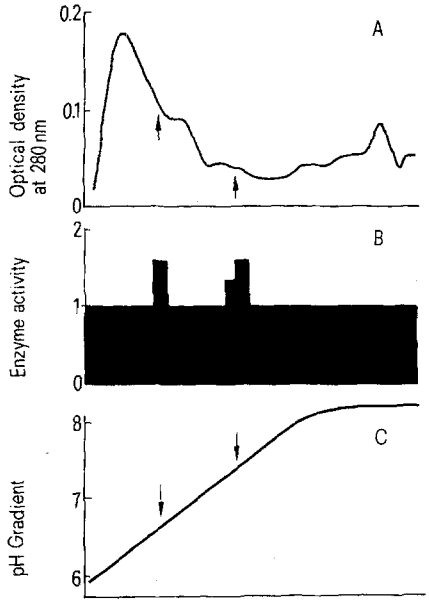

Carbonic anhydrase activities after gel electrofocussing. *A* After the electrofocussing the gel was scanned at 280 nm using an ISCO Gel Scanner, Model 659, attached to Model UA-5 Absorbance Monitor (Instrumentation Specialities Company, USA). *B* The gel was sliced into sections of 3 mm and they were immersed in 4.0 ml of 0.025 M Tris-H_2SO_4 buffer, pH 8.2. After shaking the enzyme activity was determined. Carrier ampholytes did not affect the enzyme activity in the concentration used in the present experiment. The enzyme activity was defined as the reciprocal of the ratio of the hydration rate of CO_2 to the nonenzymatic hydration rate of CO_2. The hydration rate was determined with Model F-5ss pH Meter (Horiba, Japan). *C* The 3rd gel was also sliced into sections of 3 mm and immersed in 1.0 ml of distilled water and the pH was determined with a Model HM-5A pH Meter (Toa Electronics, Japan).

performed according to the method described by Wrigley[3]. 3 columns were run simultaneously; on to one the ciliary body homogenate was loaded, on to another red cell hemolysate was loaded, and on to the 3rd sucrose solution was loaded. The 3rd column was used to check the pH gradient. Enzyme activity was determined according to Carter et al.[4].

Results and discussion. It has been a question for a long time whether the ciliary body contains CA I, CA II or both, since Wistrand and Rao[5] failed to produce a convincing result in their immunological study. The present experimental observation appears in the figure. 2 carbonic anhydrase activities were found in the pH gradient acrylamide gel. Their isoelectric point was determined to be pH 6.6 and pH 7.4, respectively. (The values for human erythrocyte CA I and CA II were reported to be pH 6.57 and pH 7.36[6]). Both activities were completely inhibited by 5×10^{-5} M acetazolamide. The same result was also obtained in rabbit red cell hemolysate. Consequently, the authors conclude that the ciliary body contains both isozymes. The total activity of the higher isoelectric point isozyme was higher than that of the lower isoelectric point isozyme.

1 T.H. Maren, in: Oxygen Affinity of Hemoglobin and Red Cell Acid Base Status, p.418, Ed. H. Røth and P. Astrup. Academic Press, New York 1972.
2 M.J. Carter, Biol. Rev. *47*, 465 (1972).
3 C. Wrigley, Sci. Tools *15*, 17 (1968).
4 M.J. Carter, D.J. Havard and D.S. Parsons, J. Physiol. *204*, 60P (1969).
5 P.J. Wistrand and S.N. Rao, Biochim. biophys. Acta *154*, 130 (1968).
6 S. Funakoshi and H.F. Deutsch, J. biol. Chem. *244*, 3438 (1969).

Enzymic 'imprinting' as the result of early postnatal administration of enzyme inducers to animals

R.I. Salganik, I.M. Gryaznova, A.L. Markel, N.M. Manankova and N.A. Solovyeva

Institute of Cytology and Genetics of the Siberian Branch of the USSR Academy of Sciences, Novosibirsk 630090 (USSR), 29 March 1979

Summary. Early postnatal treatment of rats or mice with different enzyme inducers (cortisol, galactose, steroid which induces mixed function oxidases) results in long-lasting changes in the activity of corresponding inducible enzymes.

