composition of its satellite DNA, comprising about 10% of total DNA, is known to be significantly AT-richer than that of the main band[4].

Materials and methods. DNA was isolated from murine leukemia L5178Y lymphoblasts according to Marmur[5]. Histone F1 was prepared from calf thymus by extraction with 5% perchloric acid according to Johns[6]. Histone F1-DNA complexes were obtained by mixing suitable volumes of the F1 solution with the DNA solution, both in 2.0 M NaCl, to give required weight ratio and gradual dialysis down to 0.4 M NaCl (4 h), 0.3 M NaCl (2 h) and 0.15 M NaCl (overnight) at 4 °C; all the NaCl solutions were buffered with 0.013 M sodium phosphate (pH 6.8), as described by Šponar and Sormová[2].

The precipitates formed were centrifuged for 30 min at 10,000 × g. The fraction of DNA in the complex was determined from the difference in absorbancy at 260 nm between the corresponding DNA solution before the addition of F1 and the supernatant after F1-DNA centrifugation, corrected for changes of DNA concentration during dialysis. For estimation of the base composition of the DNA in the histone-DNA complexes DNA was hydrolysed in perchloric acid and its bases separated by the paper chromatography on Whatman No. 1 paper with a solvent system composed of propan-2-ol-12 M HCl-water (85:22:18) or by thin layer chromatography on DEAE cellulose with a solvent system n-butanol-methanol-conc. ammonia-water (60:20:1:20) as described elsewhere[7]. The bases were located under UV-light and their content was estimated in the 0.1 M HCl eluates by UV-absorption properties. Distribution of radioactivity in the chromatograms of the labelled DNA hydrolysates was determined in the Packard Tri Carb liquid scintillation counter in PPO and POPOP solution in toluene.

Results and discussion. The fractionation of mouse DNA by histone F1 was investigated under the following conditions: to the solution of whole DNA in concentration of about 100 μg/ml suitable volume of F1 (approximately 2 mg/ml) was added to give the required histone-DNA weight ratio. The mixture was subsequently dialysed and centrifuged as described in Materials and methods. In the obtained supernatant salt concentration was increased to 2.0 M NaCl and afterwards the next dose of F1 solution was added. The mixture was then dialysed and centrifuged. The whole procedure was usually repeated 4 times. In DNA precipitates obtained at every step of fractionation the base composition was estimated by 2 different methods. The table shows that in the result of the fractionation procedure described above, 5 fractions of DNA differing in base composition were obtained. Those results are in line with the suggestions by Šponar and Šormová[2] that, in the interaction of histone F1 with DNA under the applied experimental conditions, we are dealing with a high degree of selectivity making it possible to distinguish between sequences of only slightly different composition, regardless of the type of DNA molecules present in the DNA solution to be fractionated. Several trials of the fractionation procedure performed by us, using various concentrations of DNA of the initial solutions, have lead us to the conclusion that the greater concentration of DNA the lower selectivity of histone-DNA binding occurs. On the other hand, under strictly defined conditions, the reproducibility of the course of fractionation was very high. It thus seems that the effect of selective precipitation of defined DNA molecules by histone F1 might be of practical use in subtle fractionation of various sequences of the genome differing slightly in base composition.

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Erythrocyte catechol-O-methyltransferase and plasma dopamine-β-hydroxylase in human umbilical cord blood[1]

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Summary. Plasma dopamine-β-hydroxylase enzymatic activity and immunoreactive protein levels in human umbilical cord blood are only about 2% as great as values in the blood of older subjects. Erythrocyte catechol-O-methyltransferase activity levels in umbilical cord blood are very similar to those in the blood of adult subjects.

Several new potential biochemical measures for the characterization of the status and function of the human sympathetic nervous system have been developed recently. Included among these are determinations of plasma dopamine-β-hydroxylase (DBH, E.C. 1.14.17.1) activity and of erythrocyte (RBC) catechol-O-methyltransferase (COMT, E.C. 2.1.1.6) activity. The regulation of these enzyme activities in human blood must be understood to make possible the interpretation of the results of clinical studies in which COMT and DBH are measured. For example, genetic factors play an important role in the regulation of both COMT and DBH activities in blood[4,5]. Changes in human plasma DBH activity during growth and development have also been described[6,7]. As one step in the further study of the effects of growth and development on human plasma DBH and RBC COMT activities, we have measured these enzyme activities in umbilical cord blood samples from a group of randomly selected infants.

DBH activity was determined by the method of Molinoff et al. as modified to measure plasma enzyme activity[8–10]. 1 unit of enzyme activity represented the production of 1 nmole of β-phenylethanolamine (β-phenylethylamine as substrate) or 1 nmole of octopamine (tyramine as substrate) per h per ml. Immunoreactive DBH (IDBH) was determined by radioimmunoassay with ^{125}I radio-iodinated human pheochromocytoma DBH as antigen and rabbit anti-human pheochromocytoma DBH as antibody[11]. Erythrocyte COMT activity was measured by the method of Raymond and Weinshilboum, an assay that includes a

step in which calcium, an inhibitor of COMT, is removed from blood lysates[12,13]. COMT activity was expressed as nmoles of 4-hydroxy-3-methoxybenzoic acid formed per h per ml of packed RBC. Blood samples in heparin tubes were obtained from the umbilical cords of 32 randomly selected white infants after normal full-term uncomplicated vaginal deliveries.

