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SEPIAPTERIN REDUCTASE IN BLOOD OF VARIOUS ANIMALS AND OF LEUKEMIC RATS

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SUMMARY

The level of sepiapterin reductase (EC 1 1 1 153) activity in blood of various animals was examined with special reference to dihydrofolate reductase activity Both enzyme activities in liver were determined for comparison

In all animal species tested, the activity of sepiapterin reductase was only found in erythrocytes though dihydrofolate reductase was found in both erythrocytes and leucocytes The rat seemed peculiar in its high activity of sepiapterin reductase in erythrocytes and liver

Both enzyme activities were also examined in blood corpuscles and ascites tumor cells of three lines of rat with transplantable leukemias In leucocytes of leukemic rats, no measurable activity of sepiapterin reductase was found, while the level of dihydrofolate reductase was about 2 times higher than that of normal Ascites tumor cells showed significant activities of both enzymes

INTRODUCTION

Unconjugated pterin, reduced biopterin, participates as a cofactor (electron donor) in the phenylalanine hydroxylation system of rat [1] this pterin has been isolated from rat liver extracts in dihydro form and it is considered that it is reduced to the tetrahydro form by dihydrofolate reductase (EC 1 5 1 3), an enzyme with an established role in one-carbon metabolism, before it can take part in the system as a cofactor Although the origin of such unconjugated pteridine in mammals has not yet been elucidated, it was demonstrated that reduced biopterin was synthesized via sepiapterin from GTP in lower vertebrates [2] It was also suggested that the supply of reduced biopterin to the hydroxylation system in rat liver would be regulated by sepiapterin reductase which catalyzes the reversible reduction of sepiapterin (2-amino-4-hydroxy-6-lactyl-7,8-dihydropteridine) to dihydrobiopterin (2-amino-4-hydroxy-6-dihydroxypropyl-7,8-dihydropteridine) by NADPH [3, 4]

Concerning the occurrence of sepiapterin reductase, it has been reported that the enzyme is found in liver of several animals [5] and in other tissues of rat, especially in blood, with high activity [6] Almost all of the activity of sepiapterin reductase in

rat blood was found in erythrocytes [6], but the activity of the enzyme has not yet been measured in leucocytes As is well known in dihydrofolate reductase, the enzyme is localized in various tissues of animals such as liver and kidney and is also found in erythrocytes and leucocytes of various animal species [7] The activity of dihydrofolate reductase in leucocytes from human blood was quite high in patients with acute leukemia or with chronic myelogenous leukemia, though it was present in trace amounts in normal subjects [8]

It seemed desirable to investigate the occurrence of sepiapterin reductase in blood of various animal species in order that a physiological role of this enzyme in blood might be clarified The present paper describes the experiments on the determination of sepiapterin reductase activity in rat erythrocytes and leucocytes by photometric measurement, and also describes the level of this enzyme activity in blood corpuscles of other animal species together with the level of dihydrofolate reductase The activities in liver will be presented for comparison In addition, both enzyme activities in blood and ascites tumor cells of three lines of leukemic rat will be given

MATERIALS AND METHODS

Chemicals

Crystalline sepiapterin and isosepiapterin [9], dihydrobiopterin [10] and 6-carboxypterin [11] were prepared by the methods described in listed references Dihydrofolate was prepared by the reduction of folic acid with sodium dithionate according to the method of Futterman [12] NADPH and glucose 6-phosphate dehydrogenase were the products of Oriental Yeast Co (unless otherwise specified) and dextran (mol wt 170 000) was that of BDH Chemicals All other chemicals were obtained from commercial sources

