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TIME COURSE OF THE ESTABLISHMENT OF UTERINE SEAWATER CONDITIONS IN LATE-TERM PREGNANT SPINY DOGFISH (*SQUALUS ACANTHIAS*)

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Summary

The gestation period for embryos of the spiny dogfish, *Squalus acanthias* (L.) lasts for nearly 2 years. During the latter part of this period the pups remain in the uterus and the fluid surrounding the embryos resembles sea water with respect to the major ions, but is low in pH (approx. 6), high in partial pressure of carbon dioxide (approx. 3 mmHg; 1 mmHg = 133·3 Pa), low in total carbon dioxide content (approx. 0·2 mmol l^{-1}), and may have a total ammonia concentration of up to 22 mmol l^{-1}. Thus the conditions under which the pups complete their development *in utero* is quite remarkable.

The derivation of these conditions was examined in late-term pregnant females, from whose uterine horns the pups had been removed, by monitoring changes that occurred in instilled uterine sea water. The mother is responsible for reducing the pH, reducing the total carbon dioxide content and elevating the partial pressure of carbon dioxide to the levels observed in fresh-caught females, in less than 24 h. The ammonia concentration is also elevated, but this takes rather longer. The decreased pH is responsible for the accumulation of ammonia in the uterine sea water, and it also serves to protect the pups from the toxic effects of NH_3, by converting it to the relatively non-toxic ionic form, NH_4^+. The reasons for the establishment of these uterine seawater conditions are still not evident.

Introduction

Spiny dogfish (*Squalus acanthias* L.) possess the rather unspecialized form of viviparity termed 'lecithotrophy' (Wourms, 1981). Fertilized eggs remain encapsulated for about 4–6 months *in utero*. The delicate egg case surrounding these 'candles' then ruptures, and the pups, which possess yolk sacs, complete their

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period of gestation which lasts nearly 2 years (Nammack, Musick & Colvocoresses, 1985; Woodhead, 1976). In the last 10–15 months of gestation these pups, numbering typically 2–6 per uterine horn, reside in a solution apparently derived from sea water (Evans, Oikari, Kormanik & Mansberger, 1982; Kormanik & Evans, 1986), and ventilate while *in utero* (G. A. Kormanik, personal observations). In fact, pups can be removed from the uterus and, if properly cared for, will survive indefinitely in sea water (Jones & Price, 1967; D. H. Evans, personal observations).

The lining of the uterus is thin in non-gravid females, but during pregnancy, becomes highly vascularized (Jollie & Jollie, 1967). The uterine artery increases in size to allow an increased flow of blood to the uterine horns (Fuller, Griendling & Kent, 1983). The high degree of vascularization and extensive blood flow have led several investigators (Burger, 1967; Jollie & Jollie, 1967; see also Wourms, 1981) to suggest that the uterine circulation might play some role in supplying O_2 and removing CO_2, and thus aid the pups in respiration. In addition, Burger (1967) has reported that the uterine horns of the pregnant female are periodically flushed with sea water, presumably to aid the pups in respiration and the removal of waste products. The relative importance of the maternal circulation and seawater flushing of the uterus in maintenance of the pups' environment is not clear.

Although the major ion concentrations of the uterine sea water resemble normal sea water (Evans *et al.* 1982), the uterine sea water is relatively acid, with a pH of about 6. The total CO_2 content is only a few tenths of a mmol l^{-1}, the partial pressure of CO_2 (P_{CO_2}) is elevated to the level found in maternal blood and ammonia concentration is extremely high, approaching 22 mmol l^{-1} (Kormanik & Evans, 1986). Thus it would appear that the uterine horns are not flushed with sea water as frequently as previous investigators have suggested (Burger, 1967). The pups appear to be in an environment which would normally be considered toxic to water-breathing organisms, yet they apparently thrive.

Neither the origins of these uterine conditions nor the reasons for their establishment are clear. For example, the acidity and ammonia may arise from the pups, since Kormanik & Evans (1984) have demonstrated that late-term pups show net excretions of ammonia and titrable acidity (G. A. Kormanik & D. H. Evans, unpublished observations). Alternatively, the pups may excrete ammonia, but the acidity may be derived from the mother, in order to convert the NH_3 into the relatively less toxic ionic form, NH_4^+ (see review by Colt & Armstrong, 1981). Lastly, the levels of ammonia and acidity in the uterine sea water may arise from both the mother and the pups. A first step in understanding the reason for these uterine conditions and their significance to the development of the pups is a determination of how they are derived. To examine the origin of these uterine seawater conditions as well as the time course of their establishment, we monitored the changes in concentration with time of several uterine seawater constituents in late-term pregnant female *Squalus acanthias* from which the pups had been removed. In addition, we determined the gradients developed from maternal blood to uterine sea water. In this manner, it was possible to examine the

contributions of the mother alone in establishing the uterine environment for the developing pups.

