TABLE 4

DDT Incorporation and Distribution in Fundulus[a]
(two 24 hour DDT doses)

| Tissue | 1 Day Post DDT Dose | | 8 Days Post DDT Dose | |
|---|---|---|---|---|
| | ppm DDT | % absorbed dose in tissue | ppm DDT | % absorbed dose in tissue |
| Intestine | 14.24 ± 4.42[b] | 18.07 ± 3.80 | 2.57 ± 0.30 | 5.69 ± 1.51 |
| Liver | 8.18 ± 1.03 | 7.43 ± 1.17 | 5.19 ± 0.31 | 6.67 ± 0.33 |
| Ovaries | 6.80 ± 0.82 | 15.20 ± 1.12 | 10.40 ± 3.00 | 25.16 ± 6.29 |
| Brain | 3.59 ± 0.60 | 0.77 ± 0.10 | 3.07 ± 0.53 | 0.78 ± 0.14 |
| Heart | 2.39 ± 0.18 | 0.17 ± 0.03 | 1.82 ± 0.42 | 0.20 ± 0.04 |
| Spleen | 1.84 ± 0.38 | 0.15 ± 0.02 | 0.94 ± 0.20 | 0.17 ± 0.07 |
| Gills | 2.50 ± 0.29 | 0.98 ± 0.16 | 2.32 ± 0.40 | 1.41 ± 0.33 |
| Muscle | 1.76 ± 0.33 | -------- | 1.23 ± 0.16 | -------- |
| Carcass | 1.73 ± 0.09 | 55.58 ± 2.90 | 1.35 ± 0.15 | 56.17 ± 7.58 |

[a]Dosage and assay performed as described in the text.
[b]Standard deviation of the mean. 5 fish used in each study.

egg membranes. Experiments with male fish show incorporation of DDT into testes at levels similar to those shown for eggs. Furthermore it should be noted that a major portion of the labelled material is still present in the fish eight days following removal from the DDT-containing medium. It therefore would appear that suitable preloading of fundulus eggs with DDT can be accomplished by administration of the pesticide to females *via* uptake from the water. Furthermore, our results suggest that more DDT is absorbed with less maternal toxicity if each 24-hour exposure is followed by 24 hours in DDT-free water.

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## THE DISSOCIATION BETWEEN RENAL $HCO_3^-$ REABSORPTION AND $H^+$ SECRETION

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Using micropuncture technique and the appropriate kinetic data, it has been possible to study the rates of $H^+$ secretion in the kidney of the skate *Raja erinacea* and to compare these with rates of $HCO_3^-$ reabsorption. *Raja* and other sea water fish offer particular advantages for such a study since they have a fixed urinary pH (5.8) and no renal carbonic anhydrase. In this situation, *Squalus* acanthias excretes (per kg fish) about 15 $\mu$eq $H^+$ per hour and reabsorbs essentially all of the filtered $HCO_3^-$, 10 $\mu$eq per hour (J. Hodler, H.O. Heinemann, A.P. Fishmann and H.W. Smith, Am. J. Physiol. 138:155, 1955). Both of these basal rates are susceptible to increase: $H^+$ output by administration of phosphate; and $HCO_3^-$ reabsorption when large amounts of $NaHCO_3$ are injected, none of which appears in the urine. The problem is to find the mechanism underlying each of these processes.

Eleven male or female skates weighing between 0.5 and 1.2 kg were anaesthetized by injecting Na pentobarbital (0.2 mg $Kg^{-1}$) and curare (0.3 mg $Kg^{-1}$) into the tail artery or a lateral tail vein. The fish were placed dorsal side up on a board. The spiracles were perfused with fresh sea water with an average perfusion temperature of 16° C.

The right kidney was exposed through a dorsal paramidline incision and the connective tissue on top of the kidney surface removed gently. A modification of the split oil-column technique was applied. A pulled glass capillary pipette with a tip diameter ground to 12-15$\mu$ was filled with mineral oil and inserted into a surface loop of the proximal tubule (Segment III in Bull. MDIBL 10: 5, 1970), using a Leitz micromanipulator. A column of oil of about the length of 10 tubular diameters (500$\mu$) was injected. With a second micromanipulator, a capillary containing the test solution was inserted into the oil column and a droplet of about the length of 20 tubular diameters was injected to split the oil into two parts.

