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RAT THIOPURINE METHYLTRANSFERASE ASSAY PROCEDURE, DEVELOPMENTAL CHANGES AND STRAIN VARIATION*

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Abstract—Thiopurine methyltransferase (TPMT) is one of the enzymes involved in the catabolism of 6-mercaptopurine and azathioprine. An assay procedure was developed for rat TPMT, and the activity of the enzyme was measured in erythrocyte lysates and in homogenates of three solid tissues of Sprague–Dawley rats. Apparent Michaelis–Menten (K_m) values for the two co-substrates of the reaction, 6-mercaptopurine (6-MP) and *S*-adenosyl-L-methionine (SAM), were 2.4, 1.8, 1.4 and 1.5×10^{-3} M for 6-MP and 5.3, 3.3, 4.3 and 4.3×10^{-6} M for SAM in erythrocyte, intestine, kidney, and spleen respectively. The pH optima were 6.7 in all four tissues. Sprague–Dawley rat kidney TPMT activity increased 28-fold and spleen activity increased 2-fold from birth to adulthood when expressed per gram of tissue. The increases were 13- and 2.2-fold, respectively, when expressed per milligram of protein. Enzyme activity was measured in tissues of adult male animals of nine inbred and two outbred rat strains. The rank order of tissue TPMT activity in all strains was: kidney > intestine > spleen > blood. There was a significant correlation between relative erythrocyte and relative kidney enzyme activities. Two-fold variations among strains in renal TPMT activity were found.

Thiopurines such as 6-mercaptopurine and azathioprine are used in the treatment of neoplastic and autoimmune diseases [1]. The two major catabolic pathways for thiopurines are oxidation by xanthine oxidase and thiol methylation by thiopurine methyltransferase [1]. The *S*-methyl derivative of the ribonucleotide of 6-mercaptopurine is an "active" metabolite that is a potent inhibitor of purine biosynthesis [2, 3]. Thiopurine methyltransferase (TPMT) catalyzes the *S*-methylation of thiopurines and thiopurine ribonucleotides with *S*-adenosyl-L-methionine as a methyl donor [4, 5]. This enzyme is also thought to catalyze the *S*-methylation of thiopyrimidines [4] and, although the nature of endogenous substrates for TPMT is not known, there are preliminary reports that endogenous substrates may accumulate in the plasma of patients with renal failure [6, 7]. TPMT is a cytoplasmic enzyme and was first characterized in partially purified form isolated from rat and mouse liver and kidney [4]. TPMT activity has been detected recently in lysates of human erythrocytes [8]. There is a 4-fold variation in human erythrocyte (RBC) TPMT activity; variation in human RBC TPMT activity is due almost entirely to a monogenically inherited (mendelian) variation in enzyme activity [9, 10]. Approximately 90 per cent of subjects are homozygous for an allele for high enzyme activity, $TPMT^H$, and about 10 per cent are heterozygous for $TPMT^H$ and an alternative allele for low activity, $TPMT^L$ [9, 10]. Heterozygous subjects have about half the RBC enzyme activity found in subjects homozygous for $TPMT^H$. One of every three hundred subjects is homozygous for $TPMT^L$ and lacks the RBC enzyme activity [9, 10]. These observations raise the possibility that individual variations in therapeutic response to thiopurines and thiopyrimidines, or the occurrence of toxic reactions to these drugs may result, in part, from "pharmacogenetic" variations in TPMT activity.

Unfortunately nothing is known of the regulation of this important drug-metabolizing enzyme in experimental animals. If studies of TPMT are to move beyond the purified enzyme to include experiments designed to study the regulation of the enzyme, optimal conditions for the measurement of TPMT in tissue homogenates of experimental animals must be determined. We wish to report determination of optimal assay conditions for the measurement of rat TPMT activity in lysates of erythrocytes and in homogenates of three other tissues—the spleen, the kidney, and the intestine. Since human pharmacogenetic studies were performed with RBC lysates, it was important to compare the biochemical properties and regulation of the erythrocyte activity with that in other tissues. Studies of developmental changes in rat TPMT from birth to adulthood have been carried out with this assay. Finally, TPMT activity has been measured in several inbred strains of rat to determine whether significant strain variations in enzyme activity exist, variations that might be useful in future pharmacologic, pharmacogenetic, and biochemical genetic studies of TPMT activity in experimental animals.