Materials and methods

Preparation of animals

Pregnant female spiny dogfish (*Squalus acanthias*) were caught by gill nets from Frenchman Bay, and held in floating live cars for several days prior to use to allow them sufficient time to recuperate from the trauma of capture. The presence of late-term pups in the uterine horns was confirmed by digital examination. Prior to use in experiments, late-term females were removed from the live cars, and a small incision was made on the tip of the snout. The fish was then pithed by the insertion of a wire (approx. 1·5 mm × 1 m) through the incision into the brain and down the length of the spinal column. The wire was then manipulated to effect destruction of the nervous tissue. The fish was immediately immersed in sea water, and water forced over the gills until at least partial ventilation was re-established. This procedure was performed at the live car, and the fish was then immediately transferred to a running seawater holding tank where ventilation of the gills with fresh aerated sea water was commenced *via* intubation of the mouth and spiracles. In most experiments, spontaneous bilateral ventilation was re-established. In some of the experiments, ventilation was weak or absent on one side. In these animals, ventilation of the gills by intubation was maintained throughout the course of the experiment. The female was loosely strapped to an operating board, dorsal side down, and the head and body anterior to the cloaca were immersed in sea water (15 ± 1 °C). Running sea water was directed over the remaining exposed portions of the body to prevent drying and keep the exposed portion of the fish cool.

Both uterine horns were perfused with fresh aerated sea water to maintain the pups temporarily. A blood catheter (PE 60) was installed in the dorsal aorta of the tail of the mother using a Tuohy needle (17 gauge thin wall, Popper & Sons, NY). Tonus of the cloaca and uterine horns was sufficiently reduced after about 30 min that, when the uterine horns were filled with sea water and slight pressure was applied to the abdomen, the pups could be expressed alive and intact from the uteri. Removal of all the pups from the uteri was confirmed by digital examination and also by dissection of the females, all of which were killed at the termination of the experiments. Water-inflated balloons were installed in the intestine and openings to the uterine horns to block any flow of intestinal contents into the cloaca and prevent contamination of the uterine fluids. In most experiments, the urinary papilla was also catheterized to help prevent retrograde flow of urine into the cloaca. The experimental period was commenced when maternal blood pH (see below) was rising and approaching normal levels (approx. 7·7). This usually occurred a few hours after surgery. Data from females with abnormally low blood pH, which was taken to indicate a lack of recovery from the surgical procedures, were discarded.

At the start of the experiment, each uterine horn was rinsed several times and then filled with 120 ml of fresh aerated sea water, about the volume typically found in fresh-caught pregnant females (Kormanik & Evans, 1986). Seawater samples (2 ml) were removed from the uterine horns by suction using a large syringe and a blunt rubber catheter. The samples were analysed for total CO_2 (T_{CO_2}), pH and total ammonia (T_{Amm}). Heparinized blood samples were removed from the female *via* the aortic catheter, and all samples were handled as previously described (Kormanik & Evans, 1986).

