A GENETIC STUDY OF ERYTHROCYTE ARGININE-tRNA SYNTHETASE ACTIVITY IN MAN

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To search for evidence of genetic variation among the aminoacyl-tRNA synthetases, a semi-automated assay procedure employing a Technicon AutoAnalyzer was used to measure erythrocyte arginine-tRNA synthetase activity in samples obtained from normal human twins of various ages. Variation in enzyme activity within the older DZ twins was five times that of the MZ twins suggesting the existence of genetically determined variation in enzyme activity. Higher enzyme activity was observed in newborn DZ unlike-sexed twins than in like-sexed twins of either zygosity. Possible explanations for this observation are discussed.

INTRODUCTION

The aminoacyl-transfer RNA synthetases play a key role in protein synthesis. They must recognize both the genetic information stored in the four nucleotide bases of nucleic acids and the twenty amino acids found in proteins. In addition, the activating enzymes may participate in such diverse cellular processes as differentiation (Strehler et al. 1967, Elska et al. 1971), development (Rennert 1969, Spadafora et al. 1973), senescence (Bick and Strehler 1971), and the changes attending various disease states such as viral infection (Marchin et al. 1972), cancer (Gallo and Pestka 1971, Goldman et al. 1969), and diabetes (Germanyuk and Mironenko 1969). Most studies of these synthetases have involved experimental animals, and, despite the recognized importance of the enzyme, few investigations have been performed on human material. Several reports have dealt with synthetases derived from human leukemic lymphocytes (Anderson 1969, Tchou et al. 1971), from biopsy specimens (Neth et al. 1968) and from placenta (Matthaei and Schoech 1967); however, no previous studies have sought for evidence of genetic variation in these enzymes in normal human subjects.

In this paper, a semiautomated assay procedure, employing a Technicon AutoAnalyzer, was used to quantitate arginine-tRNA synthetase activities in erythrocytes from twins of various ages. A statistical analysis of the resulting data showed evidence for genetic variation in the enzyme activity.

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MATERIALS AND METHODS

Erythrocytes were obtained from venous blood drawn into vacutainers containing EDTA. Twins of various ages were ascertained from the Indiana University Twin Panel, and newborn cord blood was obtained from a study of consecutive newborn twins that was conducted at three participating hospitals in Indianapolis. Twin zygosity was determined by the similarity method and by typing of the twins and their parents for more than twenty heritable blood and salivary factors (Smith and Penrose 1955). Dermatoglyphic studies of the older twins and their parents, as well as placental information, were obtained whenever possible to confirm zygosity.

L-arginine (^{3}H) was purchased from Swartz/Mann at a specific activity of 7 Ci/mole. Adenosine 5′ triphosphate (ATP) was obtained from Sigma. 2,5-diphenyloxazole (PPO) and 1,4-bis-(2-(5-phenyloxazolyl)benzene) (POPOP) were purchased from New England Nuclear. All other chemicals were of analytic purity.

In order to prepare crude mixtures of erythrocyte aminoacyl-tRNA synthetases, the erythrocytes were suspen) ded in 2.5 volumes of a 0.025 M Tris-HCl buffer (pH 7.6) containing 0.0025 M $MgCl_2$ and homogenized for 60 seconds in a power-driven teflon homogenizer (Strehler et al. 1967). The homogenates were then centrifuged for one hour at 105,000 × g in a Model L Beckman Preparative Ultracentrifuge. One volume of glycerol was added to four volumes of the resultant supernatant solutions, and these preparations were stored at —20° C until analysis. The protein content was determined by the method of Warburg and Christian (1941). The enzymes were not purified further so that loss or denaturation of minor isozymes would be avoided.

The method of Sein et al. (1969) was used to isolate tRNA from rat liver and monkey liver. The tissue was homogenized with phenol, precipitated with ethanol and then separated from the remaining nucleic acids by isopropanol fractionation.

To assay arginine-tRNA synthetase activity the basic aminoacylation reaction mixture consisted of 0.02 M phosphate buffer (pH 7.5), 0.02 M ATP (pH 7.0), and 0.04 M $MgCl_2$. To the basic components, 100 moles of ^{3}H-arginine, 0.16 mg of rat liver tRNA or 0.08 mg monkey liver tRNA, and crude enzyme extract (less than 2.5 mg of protein) were added to a final volume of 0.5 ml of standard binding reaction mixture (McCune 1974). Enzyme activity was expressed as cpm/mg protein. The assays being used measured initial rates for arginine-tRNA synthetase activity.

