thyroid when the anti-thyroglobulin antiserum was absorbed with thyroglobulin or replaced by normal rabbit serum. Neither was a reaction evident in other tissue such as parathyroid, anterior and posterior lobes of pituitary and pars tuberalis after incubation with the antiserum.

Discussion. From the late 1950's to early 1960's, numerous immunofluorescence studies using anti-thyroglobulin antiserum were performed on the thyroid. However, since the existence of parafollicular cells in the thyroid was not noticed by previous investigators, there was no report on the immunoreactivity of these cells to antithyroglobulin. The present study is the first to demonstrate that the parafollicular cells contain thyroglobulin-like or immunologically similar protein, which seems to imply a close metabolic relationship between the parafollicular and follicular cells.

It is well-known that the thyroid gland shows conspicuous hyperplastic changes following the application of antithyroid drugs. One of the authors[12] previously found that the injection of thiourea for 1–4 months causes cytological changes not only in the follicular but also in the parafollicular cells of the canine thyroid: decrease in secretory granules, enlarged cell bodies and dilation of cisterns of rough endoplasmic reticulum. The significance of the parafollicular cell reaction to antithyroid drug could not be explained at that time. However, if the synthesis of the thyroglobulin-like substance takes place in rough endoplasmic reticulum-Golgi complex system of the parafollicular cells and if it is affected by the antithyroid drug, this parafollicular cell reaction, similar to that of the follicular cells, might well be acounted for.

It has been reported recently that various polypeptide hormones may be synthesized by way of prohormones, as in the case of proinsulin and insulin. Neurophysins associated with oxytocin and vasopressin in neurosecretory granules have been known as carrier proteins. Thus, the existence of inactive proteins in addition to active polypeptide hormone has been attracting more and more attention in different polypeptide hormone-secreting cells. It will be necessary to elucidate the cell-biological significance of the thyroglobulin-like immunoreactivity in the parafollicular cells as well as its relation to calcitonin.

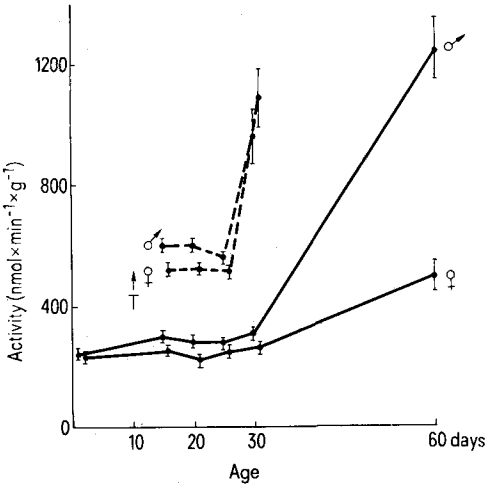
Androgen dependency of hepatic hydroxysteroid dehydrogenases in the rat: Prepubertal responsiveness and unresponsiveness towards exogenous testosterone[1]

H.-G. Hoff, R. Ghraf, E. R. Lax and H. Schriefers

Institut für Physiologische Chemie und Medizinische Klinik der Universität Essen, Hufelandstrasse 55, D–43 Essen (Federal Republic of Germany), 18 October 1976

Summary. The prepubertal responsiveness of 3 typical androgen-dependent enzyme activities, namely 20-ketoreductase, 3α- and Δ^4-3β-hydroxysteroid dehydrogenase, towards a large dose of testosterone was investigated. The androgen induced the activity of the 3α-enzyme prepubertally in both sexes.

A phase of sexual indifference, lasting from day 1 to 30 of life, is observed in the ontogenesis of the sexually differentiated enzyme activities of hepatic steroid metabolism[2–4]. Androgen or oestrogen-dependency becomes apparent only after this phase has been completed (for criteria of androgen or oestrogen dependency see Lax et al. and Ghraf et al.[5,6]). In order to test whether this prepubertal indifference is due to either insufficient levels of circulating androgen or androgen unresponsiveness, 3 typical androgen-dependent enzyme activities were investigated after administration of a large dose of testosterone between day 10 and 13 of life.



