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DEVELOPMENTAL ASPECTS OF STEROID-INDUCED AMMONIA RELEASE FROM ISOLATED SECTIONS OF RAT INTESTINE

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SUMMARY

1. Release of ammonia from isolated intestinal sections of adult male rats is higher than that measured using immature animals.

2. The increase appears to be Na^+ dependent and develops during the spurt of growth at puberty.

3. Developmental changes in Na^+-dependent ammonia release from isolated sections of the intestine and growth of the small intestine in male and female rats have been compared.

4. Intestinal growth increases far more rapidly than body weight and in the males critical developmental changes occur early during weaning and during puberty.

5. In females the major change is at weaning and little further change occurs during puberty.

6. Treatment of young animals with aldosterone or testosterone increases the Na^+-dependent ammonia release precociously.

7. Dose–response effects of testosterone and aldosterone in distal sections of the small intestine have been compared.

INTRODUCTION

The general pattern of intestinal development is similar in all mammals (Moog, 1953; Henning & Kretchmer, 1973). By the time of birth the intestine has acquired digestive and absorptive functions necessary to cope with the neonatal diet. The formation of crypts and villi begins before birth (Dunn, 1967), the generation time for the crypt cells being of the order 10–24 h and transit time for migration of epithelial cells along the length of the villus in young animals at least 96 h (Koldovsky, Sunshine & Kretchmer, 1966; Sunshine, Herbst, Koldovsky & Kretchmer, 1971).

In rats many developmental changes occur during the third week of life, and there is strong evidence that glucocorticoids may be responsible for a variety of these changes (Henning & Kretchmer, 1973). At this time 'closure' to the absorption of macromolecules occurs (Clarke & Hardy, 1969), an effect that can be induced precociously by treatment with steroids (Morris & Morris, 1974). The small intestine becomes sensitive to glucocorticoids at a crucial stage (17–18 days old) when jejunal sucrase, for example, increases rapidly, remaining high in adult animals (Henning, Ballard & Kretchmer, 1975). Henning & Jefferson (1979) suggest that the gluco-

corticoids must cause a permanent change in the gene expression of the epithelial germ cells.

Ferguson, James, Patterson, Saunders & Smith (1979) have shown that aldosterone-dependent development changes in intestinal Na^+ transport occur at birth when young animals must absorb and conserve large amounts of Na^+ in order to sustain rapid growth.

In the present study we have investigated changes in Na^+-dependent ammonia release from isolated sections of rat intestine during the early developmental period. In rat kidney a direct Na^+/NH_4^+ exchange has been suggested by Snart & Taylor (1978) on the basis of effects of aldosterone on the release of ammonia from isolated kidney cortical slices and there is generally considered to be a close relationship between epithelial Na^+ uptake and ammonia release (Maetz, 1973; Frazier & Vanatta, 1973).

METHODS

Male and female Wistar rats from two separate colonies were used in this study.

Groups of eight animals of each sex from each colony were taken at various stages just prior to and after weaning. The animals were weighed and then killed by a sharp blow to the head.

Immediately after killing, the thoracic cavity of each animal was opened and a blood sample (2–3 ml) withdrawn from the heart with a heparinized syringe. The blood was transferred to heparinized tubes and the plasma separated using an MSE Minor centrifuge. The plasma was then removed and stored as a pooled sample from eight animals at −4 °C prior to corticosterone assay using the method of Gross, Ruder, Brown & Lipsett (1972). The small intestine from stomach to caecum and colon were rapidly excised and placed on ice. They were then washed through with 0·9% saline before being slit open longitudinally. The small intestine was then divided into five sections of equal length, designated I–V, and weighed.

Following this procedure each section of small intestine and the colon was placed in a vessel containing 10 ml phosphate Ringer solution (in mM: NaCl, 133; KCl, 1; $CaCl_2$, 0·7; NaH_2PO_4, 0·6; Na_2HPO_4, 3·1; Na acetate, 0·01; pH 7·4) maintained at 37 °C in a water-bath. The intestinal sections were incubated for 15 min with shaking, after which the tissue was removed from the medium.

Kidneys were excised, decapsulated and sliced using a Stadie–Riggs microtome (0·5 mm). Four slices from each animal were weighed and incubated in phosphate Ringer solution (10 ml) for 15 min. The tissue was then removed and ammonia release assayed.

