Heparin-releasable lipase activity of rat adrenals, ovaries and testes

Hans JANSEN* and Wim J. DE GREEF†

*Department of Biochemistry I and Internal Medicine III and †Department of Physiology II, Medical Faculty, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

(Received 8 December 1980/Accepted 20 February 1981)

The presence of NaCl-resistant, neutral triacylglycerol hydrolase (lipase) activity in rat adrenal gland, ovary and testis was studied. Both adrenals and ovaries but not testes were found to contain such a lipase. The activity of the enzyme in the adrenal gland was lowered during cortisol treatment and hypothyroidism. An elevated adrenal lipase activity was found during hyperthyroidism. Pseudo-pregnant and lactating rats had higher ovarian lipase activities than cyclic rats. Ovarian lipase activity in lactating rats was positively correlated with the serum concentrations of progesterone and of 20 α -hydroxyprogesterone and negatively correlated with the high-density-lipoprotein non-esterified cholesterol concentration. The lipase activity of adrenals and of ovaries was largely releasable from these organs by heparin and could be inhibited by an antibody against heparin-releasable liver lipase. This indicated that the lipase is extracellularly located and is similar to 'liver' lipase. A possible role of this lipase in adrenals and ovaries is discussed.

Steroid hormones are synthesized from cholesterol, acquired by synthesis *de novo*, by mobilization of intracellular cholesterol pools or by uptake from the serum compartment. Which mechanism prevails is dependent on the metabolic condition studied. During short-term activation of corticosterone production mobilization of endogenous cholesterol pools and synthesis *de novo* may occur. During long-term stimulation the adrenal gland may mainly rely on cholesterol uptake from the serum compartment (Brown *et al.*, 1979). From which lipoprotein class the cholesterol is obtained then seems to be dependent on the species. In the rat, the high-density-lipoprotein fraction is probably the main source for cholesterol in the adrenal gland (Gwynne *et al.*, 1976; Balasubramian *et al.*, 1977; Andersen & Dietschy, 1978; Gwynne & Hess, 1978), the ovaries (Andersen & Dietschy, 1978) and the testes (Andersen & Dietschy, 1978). The mechanism by which cholesterol is taken up in the organs is presently not known. The presence of high-affinity binding sites for different lipoproteins on rat adrenal cells (Gwynne & Hess, 1978; Kovanen *et al.*, 1980) suggests that binding and internalization of the lipoproteins can occur, analogous to the receptor-mediated uptake of low-density lipoproteins in other cells as described by Brown *et al.* (1979).

Recently, evidence has been offered that a lipase, releasable from the liver with heparin (liver lipase),

may be involved in the metabolism of high-density lipoproteins (Jansen, 1979; Kuusi *et al.*, 1979; Jansen *et al.*, 1980b). Under influence of the lipase, surface material (e.g. phospholipids) of high-density lipoproteins can be hydrolysed, thereby inducing a flux of (non-esterified) cholesterol to the lipase-containing tissue. In this mechanism the lipoproteins are not internalized, but converted into particles of higher density (Jansen & Hülsmann, 1980). An enzyme with characteristics similar to 'liver' lipase was found to be present in rat and human adrenals (Jansen *et al.*, 1980a). Therefore, cholesterol uptake by the adrenal gland can possibly occur via the same lipase-mediated mechanism. To test whether this also holds for other steroid-hormone-secreting organs in the rat, we studied the presence of 'liver' lipase-like activities in ovaries and testes. The activity of the enzyme in adrenals and ovaries was further studied in conditions of decreased and stimulated steroidogenesis.

Methods and materials

Animals

Female rats used were locally bred from two Wistar substrains. The animals were 3–6 months old and housed under controlled conditions (temperature 20–23°C; lights on 03:00–17:00 h). They had free access to pelleted food and tap water. Vaginal smears were taken 6 days per week and only those

rats showing regular 5-day oestrous cycles were used for experimentation.

Male rats used were of the Wistar strain and housed under the same conditions as the female rats, except that lights were on between 07:00h and 19:00h.