Animals

Three lines of rat with transplantable leukemias (DBLA-1, DBLA-10 and SLA-103) were kindly supplied from Drs Odashima and Maekawa of National Institute of Hygienic Science (Tokyo) These lines were myelogenous leukemias induced by *N*-nitrosobutylurea in Donryu rat (DBLA-1 and DBLA-10) [13] and of Sprague–Dawley (SLA-103) (Maekawa, A , in preparation) One- (DBLA-1) or two- (DBLA-10 and SLA-103)-week-old tumor ascites (0 05–0 1 ml containing 1 10^8–3 10^8 tumor cells/ml) were transferred intraperitoneally into the adult male rats (200–300 g) All other animals were obtained from commercial sources Young adult male animals were used throughout the work

Assay of enzymes

Sepiapterin reductase activity was determined at 37 °C by measuring decrease in absorbance at 420 nm [6] The assay mixture contained the following components (in μmoles) potassium phosphate buffer (pH 6 4), 300, NADPH, 0 3, sepiapterin, 0 15, and enzyme solution, in a final volume of 3 0 ml The reaction mixture without NADPH served as control The activity was also observed by paper chromatography identifying the reaction product as dihydrobiopterin after incubation assay (in the system of *n*-propanol–ethyl acetate–water (7 1 2, by vol))

Dihydrofolate reductase activity was assayed at 37 °C by measuring decrease

in absorbance at 340 nm [14] The standard assay system contained the following components (in μmoles) Tris–HCl buffer (pH 7 5), 150, NADPH, 0 3, dihydrofolate, 0 15, 2-mercatoethanol, 20, and enzyme solution, in a final volume of 3 0 ml The reaction mixture without dihydrofolate served as control

Both reactions were started by the addition of NADPH, and each specific activity was expressed as μmoles of substrate reduced per h per mg protein (mg hemoglobin for erythrocyte fraction) The concentrations of sepiapterin and dihydrofolate in the reaction system were determined using the extinction coefficients of 1 04 10^4 M^{-1} cm^{-1} (at 420 nm) [6] and 1 2 10^4 M^{-1} cm^{-1} (at 340 nm) [15], respectively Absorbance readings were made automatically with a double beam spectrophotometer (Shimazu 40-R)

Perfusion of liver

A rat was anesthetized with diethyl ether The median lobe of liver was perfused 3 times with 50 ml of cold 0 85% NaCl solution, and other lobes of liver were prevented from perfusion by stopping the hepatic veins and arteries with Kocher's forceps Perfused or intact liver was homogenized as described below

Preparation of blood corpuscle and ascites cell fractions

As reported [6], very high activity of sepiapterin reductase was found in rat blood, with almost all of the activity occurring in erythrocytes The leucocyte is, then, presumed to have little or no activity of this enzyme For preparation of as many leucocytes (free from erythrocytes) as possible to be enough to detect probably low activity, the blood must be collected together from several small animals (3–6 animals of same growth stage) of the same species Fractions of erythrocytes and other tissues of various animal species were also prepared as the mixed homogenate from several animals In the case of large animals and of humans, one individual of each species was examined All operations were performed at 4 °C Blood plasma and erythrocytes were separated from heparinized whole blood (5 mg heparin per 100 ml of blood) by the method described previously [6] Since hemoglobin of certain animals was easily crystallized during the hemolyzing process, erythrocytes were mixed with 9 vol of 0 001 M potassium phosphate buffer (pH 7 5) and the mixture was stirred for 30 min in an ice bath The resulting hemolysate was centrifuged at 15 000 $\times$ g for 30 min and the supernatant solution was used as a erythrocyte fraction

Leucocytes were isolated after the method of Bertino et al [8] with the following modifications 5 vol of heparinized blood was mixed with 1 vol of 5% dextran solution in 0 15 M NaCl and the mixture was immediately poured into narrow glass tubes (inner diameter 5 mm) and kept standing 1 h after a short centrifugation (70 $\times$ g for 5 min) The resulting supernatant fluid containing most of the leucocytes was centrifuged at 800 $\times$ g for 5 min