Measurement of intestinal ammonia release during development

A 200 μl sample of medium was taken from each vessel and pipetted into a 3 ml spectrophotometer cuvette containing 2 ml Nessler's Reagent (B.D.H.), and the colour allowed to develop for 10 min. The absorbance of the resulting solution was read at 420 nm using a Unicam SP 600 spectrophotometer and the concentration of ammonia in each sample calculated from standard curves. Tests in which ammonia was first displaced with saturated potassium carbonate confirmed results obtained by direct assay.

In order to estimate the extent to which release of ammonia from the intestine was Na^+ dependent, an isotonic mannitol solution was used in place of phosphate Ringer solution in the incubation procedure. The same procedure was employed, except that the colour development of the Nessler's Reagent was allowed to proceed for just 3 min before reading because mannitol reduced the Nessler's Reagent after a lag period of 4–5 min.

Results obtained using standard ammonium chloride solutions in mannitol were similar to those measured in phosphate Ringer solution.

Operations and steroid treatment

Bilateral adrenalectomy/castration was performed on rats under ether anaesthesia. Following surgery, the rats were maintained on standard Labsure P.M.D. diet with 0·9% saline as drinking water for 4–14 days.

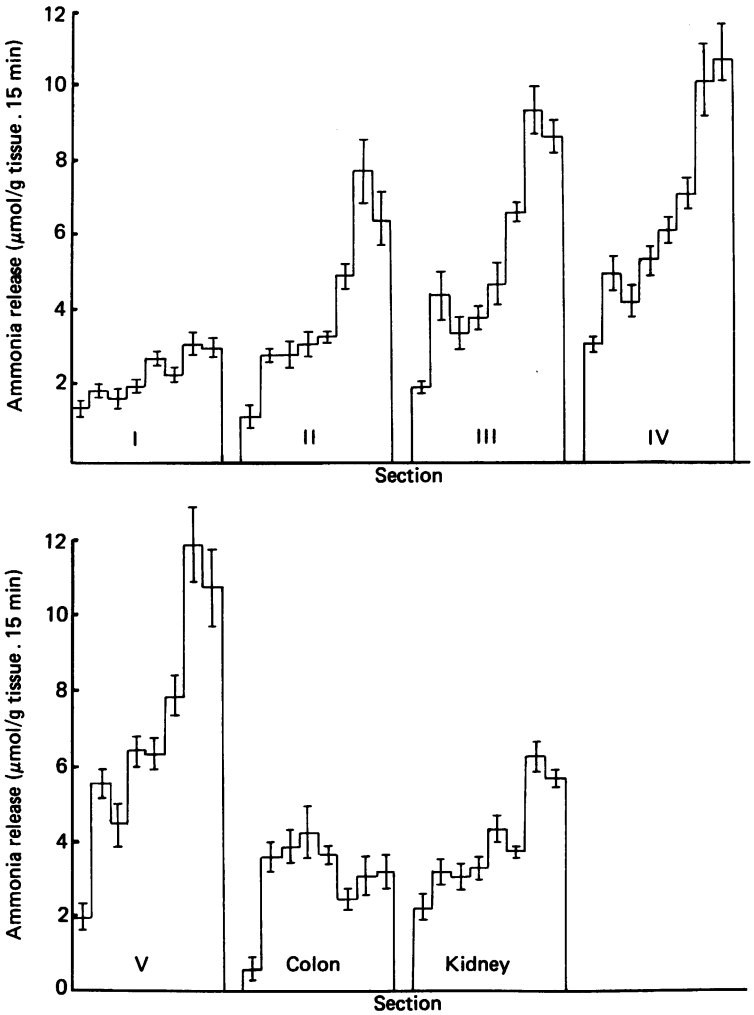

Fig. 1. Ammonia release (μmol/g tissue . 15 min) measured in isolated sections of male rat intestine at 3, 5, 6, 7, 8, 10, 14 and 18 weeks old. The histograms for each section represent the mean ± S.E. of mean at each age for eight determinations. The animals were weaned 4 weeks after birth.

Testosterone, estradiol, corticosterone and aldosterone samples were obtained from the Sigma Chemical Company. They were dissolved (100 μg/ml) in 1 % alcohol solution in phosphate Ringer. This solution was further diluted with phosphate Ringer solution to allow various amounts of steroid to be injected intraperitoneally (0·1 ml) in young animals (approx. weight 50 g) 4 h prior to killing or as indicated in the text.