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MATERIALS AND METHODS

Animals

Adult male Sprague–Dawley rats from the Hormone Assay Corp., Chicago, IL, were used to develop an assay for rat TPMT and to compare selected biochemical characteristics of the enzyme in kidney, spleen, intestine, and erythrocytes. Pregnant female Sprague–Dawley rats from the same source were used to obtain offspring for the studies of developmental changes in the enzyme activity. Nine inbred and two outbred rat strains were used to compare relative TPMT activities among strains. The inbred strains included ACI, BN, Buffalo, Fischer-344, Lewis, MAXX, and Wistar-Furth from Microbiological Associates, Bethesda, MD; and spontaneously hypertensive rats (SHR) and Wistar–Kyoto animals from the Charles River Corp., Boston, MA. The outbred strains used in these experiments were Sprague–Dawley rats from the Hormone Assay Corp. and Long–Evans animals from the Charles River Corp. All of these animals were males between 10 and 11 weeks of age.

Tissue preparation

Blood. For all experiments except those involving animals from birth to 3 weeks of age, rats were starved with free access to water for 12–14 hr before they were decapitated. Blood was collected in plastic tubes that contained 0.8 ml of sodium heparin (1000 USP units/ml). Plasma was separated from the formed elements of blood by centrifugation at 2200 *g* for 10 min in a refrigerated centrifuge. The erythrocytes were resuspended in a volume of 0.9% saline equal to one-half of the original blood volume and were "washed" twice by resuspension, centrifugation, and aspiration of the supernatant fraction. The washed cells were finally resuspended in a volume of normal saline equal to that of the pellet, and the hematocrit of the resuspended blood was determined in 1.1 × 75 mm heparinized capillary tubes after centrifugation for 10 min at 5500 *g* in an International model 428 centrifuge. The results of the assay could then be expressed per milliliter of packed erythrocytes. One volume of the resuspended washed erythrocytes was added to 4 volumes of ice-cold water in a conical plastic centrifuge tube to lyse the red blood cells. The sample was mixed on a vortex mixer for 10 sec, and the lysate was centrifuged at 10,000 *g* for 10 min. The supernatant fraction was removed and was used for the enzyme assay. The adequacy of the lysis step was confirmed by microscopic examination.

Solid tissue. The kidneys and spleen were removed from decapitated animals and were placed on aluminium foil on dry ice. The small intestine (from the ligament of Treitz to the ileocecal valve) was opened, and the intestinal contents were removed by washing with ice-cold 0.9% saline. The intestine was blotted dry and was placed on dry ice. The organs were homogenized with a Polytron homogenizer for 15 sec in 9 volumes of 5 mM potassium phosphate buffer, pH 7.5. The homogenates were centrifuged at 10,000 *g* for 10 min in a refrigerated centrifuge, and the supernatant fractions were centrifuged at 100,000 *g* for 60 min. The high-speed supernatant fractions were diluted with 5 mM potassium phosphate buffer, pH 7.5, that contained 0.25% bovine serum albumin (supernatant fraction: buffer, v:v): kidney 1:9; intestine, 1:7; and spleen 1:2. All studies of TPMT activity in different rat strains and studies of the effects of growth and development were performed with freshly homogenates. Experiments for the development of the assay procedure were performed with fresh homogenates. Experiments for the development of the assay procedure stored at −85° for a maximum of 4 days. No change in enzyme activity occurred under these conditions of storage.

