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ENDOTOXIN-INDUCED CHANGES IN SEX STEROID HORMONE LEVELS IN MALE RATS

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Summary—Intravenous administration of *Escherichia coli* endotoxin (ENDO) was found to induce profound time and dose dependent changes in the serum steroid hormones, oestrone (E_1), oestradiol (E_2), corticosterone (B), progesterone (P_4), 17α-OH progesterone ($17\alpha OHP_4$), and testosterone (T) of intact male rats. These changes were rapid, with a maximal response at 2 h and a return to close to normal values by 4 h. Non-lethal doses (0.01–2 mg/kg) of ENDO induced large increases in oestrogens (3–9-fold), P_4 (4-fold) and B (2–3-fold) and decreased serum T (2-fold). The greatest increase in E_2 level was seen with an ENDO dose of 2 mg/kg. Serum E_1, E_2 and T did not change in response to lethal ENDO doses (4–8 mg/kg); B, P_4 and $17\alpha OHP_4$ levels alone were moderately elevated. Systemic mean arterial pressure was unchanged, except at the highest ENDO dose used. Thus, the hormonal responses are unlikely to be the result of hemodynamic changes. Low doses of ENDO did not produce an increase in serum E_1 and E_2 in adrenalectomized or orchidectomized rats. These results indicate that oestrogens are largely produced in the testis. The aromatization of the testicular and adrenal androgens can be stimulated by glucocorticoid.

INTRODUCTION

A dramatic increase in female hormones, particularly oestrone (E_1) and oestradiol (E_2), has been observed in the human male in septic shock [1]. The increase in oestrogens in this pathophysiological situation also appears to be closely correlated with the severity and prognostic indices of the shock itself. The release of glucocorticoid as a result of stimulation of the adrenal cortex by ACTH in septic shock is well documented [2–4]. However, the response pattern and the origin of female sex hormones, as well as the part they play in the response to shock remain to be defined.

The present study was designed to follow the temporal changes in serum steroid hormone levels following non-lethal and lethal doses of *Escherichia coli* endotoxin (ENDO) in an experimental animal model, the rat. The participation of the adrenal glands and testes in the response was assessed by following the effect of ENDO on adrenalectomized (ADX) or orchidectomized (ORCHI) male rats.

EXPERIMENTAL

Animals

One hundred and fifty-five intact male Wistar rats weighing 250–300 g, sixty adrenalectomized (ADX) male rats and forty orchidectomized (ORCHI) male rats were used. Rats were adrenalectomized or orchidectomized under mild ether anesthesia. They were allowed to recover from surgery for a period of 1 week, during which time the ADX animals were given 0.9% NaCl solution as drinking water to prevent serum hormonal and hemodynamic changes resulting from NaCl depletion [5]. All experiments were conducted under urethane (1.2 g/kg, i.p.) anesthesia, as this anesthetic has been shown to be without effect on the response to ENDO [6].

Endotoxin administration and lethality

Various doses of *Escherichia coli* ENDO (0127 B8, Sigma) were administered i.v. at 10 a.m. to groups of 7–15 male rats. Intact, ADX and ORCHI control rats were given vehicle (0.9% saline, 0.1 ml/100 g body wt). Preliminary experiments showed that the highest doses were not lethal until 4 h after injection and that the rats do not develop the shock syndrome of severe systemic hypotension in response to the doses of ENDO used. As previously reported [6], the lethal doses were from 2 mg/kg (sub-lethal dose) in normal and ORCHI rats ($LD_{50} = 9$ mg/kg) and 0.01 mg/kg in ADX rats ($LD_{50} = 0.025$ mg/kg).

Systemic arterial pressure measurements

A polyethylene catheter was introduced into the right carotid artery and the mean arterial pressure was measured with a pressure transducer (Statham P13) connected to a Beckman Dynograph recorder.

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Recordings were made at 1, 2 and 4 h after vehicle or ENDO injection.