Treatment of results

The results are expressed as the mean ± S.E. of mean with the number of animals in each group indicated. An unpaired t test has been used to establish the level of significance of the difference between the means, the difference being taken as significant when $P \leqslant 0{\cdot}05$. In the case of the mean relative change (M) in body weight or small intestine weight, the standard error of each value (S) has been calculated using the relationship $S = M\sqrt{(S_1/M_1)^2 + (S_2/M_2)^2}$, where M_1 and M_2

represent mean weights and S_1 and S_2 the standard errors determined at a particular age and 14 weeks old respectively. In the case of plasma corticosterone assays the results represent single determinations on pooled plasma samples. The accuracy of the method was established by adding known amounts (20, 100 and 200 ng/ml) of corticosterone to a series of plasma samples. The elevations of corticosterone were found to be 20 ± 6 ng/ml (12), 98 ± 6 ng/ml (12) and 190 ± 6 ng/ml (10) respectively.

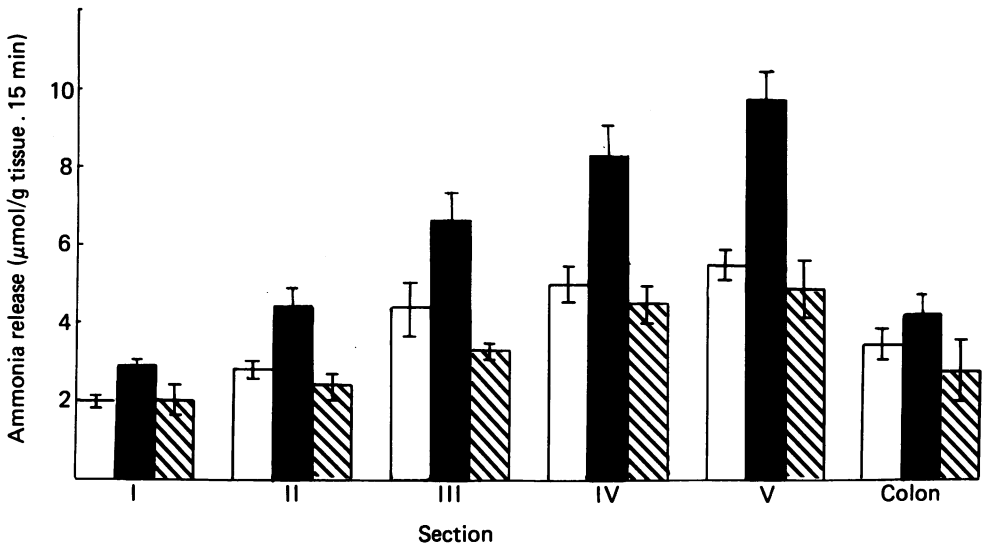

Fig. 2. The open bars represent ammonia release (μmol/g tissue . 15 min) from isolated sections of rat intestine taken from newly weaned rats (4 weeks old) in Na^+ Ringer solution (control). Corresponding values obtained using rats injected 4 h previously with 10 μg aldosterone are shown by filled bars (Na^+ Ringer solution) and hatched bars (isotonic mannitol). Each result represents the mean $\pm$ S.E. of mean for eight determinations.

RESULTS

Release of ammonia from isolated sections of intestine is greater in mature than it is in immature rats. This developmental increase appears to be entirely Na^+ dependent and occurs at about 10 weeks old (Fig. 1) during the period of rapid growth at puberty. The increase may be stimulated precociously in immature animals by treatment with aldosterone (Fig. 2), or testosterone but not to an equivalent extent by corticosterone or oestradiol at the same concentration. The effect of the steroid is most noticeable in distal sections of the small intestine where dose–response effects of testosterone and aldosterone have been compared (Fig. 3). The results indicate that testosterone is effective at physiological concentrations, suggesting a physiological role in the control of intestinal and kidney ion transport at puberty. The dose–respone curve is normal in that the response occurs over a dose range covering two orders of magnitude. Aldosterone, at physiological concentrations, stimulates ammonia release to a lesser extent than testosterone. Higher concentrations stimulate ammonia release further, reaching levels equivalent to those obtained with physiological