A number of hormones, nutrients and xenobiotics act as inducers of adaptive enzymes which mediate the effects of hormones, or metabolize nutrients and xenobiotics[1-9]. Induction is provided by DNA-dependent synthesis of RNA molecules which control, in turn, the synthesis of the respective enzymes. Thus, glucocorticoids stimulate the synthesis of the key gluconeogenic enzymes in rat liver[1]; gastrin (pentagastrin) induces histidine decarboxylase in rat gastric mucosa[2,3]; administration of galactose leads to induction of rat liver enzymes converting galactose into glucose[4]; amino acids are responsible for the induction of enzymes of the urea cycle[5]; certain xenobiotics induce microsomal enzymes in liver, ensuring the elimination of these foreign compounds[6]. A series of experimental papers on this subject has been published and recent reviews have been concerned with the patterns of enzyme induction in animals[6-9].

It has been shown earlier in this laboratory that long-term treatment of adult rats with cortisol[10], insulin[11], pentagastrin[12] or galactose[13], after an induction of adaptive enzymes, produces a stable refractoriness to the administered inducers. In young animals, unresponsiveness to an inducer develops more readily and is retained longer than in adults. It was suggested that the administration of an inducer in the early postnatal period would have more pronounced and continuous after effects.

To verify this suggestion, rats and mice were treated with inducers in the early postnatal period, and the activities of the inducible enzymes were assayed when they were adults.

Materials and methods. Cortisol in doses of 50 g in 0.1 ml of saline per animal was injected daily i.p. to Wistar rats from days 1 to 16 after birth. Rats of the same strain were injected i.p. with galactose (1 mg in 0.1 ml of saline per g b.wt daily) from days 1 to 14 of postnatal life. Neonatal mice of the hypercholesterolemic SWR/y strain were treated with 16 α-isothiocyanopregnenalone-3-acetate (IPA), a potent inducer of microsomal mixed function oxidases[14]. Each animal received orally 0.2 mg of IPA from days 2 to 8

Table 1. Activities of tyrosine aminotransferase (TAT) in the liver of adult rats after neonatal treatment with cortisol

	2-month-old rats	2-month-old rats 5 h after cortisol administration	4-month-old rats	4-month-old rats 5 h after cortisol administration
Untreated rats	6.8±0.58(7)	24.7±2.35(7)	6.3±0.12(5)	20.5±0.38(10)
Control	5.8±0.25(4)	18.8±1.7(4)	9.3±1.17(7)	22.0±0.3(10)
Neonatally cortisol-treated rats	10.6±0.55(7)	17.0±1.86(7)	14.0±1.21(5)*	15.8±0.24(10)**

Wistar rats of the experimental group were injected i.p. with cortisol (50 µg dissolved in 0.1 ml of saline; daily) from days 1 to 16 after birth; control rats received i.p. saline (0.1 ml, daily). Untreated rats served as controls of rats stressed by neonatal injections of saline. The activity of TAT was expressed in µmoles of p-hydroxyphenylpyruvate formed per h per mg of protein[16]. The number in parentheses represents the numbers of rats used. Values given as mean ± SEM. * The differences are significantly different from controls by Student's t-test ($p = 0.01$). ** The differences are not statistically different from 4-month-old rats, neonatally treated with cortisol.

Table 2. Activities of arylhydrocarbon hydroxylase (AHH) in the liver of adult SWR/y mice treated neonatally with IPA, an inducer of mixed function oxidases

	AHH activities in the liver of rats 1-month-old	2-month-old	4-month-old	8-month-old
Control	1.76±0.06(7)	2.21±0.43(8)	2.64±0.13(10)	4.98±0.63(11)
Neonatally IPA-treated rats	2.98±0.08(7)*	3.80±0.09(8)*	3.31±0.03(10)*	10.26±0.99(12)*