Mean arterial pressure was 89 ± 5 mmHg at 1 h post-ENDO (2 mg/kg), 91 ± 7 mmHg at 2 h and 109 ± 6 mmHg at 4 h. This final figure was slightly, but significantly ($P < 0.01$) larger (15 mmHg) than the control value (94 ± 3 mmHg) at 4 h.

Mean arterial pressure was measured 2 h after saline or ENDO injection. Except for the highest lethal dose (8 mg/kg), which caused a statistically significant ($P < 0.01$) decrease (20 mmHg), injection of ENDO did not alter mean arterial pressure.

The mean arterial blood pressure of control ADX rats was not significantly different from that of intact rats because the ADX rats were maintained on drinking water containing sodium chloride. The lowest dose of ENDO used (0.01 mg/kg) induced a drop in blood pressure 1 h after ENDO injection (70 ± 6 mmHg) ($P < 0.001$) which had returned to normal by 2 h (84 ± 8 mmHg) and risen above normal by 4 h [106 + 4 mmHg] ($P < 0.01$). Thus, the variations in blood pressure were relatively small (−23 to +13 mmHg).

Blood sampling

Animals were bled at 1, 2 and 4 h after injection. A single blood sample was taken from each animal to minimize hemodynamic changes resulting from repeated stress or blood loss. The blood samples were allowed to coagulate before separation of the serum by centrifugation (1500 *g* for 10 min at 4°C). Serum samples were stored at −20°C until assayed.

Steroid extraction and chromatographic fractionation

Serum samples (1 ml) were extracted three times with organic solvent (ethyl acetate–cyclohexane, v/v) and the aqueous phase removed by freezing (−20°C). The organic extracts were evaporated to dryness, taken up in 1 ml of solvent-system I (benzene–ethanol, 95:5) and placed on Sephadex LH20 microcolumns (0.5 × 6 cm). Free fatty acids, P_4 and androstenedione were first eluted with 3 ml of solvent I. E_1 and B were then eluted with 2.5 ml of solvent I, followed by 1.5 ml of solvent II (benzene–ethanol, 90:10). Finally, E_2 was eluted with 6 ml solvent II.

T and 17αOHP$_4$ were eluted from a second column of Sephadex LH20 with 5.5 ml of solvent I followed by 1 ml of solvent II. The fractions were evaporated to dryness and dissolved in RIA buffer for steroid hormone assays.

The yields from these extraction and purification steps were between 70 and 95%.

Radioimmunoassay (RIA) of steroids

Sephadex LH20-purified samples of E_1, E_2, P_4, 17αOHP$_4$, B, T and adrostenedione were assayed by RIA using rabbit anti E_1 6-thyroglobulin serum (Miles, Yeda Ltd, Israel); anti 17βE_2 6–CMO BSA serum (Institut Pasteur, France); anti P_4 11 hemisuccinate BSA serum (Institut Pasteur, France); anti 17αOHP$_4$-BSA (Miles, Yeda Ltd, Israel); anti B 21 hemisuccinate -BSA serum (Institut Pasteur, France); anti T 7αBSA serum (Miles, Yeda Ltd, Israel); anti androstenedione-7αBSA serum (Miles, Yeda Ltd, Israel).

At least two determinations were made on each serum sample and 5–10 samples were used for each experimental point. The detection limit was 37 pmol/l in all cases.

The tritiated steroids [2, 4, 6, 7-^{3}H]E_2 (91 Ci/mmol); [2, 4, 6, 7-^{3}H]E_1 (98 Ci/mmol); [1, 2, 6, 7-^{3}H]T (107 Ci/mmol): [1, 2, 6, 7-^{3}H]P_4 (104 Ci/mmol); [1, 2, 6, 7-^{3}H]B (91 Ci/mmol); [1, 2, 6, 7–^{3}H]17α-OH P_4 (80 Ci/mmol); [1, 2, 6, 7-^{3}H]androstenedione (90 Ci/mmol) were purchased from the Radiochemical Centre, Amersham. All were 99% pure; purity was checked by thin-layer chromatography.

