on the contrary 5-HTPH left in the ME for 5 days induces a very significant ($P \leq .001$) decrease in the pituitary content of LH; the reduction in pituitary LH stores observed after placement of 5-HTPH in the ME is more pronounced than that obtained (*5*) after the implantation of comparable amounts of melatonin (Fig. 1). When placed in the reticular formation of the midbrain 5-HTPH was as effective as melatonin in reducing pituitary LH content (Fig. 2); the implantation of 5-MTPH in the midbrain resulted also in a significant decrease in pituitary LH stores. No one of the compounds tested was able to modify the pituitary reserve of LH when placed in the cerebral cortex or in the pituitary.

These data confirm that the CNS contains receptors which are sensitive to changing concentrations of indole compounds, and that these receptors may participate in the regulation of synthesis, or release of LH, or both. It is interesting to note that 5-HTPH, a compound which was believed to be inactive on endocrine phenomena (*8*), proved effective in modifying pituitary stores of LH after being placed in either the ME or the midbrain. It is quite possible that the activity of 5-HTPH was not discovered in previous experiments (*8*), because the compound cannot cross the blood-brain barrier when given systemically; of course this factor is not significant when the compound is implanted directly in the brain. It appears surprising that the receptors in the ME and those in the midbrain have a different sensitivity for the various compounds tested; ME structures are sensitive to melatonin and to 5-HTPH but not to 5-MTPH, whereas midbrain elements respond to melatonin, to 5-HTPH, and to 5-MTPH.

When placed in the ME, 5-HT was inactive in spite of the fact that it has been reported that 5-HT is metabolized to 5-HTPH by brain tissue (*15*); our results suggest that this conversion does not take place in the ME.

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Fluid Transport and Tubular Intercellular Spaces in Reptilian Kidneys

Abstract. *Renal tubules of crocodiles, lizards, snakes, and turtles have intercellular spaces similar in type to those observed in the mammalian gall bladder, but different from those of mammalian renal tubules. The fluid movements across renal tubules of reptiles are correlated with the width of the tubular intercellular spaces. In the proximal tubules, where transport is always isosmotic, the spaces are open whenever the tubular epithelium is tranporting, but closed when no transport is taking place. In distal tubules, intercellular spaces are wide open when the osmolality of the urine is close to that of the blood, that is, when the fluid resorbed is almost isosmotic to the tubular fluid. The apical two-thirds of the intercellular spaces are closed when the urine is hypoosmotic. They are also closed when the tubules are not transporting, as in collapsed tubules or tubules poisoned with ouabain. Thus, as in the gall bladder, the open intercellular spaces appear to be found whenever there is fluid transport across the epithelium.*

In the proximal renal tubules, as well as in the intestine and gall bladder of vertebrates, a single layer of epithelial cells transports salt and water isosmotically from the mucosal to the serosal side. Studies of gall bladder and intestinal epithelia in vivo and in vitro have shown that even when the mucosal fluid is hyperosmotic to the serosal fluid, transport of fluid may occur from the mucosal to the serosal side. This movement of water against its gradient was explained experimentally and theoretically as a solute-linked water transport involving a three-compartment system (*1*).

Electron microscopy of gall bladder fixed rapidly in osmium tetroxide or glutaraldehyde showed that an extra compartment does exist and that it appears to consist of the lateral intercellular spaces (*2*). The spaces were found to be wide open when fluid was transported across the gall bladder epithelium from mucosal to serosal side, but tightly closed when fluid transfer was inhibited in several different ways. Furthermore, the opening or closing of the spaces was not a result of swelling or shrinking of the cells.

Electron microscopy of the kidney of the crocodile *Crocodylus acutus* (*3*) showed open lateral intercellular spaces in the proximal and distal tubules, remarkably similar to those observed in the mammalian gall bladder. There were no basal infoldings, but there were thin, finger-like projections of the lateral membranes between the cells. This was surprising since teleost (*4*), amphibian (*5*), and mammalian kidneys (*6*) have proximal and distal tubular cells in which the cellular interdigitations give rise to the so-called basal infoldings.

These findings raised two major questions: First, do all reptilian kidneys show these characteristics of the lateral borders of the tubular cells, or do they differ with the renal adaptations to different habitats? Second, could the attractive hypothesis that the intercellular spaces are related to fluid transport be tested in reptilian kidneys by exposing the animals to various conditions that would further or stop fluid transport across the renal epithelium?

