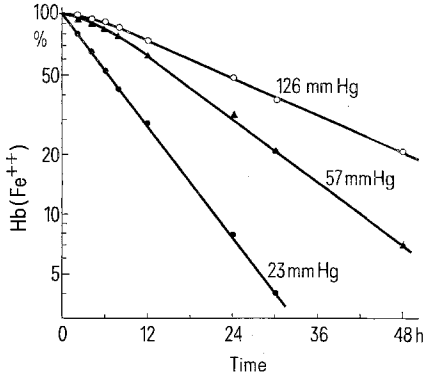
6.2% CO_2 and nitrogen, corresponding to oxygen tensions of 23, 57 and 126 mm Hg, respectively. Aliquots were sampled at fixed intervals for analysis. Oxygen content of the solution was determined by gas chromatography[12], and the O_2-saturation of hemoglobin was computed. For methemoglobin determination, calibration curves were constructed from absorbances of known mixtures of oxyhemoglobin, methemoglobin and plasma, which were pre-incubated for 1 h at room temperature. Identical results were obtained with two calibration curves, using differences A_{577}–A_{505} and A_{577}–A_{630}, respectively.

Results and discussion. Oxygen saturation was determined following 30 min of equilibration with the gas mixture. The resulting oxygen dissociation curve shows a marked shift to the left when compared with the standard dissociation curve of whole human blood: HbO_2 (126 mm) = 99%; HbO_2 (57 mm) = 92%; HbO_2 (23 mm) = 54%. The observed high affinity for oxygen is in accord with results of BUNN et al.[13] who demonstrated a P_{50} of 17 mm Hg under similar conditions. Auto-oxidation rate at 126 mm Hg seems to increase with the time of incubation, reaching a constant value after 12 h (Figure). This time-dependence of oxidation rate can be explained by either inactivation of a protective mechanism or release of an auto-oxidation enhancing principle. Red cell hemolysate and plasma contain a number of substances which are known to affect the oxidation rate of hemoglobin by dissolved oxygen, i.e. organic phosphates[8], copper ions[14], glutathione[15] and the complex enzymatic system for methemoglobin reduction[16]. Further investigations are necessary to recognize the relative importance of these constituents in auto-oxidation of plasma hemoglobin under physiological conditions. Slow production of methemoglobin in the initial period is comparable to that demonstrated by BUNN et al.[13]. Half-life of the fully oxygenated hemoglobin in plasma ($T_{1/2}$ = 20 h) seems to be half as long as that found in dilute buffer solutions[7]. In addition, the auto-oxidation curve in buffer showed a biphasic course, the initial rate being faster than the consecutive[7], a finding at variance with our results with plasma. Half-lives of oxyhemoglobin at 57 and 23 mm Hg were 12 and 7 h, respectively. These values again indicate a considerably faster oxidation of hemoglobin in plasma than in a buffer solution[7]. Provided that an extrapolation of these results is admittable, a rather fast in vivo oxidation of free hemoglobin has to be expected. However, a reliable estimation of the in vivo oxidation rate is difficult, since the oxygen tension of circulating blood fluctuates in the vascular bed. If 60 mm Hg is a reasonable assumption of the mean intravascular O_2-tension, free hemoglobin may undergo auto-oxidation with an approximate half-life of 12 h.



Auto-oxidation of hemoglobin in plasma at pH 7.25 and 37 °C.

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Effects of Two Kinds of Social Deprivation on Testosterone and Estradiol-17 β Plasma Levels in the Male Rat

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Summary. The effect of complete and tactile isolation on plasma testosterone and estradiol-17 β was studied in male rats at the age of 60 and 180 days. A decrease in the plasma levels of the two hormones was observed.

In recent years many investigations produced evidence that modifications of the social environment can affect endocrine parameters in rodents. It is well established that adrenal[2–7] and gonadal[4, 6, 8–10] functions are involved in the effects produced by social deprivation, and particularly by isolation.

Isolation influences also many behavioural traits, particularly sexual behaviour[11–14]. GERALL et al.[13] and SPEVAK et al.[14] found differences in sexual behaviour between socially reared and isolated rats, but did not find differences between subjects reared in total and in contact isolation.

The purpose of this investigation is to study the modifications of testosterone and estradiol-17 β plasma concentration induced at 60 and 180 days of age by varying degrees of social deprivation in the rearing of the male rat. For this study, 48 Sprague Dawley rats from a stock maintained in our laboratory (originally obtained from Charles River) were used. At the age of 21 days, subjects were weaned and randomly divided into 3 groups of 16 rats each.

