J. steroid Biochem. Vol. 17, pp. 401 to 406, 1982
Printed in Great Britain. 

0022-4731/82/100401-06$03.00/0


# *IN VITRO* METABOLISM OF TESTOSTERONE TO 17β-HYDROXY-5α-ANDROSTANE-3-ONE AND 5α-ANDROSTANE-3α,17β-DIOL BY THE RAT INTESTINE

PER STENSTAD and KRISTEN B. EIK-NES
Institute of Biophysics, University of Trondheim, N7034 Trondheim NTH, Norway

(*Received* 23 *February* 1982)

SUMMARY

The metabolism of testosterone to 17β-hydroxy-5α-androstane-3-one and 5α-androstane-3α,17β-diol by the 800 *g* supernatant fraction by different parts of the gastrointestinal tract from male rats was investigated. This metabolism tended to be higher in immature than in mature animals. Administration of dexamethasone or long-acting ACTH to immature and mature rats increased testosterone metabolism to 17β-hydroxy-5α-androstane-3-one and 5α-androstane-3α, 17β-diol by ileum tissue. No such effect could be observed following administration of progesterone, estradiol, prolactin, LH or FSH in mature animals. Development of the gastrointestinal tract from the immature to the mature stage was associated with augmented metabolism of testosterone to 17β-hydroxy-5α-androstane-3-one and 5α-androstane-3α,17β-diol in the ileum.

## INTRODUCTION

It is generally held that 17β-hydroxy-5α-androstane-3-one is the active form of testosterone at the cellular level. Conversion of the former steroid to 5α-androstane-3β,17β-diol and to 5α-androstane-3α,17β-diol will occur in many tissues and 5α-androstane-3α,17β-diol is also an active androgen [1]. 5α-Reduction of testosterone *in vitro* is low in intestinal tissue of the dog [2] and the pike [3]. During development from the immature to the mature stage several enzymic activities of the rat intestine undergo dramatic increases [4, 5] which can be prevented by hypophysectomy [4] or by adrenalectomy [6]. In the maturation process of epithelium from the small intestine, the enzyme ornithine decarboxylase may play an important role [5]. This enzyme is stimulated by testosterone in many tissues [7, 8]. It was therefore decided to investigate the formation of 17β-hydroxy-5α-androstane-3-one and 5α-androstane-3α,17β-diol from testosterone in the gastrointestinal tract from immature and mature male rats and to study whether pretreatment of the animals with steroid or peptide hormones had any effect on this formation.

## MATERIALS AND METHODS

### *Materials*

Radioactive steroids were obtained from the Radiochemical Centre, Amersham, U.K.: [1,2-$^3$H]-testosterone (47 Ci/mmol), [1,2-$^3$H]-17β-hydroxy-5α-androstane-3-one (78 Ci/mmol) and [1,2-$^3$H]-5α-androstane-3α,17β-diol (71 Ci/mmol). All radioactive steroids were purified by t.l.c. before being used as substrates in incubation studies.

Synacten Depot was supplied by Ciba-Geigy, Basel, Switzerland, NADP, glucose 6-phosphate, glucose 6-phosphate dehydrogenase (type XV, 230 units/mg protein) were obtained from Sigma Chemicals Corp. LH (NIH-LH-S17), FSH (NIH-FSH-S9) and prolactin (NIH-P-B5) were gifts from NIAMDD, Bethesda, Maryland, U.S.A.

Precoated silica gel 60 F-254 t.l.c. plates were purchased from E. Merck. T.l.c. ready plastic sheets precoated with silica gel (F-1500, LS 254 W, Order No. 355099) were purchased from Schleicher and Schüll. Liquid scintillation cocktail was obtained from Koch-Light. Other chemicals were of analytical grade and purchased from E. Merck. Non-radioactive steroids were supplied by Steraloids Inc.

### *Animals*

Male Wistar rats were obtained from Institutt for Folkehelse, Oslo, Norway, and kept in the animal quarters at Regionsykehuset in Trondheim. In these quarters the rats were maintained under controlled light (14 h light and 10 h darkness) and temperature (19–21°C) conditions. Rat chow and tap water were provided *ad libitum*. Immature rats were kept with their mother to different ages as stated in the result section. When such animals were removed from their mother, they were fed as mature rats.

