPH: oxidized glutathione oxidoreductase, EC 1.6.4.2), and protein.

GSH determination. GSH in whole blood was determined spectrofluorometrically using a modification of the procedure of Cohn and Lyle (17) (R. B. Brandt, M. G. Waters, M. H. Bloch, and P. Fine, unpublished data). *o*-Phthaldialdehyde in 0.1 *M* Bicine (*N,N*-bis(2-hydroxyethyl)glycine) at pH 8.3 from Boehringer-Mannheim with 0.26 m*M* EDTA, was used to form the fluorophore. Whole blood was lysed by adding 0.2 to 1.0 ml 0.26 m*M* EDTA and 0.6 ml of the lysate was added with mixing to 0.2 ml 8.8% HPO_3–0.26 m*M* EDTA for deproteinization. A protein-free supernatant fluid for analysis was obtained by centrifugation at 10,000*g* for 10 min in a Sorvall RC2-B.

Glyoxalase I and II and glutathione reductase analysis. For enzyme and protein determinations, a 1/10 hemolysate was prepared by adding 0.3 ml of whole blood to 2.7 ml H_2O. The resulting lysate was mixed and centrifuged at 27,000*g* for 20 min to remove the cell membranes. This cell-free supernatant fluid was assayed for activity of the glyoxalase enzymes and glutathione reductase. The glyoxalase I assay used was a discontinuous spectrophotometric method with alcoholic, acidic 2,4-dinitrophenylhydrazine as a reagent to follow the disappearance of MeG over time, with the absorbance of formed MeG bis-hydrazone measured at 432 nm (18). Glyoxalase I activity was the reaction velocity (v) measured at the highest substrate concentration, 3.52 m*M* MeG with 3.33 m*M* GSH (1.38 m*M* hemimercaptal), expressed as MeG used or SLG formed in $\mu mole \cdot min^{-1} \cdot ml^{-1}$ of red blood cells or $\mu mole \cdot min^{-1} \cdot g^{-1}$ of protein. K_m and V were calculated by the double-reciprocal method of Lineweaver–Burk

(19) from v at four different concentrations of MeG from 0.88 to 3.52 mM and 3.33 mM GSH. Glyoxalase II was determined using a modification of the procedure of Racker (16). This continuous spectrophotometric method is based on a decrease in absorbance by the substrate, SLG, at 240 nm. Glyoxalase II activity was the v measured at the highest substrate concentration (0.4 mM SLG) expressed as SLG used in μmole$\cdot$min$^{-1}\cdot$ml^{-1} of red blood cells or g^{-1} protein. K_m and V were calculated by the double-reciprocal method (19) from v at four different concentrations of SLG from 0.1 to 0.4 mM. Glutathione reductase activity expressed as NADPH used in μmole$\cdot$min$^{-1}\cdot$ml^{-1} of red blood cells or g^{-1} of protein, was determined in 0.1 M Tris (pH 8.0) using the method of Bergmeyer (20). All enzyme reactions were measured at 30°. Protein was measured using the biuret method (21).

D-Lactate analysis. Blood was centrifuged at 1400g to remove cells and the plasma D-lactate was determined spectrophotometrically using a specific NAD$^+$-linked D-lactic dehydrogenase (D-LDH) from *Lactobacillus leichmannii* (D-lactate: NAD$^+$ oxidoreductase; EC 1.1.1.28) by measurement of NADH formed at 340 nm (22).

General. Spectrophotometric measurements were obtained with a Gilford 252 modified Beckman DU equipped with a strip chart recorder and a Lauda K-2/R constant temperature bath and automatic cuvette positioner. Statistical data analysis was performed using the IBM 370 computer with the SAS software package and a TI 59 programmable calculator.

Results. The rats studied ranged in age from 20 to 89 days. This group represented rats from the time just after weaning to young adulthood. Sexual maturity is attained around 60 days of age (23). Body weight ranged from 24 to 442 g. Body weight increased steadily with age, as shown in Fig. 2 and Table I. These rats were then used for the analysis reported here.