Cord blood plasma DBH enzymatic activities are very low when compared with results obtained from the blood of adults or children over age 6 (table). The mean DBH activity reported in an earlier study of 841 children aged 6–12 was 686 ± 14.6 units (mean ± SEM, phenylethylamine as substrate) and that found in blood of 227 randomly selected adult blood donors was 682 ± 28 units[4]. All cord blood values were less than 50 units. There were no significant differences between cord blood values of boys and girls, with DBH activity of 22.9 ± 3.9 in cord blood from boys (mean ± SEM, N = 13), and activity of 20.4 ± 2.5 in blood from girls (N = 19). DBH activity in cord blood was also determined with tyramine as a substrate to increase the sensitivity of the assay[8]. All subjects had easily detectable DBH activity when tyramine was used as a substrate (table 1). The average value of 20 units is very similar to the DBH activity in the plasma of adult rats and other experimental animals when tyramine is used as substrate[14]. Immunoreactive DBH (IDBH) was measured in 8 consecutive samples of cord blood with radioimmunoassay (table). All of the cord blood samples contained less than 100 ng/ml of IDBH. These values were also very low when compared with the mean IDBH value in blood from a randomly selected group of 134 adolescents (16–18 years old) of 824 ± 38 ng/ml (mean ± SEM)[11].

RBC COMT activity in cord blood, in contrast to DBH activity, was not greatly different from values in blood from a randomly selected population of adolescents and adults. The average RBC COMT in 577 blood samples from 315 randomly selected adolescents aged 16–18 and 262 adult blood donors was 11.98 ± 0.19 units (mean ± SEM)[5]. The value of 9.56 ± 0.62 units in 24 cord blood samples (table) does not differ greatly from this value. As was also the case in older subjects, COMT values in umbilical cord blood showed no dramatic sex differences with values in blood from boys of 8.89 ± 1.03 (mean ± SEM, N = 9) and in blood from girls of 10.02 ± 0.71 (N = 15). The range of COMT values in human umbilical cord blood was from 4.5 to 17 units, and the primary difference between RBC COMT in cord blood and in blood from adults was the absence of subjects with values as high as 25 units among infants[5].

This study demonstrates differences in the effects of growth and development on plasma DBH and erythrocyte COMT, 2 new potential biochemical measures of adrenergic neuron status and function in man. Both DBH enzymatic activity and IDBH values are much lower in cord blood than in blood from older children and adults. RBC COMT activity, however, is very similar in cord blood and blood of adults. These observations have both theoretical and clinical implications. Genetic factors play an important role in the determination of both of these enzyme activities in blood of older children and adults[4,5]. There is an allele for very low DBH activity (d) with a gene frequency of about 0.2 that is inherited as an autosomal recessive trait[4]. Approximately one third of the population is heterozygous (Dd) for this trait, and about 4% of a randomly selected population is homozygous (dd). Homozygotes have DBH enzymatic activity of less than 50 units (phenylethylamine substrate) and IDBH values of less than 100 ng/ml, both similar to values present in cord blood samples[4,11]. The biochemical basis of the increase in plasma DBH activity during growth and development in most subjects, and the relationship, if any, of this process to the biochemical basis of the action of the allele for very low plasma DBH remains to be determined. In like fashion there is an allele for very low RBC COMT (C_L) with a gene frequency of about 0.5 that is inherited as an autosomal recessive trait[5]. Approximately half of the population is heterozygous for this trait, and 20–25% of the population is made up of homozygotes, subjects with COMT activity of less than 8 units. One third of the relatively small number of cord blood samples tested had less than 8 units of COMT activity.

In practical terms, the results of these studies emphasize that measurements of serum DBH activity in children during the first years of life may be very difficult to interpret. During these years there is a dramatic change in DBH activity in the blood of most subjects. The biochemical and physiologic basis of this change and its relationship to genetically determined variations in enzyme activity are not clear. However, RBC COMT activity in cord blood is similar to that in blood of adults. Therefore, it may be possible to interpret the results of studies of RBC COMT in blood from young children much more easily than is true of studies of plasma DBH.

Plasma DBH, IDBH and RBC COMT in human umbilical cord blood

	DBH (phenylethylamine substrate) units/ml	DBH (tyramine substrate) units/ml	IDBH ng/ml	COMT units/ml packed RBC
Mean	8.3	23.0	17.7	9.56
SD	± 10.1	± 15.0	± 11.8	± 3.00
SEM	± 1.8	± 2.7	± 4.2	± 0.62
N	32	32	8	24

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