Radioactivity was determined on samples dissolved in 4 ml PCSII (Amersham) by counting in an Intertechnique PG4000 scintillation spectrometer, using the internal standard for quench correction.

Statistics

All data were analysed by analysis of variance (ANOVA), followed by the Duncan–Kramer test [7, 8]. Results were considered significant when the probabilities were: $*P < 0.05$: $**P < 0.01$; $***P < 0.001$.

RESULTS

Response of normal rats to ENDO

Time-course of the hormonal response (Fig. 1). The levels of all the steroid hormones tested, except T, increased in response to a single sub-lethal dose of ENDO (2 mg/kg), and were maximal at 2 h after

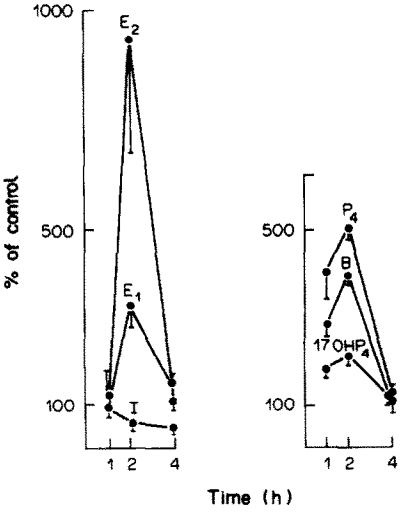

Fig. 1. Time-course of the hormone response in intact male rats. Hormone levels (% of control) were determined by RIA 1, 2 and 4 h after ENDO injection (2 mg/kg). E_1, oestrone; E_2, oestradiol; T, testosterone; 17αOHP$_4$, 17α-hydroxyprogesterone; B, corticosterone; P_4, progesterone.

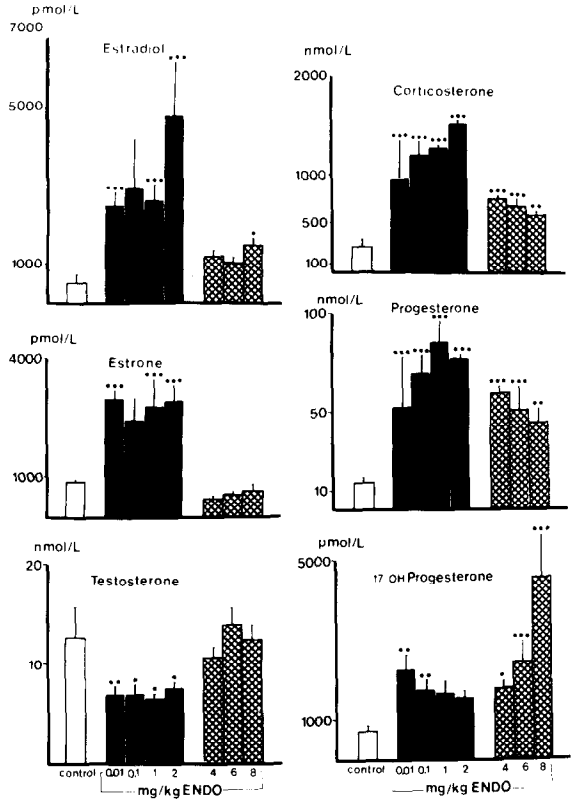

Fig. 2. Hormone levels 2 h after adminstration of non-lethal (0.01–2 mg/kg) and lethal (4–8 mg/kg) doses of ENDO to intact rats. Hormone concentrations, expressed as pmol/l and nmol/l, were determined by RIA. Each point represents the mean ± SEM of 5–8 determinations. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

ENDO injection. The levels of B, P_4 and $17\alpha OHP_4$ increased faster than those of E_1 and E_2, but the largest increase was seen in E_2 (+900%), while the smallest was $17\alpha OHP_4$ (+200%). Most levels had returned to baseline by 4 h post-ENDO. However, T slowly decreased throughout the test period (−50%).