TPMT assay

TPMT activity was assayed by a modification of the procedure of Weinshilboum *et al.* [8]. 6-Mercaptopurine (6-MP) was used as a substrate for the reaction and [^{14}C-methyl]-*S*-adenosyl-L-methionine (SAM) was used as the methyl donor. 6-MP was converted to radioactively labeled 6-methylmercaptopurine (6-MeMP) by TPMT, the reaction product was separated by organic solvent extraction, and its radioactivity was measured in a liquid scintillation counter. Specifically, 1 volume of a suspension of the solid chelating resin, Chelex-100, prepared as described elsewhere [11], was added to 9 volumes of the diluted tissue preparation immediately prior to the assay. The suspension was mixed by gentle rotation at 12 rpm for 1 hr, and the resin was separated by centrifugation at 5000 *g* for 10 min. Samples (100 μl) of the tissue preparation were then placed in 15-ml conical stoppered glass centrifuge tubes, and 25 μl of 400 mM potassium phosphate buffer, pH 6.2, was added to each tube. Then 10 μl of either dimethylsulfoxide (DMSO) or 6-MP (23.6 mg/ml) in DMSO was added; the samples without 6-MP served as blanks. The reaction was initiated by the addition of 25 μl of a mixture of the following reagents (final concentration in 150 μl indicated): [^{14}C]-*S*-adenosyl-L-methionine (sp. act. 49.8 mCi/mmole), 12.5×10^{-6} M; non-radioactive *S*-adenosyl-L-methionine HCl, 12.5×10^{-6} M; dithiothreitol, 10^{-2} M; and allopurinol, 5×10^{-5} M. The reaction tubes were incubated for 30 min at 37° in a shaker water bath, and the reaction was stopped by the addition of 0.5 ml of 0.5 M borate buffer, pH 10. Two and one-half milliliters of 20% isoamyl alcohol in toluene was added, and the contents were mixed on a vortex mixer for 10 sec. After centrifugation at 700 *g* for 10 min in an International model K centrifuge, 1.5 ml of the organic phase was removed and was placed in a 6-ml polyethylene liquid scintillation counting vial that contained 0.5 ml of absolute ethanol and 3.5 ml of toluene fluor (5 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis-2-[5-phenyloxazolyl]-benzene per liter in toluene); radioactivity was measured in a Packard 3385 liquid scintillation counter. All results were corrected for counting efficiency and for the extraction of 6-methylmercaptopurine into the organic phase (28%). One unit of enzyme activity represented the formation of 1 nmole of 6-methylmercaptopurine/hr. Results were expressed as units per gram of tissue or per milligram of protein for intestine, kidney, and spleen or as units per milliliter of packed red blood cells for blood.

Protein assay

Protein concentrations were measured by the method of Lowry *et al.* [12] with bovine serum albumin as a standard.

Thin-layer chromatography

Reaction products were identified by thin-layer chromatography on Eastman Chromagram sheets of silica gel as described elsewhere [8].

Purification of rat kidney TPMT

Rat kidney TPMT from Fischer-344 animals was partially purified by a modification of the method of Remy [4] as described elsewhere [8]. The final specific activity of the partially purified enzyme was 114 nmole.(mg protein)$^{-1}$.hr^{-1}. This represented a 15-fold purification from the high-speed supernatant fraction.

Kinetic analysis

Michaelis–Menten values were determined by the method of Wilkinson [13] with a Fortran program written by Cleland [14]. A Control Data Corporation 3500 Computer was used for the calculations.

Materials

[^{14}C-Methyl]-*S*-adenosyl-L-methionine (sp. act. 49.8 mCi/mmole) was purchased from the New England Nuclear Corp., Boston, MA. *S*-Adenosyl-L-methionine HCl, Tris(hydroxymethyl)aminomethane base, 6-mercaptopurine, and 6-methylmercaptopurine were purchased from the Sigma Chemical Co., St. Louis, MO. Dithiothreitol (Cleland's reagent) was obtained from CalBiochem, San Diego, CA. Chelex-100 and Bio-Gel A-1.5 M were purchased from BioRad Laboratories, Richmond, CA.

RESULTS

Effects of increasing tissue concentration and incubation time on TPMT activity

TPMT activity increased in a linear fashion with increasing quantities of tissue homogenate or erythrocyte lysate for all of the rat tissues studied. The relationship between enzyme activity and quantity of tissue homogenate or lysate was linear up to at least 10 μl of packed erythrocytes per assay for blood and up to 2.5 mg, 1.8 mg and 5.0 mg per assay for intestine, kidney and spleen respectively (Fig. 1). In all cases, the quantity of tissue used for the experiments described below fell within these linear ranges.

TPMT activity increased in a linear fashion with increasing incubation times for up to 40 min with RBC lysates and with homogenates of all three tissues. An incubation time of 30 min was used in every experiment.

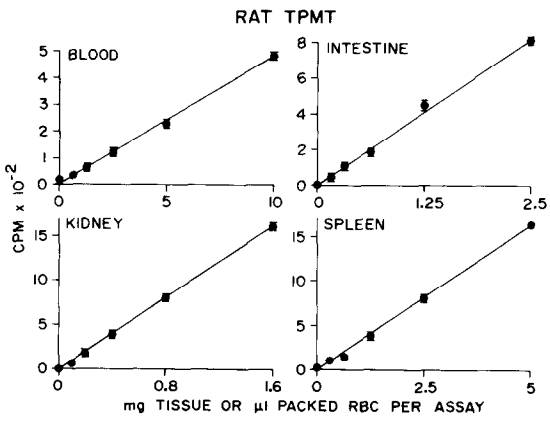

Fig. 1. Effect of increasing tissue concentration on TPMT activity measured as counts per minute (CPM) incorporated into 6-methylmercaptopurine in four tissues of Sprague–Dawley rats. Each point is the mean ± S.E.M. of three determinations.