### *Injections*

Injections of the different compounds in 0.9% NaCl were performed in the following fashion: solutions of Synacten depot, LH, FSH and prolactin were injected i.m. while suspensions of dexamethasone, estradiol and progesterone were injected s.c., every day for 4

days (estradiol and prolactin being given for 7 days). These compounds were given in doses/100 g. body wt/d of: 100 μg Synacten depot, 100 μg prolactin, 200 μg FSH, LH or desamethasone, and 100 μg estradiol or progesterone. Control rats received (i.m. or s.c.) 0.9% NaCl only.

*Preparation of homogenates*

The rats were killed by decapitation and intestinal tissue was taken out immediately. The fecal mass was removed and the intestine was washed thoroughly with ice-cold 0.9% NaCl. The tissues were suspended in 50 mM Tris–HCl buffer (pH 7.35), 1 ml per cm intestine. Homogenates were prepared using a Braun Melsungen homogenizator at speed of 1500 rev./min. The homogenate was centrifuged for 20 min at 800 *g* in a Beckman J-21 refrigerated centrifuge. The supernatant fraction was used for the incubation experiments. The protein concentration of this preparation was estimated by employing a Bio-Rad Protein Assay kit.

*Conditions of incubation*

The radioactive steroid substrate in question (500,000 c.p.m.) was dissolved in 500 μl Tris–HCl buffer (pH 7.35), and 50 μl 2.5 mM $NADP^+$, 100 μl 12.5 mM glucose 6-phosphate and 50 μl glucose 6-phosphate dehydrogenase (250 μg/ml) were added (all dissolved in Tris–HCl buffer). In incubations with $NADP^+$ as cofactor, only 50 μl 2.5 mM $NADP^+$ was incorporated. The reaction was started by adding 500 μl of the 800 *g* supernatant fraction (1.0–2.5 mg protein) and the mixture was incubated at 37°C for 60 min in an atmosphere of 96% $O_2$ + 4% $CO_2$. All tissue samples were analysed in duplicate. The reaction was stopped by addition of 2 ml of ethyl acetate followed by vigorous stirring. A blank sample was prepared by adding ethyl acetate before addition of homogenate at the start of the incubation.

*Separation of steroids*

The reaction mixture was extracted twice with 2 ml ethyl acetate each time. In order to achieve complete steroid recovery (98–100%) during extraction, the extracting solvent contained the following carrier steroids (5 μg/ml): testosterone, 17β-hydroxy-5α-androstane-3-one, 4-androstene-3α,17β-diol, 4-androstene-3β, 17β-diol, 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol. The extracted steroids were separated by t.l.c. as described [9, 10]. The radiochemical purity of the isolated steroids was confirmed by crystallization to constant specific activity [9].

The radioactivity fractions were scraped off the t.l.c. plates and counted in a liquid scintillation counter (Isocap/300). The data given in the tables are net data (the blank sample subtracted).

## RESULTS

Testosterone metabolism *in vitro* to the active androgens 17β-hydroxy-5α-androstane-3-one and 5α-androstane-3α,17β-diol by different parts of the rat gastrointestinal tract is given in Table 1. With a NADPH generating system the 800 *g* supernatant of these organs in adult rats formed 5α-androstane-3α,17β-diol from testosterone in detectable amounts (limit of detection: 0.05 ng). 5α-Reduction of testosterone was higher in immature than in mature rats (Table 1), and in mature rats 17β-hydroxy-5α-androstane-3-one could only be detected as a metabolite of testosterone in duodenum (Table 1). Since ileum showed a low conversion of the metabolites in question (Table 1), we concentrated our studies on formation of these two 5α-reduced metabolites of testosterone in this tissue. In addition to the metabolites recorded in Table 1, the gastrointestinal tract produced large amounts of 4-androstene-3α,17β-diol from testosterone [11]. The biological significance of this testosterone metabolite has recently been discussed by our laboratory [12].