Pup ammonia gradient experiments

In this series of experiments the role of seawater acidity was assessed in protecting the pups from the build-up of ammonia in the uterine sea water. Late-term pups were collected from killed females and acclimated to fresh sea water for about 1 day prior to experiments. In the first series of experiments five pups (approx. 40 g each) were placed into 2 l of well-aerated sea water (15 ± 2°C) that had been adjusted to a total ammonia concentration of 10 mmol l^{-1} (as the Cl^- salt), approximately the average concentration found in uterine sea water (see Kormanik & Evans, 1986). The pH was 8·14 at the start of the experimental period, similar to that of normal sea water (Kormanik & Evans, 1986). Heparinized blood samples were taken from the pups by ventral puncture of the dorsal aorta in the tail region at 3, 16 and 42 h. After the 16 h sample, the sea water was replaced with a fresh solution at 10 mmol l^{-1} total ammonia, and a pH of 8·2. In a second series of experiments, pups were set up as before, but were exposed to sea water with ammonia at 10 mmol l^{-1} with the pH adjusted to less than 5 with HCl. It was necessary manually to add more acid every few hours to keep the pH low and in the established range. Over one night the pH increased to 5·5 and was then maintained at that level for the duration of the experiment. Although this manual method of pH maintenance was rather crude, the pH of the seawater bath was nevertheless maintained slightly below that found normally in the uterine horns, for the duration of these experiments. Blood and seawater pH, and ammonia levels in sea water were determined as described below. NH_4^+ concentration and the partial pressure of ammonia (P_{NH_3}) were calculated from total ammonia concentration and pH for blood and sea water using the Henderson–Hasselbalch equation (see Kormanik & Cameron, 1981) and values for pK′ and blood and seawater solubility from Cameron & Heisler (1983). Since the blood pH could not be measured in all pups at all times, due in part to their small size and a desire not to disturb them too much during the course of the experiment, an average value was determined (= 7·8), and was used in the gradient calculations.

Analyses of samples

Total CO_2 levels in sea water and blood plasma were determined using a Capnicon II (Cameron Instrument Co.). The pH values of blood and sea water were determined using an IL Micro 13 (Instrumentation Laboratories) and a Orion 601A, with electrodes thermostatted to 15°C.

Ammonia in sea water was analysed using the phenol–hypochlorite assay (Solorzano, 1969), and in blood plasma using an ammonia-specific enzymatic assay (kit no. 170-UV, Sigma Chemical Co.).

Lactate was analysed in uterine seawater samples by a modification of a standard method used for plasma (kit no. 826-UV, Sigma Chemical Co.). Uterine seawater samples were removed from pregnant females and analysed immediately for lactate. 0·5 ml of uterine sea water was added directly to 2 ml of the reaction mixture. These unknowns were measured against a standard curve made up from a set of standards with 0·5 ml of fresh sea water added to each reaction mixture. The addition of an equivalent amount of fresh sea water to the standards eliminated any possible error contributed by using uterine sea water instead of plasma in this assay.

Calculations

The P_{CO_2} in the seawater samples was calculated from the Henderson–Hasselbalch equation, using the values we determined for pH and T_{CO_2}, and the appropriate values for CO_2 solubility and 'apparent' pK′ for sea water calculated using the empirically established polynomials of Boutilier, Heming & Iwama (1984). P_{CO_2} values for blood were calculated in a similar manner, from the T_{CO_2} and pH we had determined. Values for apparent pK′ and CO_2 solubility were calculated for elasmobranch blood from the data of Albers & Pleschka (1967), Albers (1970) and Pleschka & Wittenbrock (1971), again using the empirically established polynomials of Boutilier *et al.* (1984). Temperature of the sea water measured during the experiments and used in the calculations was 15 ± 1 °C. Student's *t*-test was used in the statistical comparisons, for paired and unpaired data, where appropriate.

Results

The results of the determinations made for maternal blood and uterine seawater pH and P_{CO_2} are presented in Fig. 1A. Maternal blood pH was relatively stable throughout the course of the experiments, increasing slightly with time in the first 30 h and then stabilizing for the remainder of the experiment (30–80 h) at a pH of $7{\cdot}81 \pm 0{\cdot}01$ ($N = 17$, Fig. 1A). The surgical procedures used in this investigation allowed for a relatively rapid return of the fish to normal blood pH values and most experiments could be started within a few to 24 h after surgery. Thus the females were stable with respect to acid–base status throughout the course of the experimental period, and yielded blood values similar to those of intact, rested fish at 15°C (see Heisler, Neumann & Holeton, 1980). Most fish remained in stable acid–base status beyond 48 h, and three fish lasted over 80 h under this experimental protocol. Uterine seawater values are not as complete, however, since females occasionally ejected the sea water.