The final leucocyte pellet of original whole blood (free from platelets) was suspended in 0 1 vol of 0 01 M potassium phosphate buffer (pH 6 8) and homogenized for 5 min at 20 000 rev /min in a NK micronizer (Japan Precise Instrument Co) The homogenate was centrifuged at 15 000 $\times$ g for 30 min and the supernatant solution was used as a leucocyte fraction

Ascites tumor cells of leukemic rat were obtained from the peritoneal cavity by washing with 0 85% NaCl Packed cells were obtained by centrifugation Selective

lysis of the contaminated erythrocytes in the packed cells and homogenization of the cells were performed in the same manner as in leucocytes [8]

The smears of blood corpuscles or ascites cells were stained with May–Giemsa solution The complete breaking of leucocytes or ascites cells by the homogenizer was confirmed microscopically

Preparation of liver and spleen homogenate

The tissues were washed with cold 0 85% NaCl immediately after removal from the body and homogenized with 3 vol of 0 01 M potassium phosphate buffer (pH 6 8) in a glass homogenizer After standing for 10 min, the homogenate was centrifuged at 8500 × *g* for 30 min and the supernatant was used for enzyme assay All operations were performed at 4 °C

Protein determination

Protein was determined by the method of Lowry et al [16] using crystalline bovine serum albumin as the standard Hemoglobin was determined spectrophotometrically at 540 nm for the erythrocyte fraction using the extinction coefficient of 1 42 10^4 M^{-1} cm^{-1}

RESULTS AND DISCUSSION

Activities of sepiapterin reductase and dihydrofolate reductase in perfused liver

Sepiapterin reductase activity was found in various tissues of rat, especially in liver and blood [6] Table I shows comparison of sepiapterin reductase activity between perfused and intact liver No decrease in the enzyme activity was observed

TABLE I

ACTIVITIES OF SEPIAPTERIN REDUCTASE AND DIHYDROFOLATE REDUCTASE IN PERFUSED RAT LIVER

The activities in perfused liver of rat (Sprague–Dawley) were assayed under standard conditions The amounts of protein of perfused and intact liver in the reaction mixture were 2 14 and 2 46 mg, respectively

	μmoles/h per mg protein	
	Sepiapterin reductase	Dihydrofolate reductase
Perfused liver	0 136	0 235
Intact liver	0 133	0 234

in the perfused liver As mentioned below (Table III), sepiapterin reductase was not detected in blood plasma From these results, it is apparent that the enzyme in liver does not originate from blood The similar result was obtained for dihydrofolate reductase activity

Determination of activity of sepiapterin reductase in erythrocyte fraction

The presence of sepiapterin reductase and a reasonable photometric assay method for this enzyme has been established with rat liver [3, 17] The presence of

this enzyme activity in rat blood, in the previous study [6], was observed by measuring the decrease in absorbance at 420 nm by the method of Matsubara and Akino [5] and by paper chromatographic identification of the reaction product as dihydrobiopterin after incubation In the former case, the reaction was stopped by the addition of cold trichloroacetic acid and absorbance at 420 nm in the neutralized supernatant solution was measured However, this method was not suitable for determination of initial velocity and for detection of low activity Moreover, it has recently been demonstrated that, when this method is applied to assay of the enzyme in hemolysates, non-enzymatic quenching of sepiapterin takes place, presumably due to the presence of hemoglobin and trichloroacetic acid (Katoh, S and Hasegawa, H , unpublished) Consequently, in the present study, the continuous spectrophotometric method described in Materials and Methods was adopted throughout the work on the basis of the following results

