pregnancy promotes, while treatment with corticosterone daily from tenth day of pregnancy retards, the
differentiation of the adrenocortical cells of rat embryos from the fifteenth day of foetal life.

Now, it was studied if adrenalectomy of rats on the sixth day of pregnancy influenced the fine
structure of cortical cells in the adrenal and ACTH-producing cells in the pituitary, and the plasma
corticosterone level in their embryos. It was shown that the fine structure of adrenocortical cells (first the
mitochondria and endoplasmic reticulum) were more differentiated on the fifteenth day of pregnancy than in
the corresponding controls. The level of plasma corticosterone in adrenalectomized mothers on the
eighteenth day of pregnancy was lower than that in their embryos.

The fine structure of the ACTH-producing cells in the pituitary appeared to be more differentiated,
they occurred more frequently and their immunohistochemical stainability seemed to be more intense.

It could be supposed that the hypophyseo-adrenocortical axis of rat embryos has a capability for
functioning on the fifteenth day of pregnancy.

115. Comparative Studies on Hormone Patterns in Domestic Pigs under Applied Aspects. HERBERT HAID
AND ULRICH ENSINGER, Arbeitsgruppe Endokrinologie, Institut für Zoophysiologie, Universität
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Disorders in hormonal regulation seem to be responsible for deficiencies in modern pig races,
especially for insufficient meat quality like PSE-meat (pale, soft, exudative). Different races of pigs with a
body weight of about 100 kg were examined for these aspects. Piétrain pigs are considered to have severe
constitutional defects. The Edelschweine (German large white/Yorkshire) seem to be less susceptible, while
the German Landrace is mediocre in this point of view. The following parameters were examined under
standardized conditions: blood levels of cortisol, thyroxine and insulin; the pH value in the Musc.long.dorsi
45 min postmortem and the Gofo-value 48 hr postmortem as measures for meat quality. The Piétrains have
the highest cortisol blood levels before and after a stress situation. The levels of German Landrace and
Edelschwein are significantly lower. The thyroxine levels are distinguished in like manner. Insulin blood
levels of Piétrains are comparatively low after about 1.5 days fasting. The application of adrenaline lowers,
while the application of the β-receptor blocking agent Viskin raises the pH value of the Musc.long.dorsi
These results point out that the hormonal system in different races might possibly influence the meat quality
of pigs. Our preliminary interpretation is, that adrenaline quite obviously has a causative function in the
development of PSE-meat by stimulation of the glycogenolysis in the muscle while the significance of
thyroxine and insulin seems to be of a more permissive character. Races with a high adrenaline response to
stress, high cortisol, high thyroxine and low insulin levels seem to have more deficiencies in meat quality.

XII. Osmotic and Ionic Regulation and Nitrogen Metabolism

116. Morphometrical Study with Light and Electron Microscope of the Role in Ionic Regulation of Two
Endocrine Cell Types in Stannius Bodies of Sticklebacks. S. E. WENDELAAR BONGA, J. A. P
GREVEN, C. MEIN, AND M. VEENHUIS, Zoological Laboratory, University of Groningen, The
Netherlands.

The Stannius bodies (SB) of three spined sticklebacks and European eels, both euryhaline teleosts
contain two secretory cell types (types I and II) of presumptive endocrine nature. This is in contrast with
several freshwater and seawater species, where only one cell type was found which was structural
comparable to type I (Wendelaar Bonga and Greven, in press). The function of both cell types was studied
in sticklebacks (Gasterosteus aculeatus trachurus), using quantitative methods for light and electron
microscopy (including thin-sectioning and freeze-etching). In seawater-adapted sticklebacks type I cells are
more active, while type II cells are less active, than in freshwater adapted specimens. Experiments include
exposure of fishes to artificial seawater, modified by changing the concentration of the eight most com-
mons present, showed that type I cells are activated by increasing Ca²⁺ content, while type II cells are
activated by decreasing K⁺ concentration. The substance produced by type I cells is probably comparable
to hypocalcin, the endocrine factor isolated from the SB of cod and killifish by Pang et al. (Endocrinology
548, 1974). It is suggested that type II cells are involved in the endocrine control of K⁺, especially
environments with low K⁺ content. Extirpation of the SB leads to reduction of the epithelial cells of
the second proximal tubule of the nephron, as established ultrastructurally by lineal integration. Accordingly,
is indicated that the kidney is a target for one or both Stannius hormones.
117. **Hormonal Control of Na Cl Exchange in the Flounder Platichthys flesus.** N. A. A. Macfarlane, Groupe de Biologie Marine, Commissariat a l’Energie Atomique, Station Zoologique, Villefranche-sur-Mer, 06230-France.