A semiautomated assay procedure employing a Technicon AutoAnalyzer with a continuous filter paper strip attachment was used in this project (McCune 1974). Two samples from each twin were placed in the sampler tray, the samples from each pair occupying four consecutive slots. The order within a set was randomized by assigning numbers to the twins and then using a random number table to determine their order in the tray.

RESULTS

The twins were classified by zygosity and sex and subdivided into two age groups for analysis: newborn twins and older twins, ranging in age from 6 to 76 years. Table 1 gives the means and standard deviations for arginine-tRNA synthetase activities of the various classes of older twins using rat and monkey liver tRNA in the assay. The set of older female DZ twins had a lower mean enzyme activity than did the other twin sets in that age group; however, this result may be attributable to the smaller number of DZ females in the study. The Figure is a graph of newborn-twin enzyme activity plotted against birth weight. A striking observation was that both the male and female members of unlike-sexed DZ twin pairs had a higher level of enzyme activity than did like-sexed twins of either sex or zygosity type, there being no overlap in the distribution of values.

To search for evidence of genetic variation in enzyme activity, the within and among pair mean squares of the 35 pairs of older male twins were calculated using a twin analysis program written by K.W. Kang (Christian et al. 1974) (Table 2). Other classes of twins were

Table 1. *Means and standard deviations of erythrocyte arginine-tRNA synthetase activities of various classes of older twins*

Type of twin	Male pairs		Female pairs	
	N	Activity (cpm/mg protein)	N	Activity (cpm/mg protein)
MZ twin pairs	18		13	
Rat liver tRNA		4544 ± 1753		5216 ± 1528
Monkey liver tRNA		3841 ± 1219		4224 ± 1236
DZ twin pairs	15		3	
Rat liver tRNA		4339 ± 1637		3003 ± 866
Monkey liver tRNA		3625 ± 1288		2054 ± 896

Table 2. *Erythrocyte arginine-tRNA synthetase activity: analysis of variance in male twins*

Twins class	N	Mean squares		*F*	*H*
		Among pairs	Within pairs		
Rat liver tRNA					
DZ twin pairs	16	29,281,327	6,224,772	5.53*	0.819
MZ twin pairs	19	36,937,754	1,125,178		
Monkey liver tRNA					
DZ twin pairs	16	19,055,721	4,333,920	4.78**	0.790
MZ twin pairs	19	17,746,172	905,932		

* $p < 0.0004$; ** $p < 0.001$

omitted from the analysis because of small sample size. There was no significant difference in the total variance of MZ and DZ twins, but the within pair variance of the DZ twins was about five times that of the MZ twins, providing strong evidence for the existence of a significant genetic component of variation in enzyme activity. From Holzinger's formula (Newman et al. 1937), the estimated heritability of arginine-tRNA synthetase activity was 0.82 with rat liver tRNA in the assay system and 0.79 with monkey liver tRNA.

The mean enzyme activities were found to be significantly higher in the newborn DZ unlike-sexed twins when compared with like-sexed twins of either zygosity. Unfortunately, samples were not available on older unlike-sexed twins to determine the consistency of the apparent difference with age. Although the conditions of cord blood collection were not rigorously controlled, we can think of no systematic bias that could explain the observed results. The average length of storage of the unlike-sexed twin samples prior to assay was slightly, but not significantly, longer than that of the like-sexed samples. Moreover, since enzyme activity was observed to decrease somewhat with storage, variation in length of storage cannot account for the observed result. Nor can variation in placental type explain the difference, since the DZ like-sexed and half of the MZ twins had the same dichorionic-diamniotic pla-

centation as did the DZ unlike-sexed twins; analysis by placental type substantiated the finding of lower enzyme levels in the like-sexed twins ($p < 0.05$). Birth weight was considered as a possible causative factor for the difference, and in the Figure the twin pairs' enzyme activities have been plotted against birth weight. The unlike-sexed twins had somewhat greater birth weights, but the mean weight was not significantly different from that of the like-sexed twins.