IPA was given daily per os: 0.2 mg IPA from days 2 to 8 and 0.4 mg IPA from days 9 to 15; IPA was dissolved in 0.1 ml of distilled water with Tween 80 (1:1000). Control mice received distilled water (0.1 ml) with Tween 80 (1:1000) per os. The activity of AHH is expressed in nmoles of 3-hydroxy-^{3}H-benz(a)pyren formed per mg of protein per 20 min. Values given as mean ± SEM. * The differences between control and neonatally treated mice are statistically significant ($p < 0.001$) by Student's t-test.

after birth and 0.4 mg of the compound from days 9 to 15; IPA was dissolved in 0.1 ml of distilled water with Tween 80 (1:1000). The activity of tyrosine aminotransferase (TAT) in the liver of adult rats which had been postnatally treated with cortisol was measured by the method of Diamondstone[15], the activities of galactose-1-phosphate uridyltransferase (Gal-1-PUT) and glucose-6-phosphate dehydrogenase (G-6-PD) were estimated according to Beutler and Baluda[16] and Kornberg and Horecker[17], respectively; the activity of arylhydrocarbon hydroxylase (AHH) in the liver of adult rats pretreated postnatally with IPA was determined as outlined by Nebert and Gelboin[18].

Results and discussion. It is well known that cortisol, along with other gluconeogenic enzymes, induces TAT in rat liver[19]. The salient finding of our experiments was that cortisol, when given postnatally to rats for 2 weeks, results in a 2-fold rise in TAT activity in adults (table 1). When cortisol was injected into adult rats (4 months old) the TAT activities in the liver increased more than 3-fold 5 h after the injection. When it was given to adults which had been postnatally treated with the hormone, cortisol failed to induce TAT (table 1). Postnatal treatment of SWR/y mice with the inducer of microsomal enzymes, IPA, caused a remarkably stable rise in AHH activity during a large span of adult life (table 2).

Postnatal injections of galactose in newborn rats had a marked effect on the activities of G-6-PD and Gal-1-PUT. In these experiments the activity of G-6-PD in adult rats (7–10 month old) was consistently higher than in controls; concomitantly, there was a decrease in the activity of Gal-1-PUT (table 3).

Evidence for similar effects of putative inducers is scanty. It has been reported that tryptophan, when injected into newborn rats, suppresses the activity of tryptophan pyrrolase in the livers of 18-day-old rats[20].

The genetic apparatus of animals during the early postnatal period seems to show specific response patterns to enzyme inducers. A prolonged effect of an inducer appears to be of significance not only for cell differentiation during development, but perhaps also for adaptation to novel long-lasting conditions in the environment.

Thus, it has been demonstrated recently in this laboratory that repeated short-term exposure of rats to cold for 14 days after birth produces a sharp and stable increase in the content of corticosterone, insulin and triiodothyronine in the blood of adult animals[21]. These hormonal shifts may have an adaptive role. It seems quite plausible that they are also based on stable changes in the activities of certain enzymes responsible for the synthesis and metabolism of these hormones. Taken together, these phenomena are reminiscent of immunological tolerance, an unresponsiveness induced by contact with an antigen in foetal or early postnatal life[22]. Likewise, visual stimuli at an early critical period elicit long-term imprinting of the objects of instinctive behavioural patterns[23]. This resemblance does not seem to be superficial. Antibodies are produced under the effect of an antigen-challenged DNA-dependent synthesis of RNA[24], and behavioural imprinting is the result of induced RNA synthesis in brain cells[25].

Inducers of differentiation during early development provide a stable expression of appropriate genes and thereby direct the specialization of cells. The stable changes in the activities of enzymes caused by genetic inducers at an early postnatal period, resemble the patterns of cell differentiation.