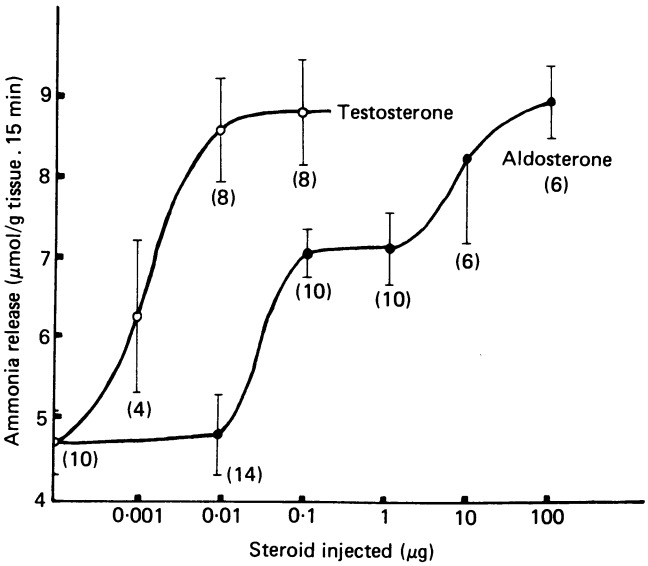

Fig. 3. Dose–response curves for testosterone- and aldosterone-stimulated ammonia release in distal (section V) regions of the small intestine taken from 4-week-old rats. Each point represents the mean of several determinations (number shown in parentheses), the standard error also being illustrated.

TABLE 1. Adrenalectomy or adrenalectomy together with castration of adult rats fails to reduce the elevated intestinal ammonia release significantly (μmol/g tissue . 15 min) within 4–14 days of operation. Animals initially 8 weeks old or 14 weeks old were saline compensated after the operations

	Adrenalectomized (4 days)	Adrenalectomized (14 days)	Adrenalectomized/ castrated (14 days)	Adrenalectomized/ castrated (4 days)
Section I	2·86±0·89 (4)	3·52±0·29 (4)	3·42±0·28 (4)	3·64±0·67 (4)
II	5·38±1·59	4·22±0·55	4·71±0·98	4·68±0·81
III	4·97±1·30	4·83±0·52	7·70±1·12	7·79±1·75
IV	7·27±1·32	7·26±0·58	11·04±1·58	8·75±2·37
V	7·07±0·74	10·07±0·52	11·92±1·50	10·18±1·88
Colon	3·56±0·79	4·53±0·96	6·93±1·25	5·10±0·72

concentrations of testosterone or corticosterone. The dose–response characteristic for aldosterone has been analysed in terms of two dose–response curves.

The steroid-stimulated increase which was evident 4 h after intraperitoneal injection with testosterone (10 μg), aldosterone (100 μg) or corticosterone (100 μg) is maintained for up to 14 days following steroid treatment.

Subcutaneous injections of testosterone (10 μg) were ineffective within 4 h but the full effect was evident 3 days after treatment. The effect of steroids on adult animals could not be systematically tested as ammonia release from isolated sections of older animals tends to remain high, and increased intestinal ammonia release is unaffected by saline-compensated adrenalectomy and castration (Table 1). Female rats show an