The formation of 5α-androstane-3α,17β-diol and 17β-hydroxy-5α-androstane-3-one from testosterone increased significantly in ileum of adult rats following treatment of the animals with long-acting ACTH or dexamethasone (Table 2). In ileum tissue from such animals the conversion of testosterone to 17β-hydroxy-5α-androstane-3-one showed a $V_{max}$ of 0.417 (nmol/mg protein/h) with $K_M$ of 2.0 (nmol/mg protein) under the incubation conditions described. Only 2

Table 1. Metabolism of [$^3$H]-testosterone by 800 *g* supernatant fraction from different parts of the gastrointestinal tract in mature and immature male rats

| Metabolites | Stomach | Duodenum | Jejunum | Ileum |
|---|---|---|---|---|
| | | *Mature rats (70 days old)* | | |
| 17β-Hydroxy-5α-androstane-3-one | <0.05 | 0.06 ± 0.02 | <0.05 | <0.05 |
| 5α-Androstane-3α,17β-diol | 0.63 ± 0.03 | 1.5 ± 0.92 | 0.93 ± 0.21 | 0.35 ± 0.14 |
| | | *Immature rats (30 days old)* | | |
| 17β-Hydroxy-5α-androstane-3-one | 0.10 ± 0.05 | 1.0 ± 0.50 | 0.26 ± 0.07 | 0.14 ± 0.05 |
| 5α-Androstane-3α,17β-diol | 0.82 ± 0.35 | 3.6 ± 0.31 | 4.2 ± 2.71 | 2.4 ± 1.30 |

The supernatant fractions were incubated with [$^3$H]-testosterone (500 ng) and a NADPH-generating system for 60 min at 37°C in an atmosphere of 96% $O_2$ and 4% $CO_2$. Mean data ± SD ($n$ = 3) are given as ng/mg protein incubated.

Table 2. Effect of treatment with various compounds on [$^3$H]-testosterone metabolism in ileum from mature male rats

| Metabolites | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Synacten depot | Dexamethasone | Estradiol | Progesterone | FSH | LH | Prolactin |
| 17β-Hydroxy-5α-androstane-3-one | <0.05 | 1.1 ± 0.90 | 1.3 ± 0.50 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| 5α-Androstane-3α,17β-diol | 0.45 ± 0.10 | 14.1 ± 3.60* | 16.4 ± 4.10* | 0.82 ± 0.52 | 0.42 ± 0.05 | 0.45 ± 0.05 | 0.40 ± 0.10 | 0.36 ± 0.20 |

*Significantly greater than control ($P < 0.005$).
Rats were treated with various compounds as described in the text. The 800 *g* supernatant fractions of ileum were incubated with [$^3$H]-testosterone (500 ng) and a NADPH-generating system for 60 min at 37°C in an atmosphere of 96% $O_2$ and 4% $CO_2$. Mean data ± SD ($n = 3$) are given as ng/mg protein incubated.

days after the start of dexamethasone treatment metabolism of testosterone to the two 5α-reduced steroids in question was significantly increased [11]. In the doses given no effect of estradiol, progesterone, prolactin, FSH or LH pretreatment could be obtained on this metabolism in ileum. Moreover, pretreatment with deoxycorticosterone (1 mg/100 g body weight/day for 4 days) had no effect on 5α-reduction of testosterone in ileum (data not shown).

Augmented formation of 17β-hydroxy-5α-androstane-3-one and 5α-androstane-3α,17β-diol from testosterone was found in ileum from 17-day-old rats treated with dexamethasone or long-acting ACTH on days 15 and 16 of extrauterine life (Table 3). In immature rats the amount of 17β-hydroxy-5α-androstane-3-one produced from testosterone by the ileum preparation employed was higher both in control and in dexamethasone or ACTH treated animals than that in mature rats. (Tables 1 and 3). Augmented 5α-reduction of testosterone was also found in intestine *in vitro* of 25-day-old rats (removed from their mother on day 21) compared with 25-day-old rats staying with their mother also during these last 4 days (Table 4). At day 28 there was no significant difference with regard to testosterone metabolism by ileum from rats removed from their mother on day 21 and rats staying with their mother from birth to day 28 (data not shown).

Metabolism of 17β-hydroxy-5α-androstane-3-one by ileum is presented in Table 5. High amounts of 5α-androstane-3α,17β-diol were formed. This conversion was lower in ileum from dexamethasone treated animals (Table 5).

5α-Androstane-3α,17β-diol was metabolized to 17β-hydroxy-5α-androstane-3-one and to 5α-androstane-3β,17β-diol by ileum (Table 6). Dexamethasone treatment of the rats led to a marked increase in 17β-hydroxy-5α-androstane-3-one production from 5α-androstane-3α,17β-diol (Table 6).

