3 Zeller, E.A., in: The Enzymes, vol. 8, p. 314. Eds P.D. Boyer, H. Lardy and K. Myrbäck. Academic Press, New York and London 1963.
4 Blaschko, G., in: The Enzymes, vol. 8, p. 337. Eds P.D. Boyer, H. Lardy and K. Myrbäck. Academic Press, New York and London 1963.
5 Buffoni, F., Pharmac. Rev. *18* (1966) 1163.
6 Rinaldi, A., Floris, G., and Finazzi-Agrò, A., Eur. J. Biochem. *127* (1982) 417.
7 Mondovì, B., and Finazzi Agrò, A., in: Structure and Function Relationships in Biochemical Systems, p. 141. Eds F. Bossa, E. Chiancone, A. Finazzi-Agrò and R. Strom. Plenum Press, New York 1982.
8 Oratore, A., Guerrieri, P., Ballini, A., Mondovì, B., and Finazzi-Agrò, A., FEBS Lett. *104* (1979) 154.
9 Oratore, A., Banchelli, G., Buffoni, F., Sabatini, S., Mondovì, B., and Finazzi-Agrò, A., Biochem. biophys. Res. Commun. *98* (1981) 1002.
10 Goa, J., Scand. J. clin. Lab. Invest. *5* (1953) 218.
11 Okuyama, T., and Kobayashi, Y., Archs Biochem. Biophys. *95* (1961) 242.
12 Rinaldi, A., Vecchini, P., and Floris, G., Prep. Biochem. *12* (1982) 11.
13 Ouchterlony, O., Acta path. microbiol. scand. *26* (1956) 507.
14 Andersson, A.C., Henningsson, S., and Rosengren, E., Agents Actions *9* (1979) 44.
15 Sessa, A., Desiderio, M.A., Bairini, M., and Perin, A., Cancer Res. *41* (1981) 1929.
16 Thomasset, N., Quash, G., and Doré, J.F., Br. J. Cancer *46* (1982) 58.
17 Baylin, B.S., Mendelsohn, G., Weisburger, W.R., Gann, D.S., and Eggleston, J.C., Cancer *44* (1979) 1315.
18 Mondovì, B., Riccio, P., Riccio, A., and Marcozzi, G.S., in: Advances in Polyamine Research, vol. 4, p. 183. Eds U. Bachrach, A. Kaye and R. Chayen. Raven Press, New York 1983.
19 Bardsley, W.G., Ashford, J.S., and Hill, C.M., Biochem. J. *122* (1971) 557.
20 Mondovì, B., Rotilio, G., Costa, M.T., and Finazzi-Agrò, A., Meth. Enzym. *17* (1971) 735.
21 Kluetz, M.D., and Schmidt, P.G., Biochemistry *16* (1977) 5191.

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Isoelectrically focused carboxyesterases as a biological marker in chimeras

M. Grim, B. Christ, H.J. Jacob, J. Kulich and J. Pařízek

Institute of Anatomy, Charles University, 12800 Praha 2 (Czechoslovakia), Institute of Anatomy, Ruhr-University, D-4630 Bochum (Federal Republic of Germany), and Department of Anatomy, Charles University, Hradec Králové (Czechoslovakia), 7 October 1983

Summary. Species-specific multiple forms of carboxyesterases (CE) were determined in zymograms obtained by isoelectric focusing (IEF) using homogenized wing zeugopodal tissues of chick, quail and quail-chick chimeras. The validity of the CE pattern of chimeric tissues was verified by the nuclear marker technique. Analytical IEF of CE was found to be useful for investigation of the origin of tissues in chimeras.
Key words. Chimeras, quail-chick; multiple enzyme forms, carboxyesterases, nuclear marker technique; zymograms.