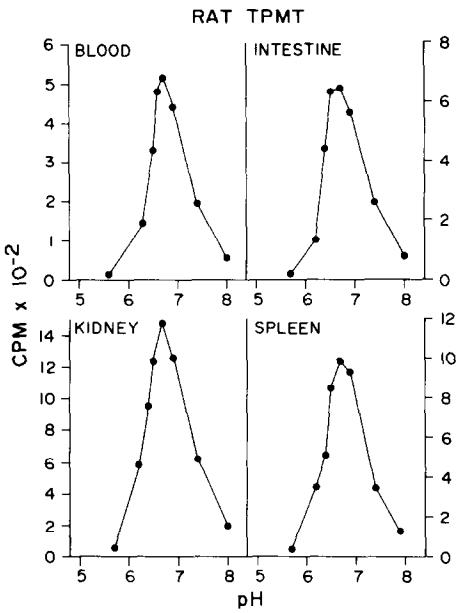

Fig. 2. Effect of pH on TPMT activity measured as in Fig. 1 in four tissues of Sprague–Dawley rats. Each point is the mean of three determinations.

Effect of pH on TPMT activity

TPMT was assayed at different pH values with potassium phosphate buffer. The pH was measured at 20° in the presence of tissue homogenates or lysates and all components of the final reaction mixture. The pH optima were approximately 6.7 for all tissues (Fig. 2).

Table 1. Michaelis–Menten (K_m) values for 6-mercaptopurine (6-MP) and *S*-adenosyl-L-methionine (SAM), the two co-substrates of the TPMT reaction

Tissue	6-MP K_m ± S.E.M.	SAM K_m ± S.E.M.
Blood	2.4(±0.2) × 10^{-3} M	5.3(±0.5) × 10^{-6} M
Intestine	1.8(±0.1) × 10^{-3} M	3.3(±0.3) × 10^{-6} M
Kidney	1.4(±0.1) × 10^{-3} M	4.3(±0.2) × 10^{-6} M
Spleen	1.5(±0.1) × 10^{-3} M	4.3(±0.4) × 10^{-6} M

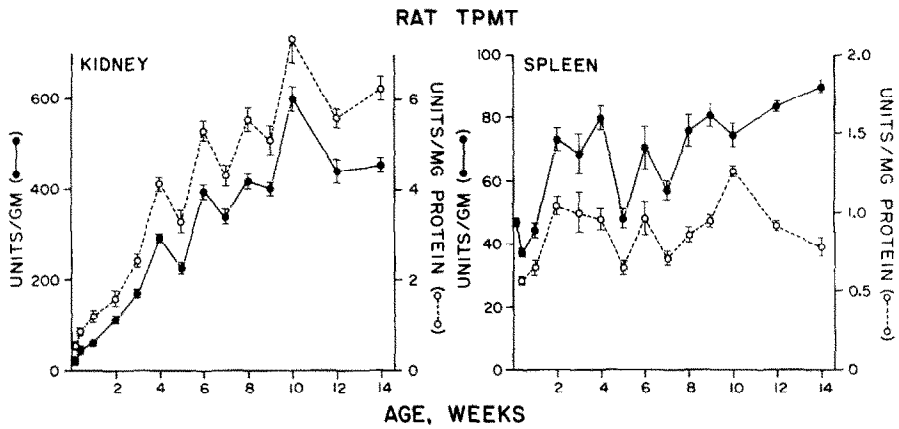

Fig. 3. Effect of growth and development on rat kidney and spleen TPMT. Each point is the mean ±S.E.M. of values for five individual animals. The results are expressed as both activity per gram of tissue (●—●) and per milligram of protein (○ - - - ○).