While a section of a nephron was filled with the colored test solution, the luminal diameter as well as the wall thickness were measured by an eye piece micrometer.

The test solution was isotonic to the fish plasma and made up to prevent any net flux of NaCl and water (NaCl 220 mM, mannitol 530 mM). Thus, the solution contained NaCl in a 35 mM lower concentration than the plasma. With such a solution there was no detectable intratubular volume change over observation periods as long as 40 minutes. As a buffer and indicator, the test solution contained bromcresol purple (BCP) in concentrations between 0.55 and 1.5 mM. BCP is a weak organic acid (pKa 6.2) which changes color in the lumen from dark purple (alkaline) to yellow (acid). $NaHCO_3$ (5 or 12 mM) or $K_2HPO_4$ (9 mM) were added as additional buffers.

The standard experiment for measurement of the acidification rate was based on the *in vivo* titration of BCP to pH 5.8. In a typical case two nanoliters of 1.1 mM BCP at pH 7.5 were injected into the lumen between two oil columns. The time to bring the color from purple (pH 7.5) to yellow (pH 5.8) was recorded. This involves the protonation of 66% of the dye, through its maximum buffer range, determined by direct acidimetric titration equivalent to $720 \times 10^{-6}$ moles $H^+$/L (Fig. 2). Thus, the amount of $H^+$ secreted into the droplet is $720 \times 10^{-6}$ moles/L • $2 \times 10^{-9}$ L = $1.44 \times 10^{-12}$ moles. This amount was produced by a cell volume calculated to be $6 \times 10^{-9}$ L so that the cells were secreting $240 \times 10^{-6}$ moles for each liter of their volume. The average time for this secretion was about six minutes, hence the rate of $H^+$ output was about 40 $\mu$moles/L of cells per minute. The first column of Table 1 shows these experiments, and the second those in which the concentration

TABLE 1

ACIDIFICATION OF LUMINAL FLUID CONTAINING BUFFER DYE (BROMCRESOL PURPLE, BCP)

| | $\mu$moles $H^+$ secreted/L cell volume per minute | |
|---|---|---|
| | BCP 1.1 mM | BCP 0.6 mM |
| Mean ± S.E. | 38.4 ± 1.1 | 39.3 ± 1.9 |
| Number of Obs. | 13 | 13 |
| Number of Fish | 6 | 3 |

All experiments carried out in the skate, *Raja erinacea*

of BCP was lowered. This shortens the transition time proportionately, yielding the same value for $H^+$ secretion rate. Spontaneous differences in luminal radius (r) yield changes in tubular volume (considered as a cylinder, $V = \pi r^2 \cdot$ length) proportional to $r^2$. In such changes however cellular volume remains constant at $6 \times 10^{-9}$ L, standardized for 1 millimeter tubular length. Figure 1 shows that variations in tubular volume do not affect $H^+$ secretion, calculated in terms of the volume of the secreting cells.