The effects of hypophysectomy on Cl exchange and of prolactin and cortisol on Na and Cl exchanges have been studied with $^{23}$Na and $^{36}$Cl. In sea water (SW) hypophysectomy did not alter plasma Cl, Cl space or Cl efflux. Hormonal effects were investigated in a transfer experiment. Na and Cl fluxes were determined simultaneously in SW, for 10 min after rapid transfer into fresh water (FW), then for 10 min after the addition of external K$^+$ (as K$_2$SO$_4$, to 10 mM K$^+$ as in SW). Control fish were noninjected. Following prolactin treatment (6 injections, 5 µg/day/g) Na efflux in SW and K$^+$-induced Na efflux were depressed slightly below control levels, though not significantly. However corresponding Cl effluxes were reduced significantly (SW: 1062 ± 121 (µeq/hr/100 g (n = 7) P < 0.05; K$: 99 ± 18 to 0 ± 18, P < 0.01) suggesting that prolactin interferes with the Cl transport mechanism. Both plasma Na and Cl were increased by prolactin (Na: +20.2 ± 6.84 meq/liter, Cl: +12.8 ± 4.17 meq/liter P < 0.05). Cortisol (25 µg/day/100 g) was tested in the short term (1 hr after first injection) and in the long term (6 injections). In SW Na and Cl effluxes and plasma Na and Cl were unaffected in each case. K$^+$-induced Na and Cl effluxes were increased by chronic treatment, though not significantly. In contrast acute treatment reduced the Na and Cl "passive leak" fluxes in FW (Na: 306 ± 32 to 211 ± 18 µeq/hr/100 g, Cl: 242 ± 27 to 165 ± 14, P < 0.05). Comparison of short- and long-term treated fish revealed a marked increase (25%) in SW Na efflux (+609 ± 208 µeq/hr/100 g, P < 0.05). Thus prolactin and cortisol exert some influence on NaCl exchange in the flounder but apparently to a lesser extent than in various other species such as the eel or killifish.

118. **Renal Response to Transfer of the Rainbow Trout from Fresh Water to Sea Water: Possible Involvement of Neurohypophysial Hormones.** R. J. H. Sinnott and J. C. Rankin, Department of Zoology, University College of North Wales, Bangor, U.K.

In rainbow trout adapted to fresh water the kidney removes water which enters the fish osmotically. On transfer of the fish to sea water the role of the kidney changes abruptly, reducing urine flow dramatically as the fish loses water by osmosis. Neurohypophysial hormones have been shown to be antidiuretic in low doses in the eel (Henderson, I. W., and Wales, N. A. M., 1974. *J. Endocrinol. 61*, 487-500) where they may be involved in the renal response to transfer to sea water (Babiker, M. M., and Rankin, J. C., 1972. *J. Endocrinol. 57*, xi-xii). The work described here is part of a study to see if the above results are peculiar to the eel or likely to be true of euryhaline teleosts in general. rainbow trout were prepared with chronically implanted bladder catheters and dorsal aortic cannulae. Glomerular filtration rate (GFR) was measured as the clearance of $^{31}$Cr EDTA and/or $^{3}$H inulin. In the first hour after transfer to sea water, urine flow dropped to about 25% of the fresh water value and the decline continued for 4 hr after which it remained almost constant at about 1% of the fresh water value. GFR fell to 20% after 1 hr and 2% after 4 hr, tubular water reabsorption being increased. Plasma osmorality increased by 50 to 65 mOsm/liter during the first hour and urine osmorality increased gradually from 25-40 mOsm/liter to 200-300 mOsm/liter over the first 12 hr. Dorsal aortic blood pressure was reduced for the first few hours but then increased. Neurohypophysial hormones were injected into fresh water adapted trout to see if they produced effects similar to those produced by transfer of the fish to sea water. Oxytocin and AVT at doses of less than 1 ng/kg body wt. were antidiuretic but in the range 1-200 ng/kg responses were variable, either diuresis or antidiuresis occurring. Higher doses were usually diuretic. Vasopressin was antidiuretic.