Effect of dithiothreitol on TPMT activity

Sulfhydryl groups play an important role in the activities of many methyltransferase enzymes [15]. These enzyme activities are often increased in the presence of sulfhydryl reducing agents such as 2-mercaptoethanol or dithiothreitol (DDT). Therefore, the effect on TPMT activity of DTT at six concentrations from 0.5 to 20 mM was determined. Enzyme activities in RBC lysates and intestinal homogenates were changed little at any of these concentrations of DTT, but there was a 10–20 per cent increase in TPMT activity in homogenates of kidney and spleen in the presence of 10 mM DTT. A final DTT concentration of 10 mM was used in all reaction mixtures.

Table 2. Relative TPMT activities of four tissues from eleven rat strains*

Strain	Kidney (%)	Intestine (%)	Spleen (%)	Blood (%)
BN	100	71.6	24.2	4.7
BUFF	100	46.7	20.1	4.9
ACI	100	38.2	19.0	4.2
L-E†	100	43.1	15.9	4.0
MAXX	100	37.3	16.6	3.7
S-D†	100	36.5	19.4	5.1
F-344	100	41.3	14.6	4.0
W-F	100	39.3	17.2	4.2
Lewis	100	33.9	16.3	3.9
SHR	100	31.5	11.2	3.9
WKY	100	29.3	11.1	3.2

* Intestine, spleen and blood activities are expressed as a percentage of the kidney enzyme activity. Results for all solid tissues are expressed as units per gram of tissue, and erythrocyte activities are expressed as units per milliliter of packed RBC. Abbreviations: BUFF, Buffalo; L-E, Long-Evans; S-D, Sprague-Dawley; F-344, Fischer-344; W-F, Wistar-Furth; SHR, spontaneously hypertensive rats; and WKY, Wistar-Kyoto.

† Outbred strain.

Effect of allopurinol on TPMT activity

Xanthine oxidase might compete with TPMT for 6-MP [1]. Since xanthine oxidase is inhibited by allopurinol, the effect on TPMT activity of allopurinol at five concentrations from 0.01 to 0.16 mM was determined in all four tissues. This concentration range has been reported to result in inhibition of xanthine oxidase [16]. No effect of allopurinol on TPMT activity was observed in any tissue except the kidney. There was a 70 per cent increase in TPMT activity in renal homogenates in the presence of 0.04 mM allopurinol. Thin-layer chromatography of the reaction products (see below) in all tissues showed only radioactive 6-MeMP and no other product.

Effect of substrate on TPMT activity

S-Adenosyl-L-methionine (SAM) and 6-MP are the two co-substrates for the TPMT reaction. The effect of eight concentrations of 6-MP from 0.3 to 40 mM and of seven concentrations of SAM from 0.8 to 50 μM on TPMT activity in red blood cell lysates and homogenates of intestine, kidney, and spleen was determined. Apparent Michaelis–Menten (K_m) constants for SAM and 6-MP were calculated from these data (Table 1). Although the apparent K_m values for SAM and 6-MP were slightly higher for erythrocyte lysates, K_m values were similar in all four tissues. The concentrations of both substrates used in the assay procedure were five to ten times the calculated apparent K_m values. In all four tissues the enzyme activity decreased slightly when the 6-MP concentration was increased to values above 20 mM.

Identification of reaction product

The product of the reaction was identified by thin-layer chromatography of toluene extracts that were dried under a stream of nitrogen. In all cases only a single radioactive product was formed, and the R_f value of the radioactive product was identical with that of authentic 6-methylmercaptopurine in two different solvent systems: *n*-butanol, acetone,

