the hexose monophosphate shunt show quantitative rather than qualitative changes during thermal acclimation in the blue crab, *Callinectes spaidus*[8]. It is of interest that the muscle and hepatopancreas of the crayfish, *Cambarus bartoni*, respond differentially to thermal acclimation. Lactate dehydrogenase activity increases in muscle but decreases in the hepatopancreas following cold acclimation[9].

The changes in lactate dehydrogenase activity in *C. irroratus* larvae indicate that metabolic rate compensation is not directed at the mean temperature of the cycle (15 °C). If this were the case, enzyme activities and respiratory rates measured at 15 °C should be very similar in constant and cyclic temperature animals. Since animals cultured under a cyclic thermal regime are subjected to both a diurnal high and low temperature, adjustment may be cued to exposure to 1 particular thermal extreme. A uni-directional extension of the zone of thermal insensitivity of respiration was observed in *C. irroratus* cultured under cyclic thermal conditions[4]. The ontogeny of metabolic rate adjustment may be demonstrated by the observation that the divergence of lactate dehydrogenase activity did not occur until later in development. Lactic acid production has been demonstrated to be an important feature of metabolism in crab tissues[10]. The elevated lactate dehydrogenase activity observed in *C. irroratus* larvae cultured under cyclic temperatures indicates increased capacity for producing and dealing with lactic acid.

8 M. Robert and I. Gray, Comp. Biochem. Physiol. *42B*, 377 (1972).
9 J. D. Popham and J. N. T. Dandy, Comp. Biochem. Physiol. *53B*, 97 (1976).
10 A. K. Hoggins, Comp. Biochem. Physiol. *18*, 283 (1966).

Fractionation of mouse DNA by precipitation with F1 histone into fragments differing in their base composition[1]

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Summary. Several fractions of mouse DNA were obtained by gradual precipitation with histone F1. The analysis of their base composition revealed that histone interacted selectively with sequences of DNA rich in adenine plus thymine, regardless of the type of DNA molecules present in the DNA solution to be fractionated.

According to Šponar and Šormová[2], gradual dialysis of DNA and histone F1 solution from a high ionic strength to 0.15 M NaCl at neutral pH is accompanied by a selective interaction of histone with sequences of native DNA rich in adenine plus thymine. The effect was observed in artificial mixtures of bacterial DNAs differing in their base composition as well as in calf thymus DNA. In the latter case, a gradual accumulation of the GC-rich satellite DNA was noted in the supernatants obtained after centrifugation of the histone-DNA complexes. However, Płucienniczak et al.[3] found subsequently that DNA sequences precipitated by F1 within the range of 5–50% of the weight of the initial DNA, and presumably corresponding to the main calf thymus DNA, do not undergo fractionation despite their heterogeneity in base composition. This observation led to the assumption that main band DNA molecules of calf thymus DNA obtained by density gradient centrifugation regardless of their base composition, differ from some GC-rich fractions, possibly those of satellite DNA, in respect of the amount of specific areas to which the F1 histone attaches in the experimental conditions applied.

It was the aim of our work to investigate the course of fractionation of some other type of mammalian DNA by gradual precipitation with successive doses of F1. For this purpose the mouse DNA was chosen because the base

1 Acknowledgments. This research was supported by the Polish Government Grant No. PRBR-1317/13.
2 J. Šponar and Z. Šormová, Eur. J. Biochem. *29*, 99 (1972).
3 J. Płucienniczak, J. Bartkowiak, A. Krzywiec and H. Panusz, Biochem. biophys. Res. Commun. *56*, 799 (1974).

Precipitation of histone F1-DNA complexes in 0.15 M NaCl and base composition of obtained DNA fractions

DNA fraction	1	2	3	4	5	6
Complex I	100	0.1	0.11 ± 0.02	11.4 ± 1.2	1.76 ± 0.09	1.79 ± 0.05
Complex II	80.0 ± 2.0	0.1	0.17 ± 0.02	15.0 ± 1.5	1.60 ± 0.09	1.62 ± 0.05
Complex III	58.0 ± 2.6	0.1	0.19 ± 0.02	13.9 ± 1.0	1.46 ± 0.09	1.51 ± 0.06
Complex IV	43.3 ± 4.2	0.2	0.41 ± 0.02	24.5 ± 0.7	1.35 ± 0.07	1.39 ± 0.05
Final supernatant	21.5 ± 2.0	–	0.59 ± 0.02	35.2 ± 2.9	1.24 ± 0.08	1.24 ± 0.06
Whole DNA	–	–	–	–	1.40	1.44

The results reported in columns 1, 3, 4, 5 und 6 are mean values from 3 experiments ± SD. Column 1: concentration of DNA solution before dialysis (μg/ml); column 2: weight ratio of F1-DNA before dialysis; column 3: the fraction of DNA in the precipitated complex, calculated at each step of the fractionation course from the content of DNA in the solution before dialysis and in the corresponding supernatant; column 4: the recovery of DNA in the precipitated complex expressed as the percentage of total DNA present in the initial solution to be fractionated; column 5: composition of 4 main bases in M% expressed as A + T/G + C coefficient; column 6: relative value of adenine and guanine content, expressed as A/G coefficient, measured by the distribution of radioactivity in purine bases of DNA prelabeled with sodium-C^{14}-formate by growing the L5178Y cells in vitro in the presence of this precursor.

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.

4 W. G. Yasmineh and J. J. Yunis, Exp. Cell Res. *59*, 69 (1970).
5 J. Marmur, J. molec. Biol. *3*, 208 (1961).
6 E. W. Johns, Biochem. J. *92*, 55 (1964).
7 J. Malec, M. Wojnarowska and L. Kornacka, Acta bioch. pol. *21*, 291 (1974).

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