Hormone levels 2 h after administration of ENDO. Serum hormone levels were measured 2 h after single doses of ENDO ranging from 0.01 to 8 mg/kg. The results obtained for non-lethal doses (0.01–2 mg/kg) and lethal (4–8 mg/kg) ENDO doses are shown in Fig. 2.

Oestrogen levels were extremely sensitive to ENDO, being raised between 3.5 and 5-fold by even the lowest dose used (0.01 mg/kg). However, there was little further increase in oestrogen level throughout the sub-lethal dose range, except for E_2, which showed a sharp peak at 2 mg/kg. Lethal doses (4–8 mg/kg) did not produce increased plasma oestrogen levels.

The dose–response for T was almost the mirror image of those for the oestrogens. The level of T remained lower (50%) than control with non-lethal doses and was not different from control at the highest doses (4–8 mg/kg). The levels of B and P_4 were both increased approx. 3-fold by the lowest ENDO dose given and showed a graded increase with ENDO doses up to 1–2 mg/kg.

Increasing ENDO doses in the lethal range (4–8 mg/kg) produced progressively smaller increases in B and P_4. $17\alpha OHP_4$ was elevated to essentially the same extent by all non-lethal doses (up to 2 mg/kg), with maximum stimulation (6-fold increase) at the highest lethal dose.

Response of adrenalectomized rats to ENDO

Table 1 summarizes the hormonal variations in ADX rats 2 h after saline (control ADX) or ENDO injections. The B and P_4 levels in control ADX rats were markedly depressed, while E_2 levels were increased 3-fold ($P < 0.01$) compared to sham ADX rats.

Previous studies have shown that ADX rats are 200 times more responsive to the lethal effects of ENDO [9]. Thus the 0.01 mg/kg dose corresponds to a non-lethal dose and the 0.02 and 0.03 mg/kg doses to lethal doses. Hormone levels (E_1, E_2, P_4, B, $17\alpha OHP_4$ and T) were not significantly changed compared to control ADX rats, 2 h after injection of either lethal or non-lethal doses of ENDO.

Response of orchidectomized rats to ENDO

The levels of the androgens, T and androstenedione, were very low in ORCHI rats compared to those of sham-ORCHI rats (Table 2). The injection of ORCHI rats with ENDO did not induce significant increases in oestrogen levels and caused no significant changes in the serum levels of $17\alpha OHP_4$, T or androstenedione. Only the level of B was significantly increased (2-fold) by a lethal dose of ENDO, while the level of P_4 decreased (2-fold, $P < 0.001$) following both lethal and non-lethal doses of ENDO.

Table 1. Hormonal response of ADX rats to endotoxin administration

		P_4 nmol/l	$17\alpha OHP_4$ pmol/l	B nmol/l	T nmol/l	E_1 pmol/l	E_2 pmol/l
Sham ADX		17 ± 1	1280 ± 120	426 ± 50	13 ± 1.7	890 ± 54	510 ± 210
ADX		2.5 ± 0.3	1436 ± 494	15 ± 6	11.2 ± 1.5	1050 ± 215	1790 ± 198**
ENDO	0.01 mg/kg	9.5 ± 3	918 ± 200	62 ± 26	10 ± 1.5	1076 ± 314	1156 ± 92
	0.02 mg/kg	3.2 ± 0.6	1366 ± 433	16 ± 6	16.5 ± 1.5	1117 ± 288	1545 ± 271

Serum hormone levels in sham ADX, control ADX and test ADX male rats were measured 2 h after administration of increasing non-lethal (0.01 mg/kg) and lethal (0.02 mg/kg) doses of ENDO. Each value represents the mean ± SEM of 5–8 determinations.

$**P < 0.01$.