The quail-chick marker technique[1] represents an important tool in experimental embryology. Since the interphase nuclei of Japanese quail cells are characterized by a large mass of perinucleolar heterochromatic DNA which does not exist in chick nuclei, it is possible to use quail cells as stable histological markers in interspecific grafting experiments. By the use of this technique various systems of migrating cells have successfully been analyzed in chimeric embryos; for example the migration of limb muscle precursor cells[2]. The employment of such a technique is limited to those species which exhibit distinctly different patterns of heterochromatin.

In order to solve some problems of morphogenesis it may be necessary to produce chimeras of species whose cells cannot be distinguished by nuclear markers. The analysis of such chimeras would require the application of non-histological techniques which could also help to elucidate biochemical aspects of developmental processes. It has been shown that the cellular genotypes in allophenic mice can be distinguished, for example, on the basis of electrophoretic variants of the enzyme glucosephosphate isomerase[3,4]. To achieve a more general employment of such a method one has to look for enzymes which occur ubiquitously and which are characterized by distinct species-specific features. It is well known that the number of multiple forms of carboxyesterases (CE) varies greatly between different species, even when they are phylogenetically closely related[5].

The aim of this study is a) to look for the differences of CE pattern of chick and quail tissues; b) to analyze the origin of tissues within chimeras using the species-specific patterns of CE and to prove the validity of this method. Different molecular forms of CE were separated by isoelectric focusing (IEF) of homogenized chick, quail and chimeric wing muscles. Zymograms were obtained by subsequent staining for CE. The validity of this method was tested using the quail-chick marker technique on sections from parallel tissue samples.

Material and methods. White Leghorn (Velaz, Praha) and Japanese quail (own hatchery) were used throughout the investigations. Eggs were incubated at 38 ± 1 °C. In order to obtain chimeric wings unilateral replacements of chick brachial somites by those of quail were performed. Two-day-old embryos at stages 13 and 14 according to Hamburger and Hamilton[6] were used. The details of experimental procedures and their results have been described previously[2]. The chimeric wing muscle contains muscle cells of quail origin and connective tissue of chick origin. Such wings represent a well-defined model where cells from two different species are intermingled. Embryos were sacrificed 13–17 days after somite replacement. Altogether 15 chimeras were evaluated. Muscles of the wing stylopodes were removed and fixed in Serra's fluid, embedded in paraplast, sectioned, treated with the Feulgen-reaction[7] and post-stained with light green. Zeugopodal muscles containing muscle fibers, connective tissue, vessels and nerves were processed for detection of CE by zymograms. Corresponding muscles of the contralateral wing were used as controls. Muscle samples ranging from 20 mg to 50 mg were homogenized and diluted with distilled water containing 1% Triton X-100

(Serva, Heidelberg, FRG) to obtain 5% or 10% homogenates (wet weight/volume). Tritonized samples were frozen and thawed 3 times to achieve a better solubilization of structure-bound enzymes. After centrifugation of homogenates (10,000 g 4 °C, 5 min) 25 µl of supernatants were applied by means of application strips on Ampholine PAG plates pH range 3.5–9.5 (LKB, Bromma, Sweden) according to the instructions of the producer. Total content of proteins evaluated by the biuret-reaction[8] ranged from 2.1 to 2.9 mg/ml supernatants of 10% homogenates. IEF of samples was performed using an LKB 2117 Mulitphor apparatus with CD power supply 3371 E, and lasted 90 min after previous preparation of pH-gradient. Carboxyesterases were visualized using the histochemical method as described by Lojda et al.[9]. As a substrate α-naphthylacetate (20 mg in 0.5 ml acetone) diluted with 50 ml 0.1 M phosphate buffer (pH 7.4) containing 25 mg Fast Red TR salt was used. The plates were incubated for 30 min (20 °C). Afterwards zymograms were washed in water, treated and stored as described previously[10]. By this means 24 samples were focused and stained simultaneously so that the results were exactly comparable. Densitometric evaluations of zymograms were performed by Quick Scan II (Helena Laboratories, Beaumont, Tx, USA). In addition, various other wing tissues of chick and quail at different developmental stages were used for detection of CE.