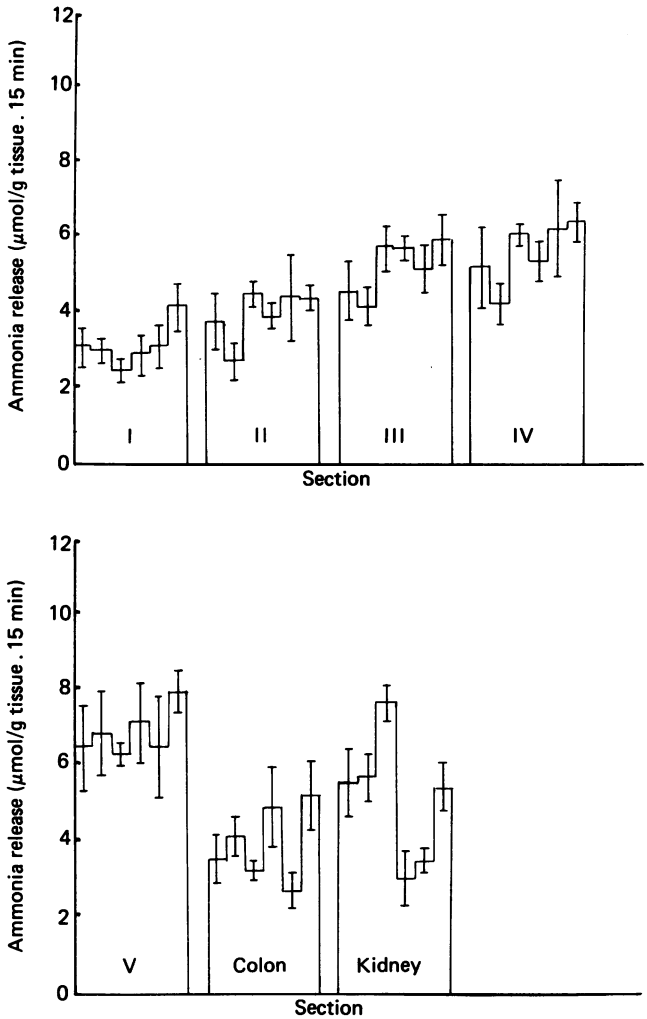

Fig. 4. Shows the ammonia release (μmol/g tissue . 15 min) measured in isolated sections of female rat intestine at 5, 6, 7, 8, 10 and 14 weeks old. The histograms for each section represent the mean ± S.E. of mean at each age for eight determinations. The animals were weaned at 4 weeks old.

increased intestinal and renal release of ammonia at weaning (Fig. 4) but little further change occurs during puberty. Plasma corticosterone concentrations measured in male and female rats increase early during weaning and a further increase occurs during the spurt of growth at puberty. At this time (10 weeks old) female animals have higher plasma corticosterone concentrations (580 ng/ml) than corresponding male animals (206 ng/ml).

The growth rate of the small intestine and total body weight measured over the period of development have been compared (Fig. 5) in groups of male and female rats. Intestinal growth proceeds at a faster rate than over-all body weight in both sexes.

However, during puberty the growth of the small intestine in male rats proceeds at a much faster rate than in female rats. The difference, expressed in terms of relative weight change, is significant at 6 weeks old, and this pattern of change has been confirmed in both colonies of rats.

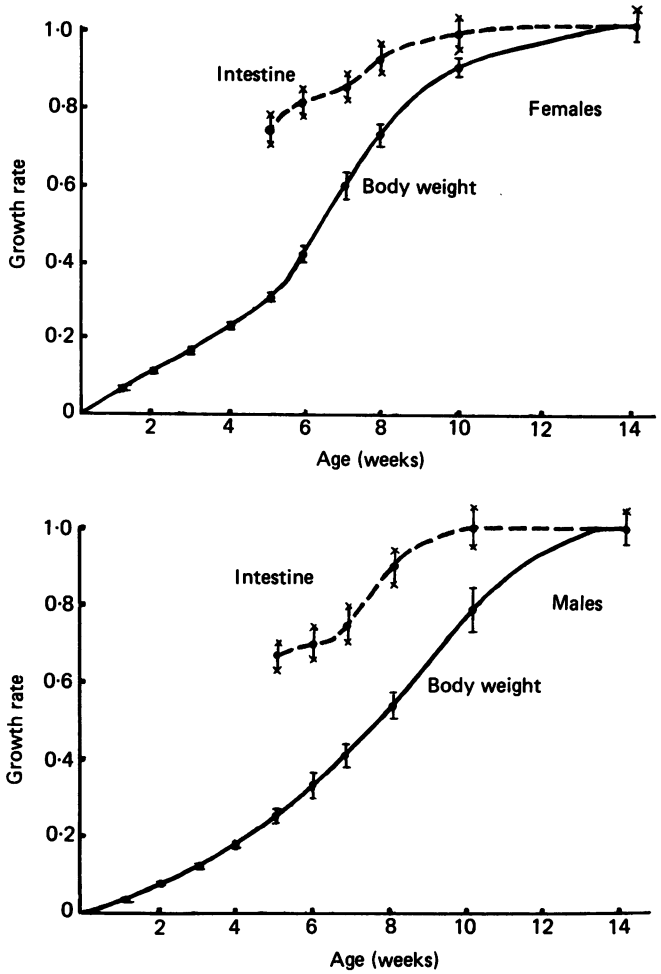

Fig. 5. Compares the relative intestinal growth and body weight in male and female rats in order to demonstrate the differential growth after weaning at 4 weeks old. The mean ± S.E. of mean values have been calculated as described in the text.

