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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Number of transverse sections (8 μm thickness) of the lacrymal bones of left hand side (LHS) and right hand side (RHS) of controls and tri-iodothyronine treated alevins of brown trout

Age (days)		13	15	17	23	31
	Numbers of sections					
Controls	LHS	0	0	0	—	51
	RHS	0	0	0	14	51
		($n = 1$)	($n = 3$)	($n = 1$)	($n = 1$)	($n = 7$)
8×10^{-7} Tri-iodothyronine treated	LHS	0	14	20	56	99
	RHS	0	15	31	54	94
		($n = 1$)	($n = 3$)	($n = 1$)	($n = 1$)	($n = 6$)
4×10^{-7} Tri-iodothyronine treated	LHS	0	24	21	54	87
	RHS	0	23	24	51	83
		($n = 1$)	($n = 2$)	($n = 1$)	($n = 1$)	($n = 5$)

n = Numbers of fish examined.

the first 31 days after hatching[2]. A need then arose for the study of the effects of T3, as well, on the skeletal tissue of the fish and also to see if T3 is more potent than T4 as it has been found in the case of the rat[3,4].

The present report is of the work carried out on the effect of T3 on the growth of skeletal tissue of *Salmo trutta* alevin. The procedure of analysis in this study was the same as that of BARRINGTON and RAWDON[2]. The fish was treated with 8×10^{-7} and 4×10^{-7} dilutions of T3 in tap water. Transverse sections of the head of the fish, from samples fixed in Bouin's solution at day 13, 15, 17, 23 and 31 were prepared and stained with the Azan technique. At day 13, are fish from each of the control, 8×10^{-7} and 4×10^{-7} groups was examined. The sections of the lacrymal bones or of their preosseus matrix appeared in none of the fish representing the 3 groups at this stage. At day 15 three fish from the control, 3 from 8×10^{-7} T3 and 2 from 4×10^{-7} T3 treatment groups were examined. In the control animals no sign of the bone or preosseus matrix was seen. In the fish of the 8×10^{-7} T3 treatment group 14 and 15 sections of the lacrymal bones were found on the left hand side (LHS) and right hand side (RHS) respectively (a result averaged from 3 individuals), whereas in the fish of 4×10^{-7} T3 treatment group 24 and 23 sections of lacrymal bone on the LHS and RHS were found (an average of 2 individuals). At day 17 one fish from each of the 3 groups was examined. In the control group there was no sign of bone. In the 8×10^{-7} T3 treated fish 20 to 31 sections of the LHS and RHS were found and in the 4×10^{-7} T3 treated fish 21 and 24 sections of the lacrymal bones of the LHS and RHS were observed. At day 23 one fish from each of the 3 groups was studied. The control on the RHS had 14 sections of the lacrymal bone (the LHS was damaged therefore it was not taken into account). The fish of 8×10^{-7} T3 group had 56 and 54 on the LHS and RHS respectively, whereas in the fish of the 4×10^{-7} T3 group 54 and 51 sections on the LHS and RHS respectively were observed. Finally at day 31 seven of the control, 6 of the 8×10^{-7} T3 and 5 of the 4×10^{-7} T3 groups were examined. On average the lacrymal bone in the control

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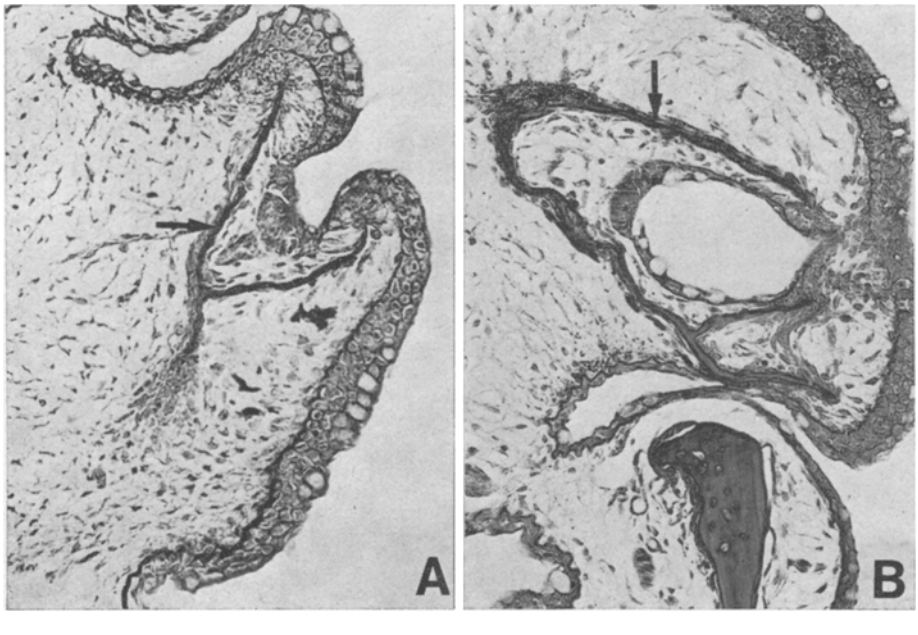

A portion from a transverse section of the head of a 31-day-old trout alevin to show the development of lacrymal bones (arrow marked). Azan, ×900. A) Control; B) tri-iodothyronine treated, alevins.

fish extends to 51 and 51 sections, in the 4×10^{-7} T3 group to 87 and 83 sections and in the 8×10^{-7} T3 group to 99 and 94 sections on the LHS and RHS respectively. These results are summarized in the Table.