The values determined for uterine sea water pH are also shown in Fig. 1A. Uterine seawater pH declined from normal seawater pH values (approx. 8·2) to

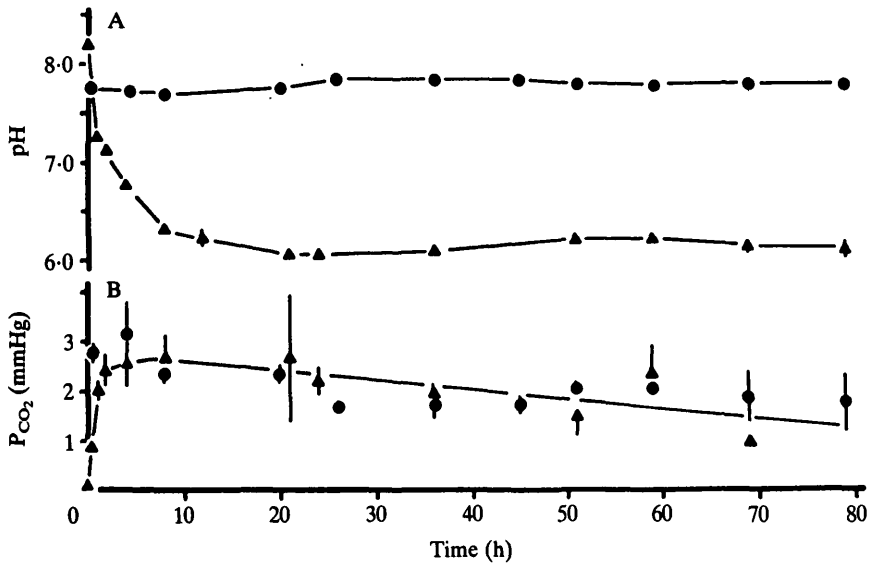

Fig. 1. Changes in pH (A) and partial pressure of CO_2 (P_{CO_2}) (B) of the blood (filled circles) and uterine sea water (filled triangles) of late-term pregnant females. Vertical bars (in some cases obscured by the symbol) indicate S.E. $N = 2$–12.

about 6 in the first 20 h, and remained at about that level for the duration of the experiment. Maternal blood T_{CO_2} (not shown) was determined in these experiments at the sampling times indicated for the pH, and blood P_{CO_2} was calculated as described above. These values are reported in Fig. 1B. Blood P_{CO_2} declined slowly during the course of the experiment, from slightly over 3 mmHg to about 2 mmHg at 80 h. Although the values for maternal blood P_{CO_2} determined at the beginning of the experiments are slightly elevated over those for well-rested fish (approx. 2 mmHg), more typical values were achieved after about 48 h. These data indicate that ventilation of the mother's gills, whether spontaneous or aided by forced seawater flow, was sufficient to alleviate build-up of respiratory CO_2 and a stable respiratory status of the females under our experimental protocol was achieved. Uterine seawater P_{CO_2} was also calculated, from the uterine seawater pH determined (Fig. 1A) and the T_{CO_2} values measured (Fig. 3). These values of P_{CO_2} for uterine sea water are also presented in Fig. 1B. The P_{CO_2} of the uterine sea water increased rapidly from the values for sea water equilibrated with atmospheric P_{CO_2} and instilled in the uterine horns (approx. 0·2 mmHg) to nearly 3 mmHg within the first few hours. Thus uterine seawater P_{CO_2} rapidly reached equilibrium with the P_{CO_2} of the blood, and by the 8 h samples, they were not significantly different ($P > 0{\cdot}1$). After the attainment of equilibrium, both blood P_{CO_2} and uterine seawater P_{CO_2} then declined with time. The P_{CO_2} of the uterine sea water therefore appears to 'track' values for blood P_{CO_2}, a further indication that equilibrium between the two fluid compartments had been achieved.

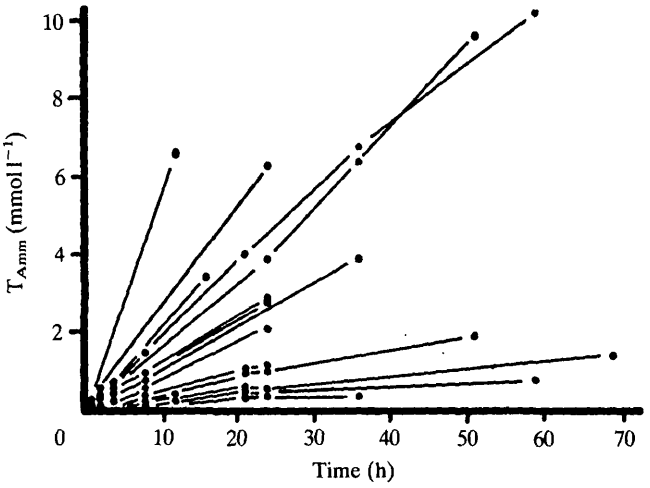

Fig. 2. Changes in total ammonia concentration (T_{Amm}) with time in the uterine sea water of late-term pregnant females. Individual determinations are indicated.