There may be no basic differences in the nature of these 2 phenomena. Being different, the postnatal stimuli, obvious-

Table 3. Activities of galactose-1-phosphate uridyltransferase (Gal-1-PUT) and glucose-6-phosphate dehydrogenase (G-6-PD) in the liver of adult (7–10 months old) rats after neonatal treatment with galactose

	Gal-1-PUT activities	G-6-PD activities
Control	71.1±8.1(8)	8.4±0.6(8)
Neonatally galactose-treated rats	41.6±8.6(8)*	11.1±0.8(8)*

Control rats received saline (0.1 ml. daily) i.p.; the experimental group was treated with galactose (1 mg dissolved in 0.1 ml of saline, daily, i.p.). Galactose and saline were injected daily from days 1 to 14 after birth. The activities of Gal-1-PUT[15] and G-6-PD[16] are expressed in µmoles of NADP-H_2 formed per h per mg of protein. Each value represents the mean ± SEM; the number in parentheses indicates the numbers of rats.

* Statistically significant ($p < 0.05$) differences (by Student's t-test) were obtained between controls and neonatally treated rats.

ly, elicit stable changes in the activities of various enzymes associated with both favourable and unfavourable consequences. In our experiments with hypercholesterolic SWR/y mice, postnatal administration of IPA resulted in a long-term induction of the activities of microsomal enzymes and 7-cholesterol hydroxylase which caused a stable decrease in blood cholesterol level[26]. Postnatal treatment of galactosemic rats with galactose elevated the activity of G-6-PD, enhanced galactose oxidation and considerably alleviated the symptoms of galactesemia such as cataracts, hepatomegaly and splenomegaly[27]. By contrast, when administered to rats, cortisol suppressed metabolic responses to the hormone in adults and weakened their protective reactions to stressing agents[28]. The data obtained indicate that postnatal induction may serve as an efficient means for long-term control of gene expression providing obvious beneficial effects. However, uncontrolled exposure to some genetic inducers during early postnatal life may be deleterious.

1 G. Weber, R.L. Singhal, N.B. Stamm and E.A. Fischer, Adv. Enzyme Regul. *4*, 43 (1964).
2 R.I. Salganik, S.V. Argutinskaya and R.I. Bersimbayev, Experientia *27*, 53 (1971).
3 R.I. Salganik, R.I. Bersimbayev, S.V. Argutinskaya, E.V. Kiseleva, N.B. Khristolyubova and V.I. Deribas, Molec. Cell Biochem. *12*, 181 (1976).
4 R.I. Salganik, N.A. Solovyeva and V.F. Drevich, Dokl. Akad. Nauk. SSSR *209*, 489 (1973).
5 R.T. Schimke, Cold Spring Harbor Symp. Quant. Biol. *26*, 363 (1961).
6 D.V. Parke, in: Enzyme Induction, p.207. Ed. D.V. Parke. New York 1975.
7 G.M. Tomkins and T.D. Gelehrter, in: Biochemical Actions of Hormones, vol.2, p.1. Ed. G. Litwack. New York 1972.
8 H.C. Pitot and M.B. Jatvin, Physiol. Rev. *53*, 225 (1973).
9 R.I. Salganik, in: The Cell Nucleus, vol.7. Ed. H. Busch. Academic Press, New York 1979.
10 R.I. Salganik, N.P. Mertvetsov and S.V. Argutinskaya, J. Steroid Biochem. *3*, 791 (1971).
11 R.I. Salganik, N.P. Mertvetsov, O.E. Gordienko, V.N. Chesnokov and L.A. Semenova, Acta endocr. *76*, 319 (1974).
12 R.I. Salganik, S.V. Argutinskaya, R.I. Bersimbayev and T.V. Zimonina, Vop. med. Khim. *17*, 390 (1972).
13 R.I. Salganik and N.A. Solovyeva, Vop. med. Khim. *17*, 472 (1972).
14 S.V. Nedelkina, S.V. Argutinskaya, L.P. Kusmartseva, A.V. Kemernitsky, A.M. Turuta and R.I. Salganik, Vop. med. Khim. *20*, 142 (1974).
15 T.J. Diamondstone, Analyt. Biochem. *16*, 395 (1966).
16 E. Beutler and M.J. Baluda, Lab. clin. Med. *68*, 137 (1966).
17 A. Kornberg and B.L. Horecker, in: Methods in Enzymology, vol.1, p.323. Eds. S.P. Colowick and N.O. Kaplan. Academic Press, New York 1955.
18 D.W. Nebert and N.U. Gelboin, Archs Biochem. Biophys. *134*, 76 (1969).
19 E.C. Lin and W.E. Knox, Biochim. biophys. Acta *26*, 85 (1957).
20 D.W. Van Bekkum and U.T.M. Niewerkerk, Science *149*, 548 (1965).
21 R.I. Salganik and V.C. Selatitskaya, in press.
22 R.E. Billingham, L. Brent and P.B. Medawar, Nature *17*, 603 (1953).
23 K. Lorenz, Studies in Animal Behaviour, vol.1. Harvard University Press, Cambridge, Mass. 1970.
24 B. Mach and P. Vassceli, Proc. natl Acad. Sci. USA *54*, 975 (1965).
25 S.P.R. Rose, P.P.G. Bateson, A.L.D. Horn and G. Horn, Nature *225*, 650 (1970).
26 N.M. Manankova, E.A. Nesterenko, S.V. Argutinskaya and R.I. Salganik, Vop. med. Khim. *25*, 456 (1979).
27 N.A. Solovyeva, V.V. Kandaurov and R.I. Salganik, in press.
28 R.I. Salganik, A.L. Markel, N.I. Baginskaya and I.M. Gryaznova, Ontogenez *9*, 193 (1978).