DISCUSSION

Our developmental studies of ammonia release from isolated sections of male rat intestine and kidney indicate that an increased Na^+-dependent ammonia release occurs about 55 days after birth, reaching adult levels by about 100 days old. This change may be associated with the spurt of growth at puberty and is less evident in female rats. It appears to be under steroid control, and we have demonstrated that

testosterone is the most effective of the various steroid tests in precociously stimulating the increase.

Testosterone is not normally associated with ion transport control but has a well-established role in intestinal cell proliferation and differentiation (Wright, Morley & Appleton, 1972). It is believed to act through metabolites, and recent work by Stenstad & Eik Nes (1982) has shown that glucocorticoid activation of the intestine augments the metabolism of testosterone into its biologically active forms.

Our measurements of plasma corticosterone levels have demonstrated that during the spurt of growth at puberty female animals have higher plasma corticosterone concentrations. We therefore conclude that in female rats increasing adrenal activity at weaning and during puberty is sufficient to account for the limited stimulation of intestinal ammonia release. In males the increase in intestinal and kidney ammonia release, at this crucial period, is believed to reflect an important role for testosterone in Na^+ absorption and conservation.

The mammalian kidney has been identified as a target tissue for testosterone (Riggs & Walker, 1963; Ohno, Stenius, Christian, Harris & Ivey, 1970; Mainwaring & Morgan, 1973). Feldman & Funder (1973), Feldman, Funder & Edelman (1973) and Funder, Feldman & Edelman (1973) have, however, failed to demonstrate an affinity of dihydrotestosterone for the 'mineralocorticoid' or 'glucocorticoid' receptors characterized in rat kidney. They none the less believe that receptor heterogeneity suggests that certain 'glucocorticoid' receptors might normally be activated by an untested steroid. In the present study the dose–response relation obtained for aldosterone-stimulated ammonia release in distal sections of the small intestine with young animals suggests the presence of two types of steroid receptor in the small intestine: a high-affinity 'androgen/mineralocorticoid' receptor which responds to testosterone or aldosterone at physiological concentrations and a second high-affinity 'androgen/glucocorticoid' receptor which responds to testosterone or corticosterone at physiological concentrations. Failure to establish specific binding of testosterone or its active metabolite to kidney or intestinal receptors (Funder *et al.* 1973; Pressley & Funder, 1975; Henning *et al.* 1975) suggests that testosterone may be active at some point beyond steroid–receptor activation (Jost & Averner, 1975). However, it is possible that different steroids are activating the tissue at critical developmental stages, and further investigation of their binding characteristics at each stage is required.

Intestinal steroid effects on ion transport are long term, but there is no suggestion that they are permanent, and several workers have demonstrated transport effects of steroids in adult rat intestine (Crocker & Munday, 1969; Edmonds, 1972). Transport effects are known to be dependent on diet, and hormonal studies on adult animals have proved inconclusive (Levin, 1969; Crocker & Munday, 1970). In young animals, however, steroid effects reflect developmental changes within the gut. It is believed that chromatin activation within the germ cells of the crypt may induce long-term changes affecting the epithelial cells, but these effects may not be permanent.

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REFERENCES

CLARKE, R. M. & HARDY, R. N. (1969). An analysis of the mechanism of cessation of uptake of macromolecular substances by the intestine of the young rat ('closure'). *Journal of Physiology* **204**, 127–134.

CROCKER, A. & MUNDAY, K. A. (1969). Factors affecting mucosal water and sodium transfer in everted sacs of rat jejunum. *Journal of Physiology* **202**, 329–364.

CROCKER, A. & MUNDAY, K. A. (1970). The effect of the renin–angiotensin system on mucosal water and sodium transfer in everted sacs of the rat jejunum. *Journal of Physiology* **206**, 323–333.

DUNN, J. S. (1967). The fine structure of the absorbtive epithelial cells of the developing small intestine. *Journal of Anatomy* **101**, 57–68.

EDMONDS, C. J. (1972). Effect of aldosterone on mammalian intestine. *Journal of Steroid Biochemistry* **3**, 150–154.

FELDMAN, D. & FUNDER, J. W. (1973). The binding of 18-hydroxydeoxycorticosterone and 18-hydroxycorticosterone to mineralocorticoid and glucocorticoid receptors in the rat kidney. *Endocrinology* **92**, 1389–1396.

FELDMAN, D., FUNDER, J. W. & EDELMAN, I. S. (1973). Evidence for a new class of corticosterone receptors in the rat kidney. *Endocrinology* **92**, 1429–1441.