The linear decrease in absorbance at 420 nm of sepiapterin was observed in the complete reaction system of sepaipterin reductase ($-\Delta A_{420} = 0\ 230/10$ min) with reference to both NADPH- and sepiapterin-omitted systems (the complete system contained (in μmoles), in a final volume of 2 0 ml at 37 °C, potassium phosphate buffer (pH 6 4), 200, sepiapterin, 0 1, NADPH, 0 2 and rat (Sprague–Dawley) erythrocyte fraction, 0 9 mg hemoglobin) The sepiapterin-omitted system and NADPH-omitted system exhibited very little decrease in absorbance (less than $-\Delta A_{420} = 0\ 007/10$ min) and this showed that hemoglobin in the erythrocyte fraction in the reaction system did not result in a significant change in the presence or absence of NADPH Therefore, the linear decrease in absorbance in the complete system is dependent on rat erythrocyte fraction and NADPH Moreover, the decrease of absorbance at 420 nm in the complete system was inhibited by the specific inhibitors of liver sepiapterin reductase [6], $5\ 10^{-5}$ M of isosepiapterin (2-amino-4-hydroxy-6-propionyl-7,8-dihydropteridine) and 6-carboxypterin (2-amino-4-hydroxy-6-carboxylpteridine) inhibited the reaction 77 and 33%, respectively, and these rates were quite similar to those of liver The reaction was not affected by the addition of 10^{-4} M aminopterin which is one of the specific inhibitors of dihydrofolate reductase

The stoichiometric relationship between the decrease of sepiapterin and of NADPH was examined according to the method of Nagai [17] The experiment is described in Table II Sepiapterin consumption was determined by decrease of absorbance at 420 nm Oxidation of NADPH could not directly be observed by decrease of absorbance at 340 nm because sepiapterin was converted to a colorless product with maximum absorbance at 330 nm in this system Therefore, NADPH oxidation was determined indirectly by the addition of a glucose 6-phosphate dehydrogenase system Table II shows a 1 1 relation between the decrease of sepiapterin and of NADPH This result is the same as in the case of sepiapterin reductase from rat liver [17]

The colorless reaction product formed from sepiapterin was next isolated by column chromatography on Sephadex G-25 as described in Fig 1 The reaction mixture containing sepiapterin, NADPH and rat erythrocyte fraction was incubated until the yellow color of sepiapterin almost disappeared and applied to the column in the dark The violet fluorescent reaction product was obtained by elution with distilled water ($K_d = 2\ 1$) with about 80% yield based on the decrease in sepiapterin Spectra of the product shown in Fig 1 were very similar to those of authentic dihydrobiopterin [17]

TABLE II

RELATIONSHIP BETWEEN THE DECREASE OF SEPIAPTERIN AND NADPH BY THE ACTION OF THE RAT ERYTHROCYTE FRACTION

The reaction mixture contained the following components (in μmoles) potassium phosphate buffer (pH 6 8), 300, NADPH (Sigma, chemically reduced), 0 3, sepiapterin, 0 15, and rat (Sprague–Dawley) erythrocyte fraction (1 75 mg hemoglobin), in a final volume of 3 0 ml The system lacking sepiapterin was used as a control throughout the experiment After incubation at 37 °C for 9 min ($-\Delta A_{420\ nm} = 0\ 255$), the reaction was stopped by heating in boiling water for 1 min To 1/3 vol of the deproteinized supernatant solution the following components (μmoles) were added potassium phosphate buffer (pH 7 5), 200, sodium glucose 6-phosphate, 0 2, $MgCl_2$, 40, and glucose 6-phosphate dehydrogenase, 1 unit in a final volume 2 0 ml The increase in absorbance at 340 nm at 37 °C was determined as NADPH consumption ($\Delta A_{340\ nm} = 0\ 074$)