Males of *Gasterosteus aculeatus* have impressive osmoregulatory capacities which enable them to migrate between sea and fresh water and, moreover, to live in fresh water without normal functioning kidneys. In the reproductive period, under the influence of testosterone, 90% of the cells of the renal tubules produce mucous substances for nestbuilding. To obtain information about renal and extrarenal osmoregulation in maturing males, we carried out light and electron microscopical studies of kidney, bladder, digestive
tract and gills and estimated the activity of ion dependent ATPases. In the glomeruli the mesangial layer (probably a filtration barrier) increases greatly in thickness, while the lumina of the glomerular capillaries decreases. This suggests a decrease of glomerular filtration rate. The cell height in the first proximal segment of the nephron decreases slightly. Conversely, the cells of the second proximal segment, the collecting tubule and part of the ureter increase in height and produce numerous granules containing mucoproteins and mucopolysaccharides. The basal folds (site of ion transporting systems) disappear completely. Freeze-etch studies showed a decreased number of particles associated with the outer cell membrane. These morphological changes take place within 3-4 days after administration of testosterone to castrates. The bladder and the distal parts of the ureter remain unchanged. The activities of ouabain sensitive Na+/K+-ATPase and of Mg2+-ATPase decrease in the kidneys, but increase in intestinal musosa. No changes were found in the gills. Ultrastructural analysis of the intestine showed in almost all epithelial cells the presence of basal folds together with the mitochondria, as are normally present in renal tubular cells. The results indicate that in mature males the intestinal epithelial cells increase ion transport activity synchronously with the diminished ion transport in the renal tubular cells. [Supported by the Netherlands Organisation for the Advancement of Pure Research (Z.W.O.).]

120. Adaptation of the Neotenic Urodele, Axolotl (Ambystoma mexicanum) to a Hyperosmotic Medium. M. P. IRELAND and I. M. SIMONS, Department of Zoology, University College of Wales, Aberystwyth, Wales, U.K.

In a previous paper [Ireland, Comp. Biochem. Physiol. 46A (1973)] the aquatic anuran Xenopus laevis subjected to hyperosmotic media was shown to have elevated blood electrolytes, reduced water uptake and urine volume and changes in the pars intermedia cells of the pituitary gland. Using the same techniques a comparison was made with an aquatic urodele, the axolotl. A. mexicanum maintained in 30% sea water for 5 days showed a significant reduction in water uptake and urine volume, compared with axolotl adapted to fresh water, of 66-70%. Serum osmotic concentration increased by 152 mOsm/liter due to elevation of Na+, Cl- and urea. The electrolytes constituted about 97% of the total difference. Ambystoma maintained in 30% sea water for 35 days showed a significant decrease in serum Na levels. After both periods of sea water adaptation the blood osmotic concentration did not exceed that of the external medium. In sea water treated axolotl the pars intermedia showed large intercellular spaces, reduced cytoplasm and the cell nuclei were significantly reduced in size (fresh water, 80.6 μm²; sea water, 58.8 and 59.2 μm²). The response of A. mexicanum to dilute sea water appears to be similar to X. laevis in that the principal contributors to the increased osmotic concentration of the blood are Na and Cl ions with a decreased water uptake and urine volume. A reduction in the size of the pars intermedia cells may be an indication of inactivity.

121. Some Morphological Aspects of the Renin-Angiotensin System in Amphibians. A. P. M. LAMERS, M. E. A. VAN MANSFIELD, and A. B. M. KLAASSEN, Department of Biology, Medical Faculty, University of Nijmegen, The Netherlands.