Table II shows the mean value ± SE with the number of observations for each of the parameters measured. The mean V for glyoxalase I was 81 μmol$\cdot$min$^{-1}\cdot$ml^{-1} and 48 μmole$\cdot$min$^{-1}\cdot$ml^{-1} for glyoxalase II. The glyoxalase II catalysis of SLG to D-lactate

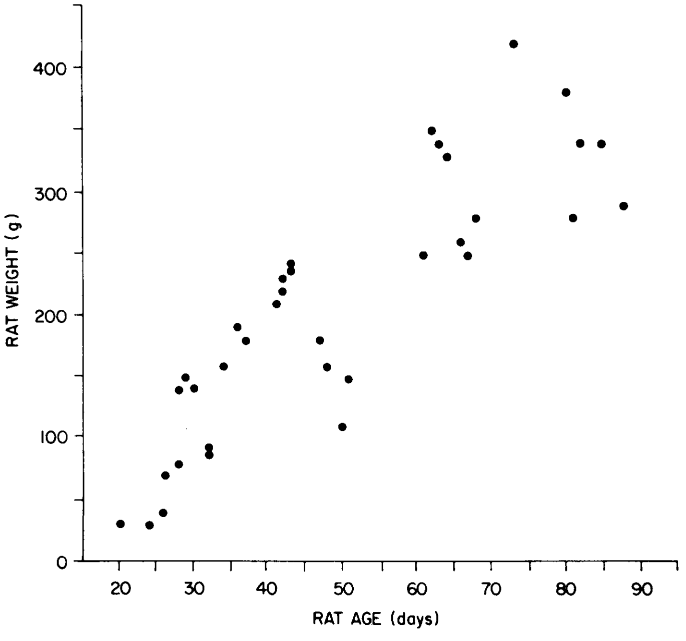

FIG. 2. Rat age vs weight.

TABLE I. CORRELATION OF BODY WEIGHT, HEMATOCRIT AND PROTEIN WITH RAT AGE

Parameter X	r[a]	P[b]	N[c]
Body weight (g)	0.874	0.001	37
Hematocrit (% red blood cells)	0.623	0.001	37
Protein (mg/ml blood)	0.473	0.005	36

[a] Simple linear correlation coefficient between X and age.
[b] P-value, determined by two-tailed t test.
[c] Number of observations, rats age 20 to 89 days.

appears to be the rate-limiting reaction in the catabolism of MeG in blood. The average K_m for glyoxalase I for MeG (at 3.33 m*M* GSH) was 0.9 m*M* and for glyoxalase II was 1.2 m*M*. GSH mean concentration in red blood cells was 73 mg/100 ml (2.4 m*M*). Mean plasma D-lactate concentration was 0.19 mg/100 ml (0.02 m*M*). Hematocrit and total blood protein concentration mean values were 48% and 129 mg/ml, respectively, and the simple correlations with age are shown in Table I.

Since the object of our study was to determine if any of the parameters we measured changed with age, linear regression and correlation coefficients for each of the parameters against rat age were determined. The results are shown in Table III. A two-tailed t test was used to test against the hypothesis that the correlations were zero (Ho: $R = 0$) with a $P < 0.05$ being accepted as minimal significance. Rat age and rat weight were strongly correlated ($r = 0.874$, $n = 37$, $P < 0.001$) (Table I) as was expected.

Significant simple correlations ($P < 0.05$) were found between rat age and hematocrit, protein, activities of glyoxalase I and II, and activity of glutathione reductase (Table III). Hematocrit and protein concentration had the highest correlation with age (Table I). With increasing age, both hematocrit and protein increased, but the enzyme activities, expressed per milliliter of red blood cells per gram of protein, decreased with age. This suggested that the correlations between age and enzyme activity may have been a consequence of the relationships between age and increasing percentage red blood cells or protein. To test this possibility, partial correlation coefficients were determined according to the method of Snedecor and Cochran (24), thus allowing for consideration of correlations between the glyoxalase system parameters against age while controlling for the effects of hematocrit and protein concentration. The results are shown in Table III. The partial correlation coefficients between age and