Results. After IEF staining with α-naphthylacetate, zymograms of normal wing zeugopodal musculature homogenates of quail and chick embryos at 17th day of incubation exhibit several distinct and deeply stained bands, representing high activities of CE focusing in the range from pH 4.8 to pH 5.6, while weakly stained bands representing low activities are found within the remaining ranges on the plates. All the bands correspond to multiple forms of CE representing its various physico-chemical species[11]. The number, the position and the activities of individual bands form patterns different for chick and quail wing zeugopodal muscles.

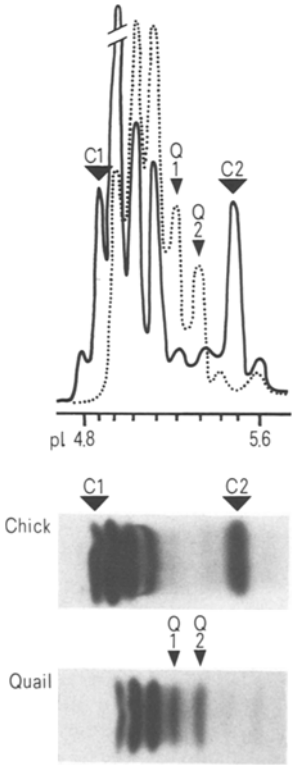

Figure 1. CE zymograms and densitograms of chick and quail zeugopodal wing muscles (5% homogenate): chick ——, quail ·····. Distinct species-specific bands and peaks of chick (C1, C2) and quail (Q1, Q2); pI, isoelectric point.

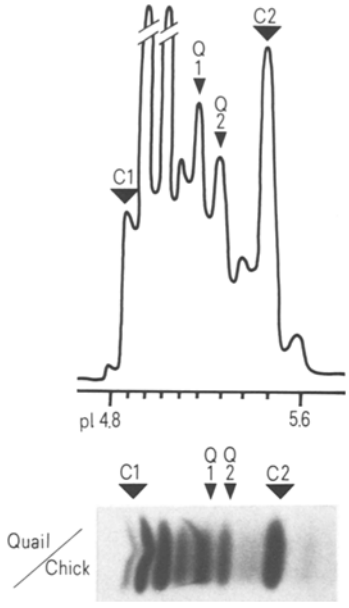

Figure 2. CE zymogram and densitogram of quail-chick chimeric muscles (10% homogenate) of the wing zeugopod. Species-specific bands and peaks of chick (C1, C2) and quail (Q1, Q2).

As shown in figure 1, 2 distinct bands with high activity (C1 and C2) are present in the zymograms obtained from chick wing zeugopodal muscles, and 2 other distinct bands (Q1 and Q2) are found to be characteristic of quail samples. The remaining bands partly common to both species seem to be less useful as species-specific markers.

The species-characteristic CE bands (C1 and C2, Q1 and Q2) are found not only in muscles, but in the other components of the wing, like skin, adipose tissue, blood, peripheral nerves and blood vessels where they show different distinctiveness. So, the band C2 is weaker in muscles than in the other wing tissues. On the other hand Q1 is more distinctive in muscles and blood than in the remaining wing components. In general, C2 and Q2 are found to be the outstanding CE bands which already appear in early wing buds.

In zymograms obtained from chimeric wing muscles, all chick and quail characteristic bands of CE are expressed (fig. 2). The histological quail-chick marker technique makes it possible to determine the interspecific composition of wing muscles. Typical quail nuclei are found within muscle fibers, whereas the other tissues consist of chick cells (fig. 3). In those chimeric muscles in which the fibers contain quail nuclei as well as chick nuclei the corresponding zymograms show weakly stained quail characteristic bands.