To answer these questions we studied the function and ultrastructure of the kidneys of reptiles from each of the four major groups of present-day living

reptiles, that is, snakes, lizards, turtles, and crocodiles. Experiments were performed on six freshwater and six saltwater crocodiles (*Crocodylus johnstoni* and *Crocodylus porosus*, respectively), nine blue-tongued lizards (*Tiliqua scincoides*; terrestrial but not arid habitat), two banded sea snakes (*Laticauda colubrina*; marine), and two green turtles (*Chelonia mydes*; marine). To determine glomerular filtration rate, inulin-C^{14} (120 μc per kilogram of body weight)

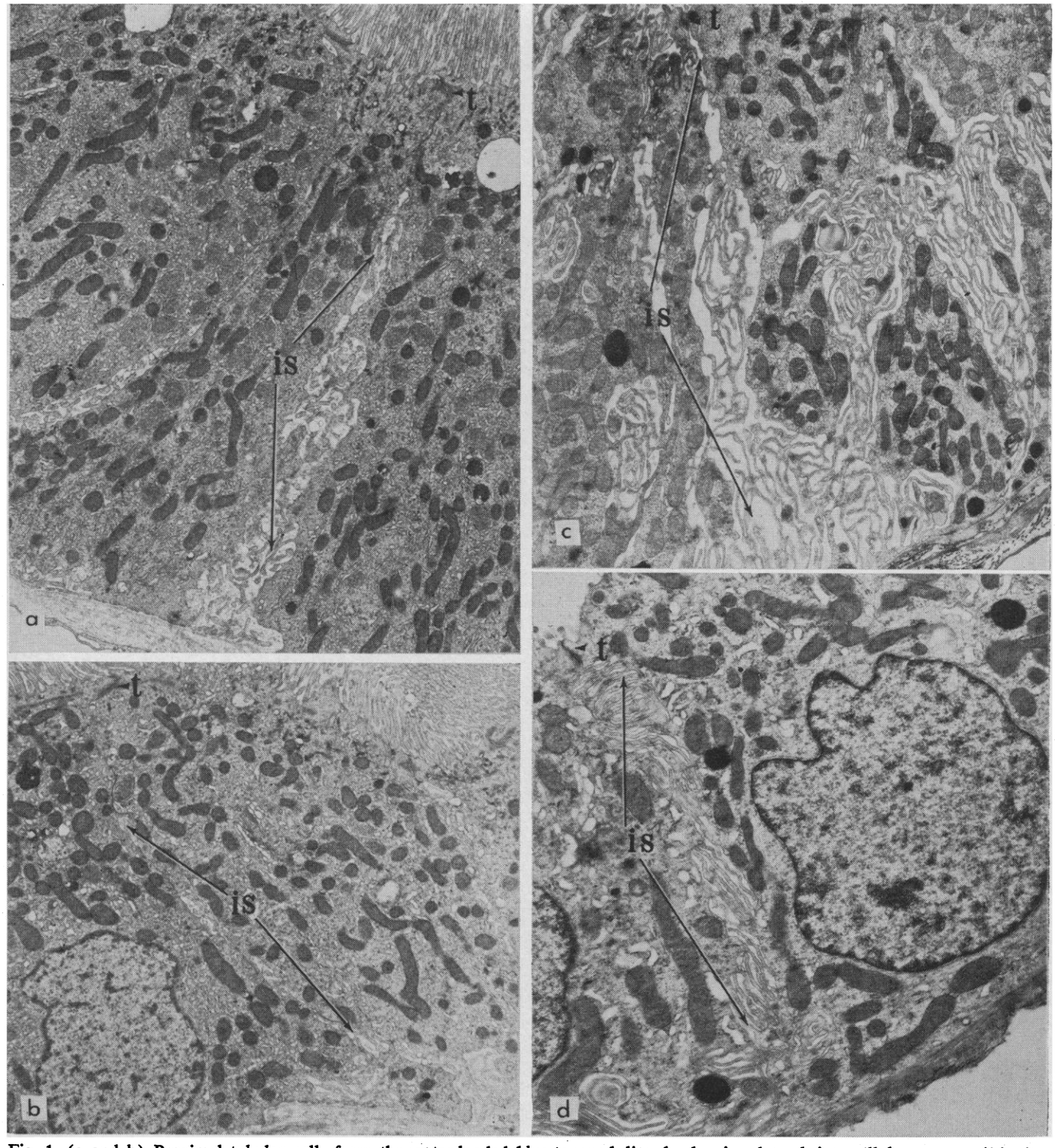

Fig. 1. (a and b) Proximal tubular cells from the water-loaded blue-tongued lizard, showing lateral intercellular spaces (*is*) (× 5900). (a) The microvilli (top right) extend into the tubular lumen (partly shown) which in this case in open. Note that the lateral intercellular space (*is*) from the end of the terminal bar (*t*) to the basal lamina (bottom left) is also open. (b) The tubular lumen shown partly at top is closed. The lateral intercellular spaces (*is*) are also closed from the terminal bar (*t*) to the basal lamina (bottom right). (c and d) Distal tubular cells of blue-tongued lizards. (c) From a dehydrated lizard with a urine osmolality 75 percent of that of the blood. The tubular lumen shown partly at top is open. Note that the extensive lateral intercellular spaces (*is*) are open all the way from the terminal bar (*t*) to the basal lamina (× 6350). (d) From a water-loaded lizard with a urine osmolality 43 percent of that of the blood. The tubular lumen shown partly at top is open. The lateral intercellular spaces (*is*) from the terminal bar (*t*) to the basal lamina (bottom) are closed (× 8470).

was injected subcutaneously the night before the experiment, and the animal was left unrestrained.