Table 2. Hormonal response of ORCHI rats to endotoxin administration

		P_4 nmol/l	$17\alpha OHP_4$ pmol/l	B nmol/l	Androstenedione nmol/l	T nmol/l	E_1 pmol/l	E_2 pmol/l
Sham ORCHI		17.8 ± 1.6	1424 ± 176	354 ± 26	1.5 ± 0.1	10.4 ± 1.2	520 ± 52	360 ± 54
ORCHI		28.3 ± 3.2	1272 ± 148	522 ± 57	0.9 ± 0.1	1.8 ± 0.1	480 ± 25	310 ± 50
ENDO	0.01 mg/kg	14.6 ± 1.4	1654 ± 144	630 ± 81	0.9 ± 0.1	1.9 ± 0.1	505 ± 40	340 ± 48
	2 mg/kg	11.1 ± 0.8***	1430 ± 118	1031 ± 86***	0.7 ± 0.1	1.9 ± 0.1	560 ± 52	310 ± 50
	6 mg/kg	12.7 ± 1.3***	1345 ± 80	815 ± 47**	0.7 ± 0.1	1.9 ± 0.1	410 ± 20	250 ± 65

Serum hormone levels were measured 2 h after administration of increasing non-lethal doses (0.01 and 2 mg/kg) and lethal doses (6 mg/kg) of ENDO. Each value represents the mean ± SEM of 10 determinations.

P_4, progesterone; $17\alpha OHP_4$, 17α OH-hydroxyprogesterone; B, corticosterone; androstenedione; T, testosterone: E_1, oestrone; E_2, oestradiol.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

DISCUSSION

The results indicate that acute i.v. administration of ENDO to male rats induces profound changes in the serum steroid hormone profile, most notably in the levels of female sex hormones. These modifications are time and dose-dependent. The changes in steroid hormone levels are rapid, with significant changes at 1 h, a maximal response at 2 h and a return to close to normal values by 4 h.

A marked discontinuity in the response to increasing doses of ENDO was observed. Non-lethal doses (0.01–2 mg/kg) induced large increases in oestrogens (3–9 fold), P_4 (4-fold) and B (2–3-fold) and decreased serum T (2-fold). A fall in T level has been reported in certain situations [10–12], and a parallel increase in oestrogen levels has been described during physical exercise [13], but not in septic shock. The most striking increase in E_2 level, up to 4750 ± 1350 pmol/l, was seen with an ENDO dose of 2 mg/kg. The large variability in the response, as reflected in the standard deviation (SD), suggests a certain population heterogeneity. Some animals appeared to be very responsive, and other less so, to this critical dose, which seems to be the transition point between non-lethal and lethal ENDO effect.

The response to lethal ENDO doses (4–8 mg/kg) was quite different, in that no increase in serum oestrogen or testosterone were seen, even within 1 h after ENDO injection (results not shown), B, P_4 and $17\alpha OHP_4$ levels alone were elevated compared to control values. These changes in steroid hormone levels were all accompanied, except at the very highest ENDO dose used (8 mg/kg), by no change in the systemic mean arterial pressure. Thus, the observed hormonal responses is unlikely to be the result of hemodynamic changes.

The action of ENDO on steroid synthesis is only partially documented. The observed discontinuity on hormonal response to non-lethal and lethal dosed of ENDO in male rats suggests that different mechanisms of action of ENDO on steroid metabolic pathways may be involved. Thus, non-lethal doses of ENDO could stimulate the adrenal cortex of male rats by an enhanced production of ACTH and PgI2 or a release of pro-opiomelanocortin-related peptides (POMC) in the testis [12, 14–17] leading to increased glucocorticoid and androgen production. The observed increases in oestrogen and decreases in T may result from potentiation of testicular aromatase activity by glucocorticoid. This does not exclude the possibility of participation of POMC peptides synthesized by testicular Leydig cells and their receptors in the modulation of testosterone secretion by the testis, as has been recently demonstrated [12, 17].