Discussion. Carboxyesterases were chosen for this study as they are ubiquitously present enzymes occurring in numerous multiple forms which vary greatly in different species[5,12]. After analytical IEF of chick and quail material, performed on Ampholine PAG plates, and subsequent histochemical staining, multiple forms of CE can be seen as species-specific band patterns. For each species at least 2 distinct bands are found to be characteristic. IEF zymograms obtained from wing muscles of quail-chick chimeras show both the chick characteristic bands and the quail characteristic ones. In accordance with this finding the histological examinations reveal the interspecific composition of parallel tissue samples. The quantitative distribution of chick and quail cells was found to be in good correspondence with the distinctiveness of the species-

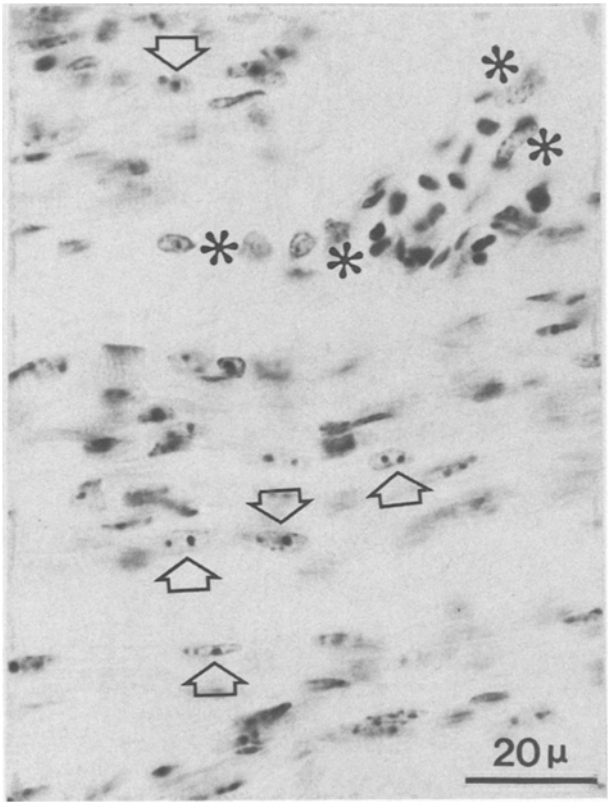

Figure 3. Feulgen-stained section of a chimeric wing muscle corresponding to that demonstrated in figure 2. Arrows: numerous quail nuclei of muscle fibers; asterisks; connective tissue cells of chick origin.

specific bands. However, quantitative evaluations of CE zymograms obtained from chimeric material are restricted to identical kinds of tissue because of the tissue specific expression of CE-activities. For chick and quail embryos used throughout these investigations, it must be mentioned that the activity of species-specific CE appears in the undifferentiated wing bud and increases during histogenesis of wing tissues. The results show that the quail cells do not change their species-specific CE pattern when developing in a host environment. Considering that the species-specific bands of CE were expressed in different structures of chick and quail embryos (skin, adipose tissue, connective tissue, blood, peripheral nerves and blood vessels) this method may be applied to other chimeric organ systems. It has to be mentioned that in samples taken from various tissues the quail typical band Q1 and the chick typical band C1 appear with different distinctiveness, whereas the bands Q2 and C2 were found to be very distinctive markings. Because of its high resolution ability, which is superior to that obtained by other separation methods[11], and because it is quick and easy to employ, the application of IEF of CE and probably other enzymes, too, was found to be a useful method for analyzing chimeric tissue. Since the numerous multiple forms of CE vary greatly in different species[5,12] it may be possible to analyze other chimeras also using this method. It can be employed with a small amount of material or with cryostat sections, giving the possibility of analyzing the histotopography of enzymes (unpublished data by J. Kulich).