The experiments lasted from 4 to 36 hours depending on the type of experiment. Urine and blood samples were collected as described previously (*7*). Urine was usually collected every 1 to 2 hours. At the end of the experiment, 60 mg of Diabutal per kilogram of body weight was injected into the heart. The abdomen was opened quickly, and the renal portal veins were injected with ice-cold glutaraldehyde. Procedures for electron microscopy were the same as described previously (*3*). Urine and blood samples were analysed for radioactivity with a liquid scintillation counter (Nuclear-Chicago), and osmolality was determined with a Fiske osmometer (*8*).

Crocodiles and lizards were studied when they were "normal", salt-loaded, water-loaded, dehydrated, or poisoned with ouabain. The snakes and turtles were studied during "normal" conditions only. "Normal" animals received food and water until the time of the experiments and no further treatment during the experiment. For salt-loading, a 9 percent NaCl solution was injected subcutaneously (10 percent of body weight). For water-loading, water was given by stomach tube (10 to 15 percent of body weight). For dehydration, the animals were deprived of water until they had lost about 10 percent of the body weight. For poisoning of the tubular sodium transport mechanism, ouabain (3 ml of a 2 m*M* solution per kilogram of body weight) was injected into the ventral tail vein 5 minutes before the animal was killed. The veins from the tail drain directly into the renal portal veins, and the poison thus reached the kidney before entering the general circulation.

The electronmicrographs showed that the lateral intercellular spaces were of a similar type in saltwater and freshwater crocodiles and in lizards, snakes, and turtles. Proximal as well as distal tubular cells have lateral finger-like projections into the intercellular spaces (Figs. 1–3), but the basal plasma membrane is without basal infoldings.

In the proximal tubule of mammals and other vertebrates, the tubular fluid always remains isosmotic to the blood, even when a considerable fraction of the fluid is resorbed. Thus in this respect the proximal tubules function in a manner similar to that of the gall bladder or intestinal epithelium.