Animal preparations

Pseudo-pregnancy was induced by caging pro-oestrous females with vasectomized males. Pseudo-pregnancy in the rat is characterized by persistent corpus-luteum function and by interruption of cyclic ovulation (cf. Welschen *et al.*, 1975). The females were used 6–8 days later.

Another group of females was impregnated by male rats of proven fertility. Near the end of pregnancy, the animals were caged individually and inspected twice daily for the presence of pups (day 1 of lactation). The rats were used between day 8 and 14 of lactation.

The effect of cortisol on adrenal lipase activity was studied in age-matched male rats, weighing 247 ± 13 g (mean $\pm$ S.D.). The rats were divided into six groups of three rats. Hydrocortisone sodium phosphate was added to the drinking water (50 mg/500 ml). Rats received cortisol for 1 week ($n = 6$) or 4 weeks ($n = 6$), whereas one group of six rats served as controls. Drinking water was renewed every other day. All rats were weighed every week. At the day of the experiment, the weights of the control, 1 week-treated and 4 week-treated animals were 301 ± 20 g, 291 ± 21 g and 259 ± 19 g respectively.

Hypothyroidism was induced in male rats by thyroidectomy and subsequent addition of methimazole (0.5 mg/ml) to the drinking water (Hülsmann *et al.*, 1977); CaCl_2 (9 g/litre) was given to prevent hypocalcaemia. Rats were made hyperthyroid by the addition of L-tri-iodothyronine or L-thyroxine (0.6 $\mu\text{g}/\text{ml}$ and 6.6 $\mu\text{g}/\text{ml}$ respectively) together with 0.03% bovine serum to the drinking water. After 6 weeks of treatment these rats plus a control group of age-matched non-treated rats were used. Serum L-tri-iodothyronine and L-thyroxine concentrations (in ng/ml of plasma) in the rats at the time of the experiment were: control group, 0.91 ± 0.12 ng of L-tri-iodothyronine, 36.13 ± 11.42 ng of L-thyroxine; hypothyroid group, 0.28 ± 0.09 ng of L-tri-iodothyronine, 1.39 ± 1.16 ng of L-thyroxine; L-tri-iodothyronine-treated rats, 23.96 ± 7.21 ng of L-tri-iodothyronine, 14.00 ± 2.95 ng of L-thyroxine; L-thyroxine-treated group, 2.94 ± 0.38 ng of L-tri-iodothyronine, 321.3 ± 14.2 ng of L-thyroxine.

Tissue preparations

Rats were anaesthetized with diethyl ether. The blood was collected from the thoracic space after removal of the lungs. Adrenals and ovaries were excised, trimmed free of fat, weighed and placed in

2 ml of ice-cold 0.15% NaCl; heparin (10 units) (Thromboliquine; Organon, Oss, The Netherlands) was added and the organs were homogenized with a Polytron PT-10 tissue homogenizer (10 s). The homogenates were centrifuged in plastic reaction vessels in an Eppendorf 3200 centrifuge at 4°C for 2 min at 8000 g. The fatcake at the top of the tubes was removed and the clear infranatant was used for enzyme determinations. These preparations are referred to as 'tissue extracts'. In the experiment on the effect of heparin administration the rats were functionally hepatectomized by ligation of the hepatic artery and portal vein just before the injection of heparin. This was done to prevent lipase activity released from the liver influencing the measurements of the lipase activity in adrenals and ovaries. Rats were killed between 09:00h and 11:30h.

Biochemical measurements

Lipase activities were measured in the tissue extracts by determination of the amount of non-esterified fatty acids released from a trioleoyl-glycerol emulsion stabilized with gum arabic as described before (Jansen *et al.*, 1978), except that NaCl was added to a final concentration of 0.6 M during the assay. All assays were carried out in duplicate and performed within 2 h after killing the animals. Lipase activities are expressed in m-units; 1 m-unit represents the liberation of 1 nmol of fatty acid per min from the substrate.