Figure 1

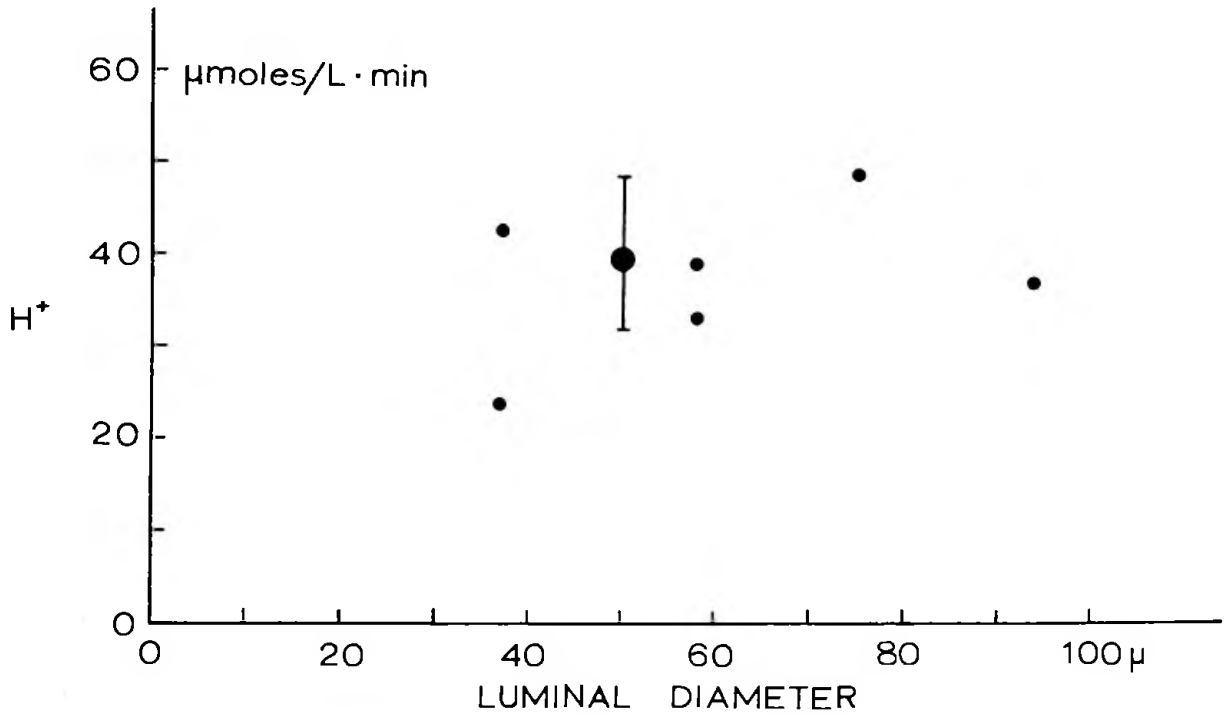


Figure 1. Showing that the $H^+$ secretion per unit cell volume in segment III of *Raja* kidney (ordinate is independent of luminal volume.

The observed rate of 40 μmoles/L per minute is now compared to the uncatalyzed rate of the reaction

$$CO_2 + H_2O \xrightarrow{k_1} H^+ + HCO_3^- \qquad (1)$$

which takes place within renal cells, $H^+$ being secreted and $HCO_3^-$ reabsorbed. At 18° $k_1$ is 1.3 $min^{-1}$ (E. Magid and B.O. Turbeck, Biochim. Biophys. Acta 165:515, 1968). The concentration of gaseous $CO_2$ within the cells of *Raja* is 0.2 mM (4.4 mm Hg) and if this is taken as the substrate, the theoretical chemical rate becomes 260 μmoles/L per minute. However this is a maximal rate and supposes no back reaction, i.e., that there is no $CO_2$ in the lumen, clearly improbable. A more reasonable guess is that the gradient or $CO_2$ driving force is 1-2 mm Hg, between cell and lumen. The theoretical chemical rate would then be 50-100 μmoles/L cell volume per minute, which is the range observed in the fish preparation.