1 Le Douarin, N., and Barq, G., C.r. hebd. Séanc. Acad. Sci., Paris *D 269* (1969) 1543.
2 Christ, B., Jacob, H.J., and Jacob, M., Anat. Embryol. *150* (1977) 171.
3 Carter, N.D., and Yoshida, A., Biochim. biophys. Acta *181* (1969) 468.
4 Gearhart, J.D., and Mintz, B., Devl Biol. *29* (1972) 27.
5 Coutinho, H.B., Padilha, M.C.S., Gomes, J.M., and Alves, J.J.A., J. Histochem. Cytochem. *13* (1965) 339.
6 Hamburger, V., and Hamilton, H.L., J. Morph. *88* (1951) 49.
7 Feulgen, R., and Rossenbeck, H., Hoppe-Seyler's Z. physiol. Chem. *135* (1924) 203.
8 Cleland, C.W., and Flater, E.C., Biochem. J. *53* (1953) 547.
9 Lojda, Z., Gossrau, R., and Schiebler, T.H., Enzymhistochemische Methoden. Springer, Berlin-Heiderlberg-New York 1976.
10 Lojda, Z., and Kulich, J., Histochemistry *73* (1981) 311.
11 Radola, B.J., in: Isoelectric Focusing, p.119. Ed. N. Catsimpoolas. Academic Press, New York-San Francisco-London 1976.
12 Paul, J., and Fottrel, P.F., Ann. NY. Acad. Sci. *94* (1961) 668.

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Glutamine homeostasis: Role of pCO_2 in regulating arterial glutamine in metabolic acidosis[1]

V. Phromphetcharat and T.C. Welbourne

Department of Physiology and Biophysics, Louisiana State University Medical Center in Shreveport, Shreveport (Louisiana 71130, USA), 28 September 1983

Summary. In chronic metabolic acidosis arterial plasma glutamine concentration is reduced 46%. This effect is attributable to a reduction in arterial pCO_2 rather than acidemia since elevating pCO_2 returns glutamine toward the control level. The extracellular glutamine precursors ammonia and glutamate exhibit a reciprocal decline with the elevation in pCO_2. This reduction in metabolic acidosis appears to play an important role in sparing glutamine for renal base generation.
Key words. Acidosis, chronic metabolic; glutamine, plasma; pCO_2; glutamine homeostasis.

Chronic metabolic acidosis results in reduced plasma glutamine concentration in man[2,3], sheep[4] and rat[5] but not in dog[6]. A reduction in plasma glutamine has been proposed as a means of coordinating interorgan glutamine conversions to provide ammonia nitrogen for base generation in metabolic acidosis[7]. A previous study in humans suggested that acid-base induced alterations in plasma glutamine were under the influence of pCO_2 rather than either hydrogen or bicarbonate ion concentration[8]. The present study was undertaken to demonstrate this relationship in the arterial blood of rats as well as to note effects on extracellular ammonia and glutamate, glutamine precursors. The results to follow show a rise in plasma glutamine of acidotic and nonacidotic rats in response to an acute elevation of pCO_2 and a reciprocal fall in glutamate.

Methods. Experiments were performed on male Sprague Dawley rats weighing 400–450 g. Chronic metabolic acidosis was induced by maintaining the animal on 210 mM NH_4 Cl drinking solution for at least 1 week. Experiments were performed

Table 1. The effect of pCO_2 on plasma glutamine in metabolic acidosis

	[Gln] nmol/ml	pCO_2 mmHg	pH U	$[HCO_3^-]$ mM	$[NH_4^+]$ nmol/ml	[Glu] nmol/ml
Control	234[a] ± 50	34 ± 2	7.37 ± 0.01	20.0 ± 1.2	98 ± 6	115 ± 22
CO_2	375[b] ± 17	67[b] ± 9	7.14 ± 0.06	21.3 ± 2.0	82[b] ± 6	58[b] ± 12
Room air	271 ± 23	37 ± 3	7.39 ± 0.02	24.4 ± 3.0	91 ± 9	91 ± 12

[a] Results are means ± SEM from 6 rats; [b] Significantly different from control period, $p < 0.05$.