In reptilian and amphibian kidneys

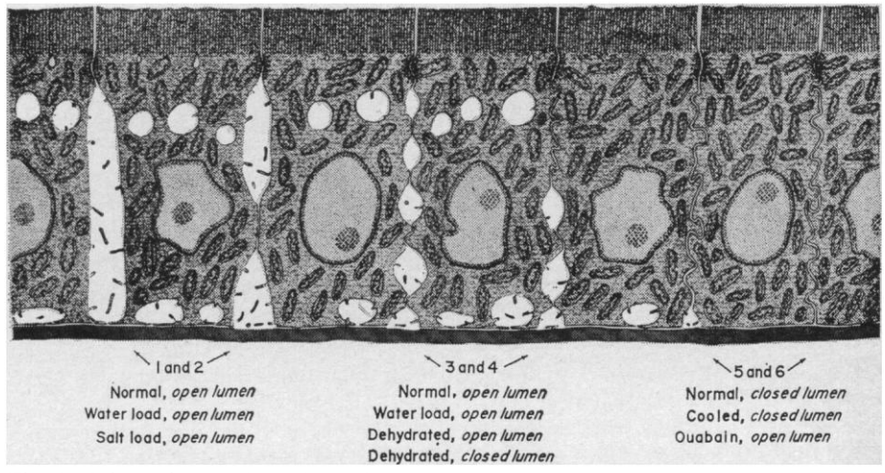

Fig. 2. Schematic drawing of the intercellular spaces of proximal tubular cells. The different types of spaces observed are represented, and the physiological conditions are given. *Lumen* refers to the tubular lumen.

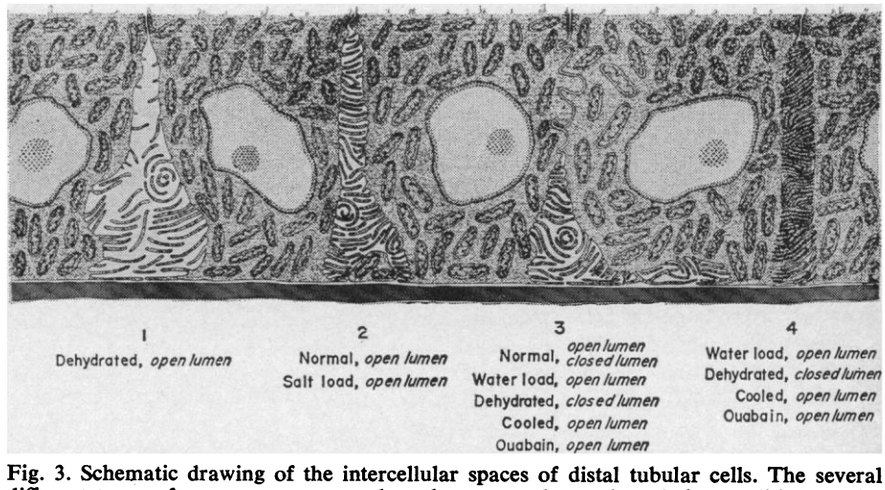

Fig. 3. Schematic drawing of the intercellular spaces of distal tubular cells. The several different types of spaces are presented as they were observed, and the conditions under which they were observed are indicated.

Table 1. Glomerular filtration rates and the ratios of urine osmolalities to blood osmolalities in the various reptiles. The data on *Crocodylus acutus* are taken from Schmidt-Nielsen and Skadhauge (*7*). The other data are from the present study. Mean values for four to six clearance periods for each animal are given. The values are from the same experimental animals from which the kidneys were fixed for electronmicroscopy, and therefore represent the physiological function of the kidneys at the time the animals were killed.

Animal	Glomerular filtration rate [ml hr^{-1} kg^{-1} (body weight)]				Osmolality, urine: plasma			
	Normal	Salt-loaded	Water-loaded	Dehydrated	Normal	Salt-loaded	Water-loaded	Dehydrated
Crocodylus acutus	9.6 ± 1.0	7.3 ± 0.8	15.2 ± 2.0	6.1 ± 0.6	0.80 ± 0.01	0.67 ± 0.02	0.82 ± 0.02	0.84 ± 0.01
Crocodylus johnstoni	6.0 ± 1.5		3.3 ± 1.1	1.9 ± .2	.71 ± .06		.83 ± .04	.94 ± .01
Crocodylus porosus	1.5 ± 0.2	2.8 ± .9	18.8 ± 2.3		.95 ± .04	.78 ± .15	.45 ± .05	
Tiliquas scincoides	15.9 ± 1.0	14.5 ± .5	24.5 ± 2.0	0.68	.50 ± .07	.66 ± .03	.43 ± .04	.79
Laticauda colubrina	0.5				.85			
Chelonia mydes	14.3				.95			

the number of functioning renal tubules varies directly with the filtration rate (*9*). Thus the filtration rate may be reduced by the closing of a number of glomeruli. When a glomerulus is closed the tubule collapses, and transport across its epithelium ceases. Functioning normal nephrons remain open, and fluid transport takes place across the tubular epithelium.