In the experiments with anti-(liver lipase) 50 μl of the tissue extracts were incubated with 10 μl of anti-(liver lipase) or control γ -globulins for 5 min at 37°C followed by 30 min at 0°C before adding the substrate. Anti-(liver lipase) was the γ -globulin fraction of an antiserum raised against liver lipase purified from heparin-containing rat liver perfusates (Jansen *et al.*, 1980b). This antibody preparation can completely inhibit liver lipase, but does not affect lipoprotein lipases from rat heart or adipose tissue. The γ -globulin fraction of a non-immunized rabbit served as control for the antiserum. Incubation of the tissue extracts with the control γ -globulins did not influence the lipase activity (see also Jansen *et al.*, 1980a).

Concentrations of progesterone were determined by radioimmunoassay as described previously (De Jong *et al.*, 1974; Meijis-Roelofs *et al.*, 1975), and 20 α -hydroxyprogesterone concentrations were measured by radioimmunoassay with an antiserum (product code P 003) purchased from Stevanti Research, St. Albans, Herts., U.K. At least two different volumes of each plasma sample were assayed. Intra- and inter-assay variations were between 5 and 11% for both assays.

L-Tri-iodothyronine and L-thyroxine concentrations were measured by radioimmunoassay as

described by Docter *et al.* (1972) and Visser *et al.* (1975) respectively. High-density lipoproteins were separated from very-low- and low-density lipoproteins by centrifugation as described by Bronzert & Brewer (1977). Rat serum (150 μ l) was mixed with 25 μ l of KBr solution ($d = 1.32$) to a final density of 1.050. This sample was centrifuged for 3 h at 160 000 g in an air-driven centrifuge (Airfuge; Beckmann Instruments) at 4°C. The upper layer was removed by suction and in the bottom fraction, containing high-density lipoprotein, non-esterified and esterified cholesterol was measured by an enzymic assay (Boehringer Testkit Combination cat. no. 124087).

Statistical procedures

All results are given as means $\pm$ s.d.. Two-tailed Student's *t* test was used for comparing groups. Coefficients of correlation were calculated using linear regression analysis.

Chemicals

All chemicals used were of analytical grade.

Results

Neutral lipase activity in rat adrenals under various conditions

The neutral NaCl-resistant lipase activity of rat adrenals (adrenal lipase) was determined in conditions of decreased (cortisol treatment, hypothyroidism; Peterson, 1958) or accelerated (hyperthyroidism; Peterson, 1958) corticosterone secretion. Adrenal lipase activity of rats that had received cortisol during 1 week was significantly lower (39%) than of control rats. The decrease was found in the total activity of two adrenals as well per g of tissue wet. wt. (Table 1). Treatment of rats with cortisol for

4 weeks resulted in a further decrease of adrenal weight and, to the same extent, of adrenal lipase activity.

Also in hypothyroid rats, a significantly lowered adrenal lipase activity was found (76% of controls). No change in the adrenal weights was observed. In contrast, treatment with thyroid hormones (L-tri-iodothyronine or L-thyroxine) led to increased adrenal weights and adrenal lipase activity, expressed per organ as well as per tissue wet weight (Table 1).

Since diethyl ether exposure may affect adrenal function, we studied whether this could also affect the adrenal lipase activity. Six male rats were killed by decapitation with ($n = 3$) or without ($n = 3$) previous diethyl ether exposure during 10 min. No difference in the adrenal lipase activity of both groups was found (6.4 ± 0.5 m-units versus 6.3 ± 0.7 m-units respectively). Thus diethyl ether exposure for 10 min does not alter adrenal lipase activity.