Uterine seawater total ammonia (T_{Amm}) is shown in Fig. 2. The rates of increase in the uterine ammonia concentration observed were highly variable, but in all determinations increased with time and, during the course of this investigation, approached values of over 10 mmol l^{-1}. At the 24 h sampling time, the average total ammonia concentration of the uterine sea water was $2{\cdot}1 \pm 0{\cdot}6$ mmol l^{-1} ($N = 10$), or about 20 % of the average value (10 mmol l^{-1}) we observed in fresh-caught late-term pregnant females (Kormanik & Evans, 1986). Two fish reached about 10 mmol l^{-1} ammonia at about 50 h, indicating that the average ammonia levels we observed in fresh-caught pregnant females can be achieved in little more than 2 days.

Changes occurring in the uterine seawater T_{CO_2} are reported in Fig. 3. The values determined for T_{CO_2} in the uterine sea water declined with time, and by the twelfth hour achieved a level of about 0·2 mmol l^{-1} and remained there for the duration of the experiment (Fig. 3). At this P_{CO_2} (about 2·5 mmHg, see Fig. 1B), the dissolved CO_2 ($0{\cdot}052$ mmol l^{-1} $mmHg^{-1} \times 2{\cdot}5$ mmHg $= 0{\cdot}13$ mmol l^{-1}) represents a substantial portion of the T_{CO_2}, thus the bicarbonate concentration of the uterine sea water had been reduced to less than 0·1 mmol l^{-1} (0·2 mmol l^{-1} T_{CO_2} minus 0·13 mmol l^{-1} dissolved CO_2).

To determine if the decline in the observed pH of uterine sea water might be attributable to the secretion of lactic acid, a common organic metabolite, a few analyses were made for lactate. Uterine seawater samples were removed both from late-term females which were being held in the live cars (conditions comparable to those reported by Kormanik & Evans, 1986) and from the experiments described above. The results are reported in Table 1. Lactate levels in the uterine sea water of the females from the live car, although quite low, are more than double that of the females which had lasted the longest in our experiments

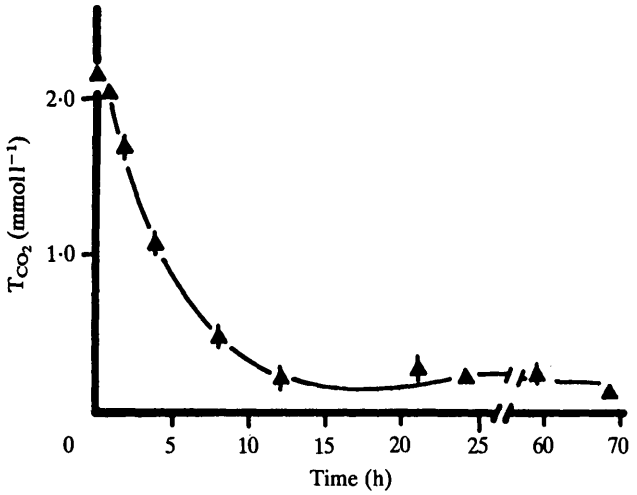

Fig. 3. Changes in the total CO_2 content (T_{CO_2}) with time in the uterine sea water of late-term pregnant females from which the pups had been removed. Vertical bars indicate S.E. $N = 2$–12.

Table 1. *Lactate concentrations in uterine sea water of fresh-caught late term* Squalus acanthias *females (with late-term pups residing in the uterine horns) removed from the live car*

	Live car	Experimental (at 75–80 h)
Lactate (μmol l^{-1})	116 ± 16	48 ± 6
N	3	3
Significance		$P < 0{\cdot}01$

Values are compared to pithed experimental females (without pups, as described in the text) near the end of the experimental period.

(i.e. 75–80 h). The difference is significant for these unpaired data. However, a reduction of uterine HCO_3^- concentration by nearly 2 mmol l^{-1}, as demonstrated in Fig. 3, would require an equivalent proton secretion of nearly 2 mmol l^{-1} to titrate the HCO_3^- of the uterine sea water one for one, with concomitant resorption of the CO_2 generated across the uterine epithelium. Thus, even if the appearance of lactate, as determined by this assay, represents the net secretion of lactic acid into the uterine sea water (the secretion of the lactate ion by itself would not affect pH), the amount would be quite inadequate to account for the decreased pH observed in this investigation.