Another possible explanation of this phenomenon could be the existence of some type of synergism between the sexes *in utero*. Sex hormones from one twin might stimulate synthe-

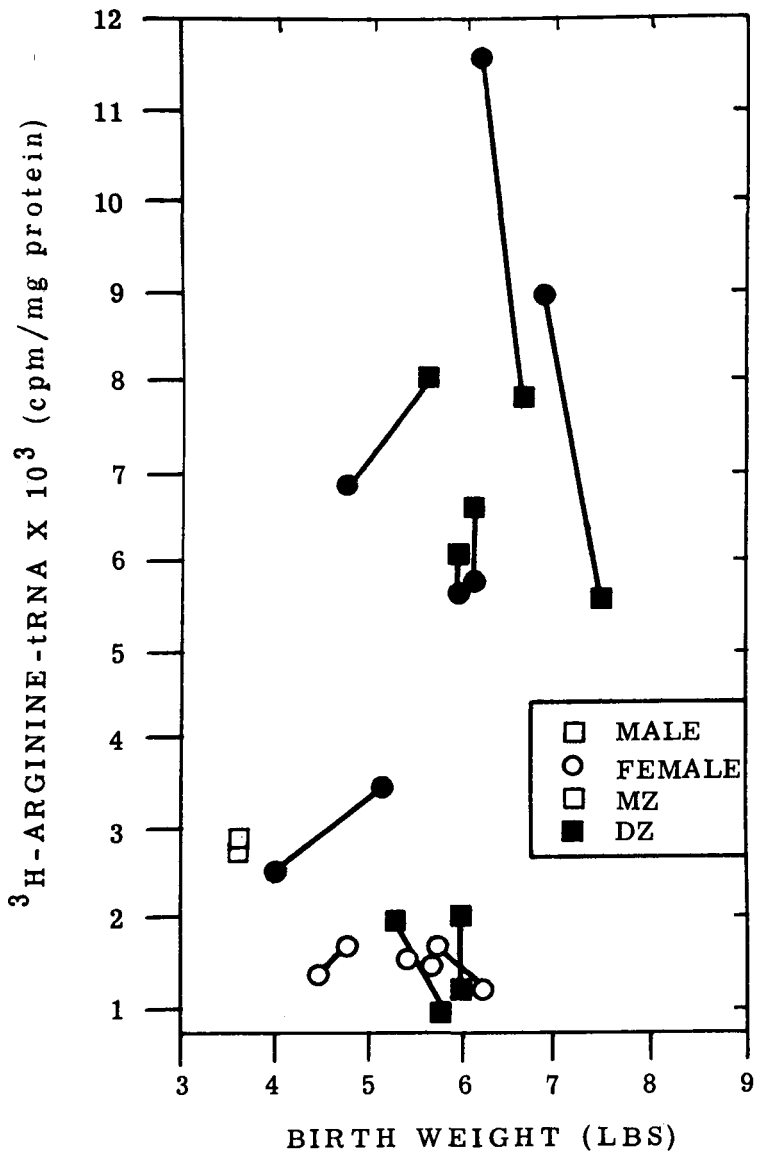

Figure. Erythrocyte arginine tRNA synthetase activity of newborn twins with rat liver tRNA vs. birth weight. Line connects twins.

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tases in the other twin and vice versa. Busby and Hele (1970) demonstrated an increase in acylation of lysyl, serine, and arginine-tRNA in immature hens and cocks which had been treated with diethylstilbesterol. He attributed the increase in acylation to production of more tRNA; however, his work did not rule out the possibility of a steroid-dependent stimulation or activation of aminoacyl-tRNA synthetases.