Table 2. The effect of pCO_2 on plasma glutamine in nonacidotic rats

	[Gln] nmol/ml^{-1}	pCO_2 mmHg	pH U	$[HCO_3^-]$ mM	$[NH_4^+]$ nmol/ml	[Glu] nmol/ml
Control	508[a] ± 33	41 ± 3	7.40 ± 0.02	27.4 ± 1.5	88 ± 3	116 ± 7
CO_2	621[b] ± 48	86[b] ± 10	7.10[b] ± 0.05	27.2 ± 2.0	103[b] ± 9	59[b] ± 9
Room air	527 ± 55	38 ± 4	7.38 ± 0.02	23.2 ± 2.9	122 ± 21	124 ± 22

under inactin anesthesia, 80 mg/kg, from 10.00 to 12.00 h. The animals were placed on a heated animal board and their body temperature maintained at 37 °C throughout the experiment. Cannulas, PE260 and PE50, were placed in the trachea and femoral artery followed by 30 min of spontaneous respiration after which 0.5 ml of arterial blood was drawn into a heparinized ice chilled syringe. The tracheal cannula was then connected to a 5 liter bag containing 5% CO_2:95% O_2 and a second 0.5 ml blood sample drawn after 30 min. A consecutive 30-min-period of respiring room air was followed by a third sample. Drawn blood was immediately analyzed for pH, pCO_2[8] and ammonia[7]; the sample was then centrifuged, 10,000 × g at 4 °C for 10 min, and plasma aliquots immediately analyzed for glutamine and glutamate[7].

Results. The effect of chronic NH_4Cl ingestion on acid base parameters and arterial plasma glutamine can be seen in comparing table 1 and 2. NH_4Cl ingestion produces a metabolic acidosis, $[HCO_3^-]$ 27.4 ± 1.5 versus 20.2 ± 1.2 mM compensated by a reduction in pCO_2, 41 ± 3 versus 34 ± 2 mmHg. Plasma glutamine concentration in acidosis was reduced from 508 ± 33 to 234 ± 50 nmol/ml, $p < 0.025$, similar to previous reports[2–5]; ammonia and glutamate concentration were not significantly different. After 30 min of 5% CO_2, blood pCO_2 increased with a rise in plasma glutamine, 234 ± 50 to 375 + 17 nmol/ml, $p < 0.025$ (table 1); elevating pCO_2 in the nonacidotic group also increased plasma glutamine, 508 ± 33 to 621 ± 48 nmol/ml^{-1}, $p < 0.025$. In agreement with the previous study in man[8] the factor influencing glutamine is the pCO_2 rather than H^+ or HCO_3 concentration since glutamine levels fall in chronic metabolic acidosis and rise in acute respiratory acidosis; plasma HCO_3-concentration was not significantly altered in these acute studies. Plasma glutamine precursors ammonia and glutamate both declined in the acidotic group as the glutamine rose (table 1); although the relationship was reciprocal the amounts are not stoichiometric. In nonacidotic animals (table 2), glutamate declines with the rise in plasma glutamine but ammonia does not. After 30 min of room air the blood pCO_2 has fallen to control levels and plasma glutamine declines, in both nonacidotic and acidotic groups (tables 1 and 2) with a reciprocal rise in blood ammonia and glutamate.

Discussion. Plasma glutamine concentration can be acutely modulated by raising or lowering the prevailing pCO_2. Such a mechanism may explain, at least in part, the reduction in plasma glutamine during chronic metabolic acidosis in man[2,3], rat[5] and sheep[4]. The significance of glutamine concentration set at a lower level may be related to the relative concentration dependence of extrarenal glutamine utilization[7,10] in contrast to the kidney[11]. This in turn may explain the unchanged glutamine turnover during chronic acidosis in the latter 2 species[4,12] and presumably in man; however, acidotic humans given a standard oral glutamine load exhibit plasma concentrations half those of control nonacidotic individuals[3] suggesting a greater rate of hepatic utilization in the former[3]. Whether extrarenal glutamine utilization is enhanced in metabolic acidosis but not expressed because of reduced substrate availability remains to be demonstrated. In dog, one might hazard the prediction that glutamine turnover is accelerated in view of the maintained plasma concentration and increased extrarenal utilization[13].