Figure 2

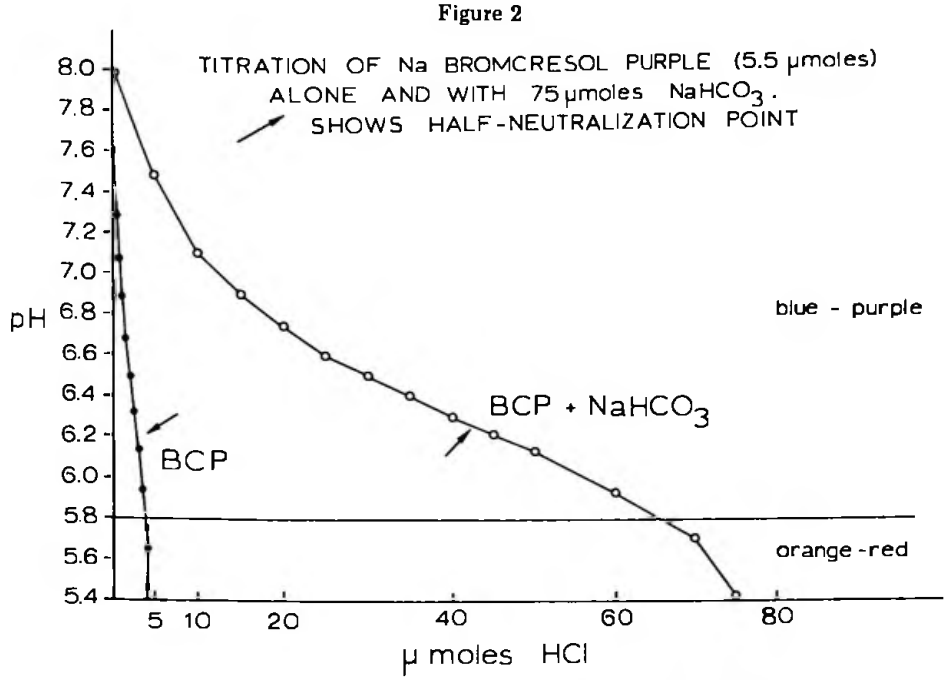


Figure 2. *In vitro* titration of BCP (as sodium salt) alone and with $NaHCO_3$. For the actual amount
amounts of BCP used in the fish experiments of Table 2, A vs. C, the $\frac{NaHCO_3\text{-}BCP}{BCP}$ ratio of titratable base to pH 5.8 is 14.

TABLE 2

CHEMICAL TITRATION AND TIMES FOR *IN SITU* ACIDIFICATION OF BUFFERS BY *RAJA* KIDNEY

| Buffer Experiment | 1<br>Conc. | 2<br>$H^+$ titration for 2 nL buffer pH 7.8 to 5.8 | 3<br>Observed time *in situ* for color change | 4<br>Calculated time *in situ* for titration of all buffers, based on rate of Experiment A | 5<br>Calculated time *in situ* for titration of non-bicarbonate buffers, based on rate of Experiment A |
|---|---|---|---|---|---|
| | mM | picamoles | minutes | minutes | minutes |
| A. BCP | 1.1 | 1.5 | 6 | (6) | (6) |
| B. BCP (1 mM) + $NaHCO_3$ (5 mM) | 6.0 | 8.2 | 6 | 33 | 5.4 |
| C. BCP (0.9 mM) + $NaHCO_3$ (12 mM) | 12.9 | 21 | 6 | 87 | 5.0 |
| D. BCP (0.9 mM) + $NaHCO_3$ (12 mM) + $K_2HOP_4$ (9 mM) | 21.9 | 38 | 29 | 157 | 75 |

Experiments B - D based on 3-5 observations in 1 fish each.

Acidification was then studied when $NaHCO_3$ was added to the intraluminal dye solution. Figure 2 shows the chemical titration of such a BCP-$NaHCO_3$ mixture; an important point is that the pK is the same as that for BCP alone. Table 2, rows B and C, shows the titratable alkalinity of two BCP-$NaHCO_3$ solutions which increase the buffer strength 5.5 and 14-fold respectively over BCP alone (column 2). Despite this the observed time for *in situ* color change in *Raja* kidney was unchanged compared to BCP alone (column 3). If $HCO_3^-$ were being titrated at the same rate as BCP, the time would have been greatly increased, as shown by column 4. However since the BCP component was but slightly changed in these solutions, the expected time if $HCO_3^-$ were not being titrated would be essentially unchanged (column 5), which was the observed (column 3) result.