TABLE II. MEAN AGE, BODY WEIGHT, AND BLOOD VALUES OF MALE SPRAGUE–DAWLEY RATS, AGES 20–89 DAYS

	Mean ± SE (N)[a]	
Age (days)	51.5 ± 3.5	(37)
Body weight (g)	217 ± 18	(37)
Hematocrit (% red blood cells)	48.1 ± 0.01	(37)
Protein (mg/ml whole blood)	129 ± 6.2	(36)
(v) Glo. I (μmole·min^{-1}·ml^{-1} red blood cells)	64.8 ± 1.2	(36)
(v) Glo. I (μmole·min^{-1}·g^{-1} protein)	254 ± 13	(36)
K_m for Glo. I (m*M*)	0.86 ± 0.04	(31)
V for Glo. I (μmole·min^{-1}·ml^{-1} red blood cells)	80.6 ± 1.8	(30)
(v) for Glo. II (μmole·min^{-1}·ml^{-1} red blood cells)	10.3 ± 0.38	(37)
(v) Glo. II (μmole·min^{-1}·g^{-1} protein)	40.6 ± 2.1	(36)
K_m for Glo. II (m*M*)	1.22 ± 0.16	(35)
V for Glo. II (μmole·min^{-1}·ml^{-1} red blood cells)	47.7 ± 5.1	(33)
(v) GSSG reductase (μmole·min^{-1}·ml^{-1} red blood cells)	0.19 ± 0.01	(37)
(v) GSSG reductase (μmole·min^{-1}·g^{-1} protein)	0.74 ± 0.05	(36)
GSH (mg/100 ml whole blood)	34.8 ± 1.8	(36)
GSH (mg/100 ml red blood cells)	72.7 ± 3.8	(36)
D-Lactate (mg/100 ml plasma)	0.19 ± 0.02	(33)

[a] In some cases, there were missing values for certain determinations. Several extreme values were also excluded by a test of outliers (24).

TABLE III. PARTIAL CORRELATION OF AGE AND BLOOD VALUES, HEMATOCRIT AND PROTEIN CONSTANT

X[a]	r_{AX}[b]	r_{HX}[c]	r_{PX}[d]	$r_{AX\text{-}HP}$[e]
(v) Glo. I (red blood cells)	−0.509[g]	−0.675[h]	−0.394[f]	−0.099
(v) Glo. I (protein)	−0.316	−0.330[f]	−0.867[h]	0.240
V Glo. I (red blood cells)	−0.351[f]	−0.491[g]	−0.323	−0.015
K_m, Glo. I	0.092	0.158	−0.123	0.060
(v) Glo. II (red blood cells)	−0.321	−0.217	−0.037	−0.275
(v) Glo. II (protein)	−0.413[f]	−0.169	−0.714[h]	−0.260
V Glo. II (red blood cells)	0.027	0.015	−0.250	0.122
K_m Glo. II	0.166	0.051	−0.274	0.304
(v) GSSG Reductase (red blood cells)	−0.296	−0.130	−0.062	−0.288
(v) GSSG reductase (protein)	−0.410[f]	−0.147	−0.586[h]	−0.295
GSH (whole blood)	−0.079	0.162	0.053	−0.242
GSH (red blood cells)	−0.240	−0.087	−0.032	−0.252
D-Lactate (plasma)	0.512[g]	0.161	0.162	0.528[g]

[a] Blood value of interest, N for each given in Table II.

[b] Coefficient for the correlation between age and X.

[c] Coefficient for the correlation between hematocrit and X.

[d] Coefficient for the correlation between protein and X.

[e] Coefficient for the correlation between age and X, holding hematocrit and protein constant.

[f] $P < 0.05$, all values without notation are nonsignificant.

[g] $P < 0.005$.

[h] $P < 0.001$.

the various blood values shown in Table III ($r_{AX\text{-}HP}$) show that only plasma D-lactate correlated ($r_{AX\text{-}HP} = 0.528$, $P < 0.005$) with age among the parameters studied. The remainder of the significant simple correlations were eliminated when the hematocrit and protein concentration effects were held constant.

Multiple regression analysis of the data using age as the dependent variable and body weight, hematocrit, enzyme activities, GSH, protein, and D-lactate concentration as the independent variables indicated that body weight and total protein concentration were the parameters of most value in predicting variation with age. The next most important parameter was glyoxalase I activity per milliliter RBC. The multiple regression model was able to account for 87% of the variation when age was used as the dependent variable ($r^2 = 0.87$).

Discussion. From the data in Table II, the mean age was 51.5 days for the rats from Fig. 2. The linear regression equation for age vs weight, $y = 4.520X - 15.993$ ($r = 0.874$) is significant at the $P < 0.001$ level for the 20- to 89-day-old rats studied. Our investigation determined that both glyoxalase I and II enzyme activities decreased with the age of the rats as shown in Table III. In addition the erythrocyte marker enzyme, glutathione reductase, was also found to decrease with rat age. However, these associations were found to be dependent on hematocrit and protein concentration, which increased with age. This is reflected by the partial correlation coefficients ($r_{AX\text{-}HP}$) in Table III. This would indicate that there is a constant amount of these enzymes per volume of blood which then becomes a decreasing amount of the total blood protein as the total protein concentration increases with age. Thus, as the protein concentration increases with age, the enzyme activities expressed per gram of blood protein decrease. This is clearly shown by the high negative correlation coefficients for glyoxalase I, glyoxalase II, and glutathione reductase activities (per gram protein) vs blood protein concentration (Table III).