Prepubertal responsiveness of NAD-dependent microsomal 3α-hydroxysteroid dehydrogenase activity of rat liver towards exogenous testosterone (T); (—), untreated animals; (---), treated animals. Bars represent means ± SD.

Material and methods. Male and female rats of the strain Chbb: THOM with intact gonads were used. Between day 10 and 13 of life, treated animals were administered 2 doses of Testoviron-Depot® s.c. (corresponding to a total amount of 100 mg testosterone) and the activities of the following microsomal liver enzymes were tested on day 15, 20, 25 and 30: NAD-dependent 3α-hydroxysteroid dehydrogenase[7], NADP-dependent Δ^4-3β-hydroxysteroid dehydrogenase[8] and NADP-dependent 20-ketoreductase[2]. Microsomal protein was estimated by the method of Lowry et al.[9]. The degree of significance between 2 mean

1 This investigation was supported by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 87, Endokrinologie).

2 H. Schriefers, R. Ghraf, H.-G. Hoff and H. Ockenfels, Hoppe-Seylers Z. physiol. Chem. *352*, 1363 (1971).
3 H.-G. Hoff, R. Ghraf, M. Raible and H. Schriefers, Hoppe-Seylers Z. physiol. Chem. *354*, 306 (1973).
4 R. Ghraf, E. R. Lax and H. Schriefers, Acta endocr., Copenh. *71*, 781 (1972).
5 E. R. Lax, H.-G. Hoff, R. Ghraf, E. Schröder and H. Schriefers, Hoppe-Seylers Z. physiol. Chem. *355*, 1325 (1974).
6 R. Ghraf, E. R. Lax and H. Schriefers, Hoppe-Seylers Z. physiol. Chem. *356*, 127 (1975).
7 H.-G. Hoff and H. Schriefers, Hoppe-Seylers Z. physiol. Chem. *354*, 507 (1973).
8 E. R. Lax and H. Schriefers, Eur. J. Biochem. *42*, 561 (1974).
9 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1951).

Prepubertal unresponsiveness of androgen-dependent microsomal enzyme activities* of rat liver towards exogenous testosterone

	Day of life	Male rats Untreated	Treated	Female rats Untreated	Treated
Δ^4-3β-Hydroxysteroid dehydrogenase	15	26 ± 6	42 ± 7	27 ± 8	45 ± 7
	20	38 ± 5	28 ± 13	35 ± 6	35 ± 8
	25	34 ± 8	31 ± 10	26 ± 8	26 ± 8
	30	45 ± 3	38 ± 6	45 ± 6	56 ± 3
	75	323 ± 46		48 ± 15	
20-Ketoreductase	15	4.6 ± 0.4	5.1 ± 0.9	3.9 ± 1.2	3.9 ± 1.5
	20	5.4 ± 0.9	4.0 ± 0.7	4.2 ± 0.7	3.7 ± 1.3
	25	7.8 ± 1.1	6.6 ± 1.1	8.6 ± 0.6	7.1 ± 1.3
	30	8.3 ± 0.6	6.4 ± 1.4	9.1 ± 1.3	6.7 ± 1.3
	75	28.0 ± 3.0		8.0 ± 1.2	
Seminal vesicles**	30	12 ± 4	125 ± 17		

*nmol × min^{-1} g^{-1} liver wet weight; means ± SD; in each group liver was pooled from 8 rats, 6 determinations per pool. **Wet weight, mg.

values was determined by Student's t-test. The degree of significance was set at $p < 0.001$.