**Figure 3**

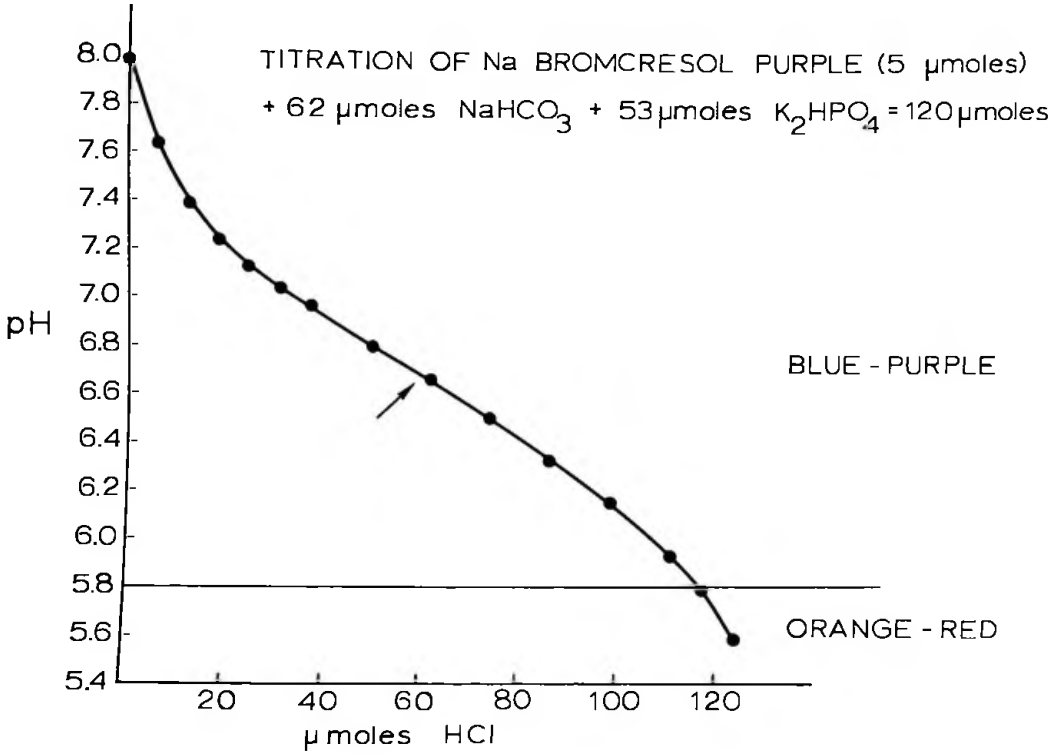


**Figure 3.** *In vitro* titration of BCP (as sodium salt) + $NaHCO_3$ + $KH_2PO_4$.

Since it was at least theoretically possible that these results could be based on greatly accelerated $H^+$ secretion in the presence of the added $HCO_3^-$ buffer, an additional experiment was done which included phosphate buffer. Figure 3 shows the titration curve of a BCP-$NaHCO_3$-$K_2HPO_4$ solution; the pK is slightly higher than in Figure 2 but buffering occurs through the region of *in situ* acidification. Table 2, row D, shows the titratable alkalinity of this solution (column 2) and that the time for *in situ* color change now is increased five fold over those for solutions containing bicarbonate only added to

BCP (column 2). Thus it appeared that phosphate, unlike $HCO_3^-$, is being titrated by the $H^+$ produced in renal cells. The observed time for the phosphate solution (29 minutes) is less than the calculated time (75 minutes) extrapolated from BCP buffer strength (excluding bicarbonate, column 5), but this can reasonably be ascribed to the stimulation of acid secretory rate by phosphate in this class of fish (Hodler et al., Am. J. Physiol. 183:155, 1955).

TABLE 3

RATES OF *IN SITU* ACIDIFICATION IN *RAJA* KIDNEY

| Buffer Experiment | | 1<br>Apparent $H^+$ titration *in situ* referred to 1 L cells* | 2<br>Observed time *in situ* for color change | 3<br>Rate* |
|---|---|---|---|---|
| | | $\mu$moles/L | minutes | $\mu$moles/L • minutes |
| A. | BCP | 240 | 6 | 40 |
| C. | BCP + $NaHCO_3$ | 3500 | 6 | 580 |
| D. | BCP + $NaHCO_3$ + $K_2HPO_4$ | 6800 | 29 | 220<br>103† |