When adult female rats were treated with dexamethasone as described (Table 2), significant augmentation of the metabolism of testosterone to 17β-hydroxy-5α-androstane-3-one and 5α-androstane-3α,17β-diol by the 800 *g* supernatant fraction of ileum was found (data not shown).

## DISCUSSION

More 5α-reduced metabolites of testosterone were produced in the gastrointestinal tract from immature than from mature rats (Table 1). The same variation in 5α-reduction of testosterone between immature and mature rats has been found in the testis [13]. ACTH administration to rats resulted in an elevation of 5α-reduction of testosterone in ileum (Table 2). ACTH could be effective either by direct stimulation of the intestine or via stimulation of corticoid production in the adrenal gland. The effect of dexamethasone administration on 5α-reduction of testosterone strongly indicates that this ACTH effect was obtained via the adrenal cortex. Moreover, only long-acting ACTH

Table 3. Effect of ACTH or dexamethasone treatment on [$^3$H]-testosterone metabolism in ileum from immature male rats (17-day-old)

| Metabolites | Treatment | | |
|---|---|---|---|
| | Control | ACTH | Dexamethasone |
| 17β-Hydroxy-5α-androstane-3-one | 3.2 ± 0.8 | 9.1 ± 1.9† | 6.1 ± 1.7‡ |
| 5α-Androstane-3α,17β-diol | 2.4 ± 1.0 | 14.6 ± 5.3* | 13.0 ± 3.5† |

Significantly greater than control; *$P < 0.01$; †$P < 0.005$; ‡$P < 0.05$.

Rats were treated with ACTH (Synacten depot, 100 μg/100 g body wt/day) or dexamethasone (200 μg/100 g body wt/day) on day 15 and day 16. The 800 *g* supernatant fractions of ileum were incubated with [$^3$H]-testosterone (500 ng) and a NADPH-generating system for 60 min at 37°C in an atmosphere of 96% $O_2$ and 4% $CO_2$. Mean data ± SD ($n = 3$: are given as ng/mg protein incubated.

Table 4. [$^3$H]-Testosterone metabolism in ileum from young male rats removed from their mother versus that of rats staying with their mother

| Metabolites | Removed from mother | Staying with mother |
|---|---|---|
| 17β-Hydroxy-5α-androstane-3-one | 6.0 ± 2.20† | 0.53 ± 0.32 |
| 5α-Androstane-3α,17β-diol | 12.7 ± 4.10* | 1.5 ± 0.70 |

Significantly greater than control; *$P < 0.005$; †$P < 0.01$.

Rats were removed from their mother on day 21 and used in experiments on day 25. Control rats were of the same age, but stayed with their mother all the time. The 800 *g* supernatant fractions of ileum were incubated with [$^3$H]-testosterone (500 ng) and a NADPH-generating system for 60 min at 37°C in atmosphere of 96% $O_2$ and 4% $CO_2$. Mean data ± SD ($n = 3$) are given as ng/mg protein incubated.

Table 5. Effect of dexamethasone treatment on [$^3$H]-17β-hydroxy-5α-androstane-3-one metabolism in ileum from mature male rats

| Metabolites | Treatment | |
|---|---|---|
| | Control | Dexamethasone |
| 5α-Androstane-3α,17β-diol | 2600 ± 580 | 1540 ± 310* |
| 5α-Androstane-3β,17β-diol | 24 ± 12 | 20 ± 15 |

*Significantly lower than control ($P < 0.025$).

Rats were treated with dexamethasone (200 μg/100 g body wt/day) for 4 days. The 800 *g* supernatant fractions of ileum were incubated with [$^3$H]-17β-hydroxy-5α-androstane-3-one (10 μg) and a NADPH-generating system for 60 min at 37°C in an atmosphere of 96% $O_2$ and 4% $CO_2$. Mean data ± SD ($n = 3$) are given as ng/mg protein incubated.

Table 6. Effect of dexamethasone treatment on [$^3$H]-5α-androstane-3α,17β-diol metabolism in ileum from mature male rats

| Metabolites | Treatment | |
|---|---|---|
| | Control | Dexamethasone |
| 17β-Hydroxy-5α-androstane-3-one | 18.0 ± 4.0 | 777.0 ± 141.0* |
| 5α-Androstane-3β,17β-diol | 4.4 ± 3.8 | 13.3 ± 0.8† |

Significantly greater than control: *$P < 0.001$; †$P < 0.01$.