Neutral lipase activity of ovaries during pseudo-pregnancy and lactation

Rat ovaries of cyclic rats during di-oestrus 1 or 2 were found to contain a low neutral lipase activity (Table 2). In pseudo-pregnant and lactating rats the ovarian lipase activity was considerably higher (up to 4-fold; Table 2). Serum concentrations of progesterone and 20 α -hydroxyprogesterone were also largely elevated in the pseudo-pregnant and lactating rats. The possible correlation between ovarian lipase activity, steroid concentration and high-density lipoprotein cholesterol was studied in lactating rats. Progesterone and 20 α -hydroxyprogesterone serum concentrations were positively correlated with ovarian lipase activity (Table 3). Ovarian lipase was negatively correlated with the serum high-density-

Table 1. Adrenal lipase activity during cortisol treatment and hypo- and hyper-thyroidism

Normally fed male rats were used. Cortisol treatment was given and hypo- and hyper-thyroidism were induced as described in the Methods and materials section. Lipase activities were measured in the tissue extracts of two adrenals. In Expt. II the extracts were pre-incubated with anti-(liver lipase) or control γ -globulins before lipase activities were determined. Total lipase activity per two adrenals and per g of tissue wet wt. are given. The values were compared with those of the non-treated animals. Abbreviations used: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$; n.d., not determined.

Expt. no.	Treatment of rats	<i>n</i>	Adrenal weight (mg)	Adrenal lipase activity		Inhibition by anti-(liver lipase) (%)
				(m-units in two adrenals)	(m-units/g wet wt.)	
I	None	6	40 $\pm$ 12	7.0 $\pm$ 2.0	179 $\pm$ 24	n.d.
	Cortisol (1 week)	6	31 $\pm$ 8	4.2 $\pm$ 1.2*	138 $\pm$ 21**	n.d.
	Cortisol (4 weeks)	6	22 $\pm$ 8*	2.6 $\pm$ 1.8***	118 $\pm$ 54	n.d.
II	None	4	45 $\pm$ 2	7.4 $\pm$ 1.2	164 $\pm$ 24	82 $\pm$ 6
	Hypothyroid	4	47 $\pm$ 6	5.6 $\pm$ 0.8*	117 $\pm$ 12*	78 $\pm$ 3
	Hyperthyroid (L-tri-iodothyronine)	4	62 $\pm$ 10*	14.2 $\pm$ 1.2***	229 $\pm$ 26**	75 $\pm$ 6
	Hyperthyroid (L-thyroxine)	4	65 $\pm$ 4*	14.8 $\pm$ 1.6***	227 $\pm$ 36*	74 $\pm$ 5

Table 2. *Ovarian lipase activity and serum concentrations of progesterone and 20 α -hydroxyprogesterone in cyclic (dioestrus), pseudo-pregnant and lactating rats*

Female rats during dioestrus, pseudo-pregnancy or lactation were bled under diethyl ether narcosis. The ovaries were weighed and lipase activity was determined in the tissue extracts after pre-incubation with anti-(liver lipase) or control γ -globulin. The effect of anti-(liver lipase) on the lipase activity is given as a percentage of the total activity in two ovaries. In the sera progesterone and 20 α -hydroxyprogesterone concentrations were measured. Results indicated by *a* were significantly different from those indicated by *b* at $P < 0.001$.

Endocrine state of the rats	<i>n</i>	Ovarian lipase activity		Lipase activity inhibitable by anti-(liver lipase) (%)	Serum progesterone (ng/ml)	Serum 20 α -hydroxyprogesterone (ng/ml)
		(m-units in two ovaries)	(m-units/g wet wt.)			
Control (dioestrus)	4	3.8 $\pm$ 0.8 ^a	36 $\pm$ 4 ^a	53 $\pm$ 18	28 $\pm$ 4	61 $\pm$ 8
Pseudo-pregnant	4	10.5 $\pm$ 1.8 ^b	100 $\pm$ 10 ^b	89 $\pm$ 7	137 $\pm$ 7	64 $\pm$ 13
Lactating	5	14.2 $\pm$ 2.8 ^b	122 $\pm$ 7 ^b	84 $\pm$ 3	117 $\pm$ 27	133 $\pm$ 25

Table 3. *Serum high-density-lipoprotein cholesterol, progesterone and 20 α -hydroxyprogesterone concentrations and ovarian lipase activity in lactating rats*

A group of 29 lactating rats was bled under diethyl ether narcosis. Tissue extracts were prepared from the ovaries. In the extracts lipase activities were measured. In the serum progesterone and 20 α -hydroxyprogesterone concentrations were determined. In 20 rats non-esterified and esterified cholesterol concentrations were measured in the serum lipoprotein fraction $d > 1.050$ (high-density lipoproteins). Correlations between all parameters of the different rats were calculated. The correlation coefficients and levels of significance are given for those regression lines with $P < 0.05$.