Among the reptiles investigated the blue-tongued lizard showed the widest variations in the filtration rate: from 0.7 ml/hr per kilogram of body weight, during dehydration, to 25 ml/hr per kilogram of body weight during water-loading (Table 1). In the saltwater crocodile a water load similarly increased the filtration rate, whereas in the freshwater crocodile little variation was seen in the filtration rate as a result of dehydration or water-loading. A survey of many sections from kidneys of all the reptiles in different physiological conditions showed a rough correlation between the number of open to closed tubular lumina and the glomerular filtration rate.

In the tubules with open lumina from normal, dehydrated, and salt-loaded lizards, the intercellular spaces were also open, but they exhibited different forms (Fig. 1, a and b; Fig. 2). Sometimes the space was widely expanded from the tight junction of the terminal bar to the base of the cell; in other tubules part of the space was closed. No particular shape could be correlated with the physiological conditions. In the tubules with closed lumina the intercellular spaces were closed. In the ouabain-treated lizard, in which active sodium transport presumably was stopped, most proximal tubules had open lumina, but the intercellular spaces were tightly closed. In summary, in proximal tubules the intercellular spaces were closed when the transport was stopped either by no filtration or by poisoning of the sodium pump with ouabain. They were open whenever fluid transport across the epithelium took place.

Urine osmolality varies relatively little in reptiles. Their kidneys do not function as countercurrent multiplier systems, and reptiles can therefore only excrete a urine that is either hypoosmotic or isosmotic to the blood (*10*). The osmolality of the urine is regulated by the distal tubules and the cloaca. In our experiments urine was collected directly from the ureters, and it is therefore the distal tubular function that has determined its osmolality. In the blue-tongued lizard, the urine osmolality varied from 43 percent to 79 percent of the blood osmolality. This means that the resorbed fluid was hyperosmotic to the tubular fluid in the water-loaded lizard, but more closely approximated the osmolality of the tubular fluid in the dehydrated lizard. The osmolality varied over a similar range in *Crocodylus porosus* and *C. johnstoni* and varied in both of them more than in *Crocodylus acutus* (Table 1).

The intercellular spaces of the distal tubular cells appeared in many different shapes (Fig. 1, c and d; Fig. 3). The width of the spaces could not be related to the rate of fluid resorption, but, when transport was stopped completely, two-thirds of the apical part of the spaces were closed. This was the case in tubules with closed lumina from normal and dehydrated animals, and in tubules with open lumina from animals treated with ouabain. The shape of the spaces also seemed conspicuously related to the osmolality of the urine. In the water-loaded lizard when the urine osmolality was 45 percent of the blood osmolality the spaces were tightly closed from the terminal bar to the basal lamina (Fig. 3).

Thus, the intercellular spaces in distal as well as proximal renal tubules of reptiles appear to be open when water and solute are resorbed in about the same concentrations as in the lumen. These spaces however are closed when no transport takes place, as in tubules with closed lumina or in tubules poisoned with ouabain. When the urine is significantly hypoosmotic to the blood (when the resorbed fluid is hyperosmotic to the luminal fluid) the spaces between the distal tubular cells are closed apically, or all the way to the basal lamina. This finding seems to contradict the predictions by Diamond and Bossert (*11*) that, when the resorbed fluid is hyperosmotic, the spaces should be wide and short whereas an isosmotic resorbed fluid should require long and narrow intercellular spaces. The findings, however, could be consistent with the predictions that active transport into the basal parts of the intercellular spaces makes the resorbed fluid more hyperosmotic.

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Synthesis of a Sulfur-Containing Amino Acid under Simulated Prebiotic Conditions

Abstract. *Ultraviolet irradiation of an aqueous solution of ammonium thiocyanate produces the sulfur-containing amino acid methionine. Synthesis of this class of biocompound fills another important gap in development of an overall picture of how prebiological chemistry may have evolved on primitive Earth.*

The primordial appearance of nearly all classes of biomonomers can now be accounted for on the basis of several experiments carried out under hypothetical conditions on primitive Earth (*1*). However, one very important group that has persistently resisted synthesis thus far is the naturally occurring sulfur-containing amino acids (*2*). Electron-beam irradiation of a gaseous mixture of ammonia, methane, water, and H_2S has led to isolation and identification of the nonproteinaceous, oxidized amino acids taurine, cysteic acid, and cystamine (*3*). [The use of H_2S in a "primitive atmosphere" is rea-