FERGUSON, D. R., JAMES, P. S., PATTERSON, J. F., SAUNDERS, J. C. & SMITH, M. W. (1979). Aldosterone-induced changes in colonic sodium transport occurring during development in the neonatal pig. *Journal of Physiology* **292**, 495–504.

FRAZIER, L. W. & VANATTA, J. C. (1973). Characteristics of H^+ and NH_4^+ excretion by the urinary bladder of the toad. *Biochimica et biophysica acta* **311**, 2073–2079.

FUNDER, J. W., FELDMAN, D. & EDELMAN, I. S. (1973). Glucocorticoid receptors in rat kidney: the binding of tritiated dexamethasone. *Endocrinology* **92**, 1005–1104.

GROSS, H. A., RUDER, H. J., BROWN, K. S. & LIPSETT, M. B. (1972). A radio-immunoassay for plasma corticosterone. *Steroids* **20**, 681–695.

HENNING, S. J., BALLARD, P. L. & KRETCHMER, N. (1975). A study of the cytoplasmic receptors for glucocorticoids in the intestine of pre- and post-weaning rats. *Journal of Biological Chemistry* **250**, 2073–2079.

HENNING, S.J. & JEFFERSON, M. A. (1979). Delineation of the glucocorticoid-sensitive period of intestinal development in the rat. *Endocrinology* **104**, 1158–1163.

HENNING, S. J. & KRETCHMER, N. (1973). Development of intestinal function in mammals. *Enzyme* **15**, 3–23.

JOST, J. P. & AVERNER, M. (1975). Gene regulation in mammalian cells: a model for the interaction of steroids and 3′,5′-cyclic AMP. *Journal of Theoretical Biology* **49**, 337–344.

KOLDOVSKY, O., SUNSHINE, P. & KRETCHMER, N. (1966). Cellular migration of intestinal epithelia in suckling and weaned rats. *Nature* **212**, 1389–1390.

LEVIN, R. J. (1969). The effects of hormones on the absorptive, metabolic and digestive functions of the small intestine. *Journal of Endocrinology* **45**, 315–348.

MAETZ, J. (1973). Na^+/NH_4^+, Na^+/H^+ exchanges and NH_3 movement across the gill of *Carassius auratus*. *Journal of Experimental Biology* **58**, 255–275.

MAINWARING, W. I. P. & MORGAN, F. R. (1973). A study of androgen receptors in a variety of androgen-sensitive tissues. *Journal of Endocrinology* **59**, 121–139.

MOOG, F. (1953). 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. *Journal of Experimental Zoology* **124**, 329–346.

MORRIS, B. & MORRIS, R. (1974). The effects of corticosterone acetate on stomach evacuation and the absorption of ^{125}I-labelled globulins in young rats. *Journal of Physiology* **240**, 79–89.

OHNO, S., STENIUS, C., CHRISTIAN, C., HARRIS, C. & IVEY, S. (1970). More about testosterone induction of kidney alcohol dehydrogenase activity in the mouse. *Biochemical Genetics* **4**, 565–577.

PRESSLEY, L. & FUNDER, J. W. (1975). Glucocorticoid and mineralocorticoid receptors in gut mucosa. *Endocrinology* **97**, 588–596.

RIGGS, T. R. & WALKER, L. M. (1963). Sex hormone modification of tissue levels and urinary excretion of α aminoisobutyric acid in the rat. *Endocrinology* **73**, 781–788.

SNART, R. S. & TAYLOR, E. (1978). Aldosterone effects on renal metabolism. *Journal of Physiology* **274**, 447–454.

STENSTAD, P. & EIK-NES, K. B. (1982). *In vitro* metabolism of testosterone to 17β-hydroxy-5α-androstane-3-one and 5α-androstane-3α,17β-diol by the rat intestine. *Journal of Steroid Biochemistry* **17**, 401–406.

SUNSHINE, P., HERBST, J. J., KOLDOVSKY, O. & KRETCHMER, N. (1971). Adaptation of gastrointestinal tract to extrauterine life. *Annals of the New York Academy of Sciences* **176**, 16–29.

WRIGHT, N. A., MORLEY, A. R. & APPLETON, D. (1972). The effect of testosterone on cell proliferation and differentiations in the small bowel. *Journal of Endocrinology* **52**, 161–175.