The role of the adrenal cortex in these steroid hormone perturbations was examined using ADX rats, which are 200 times more responsive to ENDO than intact rats [9]. The very low levels of B and P_4 in control ADX rats indicates the effectiveness of adrenalectomy. The 3-fold increase in E_2 level of control ADX rats over that of sham ADX rats injected with saline suggests that the oestrogens were of extra-adrenal origin. Similar results have been reported for female rats under surgical stress [14]. Low doses of ENDO did not produce an increase in serum E_1 and E_2 in ADX male rats, as they did in intact males. This lack of response may be because progesterone and glucocorticoids were absent, so that the aromatase system was not stimulated [18]. The difference in response between intact and ADX rats suggests that the adrenals play a crucial role in the elevation of oestrogen levels in intact rats treated with ENDO.

No changes in the serum oestrogen levels were seen following administration of non-lethal doses of ENDO to ORCHI rats. This would suggest that there is an active participation of testicular aromatase in the increase in phenol steroid levels induced by ENDO. However, the basal oestrogen level observed in ORCHI rats could result from the aromatization of androgen originating from the adrenal or peripheral tissues, such as lung or adipose tissue [18]. There was also a significant decrease in P_4 level after ENDO administration to ORCHI rats, while the P_4 level of intact rats increased. This difference may be explained by the presence, in the intact rat, of testicular

factors which could either stimulate P_4 synthesis or decrease P_4 catabolism and metabolic clearance.

The effects of lethal ENDO doses in intact male rats, i.e. normal levels of oestrogen and T, may be the result of adrenal depression [19]. This may be due to the induction by ENDO of a plasma factor inhibiting the ACTH binding to its specific adrenal receptors. The consecutive depressed stimulation of the cycloadenylate system and the consequent lower production of pregnenolone, the common precursor of glucocorticoids and androgens, could explain the unchanged levels of oestrogens and testosterone [20, 21]. Furthermore, the observed increase in $17\alpha OHP_4$ levels after a lethal ENDO dose suggests that ENDO has an effect on the steps involved in the metabolism of $17\alpha OHP_4$.

This work provides evidence for adrenal–testicular cooperation in the hormonal response to ENDO, particularly in the elevation of oestrogens and the drop in T levels. It would also appear that the changes in hormone levels depend on the size of the endotoxin stimulus. The results may lead to a better understanding of the pathophysiological mechanisms underlying the elevation of oestrogen levels observed in human septic shock and myocardial infarct [1, 22, 23] and the relationship between the adrenal glands and the testes in these conditions.

The exact metabolic pathways by which the conversion of androgens of testicular and adrenal origin to oestrogens takes place in the testis or non-endocrine tissue during endotoxic shock or in non-shock situations requires further investigation. It would also be very interesting to know whether the oestrogens and androgens have a deleterious or protective role in these pathophysiological conditions [24, 25].