	<i>n</i>	Mean value $\pm$ S.D.	Range
(1) Ovarian lipase activity (m-units/two ovaries)	29	11.1 $\pm$ 3.6	7.2–18.2
(2) Progesterone (ng/ml of serum)	29	62.1 $\pm$ 30.2	24–137
(3) 20 α -hydroxyprogesterone (ng/ml of serum)	29	48.9 $\pm$ 16.8	26–89
(4) Progesterone + 20 α -hydroxyprogesterone (ng/ml of serum)	29	110.4 $\pm$ 45.7	52–226
(5) High-density-lipoprotein cholesterol			
(a) Non-esterified (mM)	20	0.70 $\pm$ 0.17	0.42–1.05
(b) Esterified (mM)	20	1.25 $\pm$ 0.16	1.02–1.71
(c) Non-esterified + esterified (mM)	20	1.95 $\pm$ 0.19	1.70–2.33
Correlations:			
(1) versus (2)		$r = +0.59$	$P < 0.005$
(1) versus (3)		$r = +0.74$	$P < 0.001$
(1) versus (4)		$r = +0.66$	$P < 0.001$
(1) versus (5a)		$r = -0.66$	$P < 0.005$
(2) versus (3)		$r = +0.85$	$P < 0.001$
(3) versus (5a)		$r = -0.45$	$P < 0.05$
(5a) versus (5b)		$r = +0.64$	$P < 0.01$

lipoprotein non-esterified cholesterol content, but not with the cholesteryl ester concentration in these particles. A weakly negative correlation was found between high-density lipoprotein non-esterified cholesterol and 20 α -hydroxyprogesterone. Progesterone and 20 α -hydroxyprogesterone concentrations were significantly correlated ($r = 0.85$, $P < 0.001$).

Localization of neutral lipase activity in adrenals and ovaries

Extracellularly located lipases of different organs can be released from the tissues by heparin

(Augustin & Greten, 1979). To study the cellular localization of the adrenal and ovarian lipases, the effect of heparin administration on the lipase activity in the organs was determined. In adrenals excised 2 min after heparin injection the lipase activity was 80–88% lower than in the adrenals removed just before the injection (Table 4). Ovarian lipase activity of lactating rats was lowered 25% by heparin treatment. Injection of a higher dose of heparin (150 units/rat) resulted in a release of 71% of the ovarian lipase activity in 5 min. Injection of 0.15 M-NaCl without heparin did not influence adrenal or ovarian lipase activities (results not shown).

Table 4. *Effect of heparin administration on neutral lipase activity in rat adrenals and ovaries*

Normal male or lactating female rats were used. One adrenal and/or ovary was excised under diethyl ether narcosis and placed in ice-cold 0.15 M-NaCl. The hepatic artery and portal vein were ligated and heparin (50 or 150 units) was administered through the vena femoralis. After 2 or 5 min the other adrenal and/or ovary was removed. To exclude possible systematic differences between left and right adrenals and ovaries, alternatively a left and right organ was taken before heparin injection. The level of significance in the fall of lipase activity after heparin injection was calculated by using the two-tailed paired Student's *t* test, comparing adrenals and ovaries of the same rat before and after heparin administration. **P* < 0.05; ***P* < 0.001.