To determine the role of this maternally derived acidity in protecting the pups from the potential toxicity of the high levels of ammonia observed in uterine sea water, pups were exposed to high ammonia concentrations in sea water at both low

Table 2. *The effect of high ammonia level, normal pH (8·1–8·2) sea water on blood ammonia in pre-natal dogfish pups*

	3 h	16 h	42 h
Blood ammonia ($mmol\,l^{-1}$)	1·25 ± 0·33	2·32 ± 0·28	5·17 ± 0·29
N	5	5	3†
Significance		$P<0·001$	$P<0·001$
Ammonia gradients (blood–sea water)			
NH_4^+ ($mmol\,l^{-1}$)	−8·51 ± 0·32	−7·39 ± 0·28	−4·58 ± 0·29
Significance		$P<0·01$	$P<0·001$
P_{NH_3} (nmHg)*	−4860 ± 100	−5850 ± 80	−5000 ± 90
Significance		$P<0·001$	$P<0·001$
N	5	5	3†

Significance denotes a comparison with the previous time period (mean ± S.E.).
* nmHg = μTorr.
† Two pups died during the night.

Table 3. *The effect of high external ammonia level and low pH on blood ammonia in pre-natal dogfish pups*

	3 h	16 h	42 h
Blood ammonia ($mmol\,l^{-1}$)	0·38 ± 0·14	0·20 ± 0·15	0·43 ± 0·22
Significance		$P>0·1$	$P>0·1$
Seawater pH range	[-----4·1 ± 0·1-----]		[-----5·5 ± 0·2-----]
Ammonia gradients (blood–sea water)			
NH_4^+ ($mmol\,l^{-1}$)	−9·23 ± 0·13	−9·80 ± 0·14	−9·58 ± 0·22
Significance		$P<0·01$	$P>0·1$
P_{NH_3} (nmHg)*	+111 ± 40	+48 ± 43	+114 ± 65
Significance		$P>0·1$	$P>0·1$

Significance denotes a comparison with the previous time period, (mean ± S.E.). $N = 5–6$.
* nmHg = μTorr.

(approx. 5) and normal (approx. 8·1) seawater pH. The results are presented in Tables 2 and 3. Blood ammonia in pups exposed to high ammonia, normal pH sea water increased rapidly by several-fold throughout the course of the experiment and, since two of the five pups died, approached presumably lethal levels even before the last sampling period (Table 2). In all cases, very large gradients for both NH_4^+ and P_{NH_3} were directed from sea water to the blood of the pups, and resulted in a large increase in pup blood ammonia concentration. The results from a second series of experiments, in which the pups were exposed to a similar seawater ammonia concentration ($10\,mmol\,l^{-1}$), but with the pH adjusted to about 5, are presented in Table 3. Manual maintenance of a stable low pH was rather difficult

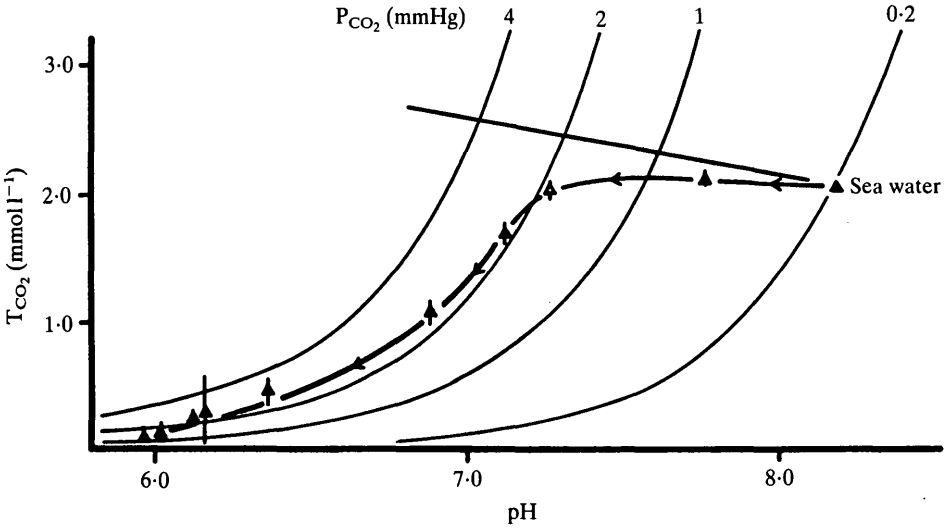

Fig. 4. Total CO_2 content (T_{CO_2}) and pH diagram for uterine sea water (filled triangles), with P_{CO_2} isobars for $P_{CO_2} = 4$, 2, 1 and 0·2 mmHg. Arrows indicate the direction of change with time. Vertical bars indicate S.E. T_{CO_2} is expressed in mmol l^{-1}. $N = 2$–12.