It is a well-known fact that, when prenatal anastomoses exist between the placentas of bovine unlike-sexed DZ twins, profound alterations in the development of the female twin occur, resulting in a stunted, sterile, " freemartin " calf (Benirschke 1970). There have been only two cases with histologically verified interplacental anastomoses in dichorionic placentas reported in man, and these were in MZ twins (Cameron 1968). However, there have been reports of heterosexual DZ twins who exhibited permanent admixtures of two types of blood cells, with normal sexual development and fertility in the female; thus, the " freemartin " effect of ruminants in apparently not exhibited in man (Beirschke and Kim 1973). It is also known that maternal cells can pass from mother to fetus and the reverse; however, the admixtures are only transient and disappear shortly after birth (El-Alfi and Hathout 1969). Therefore, it seems possible that admixture of plasma or even blood cells containing hormones, synthetases, or other stimulating factors may be a more common event in DZ twins than the infrequent occurrence of persistent red cell chimerism would suggest. If true, hormonal interactions might well account for the observed increased synthetase activity in newborn heterosexual twins. Ounsted and Taylor (1972) has suggested that a synergistic prenatal interaction between unlike-sexed DZ twins may account for their increased birth weight in comparison with that of like-sexed DZ twins. Similar interactions have also been postulated to account for the three and one half-fold increase in the incidence of cancer of the ovary in unlike-sexed as opposed to like-sexed female DZ twins (Nance 1977). It is possible that the observed enzymatic differences are a reflection of this synergism at a biochemical level. Conceivably hormones from the opposite-sexed twin stimulate an inactive synthetase to acylate another isoaccepting species of tRNA, thus increasing the total arginine-tRNA formed. Whatever the reason for the higher enzyme activity in the opposite-sexed twins, it was a very interesting finding and should be studied in greater detail under more standardized conditions of collection and storage.

Arginine-tRNA synthetase activity was consistently higher with the rat liver tRNA than with the monkey liver tRNA in the assay system. An analysis of differences in the relative synthetase activity with the different substrates in twins could provide a method of detecting genetic variation which affects the kinetic properties of the enzyme. Although the data provided strong evidence for the existence of genetic variation in enzyme activity, no evidence was found for a genetic influence on the ratio of activities with the two substrates. The observation of significant genetic variation in the quantitative levels of enzyme activity raises the possibility that polymorphic variation may exist in man, either in the structural gene which determines arginine-tRNA synthetase or in genes which regulate synthetase activity.

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RIASSUNTO

Studio Genetico sull'Attività dell'Arginina tRNA Sintetasi Eritrocitaria nell'Uomo

Al fine di valutare la variazione genetica fra aminoacil-tRNA sintetasi, è stato utilizzato un procedimento semiautomatizzato mediante impiego di un Technicon Auto-Analyzer, per misurare l'attività dell'arginina-tRNA sintetasi eritrocitaria in gemelli normali di varie età. La variazione nell'attività enzimatica nei gemelli DZ più anziani è risultata cinque volte superiore a quella dei gemelli MZ, indicando l'esistenza di un determinismo genetico dell'attività enzimatica. Una più elevata attività enzimatica è stata osservata in gemelli neonati di sesso opposto rispetto a gemelli dello stesso sesso e le possibili spiegazioni di tale reperto vengono discusse.

RÉSUMÉ

Etude Génétique sur l'Activité de l'Arginine tRNA Synthétase Erythrocytaire chez l'Homme

Dans le but d'évaluer la variation génétique des amynoacyl-tRNA synthétases, un procédé d'essai sémiautomatisé, moyennant un Technicon Auto-Analyzer, a été utilisé pour mesurer l'activité de l'arginine tRNA synthétase érythrocytaire chez des jumeaux normaux de différents âges. La variation dans l'activité enzymatique chez les jumeaux DZ plus âgés a été cinq fois plus élevée par rapport aux jumeaux MZ, ce qui indique l'existence de facteurs génétiques dans l'activité enzymatique. Les jumeaux nouveaux-nés de sexe différent ont présenté une activité enzymatique plus élevée par rapport aux jumeaux du même sexe; les possibles interprétations de cette observation sont discutées.

ZUSAMMENFASSUNG

Erbstudie über die Aktivität der tRNS-Arginin-Synthetase in menschlichen Erythrozyten

Zur Beurteilung der Erbvariation unter den tRNS-Aminoazyl-Synthetasen wurde ein halbautomatisches Verfahren unter Benützung eines Technicon Auto-Analyzers angewandt, um die tRNS-Arginin-Synthetase-Aktivität in den Erythrozyten von normalen Zwillingen verschiedenen Alters zu messen. Bei den älteren ZZ war die Variation der Enzymtätigkeit fünfmal so hoch wie bei EZ, was dafür spricht, daß die Enzymtätigkeit erbbedingt ist. Es wurde auch die Beobachtung erörtert, daß die Enzymtätigkeit bei neugeborenen Pärchenzwillingen höher war als bei gleichgeschlechtlichen Zwillingspaaren, um die Bedeutung dieser Beobachtung zu verstehen.

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