μmole per sepiapterin reductase system

Sepiapterin reduced (a)	NADPH oxidized (b)	a/b ratio
0 0736	0 0716	1 03

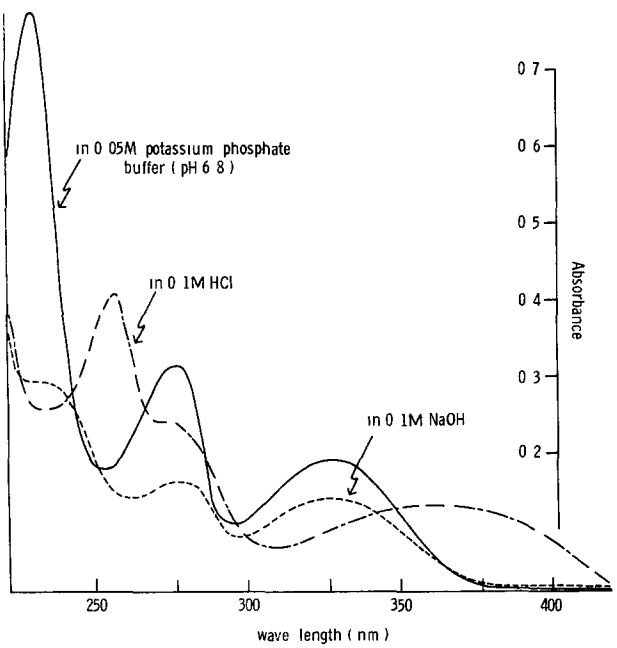

Fig 1 Absorbance spectra of the reaction product catalyzed by rat erythrocyte fraction from sepiapterin and NADPH The reaction mixture containing the following components (μmoles) potassium phosphate buffer (pH 6 4), 1000, sepiapterin, 0 5, NADPH (Sigma, chemically reduced), 1, and rat (Sprague Dawley) erythrocyte fraction (4 58 mg hemoglobin), in a final volume of 10 ml were incubated for 1 h at 37 °C The violet fluorescent product formed was isolated through a fine column of Sephadex G-25 (2 cm × 20 cm) by eluting with distilled water from the incubated reaction mixture

The R_F values of the isolated product in several solvents were also identical to authentic dihydrobiopterin 0 20 in *n*-propanol–ethyl acetate–water (7 1 2, by vol), 0 38 in *n*-propanol–1 % ammonium acetate (1 1, by vol), 0 22 in 95 % ethanol–*n*-amyl alcohol–water (7 5 3, by vol) and 0 33 in isopropanol–water (7 3, by vol)

From these results mentioned above, measurement of the decrease of absorbance at 420 nm in the reaction system containing sepiapterin, NADPH and the erythrocyte fraction is suitable for showing the activity of sepiapterin reductase in the erythrocyte fraction Purification and properties of this enzyme in rat erythrocyte will be reported elsewhere

Activity levels of sepiapterin reductase and dihydrofolate reductase in blood of various animals

As can be seen in Table III, sepiapterin reductase in rat is not detectable in leucocytes even by long incubation (for 60 min), in spite of markedly high activity in erythrocytes In all of the animal species examined, enzyme activity could not be found in leucocytes but was found to be localized in erythrocytes The activity in erythrocytes of human, pig and rabbit was very low In these case, formation of dihydrobiopterin was confirmed by paper chromatography after incubation of the reaction mixture containing enriched erythrocyte fraction (more than 50 mg hemoglobin) for 1 h Dihydrofolate reductase activity was found both in erythrocytes and leucocytes in all species examined, and the enzyme levels were quite similar to those reported by Bertino et al [7]

No significant activities of either enzyme were found in blood plasma The

TABLE III

LEVELS OF SEPIAPTERIN REDUCTASE AND DIHYDROFOLATE REDUCTASE IN BLOOD OF VARIOUS ANIMAL SPECIES

Assays were carried out under the standard conditions The protein amounts in the reaction mixture of plasma, erythrocyte and leucocyte fractions were about 7, 1 and 0 4 mg, respectively A and B mean the activities of sepiapterin reductase and dihydrofolate reductase, respectively

