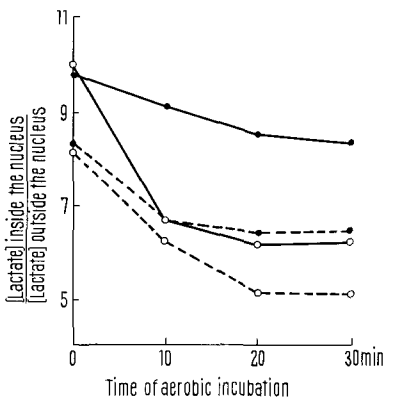
On the Effect of Insulin on Lactate Permeability through the Nuclear Membrane

This communication reports a phenomenon observed during a study on the effect of insulin in vitro on energy metabolism in isolated rat thymus nuclei. From these experiments it is concluded that insulin has no significant effect on oxygen consumption, ATP synthesis, glucose uptake, hexose monophosphate pathway activity and lactate production. A clear effect, however, was found on membrane permeability towards lactate. All methods used were exactly as described elsewhere[1-4]. From earlier[4] experiments it is known that endogenous lactate which is formed under anaerobic conditions is partly metabolized during a subsequent aerobic incubation and partly leaks out from the nucleus into the medium.

In the Figure it is demonstrated that leakage during aerobic incubation can be partly prevented by adding insulin. The ratio of lactate concentrations was influenced in the same direction when glucose was added. As can be seen in these experiments insulin had no effect on the lactate ratio during the anaerobic period. A more or less stable equilibrium of lactate inside and outside the nucleus was reached after 20 min of aerobic incubation. No information is available in literature on the influence of insulin on lactate permeability through the nuclear membrane. DANCHEVA[5] reported a slight stimulation of nuclear oxidative phosphorylation in isolated thymus nuclei by insulin in the presence of glucose and galactose and contributed this to an accelerated transport of the hexoses through the nuclear membrane. In our opinion it is unlikely that glucose entry is rate-limiting for its metabolic conversion because the nuclear membrane is extremely permeable for glucose[6]. Moreover, added glucose is rapidly catabolized to lactate by isolated thymus nuclei, while its further breakdown via the citric acid cycle is limited[2,3].



Influence of insulin on the ratio of lactate concentrations inside and outside the nucleus after anaerobic incubation. The anaerobic incubation was performed for 15 min at 30 °C and the subsequent aerobic incubation for 30 min at 22 °C. The basic medium consisted of 0.25 *M* sucrose. 3 m*M* $CaCl_2$. Broken lines illustrate experiments on endogenous substrate; solid lines experiments with 5 m*M* glucose. Open circles represent experiments without insulin; solid circles illustrate experiments in which 100 μunits insulin per ml were added.

Résumé. L'addition d'insuline aux noyaux isolés du thymus de rat peut prévenir les pertes de lactate endogène se produisant à travers de la membrane nucléaire.

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Testosterone Metabolism by Homogenates of Human and Rat Placenta

It now appears to be accepted that testosterone and its 16-hydroxylated derivative are intermediates in the placental biosynthesis of estrogens from the fetal precursors, dehydroepiandrosterone and 16-hydroxydehydroepiandrosterone, respectively[1-3]. The abundantly occurring human placental aromatase[4-7] effects a rapid conversion of these 19 carbon intermediates to the 18 carbon estrogens by a series of reactions which apparently includes oxidation of the methyl group at carbon 19 prior to its elimination[8].

In the rat, however, in vivo evidence suggests that the placenta produces little, if any, estrogen[9]; also, rat placental tissue does not appear capable of converting testosterone to estrogens in vitro[10].

Human and rat placenta appear to differ in yet another aspect. While rat tissue is capable of metabolizing testosterone to an androstane product such as 5α-androstane-3α,17β-diol[10] and thus appears to possess Δ4-reductase activity, evidence for this activity could not be found in midterm human placenta perfused in situ[11]. Recently, however, the Δ5-reduction of dehydroepiandrosterone by minced premature human placental tissue has been reported[12].

The present study compares the metabolism of 4-C^{14}-testosterone by homogenate preparations of human and rat placental tissue (both term and premature) incubated under identical experimental conditions.