Hybridization and inbreeding effects on genome coadaptation in a haplo-diploid hymenoptera: *Cothonaspis boulardi* (Eucoilidae)

C. Biémont and M. Bouletreau[1]

Département de Biologie générale et appliquée, Université C. Bernard, Lyon 1, F-69622 Villeurbanne (France), 13 February 1979

Summary. Hybridization and inbreeding effects were tested in 2 populations of a haplo-diploid parasitic wasp, *Cothonaspis boulardi.* No inbreeding depression was observed. This suggests adaptation of the genome to total homozygosity and the absence of new gene rearrangements which could be lethal after hybridization.

It has been postulated that in parthenogenetic species with a haplo-diploid system of reproduction, genomes are coadapted under conditions of hemizygosity[2] and thus indirectly under homozygosity[3]. Since disadvantageous genes are immediately revealed in haploid males, they should not be able to accumulate in such haplo-diploid systems[4,5]. With this haplo-diploid system, therefore, no inbreeding effects are expected. Nevertheless, some theoretical considerations predict that variability due to deleterious alleles is only reduced compared with that in diploid species and not completely eliminated[6]. Experimental investigations have demonstrated inbreeding effects for morphological traits, oviposition rate and honey yield of bees[7–10] and fecundity, fertility[11] and egg hatchability[12] of predaceous Acarina. However, in the latter group, arrhenotoky is now contested[13].

Though the sex locus problem has not always been excluded in these studies, the results have been interpreted as suggesting that a) lethality exists on male haploid loci in systems of balanced polymorphism[6], b) heterotic mechanisms play a role in the diploid part of the population[5], c) inbreeding apparently brings about the loss of internal genetic balance and causes low viability possibly through disruption of polygenic complexes[11,14].

These hypotheses were tested by hybridization studies. Hybridization may disturb the presumed coadapted genetic systems and destroy the favorable gene combinations exist-

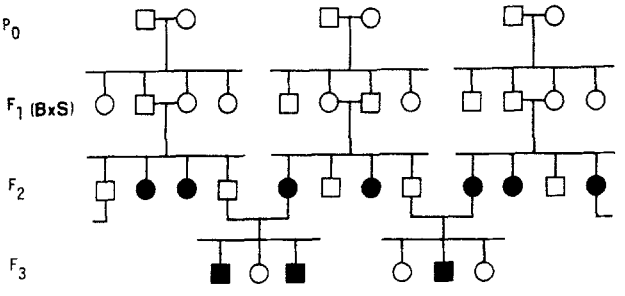

Fig. 1. Diagram of crosses. ■, inbred males and ●, inbred females. Open squares and circles are non-inbred individuals.