Organ		Amount of heparin injected (units)	Time of heparin treatment (min)	Lipase activity (m-units/adrenal or ovary)		Heparin-releasable activity (% of total)
				Before heparin treatment	After heparin treatment	
Adrenals	2 Male	50	2	4.5 (4.1, 4.9)	0.9 (0.8, 1.0)	80 (80, 80)
	4 Female	50	2	11.2 ± 3.0	1.3 ± 0.4	88**
Ovaries	5 Female	50	2	6.1 ± 1.5	4.6 ± 1.3	25*
	5 Female	150	5	7.3 ± 2.5	2.1 ± 0.1	71**

Fig. 1. *Elution pattern of ovarian and liver lipase from a Sepharose-heparin column by NaCl*

Ovaries of two lactating rats were extracted with 5 ml of heparin containing 0.15 M-NaCl as described in the Methods and materials section. Of the extract 4 ml (9.5 m-units of lipase activity) was applied to a Sepharose-heparin column (0.9 cm × 3 cm). Lipase was eluted from the column with a linear salt gradient (40 ml, 0–2 M-NaCl; ----). Of the lipase activity bound to the column (8.5 m-units) 80% was recovered in the fractions as indicated (O). For comparison the elution pattern of lipase from a rat liver acetone-powder extract (60 mg) is given (●). Experiments were carried out exactly as described previously (Jansen *et al.*, 1980a).

Nature of ovarian and adrenal lipase activity

The nature of the adrenal lipase activity has been studied using antibodies raised against heparin-releasable liver lipase [anti-(liver lipase)] and Sepharose-heparin affinity chromatography (Jansen *et al.*, 1980a). Tables 1 and 2 show that the major part of the ovarian as well as of the adrenal lipase activity is inhibited by anti-(liver lipase) in the conditions studied except for the ovarian lipase during di-

oestrus. Of the lipase activity in the extracts of the ovaries of lactating rats 85% could be bound to a Sepharose-heparin column (Fig. 1). The activity was eluted from the column by a linear salt gradient, in the same fractions as liver lipase with a maximum at 0.73 M-NaCl. About five times less lipase activity was eluted from the column when ovaries of cyclic rats were used (results not shown).

Neutral lipase in rat testis

In testis of normal rats no liver-lipase-like activity could be detected. Since only about 3% of the total testis mass consists of testosterone-producing Leydig cells, it was possible that a lipase activity, residing in these cells only, was not measurable in our assay. Therefore, lipase activity was also measured in a number of preparations enriched in Leydig cells. Neither in a testosterone-secreting Leydig cell tumour nor after dissection of tubular material of normal testis anti-(liver lipase)-inhibitable activity was detectable.

Discussion

Several organs contain extracellularly located lipase activity that can be released from the tissues by heparin (cf. Augustin & Greten, 1979). Two types of heparin-releasable lipases have been characterized as 'lipoprotein lipase' in extrahepatic tissues and 'liver lipase' in the liver. These two enzymes are distinguishable by a number of criteria. Liver lipase is resistant to high NaCl concentration *in vitro*, but lipoprotein lipase is not. Both lipases bind to Sepharose-heparin columns. However, liver lipase is eluted at a lower salt concentration (0.7 M-NaCl) than lipoprotein lipase (1.15 M-NaCl). Antibodies raised against the liver enzyme do not affect lipoprotein lipase from different organs, but inhibit

liver lipase *in vitro* and *in vivo* (Jansen & Hülsmann, 1974; Jansen *et al.*, 1980b). In the present paper it is shown that rat adrenals and ovaries contain heparin-releasable lipase activity different from lipoprotein lipase and similar to liver lipase according to the criteria mentioned above. Whether the enzymes from the different organs are identical or only share certain characteristics and immunological determinants is not clear yet. More time was needed and more heparin had to be injected to provoke the release of the lipase from the ovaries than from adrenals and from liver. This may indicate a different type of binding of lipase to the cells, caused by differences in binding sites or in the enzyme or may just reflect the relatively low blood flow through the ovaries (De Greef *et al.*, 1975). The lipases are indicated as adrenal, ovarian or liver lipase dependent on the tissue source. A more definite name may be given when the function of the enzymes has been established with certainty.