and accounts for the variations in pH shown. Nevertheless, during the course of this experiment, the pH was kept below levels normally seen in the uterine sea water (approx. 6, this report). With the reduced seawater pH, the P_{NH_3} gradient remained directed from blood to sea water throughout the course of this experiment, and the NH_4^+ gradient remained directed from sea water to blood. In these two experiments, then, only the direction of the P_{NH_3} gradient was changed while the NH_4^+ gradient always remained directed from the seawater bath to the blood. Thus, in spite of the fact that the total ammonia gradient (predominantly in the form of the ion, NH_4^+) was directed from sea water to blood by nearly 10 mmol l^{-1} (Table 3), the blood ammonia concentration remained at levels typical of pups adapted to fresh sea water.

Discussion

These preliminary data provide some insight into the changes that occur in the uterine sea water of late-term pregnant female *S. acanthias* in the absence of pups, and thus represent the contributions of the mother in establishing the uterine seawater environment.

The total CO_2 content of uterine sea water and the pH are actively reduced with time. To examine more carefully the relationship between the changes in pH, T_{CO_2} and P_{CO_2} of the uterine sea water, a total CO_2–pH diagram was constructed (Fig. 4). Seawater P_{CO_2} isopleths are presented ranging from atmospheric (approx. 0·2 mmHg, the P_{CO_2} of the sea water instilled into the uterine horns) to 4 mmHg. The seawater buffer curve shown (solid line) was determined by measuring the pH

and T_{CO_2} of fresh sea water equilibrated to known 1% CO_2 standards ($P_{CO_2} = 7{\cdot}2$ mmHg) in air. A seawater buffer curve was used since fresh sea water was instilled into the uterine horns during these experiments. Although the buffer curve of the uterine sea water can be expected to change slightly with time due to the removal of HCO_3^- by the mother, the addition of ammonia had a minimal effect (data not shown). These changes were disregarded for the following analysis.

At first the uterine seawater T_{CO_2} increased very slightly as the uterine seawater P_{CO_2} increased and the pH decreased. However, since the measured curve showed an increasing deviation from the buffer line, the T_{CO_2} was declining as the pH continued to decline. The declining T_{CO_2} then followed the P_{CO_2} isopleth for the blood at slightly more than 2 mmHg until it reached its lowest value at a pH slightly below 6 and P_{CO_2} somewhat below 2 mmHg (see also Fig. 1B). Recall that during this time period, the P_{CO_2} of the blood, as well as the uterine sea water, was declining from slightly more than to slightly less than 2 mmHg. This rapid decline in T_{CO_2}, which is predominantly a decline in the HCO_3^- concentration of the uterine sea water, is especially interesting. For a passively buffered system such as sea water (solid line in Fig. 4), one would expect to see a slight increase in the T_{CO_2} along the seawater buffer curve as the P_{CO_2} increases, with a concomitant decrease in pH. The increase in T_{CO_2} one expects with increasing P_{CO_2} would be due both to an increase in HCO_3^- and to an increase in dissolved CO_2, as predicted by the Henderson–Hasselbalch equation and solubility calculations. Thus the decrease in T_{CO_2} we observed simultaneously with an increasing P_{CO_2} and decreasing pH, suggests that the total CO_2 is reduced by extraction of CO_2 from the uterine sea water.

This rapid reduction of T_{CO_2} and decline in pH may be the result of either the active resorption of HCO_3^- (or OH^-) from the sea water in the uterine horns or the excretion of protons into the uterine sea water, with the concomitant resorption of HCO_3^- occurring by the diffusion of CO_2 gas across the uterine epithelium into the blood. If the latter is the case, the appearance of protons in the sea water would certainly not appear to be due to the secretion of lactic acid, since the concentrations of the lactate ion which were determined in the uterine sea water are very low (Table 1). Given these kinds of data, that is, measured changes in the pH, total ammonia and CO_2 content, it is impossible to distinguish among the alternatives (see Cameron, 1984, for a discussion). A more precise determination of the ion species moved and the manner by which transport occurs (i.e. Cl^-/HCO_3^- or Na^+/H^+ exchange, etc.) requires a more sophisticated analysis. Nevertheless, the reductions observed in both pH and T_{CO_2} are related at least stoichiometrically by the Henderson–Hasselbalch equation, and are probably related mechanistically in the establishment of the uterine seawater conditions we observed in experimental as well as the fresh-caught animals.