Other factors contributing to rates of glutamine production or utilization also affect plasma glutamine levels. Glucocorticoids whose levels increase in metabolic acidosis act to increase plasma levels[14] presumably through their nitrogen mobilizing effect. Ammonia infusion at rates comparable to renal venous release also increase glutamine levels[7,15]; larger doses actually reduce glutamine levels apparently by activation of hepatic glutamine uptake[9]. Consequently, these as well as other factors may act over the long term during acidosis to modify glutamine production and hence plasma levels. Although our studies clearly demonstrate an acute modulation of plasma glutamine by pCO_2 it is not apparent which site or sites of glutamine production/utilization are affected. The fall in extracellular glutamate and rise in glutamine suggests increased synthesis although subtle changes in glutamine cycling involving intracellular, intercellular as well as interorgan sites would be obviously complicated. Nevertheless, the large increments in extracellular glutamine in response to an elevation in pCO_2 may allow detection of the site(s) responsible under in vivo conditions.

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2 Owen, E.E., and Robinson, R., J. clin. Invest. *42* (1963) 263.
3 Welbourne, T.C., Weber, and Bank, N., J. clin. Invest. *51* (1972) 1952.
4 Heitmann, R.N., and Bergman, E.N., Am. J. Physiol. *234* (1978) E197.
5 Squire, E.J., Hall, D.E., and Brosnan, J.T., Biochem. J. *160* (1976) 125.
6 Pitts, R.F., Pilkington, L.A., MacLeod, M.D., and Leal-Pinto, E., J. clin. Invest. *51* (1972) 557.
7 Phromphetcharat, V., Jackson, A., Dass, P.D., and Welbourne, T.C., Kidney International *20* (1981) 598.

8 Welbourne, T.C., Eur. J. appl. Physiol. *45* (1980) 185.
9 Häussinger, D., and Seis, H., Eur. J. Biochem. *101* (1979) 179.
10 Windmueller, H.G., and Spaeth, A.E., J. biol. Chem. *249* (1974) 5070.
11 Welbourne, T.C., Am. J. Physiol. *226* (1974) 549.
12 Squires, E.J., and Brosnan, T.J., Biochem. J. *210* (1983) 277.
13 Addae, S.K., and Lotspeich, W.D., Am. J. Physiol. *215* (1968) 269.
14 Sapir, D.G., Pozefsky, T., Knochell, J.P., and Walser, M., Clin. Sci. molec Med. *53* (1977) 215.
15 Welbourne, T.C., Un. méd. Can. *102* (1973) 1451.

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Pubertal changes in the medio-basal hypothalamic area after neonatal suprachiasmatic nucleus lesions in the rat

E. Marani, W.J. Rietveld and M. Kooij

Laboratory of Anatomy and Embryology, and Department of Physiology, Leiden University, NL-2333 AL Leiden (The Netherlands), 7 June 1983

Summary. In order to get more insight into the mechanism by which the onset of puberty is controlled, a developmental study on the displacement of catalase- and dopamine-containing cells in the hypothalamic region was done in rats which received a neonatal lesion of the suprachiasmatic nucleus. The displacement of cells is delayed after these lesions. However, the time lost at the beginning of the displacement is made up at the end of the migration.
Key words. Rat hapothalamus, suprachiasmatic nucleus, neonatal lesion; pubertal changes; catalase-containing cells; dopamine-containing cells; cell displacement.

The suprachiasmatic nucleus (SCN) is a small, paired hypothalamic nucleus which is close to the midline just above the optic chiasm. It is held responsible, at least in mammals, for the generation of a number of circadian rhythms (1 for a review). The morphology and the three-dimensional extension[2] of the SCN of rats has been extensively studied. Besides its role in the control of behavioral circadian rhythms, the SCN also plays an important part in the control of endocrine rhythms. Within the SCN neurons are found that contain vasopressin, neurophysin, melatonin and LH-RH. Therefore a correlation is proposed between the SCN and the reproductive cycle[1,3-5]. Destruction of the SCN indeed leads to a loss of the estrous cycle[6].