From the data it cannot be concluded whether the liver lipase-like enzyme is the only heparin-releasable lipase in adrenals and ovaries, since by the assay system employed liver lipase is selectively measured apart from lipoprotein lipase (Huttunen *et al.*, 1975).

Previously no indication of the presence of lipoprotein lipase in rat adrenals was found (Jansen *et al.*, 1980a). Part of the lipase activity in the tissue extracts was not inhibited by anti-(liver lipase) nor bound to Sepharose-heparin. This can indicate the presence of another neutral salt-resistant lipase or another form of the same enzyme. During stimulation of the steroid production by a corticotropin- and prolactin-secreting tumour the adrenal lipase activity was found to be enhanced (Jansen *et al.*, 1980a). This led to the suggestion that the activity of the adrenal lipase is related to the level of steroidogenesis. To elaborate this idea further, adrenal and ovarian lipase activities were measured in conditions with altered steroid-hormone production rates. Thyroid hormones may increase corticosteroid turnover and synthesis (Peterson, 1958; Melby *et al.*, 1960; Labrie *et al.*, 1968). During experimentally induced hyperthyroidism adrenal lipase activity was increased, while in hypothyroidism a lowered adrenal lipase activity was found.

Adrenal corticosteroid secretion can be suppressed by glucocorticoids *in vivo* and *in vitro* (Peron *et al.*, 1960; Fekete & Georog, 1963). Treatment of rats with hydrocortisone resulted in a decreased adrenal lipase activity. Long-term enhanced steroidogenesis in the corpora lutea can be induced by (pseudo)-pregnancy and lactation (Table 2). The antibody-inhibitable ovarian lipase activity in pseudo-pregnant or lactating rats was increased proportionally to the serum concentrations of progesterone and 20 α -hydroxyprogesterone (Table

2). The metabolic clearance rate of progesterone is not influenced by the endocrine state of the female rats (Pepe & Rotchild, 1973) so that the serum concentrations of progesterone are a reflection of the hormone secretion rate by the ovaries (De Greef & Zeilmaker, 1974). Thus in all conditions studied the antibody-inhibited lipase activity of adrenals and ovaries did change concurrently with the steroid-hormone synthesis in organs. An even more direct relationship between the lipase activity and steroid hormones was found in lactating rats, where a significant positive correlation between ovarian lipase activity and serum concentrations of progesterone plus 20 α -hydroxyprogesterone was found. These data suggest again a relationship between lipase activity and the level of steroidogenesis in adrenals and ovaries. The metabolic function of the lipase is not known. It has been suggested that liver lipase plays a role in the uptake of cholesterol from plasma lipoproteins by the liver (Jansen, 1979; Kuusi *et al.*, 1979; Jansen & Hülsmann, 1980). In the rat steroid hormones are synthesized predominantly from cholesterol taken up from the serum (Andersen & Dietschy, 1978). Therefore the data support a function of the lipase in the uptake of serum cholesterol by adrenals and ovaries similar to that proposed for the liver (Jansen & Hülsmann, 1980).

We thank Dr. H. J. van der Molen for the gift of the antiserum used in the progesterone assay, Miss Paula van der Vaart and Miss Ina Kalkman for expert technical assistance, Dr. E. P. Krenning for help in the experiments with hypo- and hyper-thyroid rats, Dr. F. G. Rommerts for help in the experiments with testicular tissues and Dr. F. H. de Jong for helpful discussions. We are grateful to Dr. W. C. Hülsmann and Dr. J. C. Birkenhäger for help and advice throughout the study. Miss Cecile Hanson and Mrs. Ankie Bos are thanked for preparing the manuscript.