Rats were treated with dexamethasone (200 μg/100 g body wt/day) for 4 days. The 800 *g* supernatant fractions of ileum were incubated with [$^3$H]-5α-androstane-3α,17β-diol (10 μg) and $NADP^+$ as cofactor for 60 min at 37°C in an atmosphere of 96% $O_2$ and 4% $CO_2$. Mean data ± SD ($n = 3$) are given as ng/mg protein incubated.

could promote increased metabolism of testosterone to the two 5α-reduced metabolites measured in ileum while a fast-acting ACTH preparation had no effect (data not shown). Large concentrations of glucocorticoids appear to be needed in order to promote augmented production of 5α-androstane-3α,17β-diol and 17β-hydroxy-5α-androstane-3-one from testosterone in ileum. Dexamethasone treatment had no significant

effect on testosterone metabolism in rat prostate (data not shown). Since metabolism of testosterone to 17β-hydroxy-5α-androstane-3-one in the ileum was much slower than ileum conversion of the latter steroid to its 3α and 3β-reduced metabolites (Tables 2 and 5), 5α-reduction of testosterone is the rate limiting step also in this tissue. The same information has been obtained from the gastrointestinal tract of the pike [3].

Dexamethasone treatment of the rats led to lower conversion of 17β-hydroxy-5α-androstane-3-one to 5α-androstane-3α,17β-diol in ileum (Table 5) and to a significant increase in metabolism of 5α-androstane-3α,17β-diol to 17β-hydroxy-5α-androstane-3-one in this tissue (Table 6). All these dexamethasone effects on androgen metabolism by ileum would increase 17β-hydroxy-5α-androstane-3-one accumulation in this tissue. Since 17β-hydroxy-5α-androstane-3-one is the "active" hormone in many androgen sensitive organs, dexamethasone treatment or significant increase of adrenocortical function could lead to a state with higher androgenic activity in the ileum.

Intestinal 5α-reduction of testosterone was higher in 17-day-old rats than in mature rats (Tables 1 and 3). Augmented production of a steroid with higher activity as an androgen could be crucial for development of intestinal tissue. The shift from infantile to mature intestine in the rat is rapid, the critical period extends from about the 19th to the 23rd day. In this period alkaline phosphatase [14, 15] and disaccharidase activities [15, 16] increase in the gut in addition to structural developments [15]. This shift is controlled by the adrenal cortex [14]; adrenalectomy in the suckling rat prevents the occurrence of various maturational events in the gastrointestinal tract [6], and hypophysectomy has the same effects [15]. Cortisone treatment of hypophysectomized, immature rats increases intestinal disaccharidase and alkaline phosphatase activity [4].

The elevation of 5α-reductase in intestine of young rats removed from their mother (Table 4) was probably caused by the "stress" of being in a new environment. The acute character of this situation was demonstrated by the fact that after 7 days in this new environment with shift from the infantile to the mature state of intestinal function [4], the intestinal 5α-reductase was not significantly different from that of rats staying with their mother during this period. Augmented metabolism of testosterone to 17β-hydroxy-5α-androstane-3-one in the ileum could be involved in the rapid development of the rat intestine from the immature to the mature stage [4]. During this development intestinal ornithine decarboxylase shows increased activity [5]. This enzyme is stimulated by androgens in many organs [7, 8]. One may also wonder what role increased production of the active form of testosterone may play in gastrointestinal disturbances following stress and increased adrenocortical function [17]. Current literature shows that glucocorticosteroids have receptors in the intestinal epithelium [18] and promote increased guanylate cyclase activity in this tissue [19].

*Acknowledgements*—The authors wish to thank cand. mag. Turid Lein and cand. real. Ragnhild Lofthus for expert technical assistance and Mr Nils Nesjan, Regionsykehuset in Trondheim, for all kind help. This work was in part supported by grants from Norges Almenvitenskapelige Forskningsråd. We thank the National Institute of Arthritis, Metabolism and Digestive Diseases, Pituitary Hormone Distribution Program (Bethesda, Maryland, U.S.A.), for the gifts of prolactin, LH and FSH.