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REFERENCES

1. Benassayag C., Christeff N., Auclair M. C., Vernimmen C., Carli-Vielle C., Nunez E. A. and Carli A.: Early released lipid soluble cardiodepressant factor and elevated oestrogenic substances in human septic shock. *Eur. J. clin. Invest.* **14** (1984) 288–293.
2. Melby J. C., Egdahl R. H. and Spik W. W.: Secretion and metabolism of cortisol after injection of endotoxin. *J. Lab. clin. Med.* **56** (1960) 50–62.
3. Schumer W., Erve P. R. and Obernult R. P.: Mechanisms of steroid protection in septic shock. *Surgery* **72** (1972) 119–127.
4. Garcia R., Viloria M. D. and Municio A. M.: Influence of E. coli endotoxin on ACTH induced adrenal cell steroidogenesis. *J. steroid Biochem.* **22** (1985) 377–385.
5. Dailey J. W. and Battarbee H. D.: Effect of adrenalectomy and sodium intake on sympathetic nervous function in the rat. *Neuropharmacology* **20** (1981) 877–885.
6. Carli A., Auclair M. C., Benassayag C. and Nunez E. A.: Evidence for an early lipid-soluble cardiodepressant factor in rat serum after a sub-lethal dose of endotoxin. *Circ. Shock* **8** (1981) 301–312.
7. Duncan D. B: Multiple range and multiple F tests. *Biometrics* **11,** (1955) 1–42.
8. Kramer C. Y.: Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics* **12** (1956) 307–310.
9. Carli A., Auclair M. C., Vernimmen C. and Christeff N.: Potentiated cardiodepressant effect of serum by endotoxin in adrenalectomized rats. *Circ. Shock* **19,** (1986) 39–46.
10. Charpenet G., Tache Y., Forest M. G., Haour F., Saez J. M., Bernier M., Ducharme J. R. and Collu R.: Effect of chronic intermittent immobilisation stress on rat testicular androgenic function. *Endocrinology* **109** (1981) 1254–1258.
11. Sapolsky R. M.: Stress-induced suppression of testicular function in the wild baboon: role of glucocorticoids. *Endocrinology* **116** (1985) 2273–2278.
12. Gerendai I., Shaba C., Than R. and Bardin C. W.: Do testicular opiates regulate Leydig cell function ? *Endocrinology* **115** (1984) 1645–1647.
13. Keizer H. Z., Poorman J. and Bunnik G. S. Z.: Influence of physical exercise on sex-hormone metabolism. *J. appl. Physiol.* **48** (1980) 765–769.
14. Campbell C. S., Schwartz N. B. and Firlit M. G.: The role of adrenal and ovarian steroids in the control of serum LH and FSH. *Endocrinology* **101** (1977) 162–172.
15. Arnold G., Herman and John R. Vane.: Release of renal prostaglandins during endotoxin-induced hypotension. *Eur. J. Pharmac.* **39** (1976) 79–90.
16. Ellis E. F., Shen J. C., Sehrey M. P., Carchman R. A. and Rubin R. P.: Prostacyclin, a potent stimulator of steroidogenesis. *Prostaglandins* **16** (1978) 483–490.
17. Gerendai I., Shaba C., Gunsalves G. and Bardin C. W.: The effects of opioid receptor antagonists suggest that testicular opiates regulate Sertoli and Leydig cell function in the neonatal rat. *Endocrinology* **118** (1986) 2039–2044.
18. Simpson E. R., Ackerman G. E., Smith M. E. and Mendelson C. R.: Estrogen formation in stromal cells of adipose tissue of women: induction by glucocortico steroids. *Proc. natn. Acad. Sci. U.S.A.* **9** (1981) 5690–5694.
19. Sibbald W. J., Short A., Cohen M. P. and Wilson R. F.: Variation in adrenocortical responsiveness during severe bacterial infections. *Ann. Surg.* **186** (1977) 29–33.
20. Catalano R. D., Parameswaran V., Ramachandran J. and Trunkey D. D.: Mechanisms of adrenocortical depression during *Escherichia coli* shock. *Archs Surg.* **119** (1984) 145–150.
21. Keri G., Parameswaram V., Trunkey D. D. and Ramachandran J.: Effects of septic shock plasma on adrenocortical cell function. *Life Sci.* **28** (1981) 1917–1923.
22. Philips G. B.: Relationship between serum sex hormones and glucose, insulin and lipid abnormalities in men with myocardial infarction. *Proc. natn. Acad. Sci. U.S.A.* **74** (1977) 1729–1733.
23. Zouaghi H., Savu L., Guerot C., Gryman R., Coulon A. and Nunez E. A.: Total and unbound cortisol, progesterone, oestrone and transcortin-binding activities in sera from patients with myocardial infarction: evidence for differential responses of good and bad prognostic cases. *Eur. J. clin. Invest.* **15** (1985) 365–370.
24. Nolan J. P.: Protective action of estrogen against the lethal effect of endotoxin in the rat. *Nature* **201** (1965) 1965–1971.
25. Altura B. M.: Sex and estrogens in protection against circulatory stress reactions. *Am. J. Physiol.* **231** (1976) 842–847.