Species	Blood plasma*		Erythrocyte**			Leucocyte*	
	A	B	A	B	A/B ratio	A***	B
Rat†	0	0	0 2991	0 0018	166 17	0	0 095
Rat††	0	0	0 2628	0 0017	154 59	0	0 071
Guinea pig†††	0	0 003	0 0068	0 0079	0 86	0	0 058
Mouse§	0	0	0 0839	0 0138	6 08	—	—
Rabbit	0	0 001	0 0022	0 0020	1 10	0	0 028
Pig	0	0 001	0 0013	0 0007	1 86	0	0 011
Dog	0	0 001	0 0107	0 0001	107 00	—	—
Human	0	0	0 0010	0 0001	10 00	0	0 002
Chicken	0	0	0 0430	0 0168	2 56	0	0 294

* μmole/h per mg protein
** μmole/h per mg hemoglobin
*** Incubation for 60 min
† Sprague–Dawley
†† Donryu
††† English
§ ICR, another name is Swiss Hauschka or CD-1

ratio of activities between sepiapterin reductase and dihydrofolate reductase in erythrocytes seems to be larger than that in liver The value in rat erythrocytes was remarkably high Little discrepancy between Sprague–Dawley and Donryu rat strains was observed in levels of both enzyme activities The level of sepiapterin reductase in rat erythrocytes obtained in the present study, is about 3 times higher than that previously reported [6] This may be due to the different assay method

Levels of sepiapterin reductase and dihydrofolate reductase in liver of various animals

Levels of both enzymes were examined in livers of animals and compared with those in erythrocytes The results are summarized in Table IV Livers of all species showed easily measurable activities of both enzymes The levels were especially high in rat liver, while those in pig and dog were relatively low Data seems to indicate that the ratio of activities between sepiapterin reductase and dihydrofolate reductase in liver is smaller than that in erythrocytes

TABLE IV

LEVELS OF SEPIAPTERIN REDUCTASE AND DIHYDROFOLATE REDUCTASE IN LIVER OF VARIOUS ANIMAL SPECIES

The standard assay mixture contained about 3 mg protein A and B mean the activities of sepiapterin reductase and dihydrofolate reductase, respectively

Species	μmoles/h per mg protein		A/B ratio
	A	B	
Rat (Sprague–Dawley)	0 128	0 242	0 53
Rat (Donryu)	0 113	0 169	0 67
Guinea pig (English)	0 089	0 047	1 89
Mouse (ICR)*	0 052	0 025	2 08
Rabbit	0 034	0 147	0 23
Pig	0 014	0 017	0 82
Dog	0 033	0 012	2 75
Horse	0 023**	—	—
Chicken	0 039	0 180	0 22
Tadpole (bullfrog)	0 010***	—	—

* Another name is Swiss Hauschka or CD-1
** 25 °C [6]
*** 37 °C [6]

Levels of sepiapterin reductase and dihydrofolate reductase in blood and ascites cells of leukemic rats

Three lines of rats with transplantable myelogenous leukemias were used In the later stages of the disease in these lines, a few leukemic cells were detected in peripheral blood and proliferation of ascites, accumulation of tumor cells in ascites, and hepatosplenomegaly were observed The survival times were about 10, 20 and 15 days in DBLA-1, DBLA-10 and SLA-103, respectively [13] (Maekawa, A , in preparation)

Leukemic rats were tested at 8th, 20th and 14th days after inoculation in DBLA-1, DBLA-10 and SLA-103, respectively Each experiment with each line or

TABLE V

LEVELS OF SEPIAPTERIN REDUCTASE AND DIHYDROFOLATE REDUCTASE IN BLOOD AND ASCITES CELLS OF THREE LINES OF LEUKEMIC RATS

Leukemic rats were used on the 8th, 20th and 14th days after inoculation in DBLA-1, DBLA-10 and SLA-103, respectively About 1 mg of protein of ascites cell fraction was added to the assay mixture Other conditions were the same as those in Table III A and B mean the activities of sepiapterin reductase and dihydrofolate reductase, respectively Results are given as mean ± S D