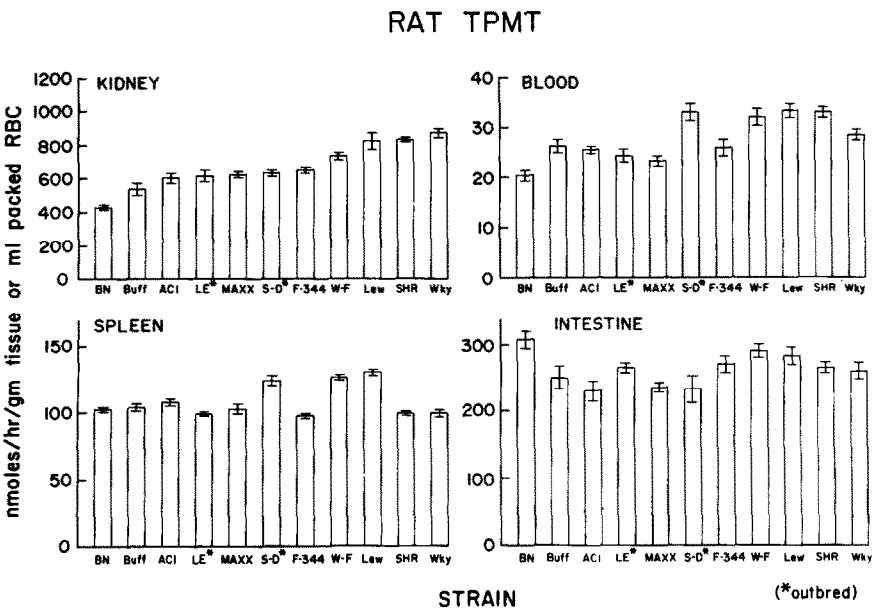

Fig. 4. Variations in the TPMT activity of four tissues among nine inbred and two outbred (*) strains of rats. Each bar represents the mean TPMT activity ± S.E.M. for five animals. The following abbreviations have been used: Buff, Buffalo; LE, Long–Evans; S-D, Sprague–Dawley; F-344, Fischer-344; W–F, Wistar-Furth; Lew, Lewis; SHR, spontaneously hypertensive rats; and Wky, Wistar–Kyoto.

NH_4OH, and water (50:40:3:15) and 1-propanolol, 1-butanol, water, and formic acid (60:20:19:1).

Effects of growth and development on rat TPMT

Thiopurines are used to treat children as well as adults. Therefore, it was of interest to determine the effects of growth and development on rat TPMT activity. The enzyme activity was measured in kidneys and spleens of male Sprague–Dawley rats from birth to 14 weeks of age (Fig. 3). The data shown in the figure have been expressed as both activity per gram of tissue and per milligram of protein. Renal TPMT increased 28-fold from birth to adulthood when expressed per gram of tissue and 13-fold when expressed as units per milligram of protein. Splenic TPMT activity increased 2-fold when expressed per gram of tissue and 2.2-fold when expressed per milligram of protein. RBC TPMT was measured in 2- and 9-week-old animals. No significant change was noted. The value for RBC TPMT was 33 1 ± 3.7 units/ml RBC (mean ± S.E.M., N = 5) at 2 weeks of age and 32.1 ± 3.1 (N = 5) at 9 weeks of age. Finally, renal TPMT activity was measured in kidneys from eight adult female Sprague–Dawley rats 4 weeks after they delivered litters. The average activity was 209 ± 4.5 units/g tissue, about one-half to one-third that found in adult male animals. Whether this striking difference was due to the

Table 3. Tissue correlations of rat TPMT activity*

Tissue pairs	Individual animals (N = 55)				Strain averages (N = 11)			
	units/g		units/mg protein		units/g		units/mg protein	
	r	P	*r*	P	*r*	P	*r*	P
RBC–Kidney	0.58	<0.01	0.55	<0.01	0.73	<0.05	0.71	<0.05
RBC–Spleen	0.41	<0.01	0.16	NS	0.53	NS	0.20	NS
RBC–Intestine	−0.04	NS	0.00	NS	−0.06	NS	−0.10	NS
Kidney–Spleen	0.18	NS	0.11	NS	0.14	NS	0.10	NS
Kidney–Intestine	0.02	NS	−0.02	NS	−0.02	NS	−0.15	NS
Spleen–Intestine	0.02	NS	−0.04	NS	0.09	NS	−0.20	NS

* Correlation coefficients of TPMT activity in eleven different rat strains are shown. Results are shown using (1) data from five rats of each strain and (2) the average values for each strain; *r* represents the correlation coefficient. NS indicates a $P > 0.05$.

effects of sex, age, or recent pregnancy remains to be determined.

The increase in renal TPMT activity during growth and development might have resulted from differences in the levels of endogenous enzyme activators, inhibitors, or competing enzyme systems. To test this possibility, TPMT activity was determined in equal volume mixtures of renal homogenates from five individual 2-week-old rats and five individual 9-week-old rats. The activities of the mixtures did not differ significantly from the expected arithmetic mean values. These results make it unlikely that the change in TPMT activity in the kidneys of Sprague–Dawley rats during growth and development can be explained on the basis of changes in the concentrations of endogenous enzyme activators, inhibitors, or competing enzyme systems.