Results and discussion. Up to day 30 of life, the activities of Δ^4-3β-hydroxysteroid dehydrogenase and 20-ketoreductase of testosterone-treated animals did not show any substantial deviation from the normal developmental course (table). In contrast, the activity of 3α-hydroxysteroid dehydrogenase responded in a biphasic manner in both sexes; within 5 days of administration, a 3fold increase had occurred and was followed by a further rise in activity between day 25 and 30 when the level normally found in mature male rats was reached (figure). No significant differences in the protein content of the microsomal fractions were noted for any of the groups, and thus the expression of the enzyme activities in terms of wet weight reflects changes in the 'specific' activities of these enzymes.

The results of this investigation indicate that the sexual indifference in the prepubertal phase of enzyme activity ontogenesis is partly the result of low androgen levels and partly the result of unresponsiveness to androgens. The refractoriness of prepubertal liver to sexual hormones has also been observed for the androgen-dependent synthesis of α_{2U}-globulin[10] and for the oestrogen-dependent synthesis of 'renin substrate'[11]. It is possible that androgen unresponsiveness may be due to the lack of androgen receptors. However, the existence of such receptors in the liver of the mature rat remains controversial[12, 13].

10 A. K. Roy, Endocrinology *92*, 957 (1973).
11 A. J. Eisenfeld, R. Aten, M. Weinberger, G. Haselbacher, K. Halpern and L. Krakoff, Science *191*, 862 (1976).
12 A. K. Roy, B. S. Milin and D. M. McMinn, Biochim. biophys. Acta *354*, 213 (1974).
13 W. I. P. Mainwaring, E. K. Symes and S. J. Higgins, Biochem. J. *156*, 129 (1976).

Catecholamine-sensitive adenylate cyclase of human fat cell ghosts. Inhibition of catecholamine stimulation by phenylephrine

H. Kather, B. Vogt and B. Simon

Klinisches Institut für Herzinfarktforschung an der Medizinischen Universitätsklinik, D–69 Heidelberg (Federal Republic of Germany, BRD), 18 October 1976

Summary. The alpha-adrenergic agonist phenylephrine (up to 1 mM) did not affect basal and NaF-stimulated adenylate cyclase activities of human fat cell ghosts, but caused a dose-dependent inhibition of cAMP formation in the presence of catecholamines.

Catecholamines are thought to act via alpha- and beta-adrenergic receptor sites[1–4]. It has been suggested that both receptor types are coupled to the membrane-bound adenylate cyclase system in adipocytes[5–9]. According to this concept, binding of catecholamines to the beta-adrenergic receptor leads to an activation of the enzyme system, whereas interaction with alpha-adrenergic receptor sites is associated with inhibition of cAMP formation. We have previously shown that the human fat cell adenylate cyclase is coupled to beta-adrenergic receptors[10]. In an attempt to clarify the role of alpha-adrenergic stimulation, the effects of phenylephrine – an alpha-adrenergic agonist – on the human fat cell adenylate cyclase activity were tested.

1 R. F. Ahlquist, Am. J. Physiol. *153*, 586 (1948).
2 E. W. Sutherland, in: Pharmacology of cholinergic and adrenergic transmission, p. 317. Ed. G. B. Koelle, W. W. Douglas and A. Carlson. Pergamon, Oxford 1965.
3 R. F. Furchgott, Fed. Proc. *29*, 1352 (1970).
4 J. Ashmore, Fed. Proc. *29*, 1386 (1970).
5 G. A. Robison, P. E. Langley and T. W. Burns, Biochem. Pharmac. *21*, 589 (1972).
6 L. A. Östman and S. Efendic, Acta med. scand. *187*, 471 (1970).
7 J. R. Turtle and D. M. Kipnis, Biochem. biophys. Res. Commun. *28*, 797 (1967).
8 G. A. Robison and E. W. Sutherland, Circulation Res. *26* and *27*, Suppl. 1, 147 (1970).
9 T. W. Burns and P. E. Langley, J. Cyc. Nucl. Res. *1*, 321 (1975).
10 H. Kather, B. Vogt and B. Simon, Klin. Wschr., submitted for publication (1976).