It is evident, from the literature, that the juxtaglomerular apparatus (JGA) in the amphibian kidney consisting of granulated media cells in the afferent arteriole of the glomerulus and a macula densa-like structure in the distal tubule, resembles the mammalian JGA in several respects and the result of bioassa procedures points to the presence of renin in amphibian blood plasma. We studied the ultrastructure of the JGA in several Amphibia, especially in Bufo bufo. It appeared that there exists species variation with respect to the structure of the JG-granules. They differ from the generally homogeneous mammalian granules. In Bufo bufo their content is lamellar. The presence of granules filled with vesicles in the neighbourhood of the Golgi system and the presence of similar vesicles near and against this system indicates that in the toad, as in mammals, the Golgi apparatus is involved in the genesis of the granule. Within the granules these vesicles seem to change into a more flocculent substance which changes into the typical lamellar structure. Histochemically, as in mammals, the granules show acid phosphatase activity. The time taken for the positive reaction for this enzyme to become manifest was shorter in animals placed for weeks in distilled water than in toads which were salt loaded for the same length of time. This indicates that these granules are involved in salt metabolism and probably are an essential part of the renin-angiotensin-aldosterone system in the toad.
122. Prolactin and Osmoregulation of the Terrapin, Chrysemys picta. K. J. BREWER and D. M. ENSOR, Dept. of Zoology, University of Liverpool, U.K.

A study has been made of the effect of prolactin on urinary excretion in *C. picta*. Injection of ovine prolactin into intact terrapins (5 IU/kg) caused an elevation in Mg²⁺ over the 24 hr period following injection. No change in the excretion of Na⁺ and K⁺ was observed during this period. Subsequent experiments showed that injection of 20 IU/kg ovine prolactin into previously dehydrated *C. picta* caused an increased reabsorption of urinary sodium (180.8 vs 264.7 mEq/liter). Further experiments using ³H-inulin clearance as a measure of glomerular filtration rate have shown that prolactin causes a significant increase in GFR, while hypophysectomy reduces the level of filtration by the kidney.


Electrolytic lesions were placed to interrupt the hypothalamo-hypophysial tract in the base of the hypothalamus and their effects on the clearance and transtubular fluxes of water and electrolytes were studied. Plasma electrolyte concentrations were unchanged in tract-operated individuals (TOP) but plasma corticosteroid levels were reduced significantly from 2.93 to 1.34 μg/100 ml (P < 0.01). During water diuresis (10 ml/100 g) TOP individuals exhibited a reduced rate of urine production, reduced osmolar and relative osmolar clearances and an enhanced tubular reabsorption of sodium ions (88.6 vs 78.4%, P < 0.02). During saline diuresis (150 mM NaCl, 10 ml/100 g) tubular sodium reabsorption was again elevated in TOP individuals (66.9 vs 50.8%, P < 0.02) and accompanied by a reduced relative osmolar clearance, but an increased inulin clearance and rate of urine production. Injections of arginine vasotocin (AVT) into TOP individuals were invariably accompanied by an antidiuresis of glomerular and tubular origin and, at most dose levels, by a reduction of both the relative osmolar and relative free-water clearances. When renal parameters were measured during prolonged saline diuresis, but employing spontaneously voided rather than ureteral urine, the differences between TOP and intact individuals were confined to an increased rate of urine production and increased clearances of inulin, solutes and water in the former. Fractional reabsorption of sodium ions did not differ significantly between TOP and intacts (89.1 vs 92.4%) indicating that differences in postrenal reabsorption of sodium had apparently rectified the disparity observed at the tubular level. These data enable one to estimate the extent of water and electrolyte reabsorption in the colon and indicate that relative sodium reabsorption from the colon was significantly reduced in TOP individuals with prolonged saline diuresis (73.2 vs 88.8%, P < 0.05). These results are consistent with the hypothesis that AVT functions as a physiological antidiuretic hormone in reptiles and may also be implicated in the regulation of postrenal sites of water and electrolyte reabsorption.