Strain variations of rat TPMT

The activities of TPMT in rat blood, intestine, kidney, and spleen were determined in nine inbred and two outbred rat strains. In each strain studied, the relative TPMT activity was greatest in the kidney, followed by the intestine, the spleen, and erythrocytes (Table 2). There were wide variations in TPMT activity among different rat strains (Fig. 4). Kidney enzyme activity varied 2-fold, blood 1.5-fold, spleen 1.4-fold and intestine 1.2-fold among these strains. Relative TPMT activities among strains were very similar when expressed as either units per gram of tissue or as units per milligram of protein.

The correlations between the relative TPMT activity of one tissue and another in these rat strains were determined (Table 3). There was a significant correlation of relative erythrocyte with relative renal activity when either the average values for each strain or the values for each of the fifty-five individual animals studied were used for the calculations (Table 3). Since the RBC contains much less activity than does the kidney, this correlation cannot be explained on the basis of contamination of renal tissue with blood. In no other tissue pairs was a striking correlation present. The lack of correlation of relative activity among other tissues is not particularly surprising since tissue variation in the regulation of other drug-metabolizing methyltransferase activities in the rat has been reported [17].

It was possible that strain variations in TPMT activity might be due to variations in endogenous enzyme activators or inhibitors rather than to variations in the enzyme activity itself. To test this possibility, two strains with very different renal enzyme activity, Lewis and BN (Fig. 4), were selected for additional studies. In the first experiment, kidney homogenates from five individual male BN rats were mixed with equal volumes of the homogenates from five individual male Lewis animals, and the TPMT activity was measured. The observed activities in the five mixtures were compared with the arithmetic mean of the TPMT activities in the homogenates used to make the mixtures. The activities in the mixtures were not significantly different from the anticipated arithmetic mean values. In the second experiment, partially purified rat kidney TPMT was added to individual renal homogenates from five male BN and five male Lewis animals. The average recoveries of the added enzyme were 90.0 ± 2.2 and 87.3 ± 1.9 per cent for BN and Lewis rats respectively. The results of these two experiments make it unlikely that the differences between TPMT activities in the kidneys of at least these two strains can be explained on the basis of variations in endogenous enzyme activators, inhibitors, or competing enzyme systems.

DISCUSSION

Thiopurine methyltransferase catalyzes the *S*-methylation of thiopurines and thiopurine ribonucleotides [1, 4, 5]. Several thiol methyltransferase activities have been described. The name "thiol methyltransferase" (EC 2.1.1.9) has usually been applied to a membrane-bound enzyme that catalyzes the methylation of aliphatic sulfhydryl compounds such as 2-mercaptoethanol [18]. Remy clearly differentiated the "soluble" thiopurine methyltransferase activity from this membrane-bound thiol methyltransferase, and Remy's nomenclature has been used here [4, 19]. *S*-Methylated thiopurine ribonucleotides are active metabolites that are potent inhibitors of purine biosynthesis [2, 3]. It is possible that one factor in individual variation in the response of patients to thiopurines, as well as in the occurrence of side effects with these drugs might be related to variations in TPMT activity. TPMT is present in the human erythrocyte and there are wide individual variations in human RBC TPMT activity [8], variations that are due to the effects of inheritance [9, 10]. These observations have raised the possibility that measurements of RBC TPMT might have value as a measure of variations in thiopurine *S*-methylation.

Since nothing is known of the regulation of TPMT activity in experimental animals and since optimal conditions for the assay of the enzyme in tissue homogenates of experimental animals had not been determined, an assay procedure for the measurement of TPMT activity in four different rat tissues was developed. It was found that the pH optima and the K_m values for the two co-substrates for the TPMT reaction, 6-MP and SAM, are similar with all four rat tissues. The assay was used to measure changes in renal TPMT activity during growth and development. In addition, the enzyme was measured in eleven strains of rat. In all strains the kidney was found to have activity higher than that in the intestine, the spleen, or the erythrocyte. Among strains a significant positive correlation of relative RBC TPMT activity with relative enzyme activity in the kidney was observed. This rat tissue TPMT assay procedure and information from its application will make it possible for the first time to begin to study the regulation of this important drug-metabolizing enzyme in an animal system. The results of such future pharmacologic, pharmacogenetic, and biochemical genetic studies of TPMT may help to clarify the role of TPMT in thiopurine and thiopyrimidine metabolism. However, since it is not possible to extrapolate results from experimental animals directly to man, similar experiments ultimately must be performed with human tissue.

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