* Assuming that the color change is due to titration of all buffers

† Rate if only BCP + $KH_2PO_4$ are titrated

Table 3 gives the data in terms of rates, so that comparison with chemical acidification rates derived from equation 1 may be made. The first row (A) gives the rate in the standard BCP experiment, calculated as described above. The second row (C) gives the rate calculated as if BCP + $NaHCO_3$ were being titrated. The third row (D) gives the rates calculated both on the basis of all buffers titrated, and that excluding $NaHCO_3$. We showed in connection with equation 1 above that the maximum possible uncatalyzed rate of $H^+$ secretion *in situ* was 260 $\mu$moles/L per minute and the probable rate dictated by the chemistry and physiology of the system was 50-100 $\mu$moles/L per minute. If this be the case the observed rates (Table 3, column 3) of total acidification (i.e., color change *in situ*) for the solutions containing $NaHCO_3$ must include a major component in addition to $H^+$ secretion. Acidification rates *in situ* of BCP and phosphate components, on the other hand, are well within the range of rates calculated from equation 1.

The conclusion appears inescapable that luminal $HCO_3^-$ is not titrated to $CO_2$ but is rapidly reabsorbed by proximal cells directly, as bicarbonate ion, i.e., by ionic movement as such. We feel that this is consistent with data from other vertebrates, including mammals, for when carbonic anhydrase is totally inhibited in rat, man or dog, 60-80 percent of filtered $HCO_3^-$ is still reabsorbed (Maren, Phys. Rev. 47:595, 1967).

The acidification rates observed in these *in situ* micropuncture experiments agree reasonably with those found in the free swimming fish. We have shown the rate to be 40 $\mu$moles/L cell volume per minute *in situ*. The excretion of titratable acid in the related elasmobranch fish *S. acanthias* is about 0.2 $\mu$moles per minute per kg body weight (Hodler et al., vide supra). Using this figure (which is probably high for *R. erinacea* because of its lower GFR and metabolism than *S. acanthias*) and a kidney cell volume of 1.5 ml for a skate weighing about 1 kg yields 133 $\mu$moles/L cell volume.

In these species lacking carbonic anhydrase it therefore appears that essentially all $HCO_3^-$ is reabsorbed as such and $H^+$ secreted by tubule cells inevitably appears in the urine as titratable acid.

We thank Mr. Erik Swenson for his fine analytical work.

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## UREA REABSORPTION BY THE SKATE NEPHRON: MICROPUNCTURE OF COLLECTING DUCTS IN *Raja erinacea.*

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Before entering a collecting duct the final thin-walled segment of the skate nephron passes in intimate apposition to coiled loops of more proximal segments (Bull. MDIBL 10:5, 1970). A similar finding in Squalus had suggested that this disposition of early and late segments might play a role in the unique handling of urea by the elasmobranch kidney (Bull. MDIBL 9:60, 1969). In the present study the urea concentration of initial collecting duct fluid was compared to that of plasma and simultaneously formed urine. Results indicate that tubular fluid entering the collecting duct has already reached its final concentration with respect to urea.

Data are presented from 13 male and female skates weighing 0.74 -1.2 Kg and examined not more than two days following capture in Frenchman Bay. Fish were anaesthetized with nembutal (0.2 mg $Kg^{-1}$) and curare (0.3 mg $Kg^{-1}$) and prepared for micropuncture as previously described (Bull. MDIBL 10:5, 1970 and Ibid. 11:91, 1971). Collecting ducts are visible and accessible at the dorsal surface of the exposed kidney. Urea determination in tubular and collecting duct fluid was by the method of Marsh (J. Clin. Path. 14:418, 1961), in plasma and final urine by the phenol-alkaline hypochlorite method. The puncture site in each case was identified by the injection of a droplet of Lissamine green or microfil after sampling was complete.

Plasma urea concentration ranged from 300-423 $mML^{-1}$ (mean and SD, 370 $\pm$ 40); urine to plasma (U/P) urea ranged from 0.14 to 0.39. We obtained 15 samples from first order (initial) collecting ducts. Fluid: plasma ratios for these varied from 0.10 to 0.42, not significantly different from final urine.

Stolte, et al, (Bull. MDIBL 11:91, 1971) found the collecting ducts to be the principal site of urinary dilution in the skate kidney. It appears therefore from the present study that urea reabsorption and the final adjustments in ionic composition occur at different sites along the nephron