18-Hydroxycorticosterone (18–OH–B), a steroid found in relatively large amounts when seal adrenals are incubated in the presence of radioactive precursors (Borruel et al. Gen. Comp. Endocrinol. 22, 1, 1974), was administered in a single dose of 3 μg to adrenalectomized rats which had been put on a standardized diet. Urine was retained for 3.5 hr in the bladder and was then analysed for Na⁺, K⁺, NH₄⁺, titratable acidity to pH 7.4 (titratable H⁺) and net acidity (H⁺ from NH₄⁺ plus titratable H⁺). Increases of excretion rates above controls for 18–OH–B treated rats were as follows: Titratable H⁺: 82% (P < 0.01). NH₄⁺: 54% (P < 0.05). Net acidity: 48% (P < 0.02). Na⁺ excretion rate was also slightly increased (P < 0.05) but K⁺ excretion rate and urinary volume remained unaffected. Aldosterone, at the same dose level had a pronounced sodium-retaining effect but no effect on the output of protons. Thus 18–OH–B increases the output of protons independently of any reabsorption of Na⁺. It is postulated that the prolonged diving capacity of seals, the high Na⁺ concentration of their marine habitat and the ability of their adrenals to synthesize 18–OH–B are related to this biological effect of the steroid.
125. *The Effects of Hormonal Substitution in Thyro-Parathyroidectomized Rats.* S. BlähsEr, Zentrum für Anatomie und Cytobiologie, Justus Liebig University, Giessen, FRG.

Growing female Wistar rats were thyro-parathyroidectomized to investigate the effects of hormonal substitution on (1) serum concentrations of different substances and (2) the epiphyseal plate morphology and histochemistry. The operated rats received daily subcutaneous injections of thyroxin, parathormone, calcitonin, thyroxin + parathormone or thyroxin + calcitonin from days 3 to 14 after the operation. Neither the operation without a following hormonal treatment nor the hormonal treatments in operated rats caused significant changes in the serum concentrations of urea, potassium, sodium, or in the activity of alkaline phosphatase. The serum concentration of inorganic phosphorus decreased and that of total protein increased after injections of thyroxin + parathormone or thyroxin + calcitonin. An increase in serum calcium concentration was observed only in the group treated with thyroxin + calcitonin. In order to study the effect of thyroxin, the distal epiphyseal plate of the femur was examined histologically and histochemically. Parathormone exerts an osteolytic action only if combined with injections of thyroxin. The strong maturation-inducing effect of thyroxin on cartilage is abolished by additional application of parathormone or calcitonin.

126. *Regulation of the Blood Calcium Concentration in the Pulmonate Snail, Lymnaea stagnalis.* N. D. De Wit and H. van der Brink, Dept. of Biology, Free University, Amsterdam, The Netherlands.

From physiological data it is suggested that with respect to blood calcium concentration at least two regulatory systems may function in this animal. One system enables the snail to maintain a blood calcium concentration more or less independent of the calcium concentration of the medium. Another system determines the level of the blood calcium concentration in response to the "physiological condition" of the animal.

The possibility that certain types of neurosecretory cells in the central nervous system could be involved in the regulation of the blood calcium concentration was investigated by ganglion extirpations and ganglion homogenate injections. Because extirpation of the various ganglia affects food intake in varying degrees, all snails were starved after being operated. The experiments showed that in starved snails one of the extirpations caused a change in the blood calcium concentration. In order to include normally fed animals in the experiment, homogenates of the various ganglia were injected via a permanent cannula in the visceral sinus. Serial blood samples were taken by way of the same cannula. Homogenates of the various ganglia from both starved and fed snails were injected into both starved and fed unoperated snails. Homogenates of any ganglion from normally fed snails produced a marked rise in the blood calcium concentration of both starved and fed snails. Homogenates of ganglia from starved snails only produced a slight rise in the blood calcium concentration, in both fed and starved snails. The experiments described above have failed to demonstrate a clear effect of any neurosecretory centre on the blood calcium concentration so far. It is suggested that neurotransmitters could be partly responsible for the results of the injection experiments. Attempts will now be made to remove the neurotransmitters from the homogenates prior to repeating the injection experiments.