References

- Andersen, J. M. & Dietschy, J. M. (1978) *J. Biol. Chem.* **253**, 9024–9032
- Augustin, J. & Greten, H. (1979) *Prog. Biochem. Pharmacol.* **15**, 41–66
- Balasubramian, S., Goldstein, J. L. & Foust, J. R. (1977) *J. Biol. Chem.* **252**, 1771–1779
- Bronzert, T. J. & Brewer, H. B. (1977) *Clin. Chem.* **23**, 2089–2098
- Brown, M. S., Kovanen, P. T. & Goldstein, J. L. (1979) *Recent Prog. Horm. Res.* **35**, 215–256
- De Greef, W. J., Zeilmaker, C. H. (1974) *Endocrinology* **95**, 565–569
- De Greef, W. J., Dullaart, J. & Zeilmaker, G. H. (1975) *J. Endocrinol.* **66**, 249–256
- De Jong, F. H., Baird, D. T. & van der Molen, H. G. (1974) *Acta Endocrinol. (Copenhagen)* **77**, 575–587

- Docter, R., Henneman, G. & Bernard, H. F. (1972) *Isr. J. Med. Sci.* **8**, 1870–1874
- Fekete, G. Y. & Georog, P. (1963) *J. Endocrinol.* **27**, 123–126
- Gwynne, J. T. & Hess, B. (1978) *Metabolism* **27**, 1593–1600
- Gwynne, J. T., Mahaffee, D., Brewer, H. B. & Ney, R. L. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4329–4333
- Hülsmann, W. C., Oerlemans, M. C. & Geelhoed-Mieras, M. M. (1977) *Biochem. Biophys. Res. Commun.* **79**, 748–788
- Huttunen, J. K., Ehnholm, C., Kinnunen, P. K. J. & Nikkilä, E. A. (1975) *Clin. Chim. Acta* **63**, 335–347
- Jansen, H. (1979) in *Edition des Colloques de l'Inserm: Obesity, Cellular and Molecular Aspects* (Ailhaud, G., ed.), vol. 87, pp. 137–147
- Jansen, H. & Hülsmann, W. C. (1974) *Biochim. Biophys. Acta* **369**, 387–396
- Jansen, H. & Hülsmann, W. C. (1980) *Trends Biochem. Sci.* **5**, 265–268
- Jansen, H., van Berkel, Th. J. C. & Hülsmann, W. C. (1978) *Biochem. Biophys. Res. Commun.* **85**, 148–152
- Jansen, H., Kalkman, C., Birkenhäger, J. C. & Hülsmann, W. C. (1980a) *FEBS Lett.* **112**, 30–34
- Jansen, H., van Tol, A. & Hülsmann, W. C. (1980b) *Biochem. Biophys. Res. Commun.* **92**, 53–59
- Kovanen, P. T., Goldstein, J. L., Chappel, D. A. & Brown, M. S. (1980) *J. Biol. Chem.* **255**, 5591–5598
- Kuusi, T., Kinnunen, P. K. J. & Nikkilä, E. A. (1979) *FEBS Lett.* **104**, 384–388
- Labrie, F., Pelletier, G., Labrie, R., Ho-Kim, M. A., Delgado, A., MacIntosh, B. & Fortier, C. (1968) *Ann. Endocrinol.* **29**, 29–43
- Meijs-Roelofs, H. M. A., De Greef, W. J. & Uilenbroek, J. Th. J. (1975) *J. Endocrinol.* **64**, 329–336
- Melby, J. C., Egdaahl, R. H., Story, J. L. & Spink, W. W. (1960) *Endocrinology* **67**, 389–393
- Pepe, G. J. & Rotchild, I. (1973) *Endocrinology* **93**, 1200–1205
- Peron, F. G., Moncloa, F. & Dorfman, R. I. (1960) *Endocrinology* **67**, 379–388
- Peterson, R. E. (1958) *J. Clin. Invest.* **37**, 736–743
- Visser, T. J., van Hout-Goemaat, N. L. & Docter, R. (1975) *Neth. J. Med.* **18**, 111–115
- Welschen, R., Osman, P., Dullaart, J., De Greef, W. J., Uilenbroek, J. Th. J. & De Jong, F. H. (1975) *J. Endocrinol.* **64**, 37–47