Since both hematocrit and protein concentration increased with age, it could be suspected that an increase in hematocrit would produce an increase in total blood protein as hemoglobin is the protein found in largest quantity in whole blood. This increase is apparently a physiological characteristic of the growing rat, and is coupled with a decreased blood volume per kilogram body weight and an increased oxygen carrying capacity per kilogram with growth (23).

During the linear growth period, in the first 90 days of life, the rats showed an increasing concentration of plasma D-lactate. D-Lactate had a high correlation with age ($r = 0.512$, $P < 0.005$) which increased when the effects of protein concentration and hematocrit were statistically controlled. The D-lactate concentration may be proportional to the amount of MeG formed in blood and other tissues, since MeG is converted rapidly and unidirectionally to D-lactate. *In vitro* experiments showed that added MeG is converted stoichiometrically to D-lactate in blood. Also, when glycolysis was inhibited with fluoride, increases in D-lactate concentration were found. *In vitro* addition of large quantities of glycolytic precursors without glycolytic inhibition led to increased plasma D-lactate (10). This indicates a potential glycolytic control function for MeG production and thus possible glycolytic control of growth. Recent work has shown that D-lactate is catabolized to carbon dioxide in various rat tissues, with virtually no catabolism in whole blood (25). This leads us to question whether D-lactate plasma levels are a reflection of the MeG levels, the speed of catabolism, or both, when considering the intact organism. Szent-Gyorgyi calculated a concentration of 0.1 *M* MeG was necessary to inhibit growth of liver cells. He also postulated that MeG is kept separate in the intact cell from the glyoxalase system by combining with other cellular components such as amino or -SH groups and shows that there may be complexed MeG approaching 0.1 *M* (26). It may be that we are measuring only a portion of the MeG produced by measuring its catabolic product D-lactate, even without consideration of the possible fallacy introduced by D-lactate catabolism. At the very least, the correlation of D-lactate concentration with age (Table III) indicates either an increased MeG turnover with age or a decreased D-lactate catabolism with age.

The K_m for the glyoxalase enzymes did not significantly correlate with age in the growing rat, but was consistent with a mean of 0.86 m*M* ± 0.04 for 31 rats. This is a 5 to 10 times difference from the 0.09 m*M* K_m reported by Vander Jagt for rat erythrocyte glyoxalase I (27) depending on whether the K_m is determined on the basis of MeG or the calculated hemimercaptal. The differences in methodology lead to the higher K_m reported here. The analytical procedure that we use (18) is readily applicable to crude tissue systems. Vander Jagt reported (27) a specific activity of 0.26 IU for rat blood homogenate which is in excellent agreement with our finding of 0.32 IU from the *V* data in Table II for 36 rats at a 5° higher assay temperature. The K_m reported here of 1.22 ± 0.16 m*M* for SLG is about 10 times that reported for rat blood (28) and may be a factor of our using the Racker (16) procedure at a higher temperature. Both the K_m for glyoxalase I and II were consistent for triplicate determinations in this study.

Our investigation found a highly positive correlation between plasma D-lactate levels and rat age. The other glyoxalase system parameters did not show this correlation when the effects of hematocrit and protein concentration were controlled (held constant). If D-lactate concentration does adequately reflect cellular MeG, the hypothesis that MeG is a natural growth inhibitor and thus would increase as organisms reach the end of their rapid growth phase would have some support.

Supported by funds from the National Foundation for Cancer Research.

---

1. Egyud LD, Szent-Gyorgyi A. Cell division, SH, ketoaldehydes, and cancer. Proc Nat Acad Sci USA **55**:388–393, 1966.
2. Greeg CH. Inhibition of mammalian cell division by glyoxals. Exp Cell Res **50**:65–72, 1968.
3. Lohmann K. Behavior of the enzymatic attack on methylglyoxal for the synthesis of lactic acid. Biochem Z **254**:332–354, 1932.