Materials and methods. Human placentas were obtained at the time of delivery and processed immediately. Rat placentas were removed after sacrifice of the animals by concussion and also used immediately. Details of the methods used are reported elsewhere[13]. In brief, all

Table I. Percentage of total radioactivity recovered in different steroid fractions after incubation of 4-C^{14}-testosterone with human and rat placental homogenates[a]

No.	Age (weeks)	Weight (g)	Protein N[b] (mg)	NADPH not added to incubation Steroid fraction			NADPH added to incubation[c] Steroid fraction		
				Testosterone	More polar	Less polar	Testosterone	More polar	Less polar
Human placentae:									
1	12	58	50.4	74	7	19	–	–	–
2	15	88	50.0	44	7	42	42	12	39
3	16	94	57.5	51	7	39	47	9	41
4	16	100	47.5	51	3	38	35	10	48
5	24	240	57.3	11	13	76	10 (16)	16 (25)	75 (58)
6	25	206	62.5	29	3	63	14	8	74
7	29	334	65.0	8	5	81	4	12	77
8	39	343	95.0	8	11	73	3	29	60
9	39	400	–	4	8	81	2	21	70
10	40	515	82.5	4	8	82	9	23	62
11	40	675	72.5	11	5	79	10	8	76
12	42	396	72.5	8	23	57	9	36	43
Rat placentae:									
	Age (days)	Average wt. (g)							
1	15	0.30	112.5	79	0	18	–	–	–
2	15	0.30	105.0	88	0	10	–	–	–
3	20–22	0.55	112.5	80	0	17	(67)	(2)	(29)
4	20–22	0.42	90.0	67	0	31	(47)	(3)	(48)
5	22	0.56	107.5	63	0	35	–	–	–
6	20–22	0.52	90.0	76	0	22	(68)	(7)	(22)
7[d]	20–22	–	40.0	67	0	26	–	–	–
8[d]	20–22	–	40.0	58	0	26	50	2	33
9[d]	20–22	–	50.0	49	0	47	36	2	55
10[d]	20–22	–	60.0	36	0	56	23	0	70

[a] Percentage figures are corrected for any radioactivity in corresponding fractions of paper chromatograms of unincubated samples. [b] Protein N content per 10 ml of incubated homogenate. [c] The amount of NADPH added was 2 mg/10 ml of homogenate except in experiments where figures are in parentheses in which 6–10 mg was used. [d] In these experiments samples of tissue were not homogenized in the usual way with a volume of Krebs-Ringer buffer equal to their weight; tissue/buffer ratio used was less than 1.

samples of tissue were homogenized with a volume of Krebs-Ringer phosphate buffer equal to their weight. The homogenates were centrifuged at 800 *g*, and 10 ml aliquots of the supernatant material were incubated in beakers containing 1 mg of non-radioactive testosterone mixed with 1.2×10^6 cpm of C^{14}-labelled testosterone. The co-factor NADPH (nicotinamide adenine dinucleotide phosphate, reduced form) was also added to one half of the samples in most experiments.

Following incubation, chloroform extracts of the incubation mixture were chromatographed on paper in a hexane-propylene glycol system. The substrate testosterone and the products of its metabolism were detected by radioautography. These steroids were then eluted from the papergrams and aliquots plated on planchets for measurement of their radioactive content in a proportional flow counter.

Results. Table I gives the percentage of 4-C^{14}-testosterone recovered following its incubation with the different placental homogenate preparations with or without exogenous NADPH. It is apparent that the capacity of human placental homogenates to metabolize testosterone was greater in the samples at 24 or more weeks of gestation than in the specimens obtained at 12–16 weeks. Virtually all the testosterone was metabolized in incubations with term human placentas, and this was the case whether or not NADPH was added to the incubates.

Under identical conditions rat placental tissue was much less capable of metabolizing testosterone than was term human tissue. In most experiments more than 50% of the testosterone was recovered unchanged following incubation with both premature (15-day) and near term (20–22-day) samples.

Table I also shows that most of the testosterone was metabolized to material less polar than itself (more rapidly moving in the particular paper chromatographic system used), in incubations with both human and rat tissues, regardless of gestational age. Also, in all experi-

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ments the major component of this less polar material was a metabolite identified as androstenedione (Table II). Other metabolites were present in $^1/_4$ or less of the amounts of androstenedione in both human and rat experiments.

Table I shows that human placental homogenates also converted testosterone to metabolites with lesser mobility in the chromatographic system used. This conversion to more polar substances was enhanced by addition of NADPH to incubation and was most notable with the term placental samples.

In contrast to the human, rat placental homogenates metabolized testosterone to more polar material in significant quantity only when NADPH was added to the incubation. Even then, the extent of this conversion was less than that observed with the term human samples although greater amounts of NADPH were used in the rat incubations.