The increase in ammonia concentration seen in the uterine sea water may be secondary to this reduction in pH and the resulting P_{NH_3} gradient established from the blood of the mother to the uterine sea water. Ammonia would diffuse from the blood of the mother to the uterine sea water in the form of NH_3. Substantial P_{NH_3}

gradients of about 3 to 1 from maternal blood to uterine sea water are still present at pH 6, even when the uterine seawater T_{Amm} approaches 10 mmol l^{-1} (Kormanik & Evans, 1986). Therefore, it is not necessary to postulate any active process for ammonia excretion into the uterine sea water, such as the excretion of NH_4^+, but such a process cannot be ruled out at this time.

The rate of increase of the ammonia concentration in the uterine seawater is far slower than the decrease in either T_{CO_2} or pH we observed in these experiments. To approach the levels of total ammonia we observed in fresh-captured females of up to 22 mmol l^{-1} ammonia (average = 10 mmol l^{-1}, Kormanik & Evans, 1986) might take nearly a week. Only a few of our females had uterine sea water ammonia concentrations approaching 10 mmol l^{-1} even after 2–3 days, and in the absence of any uterine flushing, since the latter was prevented by our experimental protocol. This period may be slightly overestimated, since the presence of pups in the uterine sea water would probably increase the rate of appearance of ammonia in uterine sea water (Kormanik & Evans, 1984). Nevertheless, incomplete uterine flushing *in situ* would certainly serve to increase the time it takes for the ammonia concentration to build up, and frequent flushing would prevent the build-up of ammonia (and acidity as well).

The experiments where pups were placed in high (10 mmol l^{-1}) ammonia sea water are especially interesting regarding the role of a low uterine seawater pH. In both series of experiments, the total ammonia concentration was the same, but the direction of the P_{NH_3} gradient was varied by adjusting the pH. The data in Tables 2 and 3 are in sharp contrast and are instructive on two points. First, high blood levels of ammonia appear to be toxic to the pups, presumably due to the elevated levels of NH_3 and/or NH_4^+ of the blood. Thus, the acidification of the uterine sea water by the mother that we observed would serve to protect the developing pups from increases in blood ammonia in the high ammonia uterine seawater environment, by maintaining a P_{NH_3} gradient directed from the blood to the uterine sea water. Second, ammonia movement appears mostly to follow the P_{NH_3} gradient, which suggests a predominance of NH_3 diffusion. Thus ammonia excretion by the pups can be maintained in the face of a steep total ammonia gradient if the P_{NH_3} gradient is directed from blood to sea water, regardless of the NH_4^+ gradient, at least in these experiments. These data support the suggestion that, at least in this elasmobranch, ammonia moves predominantly in the form of NH_3 and that the gills of the pups as well as the uterine epithelium of the mother are relatively impermeable to NH_4^+ (Cameron, 1986; Kormanik & Cameron, 1981; Kormanik & Evans, 1986).

These data certainly indicate that the uterine conditions we observed in fresh-caught, late-term pregnant females, that is low pH, low CO_2 content and high ammonia concentration (Kormanik & Evans, 1986), can occur where uterine flushing is prevented even in the absence of pups. The decline in pH and total CO_2 content in freshly introduced uterine sea water occurs rather rapidly, and ammonia builds up rather more slowly. While the acidity detoxifies ammonia, also promotes its accumulation and thus establishes a rather circular argument.

The central question then, still remains. Why are these conditions established in the first place, when it would appear to be relatively easy for the mother to flush the uterine horns periodically with fresh sea water as Burger (1967) suggested? One possible explanation is that longer retention of uterine sea water should help to reduce exposure of the developing pups to seawater-borne pathogens. The retention of uterine sea water can occur for longer intervals only if the ammonia which accumulates is detoxified by acidification. However, the results presented here demonstrate that the major source of ammonia in the uterine sea water is the mother. Therefore, a second possibility is that the uterine ammonia may act as a source of nitrogen for the pups developing *in utero*. This is currently under investigation in our laboratory.

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