The most convincing point noted in this analysis was that among the 13 control fish and the 22 treated fish studied not a single case of overlaping results was found in the 2 groups. Comparing the responses to the two levels of the hormone, not a great deal of difference was noticed except at day 31 when the treatment of 8×10^{-7} T3 caused a small but an insignificant advanced growth of the bone. Figure a and b show the state of development of the lacrymal bone in a control and a T3 treated alevins respectively at day 31 after hatching.

These results provide further evidence in support of the finding that exogenous T4 promotes synthesis and further development of lacrymal bone in *Salmo trutta* alevin during the 31 days after hatching[2]. Moreover the results of T3 treatments are consistent as unlike those of T4 treatment; no overlaping cases were found in the T3 treatments. The results also indicate that the effect of higher concentration was slightly inhibitory as compared to the lower concentration one. It is also demonstrated here that T3 is more than 8 times as potent as T4 – a situation similar to that reported in the case of the rat[3,4]. The precise reason for the high potency of T3 are not known, however, it may be that T3 is metabolized faster than T4[5], thus the T3 treatment resulted in an efficient response[6].

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Free and Sulfoconjugated Dehydroepiandrosterone, Cyclic Adenosine-3′,5′-Monophosphate, and Free Estriol in Maternal and Cord Blood

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Summary. When free DHEA, its sulfatide, and sulfate were assayed in maternal plasma as well as in umbilical cord arterial and venous plasma, rather high concentrations were found in either fraction from cord arterial plasma, reflecting the fetal contribution not only of free DHEA and DHEA sulfate, but also of the lipophile steroid sulfatide. Since high DHEA levels were associated with elevated c-AMP concentrations, a certain interrelationship of both parameters is indicated. In the course of delivery, a rapid decrease of free estriol in maternal plasma was observed. Higher concentration of free estriol in umbilical venous plasma pointed at its placental biosynthesis from fetal precursors.

The role of sulfoconjugated DHEA (dehydroepiandrosterone, 3β-hydroxy-5-androsten-17-one) as a major precursor of estrogens in the fetoplacental unit has been established beyond any doubt[1,2]. Comparatively higher concentrations of sulfoconjugated DHEA in umbilical cord arterial blood suggested the biosynthesis of this precursor in fetal adrenal tissue[3]. Since, however, under physiological conditions the predominant portion of sulfoconjugated DHEA in adult human subjects apparently occurs as a lipophile compound, e.g. a diglyceride sulfate or 'sulfatide'[4,5], it seemed of particular interest to investigate whether also the fetal adrenal produces such lipophile sulfoconjugates. Especially in view of the fact that only DHEA sulfatide affects the activity of G-6-PDH (glucose-6-phosphate dehydrogenase)[6,7] or the concentration of c-AMP in plasma[8], whereas DHEA sulfate proved to be completely inactive in the G-6-PDH inhibition test.

Therefore, DHEA was determined in the fractions of free steroids, steroid sulfatides, and steroid sulfates from umbilical cord arterial and venous plasma as well as maternal plasma before, during, and after delivery. At the same time, c-AMP and free estriol (1, 3, 5(10)-estratriene-3, 16, 17β-triol) were measured in these samples.

Material and methods. In 20 normal pregnant women, blood samples were collected during the initial stage of labour, in the expulsion period, and 2 and 48 h after delivery. From the umbilical cord, arterial and venous blood were withdrawn immediately after delivery, yielding 0.8 to 3.7 ml of heparinized plasma.

All heparinized plasma samples were assayed for the above-mentioned parameters by standard procedures. Steroid sulfatides and sulfates were isolated by ion exchange chromatography on polyamide columns and subseuqnet thin layer chromatography[9]. Following solvolysis of sulfoconjugates DHEA was separated by repeated thin layer chromatography and quantitated by densitometry of its 2, 4-dinitrophenylhydrazone[10]. c-AMP was measured by the protein binding assay of Brown et al.[11], while free estriol was determined by radioimmunoassay[12].

Results and discussion. As shown in the Table, the maternal plasma levels of DHEA varied considerably in the course of delivery. Whereas in the initial stage of labour, the concentration of total DHEA averaged 83.9 μg/100 ml, a total of 97.2 μg DHEA/100 ml were found during the expulsion period. As compared to 42.3 $\pm$ 18.9 μg/100 ml, measured in peripheral plasma of 10 normal preg-

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