Discussion. The present experiments indicate that the ability of human placenta to convert testosterone to both more and less polar metabolites was dependent upon gestational age, with term placenta being the most active. Similar age dependency has also been observed by others studying the metabolism of other steroids by human placenta. In each case, using 17β-estradiol as substrate for incubation studies, both SMITH and AXELROD[14] and RYAN and ENGEL[15] found that 17β-hydroxysteroid dehydrogenase activity (as indicated by the formation of estrone) increased with gestational age. This was evidently also the case in the present study since androstenedione was the major metabolite of testosterone in all experiments. The extent of 20α-hydroxysteroid dehydrogenase activity has also been observed to be age dependent[16].

The present report indicates yet another difference between rat and human placental activities; under identical experimental conditions homogenates of term human tissue metabolized testosterone to other substances to a considerably greater extent than did similar rat preparations. This suggests that in addition to lacking the aromatizing enzyme(s) abundant in human placenta[4–7], rat tissue is also less rich in 17β-hydroxysteroid dehydrogenase content than the human. The more polar material elaborated in the samples incubated with exogenous NADPH has previously been shown[10] to consist principally of 5α-androstane-3α,17β-diol and other unidentified ring A reduced metabolites. The presence of this cofactor evidently stimulated this reductive mode of metabolism of testosterone by rat placental homogenates.

The capacity of 15-day and term rat placental homogenates to totally metabolize testosterone did not appear to differ as markedly as did the premature and term human preparations. Histological studies, however, suggest that 17β-hydroxysteroid dehydrogenase activity in the labyrinth of the rat placenta increases towards term[17]. In contrast, DEANE et al.[18] observed that placental 3β-hydroxysteroid dehydrogenase activity reaches a peak of 15–16 days and then declines towards term.

Histological studies have indicated that while there are at least 2 estradiol 17β-hydroxysteroid dehydrogenases present in human placenta which can be characterized by their cofactor dependency and location, i.e. NAD-specific and present in syncytial trophoblast and decidual walls or, NADP-specific and located in the perivascular stroma and vessel walls[19, 20], testosterone-17β-hydroxysteroid dehydrogenase utilizes either NAD or NADP and is located only in the vessel walls[20].

The significance of the change in extent of activity with gestational age of certain enzymes in human placenta such as those involved in the metabolism of testosterone in the present experiments, and other enzymes, e.g. estradiol-17β-hydroxysteroid dehydrogenase[15], 20α-hydroxysteroid dehydrogenase[16], is not clear. It has been suggested that these coenzyme-linked steroid dehydrogenases may be implicated, with their steroid substrates, in the regulation of cellular metabolism[21].

It may also be that the increase of certain placental enzymes towards term, such as the 17β-hydroxysteroid dehydrogenase which converts testosterone to the less biologically active androgen, androstenedione, is a mechanism for protecting the fetus from the possibly damaging effects of excessive amounts of certain steroid hormones. It may be of significance in this connection that the 16α-hydroxylated derivative of testosterone was found by NEHER and STARK[22] to be one of the most abundant of neutral steroids extracted from human placenta[23].

Table II. Identification by recrystallization to constant specific activity of androstenedione derived from the metabolism of testosterone by homogenates of human and rat placental tissue

Recrystallization No.		Specific activity (disintegrations per min/mg)	
		Human experiment	Rat experiment
1	Mother liquor	3080	2130
	Crystals	3120	2180
2	Mother liquor	3200	2130
	Crystals	3070	2210
3	Mother liquor	3210	2200
	Crystals	3190	2140

Following chromatography on paper (hexane-propylene glycol system) and on silica gel thin-layer plates (developing solvent 85 parts benzene, 15 parts methanol) aliquots of samples pooled from several incubations were diluted with 100 mg of authentic androstenedione. The resulting solutions had a specific activity of 3210 and 2390 dpm/mg for human and rat samples, respectively. Recrystallizations to constant specific activity were then carried out with solvents used being mixtures of chloroform and methanol.

Résumé. Presque toute la testosterone a été métabolisée dans les incubations avec les homogenates du placenta humain à terme que le cofactor NADPH y ait été ajouté ou non. Mais, les échantillons humains d'une grossesse moins de 24 semaines et aussi les homogenates placentaires du rat à terme et prématuré étaient tous moins actifs dans ce respect.

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