Group 1. Total isolates. Animals were raised alone in a cage 15 cm wide by 12 cm high by 33 cm long. They could receive auditory and olfactory stimuli. Group 2. Contact isolates. Animals were separated by a wire mesh screen and so disposed that each subject was adjacent to a male and a female. Each compartment was 25 cm wide by 20 cm high by 25 cm long. Group 3. Socially reared. 4 males and 4 females were housed in a cage (38 cm wide $\times$ 60 cm long $\times$ 20 cm high). Litters delivered in the cage were removed at the age of 20 days.

Eight animals of each group were sacrificed at the age

Mean values (± SE) of testosterone and estradiol-17β plasma concentration (ng/ml) in male rats reared under different environmental conditions, at 60 and 180 days of age

	60 days				180 days			
	Testosterone		Estradiol-17β		Testosterone		Estradiol-17β	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Total isolates	0.43[a]	0.11	0.27[d]	0.02	0.53[g]	0.11	0.17[l]	0.02
Contact isolates	1.43[b]	0.25	0.18[e]	0.01	1.76[h]	0.62	0.18[m]	0.02
Socials	4.52[c]	0.38	0.58[f]	0.07	4.52[i]	0.83	0.36[n]	0.05

Comparison of means (F-test): a–c, b–c, d–f, e–f, g–i $p < 0.001$; a–b, l–n $p < 0.01$; d–e, h–i, m–n $p < 0.05$.

of 60 days by decapitation. The others were sacrificed at the age of 180 days. Plasma was extracted with diethyl ether and the concentration of testosterone and estradiol-17β was determined by radioimmunoassay according to COLLINS et al.[15] and EMMENT et al.[16] respectively, without chromatography.

Factorial analysis of variance of the data shows for plasma testosterone significant differences caused by treatment ($F = 39.09$; $df = 2/42$; $p < 0.001$), while age ($F = 0.06$; $df = 1/42$; $p > 0.2$) and interaction ($F = 0.09$; $df = 2/42$; $p > 0.2$) do not significantly affect this parameter.

The values of plasma estradiol-17β show significant differences for treatment ($F = 23.95$; $df = 2/42$; $p < 0.001$) and age ($F = 8.64$; $df = 1/42$; $p < 0.025$), but not for interaction ($F = 2.96$; $df = 2/42$; $p < 0.1$).

Mean values of testosterone and estradiol-17β plasma concentration (ng/ml ± SE) in subjects of 60 and 180 days are shown in the Table, with the levels of significance in the comparison between groups.

Complete social deprivation (total isolation) affects the plasma level of testosterone and estradiol-17β: this results in a significant decrement of both hormones, which is more relevant for testosterone than for estradiol-17β. The effect of tactile deprivation (contact isolation) appears to be intermediate between those of the other two conditions of rearing, except that for estradiol-17β at 60 days. Social rearing is correlated with the highest level of both hormones.

The fact that differences between total and contact isolates are statistically significant at 60 days only, and that the differences between socials and both kinds of isolates are greater at 60 days, suggests a connection with the development of the rat. A sensitive period between weaning and sexual maturity may be hypothesized in which the social environment plays a major role on control mechanism of endocrine secretion. This action seems to be reversible: actually, after sexual maturity is reached, these differences are going to diminish, or to disappear altogether.

Differences between socials and contact isolates can be accounted for by tactile stimulation and, in subjects under 60 days, by play activity, which in rats is a prominent factor in the ontogenesis of behaviour, as suggested by GERALL et al.[13] and SPEVAK et al.[14]. Play activity could be relevant in the ontogenesis of endocrine equilibrium too. On the other hand, as differences between socials, contact isolates and total isolates decline from 60 to 180 days, it appears that in this respect sexual experience and the presence of litters play a minor role.

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The Effect of Tri-Iodothyronine on the Skeletal Growth of *Salmo trutta* Alevin

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Summary. T3 treatment enhanced the growth and development of lacrymal bone in *Salmo trutta* alevin. It was also shown that T3 is more potent than T4 in the case of fish.

Thyroxine (T4) and tri-iodothyronine (T3) are known to play an important role in the normal development and growth of mammals. One of the most sensitive effects noted in mammals, is their influence in enhancing the growth and development of skeletal tissues. Attempt to demonstrate the comparable effects of the hormones in the fish has also been made[2]. Whether the hormones play some role in the normal growth and development of skeletal tissue of the fish is a matter yet to be resolved. However, it has been shown that exogenous T4 promotes the synthesis of preosseus matrix and the further development of the lacrymal bone in *Salmo trutta* alevin during

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