The SCN is known to have various connections. Among these is a direct connection from the SCN to the pituitary stalk nuclei, the arcuate nucleus and the median eminence[7]. However, studies using retrograde tracers failed to demonstrate the connection from the SCN to the median eminence[8].

Histochemical studies for catalase revealed a critical period in the development of the ventrobasal hypothalamus and the pituitary stalk area around puberty, when groups of cells undergo a migration[9-13]. In adult animals the catalase activity fluctuates according to the estrous cycle, showing maximum activity at the time of the pre-estrous[14-16]. A similar shift has been found for catecholaminergic fluorescent cells, which populate the median eminence, the intermediate area and then the arcuate nucleus[17,18] following the same time sequence.

The catalase activity is related to the onset of the production of LH-RH[12,19]; the importance of the catecholaminergic cells for the function of the tubero-infundibular system in reproduction[20] has also been demonstrated. The SCN-preoptic area subserves at least the dynamic control of the estrous cycle, and as one may assume that an efferent connection exists from the SCN to the arcuate-median eminence system, we have investigated whether prepubertal ablation of the SCN affects the development of this hypothalamic system.

Material and methods. All rats used in this study (Wistar-WU, SPF, from TNO, Delft, the Netherlands) were housed under standard conditions (22 ± 1 °C, rel. hum. 65 ± 3%, light on 08.30–20.30 h) and fed ad libitum. Male newborn rats (2 days old) from several litters were stereotaxically lesioned in the SCN nucleus. Stereotaxic coordinates for the SCN were adapted from earlier data[21]: 0.9 AP, 0.6 V, 0.1 L. Coagulation was effected with a 0.1-mm needle, isolated except at its tip. A current of 0.8 mA for 5 sec was used in each nucleus. The best results were obtained by placing young males of 8–10 g in a plastic jig in a David Kopf holder and making them hypothermic with ice. All lesioned animals survived the electrocoagulation treatment and were reaccepted in the litters and by the mother. 2 animals were sacrificed at 2-day intervals from day 3 till day 55 after birth. The animals were decapitated under

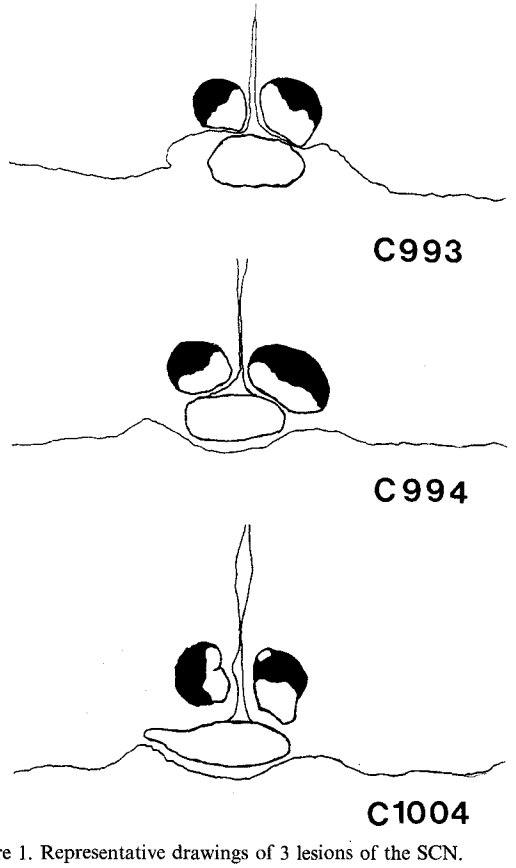

Figure 1. Representative drawings of 3 lesions of the SCN.