Leukemic lines	Blood plasma*		Erythrocyte**		Leucocyte*		Ascites cell*	
	A	B	A	B	A	B	A	B
DBLA-1	0	0 002 ± 0	0 3205 ± 0 0437	0 0026 ± 0 0007	0	0 118 ± 0 014	0 057 ± 0 039	0 083 ± 0 034
DBLA-10	0 001 ± 0	0 001 ± 0	0 2776 ± 0 0406	0 0023 ± 0 0004	0	0 150 ± 0 026	0 104 ± 0 019	0 157 ± 0 046
Normal***	0	0	0 2644 ± 0 0164	0 0021 ± 0	0	0 068 ± 0 006	—	—
SLA-103	0	0 001 ± 0	0 2192 ± 0 0414	0 0020 ± 0	0	0 153 ± 0 009	0 129 ± 0 076	0 074 ± 0 062
Normal†	0	0	0 2995 ± 0 0590	0 0018 ± 0	0	0 089 ± 0 024	—	—

* μmole/h per mg protein
** μmole/h per mg hemoglobin
*** Donryu
† Sprague–Dawley

normal rat represented 3–10 pooled tissues or blood from same stage animals bred under strictly constant conditions For each line or normal control, experiments were performed in triplicate The results are summarized in Table V Both enzyme levels in leukemic erythrocytes seemed to be consistent with that of normal in all cases In leucocytes of the three leukemic lines, some increase of dihydrofolate reductase activity is observed (about 2 times higher than normal cells), whereas no measurable activity of sepiapterin reductase is detected, as in normal leucocytes Very slight activities of both enzymes were sometimes detected in blood plasma and ascites but only in the later stages of the disease

In addition, ascites tumor cells from three lines have been found to show significant activities of both enzymes Levels of both enzyme activities shown in the table are consistant with that of rat liver but these appeared to alter according to the age of cells Further investigations are needed to obtain more detail

The level of dihydrofolate reductase was reported to be elevated in many tumor cells, including murine and human leukemic cells [8, 18–20], but there has been no information for rat leukemia Dihydrofolate reductase holds the key role in biosynthesis of purine, thimidylate and some amino acids by forming the coenzyme tetrahydrofolate This elevated enzyme activity in tumor cells is considered to be related to the number of dividing cells, or those capable of mitosis As well as dihydrofolate reductase, quite significant activity of sepiapterin reductase was found in the present study, in ascites tumor cells of rat leukemia This suggests that unconjugated pteridines may be metabolized advantageously in tumor cells Further studies must be awaited on the level of sepiapterin reductase activity in leukemia blood, especially in human leukemias

Sepiapterin reductase and dihydrofolate reductase in liver and spleen of leukemic rats

Table VI shows activities of both enzymes in liver and spleen of three lines of leukemic rats Both activities in these tissues do not exhibit noticeable difference between leukemic and normal tissues

TABLE VI

LEVELS OF SEPIAPTERIN REDUCTASE AND DIHYDROFOLATE REDUCTASE IN LIVER AND SPLEEN OF THREE LINES OF LEUKEMIC RATS

About 2 mg of protein of spleen was added to the assay mixture Other conditions were the same as in Table IV A and B mean the activities of sepiapterin reductase and dihydrofolate reductase, respectively Results are given as mean ± S D

Leukemia lines	Liver*		Spleen*	
	A	B	A	B
DBLA-1	0 122 ± 0 021	0 186 ± 0 029	0 091 ± 0 032	0 061 ± 0 017
DBLA-10	0 189 ± 0 040	0 189 ± 0 068	0 087 ± 0 022	0 062 ± 0 015
Normal**	0 106 ± 0 012	0 148 ± 0 022	0 077 ± 0 006	0 066 ± 0 019
SLA-103	0 161 ± 0 013	0 289 ± 0 047	0 112 ± 0 002	0 061 ± 0 002
Normal***	0 122 ± 0 006	0 271 ± 0 035	0 138 ± 0 020	0 129 ± 0 013

* μmole/h per mg protein
** Donryu
*** Sprague–Dawley

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