127. *The Influence of ACTH and Corticosteroids on the Activities of Enzymes Correlated with Urea Production in Ranidae.* P. Radke and W. Hanke, Zoologisches Institut, Universität Karlsruhe F.G.R.

Some amphibian species, like *Xenopus laevis* and *Rana cancrivora*, are able to react to osmotic stress with an increase of urea in the blood. Similar results are obtained after desiccation experiments with *Rana temporaria* and *Rana esculenta*. The increase of the urea level is due to accumulation of urea and high activities of the enzymes correlated with urea production. The question is, whether these changes of enzyme activities are regulated by hormones. Thyroxine increases the activities of urea cycle enzymes (for example in *X. laevis*), but it is not known whether other hormones have similar effects. For the determination of urea cycle enzymes a modified method of Brown and Cohen (1959) was used. Glutamate-oxalacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) were studied with a test-combination and glutamat
Treatment with ACTH, cortisol, corticosterone, or aldosterone for 4 days leads to a more than twofold increase in the urea level, whereas treatment with TSH has no effect and T3 and T2 must be applied in very high doses to produce the same effect as corticosteroids. Studies with 14C-urea show that the high urea levels are generated by lower excretion rates. Control animals excrete at a rate of about 3%/hr, whereas ACTH lowers the excretion rate to 1.5%/hr, cortisol and aldosterone to 1%/hr, and corticosterone to 0.7%/hr. TSH and T3 (3.2 and 2.2%/hr, respectively) do not affect the excretion rate to the same extent as corticosteroids do. Metamorphosed axolotls excrete urea at about the same rate as neotenic animals but if the animals are very well fed the excretion rate increases, especially in larval axolotls.

XIII. Vertebrate Carbohydrate Metabolism

129. Entero-Insular Endocrine Cells in Myxine glutinosa. Sture Falkmer, Yngve Östberg, and Lennart Boquist, Department of Pathology, University of Umeå, S-901 87 Umeå 6, Sweden.

Current studies on the evolution of insulin and glucagon production have revealed that whereas insulin occurs in the islet parenchyma of all vertebrates, pancreatic glucagon production seems to be limited to Gnathostomian species only. Against this background the occurrence of various types of endocrine cells in the islet organ and in the mucosa of the adjacent bile duct and intestine of an Agnathan species was supposed to be of particular interest. The Atlantic hagfish, Myxine glutinosa, was chosen, due to the fact that it seems in several respects to present the most primitive and original features of the whole endocrine system, being an extant species of a group known since the Silurian era. Correlated light-microscopical and ultrastructural techniques were applied, including differential granule staining procedures, immunofluorescence and histochemical methods, as well as transmission electron microscopy. Previously described, insulin-producing β-cells in the separate islet organ with their typical, poikiloform secretion granules were found to originate from cells budding off from the bile duct mucosa. A second, rarer type of islet parenchymal cells, showing spherical secretion granules, also seemed to bud off from the bile duct mucosa. It was found to belong to the so-called APUD-series of endocrine cells, and showed also in other respects the main features of insulin-producing β-cells. Moreover, transitional forms were observed. Neither in the islet parenchyma, nor in the bile duct mucosa, were any glucagon- or gastrin-producing cells discovered. In the gut mucosa, however, argyrophil cells with light-microscopic and ultrastructural characteristics of enteroglucagon-producing cells were found. The results indicate that there is an intimate relationship of insulin-producing cells to the bile duct mucosa, and that enteroglucagon production is phylogenetically older than pancreatic glucagon production.

130. Regulation of Blood Glucose in Intact and Hypophysectomized River Lampreys (Lampetra fluviatilis) before and during period of Sexual Maturation. Lis Olesen Larsen, Zoophysiological Laboratory A, University of Copenhagen, Denmark.

Glucose concentration in peripheral blood increased in intact male and female river lampreys at the time of spermatiation or ovulation, from 20–30 mg/100 ml to about 35 mg/100 ml (method: Boehringer’s