## REFERENCES

1. Vergans H. L. and Eik-Nes K. B.: Effects of androstenes, 5α-androstanes, 5β-androstanes and oestratrienes on serum gonadotrophin levels and ventral prostate weights in gonadectomized adult male rats. *Acta Endocr., Copenh.* **83**, (1976), 201–210.
2. Harri M.-P., Nienstedt W. and Hariala K.: Testosterone metabolism by the canine intestine. *Acta chem. Fenn.* **43** (1970) 395–399.
3. Nienstedt W., Nienstedt I. and Mannisto J.: Metabolism of testosterone and progesterone by gastrointestinal tissues of the pike (Esox lucius). *Gen. comp. Endocr.* **43** (1981) 148–156.
4. Yen K-Y. and Florence M.: Development of the small intestine in the hypophysectomized rat. I. Growth, histology, and activity of alkaline phosphatase, maltase, and sucrase. *Develop. Biol.* **47** (1975) 156–172.
5. Luk G. D., Marton L. J. and Baylin S. B.: Ornithine decarboxylase is important in intestinal mucosal maturation and recovery from injury in rat. *Science* **210** (1980) 195–198.
6. Koldovsky O., Jirosova V. and Heringova A.: Effect of aldosterone and corticosterone on β-galactosidase and invertase activity in the small intestine of rats. *Nature* **206** (1965) 300–301.
7. Puhvel S. M. and Sakamoto M.: The effect of androgen stimulation on ornithine decarboxylase activity in the pre-putial and sebaceous glands of rats. *J. Invest. Dermatol* **74** (1980) 245 (abstract).
8. Henningson S., Persson L. and Rosengren E.: Poly amines and nucleic-acids in the mouse kidney induced to growth by testosterone propionate. *Acta Physiol. Scand.* **102** (1978) 385–393.
9. Stenstad P., Østgaard K. and Eik-Nes K. B.: Androgen metabolism in relation to growth stimulation by a uterine cell line. *Mol. Cell. Endocr.* **8** (1977) 283–289.
10. Sunde A., Stenstad P. and Eik-Nes K. B.: Separation of epimeric 3-hydroxyandrostanes and 3-hydroxyandrostenes by thin-layer chromatography on silica gel. *J. Chromatog.* **175** (1979) 219–221.
11. Stenstad P.: Androgen metabolism by muscular and intestinal tissue from rats. Dr. ing. thesis from the University of Trondheim. NTH. 1980. Printed by NTH-Trykk, Trondheim, Norway 1980.
12. Stenstad P. and Eik-Nes K. B.: Androgen metabolism in rat skeletal muscle *in vitro*. *Biochim. biophys. Acta* **663** (1981) 169–176.
13. Rosness P. A., Sunde A. and Eik-Nes K. B.: Production and effects of 7α-hydroxytestosterone on testosterone and dihydrotestosterone metabolism in rat testis. *Biochim. biophys. Acta* **488** (1977) 55–65.
14. Mog F.: The functional differentiation of the small intestine. III. The influence of the pituitary-adrenal system on the differentiation of phosphatase in the duodenum of the suckling mouse. *J. Exp. Biol.* **124** (1953) 329–346.
15. Yeh K. Y. and Moog F.: Development of the small intestine in the hypophysectomized rat. II. The

influence of cortisone, thyroxine, growth hormone, and prolactin. *Develop. Biol.* **47** (1975) 173–184.
16. Rubino A., Zimnalatti F. and Auriccho S.: Intestinal disacharidase activities in adult and suckling rats. *Biochim. biophys. Acta* **92** (1964) 305–312.
17. Myhre E.: Regeneration of the fundic mucosa in rats IV. Behavior of the connective tissue under various hormonal influences. *AM. Med. Assoc. Archs Pathol.* **69** (1960) 314–322.
18. Lentze M. I., Moxey P. C. and Trier J. S.: Distribution of glucocorticoid receptors in rat intestinal epithelium. *Ped. Res.* **13** (1979) 1192 (abstract).
19. Marnane W. G., Tai Y.-H., Decker R. A., Boedeker E. C., Charney A. N. and Donowitz M.: Methyl prednisolone stimulation of guanylate cyclase activity in rat small intestinal mucosa: possible role in electrolyte transport. *Gastroenterology* **81** (1981) 90–100.