4. Brandt RB, Siegel SA. Methylglyoxal catabolism in blood. Fed Proc **38**:673, 1979.
5. Vander Jagt DL, Daub E, Krohn JA, Han LB. Effects of pH and thiols on the kinetics of yeast glyoxalase I, an evaluation of the random pathway mechanism. Biochemistry **14**:3669–3675, 1975.
6. Riddle V, Lorenz FW. Nonenzymatic polyvalent anion-catalyzed formation of methylglyoxal as an explanation of its presence in physiological solutions. J Biol Chem **243**:2708–2724, 1968.
7. Siegel SA, Brandt RB. Non-enzymatic formation of methylglyoxal from dihydroxyacetone phosphate in rat liver extract. Va J Sci **29**:99, 1978.
8. Cooper RA, Anderson A. The formation and catabolism of methylglyoxal during glycolysis in E. coli. FEBS Lett **11**:273–276, 1970.
9. Sato J, Wang Y, Van Eys J. Methylglyoxal formation in rat liver cells. J Biol Chem **255**:2046–2050, 1980.
10. Brandt RB, Siegel SA. Methylglyoxal production in human blood. In: Wolstenholme GEW, Fitzsimons DW, Whelan J, eds. Submolecular Biology and Cancer (Ciba Foundation Symposium). Amsterdam, Excerpta Medica, Vol 67:pp 211–223, 1979.
11. Alexander NM, Boyer JL. Glyoxalase activity in sham and partially hepatectomized rats. Cancer Res **31**:1875–1878, 1971.
12. Strzinek RA, Vela GR, Scholes VE, Norton SJ. Further studies on liver glyoxalase activity in mice bearing lymphosarcoma. Cancer Res **30**:334–337, 1970.
13. Winter R, Piskorska D, Jerzykowski T. Further studies on liver glyoxalase I and glyoxalase II activity in mice bearing sarcoma 180 and L1210 leukemia. Neoplasma **25**:465–470, 1978.
14. Strzinek RA, Scholes VE, Norton SJ. The purification and characterization of liver glyoxalase I from normal mice and mice bearing a lymphosarcoma. Cancer Res **32**:2359–2364, 1972.
15. Friedemann TE. The action of alkali and hydrogen peroxide on glyoxals. J Biol Chem **73**:331–334, 1927.
16. Racker E. The mechanism of action of glyoxalase. J Biol Chem **190**:685–696, 1951.
17. Cohn VH, Lyle J. A fluorometric assay for glutathione. Anal Biochem **14**:434–440, 1966.
18. Gilbert RP, Brandt RB. Spectrophotometric determination of methylglyoxal with 2,4-dinitrophenylhydrazine. Anal Chem **47**:2418–2422, 1975.
19. Lineweaver H, Burk D. The determination of enzyme dissociation constants. J Amer Chem Soc **56**:658–666, 1934.
20. Bergemeyer HU. Glutathione reductase. In: Bergemeyer HU, ed. Methods of Enzymatic Analysis. New York, Academic Press, Vol 1: pp465–466, 1974.
21. Layne E. Spectrophotometric and turbidimetric methods for measuring proteins. In: Colowick SP, Kaplan NO, eds. Methods in Enzymology, New York, Academic Press, Vol 3:pp450–451, 1957.
22. Brandt RB, Siegel SA, Waters MG, Bloch MH. Spectrophotometric assay for D-(–)lactate in plasma. Anal Biochem **102**:39–46, 1980.
23. Donaldson HH. The Rat. Philadelphia, Wistar Institute, pp18,175,198, 1924.
24. Snedecor GW, Cochran WG. Statistical Methods. Iowa State Univ Press, Ames, 1967.
25. Waters MG, Rispler MJ, Kline ES, Brandt RB. D- and L-lactate catabolism in tissues. Va J Sci **32**: 138, 1981.
26. Szent-Gyorgyi A, Egyud LG, McLaughlin JA. Ketoaldehydes and cell division. Science **155**:539–541, 1967.
27. Han LB, Davison LM, Vander Jagt DL. Purification and kinetic study of glyoxalase I from rat liver, erythrocytes, brain, and kidney. Biochim Biophys Acta **445**:486–499, 1976.
28. Ball JC. Purification and substrate specificity of a specific glutathione thiolesterase, glyoxalase-II, from rat erythrocytes. Fed Proc **38**:672, 1979.

Received November 19, 1981. P.S.E.B.M. 1982, Vol. 169.